Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

syndrome of cryptal necrosis in the intestine, resembling panleukopenia, but without lymphoid or bone marrow depletion. **Astrovirus**, enteric **coronavirus**, **calicivirus**, **rotavirus**, and other viruses are reported from the feces of cats, but are only rarely associated with diarrhea, and even less frequently associated with mortality. A **torovirus** has a questionable association with a syndrome of diarrhea and bilateral protrusion of the nictitating membrane. Diarrhea is a common sign in cats with ***Feline immunodeficiency virus*** infection, and may occur in cats with *Feline leukemia virus* infection. Bacterial infections such as **salmonellosis**, **shigellosis**, **yersiniosis**, **helicobacteriosis**, and **Tyzzer's disease** are unusual, as is significant parasitic enteritis due to **helminths**, ***Cystoisospora***, ***Toxoplasma***, ***Giardia***, or ***Cryptosporidium***. Chronic inflammatory small-bowel disease and the forms of colitis occasionally encountered in cats have been considered in previous sections.

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INFECTIOUS AND PARASITIC DISEASES OF THE ALIMENTARY TRACT

Viral diseases of the alimentary tract

Foot-and-mouth disease

Foot-and-mouth disease (FMD) is a highly contagious viral infection of all cloven-hoofed animals. It is a problem of worldwide concern, being enzootic in large areas of Africa, Asia, and parts of Europe and South America. Presence of the virus imposes serious trade restrictions that effectively thwart the development of a healthy agricultural economy.

FMD is an acute febrile condition characterized by the formation of vesicles in and around the mouth, on the feet, teats, and mammary glands. The disease is not notable for high mortality, except in sucklings, but morbidity is very high, with a concomitant loss of productive efficiency.

FMD virus (FMDV) belongs to the genus *Aphthovirus* (aphtha = ulcer) in the Picornaviridae family. The virus is highly resistant under many circumstances, but is inactivated by direct sunlight, due to drying and increase in temperature, and by moderate acidity (pH < 5.0). The acid production that accompanies rigor mortis in carcasses and meat inactivates the virus. However, the alteration in pH is not dependable and the virus survives in offal, viscera, lymph nodes, and bone marrow for an indefinite period under refrigeration. Next to the movement of infected animals, contaminated animal products are likely the most common mechanism of spread. It may survive on hay and other fomites for several weeks.

The resistance of FMDV is of epidemiological significance, especially where control policies involve slaughter rather than vaccination. The importance of a carrier state in the epidemiology of FMD is uncertain. The carrier state has been observed in cattle, sheep, goats, and African buffalo (*Syncerus caffer*), but not in pigs. The carrier state may persist for up to 2 years postinfection in cattle, even in animals with a significant level of serum-neutralizing antibody. Sheep and goats are considered to be a frequent inapparent source of dissemination of the virus. African buffalo can carry FMDV for at least 5 years. Field outbreaks have been associated with buffalo–cattle contact in Africa, but these appear to be rare. Although Asian water buffaloes (*Bubalus arnee*) may be affected in FMD outbreaks, it is not known whether they remain carriers.

Of equal importance to the persistence of the virus is its *antigenic heterogeneity and instability*. There are seven principal antigenic serotypes, namely, the classical A, O, and C types, and SAT-1, SAT-2, SAT-3, and Asia-1. These can be distinguished by serologic tests. Six of the seven serotypes (O, A, C, SAT-1, SAT-2, SAT-3) are known to occur in Africa, four (O, A, C, Asia 1) in Asia, and three (O, A, C) in Europe and South America, although recent pandemics are blurring these geographic distinctions. These serotypes are sufficiently different immunologically that *infection with one type does not confer resistance to the other six*. Within these seven major types there are antigenic subtypes, each different, to variable degrees, from the parent type. Generally, the subtypes cross-immunize to a useful degree, but exceptions do arise and become recognizable, especially when vaccination fails. *Antigenic drift* can also be demonstrated experimentally; new subtypes can be produced by passing the virus in immune or partially immune animals, or by growing the virus in vitro in the presence of immune serum. There are presently over 70 distinct antigenic strains of the virus of natural origin.

As well as differences and variability in antigenic characters, strains of the virus differ in virulence, and a given strain is probably able to vary in virulence. Certainly, comparing different outbreaks, there is considerable variation in the severity of the disease produced in a given host species. Virulence also varies among species. Although the vast majority of strains will affect a wide range of species, there have been occasional viruses that show a distinct predilection for one species. An example is the porcinophilic strain that originated in China in the late 1990s and spread to Taiwan, destroying the swine industry there.

The main portal of entry and primary site of viral multiplication are the pharynx and lung. Subsequent to the first round of replication, there is widespread viremic dissemination to surface epithelium, with subsequent development of lesions in sites of mechanical or physiologic stress, such as oral and pedal epithelium, or teats in lactating animals. FMDV probably gains entry to these areas via Langerhans cells, with replication in a contiguous group of cells in the stratum spinosum. The resulting cellular degeneration and lysis result in an **epidermal vesicle**, *which is the hallmark of the disease.*

Virus is present at high titer in the vesicular fluid, and is present in large amounts in expired air from acutely infected animals, which is the main source of spread – pigs in particular liberate large quantities of airborne virus. Virus persists in lesions for 3–8 days after the appearance of significant neutralizing titers in serum, but seldom beyond day 11 of clinical illness. It is believed that FMDV is localized in epithelial cells of the oropharynx during persistent infection, so that virus may be found in esophagopharyngeal fluid for a considerable period of time.

Within a week of development of neutralizing antibody, the titer of virus in circulation declines. Ordinarily, serum antibody titers decline progressively and fairly rapidly. The duration of persistence of antibody is correlated with the initial titer. In general, animals are resistant to reinfection with homologous strains by natural exposure for about 2–4 years; susceptibility increases as the antibody titer declines.

The characteristic **lesions** of FMD are only seen in those animals which are examined at the height of disease. Lesions heal or are obscured by secondary bacterial infection. Lesions develop mainly in areas subject to trauma: the *oral mucosa*, especially the *tongue*; the *interdigital cleft*; and the *teats* in lactating animals. In cattle, there is appreciable loss of weight and the buccal cavity may contain much saliva. In the living animal, there is diffuse buccal hyperemia and mild catarrhal stomatitis, but the hyperemia disappears at death. Vesicles form on the inner aspects of the lips and cheeks, the gums, hard palate, dental pad, and especially on the sides and rostral portion of the dorsum of the tongue. Sometimes they form on the muzzle and exterior nares. The primary vesicles are small, but coalesce to produce *bullae* which may be 5–6 cm across; these bullae rupture in 12–14 hours, leaving an intensely red, raw, and moist base to which shreds of epithelium may still adhere (Fig. 1.84A). The eroded to ulcerated area may be replaced by regenerated epithelium in less than 2 weeks. Secondary infection may complicate this course.

Foot lesions occur in the majority of cases. There is inflammatory swelling with blanching of skin of the interdigital space in ruminants, coronet in swine, and heels in all species a day or so before vesicles form. The swellings persist until the vesicles rupture and the resultant erosions heal; healing may be considerably delayed on the feet. Vesicles may also occur in the other sites, but much less frequently.

A *malignant form of the disease*, without vesiculation, does occur in young animals and occasionally in adults. In these, death is common, due to *myocarditis*. Poorly defined pale foci of variable size are seen anywhere within the ventricular muscle. Although historically referred to as "tiger-heart," these gross lesions are no different from those generated in any other syndrome of severe, acute myocardial damage, but necrosis of fibers may be striking. Chronic lesions include myocardial necrosis and scarring, pancreatitis with acinar necrosis and regeneration. Diabetes mellitus occurs in experimental cases, as does hypophysitis, leading to a constellation of endocrinopathies because of a range of expressions of pituitary dysfunction.

Sheep are, in general, less susceptible than cattle, and the infection runs a milder course, though there may be exceptions. Lesions may not develop. When they do, the *dental pad* is the preferred site in the oral cavity. Lingual lesions tend to occur on the caudal dorsal portion as underrunning necrotic erosions rather than vesicles. These are small and easily missed, and they heal within a few days. *Lameness* may be prominent in acute outbreaks. Typical vesicles develop in the interdigital cleft, on the coronet and bulb of the heel.

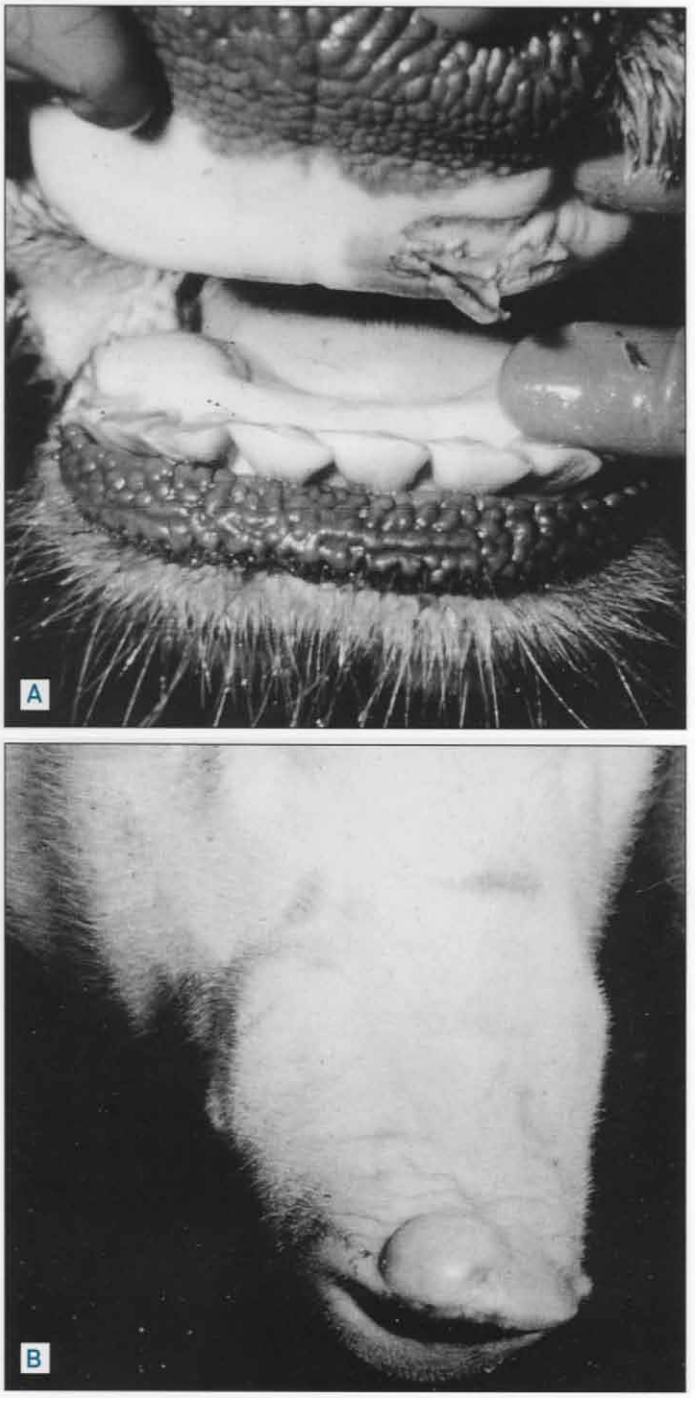

Figure 1.84 Foot-and-mouth disease. A. Ruptured vesicle on the dental pad of a cow. **B.** Vesicle on the snout of a pig.

They may occasionally involve the entire coronet and lead to eventual shedding of the hoof. Vesicles also occasionally occur on the teats, vulva, prepuce, and on the pillars of the rumen. The peracute form with myocardial necrosis may occur in lambs.

The disease in **goats** is, in general, similar to that described for sheep. Both species may be inapparent carriers, and many outbreaks worldwide have been due to transport of inapparently infected small ruminants.

In **pigs**, lesions occur in the usual sites, although more commonly on the feet than in the mouth. They may be present on the

snout and behind its rim (Fig. 1.84B), and on the teats of lactating sows. Abortion and stillbirth of infected piglets are recorded. The peracute form, with high mortality due to myocarditis, occurs in sucklings, often before vesicle formation is noticed in sows.

FMD must be differentiated from other viral vesicular diseases such as vesicular stomatitis, vesicular exanthema, and swine vesicular disease in susceptible species, and in the latter stages, from diseases producing erosive/ulcerative lesions of the oral cavity. The disease must be considered in the differential diagnosis in cases of sudden death among cloven-footed animals, especially the young. Definitive **diagnosis** requires virus isolation and characterization, demonstration of viral antigen by enzyme-linked immunosortbent assay, or detection of viral genome by polymerase chain reaction or reverse transcriptase-polymerase chain reaction in lesional material. The regulatory status of FMD dictates that this be carried out in accredited laboratories.

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Vesicular stomatitis

Vesicular stomatitis (VS) affects horses, cattle, and pigs, and may also affect wildlife species such as white-tailed deer, raccoons, feral swine, and some rodents. In humans, the virus may cause an inapparent infection or a mild influenza-like condition. Experimentally, various rodent species are susceptible and persistence has been demonstrated in hamsters. Also, persistence of viral RNA has been shown in both experimentally and naturally infected cattle, but the reservoir of the virus is unknown. *The disease is important because it causes a loss in production, especially in dairy herds, and it must be differentiated from foot-and-mouth disease in cattle and pigs.* VS is the only vesicular disease naturally occurring in horses. Sheep and goats do not appear to be susceptible to the disease, but a severe, though nonfatal, influenza-like syndrome may occur in infected humans.

VS virus (VSV) belongs to the Rhabdoviridae family, genus *Vesiculovirus* (type species: *VS Indiana virus* (VSIV)). It is an enveloped single-stranded RNA virus, bullet-shaped and about 80 × 120 nm. Apart from being inactivated by pasteurization temperatures, it shares qualities of resistance with the aphthoviruses. There are several serologically and immunologically distinct types of virus based on epitopes of the surface glycoproteins, including New Jersey, Indiana, Piry, Isfahan, and Chandipura. The two most common serotypes of VSV infecting domestic animals in the Americas are New Jersey and Indiana.

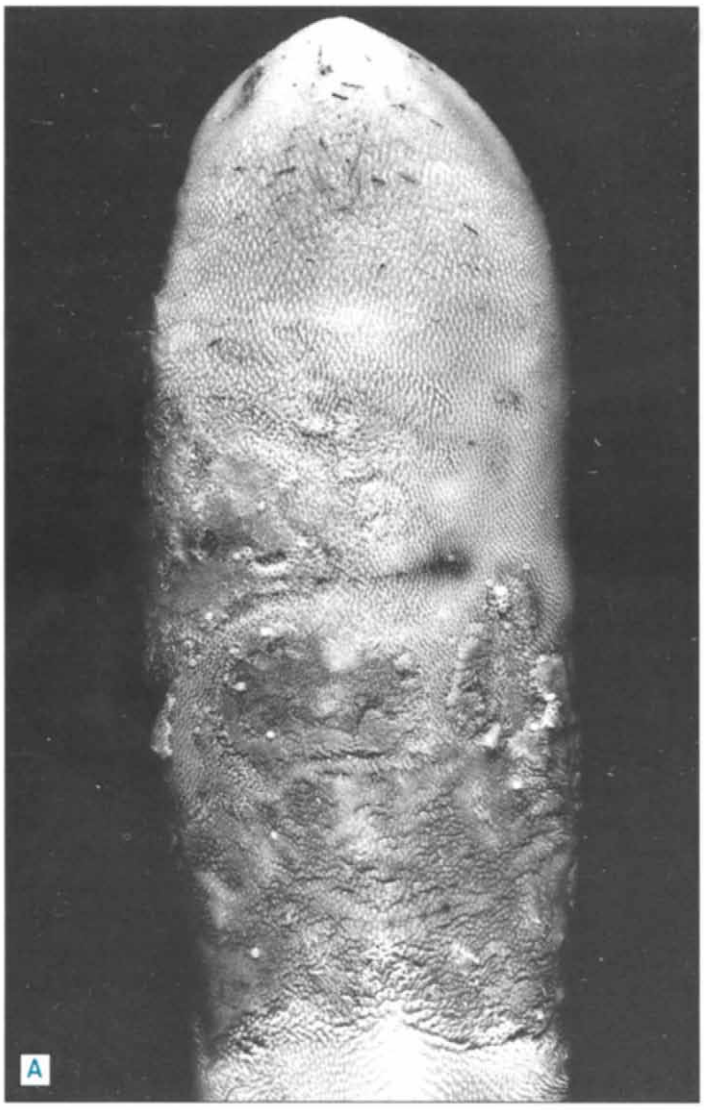
VS is enzootic in Central and South America and occurs sporadically elsewhere in the Americas. It has a seasonal occurrence; outbreaks occur in the warmer seasons and usually cease with the onset of cold weather. The seasonal nature of the disease suggests that it is transmitted by *insects*; however, insect transmission is not essential and *contact transmission* has been proven experimentally. VSV has been isolated from both biting and nonbiting insects and black flies have transmitted the virus to pigs. Biting insects most likely become infected from feeding on lesions rather than blood, since viremia is transient, if present. Nonbiting insects act as mechanical carriers of the virus. It is not known how the virus spreads from one geographic area to another. There is some indication that VSVs adapt to specific regions, developing distinct genotypes based on specific vectors or reservoirs in an ecological area. The intact mucosa is resistant to infection, but abrasions in a susceptible site readily result in infection when contaminated with saliva or exudate from a lesion. Environmental factors that increase the chance of causing abrasions to the skin, teats, or oral mucosa may predispose to infection.

Morbidity in lactating dairy cows may be as high as 100%, although only about 60% of the affected animals drool or froth around the mouth. The lesions of VS occur mainly on the *oral mucosa*; occasionally they do occur elsewhere, including on the *feet*, and in swine, foot lesions are common. This is by no means a dependable feature, and outbreaks of the disease in cattle have been described in which the lesions were predominantly on the *teats*.

The incubation period following exposure by abrasion is 24–72 hours and the viremic phase seems to be short-lived, because the virus cannot be cultured from blood. Secondary lesions are rare. In cattle, intramuscular injections will not initiate the disease, a distinguishing feature from foot-and-mouth disease. After experimental infection of swine, infectious viral particles can be recovered from a wide variety of tissues within 6 hours postinfection, including salivary gland, tonsils, snout, skin, and lymph nodes. However, infective virus, viral antigens, and nucleic acids cannot be demonstrated 6 days postinfection.

Specific serum neutralizing antibodies persist for months in swine and years in cattle. There is no evidence that animals with persistent antibodies may act as a source of infection to herd mates. Animals are immune against the homologous but not the heterologous strains of the virus.

The lesions of VS are indistinguishable from those of foot-and-mouth disease (Fig. 1.85A). Initially, in cattle, there is a raised flattened pale-pink to blanched papule a few millimeters in diameter in or near the mouth. These papules rapidly become inflamed and hyperemic. In the course of a day or so, they develop into vesicles 2–3 cm in diameter and by coalescence may involve large areas. The shallow erosions which follow rupture of vesicles heal within 1–2 weeks unless secondary infections occur; in the mouth, the latter are common. Oral and mouth lesions heal rapidly in swine, but coronary band lesions often become secondarily infected to the point where the claw may separate and slough. Serous rhinitis, with the development



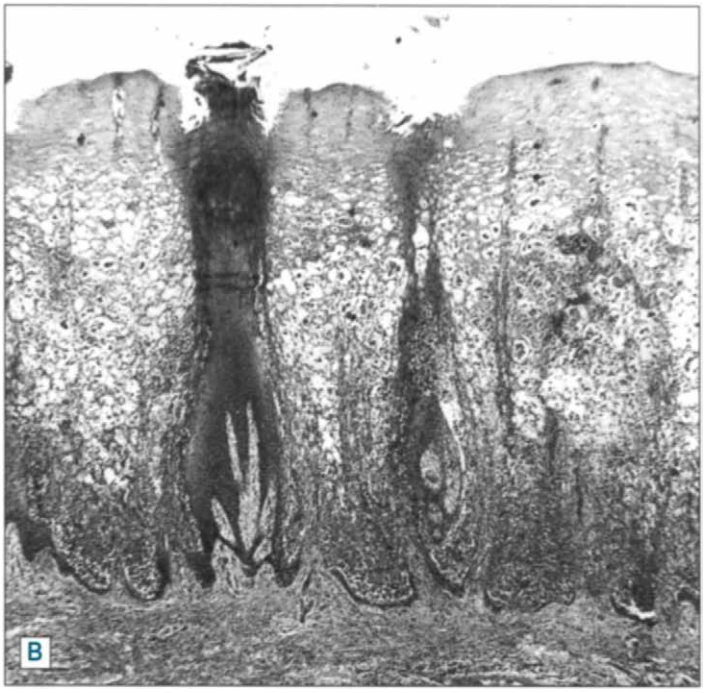


Figure 1.85 Vesicular stomatitis. A. Erosion of vesicular lesions in tongue at 4 days post-inoculation of *Vesicular stomatitis virus*. **B.** Intraepithelial vesicle formation. (Courtesy of Seibold HR, Sharp JB Jr. Am J Vet Res 1960;21:35–51.)

of tags of necrotic mucosa, has been described in experimentally infected swine.

The first microscopic changes are seen in the deeper layers of the stratum spinosum, where the virus replicates. Increasing prominence of the intercellular spaces and stretching of the desmosomes are accompanied by a reduction in volume of the cell cytoplasm (Fig. 1.85B). This dissociation of cells proceeds to distinct intercellular edema (*spongiosis*) followed by further cytoplasmic retraction until the affected epithelial cells float freely in enlarging vacuoles which in turn are loculated by strands of cytoplasmic debris (Fig. 1.86). There is no hydropic degeneration of the epithelial cells and the nuclei until now remain normal. *There are no inclusion bodies.* With the onset of epithelial cell necrosis there is a pleocellular inflammatory reaction in the mucosa and underlying lamina propria. Electron microscopic examination of epithelial cells adjacent to the vesicles confirms the intercellular edema and keratinocyte necrosis seen under the light microscope. The microscopic appearance of the lesions is not diagnostic.

In light of the similarity of VS to foot-and-mouth disease, *laboratory confirmation of VS is essential.* Vesicular fluid and mucosa from the tongue are good sources of the virus. *Diagnosis* is accomplished through virus isolation in tissue culture or embryonated eggs, fluorescent antibody techniques, complement fixation to identify viral antigen, polymerase chain reaction, and inoculation of suckling mice.

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Figure 1.86 Edge of gross vesicle in **vesicular stomatitis**. (Courtesy of Seibold HR, Sharp JB Jr. Am J Vet Res 1960;21:35–51.)

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Vesicular exanthema of swine

Vesicular exanthema (VE) of swine is an acute, febrile disease of swine that is characterized by formation of vesicles on the snout, mouth, nonhaired skin, and feet. *The lesions are indistinguishable from those of foot-and-mouth disease, vesicular stomatitis, and swine vesicular disease.* VE of swine was first diagnosed in California in the 1930s and eventually spread to most swine-producing states in the USA. An eradication campaign was undertaken and the last reported outbreak of VE was in New Jersey in 1956.

VE of swine virus (VESV) is the type species of the genus *Vesivirus*, family Caliciviridae. It has a single-stranded RNA genome and has only one major polypeptide. It is about 35–40 nm in diameter and characteristic cup-shaped structures (calyces) are evident in electron microscopic preparations. There are 13 immunologically distinct serotypes, which vary in virulence.

In 1973, a virus that is biophysically and morphologically similar to VESV was recovered from sea lions (*Zalophus californianus*) with vesicles on their flippers, off the coast of California near San Miguel island. Several strains of this virus, called *San Miguel sea lion virus* (SMSV), produce milder but otherwise identical lesions to those of vesicular exanthema when inoculated into swine, and SMSV is classified as a serotype of VESV. The host range of SMSV is very broad. One serotype, SMSV-7, has been isolated from opaleye fish (*Girella nigricans*) and it produces lesions identical to VE when inoculated into swine, with horizontal transmission to contact swine. It is now thought that VE of swine arose through the feeding of ocean fish to swine, with some adaptation of the virus allowing for very efficient spread through swine.

Most outbreaks of VE were associated with feeding of raw garbage containing pork waste, indicating that the disease was transmitted by direct contact and fomites. VESV now exists only as viral stocks archived in freezers and should be considered a *disease of historical significance only*. The possibility of a recurrence remains, however, if swine are fed uncooked tissues from ocean-origin fish or marine mammals.

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Swine vesicular disease

Swine vesicular disease (SVD) is a highly contagious viral disease of pigs that is characterized by formation of vesicles around the coronary bands and heels of the feet, and, to a lesser extent, on the mouth, lips, tongue, and teats. *Clinically, the disease is indistinguishable from the other vesicular diseases of swine*, including foot-and-mouth disease, vesicular stomatitis, and vesicular exanthema of swine.

The disease was first recognized in Italy in 1966 and it has since been reported from Hong Kong, the UK, continental Europe, and Asia. The economic importance of SVD is related less to the rather limited losses in production and more to the fact that it is difficult to differentiate from other vesicular diseases and hence restricts trade.

Swine vesicular disease virus (SVDV) is a small RNA virus that belongs to the family Picornaviridae, genus *Enterovirus*. It is a porcine variant of Human coxsackievirus B5, which is a serotype of *Human enterovirus B*. SVDV is highly resistant to environmental factors. Unlike foot-and-mouth disease virus, it is not inactivated at the low pH in muscle commonly associated with rigor mortis.

Many outbreaks of SVD appear to originate by feeding raw garbage containing pork products. Transmission within and among affected herds is by direct contact, especially during the early stages of the disease, or by exposure to the virus in the environment, where it is very persistent. The portal of entry is most likely oral or by exposure of excoriated skin. Following contact with infected pigs, vesicles develop within 2 days and consistent virus isolation from tonsil is possible for 1–7 days. Viremia lasts for 2–3 days. SVDV has a strong affinity for the epithelial cells of the coronary band, tongue, snout, lips, lymphoid follicles of the tonsils, myocardial cells, and brain. Virus titers in tissue decrease with the appearance of circulating antibodies, which peak after 2–3 weeks and apparently persist for years. Secretions and excretions have high viral titers for a period of 12–14 days. Feces may contain virus for up to 3 months.

There is some evidence that pigs may become carriers, with stress reactivating viral shedding several months postinfection.

Clinically, *vesicles are most common on the feet*. Oral lesions occur in only about 10% of affected pigs. The foot lesions appear first at the junction between the heel and the coronary band. Initially, there is a 5-mm wide, pale, swollen area that encircles the digit. In later stages a 1-cm wide band of necrotic skin is located along the coronet. Vesicles on the mouth, lips, and tongue occur in clusters and they are small, ~2.0 mm in diameter, white, and opaque. They coalesce and rupture within 36 hours and may be covered by a pseudodiphtheritic membrane due to secondary bacterial infections. Affected pigs usually recover in 2–3 weeks.

The development of vesicles tends to follow a similar course as that reported for foot-and-mouth disease. The virus infects individual epithelial cells in the stratum spinosum, which leads to focal areas of keratinocyte degeneration and vesicle formation. There is an intense leukocytic reaction in the necrotic areas, which is mainly neutrophilic. As with the other vesicular diseases, after 1 week there are indications of epithelial regeneration.

Nervous signs and lesions of *nonsuppurative meningoencephalomyelitis* have been reported in field outbreaks and reproduced experimentally in SVD. Lesions involve most areas in the brain and sometimes spinal cord, and are centered on ganglia and spinal nerve roots. Clinically, the severe lameness tends to overshadow any nervous signs that might be present.

Laboratory diagnosis depends on demonstration of the agent by virus isolation, antigen capture enzyme-linked immunosorbent assay or polymerase chain reaction, in accredited laboratories, on account of its regulatory status.

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Bovine viral diarrhea

Bovine viral diarrhea virus (BVDV) is an RNA virus of the *Pestivirus* genus in the family Flaviviridae. It is widespread in cattle populations and it or closely related viruses can infect most even-toed ungulates, including swine. As an RNA virus, BVDV is highly mutable, due to the error-prone nature of the RNA polymerases responsible for replication of viral RNA. As a result, "swarms" of viral mutants form "quasispecies" that circulate within an infected individual and among individuals in a population. While most quasispecies lack a selective advantage, or suffer deleterious point mutations, preventing them from becoming dominant, the ability to generate mutants enables BVDV to adapt to host responses, and to establish chronic or persistent infections in some circumstances. While low virulence would seem to promote prolonged viral shedding, there may be advantages in high virulence that favor the emergence of quasispecies capable of causing severe disease and high virus shedding.

Point mutation and recombination have produced a number of genotypes of *Pestivirus*, including *Classical swine fever virus* and *Border disease virus*. Two genotypes of BVDV occur, **type 1** (BVDV-1) and **type 2** (BVDV-2), each of which has a number of closely related subgenotypes, many of which circulate widely. Viral genotype may be linked to specific manifestations of BVDV infection; noncytopathic (NCP) BVDV-2 has been associated with thrombocytopenia, for instance. However, there is a range of virulence among both type BVDV-1 and BVDV-2 isolates, varying from subclinical infections or mild clinical disease to severe fatal syndromes.

Recombination of RNA from homologous (BVDV) or heterologous (other viral or host) sources, usually involving the region encoding the nonstructural protein NS2–3 of noncytopathic virus, results in a shift in biotype of either genotype of virus, from the more common **NCP biotype**, in which inapparent persistent infection is produced in cultured cells, to a **cytopathic (CP) biotype**, capable of inducing cytoplasmic vacuolation and apoptotic death of cells in tissue culture. Recombination splits NS2–3, resulting in a small NS3 protein, which induces apoptosis, and is a marker for CP BVD viruses. Reversion of CP viruses to an NCP biotype also occurs, less commonly. *Cytopathogenicity in vitro is not directly related to virulence in vivo.*

BVDV gains access to the oropharyngeal mucosa by ingestion or inhalation, and primary replication is in oropharyngeal lymphoid tissues, including tonsils. The outcome of the ensuing viremia is a product of the genotype and virulence of the virus, the immune status of the host, whether or not the animal is pregnant, and, if so, the stage of pregnancy.

Infection of immunocompetent, seronegative, nonpregnant animals usually results in subclinical infection or mild clinical disease. Affected animals develop a slight fever, leukopenia, and specific neutralizing antibodies, the outcome in 70–90% of BVDV infections. In a few situations, animals, mainly over 6 months of age, develop a more obvious clinical syndrome, with a high morbidity and low mortality – classical **bovine viral diarrhea (BVD)**. The infecting agent is usually an NCP BVDV. After an incubation period of 5–7 days, the affected animals develop a fever, leukopenia, and viremia that may persist up to 15 days. The virus is present in leukocytes (buffy coat), especially lymphocytes and monocytes, and in plasma. There is a transient decrease in the number of B and T lymphocytes and a decline in responsiveness to mitogen stimulation. Clinically, the disease is characterized by lethargy, anorexia, mild oculonasal discharge, and occasional mild oral erosions and shallow ulcers. Diarrhea may occur. In dairy herds there is a transient drop in milk production. Affected animals develop neutralizing antibodies that peak in 10–12 weeks, and probably are immune for life.

A syndrome of **severe acute BVD**, characterized by high morbidity and mortality in all age groups of susceptible animals, has been recognized since the early 1990s. Sometimes termed "BVD type 2," since it is caused mainly, though not exclusively, by primary infections with type 2 virus, this syndrome usually has a peracute to acute course, with fever, sudden death, diarrhea, or pneumonia. It should be noted that not all BVDV-2 isolates are highly virulent. In some cases a **thrombocytopenic syndrome**, characterized clinically by epistaxis, hyphema, mucosal hemorrhages, bleeding at injection sites, and bloody diarrhea, is superimposed on the alimentary

syndrome caused by type 2 BVDV, or occurs independently. The pathogenesis of BVD type 2 is most frequently linked to increased strain virulence. However, production of inflammatory cytokines, in response to widespread infection of mononuclear phagocytes, has also been postulated as a cause for the severe disease seen clinically. The mechanism of thrombocytopenia is not completely defined, although infected megakaryocytes in the bone marrow undergo necrosis.

Fetal infections may occur in pregnant immunocompetent seronegative acutely infected females, and in persistently infected counterparts. The outcome of fetal infection is primarily dependent on the stage of gestation. The most serious consequences occur if an NCP BVDV crosses the placental barrier during the first 4 months of gestation. It may result in fetal resorption, mummification, abortion, congenital anomalies, or a persistently infected calf. If the calf survives, it remains viremic for life, and it is also immunotolerant to homologous NCP BVD viruses, due to failure of the immature fetal immune system to recognize the infecting viral antigens as "not-self" or foreign.

Persistently infected (PI) calves may be clinically normal, weak, or undersized at birth. They may appear normal, but are often unthrifty, and may have a rough or curly hair coat. The prevalence of these calves in a herd is usually less than 2%, but may be as high as 25–30% in herds where a large number of naive cows, early in pregnancy, have been exposed to NCP BVDV. *Most PI calves succumb to mucosal disease* (see below), usually between the ages of 6 months and 2 years. The offspring of the few animals that reach sexual maturity and become pregnant are also persistently infected, which can result in families of animals persistently infected with BVDV. PI animals are viremic, and lack antibody to the infecting virus, which they shed constantly, acting as the *most important source of infection in the population*.

In PI animals, virus is present in a wide variety of tissues, and antigen can be demonstrated by immunohistochemistry in skin biopsies – in keratinocytes, hair follicle epithelium, hair matrix cells of the hair bulb, and dermal papillae. Use of skin biopsies for diagnosis of persistent infections by immunohistochemistry or enzyme-linked immunosorbent assay has been exploited diagnostically, but it should be recognized that acutely infected animals may have virus in skin biopsies as well. Lesions in PI animals are minimal and subclinical, in spite of the widespread infection of virtually all tissues.

Mucosal disease is a clinicopathologic syndrome occurring in PI animals that subsequently become infected with a closely related CP strain, or probably more commonly, when the virus causing persistent congenital infection spontaneously develops a recombination encoding NS3. The result is an overwhelming infection that destroys cells, and to which the animal is incapable of responding. Characterized by *low morbidity but very high mortality*, mucosal disease most commonly occurs in cattle that are 6 months to 2 years of age. While deaths may occur within a few days of illness, and almost always within 2 weeks, some cases may survive for months. The incubation period after experimental infection with a CP strain in an animal persistently infected with an NCP BVDV is usually 7–14 days, but may be considerably longer.

Basically, there are *two forms of clinically severe BVDV infection*: mucosal disease in persistently infected animals, and the more recently recognized severe acute form of BVD caused by primary infections with very virulent strains of virus. At necropsy, one cannot confidently differentiate spontaneous cases of severe acute BVD due to BVDV-1 or BVDV-2 from each other, or from cases of mucosal disease, other then by the more hemorrhagic character of some cases of severe acute BVD due to type 2 isolates. Tentative differentiation of mucosal disease from severe acute BVD rests on the epidemiologic picture, and antigenic or molecular characterization of the involved viruses is required for definitive diagnosis of the various syndromes.

Fulminant severe acute BVD or mucosal disease closely resembles rinderpest. At the onset the animal is febrile, with serous to mucoid nasal discharge. Discrete oral lesions are preceded by acute stomatitis and pharyngitis, the mucosae being hyperemic and pink and covered by a thin gray film of catarrhal exudate. There is severe diarrhea and tenesmus with feces containing little or no blood or mucus. Affected animals become lethargic, anorexic, and dehydrated; they have ptyalism, polypnea, and tachycardia, and may die quickly.

In more chronic cases, the development of the oral lesions is like that found in acute cases; however, by the time they die there is usually some evidence of healing. The watery diarrhea of the early phase gradually gives way to feces that are passed frequently, are scant in volume, and contain a large proportion of mucus flecked with blood. Late in the clinical course, there is lethargy, emaciation, ruminal stasis, and frequent attempts at defecation accompanied by severe tenesmus. Interdigital dermatitis, coronitis, and laminitis affecting all four feet may be present in chronically affected animals (Fig. 1.87A), resulting in lameness. In these too, the skin is dry and scurfy, especially over the neck, withers, back, perineal and preputial areas, and vulva, while that on the medial aspect of the thighs and forelegs becomes moist and discolored a dirty yellow.

At necropsy, the **gross lesions** vary considerably, especially in acute cases, in which either upper alimentary or intestinal lesions rarely may be absent, and less so in the chronic disease, in which a broader pathologic picture is often present, perhaps partially obscured by healing or evolution of lesions.

Crusts, erosions, and shallow ulcers are present on the muzzle and nares of many affected cattle. There is loss of epithelium from much of the oral cavity. The most conspicuous oral erosions are on the palate, on the tips of the buccal papillae (Fig. 1.87B), and on the gingiva. Many, especially on the papillae, the hard palate, and in the pharynx, are sharp punched-out ulcers, and expose a denuded, intensely hyperemic lamina propria. In more chronic cases, ulcers may have a margin of thickened proliferative epithelium. The tongue is not always affected; when present, lesions may be evident on all surfaces (Fig. 1.88A, B).

Esophageal lesions are usually present, most commonly in the upper third. In some acute cases, the lesions are shallow erosions, rather than ulcers. The erosions are more or less linear but otherwise irregular, have a dirty brown base, and little or no reactive hyperemia, and may be covered by shreds of necrotic epithelium in animals that have not been swallowing (Fig. 1.88C). In more advanced cases, discrete ulcerations occur. In many chronically affected animals, the ulcers are beginning to heal and have yellow-white slightly elevated plaques of proliferative epithelium at the periphery of the mucosal defect.

Lesions are found in the *reticulorumen and omasum*, but usually not in the esophageal groove. The ruminal content in chronically affected animals with prolonged anorexia is usually scant and dry. In most acute cases, the ruminal content is unusually liquid and putrid. The lesions on the wall of the rumen resemble those present elsewhere in the upper alimentary tract and, although they occur anywhere, they

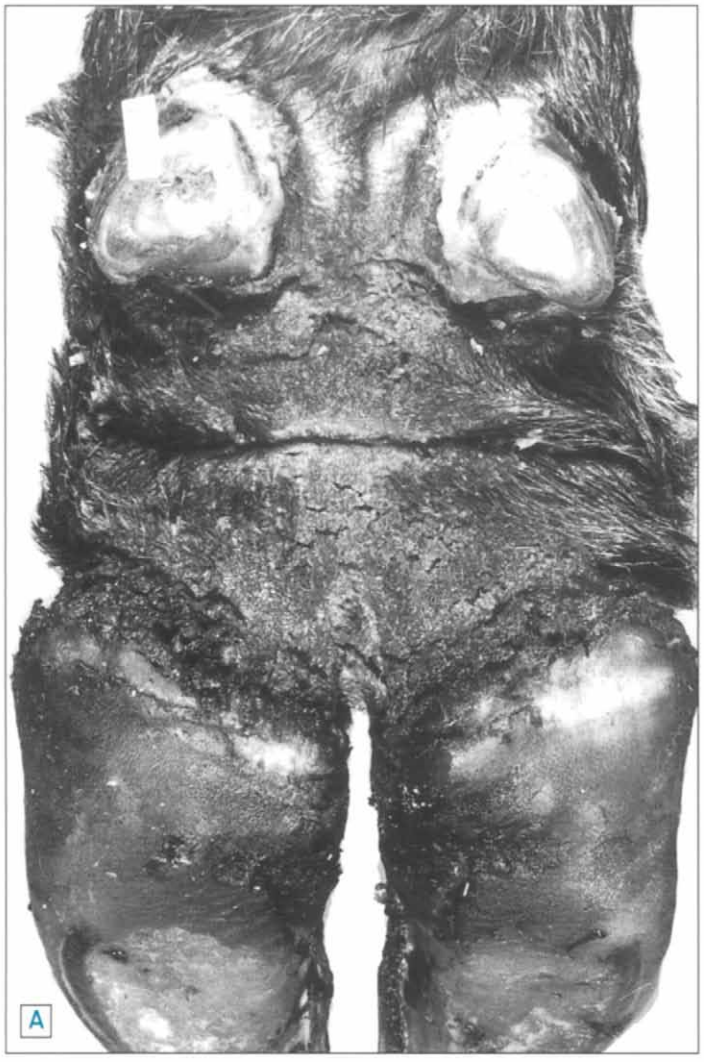


Figure 1.87 Bovine viral diarrhea. A. Coronitis and erosive-ulcerative dermatitis of pastern in BVD. **B.** Blunting of papillae on the buccal mucosa due to necrosis induced by BVDV infection. A few remaining normal papillae are long with sharp points.

are best seen on the pillars and other smooth or nonvillus portions of the mucosa (Fig. 1.89). The omasal lesions are most numerous along the edges of the leaves, sometimes causing a scalloped margin or perforation.

The morphogenesis of the lesions in the squamous mucosa of the upper alimentary tract begins with necrosis of the epithelium (Fig. 1.90). Individual cells and groups of cells deep in the epithelium are eosinophilic and swollen, with pyknotic nuclei. These foci enlarge progressively and form areas of necrosis that extend to, and may involve, the basal layer. In the early stages there is little or no inflammation of the lamina propria, but leukocytes infiltrate the necrotic epithelium. These necrotic foci enlarge progressively and by coalescence, and may form small cleavage vesicles along the proprial–epithelial junction (Fig. 1.91A), leading to erosions or ulcers as necrotic epithelium is abraded away. The ulcerations of the squamous epithelium of the upper alimentary tract are accompanied by inflammation in the lamina propria, especially where this forms papillae (Fig. 1.91B).

Changes are regularly present in the *abomasum*. The sides of the rugae bear ulcers that may be punctate to 1 cm or more in diameter (Fig. 1.92). The histological changes in the glandular epithelium of the abomasum are characterized by epithelial necrosis, mainly in the depths of the glands, and accompanying interstitial inflammation.

The mucosa of the *small intestine* often appears normal over much of its length. However, in some cases the mucosa of the small intestine may have patchy or diffuse congestion. In rare cases, fibrin casts may be in the lumen of the small bowel.

In acute cases, it is usual to find *coagulated blood and fibrin overlying and outlining Peyer's patches*, the covering of which is eroded. This, when present, is a very distinctive lesion that is only paralleled in rinderpest. Severely affected Peyer's patches are often obvious through the serosa as red-black oval areas up to 10–12 cm long on the antimesenteric border of the gut (Fig. 1.93A). Less acutely affected Peyer's patches may be overlain by a diphtheritic membrane, while in milder or more chronic cases the patches may be depressed and covered by tenacious mucus. Mesenteric lymph nodes may or may not be enlarged.

Lesions in the *large bowel* are highly variable. The mucosa may be congested, often in a "tiger-stripe" pattern following the colonic folds, a reflection of tenesmus. In acute cases there may be fibrinohemorrhagic typhlocolitis (Fig. 1.93B). In more chronic cases, fibrinous or fibrinonecrotic lesions and focal or extensive ulceration may be present at any level of the large bowel, but particularly in the cecum and rectum.

The characteristic **microscopic lesion** in the intestinal mucosa is *destruction of the epithelial lining of the crypts of Lieberkühn*. In the

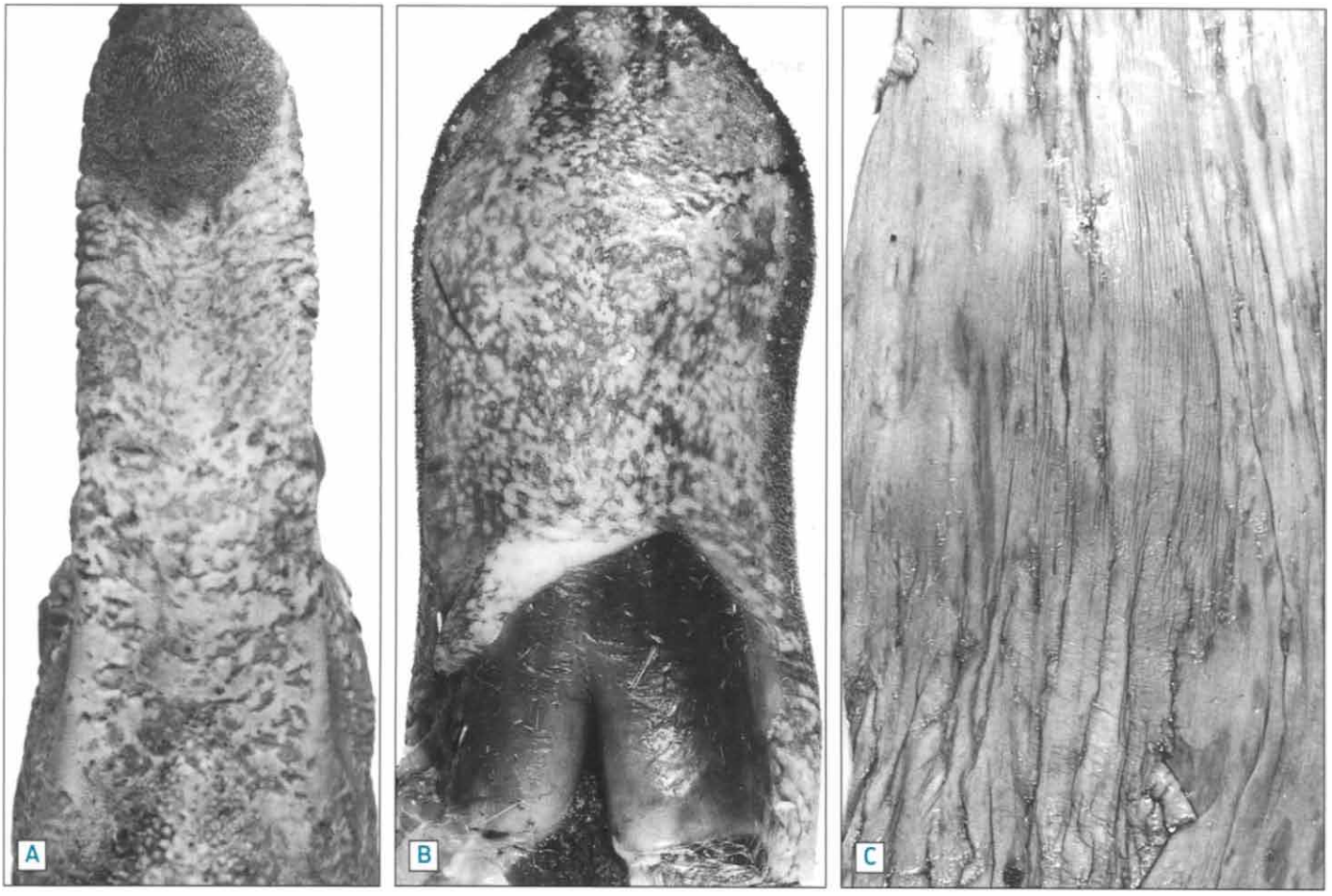


Figure 1.88 Bovine viral diarrhea. A. Dorsal surface of tongue showing multiple confluent ulcers. **B.** Ventral surface of tongue showing multiple confluent ulcers. **C.** Longitudinal erosions and ulcers on the esophagus.

duodenum, only a few crypts are affected, but more crypts are affected more severely in the lower reaches of the small intestine and in the cecum and colon. Affected crypts are dilated and filled with mucus, epithelial debris, and leukocytes. Remaining crypt-lining cells are attenuated in an attempt to cover the basement membrane. Reparative hyperplasia of crypt lining is rarely encountered. Crypt drop-out may be evident microscopically. In the cecum and colon, extensive damage to crypts and attendant collapse of the lamina propria are the probable cause of ulceration seen grossly (Fig. 1.94A). Congestion of mucosal capillaries, and in acute or ulcerated cases, effusion of fibrin and neutrophils from the mucosal surface may be evident.

The microscopic lesions of Peyer's patches are distinctive in BVD, comparable lesions being caused only by rinderpest. In the acute phase of the disease, severe acute inflammation in the mucosa over Peyer's patches accompanies almost complete destruction of the underlying glands, collapse of the lamina propria, and lysis of the follicular lymphoid tissues (Fig. 1.94B). Later in the course of the disease, dilated crypts, lined, at least in part, by cuboidal epithelium and filled with necrotic epithelial cells, mucus, and inflammatory cells appear to *herniate* into the submucosal space previously occupied by involuted lymphoid follicles. Peyer's patches should be sought assiduously at autopsy since their gross and microscopic appearance may provide useful evidence for diagnosis.

Microscopically, the mesenteric and sometimes other lymph nodes show a diminished population of lymphocytes and necrosis of germinal centers. By immunohistochemistry, there is a marked decrease in most lymphocyte subpopulations.

An important microscopic lesion is *hyaline degeneration and fibrinoid necrosis of submucosal and mesenteric arterioles* (Fig. 1.95A). A mild-to-moderate mononuclear inflammatory cell reaction is frequently present in the walls of the vessels and in perivascular areas. The vascular lesions may also be present in a variety of other organs, such as the heart, brain, and adrenal cortices, which may make it difficult to differentiate the disease from malignant catarrhal fever. The vascular lesions in acute mucosal disease are less consistently present and are usually milder, and there is *involution of lymphoid tissue in BVD*, in contrast to the lymphoproliferation characteristic of malignant catarrhal fever.

Coronitis may extend completely around the coronary band, with some separation of the skin–horn junction causing disturbance and overgrowth of the horn (see Fig. 1.87A). Dermatitis may extend from the coronet up the back of the pastern. Milder dermatitis is generalized, with scurfiness, especially from the ears to the withers. In sections of the skin of animals with chronic mucosal disease, there is hyper- and parakeratosis with focal accumulations of necrotic epithelium with intense hyperemia of the adjacent superficial dermis. The epithelial lesions are basically similar to those in the squamous mucosa of the

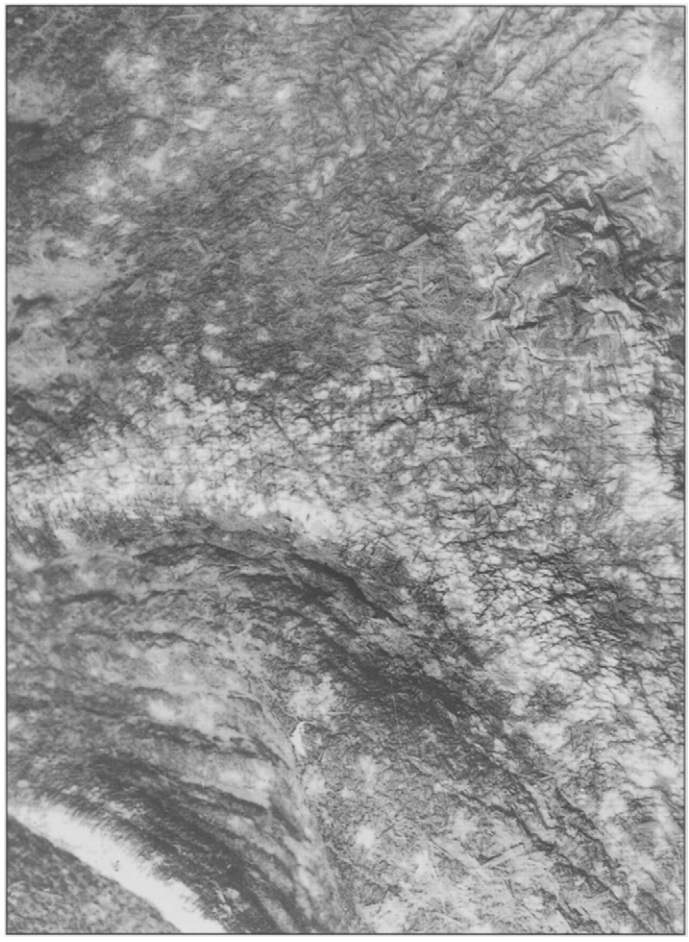


Figure 1.89 Focal and confluent often preulcerative, plaque-like lesions on mucosa of dorsal sac of rumen in **bovine viral diarrhea**.

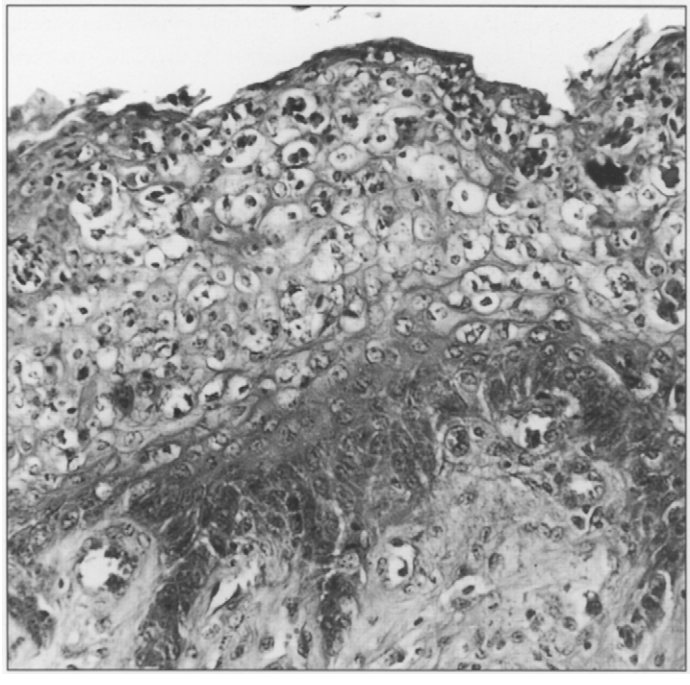
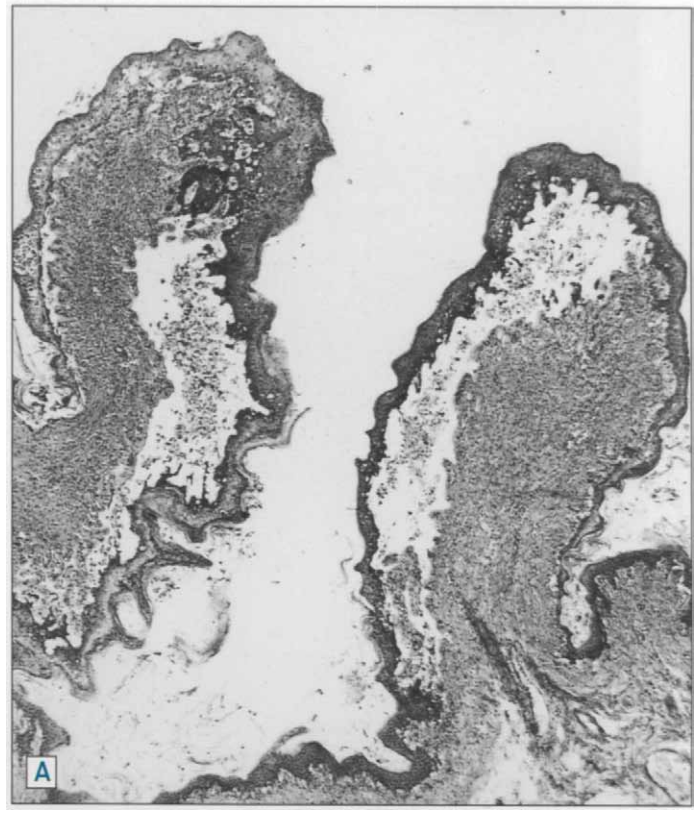


Figure 1.90 Histologic appearance of esophageal lesion in **bovine viral diarrhea**.



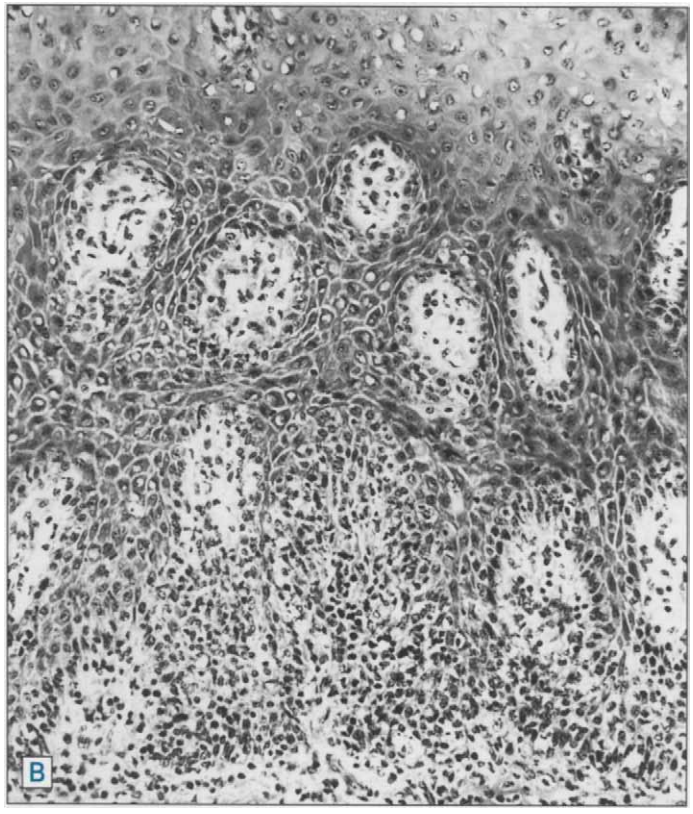


Figure 1.91 Bovine viral diarrhea. **A.** Cleavage vesicles in rumen papillae. **B.** Edema of proprial papillae, acute focal inflammation of papilla and propria. There is necrosis of scattered cells deep in the epithelium.

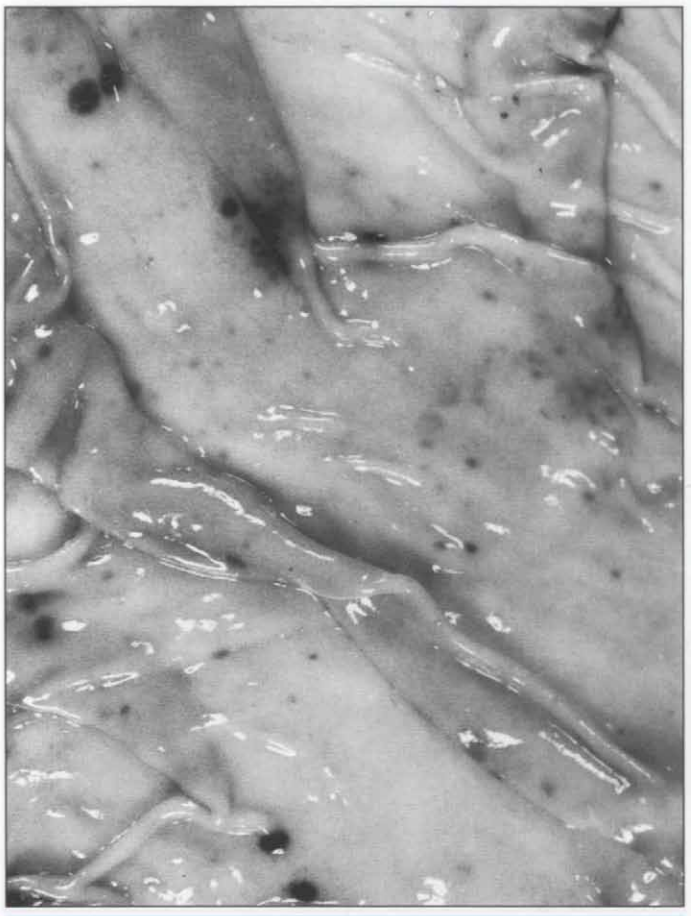


Figure 1.92 Hemorrhage and ulceration in the abomasum in **bovine viral diarrhea**.

upper alimentary tract (Fig. 1.95B). Necrosis often extends deeply to or through the basal layers; it results in minute erosions or ulcerations. There is massive infiltration of macrophages and some lymphocytes in the underlying dermis. These deeper lesions occur in the inner aspects of the legs and the perineum, and there is exudation of serum in these areas. The overlying degenerate epithelium becomes disorderly and eventually is lifted off.

Some animals with chronic disease develop *mycotic infections* secondary to lesions in the forestomachs, abomasum, and Peyer's patches. The lesions are areas of hemorrhagic necrosis involving the mucosa, submucosa, and sometimes deeper layers of the wall. Fungal hyphae are found invading the stroma and causing thrombosis in venules.

Infection of oocytes and cumulus cells in the ovaries has been well-documented, causing speculation concerning ovarian dysfunction and reduced fertility in animals surviving BVDV infection.

After *experimental inoculation with virulent BVDV-2*, animals are febrile by 7 days postinfection. Prominent clinical signs are anorexia, depression, and episodes of profuse watery and blood diarrhea that persist until the animal is moribund at 13–14 days postinfection. Pregnant animals may abort. Leukopenia and thrombocytopenia are often marked. In cases with severe thrombocytopenia, hemorrhage may be evident clinically.

Lesions are found in the digestive and respiratory systems. There is mild tracheitis, bronchitis, and bronchiolitis, which can progress to secondary bacterial pneumonia. Strains may vary in their ability to infect the pulmonary tree and result in disease. Intestinal lesions strongly resemble those seen in mucosal disease, with severe lymphoid depletion and necrosis of epithelial cells. However, with these BVDV-2 infections, there is often also a significant amount of hemorrhage evident externally, as described above, and there may

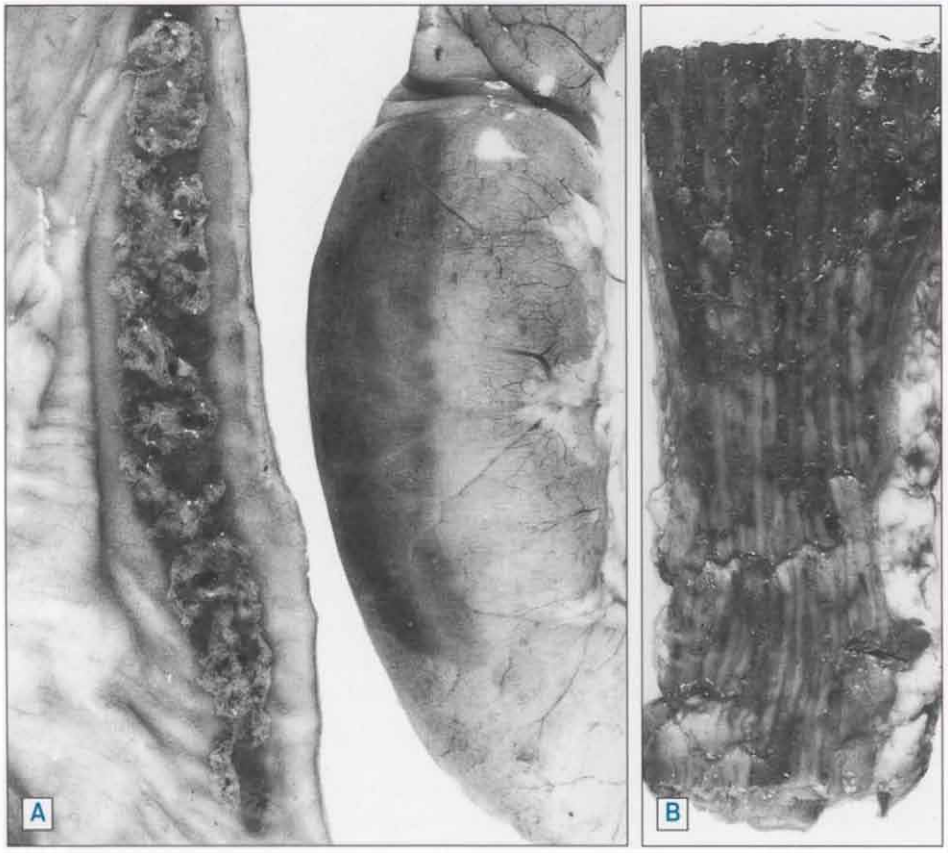


Figure 1.93 Bovine viral diarrhea. A. Fibrinohemorrhagic exudate over Peyer's patch in the ileum (left). Deep red Peyer's patch visible through serosa of small intestine (right). **B.** Fibrinohemorrhagic colitis.

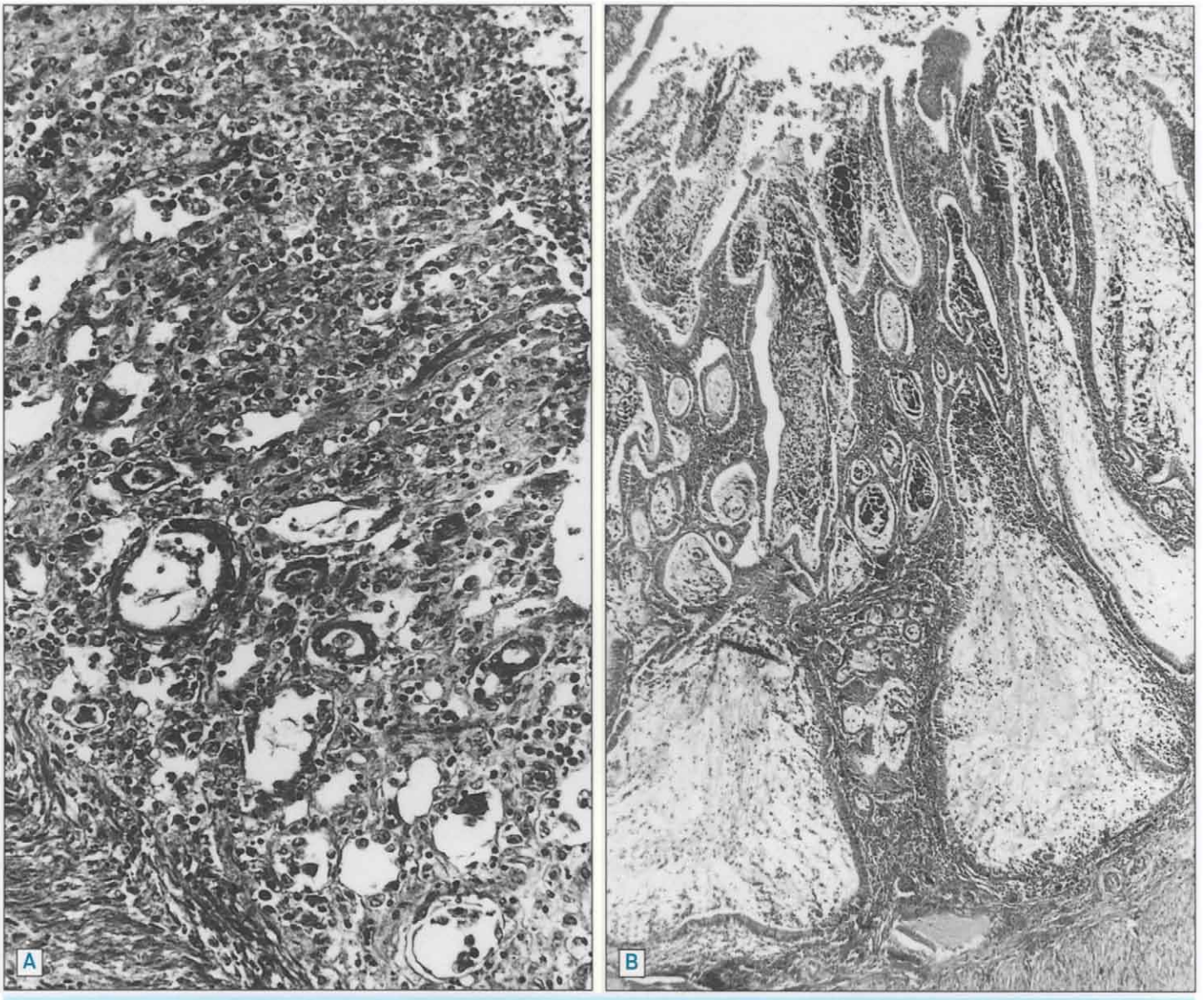


Figure 1.94 Bovine viral diarrhea. A. Colon, with dilated and denuded glands, collapse of lamina propria, and pseudomembrane formation. **B.** Herniation of crypts of Lieberkühn into the submucosa replacing necrotic lymphoid follicles in Peyer's patch. Mucus and inflammatory exudate are in the cystic glands and on the surface of the mucosa.

be extensive subserosal hemorrhages in the thoracic and abdominal cavities (Fig. 1.96). Similarly, edema may be more noteworthy in this form than in mucosal disease. Severe necrotizing vasculitis, especially arteritis, is noted in multiple organs but is most readily identifiable in lymphoid tissue. Meningoencephalitis associated with neuronal infection by BVDV-2 has been reported.

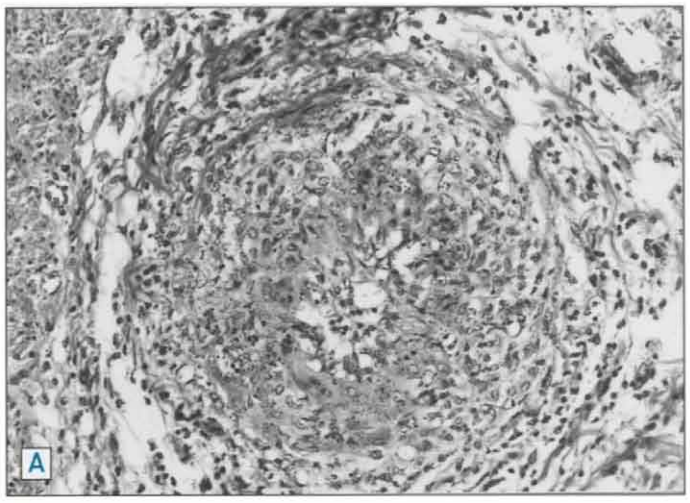
By immunohistochemistry, there is widespread viral antigen within epithelial cells (including oral and esophageal epithelium), smooth-muscle cells, and mononuclear phagocytes in multiple organs, although lesions often do not correspond to sites of antigen staining.

Bovine viral diarrhea virus and secondary infections

BVDV infection suppresses interferon production and impairs lymphocyte function, monocyte proliferation and chemotaxis, humoral antibody production, neutrophil function, and bacterial clearance. These changes are fairly persistent in chronically infected animals and in animals with mucosal disease. The failure of immunogenic response may be associated with immunotolerance, or destruction of immunocompetent cells, which is reflected in lymphopenia. In addition to a lack of humoral antibody response, there is also depression of cell-mediated immunity, as indicated by a poor response of cultured peripheral lymphocytes to various mitogens. The impairment of neutrophil function in cattle infected with BVDV may explain in part the apparent susceptibility of such cattle to *secondary bacterial infections.*

Fetal infection with *Bovine viral diarrhea virus*

In addition to the early embryonic death and abortions that can be ascribed to BVDV, infections of seronegative immunocompetent



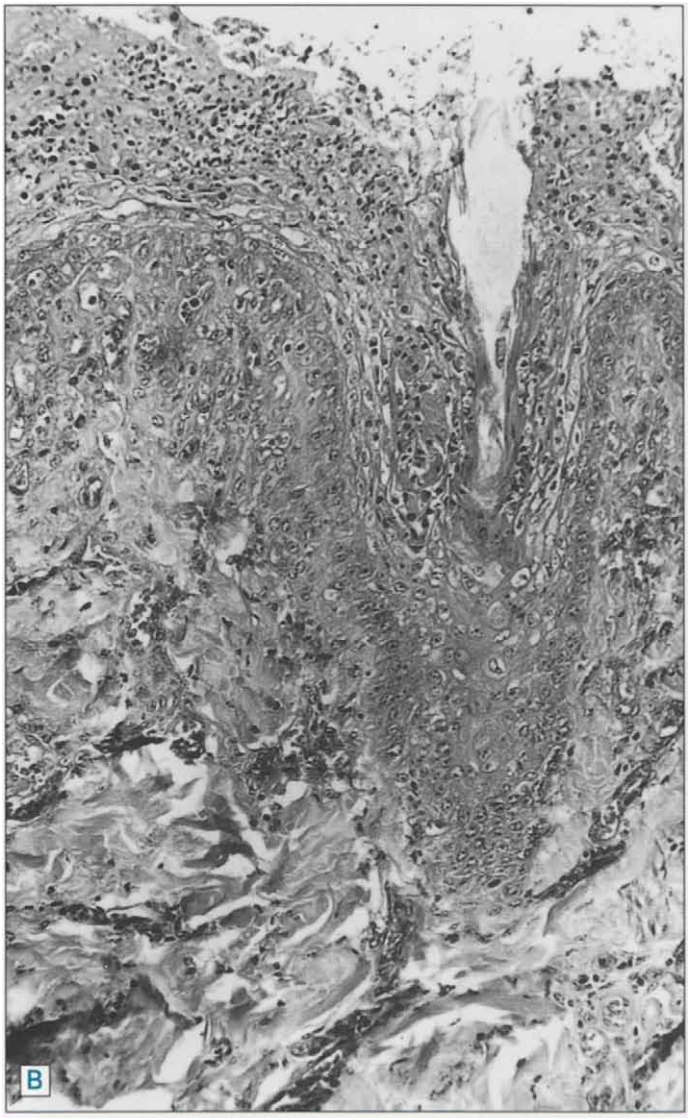


Figure 1.95 Bovine viral diarrhea. A. Fibrinoid necrosis and mild periarteritis of a mesenteric arteriole in the colon. **B.** Skin, with superficial epidermal necrosis extending into hair follicle and hyperplasia of the stratum germinativum.

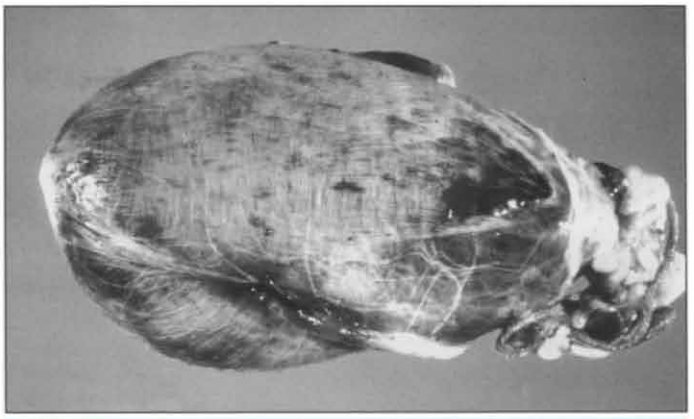


Figure 1.96 Hemorrhages on the serosal aspect of the bladder in the **thrombocytopenic form of bovine viral diarrhea**.

dams, usually between 90 and 120 days of gestation, may result in a wide spectrum of *teratogenic lesions*, including microencephaly, hypomyelinogenesis, cerebellar hypoplasia and dysgenesis, hydranencephaly, hydrocephalus, and defective myelination of the spinal cord. Ocular lesions, such as microphthalmia, cataracts, retinal degeneration, atrophy and dysplasia, and optic neuritis, have all been associated with fetal infections by BVDV (see Vol. 1, Nervous system; Vol. 1, Eye and ear; Vol. 3, Female genital system). Infections of the immunocompetent fetus, usually after 135 days of gestation, result in antibody production that is detectable in precolostral serum samples of the newborn calf.

Fetal infection later in gestation may produce lesions unrelated to teratogenesis. Alimentary tract lesions of the disease may be observed in the fetus. Punctate hemorrhages with ulcers 1–3 mm in diameter may be profuse in the oral cavity, excepting the dorsum of the tongue, and in the esophagus, larynx, trachea, conjunctiva, and abomasum. The fetal lesions of squamous epithelium evolve in somewhat the same manner as those described earlier, with focal hemorrhages in the lamina propria and epithelial necrosis beginning in the basal layer.

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Bovine viral diarrhea virus infection in pigs

The prevalence of naturally occurring antibodies to BVDV in swine in different countries varies between 2 and 40%. The antibodies may complicate the diagnosis of classical swine fever, especially in those countries considered to be free of this disease. Cattle, and modified live virus vaccines containing contaminated fetal bovine serum, are considered to be common sources of infection for swine. There are sporadic reports of disease associated with BVDV infection, including stillbirth, and poorly viable piglets, some showing tremors. Some 2–4-week-old pigs in infected herds are anemic, have a rough hair coat, growth retardation, wasting, and diarrhea. Affected pigs fail to develop neutralizing antibodies to the infecting homologous BVDV. Littermates that remain normal develop neutralizing antibodies. The suggestion is that the infections are congenital. Experimental in utero BVDV infection of sows may result in prenatal and perinatal deaths, persistently infected immunotolerant or normal pigs. Many of these conditions resemble the effects of in utero infection with NCP BVDV in cattle.

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Border disease

Border disease is a congenital infection of sheep and goats, usually with one of several NCP genotypes of *Border disease virus* (BDV), a pestivirus antigenically related to BVDV and *Classical swine fever virus*, but apparently also with some BVDV-2 strains. The disease was first reported in lambs from border areas between England and Wales. It is characterized by embryonic and fetal death, abortion, mummification, and birth of weak lambs or kids. The affected animals have an abnormal body conformation, long hairy fleece, clonic rhythmic tremors ("*hairy shakers*"), unthriftiness, and poor viability (see Vol. 1, Nervous system; Vol. 1, Skin and appendages; Vol. 3, Female genital system).

A syndrome resembling mucosal disease has been reported in lambs that survived the initial border disease; they were persistently infected with an NCP BVDV. Immunohistochemical examination of tissues from persistently infected sheep reveals viral antigen in smooth-muscle cells of hollow organs and blood vessels, epithelial cells in the gastrointestinal tract, lymphocytes, neurons, and glial cells. When cytopathic BDV is superimposed on persistent infection, affected sheep develop chronic diarrhea, wasting, nasal discharge, and polypnea. Macroscopic lesions are particularly present in the cecum and colon and in a few sheep also the terminal ileum. There is marked thickening of the gut wall due to subserosal and mucosal edema and diffuse polypoid hyperplasia of the mucosa, which is hemorrhagic and focally ulcerated.

The *microscopic lesions* in the gut are similar to those described for mucosal disease in cattle. Lymphoid cell reactions are evident in the choroid plexus, portal triads of the liver, kidney, myocardium, thyroids, lungs, spleen, and lymph nodes. In addition, some lambs have marked hypertrophy and edema of the muscularis of the terminal ileum. The lesions in the terminal ileum resemble "terminal ileitis," and BVDV should be considered as a possible cause of that syndrome.

The pathogenesis of fetal infections, resultant border disease, and related enteric lesions in sheep appear to be similar to the multitude of conditions associated with NCP BVDV prenatal infections in cattle. Seronegative ewes infected before 80 days of gestation may produce persistently infected, immunotolerant, chronically viremic lambs.

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Rinderpest

Otherwise known as "cattle plague," rinderpest is an *acute or subacute highly contagious disease of cattle, characterized by erosive or hemorrhagic lesions of all mucous membranes*. The distribution of the disease is progressively shrinking, but it is still enzootic in parts of Africa, the Middle East, and the Orient, to which places it is restricted by limitations on animal movement and the use of highly efficacious vaccine. Pandemics have occurred in the Middle East, sub-Saharan and equatorial Africa, and some of these were apparently related to a relaxation in vaccination programs.

Rinderpest virus (RPV) belongs to the family Paramyxoviridae, genus *Morbillivirus*. It is a highly pleomorphic single-stranded RNA virus with a core diameter of 120–300 nm and a spiked envelope. The virus is highly fragile under ordinary environmental conditions; it is incapable of surviving more than a few hours outside the animal body under normal circumstances.

Probably all cloven-hoofed animals are naturally susceptible to infection, but the expression of infection varies considerably. Goats and sheep do respond, but inconsistently, to experimental inoculations of RPV. Infection in Asiatic pigs may be severe but it tends to be mild in European breeds, which are considered "dead-end" hosts for rinderpest.

Although different strains of RPV vary considerably in their pathogenicity, they are grouped in a single serotype and, when suitably modified, make effective vaccines.

Control of the disease in endemic areas is impeded by difficulties inherent in systems of husbandry and by the coexistence of cattle with large populations of susceptible ungulate wildlife. The infection impacts heavily on wildlife populations in close contact with cattle, and they are important in virus spread. However, wildlife in east Africa are not clearly implicated as reservoirs of the infection. The lability of the virus is such that the *spread of infection* from endemic areas is most likely to be by live animals with mild or subclinical disease.

The disease in cattle may be mild, especially in endemic areas, but will probably be acute or peracute and severe in new foci. The different degrees of severity are in part due to real differences in virulence of strains, and largely due to differences in susceptibility of breeds or races of cattle. Rinderpest may also persist for prolonged periods as a very mild disease in endemically infected cattle and wild ungulate herds and may remain stable for years before the emergence of more pathogenic strains that induce the characteristic disease.

The *nasopharyngeal mucosa* appears to be the main portal of entry in rinderpest. The virus uses glycoproteins expressed on activate lymphocytes and monocytes and on dendritic cells as receptors, and destruction of such cells may be a means by which it causes immunocompromise. It localizes and replicates initially in the palatine tonsils and regional lymph nodes. This is followed after an 8–11-day incubation by a 2–3-day period of viremia that coincides with the fever seen clinically. In circulation, the virus is associated with mononuclear cells. After the viremic stage, the virus replicates in all lymphoid tissues, the bone marrow, and the mucosa of the upper respiratory tract and the gastrointestinal tract. Nasal, oral, and ocular secretions, as well as feces, contain high titers of the virus. In general, excretion of virus ceases by about day 9 of the clinical disease, with the onset of neutralizing antibodies. Recovered animals do not appear to be carriers, although there are reports to the contrary.

Fever and its attendant signs usher in the **clinical syndrome**, with early leukopenia. Fever reaches its peak in about 3 days and falls with the onset of diarrhea, which may be bloody. There is severe abdominal pain, anorexia, ocular and nasal discharge, tachypnea, fetid breath, occasional cough, lethargy, severe dehydration and emaciation, and prostration. Death occurs in 5–8 days. Explosive outbreaks with high morbidity and mortality are more likely to occur in naive populations. Vaccinated or recovered animals usually have lifelong immunity. Secondary bacterial, viral, protozoal, and rickettsial infections are common.

The **gross morbid anatomical changes** in rinderpest are characteristic but not pathognomonic, and are similar to mucosal disease (Fig. 1.97). The lesions in the upper alimentary tract are *necrotizing and erosive-ulcerative*.

RPV has an affinity for the alimentary epithelium. Most severely affected areas in the oral cavity are those contiguous with lymphoid aggregates. Consequently, the caudal part of the oral cavity is affected preferentially. There is some strain variation with respect to presence of oral lesions. In nonfatal cases, there is rapid regeneration of the oral mucosal lesions. Esophageal erosions are usually mild and affect the proximal portion. The forestomachs rarely exhibit any lesions.

The **histologic lesions** of stratified squamous epithelium originate in the stratum spinosum. Entrance into the epithelium may be via infected Langerhans cells that then pass virus along to adjacent cells. Immunohistochemically, irregularly shaped rafts of acanthocytes are infected with virus. These same cells then undergo degeneration and necrosis. Multinucleate syncytia form in the epithelium (Fig. 1.98) and these may have *cytoplasmic and nuclear inclusions*. Abrasion causes the necrotic tissue to lift off and produce shallow erosions or ulcers. This occurs so readily that they are usually the first lesions observed. Their margins are sharp, and the bases are reddened by the underlying congested capillaries. The initial minute erosions enlarge and coalesce to form extensive defects.

The abomasum is often severely reddened, which may just be a reflection of generalized stress for, although immunohistochemically abomasal epithelium is infected with virus, the extent of infection and resulting necrosis is far less than that seen in intestinal mucosa.

Lesions in the intestine are severe and severity correlates with amount of lymphoid tissue in subjacent areas. Consequently, greatest mucosal damage is seen in ileum and the proximal colonic patch. Peyer's patches are almost universally involved. These areas become hemorrhagic and necrotic, and are associated with necrosis of the overlying mucosa, leaving deep ulcers (Fig. 1.99A).

Microscopically and immunohistochemically, there is replication of virus at all levels of intestine, with both crypt and villus epithelium involved. Replication is associated with formation of inclusion bodies, both nuclear and cytoplasmic, degeneration, necrosis, denuding of epithelium, formation of crypt abscesses and, if prolonged enough, villus atrophy. The formation of syncytia within gut epithelium is a rare event, in contrast to the oral cavity lesions, where it is seen with some regularity.

Receptor affinity dictates that *RPV is tropic for lymphoid tissues*. Infection and replication have been documented in both lymphocytes and macrophages. Necrosis of follicular lymphocytes is extreme, and gross inspection, which reveals little abnormality of

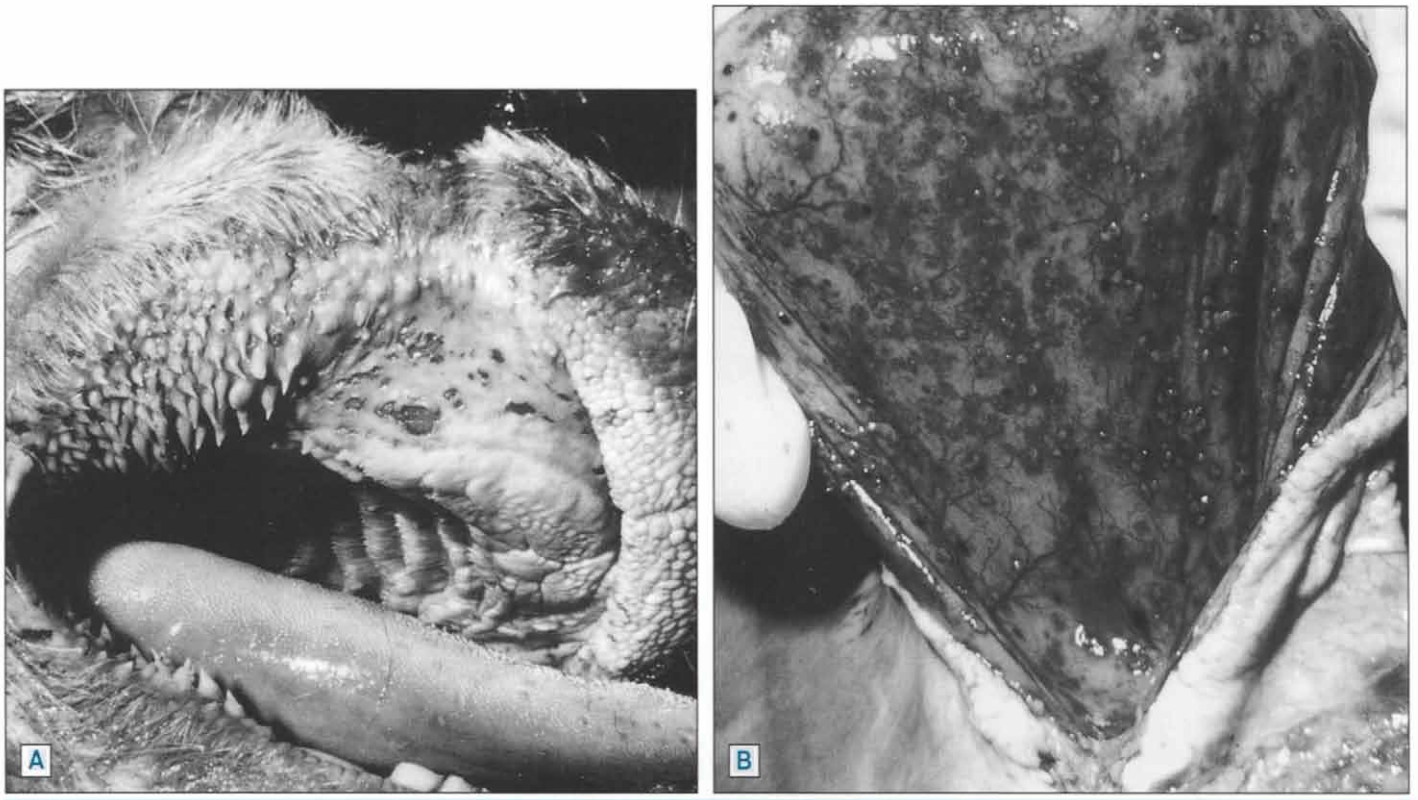

Figure 1.97 Rinderpest in a cow. **A.** Ulcers on the buccal mucosa, and small incipient ulcers on the underside of the tongue. **B.** Multifocal to coalescing fibrinohemorrhagic colitis.

nodes, is misleading (Fig. 1.99B). Multinucleate cells, similar to those in the oral mucosa, occasionally form in the lymph and hemolymph nodes. All or only some follicles may be involved and there is often an increase of other leukocytes in the sinuses. Similar lesions occur in the spleen, tonsils, and, as already noted, in the Peyer's patches.

Acute congestion and edema of the conjunctiva may be followed by purulent conjunctivitis and corneal ulceration. Petechiae are common in the mucosa of the upper respiratory tract, which is usually covered with mucopurulent exudate.

The gross lesions resemble those of severe acute bovine viral diarrhea and mucosal disease, but *rinderpest is distinguished microscopically most readily by the presence of syncytia and inclusion bodies.* Its regulatory status demands laboratory diagnosis in accredited laboratories, by virus isolation, antigen identification in fresh or fixed specimens, or polymerase chain reaction.

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Peste des petits ruminants

Peste des petits ruminants is an acute viral disease of sheep and goats that closely resembles rinderpest and is also known as kata, *stomatitis–pneumoenteritis complex*, goat plague, and pseudorinderpest. The disease was first recognized in West Africa, and is now distributed in sub-Saharan Africa, the Arabian Peninsula, Anatolia, and the Indian subcontinent, including Nepal and Bangladesh. *Peste-des-petits-ruminants virus* (PPRV) is in genus *Morbillivirus*, family Paramyxoviridae, closely related to *Rinderpest virus*, with which it shares common antigenic determinants. The virus cross-reacts with RPV in the immunodiffusion and complement fixation tests. It may be differentiated from RPV using monoclonal antibody techniques and cDNA probes.

The clinical signs, pathogenesis, and lesions of the disease in sheep and goats in general are similar to those of rinderpest (Fig. 1.100), except that the disease is more acute in onset, especially in goats, and follows a more rapid course.

Another difference is the marked involvement of the respiratory tract; affected animals have dyspnea, hyperpnea, and cough. There is

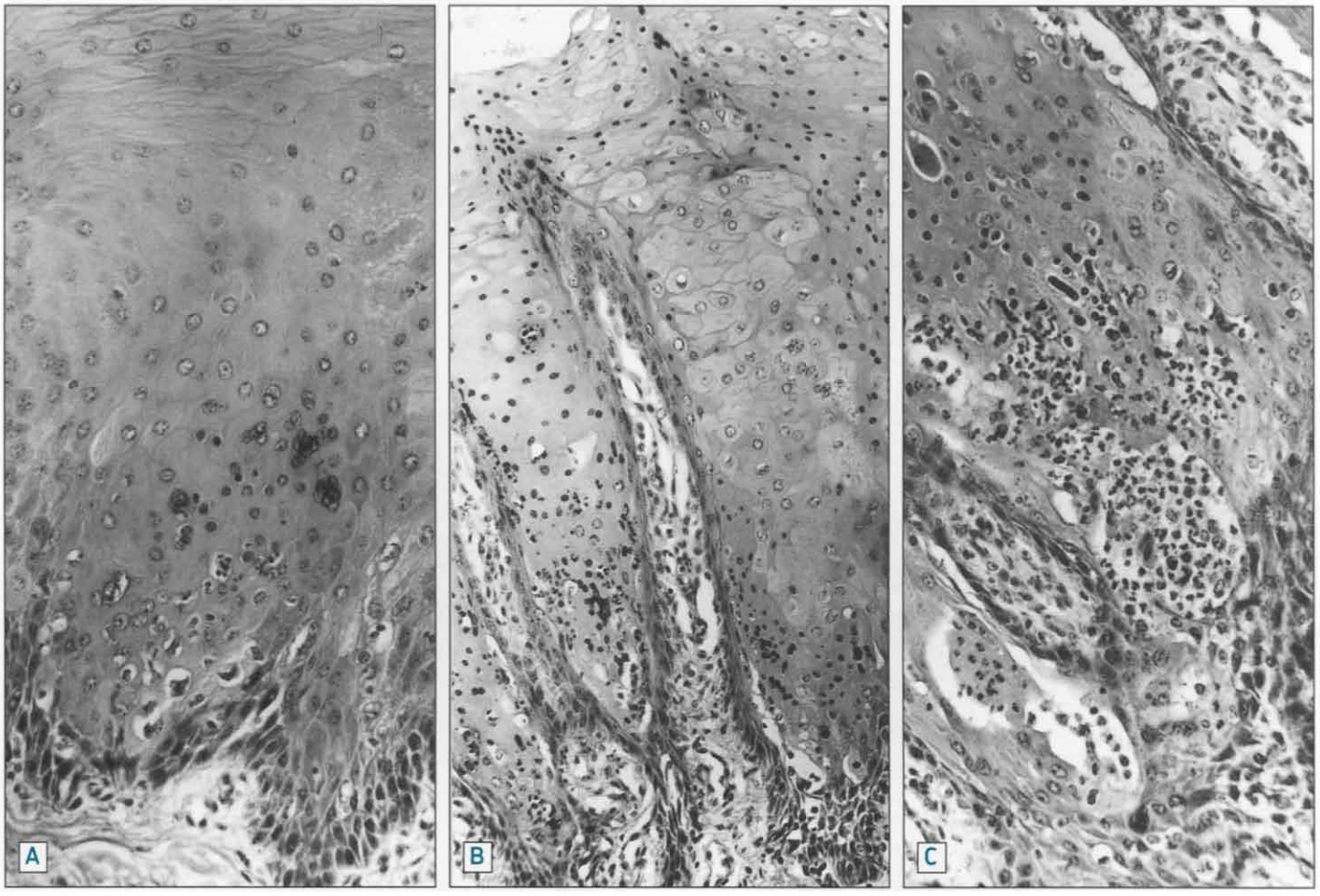

Figure 1.98 Rinderpest lesions in the tongue of an ox. **A.** Early stage of oral lesion showing disorganization of epithelium above the basal layer and formation of syncytial cells. **B, C.** Slightly later stage of (**A**) with beginning separation sparing basal cells.

also a marked serous to mucopurulent nasal and ocular discharge, and erosion/ulceration of the pharyngeal epithelium may be diffuse. The pulmonary lesions of peste des petits ruminants are similar to pneumonia due to *Canine distemper virus* in dogs and *Measles virus* infections in humans. The macroscopic pulmonary lesions include consolidation, atelectasis, and dark-red discoloration of the cranioventral lobes. Some animals have fibrinous pleuritis (see Vol. 2, Respiratory system). Microscopically, there is mild multifocal tracheitis, bronchitis and necrotizing bronchiolitis, and diffuse proliferative *interstitial pneumonia*, with formation of alveolar syncytial cells. Eosinophilic cytoplasmic and nuclear inclusions are present in the epithelial cells of the air passages, type II pneumocytes, and syncytial cells. Viral antigen may be demonstrated in the same cells with appropriate immunohistochemical techniques. The primary viral lesions are often complicated by secondary bacterial infections.

Experimental inoculation of PPRV into cattle and pigs does not produce clinical disease, but these animals will resist subsequent challenge with *Rinderpest virus*. These species are considered to be "dead-end" hosts, since they do not seem to spread the infection to other species. Natural infection or vaccination of sheep and goats with *Rinderpest virus* will protect them against PPRV.

A zoo outbreak of peste des petits ruminants that involved several species of wild ungulates has been reported, and Indian buffalo are also susceptible. The distribution of the virus in free-ranging wild ungulates has not been investigated.

Peste des petits ruminants must be distinguished from rinderpest in the laboratory based on use of monoclonal antibodies to distinguish the agents immunologically in wet or fixed tissue, or by molecular techniques.

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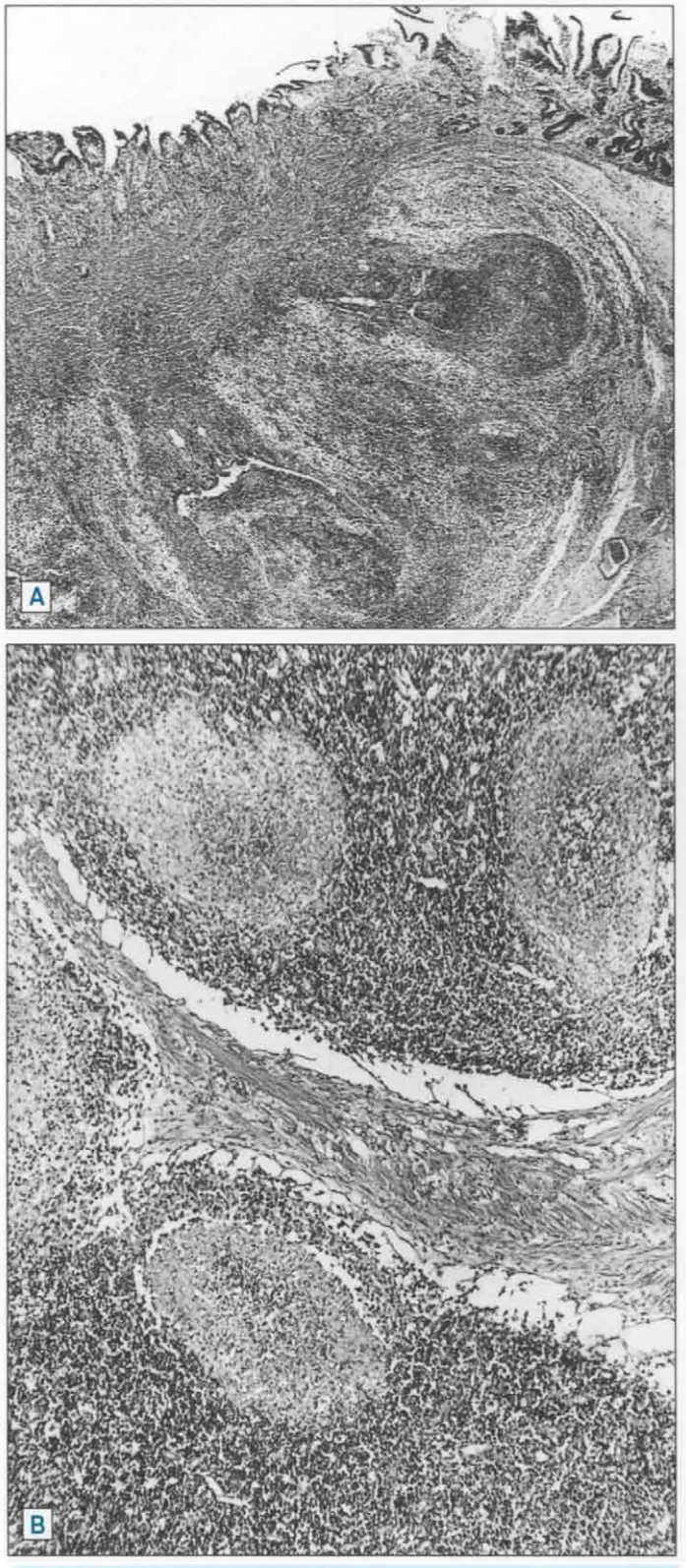


Figure 1.99 Rinderpest in an ox. **A.** Necrosis of Peyer's patch in ileum. **B.** Necrosis of germinal centers in a lymph node.

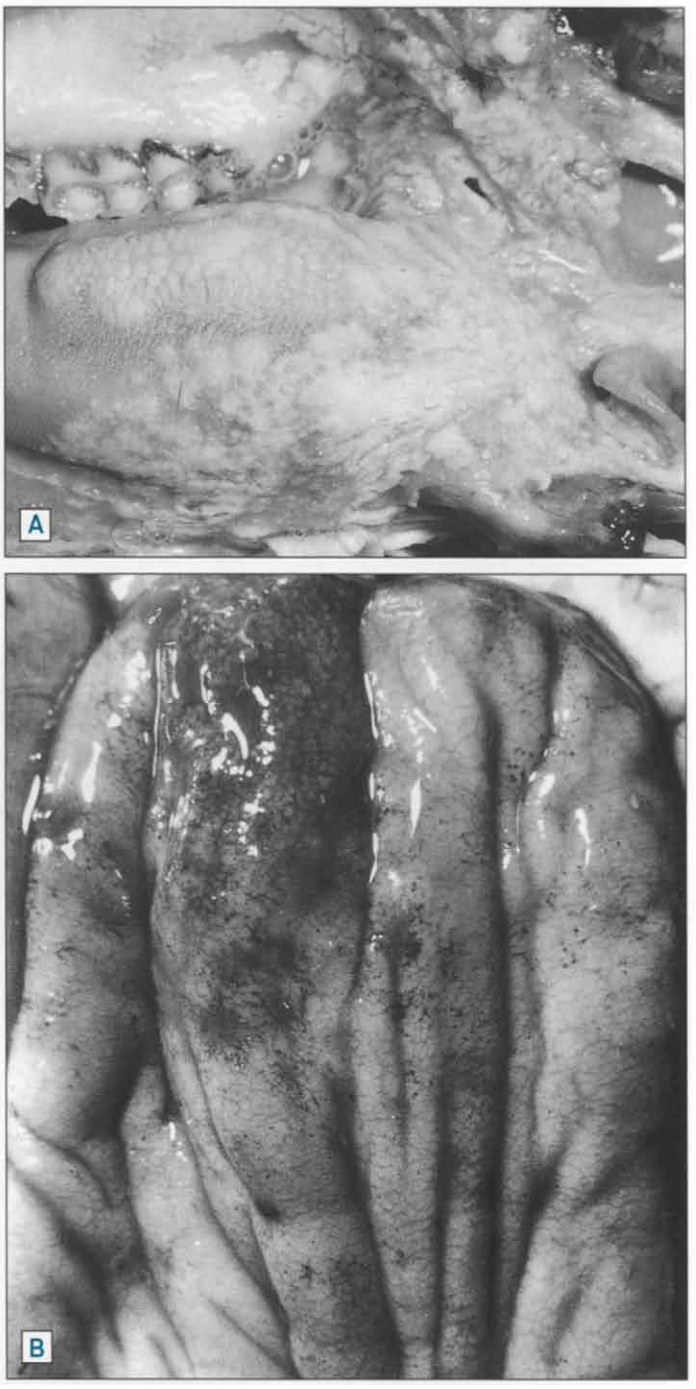


Figure 1.100 Peste-des-petits-ruminants in a goat. **A.** Palatine ulcers, and raised plaques on the mucosa of the side of the tongue and oropharynx. **B.** Diffuse edema, and focal congestion and ulceration of the cecal mucosa.

Malignant catarrhal fever

Malignant catarrhal fever (MCF) is an infectious disease of domestic cattle, some wild ruminants, and occasionally pigs, also known as malignant head catarrh, and snotsiekte. The disease is characterized by *lymphoproliferation, vasculitis, and erosive-ulcerative mucosal and cutaneous lesions.*

MCF is distributed worldwide. It is generally sporadic, although severe herd outbreaks have been reported in feedlot, dairy and

Toplu N. Characteristic and noncharacteristic pathological findings in peste des petits ruminants (PPR) of sheep in the Ege district of Turkey. J Comp Pathol 2004;131:135–141.

range cattle, in farmed bison and deer, and in zoos. Among the Cervidae, all species except fallow deer are probably susceptible. Other susceptible species of ruminants include banteng, Cape buffalo, and greater kudu. *Mortality in susceptible species approaches 100%*, although there are rare recorded cases of chronic infection and also of recovery from the disease, especially in infected goats, bison, cattle, and pigs. Although the agent is transmissible, the disease is apparently not contagious among cattle or bison by direct contact.

MCF is caused by cross-species infections with members of the MCF virus group of ruminant rhadinoviruses (genus *Rhadinovirus*, subfamily Gammaherpesvirinae), four of which are associated with clinical MCF: (1) ***Alcelaphine herpesvirus 1*** (AlHV-1), carried by wildebeest (*Connochaetes* sp.); (2) ***Ovine herpesvirus 2*** (OHV-2), endemic in domestic sheep; (3) ***Caprine herpesvirus 2*** (CpHV-2), endemic in domestic goats; and (4) a virus of undetermined origin causing *MCF in white-tailed deer* (MCFV-WTD). Related rhadinoviruses, as yet unassociated with MCF, have been detected in a variety of members of the Bovidae. Rhadinoviruses of ruminants are highly cell-associated lymphotropic herpesviruses, difficult or impossible to isolate, which are typically transmitted from adults to offspring within the first 2–3 months of life, probably via free virus shed in nasal secretions. In the natural host, infection is latent or inapparent, with intermittent virus shedding, although disease has been incited in sheep by experimental aerosol inoculation of a large dose of OHV-2.

Formerly, MCF was described as the "African" or *wildebeest-associated* (**WA-MCF**) form if it was presumed to be due to AlHV-1, and "North American" or *sheep-associated* (**SA-MCF**) if it was presumed to be due to exposure to sheep. There is no clinicopathologic difference in the disease induced by the various rhadinoviruses, and these terms have only broad epidemiologic connotations. The blue, brindled, or white-bearded wildebeest (*Connochaetes taurinus*) carries AlHV-1. Wildebeest calves become infected during the first 2–3 months of life, when they are also viremic and shed cell-free AlHV-1 in nasal and ocular secretions. Most wildebeest older than 7 months are serologically positive for AlHV-1. In utero infections have also been reported. Wildebeest are infected for life and transmit AlHV-1 to their calves without showing clinical signs. *Wildebeest calves are considered to be the main source of infection for cattle in East Africa.* They may shed infective cell-free virus, in nasal and ocular secretions, for several days. Transmission to cattle may occur even without intimate contact, suggesting aerosol spread. Viremia apparently ceases with the development of active neutralizing antibodies in animals >6 months old. It may be reactivated during late pregnancy or periods of stress, e.g., transportation. Although AlHV-1 produces MCF in many captive exotic species of ruminants, apparently most species that are exposed in their native habitat do not develop disease. AlHV-1 has been transmitted and adapted to domestic rabbits, hamsters, rats, and guinea pigs, in which it produces MCF-like lesions.

The etiologic agent of SA-MCF has never been isolated from sheep; however, polymerase chain reaction probes have permitted its differentiation. *Most sheep have polymerase chain reaction-detectable specific OHV-2 sequences in cells*, and identical sequences are detectable in spontaneous cases of SA-MCF. Experimental transmission of OHV-2 between sheep has been accomplished using an aerosol of virus-infected nasal secretions, and natural transmission from adults to offspring probably takes that route, producing very high rates of infection in the sheep population, where it can be considered ubiquitous. The other two rhadinoviruses associated with MCF have also been identified and implicated by molecular diagnostic techniques.

Attempts to reproduce MCF in cattle by inoculating sheep tissues and secretions have been unsuccessful, but MCF-like disease can be induced in rabbits, hamsters, and guinea pigs by transfer of lymphocytes or T lymphoblast cell lines derived from MCF-affected cattle and deer. The domestic rabbit is the most commonly used model to study the pathogenesis of both forms of MCF. MCF due to OHV-2 occurs spontaneously and experimentally in pigs, and the relative rarity of the disease in swine may be due to lack of exposure to ruminant rhadinoviruses under most conditions of husbandry.

SA-MCF occurs where bovids and deer come in contact with sheep. There is considerable variation in the susceptibility of various ruminant species to SA-MCF. Domestic cattle (*Bos taurus* and *B. indicus*) appear to require high levels of exposure to induce disease. Bali cattle or banteng (*B. javanicus*), the domestic water buffalo (*Bubalus bubalis*), American bison (*Bison bison*) and most species of deer, with the exception of fallow deer (*Dama dama*), seem to be highly susceptible. MCF is one of the most serious diseases of farmed deer in New Zealand, Australia, and the UK. Multiple case outbreaks have also been reported in captive North American cervids.

The *mucosa of the upper respiratory tract and/or the tonsil* is the most likely natural route of entry for the agents of MCF. Both WA-MCF and SA-MCF can be transmitted to susceptible hosts with large volumes of whole blood or lymphoid tissues administered intravenously, but not by cell-free filtrates, indicating that the agents are cell-associated, probably with lymphocytes. The incubation period of MCF is usually 2–10 weeks, but may, on occasion, be very much longer than this.

Antibodies against the rhadinovirus involved can be detected in animals with MCF, and often in herdmates, implying subclinical infection. Development of antibodies does not prevent a fatal outcome.

The **pathogenesis**, clinical signs, and lesions are similar, whatever the agent inducing MCF. Viremia in WA-MCF usually starts about 7 days before the onset of fever, and persists throughout the course of the disease. MCF is characterized by *marked T-lymphocyte hyperplasia.* A population of large granular lymphocytes appears to be infected and transformed by rhadinoviral infection, and OHV-2 genome has been detected in CD8+ T cells, the predominant cell infiltrating around vessels in the brains studied. These cells are probably cytotoxic T lymphocytes or T-suppressor cells, but the mechanism by which they mediate the lesions of MCF is unclear. Dysfunction of this cell population may result in de-repression of T-lymphocyte replication, permitting lymphoproliferation. Deranged cytotoxic T-cell activity may then create the epithelial and vascular lesions, through a type of graft-versus-host response, attacking epithelium of the respiratory and gastrointestinal systems, as well as medium-sized arteries throughout the body. This is a unifying, but unproven, hypothesis explaining the lymphadenopathy, mucosal epithelial lesions, and vasculitis characteristic of MCF. Vascular lesions may mediate infarction of some affected tissue fields, as well.

There is wide variation in the presenting **clinical syndromes**, which are potentially pansystemic. Quite consistently, affected animals have enlarged lymph nodes, although this may be less the case in bison, and there is usually some degree of ocular and oral disease, and exudative dermatitis. There is edema of the eyelids and palpebral conjunctivae and congestion of the nasal and buccal mucosae.

Photophobia is accompanied by copious lacrimation. There is conjunctivitis and an increasing rim of corneal opacity, starting at the limbus and progressing centripetally. Corneal ulceration occurs in some cases, but in those that die quickly, the infiltration of the filtration angle may be all that is seen, and this is easily overlooked. Hypopyon may be seen. In some cases there are nervous signs, such as hyperesthesia, head pressing, trembling, nystagmus, incoordination, and behavioral changes. Other animals may have gastroenteritis with diarrhea, which may in acute cases be bloody. This is most commonly seen in deer. The disease may take an acute course of about 1–3 days, particularly in animals with hemorrhagic enteritis. Those with less severe gastroenteritis, central nervous signs, or generalized disease may linger for as long as 9–10 days. Mortality in MCF has been considered to approach 100% of clinical cases, but recovery may occur, although chronic ocular lesions and vasculitis persist.

Gross morbid changes may not be present in occasional animals that die of peracute MCF, and in these the diagnosis must rest on the detection of the characteristic histologic changes, and demonstration of the genome of an implicated rhadinovirus in tissue.

The carcass is dehydrated, and may be emaciated if the course has been prolonged. Conjunctivitis may be evident. The muzzle and nares are heavily encrusted and, if wiped, often reveal irregular raw surfaces, although in some cases there may be only a slight serous discharge. Cutaneous lesions, especially in SA-MCF, are common, but often overlooked. Affected areas include the thorax, abdomen, inguinal regions, perineum, udder, and occasionally the head. There may be, acutely, more or less generalized exanthema with sufficient exudation to wet and mat the hair, and to form detachable crusts; in unpigmented skin there is obvious hyperemia. The crusts may become several millimeters thick, and there is patchy loss of hair. Sometimes these cutaneous changes begin locally about the base of the hooves and horns, the loin, and perineum; they may remain localized or become generalized. In severe cases, the horns and hooves may slough. *Caprine herpesvirus-2* has been associated with syndromes in deer that include dermatitis and alopecia, alone, or in combination with gastrointestinal or neurological disease.

The respiratory system may have minor or severe lesions (Fig. 1.101A). When the course is short, the nasal mucosa may only have congestion and slight serous exudation. Later, there is a copious discharge. Lesions are most severe in the rostral third of the nasal cavity, corresponding to the zone of stratified squamous epithelium. In some cases, fibrinous tracheobronchitis may occur (Fig. 1.101B).

The lower alimentary mucosae may have no significant lesions in the peracute disease, although *oral lesions are present in most cases of MCF*. Minor erosions are first observed on the lips adjacent to the mucocutaneous junction. Sometimes apparently normal epithelium on the surface of the tongue peels off in sheets (Fig. 1.102). Later, erosive and ulcerative lesions may involve a large area of oral mucosa, frequently occurring on all surfaces of the tongue, the dental pad, the tips of the buccal papillae, gingivae, both areas of the palate, and the cheeks. In some areas, the cheesy or tattered necrotic epithelium may not be sloughed at the time of inspection. Esophageal erosions or

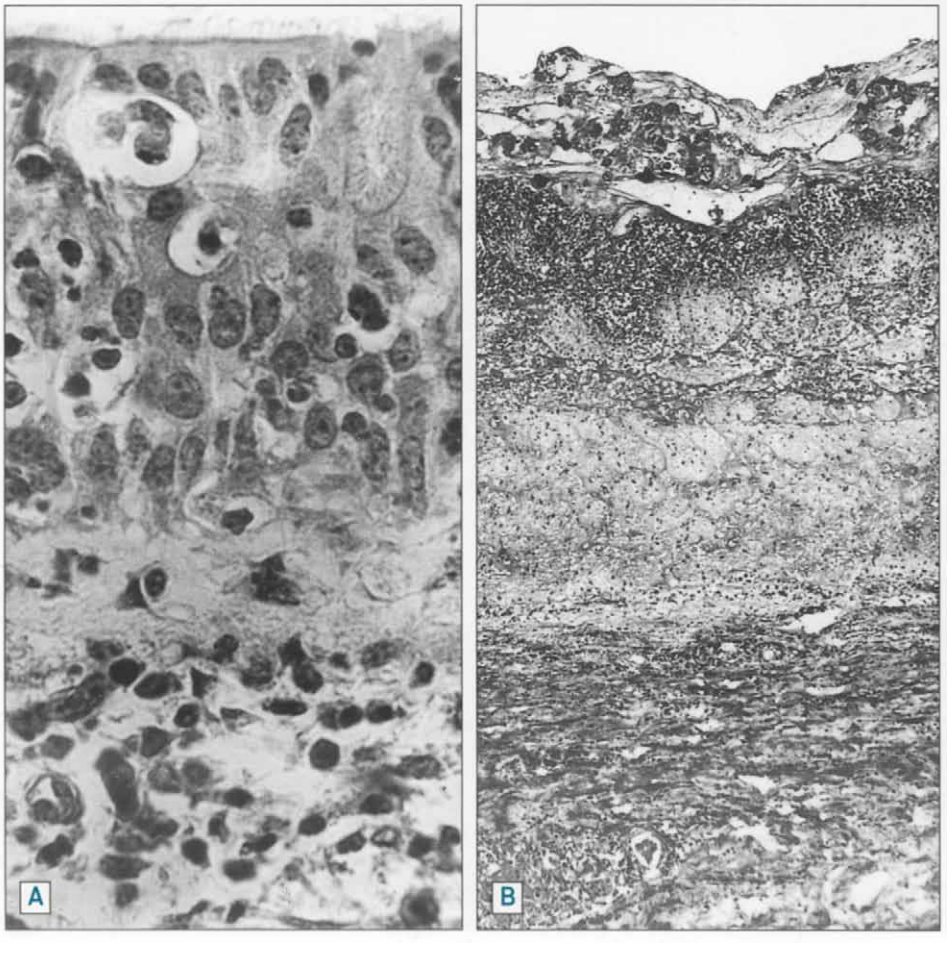


Figure 1.101 Malignant catarrhal fever. **A.** Nasal mucosa, with degeneration of epithelium and infiltration of lymphocytic cells in uncomplicated rhinitis. **B.** Pseudomembranous tracheitis.

ulcers, similar to those that occur in the other diseases causing ulcerative stomatitis, occur in MCF, and, as in rinderpest, are most consistent in the cranial portion. Lesions of the same sort may be present in the forestomachs. Focal ulceration or generalized hyperemia may be evident in the abomasal mucosa. In deer, especially, hemorrhagic or fibrinohemorrhagic typhlocolitis may be a prominent finding.

The *liver* may be slightly enlarged. Close inspection will reveal, in some cases, diffuse mottling with white foci, which are periportal accumulations of mononuclear cells (Fig. 1.103). There may be numerous small hemorrhages and a few erosions of the mucous membrane of the gallbladder.

Characteristic lesions may occur in the *urinary system*. Renal changes are not always present. They are infarcts or 2–4 mm foci of nonsuppurative interstitial nephritis (Fig. 1.104). They may be numerous enough to produce a mottled appearance, and may form slight rounded projections from the capsular surfaces. The pelvic and ureteral mucosa frequently has petechial and ecchymotic hemorrhages. Similar lesions are present on the mucosa of the urinary bladder, or there may be more severe hemorrhage erosion and ulceration of the epithelium, and hemat Superficial lesions may occur in the vagina and vulva of the oral cavity and skin.

Enlargement of lymph nodes is a characteristic les most species, perhaps except bison. All nodes may some may appear grossly normal. Affected nodes ma the normal size, and some, including hemolymph r usually too small to recognize, may become qui 1.106). There is edema of the affected nodes and connective tissue. On cross-section it is apparent th size is due to *lymphocytic hyperplasia*. Some of the noc or hemorrhagic. The spleen is slightly enlarged, ar follicles are prominent.

Most animals may have *meningoencephalitis*, as a tis, perhaps reflected grossly by meningeal edema, the most consistent histologic lesions of the diseas

The **histologic changes** must usually be relied nosis of MCF, and its differentiation from similar c access to molecular diagnosis of specific rhadinov increasing. The characteristic histologic changes ar phoid tissues and in the adventitia and walls of medi especially arteries in any organ, and these will be

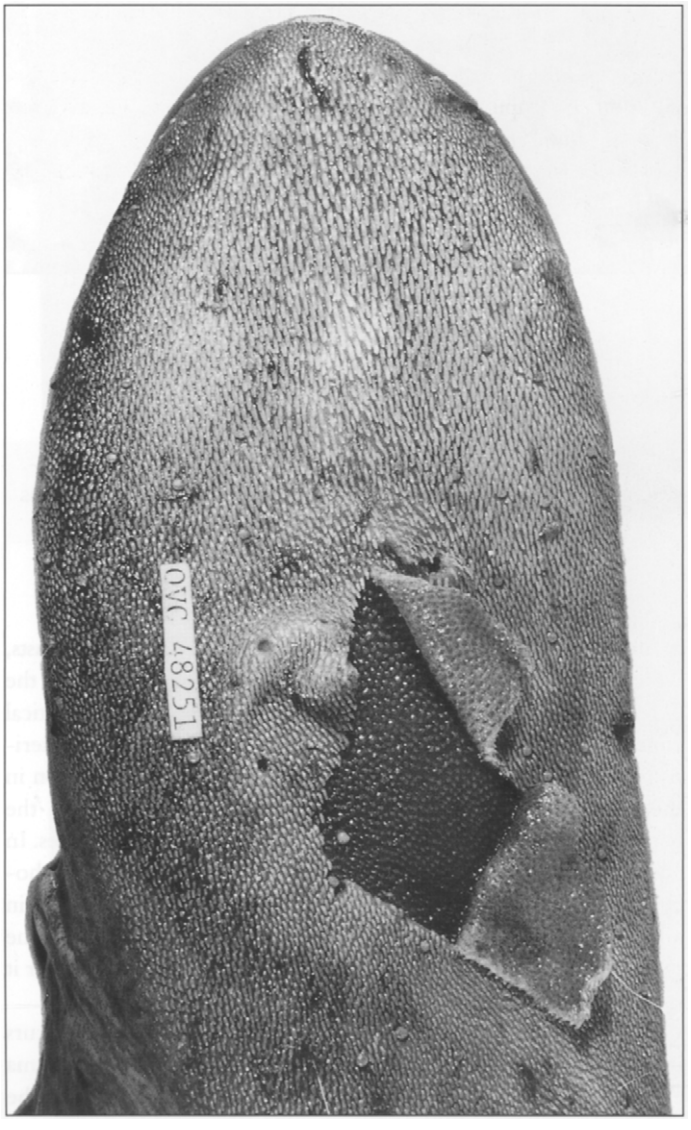


Figure 1.102 Separation of necrotic lingual epithelium from underlying propria in an ox with **malignant catarrhal fever**.

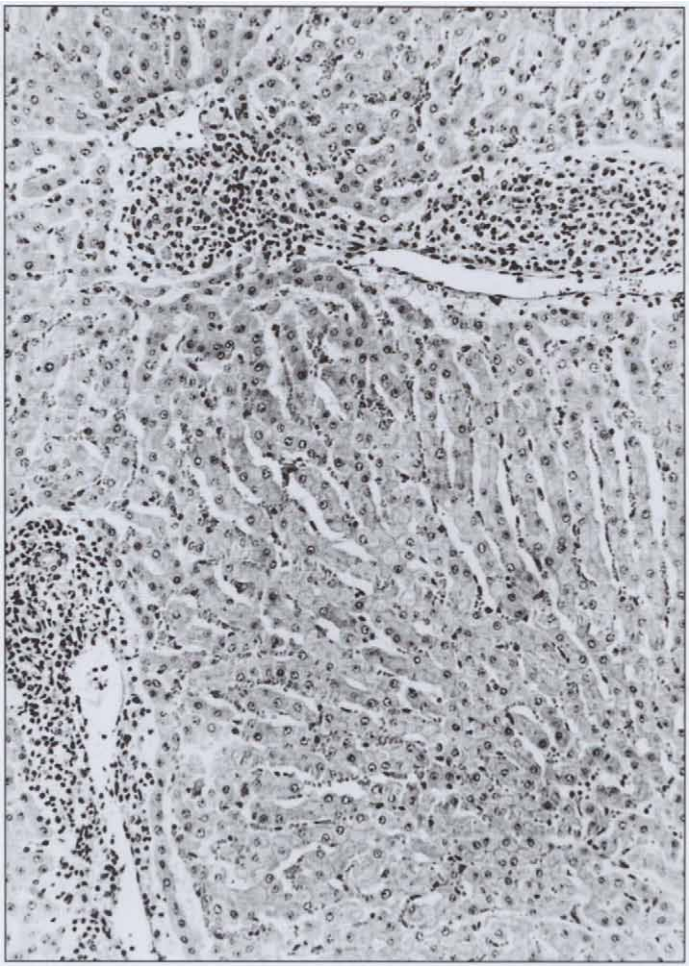
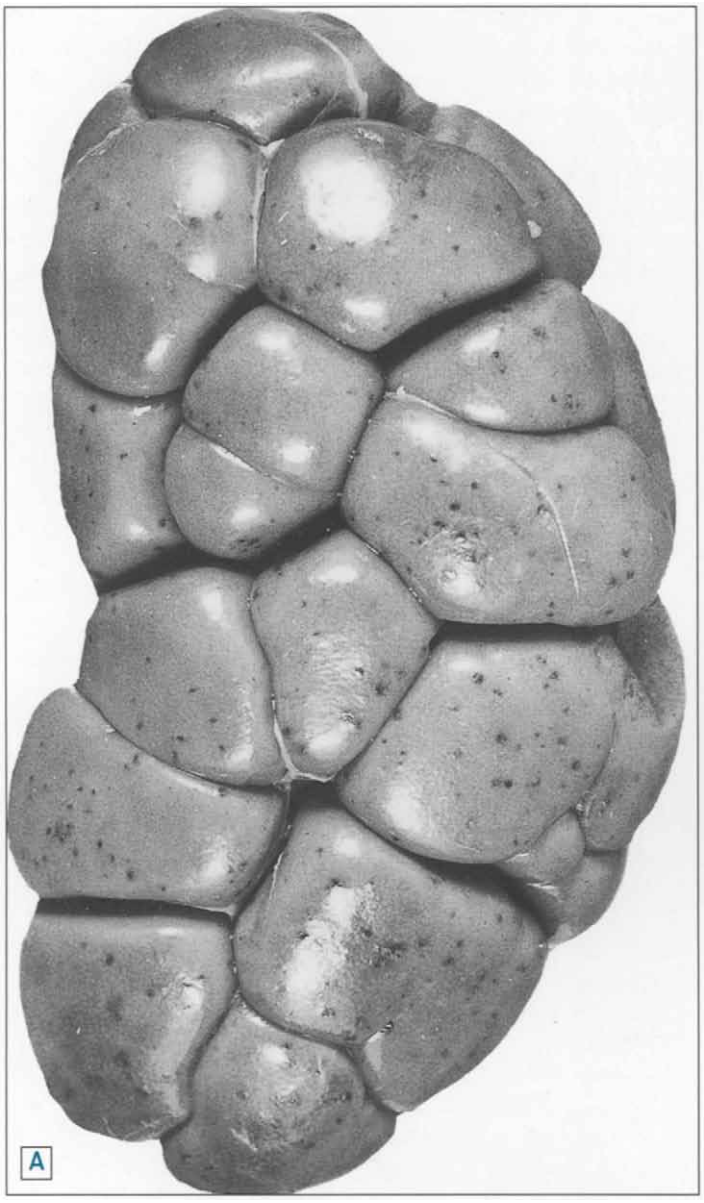


Figure 1.103 Accumulations of lymphocytes in portal triads in **malignant catarrhal fever**.



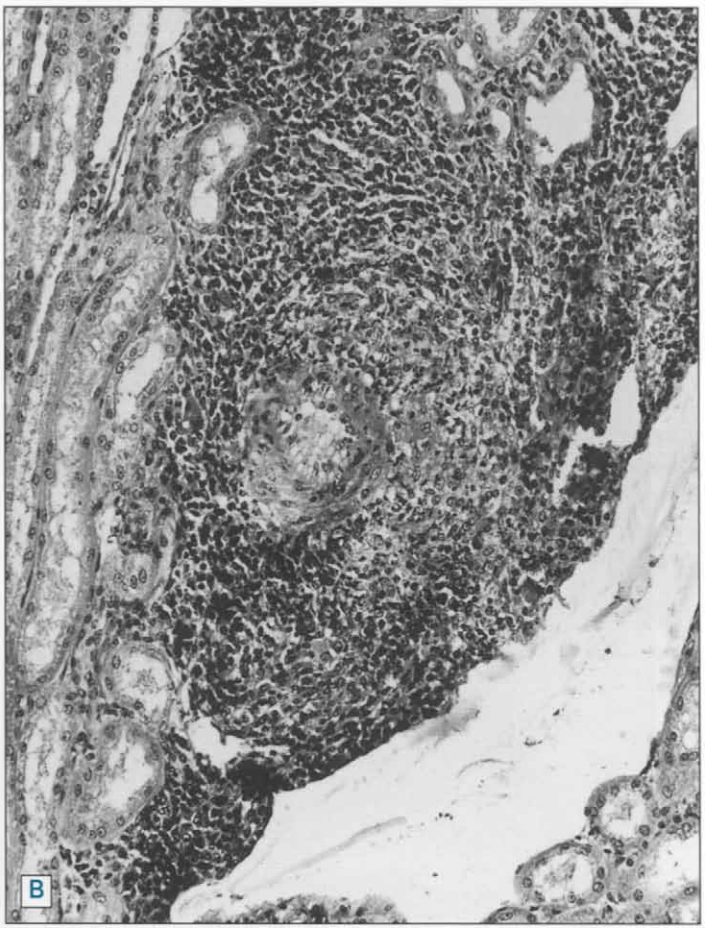


Figure 1.104 Malignant catarrhal fever. A. Focal nonsuppurative interstitial nephritis. **B.** Extensive cuff of mononuclear cells, and fibrinoid necrosis in the wall of a small arteriole in the kidney.

other lesions. They are characterized by perivascular accumulation of mainly mononuclear cells, and fibrinoid necrotizing vasculitis (Figs 1.107 and 1.108). These changes may be focal or segmental, and may involve the full thickness of the wall, or be confined more or less to one of the layers. When the intima is involved, there is often endothelial swelling. Thrombi are difficult to demonstrate in damaged vessels. The media may be selectively affected, or perhaps the adventitia alone. Severely affected segments of vessel are replaced by a coagulum of homogeneous, eosinophilic material, in which fragmented nuclear remnants are seen. The perivascular accumulation of cells is particularly characteristic. They are mainly lymphoid cells with large open nuclei and prominent nucleoli; small lymphocytes and plasma cells may be present occasionally. The vascular lesions may be more subtle in other species (especially deer and elk) than in cattle. Cattle that recover from SA-MCF also have distinctive vascular lesions 90 days after clinical onset. Concentric fibrointimal plaques, disrupted inner elastic lamina, focally atrophic tunica media, and vasculitis of variable severity are evident in many organ systems.

In *lymph nodes* there is active proliferation of lymphoblasts, which form extensive homogeneous populations of cells in the T-cell-dependent areas of the interfollicular cortical and paracortical zones. Focal areas of hemorrhage and necrosis associated with arteritis may be seen in all areas of the nodes. The lymphoid reaction in the spleen varies from marked lymphoid cell hyperplasia, in the periarteriolar sheaths, to atrophy and depletion of lymphocytes. In addition, there is marked proliferation and infiltration of lymphocytic and lymphoblastic cells, mainly perivascular in distribution, in a variety of organs. The lymphoreticular proliferation may become so severe in some organs that it is difficult to determine whether it is hyperplastic or neoplastic.

Microscopic arteritis similar to that present in other organs occurs in the nervous system of many cases. Necrotizing arteritis, plasma exudation into the meninges or Virchow–Robin space, and the predominantly adventitial lymphocytic response are, in the brain of cattle, *unique to MCF*, and allow it to be differentiated from other nonsuppurative encephalitides. Degenerative changes in nervous

Figure 1.105 Hemorrhages in mucosa of urinary bladder in **malignant catarrhal fever**.

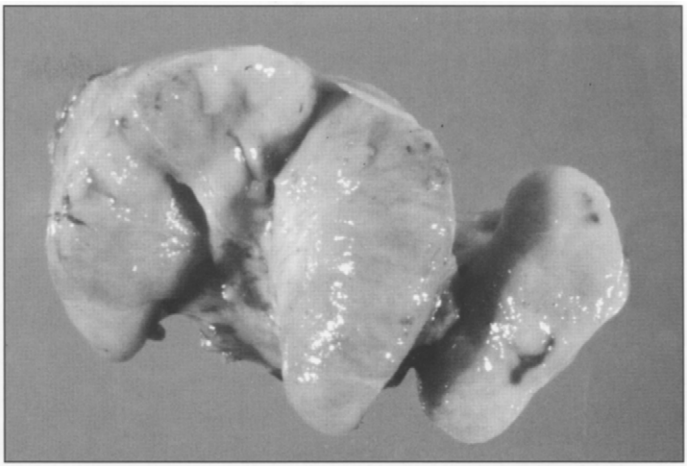


Figure 1.106 Hyperplastic enlarged lymph nodes bulging on cut surface. in cow with **malignant catarrhal fever**.

parenchyma can be explained on the basis of the vascular changes.

The lesions in *skin and squamous mucosae* of the alimentary tract consist of a lichenoid infiltrate, as the altered and proliferating lymphoid population moves into the upper dermis and then the epidermis (Fig. 1.109). Often, typical arteritis, involving small and medium-sized vessels, is present in underlying tissue. Groups of epithelial cells become necrotic, with swollen, strongly acidophilic cytoplasm; ultimately the full thickness of epithelium in affected areas undergoes necrosis and ulcerates (Fig. 1.109). Granulomatous mural folliculitis was the prominent microscopic finding in alopecic sika deer infected with CpHV-2.

The mucosa of the abomasum may be infiltrated by lymphocytes, and undergo mucous metaplasia or focal ulceration. The mucosa of the lower alimentary tract, especially the cecum and colon in deer, may similarly be heavily infiltrated by lymphocytes, often with fibrin and blood exuding into the lumen where surface and glandular epithelium has undergone necrosis and collapsed, sometimes over wide areas (Fig. 1.110). Submucosal arterioles in affected areas of abomasum and intestine are affected by the characteristic arteritis.

The *mottling of liver and the focal nephritis* seen grossly are due to the perivascular accumulation of mononuclear cells in the portal triads of the liver (Fig. 1.103) and in the cortices of the kidney. In the liver, these cuffs may be very large and invest the branches of the hepatic artery, which may undergo fibrinoid necrosis. Microscopic lesions are frequently present in the kidneys, even though gross lesions are not; they consist of vasculitis involving the smaller arteries and afferent arterioles. Extensive diffuse lymphocytic infiltrates disrupt the normal renal cortical architecture, and in some cases, infarcts appear to be associated with vasculitis involving arcuate arteries.

Ophthalmitis often occurs, and its presence is a useful differential criterion from other ulcerative diseases of the alimentary tract. Corneal edema, secondary to vasculitis, is responsible initially for the opacity (Fig. 1.111). Later there may be lymphocytic infiltration of various structures within the globe. There is retinal vasculitis and, in some cases, hemorrhagic or inflammatory detachment of the retina in focal areas. Lymphocytic optic neuritis and meningitis may be seen (see Vol. 1, Eye and ear).

Differentiation of acute severe BVD and mucosal disease from MCF is sometimes difficult, but *MCF usually affects one or more organ systems or tissues (liver, kidney, bladder, eye, brain, tracheobronchial tree) not involved in mucosal disease, and typically produces lymphoid hyperplasia in cattle, whereas lymphoid tissue in BVDV infections is expected to be atrophic.* Arteritis may be seen in some cases of BVDV infection, mainly in the submucosa in the lower alimentary tract. Fortunately, arteritis is present in one tissue or another in all cases of MCF, whether peracute, acute, or mild with recovery, though it may be necessary to examine many sections to find it. The best organs to examine for vascular lesions are the brain and leptomeninges, carotid rete, kidney, liver, adrenal capsule and medulla, salivary gland, and any area of skin or alimentary tract showing gross lesions. *A combination of arteritis, lymphoid hyperplasia, and lymphocytic infiltrates into affected epithelia is very characteristic of MCF.*

Bali cattle as well as other species of cattle and buffalo are affected with a disease that closely resembles MCF, and both diseases occur geographically together in Indonesia. **Jembrana disease** is caused by a *lentivirus* distinct from *Bovine immunodeficiency virus*, and has associated mononuclear proliferation and thrombosis in vessels of multiple organs, seemingly similar to MCF. Proliferation of T lymphocytes

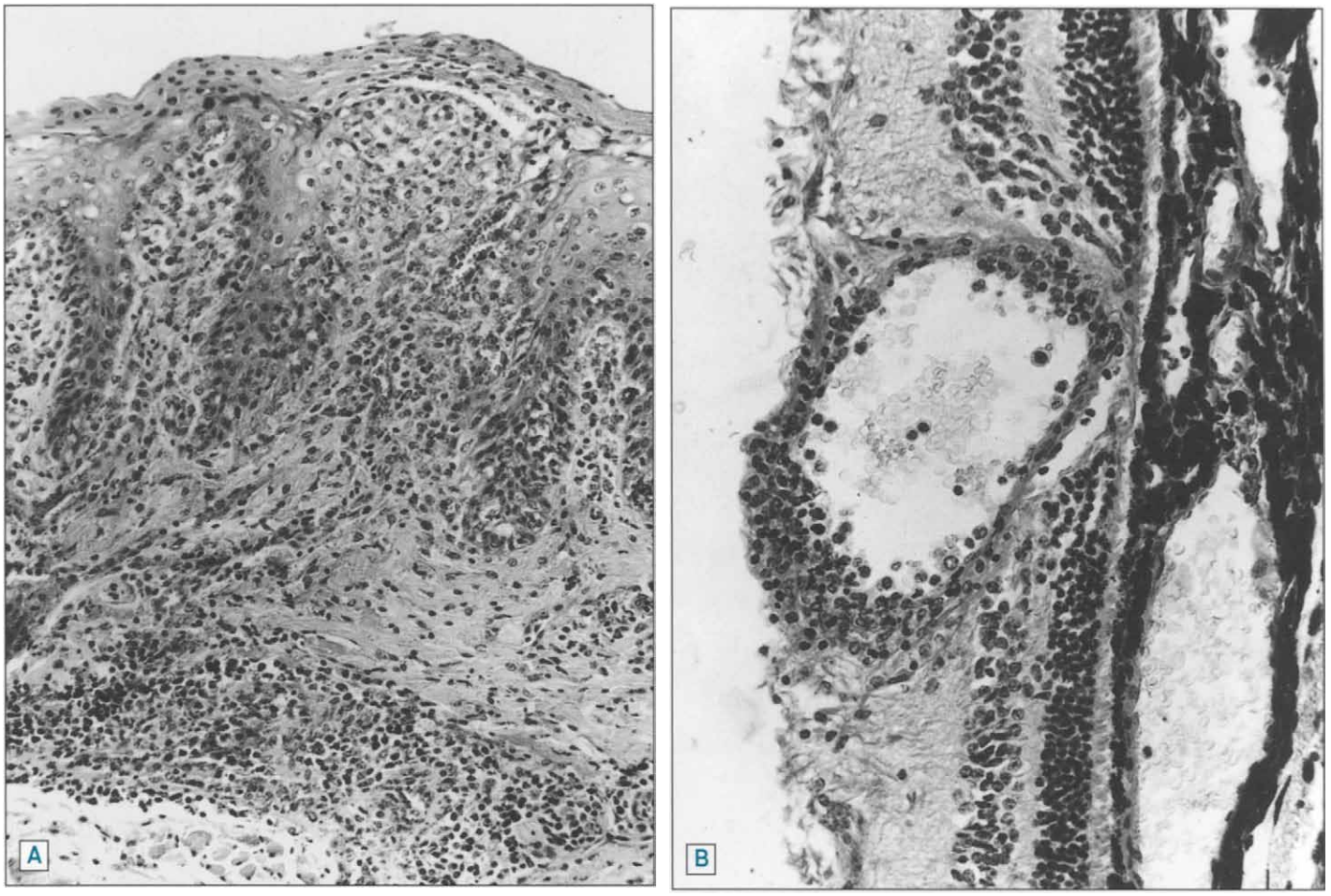


Figure 1.107 Malignant catarrhal fever. A. Vasculitis, with infiltration of lamina propria by lymphocytic cells with developing ulcer over papilla in the tongue. **B.** Vasculitis in retina.

and atrophy of follicles in lymph nodes and spleen, with lymphoid infiltrates in multiple organs in Jembrana disease, appear similar to lesions of MCF.

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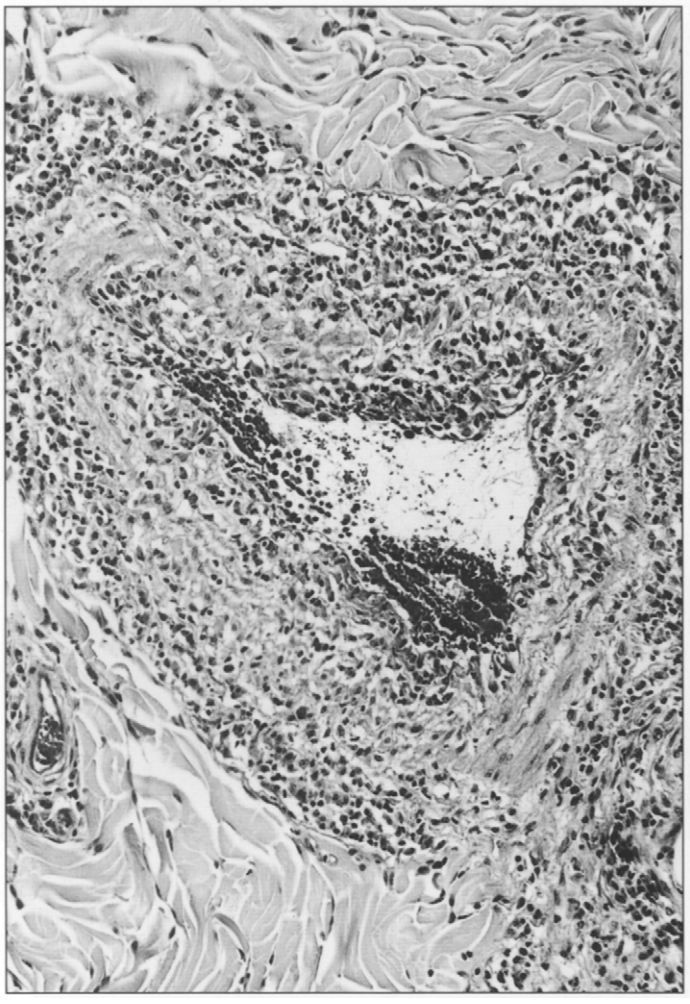

Figure 1.108 Periarteritis in the deep dermis of a cow with **malignant catarrhal fever**. (Courtesy of JA Yager.)

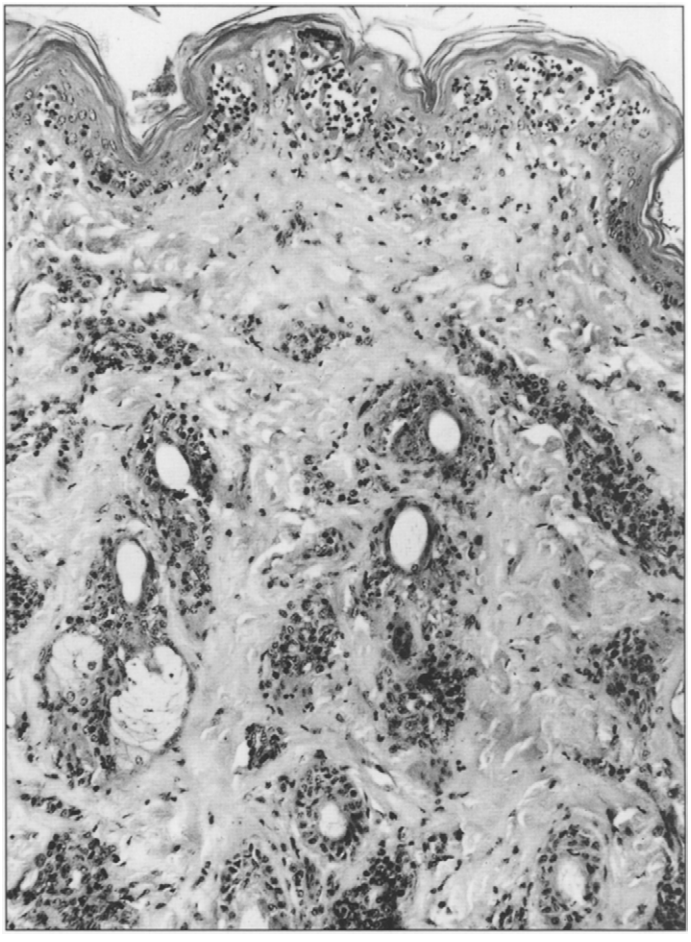

Figure 1.109 Necrosis of epithelium of skin in **malignant catarrhal fever**.

Bluetongue and related diseases

Bluetongue is caused by a reovirus of the genus *Orbivirus,* family Reoviridae. There are at least 24 recognized serotypes of *Bluetongue virus* (BTV), distinguished by serum neutralization tests. Immunity to one serotype does not confer resistance against another, and may cause sensitization, with a more severe syndrome following infection by a second type. Apparently, not all serotypes are pathogenic.

Epizootic hemorrhagic disease of deer is caused by a virus which represents another serogroup of *Orbivirus.* The virus causing *Ibaraki disease*, recognized in cattle in Japan, is a variant of *Epizootic hemorrhage disease virus* (EHDV); seropositive animals have also been found in Taiwan and Indonesia, and an identical virus has been isolated in Australia.

BTV, EHDV, and related viruses are spread by vector-competent *Culicoides* spp., also known as midges or gnats. The virus multiplies by a factor of 10^3–10^4 in the *Culicoides* within a week of the infected blood meal being ingested, and transmission can occur, following infection of the salivary glands, 10–15 days after the initial blood meal. Transovarial transmission of virus in *Culicoides* does not occur.

BTV circulates in a broad belt across the tropics and warm temperate areas, from about latitude 40°N to 35°S, with incursions or recrudescence during the *Culicoides* season, annually, or at irregular longer intervals in cooler temperate areas. The condition is enzootic or seasonally epizootic in most of Africa, the Middle East, the eastern Mediterranean basin, the Indian subcontinent, the Caribbean, and the USA. It appears sporadically in the Okanogan valley of western Canada. Recently it has made persistent incursions into the Iberian peninsula, Corsica and Sardinia, Italy, and the Balkans, perhaps associated with climate change. Seasonality of infection toward the periphery of its distribution probably reflects the unavailability of vectors, since virus may be able to overwinter in latently infected γδT cells in the skin of sheep, which express virus once vector feeding occurs again.

Sheep, goats, and cattle are the susceptible domestic species, wherever bluetongue occurs. Sheep are the domestic species most highly susceptible to bluetongue, but there is considerable variation in expression of the disease, depending on the breed, age, and immune status of the sheep, the environmental circumstances under which they are held, and the strain of virus. Typically, indigenous breeds seem more resistant to clinical disease than do exotics. Goats, though susceptible to infection, rarely show signs; however, disease has occurred in goats in the Middle East and India. Infection in cattle usually produces only inapparent infection or mild clinical disease. In Africa, a wide variety of nondomestic ungulates and some small mammals may be inapparently infected; mortality has occurred in naturally or experimentally infected topi, Cape buffalo, and kudu. In North America, wildlife species, particularly white-tailed deer, black-tailed or mule

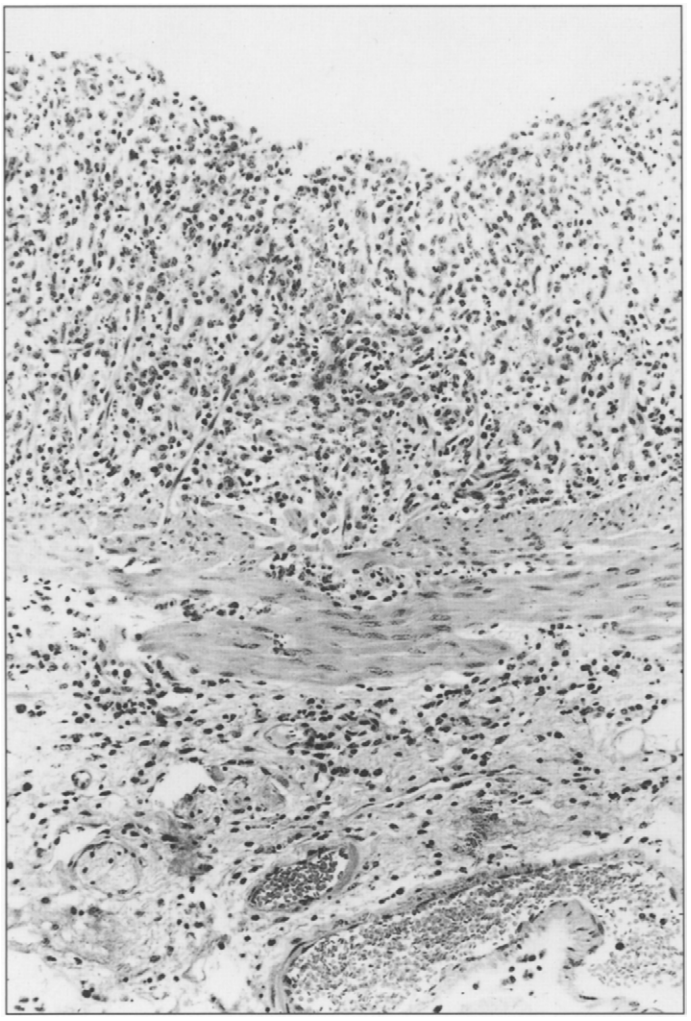

Figure 1.110 Colitis with collapse of glands and edema of submucosa in **malignant catarrhal fever**.

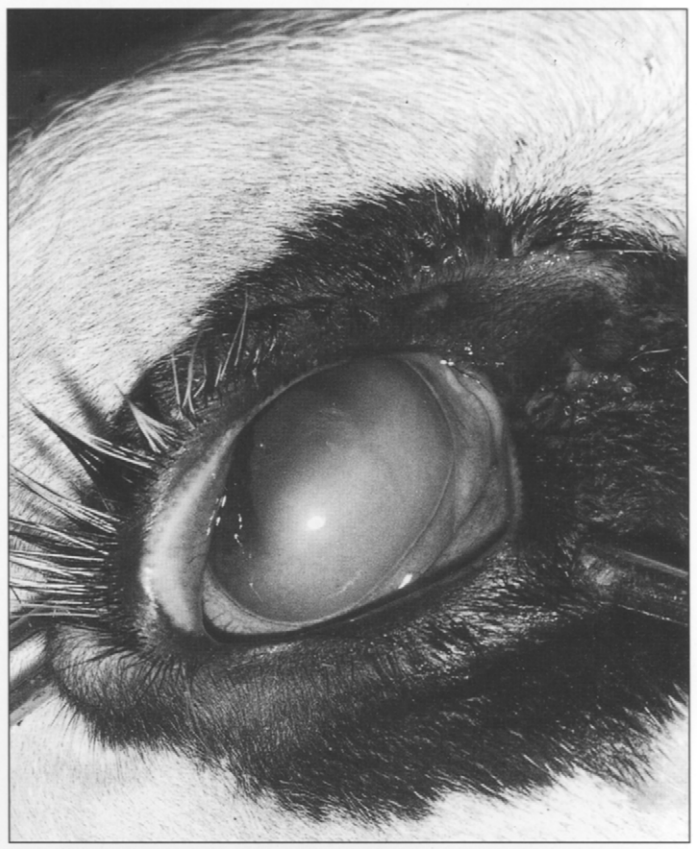

Figure 1.111 Corneal edema in **malignant catarrhal fever**.

deer, elk, bighorn sheep, bison, and pronghorn antelope are also infected. Bluetongue is responsible for significant mortality in all these species except elk, which usually develop mild or inapparent infection, and bison, which are infrequently demonstrated to be serologically positive. Vaccine inadvertently contaminated with BTV and administered to dogs caused significant mortality, but BTV is not normally considered a pathogen in dogs.

Epizootic hemorrhagic disease serogroup virus has been isolated in Nigeria, but the natural vertebrate hosts there are not known. In North America, the white-tailed deer is extremely susceptible, and widespread epizootics have occurred among this species in the USA. A single outbreak has been recognized in Alberta, Canada. The rate of survival is much higher among black-tailed deer and pronghorn antelope, and elk are only very mildly affected. Clinical disease similar to that produced by bluetongue may occur rarely in cattle. Though sheep are not considered to develop disease when infected with BTV, occasional mild clinical signs and lesions resembling bluetongue have been reported in sheep inoculated with some Australian isolates. In Japan, the *Ibaraki virus* strain of EHDV produces a clinical syndrome resembling bluetongue in cattle, but not in sheep.

BTV and EHDV circulate together in North America. Both viruses may be involved simultaneously in outbreaks of hemorrhagic disease in wild ruminants, and both have been isolated from *Culicoides* in a single locality at the same time. The role of cattle as reservoirs of BTV is uncertain. Cattle may act as reservoirs in that the virus will be associated with the erythrocytes for the lifespan of that cell. Detectable viremia in cattle is thought to be less than 9 weeks.

The **pathogenesis** of bluetongue, epizootic hemorrhagic disease, and Ibaraki disease is fundamentally similar in all species in which disease is seen. Primary viral replication following insect bite occurs in regional lymph nodes and spleen. Viremia about 4–6 days after inoculation results in secondary infection of endothelium in arterioles, capillaries, and venules throughout the body, but especially in lung microvascular endothelium. Microscopic lesions, fever, and lymphopenia begin a day or so later, about a week after inoculation. BTV in the blood appears to be closely associated with, or in, both leukocytes and erythrocytes, and it may co-circulate with antibody.

Endothelial damage caused by viral infection initiates local *microvascular thrombosis and permeability*. This is reflected microscopically by the presence of swollen endothelium, and fibrin and platelet thrombi in small vessels, with edema and hemorrhage in surrounding tissue. These lesions in turn mediate the full spectrum of gross findings. These are fundamentally *ischemic necrosis* of many tissues; *edema* due to vascular permeability; and *hemorrhage* resulting from vascular damage compounded, in severe cases, by consumption coagulopathy due to thrombocytopenia and depletion of soluble clotting factors. Differences in the expression and activity of vasoactive and pro- and

anticoagulant mediators by infected pulmonary endothelium may explain the greater propensity of sheep to show signs, in comparison with cattle.

Bluetongue in sheep is highly variable; it may cause inapparent infection or an acute fulminant disease. Typically, leukopenia and pyrexia occur, even in mild infections, coincident with viremia. The degree and duration of fever do not correlate with the severity of the syndrome otherwise.

In the early phase there is hyperemia of the oral and nasal mucosa, drooling, and nasal discharge within a day or two of the onset of fever. Hyperemia and edema of the eyelids and conjunctiva may occur, and edema of lips, ears, and the intermandibular area becomes apparent. Hyperemia may extend over the muzzle and the skin of much of the body, including the axillary and inguinal areas. Focal hemorrhage may be present on the lips and gums, and *the tongue may become edematous and congested or cyanotic*, giving the disease its name. Infarcted epithelium thickens and becomes excoriated; erosions and ulcerations develop along the margins of the tongue opposite the molars, and the mucosa of much of the tongue may slough.

Excoriation and ulceration also occur on the buccal mucosa, the hard palate, and dental pad. Affected areas of skin may also become encrusted and excoriated with time, and a break in the wool can result in parts or much of the fleece being tender or cast. The coronet, bulbs, and interdigital areas of the foot may become hyperemic. Coronary swelling and streaky hemorrhages in the periople may be evident as a result of lesions in the underlying sensitive laminae. These hemorrhages may persist in the hoof as brown lines that move down the hoof as it grows. A defect parallel to the coronet may also be evident in the growing hoof in recovered cases.

Internally, in acute cases, there is *subcutaneous and intermuscular edema*, which may be serous or suffused with blood. Superficial lymph nodes are enlarged and juicy. Bruise-like gelatinous hemorrhages and contusions, which may be small and easily overlooked if not numerous, are often present in the subcutis and intermuscular fascial planes. Focal or multifocal pallid areas of streaky myodegeneration may be present throughout the carcass, perhaps partly obscured by petechial or ecchymotic hemorrhage. Resolving muscle lesions may be mineralized or fibrous. Stiffness, reluctance to move, and recumbency seen clinically are due to these muscle lesions.

Necrosis may be present deep in the papillary muscle of the left ventricle, and elsewhere in the myocardium. The lesion which is perhaps most consistent and closest to pathognomonic for bluetongue is *focal hemorrhage*, petechial or up to 1 cm wide × 2–3 cm long, in the tunica media at the *base of the pulmonary artery*. These hemorrhages are visible from both the internal and adventitial surfaces, and may be present in clinically mild cases with few other lesions. Petechial hemorrhage may also be present at the base of the aorta and in subendocardial and subepicardial locations over the heart.

There may also be edema and petechial or ecchymotic hemorrhage in the pharyngeal and laryngeal area. In severe cases the lungs may assume a purple hue, with marked edematous separation of lobules, and froth in the tracheobronchial tree, probably due to pulmonary microvascular damage and heart failure. Animals with pharyngeal or esophageal myodegeneration suffer from dysphagia, or regurgitate, and may succumb to aspiration pneumonia.

Hyperemia, occasionally marked hemorrhage, or in advanced cases, ulceration of the mucosa may occur on rumen papillae, the pillars of the rumen, and the reticular plicae. In convalescent animals, stellate healing ulcers or scars on the wall of the forestomachs may be apparent.

Microscopically, acute lesions are characterized by microvascular thrombosis, and edema and hemorrhage in affected sites recognized at autopsy. In squamous mucosa and skin, capillaries of the proprial and dermal papillae are involved, resulting in vacuolation and necrosis of overlying epithelium. There is a mild, local neutrophilic infiltrate acutely, and a similarly mild mononuclear reaction in the dermis or propria in uncomplicated chronic lesions, which may granulate if widely or deeply ulcerated. Similar microvascular lesions are associated with necrosis and fragmentation of infarcted muscle. Muscle during the reparative phase follows the usual course of regeneration of fibers or fibrous replacement, depending on whether or not the sarcolemma retains its integrity.

In **cattle**, *clinical bluetongue is rarely apparent*; in endemic areas it may never be evident. Mortality is low, and it is often attributed to secondary infection. Clinical disease may be a function of hypersensitivity in previously exposed animals, and disease in experimentally infected animals is poorly defined. Fever, loss of appetite, and leukopenia are usually seen after an incubation period of 6–8 days, and there may be a drop in milk production in dairy cattle. There is reddening of the epithelium of the mucous membranes, and of thin exposed skin, especially notable on the udder and teats. Edema of the lips and conjunctiva may be present. Drooling may become profuse, and as the disease progresses over the next several days, hyperemia and congestion of the mucosae become more intense. Ulcerations of the gingival, lingual, or buccal mucosa occur, most consistently on the dental pad. There may be necrosis of epithelium on the muzzle. Muscle stiffness is a feature of the disease in some animals. Laminitis, characterized by hyperemia and edema of the sensitive laminae at the coronet, may be apparent, and in some cases, hooves on affected feet may eventually slough. Sloughing or cracking of crusts of necrotic epithelium may also occur on affected parts of the skin, but the ulcerative or erosive defects heal readily. Viral antigen and thrombosis are present in small vessels in affected tissues during the acute phase.

The signs and lesions of **Ibaraki disease** are similar to those of bluetongue in cattle, though more severe in some cases. As well as the signs and lesions described in cattle with bluetongue, there may be difficulty in swallowing in 20–30% of clinically affected animals, and the swollen tongue may protrude from the mouth. At necropsy, in addition to the lesions observable externally, there may be congestion, erosion, or ulceration of the mucosa of the abomasum, and less commonly, the esophagus and forestomachs. Ischemic necrosis and hemorrhage of the striated muscle in the tongue, pharynx, larynx, and esophagus cause the difficulty in swallowing seen clinically, and similar changes are seen in other skeletal muscles. Necrotizing aspiration pneumonia is a sequel to dysphagia in some animals.

The *hemorrhagic diseases* in bighorn sheep, pronghorn antelope, and white-tailed and black-tailed or mule deer in North America resemble bluetongue in sheep. White-tailed deer may develop a particularly severe and fulminant hemorrhagic disease, with high mortality. There may be necrosis of velvet antler, and hooves may slough in survivors. Bluetongue in goats, though usually inapparent, can resemble bluetongue in sheep.

Bluetongue in sheep must be differentiated from foot-and-mouth disease, peste des petits ruminants, contagious ecthyma, and photosensitization in particular. In cattle, the condition must be differentiated from foot-and-mouth disease, vesicular stomatitis,

bovine viral diarrhea, rinderpest, malignant catarrhal fever, and photosensitivity. In Japan, Ibaraki disease of cattle must in addition be differentiated clinically from ephemeral fever virus.

In addition to the systemic disease described, *abortion*, perhaps unobserved, and birth of progeny with various *congenital defects* may follow BTV infection of pregnant sheep and cattle. In sheep, BTV infection of ewes early in gestation may result in hydranencephaly. Anomalous calves produced by BTV-infected cattle have excessive gingiva, an enlarged tongue, anomalous maxillae, dwarf-like build, and rotations and contractures of the distal extremities. Porencephaly, hydranencephaly, and arthrogryposis are also reported in calves infected in utero with BTV. Antibody may be sought in neonates that have not sucked, and attempts should be made to isolate virus, since some prenatally infected animals may have immune tolerance, and persistent infection. Anomalies of the brain are considered further in Vol. 1, Nervous system.

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Parapoxviral infections

Bovine papular stomatitis

Papular stomatitis of cattle occurs worldwide. *It is generally an insignificant infection*, but needs to be differentiated from other more serious diseases affecting the oral cavity and skin. It is caused by *Bovine papular stomatitis virus* (BPSV), a member of the family Poxviridae, genus *Parapoxvirus*, that is closely related to *Pseudocowpox virus* that causes pseudocowpox in cattle and milker's nodules in humans. It is morphologically similar to, and shares antigens with, *Orf virus* of sheep and goats (see Vol. 1, Skin and appendages). However, analysis of the genome indicates that these viruses are distinct.

BPSV is relatively host-specific. As with many of the poxviruses, neutralizing antibody is not readily demonstrated. Infection does not confer significant immunity, and successive crops of lesions and relapses can occur. The disease is more common in calves than in older animals, although the susceptibility of, or recrudescence in, the latter may be increased by intercurrent debility, disease such as bovine viral diarrhea, infectious bovine rhinotracheitis, or other stressors.

The *papular lesions* of this disease occur on the muzzle and in the rostral nares, on the gums, the buccal papillae, the dental pad, the inner aspect of the lips, the hard palate (Fig. 1.112A), the floor of the oral cavity behind the incisors, the ventral and lateral (not dorsal) surfaces of the tongue, and, occasionally, in the esophagus (Fig. 1.112B) and forestomach.

The initial lesions, which are likely to be detected on the muzzle or lips, are erythematous macules, ~2 mm–2 cm in diameter. Shortly, the central portion becomes elevated as a low papule, although the elevation is not easy to see, and by the second day a gray central zone of epithelial hyperplasia has developed on which there is superficial scaliness and necrosis. A central necrotic area may slough to form a shallow craterous defect surrounded by a slightly raised red margin. Lesions may coalesce. The course of individual lesions is about a week.

Histologically, there is focal but intense hyperemia and edema in the papillae of the lamina propria, with accumulation of a few mononuclear leukocytes. The epithelium is thickened, sometimes to twice its normal depth, by hyperplasia and ballooning degeneration in the deeper layers (Fig. 1.112C). The cytoplasm of affected cells is clear, and the nucleus may be shrunken. *Dense eosinophilic inclusion bodies lie in the vacuolated cytoplasm*, especially in cells at the active margin of the lesion. In the central, more advanced part of the lesion, a mainly neutrophilic infiltrate into the superficial propria and epithelium is associated with erosion of the upper layers of necrotic cells. The basal layer survives and may be very flattened in eroded areas. Vesicles do not form.

A *chronic form* has been reported, with necrotic and proliferative stomatitis, represented histologically by extensive parakeratotic hyperkeratosis, pseudoepitheliomatous hyperplasia, and occasional intracytoplasmic inclusion bodies.

Papular stomatitis is probably more common and widespread than reports indicate. Variation in the extent and gross appearance of the lesions is to be expected, depending on the usual host–parasite factors and the nature of superimposed infections. They may predispose to the development of necrotic stomatitis, and must be differentiated from the lesions of bovine viral diarrhea, alimentary infectious bovine rhinotracheitis, and other causes of ulcers and erosions in the upper alimentary tract. The infection can be transmitted to humans to produce small papules that may persist for several weeks on the skin, usually of the fingers or forearms.

Rapid diagnosis is readily accomplished by demonstration of characteristic parapoxvirus particles in negatively stained material from lesions examined under the electron microscope.

Figure 1.112 Bovine papular stomatitis. A. Lesions at various stages of evolution in palate. **B.** Lesions in esophagus. **C.** Thickened epithelium at the margin of a lesion, with ballooning degeneration of cells in the deeper layers.

Contagious pustular dermatitis

Contagious pustular dermatitis, also called **orf** or **contagious ecthyma**, is a parapoxviral disease of sheep and goats that is characterized mainly by *proliferative scabby lesions on the lips, face, and feet* (see Vol. 1, Skin and appendages). The disease has also been reported in camels and a gazelle. Lesions may extend into the oral cavity, involving the tongue, gingiva, dental pad, and palate. Involvement of the esophagus and forestomachs occurs, but is very unusual. In general the evolution of the lesions is similar to papular stomatitis of cattle, though they are more exudative and usually much more proliferative. Morbidity may be high, and death can occur in suckling animals. In the upper alimentary tract, lesions may consist of focal red, raised areas, which coalesce to form papules followed by pustules. The latter rupture, and on the muzzle and in the mouth they may become covered by a gray to brown scab, although scab formation may not occur in the mucosa of the upper alimentary tract. Diagnosis is by demonstration or isolation of *Orf virus*.

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Infectious bovine rhinotracheitis

Bovine herpesvirus 1 (Infectious bovine rhinotracheitis virus, BoHV-1), which is in genus *Varicellovirus*, subfamily Alphaherpesvirinae, has been associated with a wide range of clinicopathologic syndromes in cattle. These include necrotizing rhinotracheitis, conjunctivitis, infectious pustular vulvovaginitis and balanoposthitis, vesicular lesions of the udder, abortions, and latent infection (see appropriate chapters). Clinical significance of BoHV-1 in other species such as bison that have serological evidence of exposure is not known.

A *systemic form* of the disease, which usually involves the alimentary tract, may occur spontaneously in neonatal calves (in which it may be congenital, or acquired shortly after birth) and in feedlot cattle. It has been reproduced experimentally in young calves.

The pathogenesis of systemic infection with BoHV-1 is poorly understood. Colostrum-deprived calves are especially susceptible,

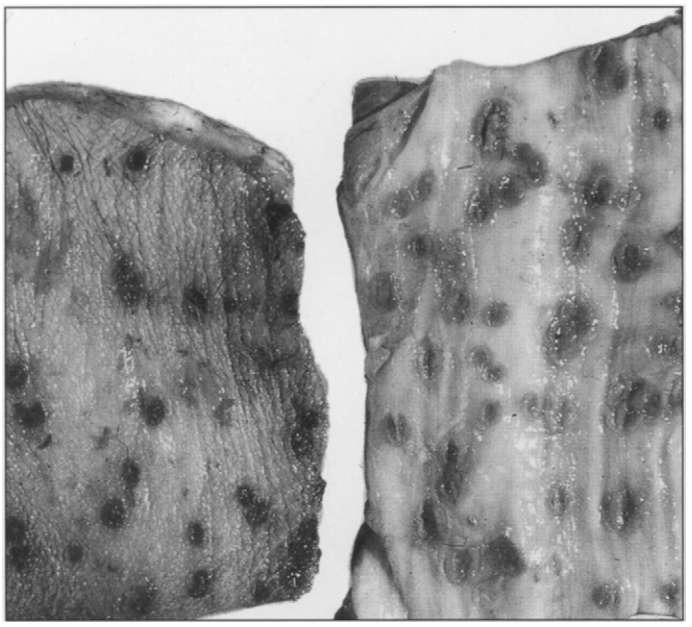

Figure 1.113 Foci of necrosis on mucosa of esophagus and rumen in a neonatal calf in **infectious bovine rhinotracheitis**.

and the disease can be prevented by feeding colostrum from actively immunized dams. The virus probably spreads from the mucosa of the upper respiratory tract to other tissues by circulating leukocytes. Peripheral blood mononuclear leukocytes may exhibit apoptosis in response to BoHV-1, but the significance of this is unknown. Experimental infection of calves with noncytopathic *Bovine viral diarrhea virus* (NCP-BVDV) followed by BoHV-1 inoculation results in dissemination of the latter to a variety of tissues. BVDV impairs cell-mediated immunity, and this may allow BoHV-1 to escape from the respiratory tract and lead to a systemic infection. Dual infections of BVDV and BoHV-1 occur under field conditions, but coinfection of these two viruses is not a prerequisite for the disease to develop.

Clinically affected animals have hyperemic oral and nasal mucosae, and focal areas of necrosis, erosion, and ulceration on the nares, dental pad, gums, buccal mucosa, palate, and the caudal, ventral, and dorsal surfaces of the tongue. Characteristically, the lesions tend to be punctate with a slightly raised margin; the necrotic areas are covered by a gray-white layer of fibrinonecrotic exudate, which leaves a raw red base when removed.

The *lesions* may extend into the esophagus, usually only the upper third, and the forestomachs. In the esophagus, the erosions and ulcers may be irregular, circular, or linear, and often they have a punched-out appearance and a hyperemic border (Fig. 1.113). The ruminal lesions, which are most commonly located in the dorsal and cranioventral sacs, vary considerably. The earliest lesions consist of foci of necrosis and hemorrhage, a few millimeters in diameter. In some cases, the necrosis may involve almost the entire surface of the ruminal mucosa, which becomes covered by a thick, dirty gray layer of exudate, resembling curdled milk, which adheres tightly to the wall (Fig. 1.114A). Similar lesions may be evident in the reticulum. Focal areas of necrosis result in the formation of holes, as large as 1.5 cm in diameter, in the leaves of the omasum. In addition, these calves may have focal areas of necrosis in the abomasal mucosal folds, which may coalesce to form areas of necrosis 2–3 cm in diameter. The intestines are red and dilated, and the serosal surface may be covered by a thin layer of fibrinous exudate.

The enteric lesions may be accompanied by changes in the upper respiratory tract. When present, the respiratory lesions are similar to those described for older cattle, although they are milder and generally limited to the nasal mucosa, larynx, and upper third of the trachea (see The Respiratory System, Volume 2, Chapter 5).

Gray to yellow necrotic foci 2–5 mm diameter may be evident macroscopically on the capsular and cut surfaces of the liver, the adrenal cortices, the spleen, and in Peyer's patches.

Microscopically, the lesions in the squamous mucosa are characterized by *focal areas of necrosis* (Fig. 1.114B), *erosion, and ulceration*. Severe necrosis may involve the entire papilla or mucosa more diffusely. *Nuclear inclusions* may be present in epithelial cells in the periphery of the lesion, although these are an inconsistent finding. They are more likely to be found if tissues are collected in the early stages of the disease and fixed in Bouin's fluid. The abomasal lesions consist of necrosis of glandular epithelial cells. Affected glands are dilated, and filled with necrotic debris. Focal necrotic lesions involving crypts and lamina propria may be present in both the small intestine and large bowel (Fig. 1.115). Abomasal and intestinal lesions may predispose to the development of *secondary mycosis*, which is a common complication.

Foci of coagulative necrosis may occur in the liver, lymph nodes, thymus, Peyer's patches, spleen, and adrenal cortices. Typically, there is little inflammation associated with the necrosis. Herpesviral inclusions are inconsistently seen in cells at the periphery of the necrotic foci.

The lesions in the upper alimentary tract of cattle associated with BoHV-1 infection must be differentiated from those of calf diphtheria, bovine papular stomatitis, and bovine viral diarrhea. The ruminal lesions must be differentiated from those of *Bovine adenovirus* infection and nonspecific rumenitis, described elsewhere in this chapter. The liver lesions may be confused with focal necrosis associated with septicemias, for example, listeriosis or salmonellosis (see Vol. 2, Liver and biliary system).

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Caprine herpesvirus

A herpesvirus that shares some antigens with, but on the basis of restriction endonuclease analysis is distinct from, *Bovine herpesvirus 1*, has been isolated from neonatal goat kids in various parts of the world. This virus has been designated *Caprine herpesvirus 1* (CpHV-1),

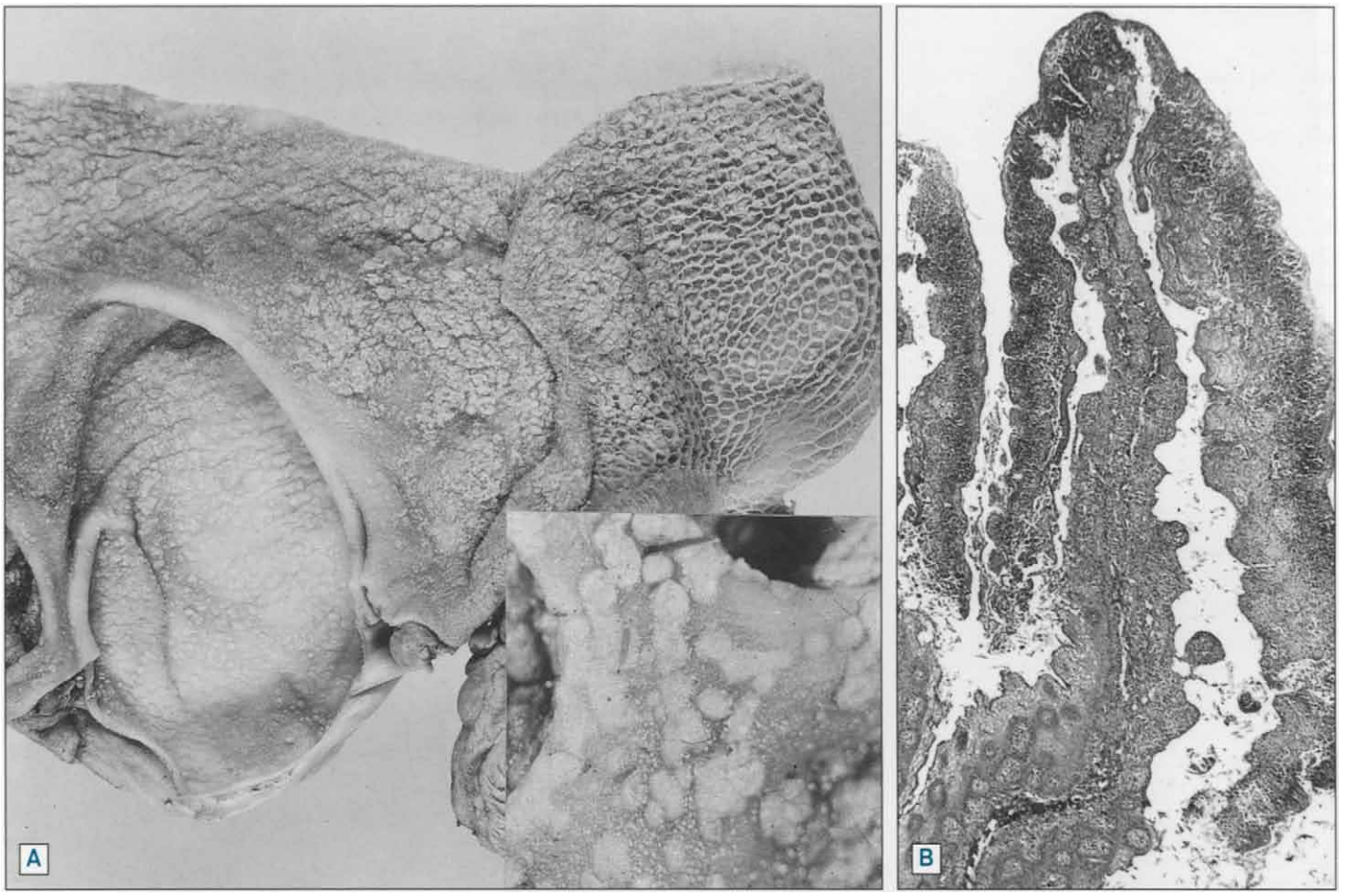

Figure 1.114 Infectious bovine rhinotracheitis in a neonatal calf. **A.** Cheesy necrotic debris in rumen and reticulum. Inset: Detail of rumen lesion. **B.** Necrosis of omasal fold.

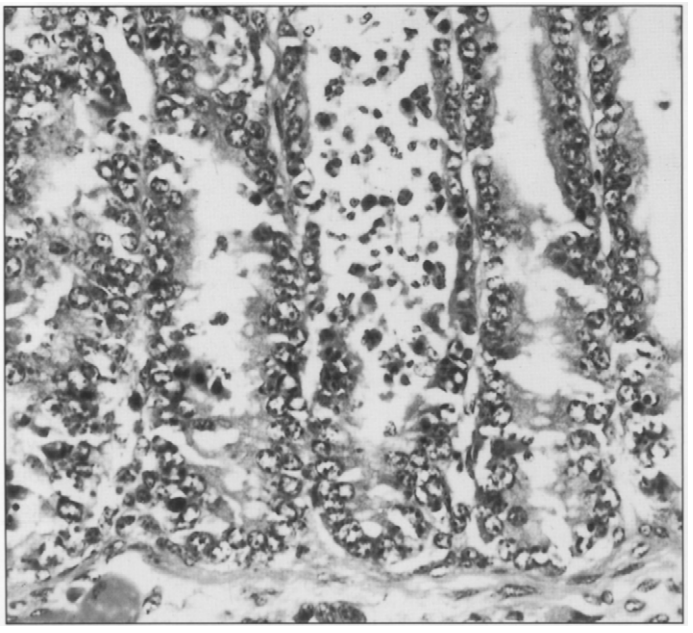

Figure 1.115 Necrosis of epithelium in the crypts of Lieberkühn in the small intestine of a neonatal calf with **infectious bovine rhinotracheitis**.

genus *Varicellovirus*, subfamily Alphaherpesvirinae. Although capable of infecting sheep, cattle, and goats, severe infection is restricted to goats. *CpHV-1 causes severe systemic disease with erosions and ulcerations of the alimentary tract in neonatal goats.* Adult goats may be latent carriers, developing vulvovaginitis or balanoposthitis (see Vol. 3, Female genital system). Infection of pregnant does causes abortion.

The disease in neonatal kids is characterized clinically by fever, conjunctivitis, ocular and nasal discharges, dyspnea, anorexia, abdominal pain, weakness, and death, usually within 1–4 days after onset of clinical signs. Affected kids have leukopenia and hypoproteinemia.

Macroscopic lesions are most obvious throughout the entire alimentary tract. Round or longitudinal erosions, which have a hyperemic border, are evident in the oral mucosa. These are particularly prominent on the gums around the incisor teeth and to a lesser extent in the pharynx and esophagus. Focal red areas of necrosis, which may be slightly elevated above the surrounding mucosa, occur in the rumen. In the abomasum, numerous longitudinal, red erosions are located in the mucosa. The most severe lesions occur in the *cecum and ascending colon*, which are dilated, with a thickened wall, and contain focal to large areas of mucosal necrosis and ulceration, frequently covered by a pseudodiphtheritic membrane. The contents are yellow and mucoid. Hemorrhagic foci may be visible in the bladder mucosa.

Microscopically, the lesions in the upper alimentary tract are typical areas of necrosis and erosion of squamous epithelial cells. The epithelial cells at the periphery of necrotic areas are swollen and vacuolated and these may contain *herpesviral inclusions*. There is marked inflammatory reaction in the underlying lamina propria. The abomasal lesions consist of acute foci of mucosal necrosis. Inclusions are particularly evident in this area. Lesions in the cecum and colon are more extensive and consist of large areas of mucosal ulceration and necrosis, which may involve the entire thickness of the wall. The submucosa is edematous and markedly infiltrated by inflammatory cells. The mesenteric nodes are edematous and germinal centers are depleted of lymphoid cells. Focal areas of necrosis with a mild inflammatory cell reaction may also be present in liver, urinary bladder, and kidney.

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Other herpesviruses

Canid herpesvirus 1 causes systemic disease of neonatal puppies characterized by foci of necrosis and hemorrhage in a wide variety of organs, especially the lungs and renal cortices (see section on Viral infections of the pregnant uterus, Vol. 3, Female genital system). Focal areas of necrosis may occur in the intestine as part of the systemic syndrome.

Felid herpesvirus 1 (Feline viral rhinotracheitis virus) causes oral lesions (see section on Inflammation of the oral cavity, above). Viruses antigenically related to *Felid herpesvirus 1* have been isolated from dogs with diarrhea, but descriptions of lesions are not available.

Natural infections with ***Suid herpesvirus 1*** (SuHV-1; Pseudorabies virus, Aujeszky's disease virus) often result in necrotizing tonsillitis. Experimental infection of pigs with SuHV-1 may cause necrotizing enteritis of the distal small intestine. The enteric lesions are characterized by focal areas of necrosis of the cryptal mucosa, muscularis mucosae, and tunica muscularis. Immunohistochemically, antigen is documented in the dome area, the lymphoid follicles of Peyer's patches, and ganglion cells of Meissner's and Auerbach's plexuses.

Necrotizing enterocolitis in adult horses due to ***Equid herpesvirus 1*** has been reported rarely. At necropsy, there are multiple areas of hemorrhage, necrosis, and ulceration, some several centimeters in diameter, of the mucosa in both small and large intestine. Microscopically, these lesions consist of erosions and ulcerations of the mucosa, and necrosis of cryptal epithelial cells in adjacent areas. Cryptal epithelial cells and some proprial mononuclear cells may have acido- and amphophilic nuclear inclusions.

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Adenoviral enteritis

The adenoviruses that have been associated with enteric infections in humans, cattle, swine, horses, sheep, and dogs belong to the Adenoviridae, genera *Mastadenovirus* and *Atadenovirus*. Serological surveys show that widely divergent serotypes occur both within and between host species and their distribution is worldwide. All serotypes are morphologically similar; the virus consists of a nonenveloped icosahedral capsid, 70–80 nm in diameter, which has 252 capsomeres. Virus neutralization tests are used to distinguish serotypes. Adenoviral infection of cells causes *intranuclear inclusions*. Adenoviruses are relatively heat-resistant and can survive for several days at room temperature. Most adenoviruses are transmitted by feces, aerosols, or possibly fomites, to susceptible, usually suckling or recently weaned, animals. Infected animals may remain carriers for weeks.

Adenoviruses are highly host-specific. Infections in both humans and animals appear, in general, to be subclinical, and disease seems to occur more commonly in immunologically compromised individuals. Most infections are systemic; certain strains have a tropism for the respiratory tract, and others for the alimentary tract, vascular endothelial cells, or hepatocytes. Their enteric manifestations will be considered here.

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Bovine adenoviruses

There are 10 serotypes of *Bovine adenovirus* (BAdV) in the genera *Mastadenovirus* and *Atadenovirus*. Infection has been mainly associated with keratoconjunctivitis and respiratory disease. Many strains have been isolated from normal cattle. Serotypes 3, 4, 7, and 10 have been associated with enteric disease. It appears that after an initial viremic stage, the virus localizes in the *endothelial cells* of vessels in a variety of organs, resulting in thrombosis with subsequent focal areas of ischemic necrosis.

Clinically, enteric infections with BAdV occur sporadically in 1–8-week-old calves and in feedlot animals. Affected animals have fever, and diarrhea that may contain blood, and some animals will die peracutely from dysentery. They are dehydrated and the mucous membranes of the muzzle and mouth are congested. Dry, encrusted exudate may cover the muzzle and there may be serous to mucopurulent ocular and nasal discharges.

Macroscopic lesions may be present in the forestomachs, abomasum, and intestine. Those in the forestomachs are characterized by irregular, raised, red-to-gray necrotic areas, 2–4 mm in diameter on the mucosa of both the dorsal and ventral sacs of the rumen. In

some cases, the areas of necrosis coalesce to give rise to diffuse necrotizing rumenitis. Ulcers up to 1.5 cm in diameter may be located on the ruminal pillars and these may be visible through the serosa. Similar lesions may be evident in the omasum. The abomasal mucosal folds are edematous and congested, with focal necrosis and ulceration in the mucosa that, like those in the forestomachs, may be visible on the serosal surface.

The intestinal lesions vary from slight dilation and distension with excessive fluid to severe multifocal or diffuse necrosis, which may be covered by a pseudodiphtheritic membrane. In young calves, the lesions are most severe in the jejunum and ileum, especially over the Peyer's patches. In feedlot cattle, the lesions may be most prominent in the colon. The mucosa is dark red (Fig. 1.116) and there is marked edema of the mesocolon. The mesenteric lymph nodes are enlarged and edematous.

Microscopically, *large basophilic to amphophilic inclusions* completely or partially fill the nuclei of endothelium in the vessels of the lamina propria and submucosa of affected areas of the rumen, abomasum, and intestine. The endothelial cells are swollen and necrotic, and some veins and lymphatics contain thrombi. Foci of ischemic necrosis are evident in the overlying mucosa, and in more advanced lesions, the necrosis extends across the muscularis mucosae (Fig. 1.117A). Fibrinocellular exudate often covers the mucosal surface. Intestinal crypts are dilated, lined by flat epithelial cells, and usually contain necrotic debris (Fig. 1.117B). There is usually marked submucosal edema, congestion, and fibrinous exudation. Foci of necrosis are evident in the lymphoid follicles of the Peyer's patches, which are also depleted of lymphocytes.

Typical inclusions may also be found in endothelial cells of vessels and sinusoids of the adrenal glands, mesenteric lymph nodes, liver, spleen, glomeruli, and interstitial capillaries in the kidney, and in the mucosa of the urinary bladder.

Confirmation of enteric BAdV infection depends on the demonstration of the virus in tissue, through electron microscopy, in situ hybridization, or isolation of the virus in tissue cell cultures. The latter is often difficult because different serotypes and strains of

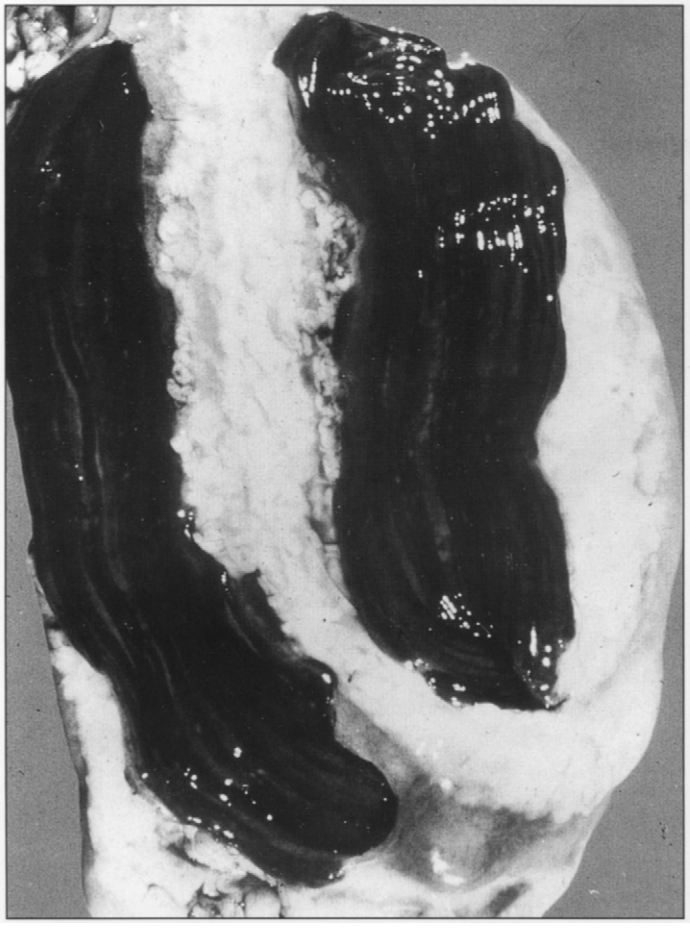

Figure 1.116 Congested and hemorrhagic colon caused by ***Bovine adenovirus*** infection.

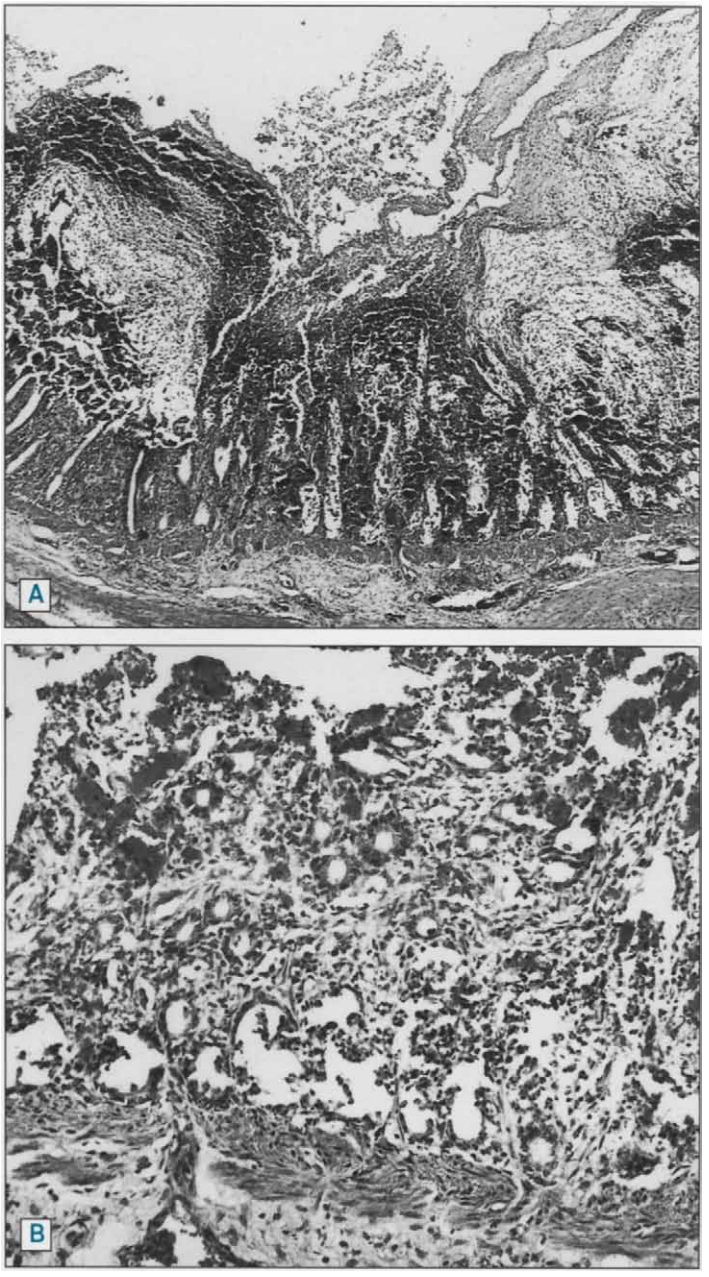

Figure 1.117 ***Bovine adenovirus*** infection. **A.** Infarctive necrosis and hemorrhage of the colon. **B.** Infarctive necrosis of crypt epithelium and hemorrhage in the colon.

the virus require specific tissue cell cultures and several blind passages may be required before cytopathic changes are evident.

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Porcine adenovirus

There are six serotypes of *Porcine adenovirus* (PAdV) which, according to serological surveys, are all common. Serotype 4 appears to be the most widely distributed strain of the virus in Europe and North America. Asymptomatic infections are most common in swine and PAdV may be isolated from feces of normal pigs; PAdVs are actually of more interest as viral vaccine vectors than as pathogens.

The importance of adenoviruses as a cause of enteric disease in the field remains controversial. When disease occurs, the macroscopic lesions in the intestine consist of excessive yellow watery to pasty contents and moderate enlargement of the mesenteric lymph nodes, which cannot be differentiated from other causes of diarrhea in neonatal pigs.

In contrast to the situation in calves, in which inclusions are located in the nuclei of endothelial cells, *inclusions in pigs are in enterocytes* in the distal jejunum and ileum, where primary viral replication likely occurs. The infected nuclei are enlarged, round, and displaced to the apical portion of the cell. The villi may be short and blunt. There may be a moderate mononuclear cell reaction in the lamina propria. Inclusions are also found in the squamous epithelial cells of the tonsils.

Ultrastructurally, infected nuclei of enterocytes are round and swollen and contain numerous typical adenoviral particles (Fig. 1.118). Affected enterocytes are cuboidal and the apical portion protrudes slightly into the lumen. The cell membrane and microvilli are irregular and the terminal web is absent. The rough endoplasmic reticulum shows local distension with formation of large multivesicular bodies. Eventually, there is complete loss of microvilli, and the cell membrane ruptures with the release of cell contents and virus particles into the gut lumen.

The significance of adenoviral inclusions in enterocytes must be interpreted with caution. A survey in Canada revealed that 4.4% of 5-day to 24-week-old pigs had adenoviral inclusions in enterocytes, mainly in the ileum. More than 50% of the pigs had diarrhea; however, other enteropathogens were found in most of these animals.

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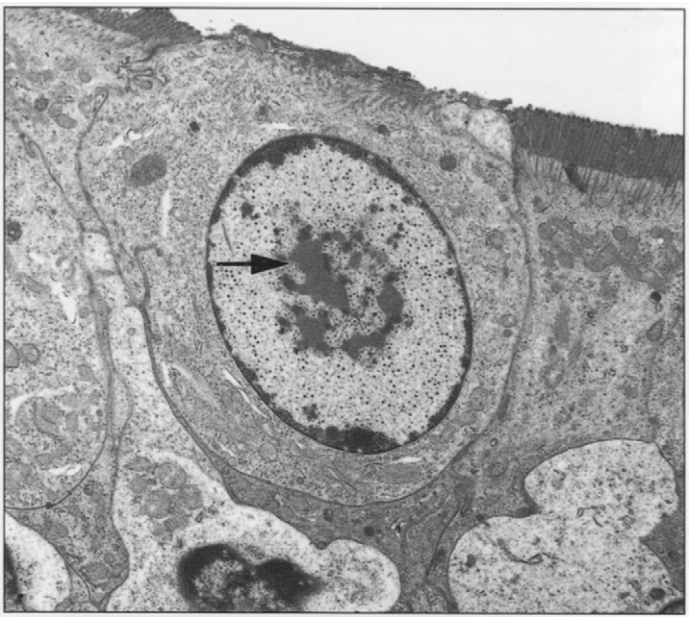

Figure 1.118 ***Porcine adenovirus***-infected cell in epithelium of dome over Peyer's patch; note inclusion (arrow). (Courtesy of DM Hoover, SE Sanford.)

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Equine adenovirus

Two serotypes of *Equine adenovirus* (EAdV) have been identified in horses. EAdV-1 has a worldwide distribution. Subclinical infections are common. Clinical disease occurs mainly as an upper respiratory infection in foals less than 3 months of age. The infection is particularly important in Arabian foals with combined immunodeficiency (CID), in which intestinal involvement is common. EAdV is capable of replication in the intestinal epithelium and produces duodenal villus atrophy after experimental infection.

Another adenovirus, proposed to be a prototype of EAdV-2, has been isolated in Australia from foals with diarrhea. *Rotavirus* was also identified in the feces of these foals. A serological survey showed that 77% of adult horses in the area had neutralizing antibodies to this particular serotype.

An unidentified alimentary tract adenoviral infection has been reported in an Arabian foal that did not have lesions of CID. The foal had diarrhea and progressive weight loss over a 2-month period. The macroscopic lesions consisted of ulcers in the distal esophagus and nonglandular mucosa of the stomach. The intestine contained soft to semifluid ingesta. Histologically, there was necrosis and ulceration of the esophageal and gastric squamous mucosa. Typical adenoviral inclusions were found at all levels of the small intestine. These were most commonly located in the villus epithelial cells, less often in the crypts, and only occasionally in the submucosal glands. There was focal-to-diffuse villus atrophy through the small intestine.

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Adenoviruses in other species

More than seven serotypes of *Ovine adenovirus* (OAdV) have been isolated from **sheep**. Serotypes 1, 2, and 3 have been recovered from feces of normal sheep, and lambs with enteritis and pneumoenteritis. Experimental inoculation of specific-pathogen-free lambs with OAdV-4 did not cause disease but the virus was re-isolated from feces and nasal secretions for several days postinfection. However, occasionally, there are reports of enteritis associated with abundant adenoviral inclusions in lambs and kids. In the former, inclusions were found predominantly in the lamina propria whereas in the latter they were mostly epithelial.

Goat adenovirus (GAdV) serotypes 1 and 2 can cause enteritis and diarrhea in **goat** kids; GAdV-1 is a serotype of *Ovine adenovirus D*, which also includes serotype 7 and isolate 287.

Two distinct but serologically related adenoviruses have been isolated from **dogs**. *Canine adenovirus 1* (CAdV-1) infection is usually subclinical, but it may cause infectious hepatitis, and diarrhea may be present in these cases. The virus has a particular tropism for hepatocytes and endothelial cells. The serosal hemorrhages in the gastrointestinal tract and possibly the diarrhea may be related to vascular damage in the serosa and mucosa respectively (see Vol. 2, Liver and biliary system). CAdV-2 is usually associated with upper respiratory infections in dogs (see Vol. 2, Respiratory system). Viruses serologically similar to CAdV-2 have been isolated from feces of diarrheic dogs. DNA fingerprinting of two of these isolates indicated that they are distinct from CAdV-2. It may be that the fecal isolates are due to swallowing of virus originating from upper respiratory tract infections.

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Enteric coronaviral infections

Coronaviruses cause disease affecting a number of organ systems in a variety of species. Among domestic mammals they mainly cause enteric infections, although coronaviruses are implicated in pneumonia in swine and cattle, and in feline infectious peritonitis.

Genus *Coronavirus* is found with the genus *Torovirus* in the family Coronaviridae. Coronaviruses have a single-stranded RNA genome. They are pleomorphic or roughly spherical and vary in size from about 70 to 200 nm in diameter, averaging ~100–130 nm. They have a phospholipid-bearing envelope, probably derived in part from host cell membrane. They gain their name from the characteristic "corona" of petal- or droplet-shaped radial surface projections ("peplomers") visible under the electron microscope in negatively stained preparations.

The coronaviruses infecting each species of host appear to be distinctive; some species are infected by more than one type of coronavirus. There are antigenic relationships among viruses from various hosts, and experimental cross-infection will occur between some host species, usually without pathologic consequences. The species *Transmissible gastroenteritis virus*, *Feline coronavirus* (with two biological types: Feline infectious peritonitis virus, and Feline enteric coronavirus), and *Canine coronavirus* are in antigenic group 1. *Bovine coronavirus* is in Group 2, antigenically related to *Porcine hemagglutinating encephalomyelitis virus*, *Mouse hepatitis virus*, and *Rat coronavirus*, among others. Persistent infections can occur.

Viral replication in the intestinal epithelium by coronaviruses is similar in all the species studied. Coronavirus infects and replicates in the apical cytoplasm of absorptive enterocytes on the tips and sides of intestinal villi. Virions are probably taken up by the apical border of the cell, by fusion with the plasmalemma. Replication and maturation appear to involve budding of virions from the cytosol through the membrane and into the lumen of vacuoles or cisternae in the smooth endoplasmic reticulum, where they accumulate. Virions are found in tubules of the Golgi apparatus. They may exit via that route from infected cells, by exocytosis at the apical cell membrane, or on the lateral cell surface, since viral particles are often seen lined up between microvilli or in the basolateral intercellular space between infected cells. Virus may also be released by lysis of infected cells. Coronaviruses will also infect some mesenchymal cells in villi and probably mesenteric lymph node.

Changes in the infected cell occur by about 12–24 hours after infection. Mitochondria in virus-infected cells swell, cisternae of smooth and rough endoplasmic reticulum dilate, the cytoplasm of infected cells loses its electron density, and cells lose their columnar profile. The terminal web is fragmented; microvilli swell and become irregular, perhaps in association with blebbing of the apical membrane. Damaged epithelium may lyse in situ, releasing virus retained in cytoplasmic vacuoles, or it may exfoliate into the lumen. Profuse diarrhea usually begins about the time that early cytologic changes are becoming apparent, but before there is extensive epithelial exfoliation.

Exfoliation of damaged epithelium may be massive over a relatively short period of time, leading to the development of *villus atrophy*, the severity of which largely reflects the degree of initial viral damage. Villi may appear fused along their sides or tips, and during the exfoliative phase some villi with denuded tips may be present. The enterocytes present on villi shortly after the initial

exfoliative episode are mainly poorly differentiated low columnar, cuboidal, or squamous cells, with stubby irregular microvilli. Within 2–3 days, villi begin to regenerate and the epithelium becomes progressively more columnar, though still lacking a well-developed brush border and its complement of enzymes. Defective fat absorption is reflected in the accumulation of lipid droplets in the cytoplasm of enterocytes on villi. This is particularly marked over the period of about 2–5 days after experimental inoculation.

With progressive epithelial regeneration from the crypts, the *villus fusion*, which may be the result of adhesion of temporarily denuded lamina propria of adjacent villi, regresses. Separation begins along the basal margins of the adhesions and progresses toward the tips of the villi. There may be focal acute inflammation in the lamina propria of temporarily denuded villi, and a mild mononuclear infiltrate in the stroma of collapsed villi. Though several cycles of viral replication may occur, poorly differentiated enterocytes appear relatively refractory to infection, and the virus titer falls, presumably as local immune mechanisms also come into play. Hyperplasia of epithelium in crypts usually results in eventual resolution of the villus atrophy, restoring normal function.

The diarrhea that occurs is a result of electrolyte and nutrient malabsorption, with some contribution by secretion by crypt cells, and probably by poorly differentiated surface epithelium in the reparative phase. Mechanisms of diarrhea in villus atrophy are discussed in the section on the Pathophysiology of enteric disease. Remission of signs occurs within about 4–6 days as regeneration of villi occurs, providing the animal survives the dehydration, electrolyte depletion, and acidosis brought about by diarrhea.

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Swine coronaviruses

Three coronaviruses cause gastrointestinal signs in swine. *Porcine hemagglutinating encephalomyelitis virus*, in group 2 species of genus *Coronavirus*, causes vomiting and wasting disease in suckling piglets; this is a condition mainly mediated by infection of the central and peripheral nervous system (see Vol. 1, Nervous system). *Transmissible gastroenteritis virus* (TGEV) and *Porcine epidemic diarrhea virus* (coronavirus 777), both in group 1 species of genus *Coronavirus*, cause syndromes of acute diarrheal disease in all age groups, and chronic diarrhea and runting in weaned pigs. In some areas, coronaviruses, especially TGEV, are the major cause of diarrhea in neonatal swine.

Transmissible gastroenteritis (TGE) may affect swine of any age, causing vomiting, severe diarrhea, and, in piglets, high mortality. The disease is recognized throughout most of the world.

The epizootiology of TGE depends on the overall immune status of the herd and of the various age groups within the herd. Introduction of TGEV into a naive herd results in rapid spread of disease with high morbidity affecting all age groups. Sows and older pigs will show transient inappetence, possibly diarrhea and perhaps vomiting. Signs may be more severe in sows exposed to high virus challenge from infected baby pigs. Agalactia may occur in recently farrowed sows, perhaps related to TGEV infection of the mammary gland. *Suckling piglets develop severe diarrhea*, and mortality may approach 100% in piglets under 10–14 days old. Older pigs usually develop less severe signs and have lower mortality. In herds with enzootic infection, high piglet mortality may occur in the offspring of recently introduced naive sows, and diarrhea with lower mortality may occur in piglets over about 2–3 weeks of age as milk intake and concomitant lactogenic immunity wane. Infected pigs in the late suckling or weanling age group may runt. TGE is more prevalent in the winter, perhaps because the virus is not resistant to summer environmental conditions of warmth and sunlight. Baby pigs that are chilled also seem less able to survive the effects of infection.

The severity of disease in baby pigs is partly related to their inability to withstand dehydration, due to their small size, and to their susceptibility to hypoglycemia. Probably as significant is the differentiation, and low rate of turnover, of small intestinal epithelium in the neonate. The surface epithelium is mature and has an extensive vesicular network in the apical cytoplasm associated with uptake of macromolecules and colostrum during the first day or two after birth. Crypts are short and relatively inactive. The population of epithelium susceptible to infection on each villus is therefore large, and the capacity to regenerate new enterocytes is small. By about 3 weeks of age, epithelium is actively proliferative. Virus production by infected enterocytes in older pigs seems less efficient, and replacement of cells lost to infection is more rapid, contributing to the relative resistance seen in swine over about 3 weeks of age.

Piglets with TGE have the nonspecific gross appearance at necropsy of undifferentiated neonatal diarrhea. The stomach may contain a milk curd or bile-stained fluid. The small bowel is flaccid and contains yellow frothy fluid with flecks of mucus; chyle is not usually evident in mesenteric lymphatics since there is fat malabsorption.

The microscopic lesions are those of villus atrophy due to exfoliation of surface enterocytes (Fig. 1.119), the severity of which is a function of the age of the pig and the stage of the disease. In young piglets, the lesions are most severe about the time of the onset of diarrhea. In later phases or in older pigs there may be subtotal to moderate atrophy, and the mucosa may be lined by cuboidal to low columnar epithelium, with irregular nuclear polarity and an indistinct brush border. Severe atrophy is readily recognized at necropsy of neonatal piglets, by examination of the mucosa under a dissecting microscope. Lesions are most common in the middle and lower small intestine, and villi in the duodenum are usually tall and cylindrical. Lesions may be patchy, and several areas of lower small intestine must be examined before atrophy is considered not to be present. In animals beyond the neonatal age group, atrophy may not be so severe and readily recognized under the dissecting microscope, and the contrast with the normally shorter villi in the duodenum of older pigs is not as marked. Histologic assessment of the gut is essential.

Porcine respiratory coronavirus (PRCoV) is genetically and antigenically extremely close to enteric TGEV. It cross-reacts serologically,

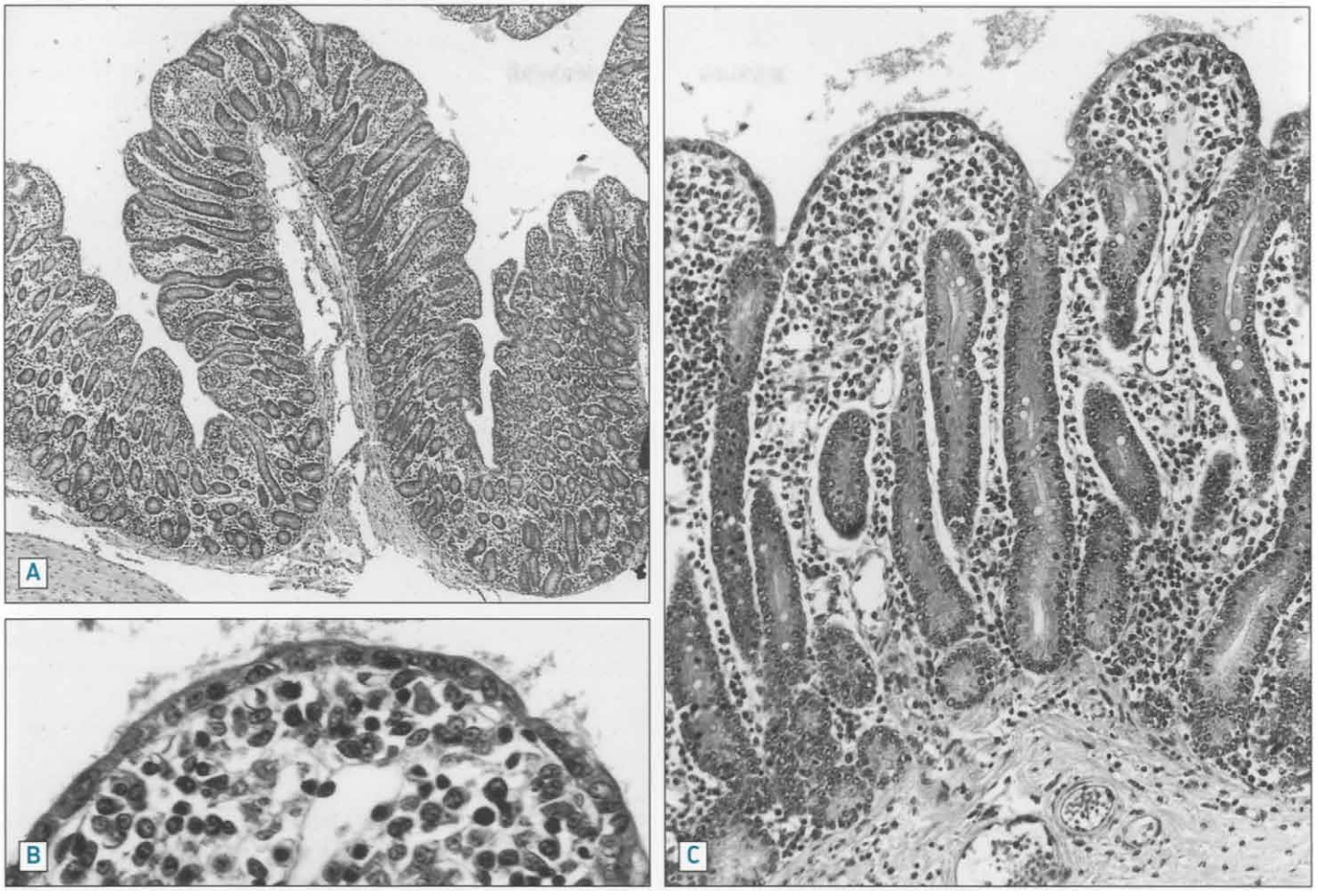

Figure 1.119 Transmissible gastroenteritis in a pig. **A.** Atrophy of villi in small intestine. **B.** Severely attenuated enterocytes on surface of an atrophic villus. **C.** Atrophy of villi, with compensatory hypertrophy of crypts of Lieberkühn. Surface epithelium is cuboidal or flattened.

and vaccinated sows successfully induce passive immunity against enteric infections. PRCoV is spread by inhalation, and infects lining cells of the upper respiratory tract. Mild bronchointerstitial pneumonia results from experimental infection, and the agent has been associated with outbreaks of respiratory disease.

Porcine epidemic diarrhea virus (PEDV), antigenically distinct from TGEV, is reported from England, continental Europe, China, and Taiwan. It causes disease that is essentially similar to TGE in epidemiology, pathogenesis, and lesions, but is milder. In addition to infection of epithelium low on villi and occasionally in small-bowel crypts, PEDV may also cause mild exfoliative lesions in colonic crypts. It is differentiated from TGEV by its distinct viral antigenicity and genome.

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Bovine coronavirus

In **neonatal calves**, *Bovine coronavirus* (BCoV) infection is a common cause of diarrhea, either alone or in combination with other agents, particularly *Rotavirus* and *Cryptosporidium*. The disease may be severe in combination with BVDV infection. BCoV is capable of infecting absorptive epithelium in the full length of the small intestine, and in the large bowel. Viral antigen is also found in macrophages in the lamina propria of villi and in mesenteric lymph nodes. In field infections, microscopic lesions are found most consistently in the lower small intestine and colon. Calves with BCoV infection usually develop mild depression, but continue to drink milk despite developing profuse diarrhea. With progressive dehydration, acidosis, and hyperkalemia, the animals become weak and lethargic; death ensues as a result of hypovolemia, hypoglycemia, and potassium cardiotoxicosis. Diarrhea in survivors resolves in 5–6 days.

At autopsy, affected animals have the nonspecific lesions of undifferentiated neonatal calf diarrhea. Rarely, mild fibrinonecrotic typhlocolitis is recognized at necropsy in calves with coronaviral infection. Mesenteric lymph nodes may be somewhat enlarged and wet.

The microscopic lesions of coronaviral infection in calves vary with the severity and duration of the infection; *villus atrophy in combination with mild colitis is typical* (Fig. 1.120). In the calf *small intestine*, villus atrophy is rarely as severe as that seen in neonatal swine with TGE. Rather, villi are moderately shortened, or have subtotal atrophy with stumpy, club-shaped, or pointed tips, and villus fusion may be common. In the early phase of the clinical disease, villi are often pointed and covered by cuboidal to squamous epithelium. Exfoliation of epithelium and microerosion may be evident. Later, the epithelium is cuboidal to low columnar, basophilic, with irregular nuclear polarity and an indistinct brush border. Cryptal epithelium is hyperplastic. The lamina propria may contain a moderate infiltrate of mainly mononuclear inflammatory cells, some of which may have pyknotic or karyorrhectic nuclei. In the early stages of infection, necrosis of cells in mesenteric lymph nodes is associated with viral replication. Peyer's patches in animals examined after 4–5 days of clinical illness often appear involuted, and are dominated by histiocytic cells. Whether this is the result of viral activity or the effect of endogenous glucocorticoids is unclear.

In the *colon* during the early phase of infection, surface epithelium may be exfoliating, flattened, and squamous or eroded in patchy areas; some colonic glands lined by flattened epithelium will contain exfoliated cells and necrotic debris. A moderate mixed inflammatory

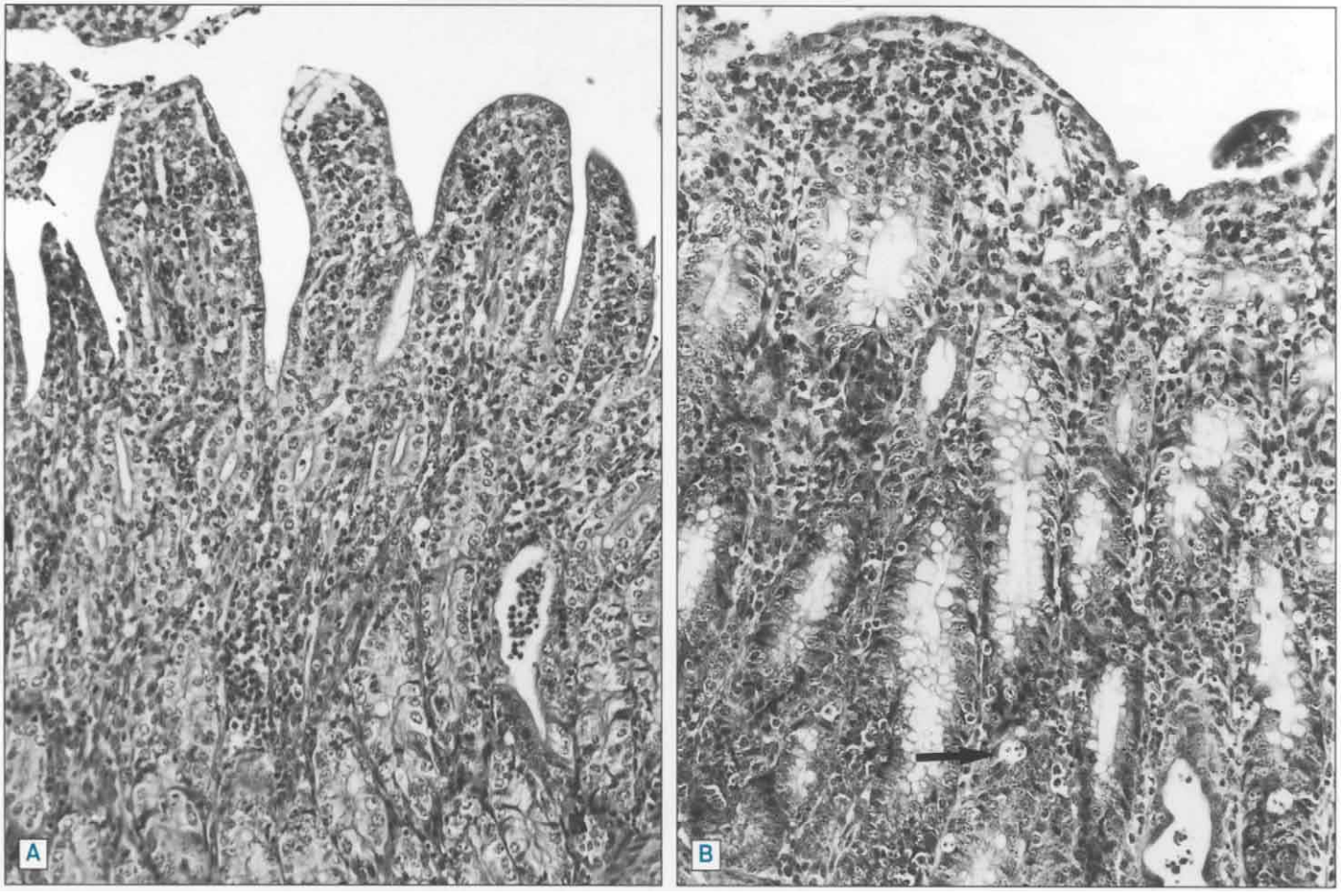

Figure 1.120 *Bovine coronavirus* infection. A. Blunt, fused villi with cuboidal surface epithelium in small intestine. **B.** Attenuation of surface epithelium and necrosis of gland epithelium (arrow), in colon. (Courtesy of M Morin.)

reaction is present in the lamina propria, and neutrophils may be in damaged glands or effusing into the lumen through superficial microerosions. Later in infection, some dilated debris-filled colonic glands will remain, but other glands will be lined by hyperplastic epithelium, and the surface epithelium will be restored to a cuboidal or low columnar cell type. Goblet cells are usually relatively uncommon. Colonic lesions may be recognizable in tissues from animals submitted dead, though postmortem change has obscured changes in the small intestine.

Live calves in the early stages of clinical disease are the best subjects for confirmation of an etiologic diagnosis. In calves becoming ill under 4–5 days of age, enterotoxigenic *Escherichia coli* is the main alternative diagnosis. *Rotavirus*, *Cryptosporidium*, and combined infections must be considered in calves 5–15 days of age. Infectious bovine rhinotracheitis, salmonellosis, and bovine viral diarrhea must also be considered. Both salmonellosis and bovine viral diarrhea may be associated with depletion of Peyer's patches and colitis that can be confused with that of coronaviral infection; neither is common in the strictly neonatal age group (under 7–14 days of age).

Respiratory tract infection also occurs in calves and feeders infected with BCoV. The virus replicates in the epithelium of the nasal turbinates and tracheobronchial tree, and *respiratory infection may precede, be concurrent with, or follow enteric infection*. Mild nasal discharge, cough, and increased respiratory rate are associated. Respiratory infections may play a role in maintaining the virus within a herd, and significant, but poorly characterized, pneumonia has been reported in some experimentally infected calves. In addition, coronaviral infection may predispose to subsequent respiratory bacterial infections or contribute to more severe respiratory disease as part of the shipping fever syndrome. Virus may be identified in tissue or nasal secretions by immunofluorescence or immunohistochemistry.

Winter dysentery is a syndrome in adult cattle that has been associated with BCoV in a number of areas around the world. Animals develop blood-tinged diarrhea, nasolacrimal discharge or cough, anorexia, and drop in milk production. Mortality is rare, but may occur. The colon of affected animals has linear congestion and hemorrhage along the crests of mucosal folds. Coronaviruses are commonly demonstrated in the feces of cattle with winter dysentery; seroconversions occur, and seroprevalence increases in affected herds; and coronavirus antigen is found in the colonic glands of affected animals, in which there is necrosis and exfoliation of epithelial cells. Certain management practices, notably housing animals in stanchions and use of equipment that handles both manure and feed, have been associated with the development of winter dysentery.

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Canine coronavirus

Canine coronavirus (CCV) is widely prevalent in the dog population. Although dogs of all ages appear to be susceptible to infection by CCV, the condition is probably most important as an *uncommon, transient, generally nonfatal, diarrhea in puppies*. Fatal infections have been reported in pups previously infected with parvovirus.

Viral replication occurs in the enterocytes of the small intestine, and in experimental infections in neonatal puppies, the lesion resembles the *villus atrophy* associated with coronaviral infection in other species. Diarrhea begins as early as 1 day after inoculation and in most animals by 4 days. Onset of signs coincides with the development of moderate villus atrophy and fusion. Enterocytes on villi become cuboidal, contain lipid vacuoles, and have an indistinct brush border. Lesions are most consistent and severe in the ileum. Resolution of villus atrophy within 7–10 days is associated with remission of signs.

Colonic infection by CCV was not demonstrated by immunofluorescence in experimental animals, though mild colonic lesions were described, including loss of sulfomucins from goblet cells and some epithelial shedding. However, in a report of lesions due to spontaneous CCV infection, colonic infection and lesions were demonstrated. There was watery content in the lumen of the small and large intestine, and in the cecum and colon fibrin mixed with some blood was evident. Mesenteric lymph nodes were enlarged and edematous. Villus atrophy in the jejunum was inconsistent, but there was necrotic debris in many glands in the cecum and colon. Virus-infected cells were exfoliating into the lumen.

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Feline and other enteric coronaviruses

Our understanding of the enteric implications of coronaviral infections of **cats** is still incomplete. It appears that *Feline enteric coronavirus* (FECV) can establish persistent infection in the intestine which, in rare cases, may be clinically apparent. Infection is very common. Diarrhea, when it occurs, is usually mild or moderate, perhaps with some blood, and kittens are most susceptible. Viral antigen is in cells on the tips of villi, and mild villus atrophy has been illustrated. Feline infectious peritonitis (see section on Peritonitis in cats, below), of far greater clinical significance, is due to viruses that arise by mutation from endemic FECV.

Coronaviruses have been recovered from the feces of **sheep** with transient diarrhea, and they have been associated with severe villus atrophy in several spontaneous outbreaks of diarrhea. No experimental confirmation of the pathogenicity of coronavirus in sheep is available.

Coronaviral infection may also be associated with diarrhea in **foals**, but again, experimental confirmation of pathogenicity is lacking.

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Rotaviral infections

Members of the genus *Rotavirus*, in the family Reoviridae, infect the gastrointestinal tract of most mammals and birds. Rotaviruses are classified according to antigenic properties with group, subgroup, and serotype categories. Segment 6 of the *Rotavirus* genome codes for the VP6 intermediate capsid protein, which is used to classify the virus into seven serogroups, A–G. *Group A rotaviruses (RV-A) are the most common* and infect all species of domestic animals, as well as humans, laboratory animals, and wildlife. RV-A can be subdivided into G and P serotypes based on VP7 and VP4 external capsid proteins respectively; there are 14 G serotypes and 20 P serotypes. The serotypes isolated most commonly from piglets with diarrhea are P[7],G5, P[6],G4, P[7],G3, and P[7],G11. Individual rotavirus serotypes have a surprisingly wide range of host susceptibility. Nongroup A rotaviruses infect pigs and ruminants, among domestic animals.

The ability to infect cells, and the serotype specificity of rotaviruses, are conferred by elements of the outer capsid layer. The viruses are probably generally host-specific, with little significant zoonotic potential. However, if epidemiologic circumstances are favorable, cross-species transmission may occur.

Rotaviruses infect the absorptive enterocytes and occasionally goblet cells on the tips and sides of the distal half or two-thirds of villi in the small intestine. Rotaviruses infect cells on the apical half (ruminants) or the entire villus (pigs), mainly in the jejunum and ileum. Virus production and the pathogenesis of infection are similar in all species studied. Rotaviruses adhere to cell receptors (e.g., integrins, sialic acid), and inner capsid components are internalized into the cell. Granular "viroplasm" containing incomplete virions is seen in the apical cytoplasm of infected cells, and virions acquire their complete capsid after budding into dilated cisternae of endoplasmic reticulum, where they accumulate. Elongate tubular structures are found in the nuclei and rough endoplasmic reticulum of some infected cells.

Virus-infected cells are most prevalent 18–24 hours after experimental infection, and they tend to diminish in number rapidly, so that by 3–4 days after infection few cells containing viral antigen are present. Infected enterocytes lose cytoplasmic electron density, and mitochondria swell, as does the cell generally. Swollen rarified cells and syncytia may occur in the enterocytes at the villus tips, but cells are fragile and will shed readily, particularly if autolysis intervenes. Syncytial cell formation has been recognized in porcine, bovine, and laboratory animal infections with rotavirus. Microvilli become irregular and somewhat stunted, and there may be some blebbing of membranes. Infected cells exfoliate into the intestinal lumen, and virus is released by lysis of damaged epithelium prior to or after exfoliation.

The *pathogenesis of diarrhea* with *Rotavirus* involves three mechanisms. First, malabsorption occurs secondary to destruction of enterocytes. Second, a vasoactive agent is released from infected epithelial cells and causes villus ischemia and activation of the enteric nervous system. Third, rotaviruses are capable of producing a nonstructural protein, NSP4, which acts as a *secretory enterotoxin*. This is the first viral pathogen known to produce a toxin.

Exfoliation of infected epithelium over a relatively short period of time results in villus atrophy. The mucosal surface is covered by cuboidal, poorly differentiated epithelium that has an ill-defined microvillus border and that may contain lipid droplets in the cytoplasm. Diarrhea is probably mediated by electrolyte and nutrient malabsorption, perhaps exacerbated by the effect of cryptal secretion. It begins about the time of early viral cytopathology 20–24 hours after infection, and may persist for a variable period, from a few hours to a week or more. Regeneration of the mucosa by epithelium emerging from crypts, and differentiating on reformed villi, is associated with remission of signs in animals surviving the effects of diarrhea.

Rotaviruses are widespread, if not ubiquitous, among populations of most species, and they are relatively resistant to the external environment. Protection against infection in neonates is apparently largely conferred by the presence of *lactogenic immunity*. Many individuals in a population probably undergo inapparent infection. Disease is seen in the various species when viral contamination of the environment is

heavy, perhaps as a result of intensive husbandry practices, and lactogenic immunity is waning or absent. Though rotaviral infection is usually associated with younger age groups, and viral receptors on cells diminish with age in some species, naive older animals may become infected, sometimes with the development of diarrhea.

Cattle rotaviral infection

Rotaviral infection is mainly implicated in *diarrhea of neonatal beef and dairy calves*, both suckled and artificially reared, though there are reports of its association with diarrhea in adult cattle. Diarrhea may be produced in calves by rotaviral infection alone, but the condition is usually considered to be relatively mild or transient in comparison with that induced by enterotoxigenic *Escherichia coli* or *Bovine coronavirus*. Combinations of agents, including *Rotavirus*, are frequently involved in outbreaks of diarrhea in neonatal calves. *Rotavirus* may be implicated in animals developing signs at any time over the period up to about 2–3 weeks of age, and it is more commonly encountered in animals over 4–5 days of age. Rotaviral diarrhea is most severe in calves that have slower enterocyte regeneration times.

The gross lesions of rotaviral infection are the nonspecific findings of undifferentiated neonatal diarrhea in calves, described previously. *Microscopic lesions in the small intestine cannot be differentiated from those of coronaviral infection.* They may vary somewhat depending on the severity of the initial viral damage and the stage of evolution of the sequelae. Blunt club-shaped villi, mild or moderate villus atrophy, and perhaps villus fusion may be present (Fig. 1.121). Villi are covered by low columnar, cuboidal, or flattened surface epithelium with a poorly defined brush border. There is usually a moderate proprial infiltrate of mononuclear cells and eosinophils or neutrophils, and hypertrophic crypts may be evident. The distribution of lesions may vary between animals and perhaps with time after infection within an individual animal, since the onset of maximal viral damage may not occur synchronously throughout the full length of the intestine. Lesions and viral antigen should always be sought in the distal small intestine, and preferably at several sites along its length. *Rotavirus* does not cause gross or microscopic lesions in the colon, in contrast to coronavirus.

Swine rotaviral infection

Rotavirus infection is widespread and enzootic in most swine herds, and subclinical infection of piglets is common. It assumes particular importance as a cause of diarrhea in pigs with reduced lactogenic immunity, either as a result of early weaning or following normal weaning. High environmental levels of virus may result in disease in piglets suckling the sow, but in these circumstances the signs are usually relatively mild. *Rotavirus* may be a cause of "3-week," "white," or postweaning scours in piglets from 2 to 8 weeks of age.

The signs may resemble those of transmissible gastroenteritis (TGE), although *Rotavirus* infection is considered to be less severe. Vomition is less commonly encountered than with TGE, but depression, diarrhea, and dehydration are usual. The character of the

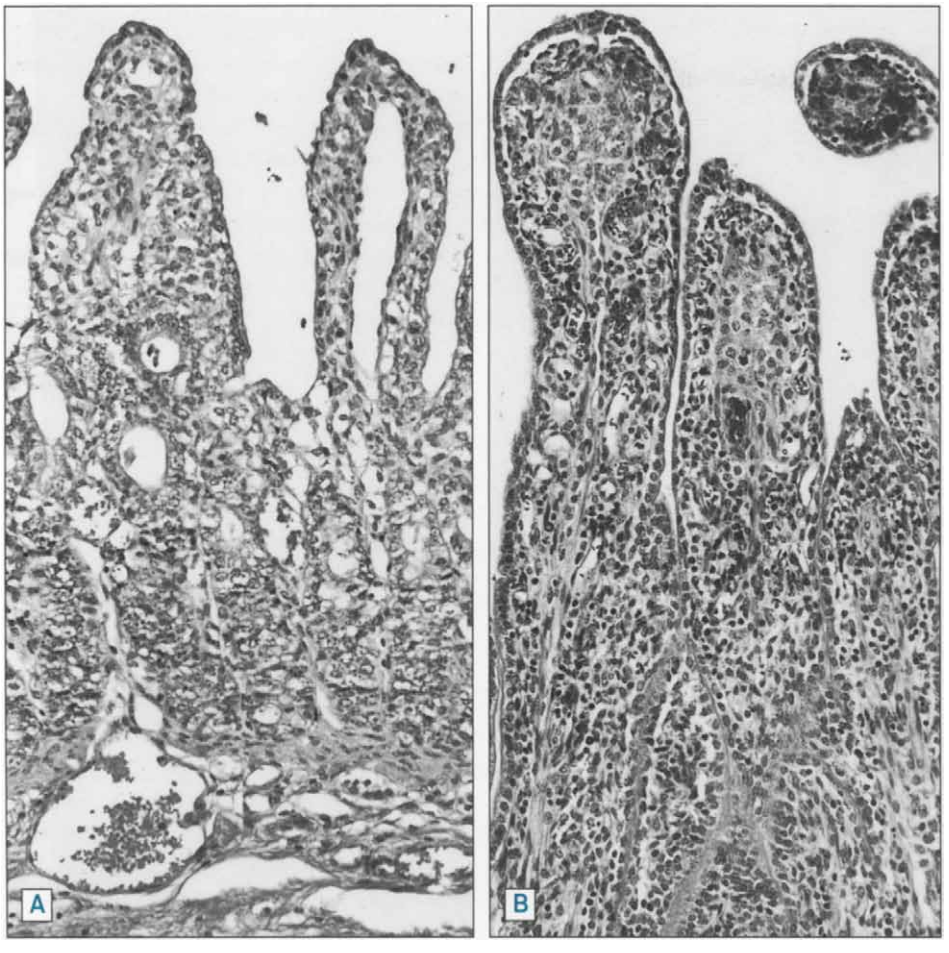

Figure 1.121 Bovine ***Rotavirus*** infection. **A.** Stumpy villi with severely attenuated surface epithelium. **B.** Club-shaped villi with cuboidal or flattened epithelium. (Courtesy of M Morin.)

feces varies with the diet. Steatorrhea occurs in white scours of suckling piglets. *Rotavirus* infection in swine may be associated with other causes of diarrhea, including *E. coli*, coccidiosis, adenoviral infection, and *Strongyloides*.

The gross and microscopic lesions and pathogenesis of rotavirus infection in pigs resemble those of TGE (Fig. 1.122). As in TGE, severity of lesions seems inversely related to age.

Rotaviral infection in other species

Neonatal lambs have proved a useful model for the demonstration of the importance of lactogenic immunity in preventing disease due to *Rotavirus*. *Rotavirus* may cause diarrhea in neonatal lambs alone or in combination with enterotoxigenic *E. coli* and *Cryptosporidium*. The pathogenesis and lesions of *Rotavirus* infection in lambs are like those caused in other species, with the exception that viral infection of the colon may occur.

In **foals** under 3–4 months of age, diarrhea may be associated with *Rotavirus* infection, though mortality is rare. Outbreaks have been reported in many areas of the world. The natural and experimental disease resembles that seen in other species, with significant viral infection limited to enterocytes in the small intestine, where villus atrophy occurs.

In young **puppies**, especially those under 1–2 weeks of age, diarrhea, occasionally fatal, may be caused by *Rotavirus* infection. In experimentally infected pups, green fluid content filled the lower small bowel and colon, and moderate villus atrophy was induced by exfoliation of epithelium from the distal half of villi.

Rotavirus has also been associated with diarrhea in **kittens**, although rotavirus can also be isolated from asymptomatically infected kittens.

Rotavirus infection should be sought in cases of diarrhea in young animals of any species and it should be particularly suspected in animals with villus atrophy in the small intestine. Rotavirus is part of the syndrome of undifferentiated neonatal diarrhea in any species.

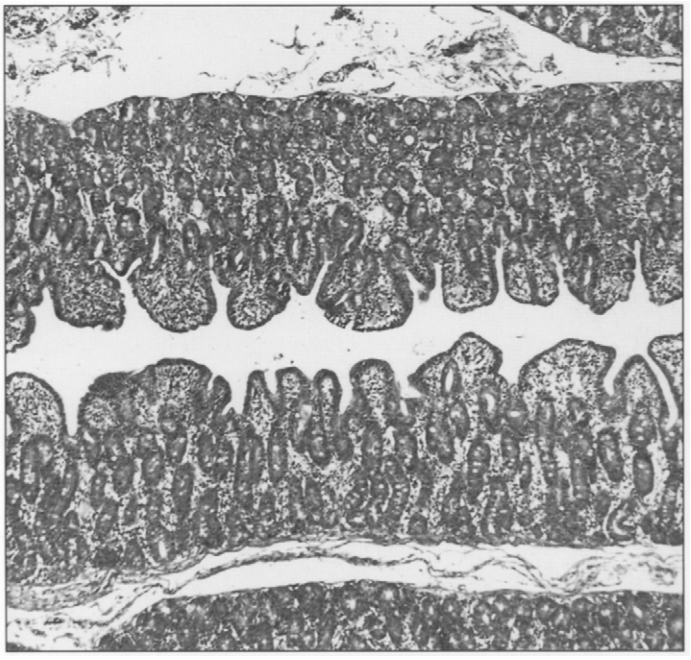

Figure 1.122 Villus atrophy, caused by ***Rotavirus*** infection, in the small intestine of a 3-week-old piglet.

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Parvoviral enteritis

The Parvoviridae are small nonenveloped viral particles about 18–26 nm in diameter, with icosahedral symmetry and a short single-stranded DNA genome. They replicate, and produce inclusion bodies, in the nucleus of infected cells. Members of the genus *Parvovirus* infect many species of laboratory and domestic animals. Among syndromes associated with parvovirus infection are: disease in cats, dogs, and mink (distinct from *Aleutian mink disease virus*) dominated clinically by enteritis; diarrhea in neonatal calves; and reproductive wastage in swine.

Feline panleukopenia virus (FPV), *Mink enteritis virus* (MEV) and *Canine parvovirus 2* (CPV-2), the agent causing parvoviral enteritis in dogs, are considered host range variants or strains of the feline parvovirus subgroup within the genus *Parvovirus*. These viruses are biologically distinct, varying in their hemagglutination characteristics, in vitro host cell ranges, infectivity, and virulence in experimentally inoculated hosts. There are subtle antigenic differences among them, detected by monoclonal antibodies; these, and differences in host specificity, are conferred by variations in only very small segments of the viral genome. Based on the nucleotide sequence in the gene for capsid proteins VP1/VP2, FPV and MEV are very closely related to each other, and somewhat less related to CPV-2. Two new antigenic types have evolved from CPV-2 – CPV-2a and CPV-2b – and are able to replicate in cats. Another variant, CPV-2c, has been isolated from leopard cats. The host ranges of FPV and CPV are determined by receptor binding, particularly to the transferrin receptor TfR. *Canine parvovirus 2* is distinct from *Canine minute virus* (CMV, or *Canine parvovirus 1*) that also has enteric lesions associated with infection.

Though autonomous parvoviruses may infect cells at any phase of the cell cycle, replication is dependent on cellular mechanisms only functional during nucleoprotein synthesis prior to mitosis. Hence, *the effects of parvoviral infection are greatest in tissues with a high mitotic rate*. These may include a variety of tissues during organogenesis in the fetus and neonate. In older animals, the proliferative elements of the enteric epithelium, hematopoietic and lymphoid tissue are particularly susceptible. At the time of virus assembly, *large basophilic or amphophilic Feulgen-positive nuclear inclusions* may be found in infected cells, especially in Bouin's fixed tissues. Parvovirus is demonstrated in these inclusions by electron microscopy. The chromatin in inclusion-bearing nuclei is usually clumped at the nuclear membrane. Inclusions are most prevalent late in the incubation period, prior to extensive exfoliation or lysis of infected cells. Hence, they are not commonly encountered in animals submitted for autopsy after a period of clinical illness culminating in death. Large nucleoli, seen in proliferative cells encountered in the intestine of parvovirus-infected animals, should not be confused with intranuclear viral inclusions.

The *pathogenesis* of FPV and of CPV-2 infection is sufficiently similar for them to be considered together here, followed by separate discussions of the specific diseases. Oronasal exposure results in uptake of virus by epithelium over tonsils and Peyer's patches. Infection of draining lymphoid tissue is indicated by isolation of virus from mesenteric lymph nodes 1–2 days after experimental inoculation. Release of virus into lymph, and dissemination of infected lymphoblasts from these sites, may result in infection of other central and peripheral lymphoid tissues, including thymus, spleen, lymph nodes, and Peyer's patches, 3–4 days after infection. Lymphocytolysis in these tissues releases virus, reinforcing cell-free viremia. Viremia is terminated when neutralizing antibody appears in circulation about 5–7 days after infection. Moderate pyrexia occurs at about this time.

Infection of the gastrointestinal epithelium is a secondary event, following dissemination of virus by circulating lymphocytes and cell-free viremia. Peyer's patches are consistently infected at all levels of the intestine, and epithelium in crypts of Lieberkühn over or adjacent to Peyer's patches usually becomes infected a day or so later. Infection of gastrointestinal epithelium at other sites in the gut is less consistent, but is usually more severe in the lower small intestine. It may be the result of virus free in circulation, or carried by infected lymphocytes homing to the mucosa. Maximal infection of cryptal epithelium occurs during the period about 5–9 days after infection.

The occurrence and severity of enteric signs are determined by the degree and extent of damage to epithelium in intestinal crypts. This seems to be a function of two main factors. The first is the availability of virus, which is influenced by the rate of proliferation of lymphocytes, and therefore their susceptibility to virus replication and lysis. The second factor influencing the degree of epithelial damage is the rate of proliferation in the progenitor compartment in crypts of Lieberkühn. If many cells are entering mitosis, large numbers will support virus replication and subsequently lyse. Destruction of cells in the crypts of Lieberkühn, if severe enough, ultimately results in focal or widespread villus atrophy and perhaps mucosal erosion or ulceration. The recognition, evolution, and sequelae of radiomimetic insult to the intestine, such as that caused by parvovirus, are described elsewhere (see section on Epithelial renewal in health and disease, above).

Regeneration of cryptal epithelium and partial or complete restoration of mucosal architecture will occur, if undamaged stem cells persist in most affected crypts, and the animal survives the acute phase of clinical illness. In some survivors, focal villus atrophy is associated with persistent dilated crypts containing cellular debris, and with local "drop-out" of crypts completely destroyed by infection. In rare animals recovered from acute disease, chronic malabsorption and protein-losing enteropathy are associated with persistent areas of ulceration caused by more extensive loss of crypts and collapse of the mucosa.

The low rate of replication of intestinal epithelium in germfree cats explains failure to produce significant intestinal lesions and clinical panleukopenia in experimentally infected animals. In spontaneous cases, the lower prevalence of parvoviral lesions in the colon and stomach, in comparison with the small intestine, reflects the relatively lower rate of epithelial proliferation in those tissues. The consistency of epithelial lesions in the mucosa over Peyer's patches probably results from high local concentrations of virus derived from infected lymphocytes in the dome and follicle. This may be coupled with local stimulation of epithelial turnover by cytokines released by T lymphocytes in the vicinity. Variations in the rate of epithelial proliferation related to age, starvation, and refeeding, or concomitant parasitic, bacterial, or viral infections, may also influence

the susceptibility of crypt epithelium to infection, and therefore affect the extent and severity of intestinal lesions and signs.

Diarrhea in parvoviral infections is mainly the result of reduced functional absorptive surface in the small intestine. Effusion of tissue fluids and blood from a mucosa at least focally denuded of epithelium probably also contributes to diarrhea. Dehydration and electrolyte depletion are the result of reduced fluid intake, enteric malabsorption, effusion of tissue fluid, and, in some animals, vomition. Hypoproteinemia is common, and anemia may occur due to enteric blood loss; both are exacerbated by rehydration. Anemia reflects hemorrhage into the gut.

Proliferating cells in the *bone marrow* are also infected during viremia. Lysis of many infected cells is reflected in hypocellularity of the marrow caused by depletion of myeloid and erythroid elements, particularly the former. Megakaryocytes may also be lost, but seem the least sensitive cell population in the marrow. The number of neutrophils in circulation drops quickly in severely affected animals. This is due to failure of recruitment from the damaged marrow, and peripheral consumption, especially in the intestine. Transient neutropenia, of about 2–3 days' duration, occurs consistently in cats, and less commonly in dogs. In surviving animals, regeneration of depleted myeloid elements from remaining stem cells restores the circulating population of granulocytes within a few days. Neutrophilia with left shift may occur during recovery.

Lymphopenia, relative or absolute, results from *viral lymphocytolysis* in all infected lymphoid tissue. Relative lymphopenia is more consistently observed in dogs than neutropenia. When lymphopenia and neutropenia occur together, *the combined leukopenia may be profound in both dogs and cats*. In dogs surviving the lymphopenic phase, circulating lymphocytes return to normal numbers within 2–5 days, as regenerative hyperplasia occurs in lymphoid tissue throughout the body. Lymphocyte numbers increase rapidly, sometimes producing lymphocytosis in recovering dogs. However, there may be transient immunosuppression in gnotobiotic pups subclinically infected with CPV-2. Transient depression of T-cell response to mitogens occurs in cats a week after experimental infection with FPV. But immunosuppression by these agents appears to be of little clinical significance.

Most infected cats and dogs do not develop clinical disease. When it occurs, signs usually begin during the late viremic phase, about 5–7 days after infection. Severe enteric damage is the major cause of mortality. Shedding of infective virus in feces begins about 3–5 days after infection, when Peyer's patches and cryptal epithelium first become infected. Virus shedding persists until coproantibody appears to neutralize virus entering the gut, about 6–9 days after infection. Virus-infected cells may still be detected in crypts and Peyer's patches at this time, and virus complexed with antibody may be found in feces or intestinal content by direct electron microscopy. However, attempts to demonstrate virus in tissues or feces after several days of clinical disease, or at death, are often thwarted by the fact that virus is neutralized by antibody present in tissue fluids. Persistent or sporadic shedding of virus by recovered animals may be the result of virus replication in cells entering mitosis days or weeks after they were infected during the viremic phase.

Infection of the fetus during late prenatal life by FPV causes *anomalies of the central nervous system*, mainly hypoplasia of the cerebellum; anomalies of the central nervous system have not been reported in puppies with CVP-2, although there is mounting evidence from polymerase chain reaction studies that central nervous system lesions in puppies can be induced by fetal CVP-2 infections. Infection of proliferating cardiac myocytes in young puppies with CPV-2 results in nonsuppurative myocarditis and sequelae of acute or chronic heart failure (see Vol. 3, Cardiovascular system), but this is rarely seen in populations with a high prevalence of maternal immunity. A tentative association has been made between infection of kittens with FPV and myocarditis, as well as subsequent cardiomyopathy.

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Feline panleukopenia

Feline panleukopenia virus (FPV) infects all members of the Felidae, as well as mink, raccoons, and some other members of the Procyonidae. FPV is ubiquitous in environments frequented by cats, and infection is common, though generally subclinical. The disease panleukopenia (infectious feline enteritis, feline distemper) usually occurs in young animals exposed after decay of passively acquired maternal antibody, but it may occur in naive cats of any age. Clinical signs of several days' duration, including pyrexia, depression, inappetence, vomition, diarrhea, dehydration, and perhaps anemia may be evident in the history. However, many cases, particularly poorly observed animals or those prone to wander, may be presented as "sudden death." The pathogenesis of panleukopenia has been considered above. Lesions of the central nervous system in kittens are considered in Vol. 1, Nervous system.

At **necropsy**, external evidence of diarrhea may be present, the eyes may be sunken, and the skin is usually inelastic, with a tacky subcutis reflecting dehydration. Rehydrated animals may have edema,

hydrothorax, and ascites due to hypoproteinemia. There is pallor of mucous membranes and internal tissues in anemic animals. Gross lesions of internal organs most consistently involve the thymus and the intestine. The thymus is markedly involuted and reduced in mass in young kittens. Enteric lesions may be subtle and easily overlooked. Hence *it is mandatory that intestine be examined microscopically despite the apparent absence of gross change.*

The intestinal serosa may appear dry and nonreflective, with an opaque ground-glass appearance. Uncommonly in cats, there may be petechiae or more extensive hemorrhage in the subserosa, muscularis, or submucosa of the intestinal wall. The small bowel may be segmentally dilated and can acquire a hose-like turgidity in places, perhaps due to submucosal edema. However, turgidity is difficult to assess in the intestine of the cat. The content is usually foul-smelling, scant, and watery, and yellow-gray at all levels of the intestine. The mucosa may be glistening gray or pink, with petechiae, perhaps covered by fine strands of fibrin (Fig. 1.123). Patchy diphtheritic lesions may be present, especially over Peyer's patches in the ileum. Flecks of fibrin and sometimes casts may be in the content in the lumen. Formed feces are not evident in the colon. Lymph nodes may be prominent at the root of the mesentery. Gross lesions elsewhere in the carcass are usually restricted to pulmonary congestion and edema in some animals, and pale gelatinous marrow in normally active hemopoietic sites.

Microscopic lesions are consistently found in the intestinal tract in fatal cases, and are usual in lymphoid organs and in bone marrow. The *intestinal lesions* vary with the severity and duration of the disease. Lesions may be patchy, and several levels of gut should be examined, preferably including ileum and, if possible, Peyer's patch. During the late incubation period and early phase of clinical disease, crypt-lining epithelium is infected. Intranuclear inclusions may be found, and damaged epithelium containing inclusions exfoliates into the lumen of crypts. Crypts are dilated and lined by cuboidal or more severely attenuated cells. The lamina propria between crypts contains numerous neutrophils and eosinophils at this time, and some emigrate into the lumen of crypts, where they join the epithelial debris.

Subsequently, severely damaged crypts may be lined by extremely flattened cells, and by scattered large bizarre cells with swollen nuclei and prominent nucleoli (Fig. 1.124A). Enterocytes covering villi are not affected. But as they progress off the villus, they are replaced by a few cuboidal, squamous, or bizarre epithelial cells, so that villi in affected areas undergo progressive collapse. If cryptal damage is severe and widespread, the mucosa becomes thin and eroded or ulcerated, with effusion of tissue fluids, fibrin, and erythrocytes. Inflammatory cells are usually sparse in the gut of such animals, and superficial masses of bacteria may be present, occasionally accompanied by locally invasive fungal hyphae. In less severely affected animals with disease of longer duration, corresponding to about 8–10 days after infection, scattered focal drop-out of crypts, or focal mucosal collapse and erosion or ulceration, may be evident. In these animals, remaining crypts recovering from milder viral damage show regenerative epithelial hyperplasia (Fig. 1.124B). Mucosal lesions are often most marked in the vicinity of Peyer's patches.

Lesions in the *colon* generally resemble those found in the small bowel, though they are often less severe or more patchy in distribution. Colonic lesions are present in about half of fatal cases of panleukopenia. Gastric lesions resulting from damage to mitotic epithelium are relatively uncommon in cats. They are recognized by flattening of basophilic cells lining the narrowed isthmus of gastric fundic glands, with some reduction in number of parietal cells in the upper portion of the neck of glands.

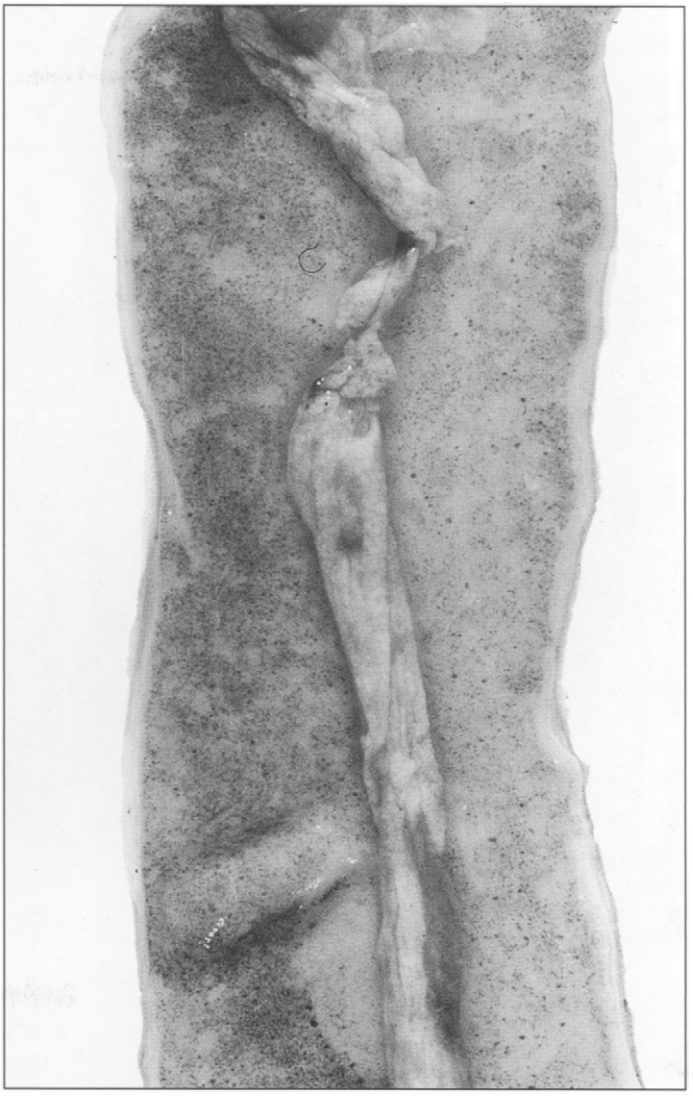

Figure 1.123 Petechiae and fibrin cast on mucosa of small intestine in **feline panleukopenia**.

Lesions of *lymphoid organs* during the early phase of the disease consist of lymphocytolysis in follicles and paracortical tissue in lymph nodes, in the thymic cortex and splenic white pulp, and in gut-associated lymphoid tissue. Lymphoid necrosis has been associated with induced apoptosis of virus-infected lymphocytes. Lymphocytes are markedly depleted in affected tissue and large histiocytes are prominent, often containing the fragmented remnants of nuclear debris. Follicular hyalinosis, the presence of amorphous eosinophilic material in the center of depleted follicles, may be seen. Erythrophagocytosis by sinus histiocytes may occur in lymph nodes, especially those draining the gut. Severely depleted Peyer's patches may be difficult to recognize microscopically. Later in the course of clinical disease, corresponding to the period beyond about 7–8 days after infection, prominent regenerative lymphoid hyperplasia may be found.

In severely affected animals at the nadir of the leukopenia, virtually all proliferating elements in the *bone marrow* may be depleted.

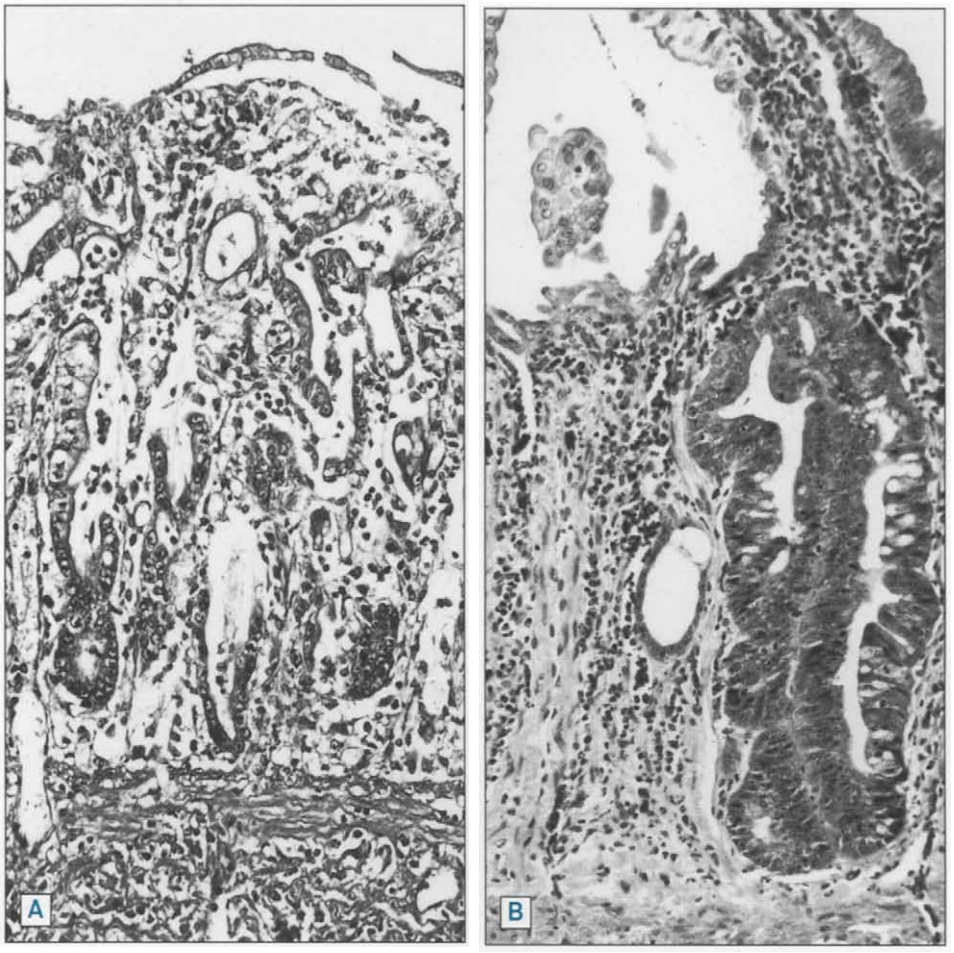

Figure 1.124 A. Severe atrophy of villi associated with damage to crypts of Lieberkühn, caused by ***Feline panleukopenia virus*** infection, in a cat. Attenuation of surface epithelium and depletion of proprial inflammatory infiltrate. **B.** Loss of crypts and collapse of proprial stroma; gland is lined by hyperplastic epithelium in a regenerating state in ***Mink enteritis virus*** infection 12 days after inoculation.

The extremely hypocellular, moderately congested marrow is only populated by scattered stem cells. Milder lesions mainly affect the neutrophil series, generally sparing megakaryocytes and the committed erythroid elements. During the later phases of the disease, marked hyperplasia of stem cells, and eventually of amplifier populations in the various cell lines, is evident.

In the *liver*, dissociation and rounding up of hepatocytes, and perhaps some periacinar atrophy and congestion, may be evident. This is probably associated with dehydration and anemia. Pancreatic acinar atrophy is also common, reflecting inappetence. The lung may be congested and edematous. In leukopenic animals, few white cells are seen in circulation in any organ.

A **diagnosis** of feline panleukopenia may be made on the basis of the characteristic microscopic intestinal lesions, in association with evidence of involution or regenerative hyperplasia of lymphoid and hematopoietic tissues. *Inclusion bodies* may be sought in these tissues, but are usually present in significant numbers only during the late incubation and early clinical period. Application of immunohistochemical techniques may identify viral antigen in tissue as late as 8–10 days after infection. Cryptal necrosis is also reported in the intestines of some cats with *Feline leukemia virus* infection, which must be differentiated.

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Canine parvovirus 2 infection

Canine parvovirus 2 (CPV-2) resulted from mutation of a closely related virus, likely an FPV-like virus from wild carnivores, such as foxes. It appeared spontaneously and virtually simultaneously in populations of dogs on several continents in 1978, and rapidly spread worldwide. Retrospective serologic studies suggest that it was circulating unnoticed in western Europe by 1976. In addition to domestic dogs, several species of wild canids, including coyotes, gray wolves, and raccoon dogs, are susceptible to infection.

Enteric disease due to this virus was epizootic for several years in naive populations of dogs, affecting animals of all ages. As the prevalence of antibody due to natural infection and vaccination increased, the problem subsided to one of an enzootic disease. It now affects those animals with reduced levels of passively acquired maternal immunity, or scattered naive individuals.

During the epizootic period, nonsuppurative viral myocarditis due to CPV-2 was prevalent in the offspring of naive bitches unable to protect pups with maternal antibody during the first 15 days of life, when replicating myocardial cells are susceptible to parvoviral

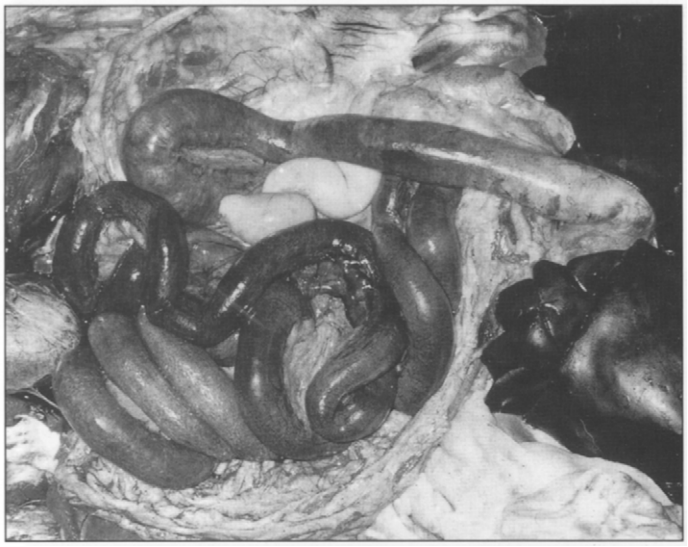

Figure 1.125 Segmental subserosal hemorrhage and mild fibrinous exudation on intestinal serosa in ***Canine parvovirus 2*** infection.

damage. Myocardial disease in pups due to CPV-2 is now fairly uncommon, as most bitches have antibody. Enteric and myocardial disease rarely occur together in the same individual or cohort of animals. Occasional cases of generalized parvoviral infection have been reported in susceptible neonates. Necrosis and inclusion bodies are found in organs such as kidney, liver, lung, heart, gut, and vascular endothelium. They are presumably related to mitotic activity during organogenesis.

Dogs with typical disease due to CPV-2 become anorectic and lethargic and may vomit and develop diarrhea, perhaps in association with transient moderate pyrexia. Relative or absolute lymphopenia or leukopenia of 1–2 days' duration may occur. Diarrhea may be mucoid or liquid, sometimes bloody, and is malodorous. After a period of 2–3 days, dogs either succumb to the effects of dehydration, hypoproteinemia, and anemia, or begin to recover.

Gross findings at autopsy of fatal cases are those of dehydration, accompanied by enteric lesions characteristic of the disease. There is often segmental or widespread subserosal intestinal hemorrhage, which may extend into the muscularis and submucosa. The serosa frequently appears granular due to superficial fibrinous effusion (Fig. 1.125). Peyer's patches may be evident from the serosal and mucosal aspects as deep red oval areas several centimeters long. The intestinal contents may be mucoid or fluid; sometimes they look like tomato soup, due to hemorrhage. The mucosa is usually deeply congested and glistening, or covered by patchy fibrinous exudate. Severe mucosal lesions may be widespread or segmental, and their distribution is irregular; thus tissues from several levels of the small intestine should be selected for microscopic examination. Gross changes in the colon are similar, but less common. The stomach may have a congested mucosa and contain scant bloody or bile-stained fluid. Mesenteric lymph nodes may be enlarged, congested, and wet, or be reduced in size. Thymic atrophy is consistently present in young animals, and the organ may be so reduced in size as to be difficult to find. The lungs often appear congested, and have a rubbery texture.

The **microscopic lesions** in stomach, small intestine (Fig. 1.126), colon, lymphoid tissue, and bone marrow due to CPV-2 infection do not differ significantly from those described above in cats with panleukopenia. Gastric lesions are perhaps more frequently encountered in dogs with parvoviral infection. Small intestinal lesions are invariably severe in fatal cases. The colon is involved in a minority of animals. Pulmonary lesions such as alveolar septal thickening by mononuclear cells, congestion, and effusion of edema fluid and fibrin into the lumina of alveoli may be related to terminal gram-negative sepsis and endotoxemia, which is common in fatal cases. Periacinar atrophy and congestion in the liver are attributable to anemia, hypovolemia, and shock, and prominent Kupffer cells probably reflect endotoxemia. Some studies have shown that viral inclusions may occur in tongue epithelium cells as well. Although these inclusions are nuclear, they often appear to be in the cytoplasm (pseudocytoplasmic). A case of erythema multiforme as a result of CPV-2 infection of keratinocytes has been described in a dog with concurrent parvoviral enteritis; viral inclusions were present in oral and skin epithelial cells.

The **diagnosis** of parvoviral enteritis in dogs follows the principles described for that of panleukopenia in cats. The disease must be differentiated from *Canine coronavirus* infection, which is very rarely fatal, and from canine intestinal hemorrhage syndrome, shock gut, intoxication with heavy metals or warfarin, infectious canine hepatitis, and other causes of hemorrhagic diathesis. Involution of gut-associated lymphoid tissue and cryptal necrosis caused by parvovirus must be differentiated from similar lesions occasionally seen in canine distemper.

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Canine minute virus

Canine minute virus (CMV, Minute virus of canines, *Canine parvovirus 1*) is most closely related to *Bovine parvovirus*, and is distinct from members of the feline panleukopenia parvovirus subgroup. Serologic prevalence in the USA is approximately 50% in adult dogs. The virus is capable of transplacental transmission to the fetus, and exposure of pregnant bitches was associated with fetal resorption, or birth of dead or weak pups. CMV causes enteric or respiratory symptoms in puppies less than 3 weeks of age. Large intranuclear viral inclusion bodies are evident in enterocytes. Interstitial pneumonia and myocarditis are variably present in naturally infected puppies, with death occurring sporadically.

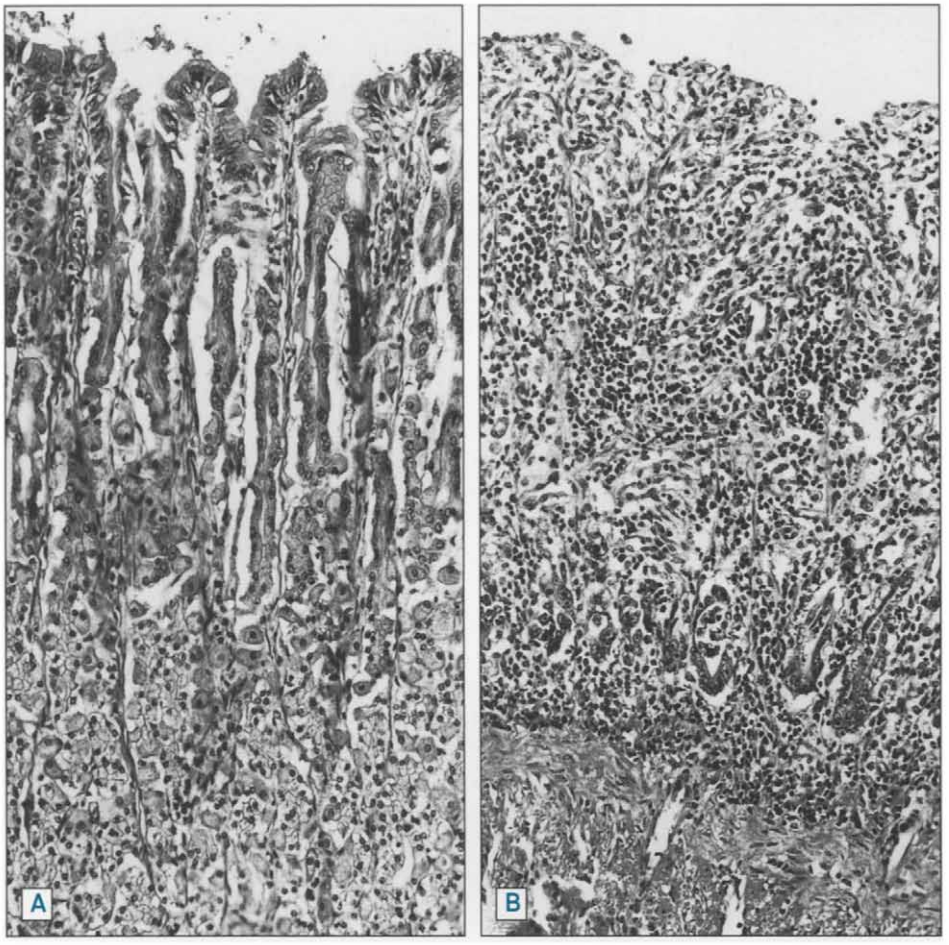

Figure 1.126 ***Canine parvovirus 2*** infection in a dog. **A.** Attenuation of epithelium lining isthmus and upper neck of fundic gastric glands. **B.** Loss of crypts of Lieberkühn and collapse of proprial stroma in small intestine. Remnants of crypt lining epithelium persist deep in lamina propria.

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Bovine parvovirus infection

The antigenically distinct *Bovine parvovirus* (BPV) has been recognized for many years and occurs widely in cattle populations on all continents. A single serotype is known. It has been isolated from the feces of normal and recently diarrheic calves as well as from conjunctiva, and from aborted fetuses. *The status of BPV as an enteric pathogen is unclear.* Virus shedding is not always associated with diarrhea, and it may be part of a mixed infection in diarrheic animals. Serological prevalence of antibodies to BPV is high, with 83% of cattle and 100% of herds being positive over 2 years of testing in one study. It is rarely diagnosed as a cause of death, and unless sought specifically by culture, direct electron microscopy, or molecular probe, would be missed as a cause of clinical diarrhea. Its significance may be greatest in neonatal calves and animals exposed while passive maternal antibody levels are waning, or in animals in the postweaning period.

The pathogenesis of infection with BPV resembles that in carnivores. Initial viral replication following oral inoculation is in tonsils and gut, with spread to systemic lymphoid tissues, resulting in transient lymphopenia. Viral antigen has been identified in the nuclei of epithelium in intestinal crypts and in cells in thymus, lymph nodes, adrenal glands, and heart muscle. Transient lymphocytolysis in infected tissues, and exfoliation of epithelium in crypts of the small and large intestine, with moderate villus atrophy, depletion of colonic goblets, and mixed inflammatory cell infiltration of the mucosa, have been seen experimentally. Intranuclear inclusions are present at the time when lesions are prevalent. Gross lesions other than abnormally fluid content in the gut are subtle, or absent. Intravenous inoculation of BPV into young calves causes severe watery diarrhea and prostration. Milder diarrhea occurs in calves infected orally. The severity of the disease may be potentiated by concurrent infection with other enteric pathogens, or other factors that may increase intestinal epithelial proliferation.

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Bacterial diseases of the alimentary tract

Virulence of bacterial pathogens

Evolutionary processes for bacterial survival, persistence, and proliferation are controlled by virulence genes and are subject to complex mechanisms of regulation of expression. Similarly, evolutionary processes for resisting the effects of bacteria on the host are determined by genetic factors and equally complex regulatory processes in the host. *Bacterial virulence can be resolved into five components*: (1) attachment; (2) colonization or entry into the host; (3) evasion of host defense; (4) multiplication and/or spread within the host, and damage to the host, by direct virulence attributes, or by stimulation of an immunoinflammatory response; and (5) transmission to other susceptible animals. The interplay between host and pathogen has been extensively studied for a number of important enteric bacterial pathogens, including *Salmonella* and *Escherichia coli*. Genes encoding a range of virulence characteristics in pathogenic bacteria, including adhesion factors, toxins, proteolytic enzymes, and other agents that promote tissue invasion, are often clustered in discrete regions of the genome known as *pathogenicity islands*. These appear to be sites of relative instability, and are thought to facilitate the horizontal transfer of virulence factors between bacteria, and their continued evolution. Similarities in the regulatory mechanisms for pathogenicity islands of important enteric pathogens, including *Salmonella*, *Shigella*, *Vibrio*, *Yersinia* and *E. coli*, are providing new insights into the reasons why strains of many genera of bacteria vary greatly in their host range and ability to cause disease.

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Escherichia coli

Escherichia coli has several virulence attributes that result in disease in animals. Principally, these promote **colonization or adhesion** to the mucosa; they cause **metabolic dysfunction or death of enterocytes**; they affect the **local or systemic vasculature**; or they promote **invasion and septicemia**. Disease syndromes caused by *E. coli* in domestic animals can be related to the combinations of virulence attributes expressed. Many terms and acronyms have been applied to the mechanisms of action of *E. coli*, with some becoming obsolete and others applying mainly to *E. coli* infections of laboratory animals and humans, rather than to domestic animals.

"**Enterotoxigenic**" *E. coli* (ETEC) cause secretory small-bowel diarrhea stimulated by enterotoxins produced by *E. coli* colonizing the mucosa of the small intestine. This condition is an important, common cause of diarrhea in neonatal animals of many species, and in postweaning pigs.

"**Enteropathogenic**" *E. coli* (EPEC) in humans may colonize the mucosa of the intestine by a mechanism involving adhesion-effacement ("**enteroadherent**" *E. coli* – EAEC, or "**attaching-effacing**" *E. coli* – AEEC). Some do not produce recognized toxins, but are associated with villus atrophy; they are an uncommon cause of disease in domestic animals.

Other strains of *E. coli*, many, but not all, of which are attaching-effacing, in addition secrete *cytotoxins* (Shiga toxins = verotoxins) that have an effect locally or systemically. Depending on the manifestation of this effect, such *E. coli* have been categorized as "**Shiga toxin-producing**" (STEC) = "**verotoxin-producing**" (VTEC), or "**enterohemorrhagic**" (EHEC). EHEC are a serious cause of foodborne illness in humans and have been incriminated as a cause of hemorrhagic enterocolitis in calves under a month of age.

Shigatoxigenic infections in swine, which are not attaching-effacing, are associated with some outbreaks of postweaning *E. coli* enteritis and also cause edema disease of weaned pigs, which is a systemic toxemia.

"**Enteroinvasive**" *E. coli* (EIEC) can be internalized by surface enterocytes and subsequently disseminate through the body to become septicemic. While EIEC are poorly documented in domestic animals, **septicemic colibacillosis** is a common manifestation of *E. coli* infection caused by strains adapted to avoid specific or innate systemic defense mechanisms, often in compromised hosts. The intestine is not necessarily the portal of entry and there may not be alimentary disease. The signs of *E. coli* septicemia are mainly referable to bacteremia, endotoxemia, and the effect of bacterial localization in a variety of tissue spaces throughout the body.

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Enterotoxigenic colibacillosis

Enterotoxigenic colibacillosis caused by enterotoxigenic *E. coli* (ETEC) is one of the major forms of diarrhea in neonatal pigs, calves, and lambs, as well as in humans.

Two major attributes confer virulence upon these strains of *E. coli*. These are the ability to colonize the intestine, and the capacity to produce toxins that stimulate secretion of electrolyte and water by the intestinal mucosa. *Colonization and enterotoxin production must occur together for disease to ensue.* The diarrhea produced by ETEC is accompanied by relatively minor microscopic evidence of inflammation,

and by little or no architectural change in the mucosa. As a result, overt enteritis is usually not evident at autopsy, and the disease is part of the syndrome of undifferentiated diarrhea of neonatal animals.

Intestinal colonization results from the adhesion of *E. coli* to the surface of enterocytes on villi in the small intestine, and proliferation there (Fig. 1.127A). By adhering to the mucosa, bacteria are able to resist the normal peristaltic clearance mechanisms. Large numbers of organisms, of the order of over 10^7 per gram of mucosa, or 20–30 per enterocyte, cover the surface of villi. The ability to attach to enterocytes is conferred upon ETEC by pili, and may be enhanced by the presence of a capsule.

Fimbriae, or **pili** (also known as **colonization factor antigens – CFA**, with specific names in transition to a system of "F" numbers) are rod-like or filamentous projections from the cell wall of *E. coli* that attach to specific glycoconjugate receptors on the surface of enterocytes (Fig. 1.127B). They are distinct from type 1 fimbriae, which do not promote colonization of the gut. Fimbriae are polymers of protein (pilin) subunits, which are coded by plasmid (F4 (K88), F5 (K99), F18; some F6 (987P)) or chromosomal (F6 (987P), F17, F41) DNA. They are antigenically distinct, permitting recognition by specific antibody.

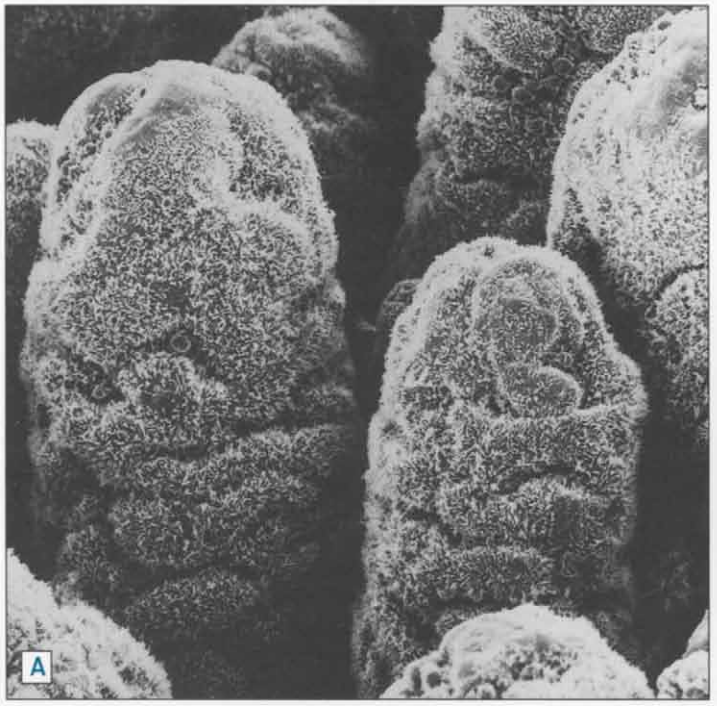
Fimbrial adhesins include **F5** (K99) and **F41** in strains affecting calves, lambs, and pigs; **F42, F165, F17, F18** in calves and pigs; and **F4** (K88), **F6** (987P), **F18** in pigs. Combinations of adhesins may be expressed by the same strain of ETEC; typically, F41 is expressed by strains also expressing F5, and seems to be of minor importance. Bacteria possessing F4 colonize the entire small bowel, whereas those with F5, F6, and F41 mainly adhere in the jejunum and ileum.

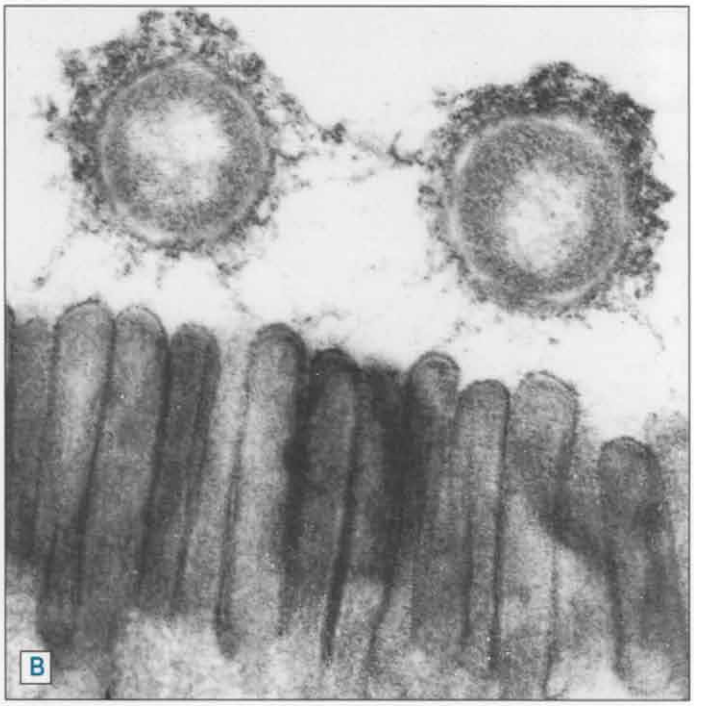
Susceptibility to bacterial fimbrial adhesins, especially F5 and F6, appears to be somewhat age-related; the ability of fimbria-bearing *E. coli* to colonize the small intestine is greatest in animals only a few days old. F5 receptors on enterocytes decline in availability with age, while receptors for F6 are shed into the lumen in older pigs, facilitating clearance of bacteria from the mucosa, and interfering with colonization. F18 receptors are not found in neonatal pigs, but are produced with increasing age to weaning. Stimulation of maternal immunity to fimbrial antigens causes secretion of lactogenic antibody, which combines with adhesins in the gut lumen, preventing colonization of the gut of suckling animals.

A nonfimbrial plasmid-encoded *adhesin involved in diffuse adherence* (**AIDA**) of *E. coli* to enterocytes in humans also occurs in strains from pigs associated with edema disease and postweaning diarrhea, often in combination with F18.

Enterotoxigenic strains of *E. coli* produce two classes of plasmid-encoded proteins – heat-labile toxin (LT) and heat-stable toxin (ST) – which act locally in the intestine to alter secretion and absorption of electrolyte and water by enterocytes.

Heat-labile toxin is a large immunogenic plasmid-encoded molecule, with two subgroups (LTI and LTII) and comprised of a small A subunit with two fragments (A1 and A2), which links A1 to a large pentamer of five B subunits. LTI is antigenically similar to cholera toxin, while LTII toxins have B subunits that differ from LTI. The B subunits bind to ganglioside receptors on the enterocyte surface; the toxin complex then dissociates, and the A1 subunit is internalized into the cell. It operates via an adenylate cyclase pathway to cause chloride secretion by enterocytes, sodium, and water following osmotically from the mucosa. Co-transport of sodium chloride by enterocytes, and associated water uptake, is probably also shut down at the same time. LT may also promote mucosal secretion by stimulation of local prostaglandin production, the enteric nervous system, and cytokine activation. LT has a latent period prior to the development of secretion, but the effects on the cell are irreversible.





Heat-stable toxin is classified as STa and STb based on biological properties, and is plasmid-encoded. STa causes an increase in cyclic guanosine monophosphate, which inhibits Na/Cl co-transport and therefore water absorption by surface enterocytes, while in crypt epithelium it promotes Cl^- and water secretion. STb, mainly produced by ETEC associated with pigs, may cause secretion by stimulation of prostaglandin E_2 and 5-hydroxytryptamine production. In pigs, STb can cause exfoliation of surface enterocytes, resulting in mild atrophy of villi.

Enterotoxigenic colibacillosis is among the commonest causes of diarrhea in **neonatal pigs**, from a few hours to about a week of age. Commonly, serogroups O8, O45, O138, O141, O147, O149, and O157, expressing F4, are involved in enterotoxigenic colibacillosis in piglets, though the prevalence of F4-bearing strains may be declining due to vaccination of sows. Less commonly, F5, F6, and F41 pilus adhesins are involved. STb is the most common toxin produced by porcine ETEC; when LT is found, it is in association with STb, which may be encoded on the same plasmid. STa also occurs in strains of ETEC in swine, alone, or in combination with other enterotoxins.

At necropsy, enterotoxigenic colibacillosis cannot be readily separated from the other common causes of undifferentiated neonatal diarrhea without laboratory assistance. Generally there is dehydration, usually with evidence of diarrhea, or a history of its occurrence in the herd. Other than the presence of characteristic fluid content in the flaccid small and large bowel, usually with clotted milk still in the stomach, the internal findings are unremarkable.

In contrast to the viruses and *Isospora*, *ETEC usually does not cause significant villus atrophy* (Fig. 1.128A). Small clumps, or a continuous layer, of bacteria may be found on the surface of enterocytes on villi in mucosal tissue sections, most consistently in ileum (Fig. 1.128B). Some neutrophils may be present in the proprial core of villi, and transmigrating the epithelium into the lumen.

The involvement of ETEC expressing F4 in postweaning diarrhea of pigs over 3 weeks of age, and distinct from postweaning colibacillosis caused by VTEC, discussed below, may be related to colonization of intestine in weaned pigs in which *Rotavirus* infection, changes in diet, or villus atrophy associated with hypersensitivity to dietary protein constituents, provide adhesin-bearing *E. coli* with a competitive advantage. It causes diarrhea for up to a week or so, with ill-thrift, but is uncommonly fatal, though a syndrome probably caused by endotoxic shock, similar to that associated with shigatoxigenic stains associated with postweaning colibacillosis, described below, may occur.

In **calves**, many cases of undifferentiated neonatal diarrhea are accounted for by enterotoxigenic colibacillosis, usually involving strains of serogroups O8, O9, O20, O64, O101 with fimbrial adhesins F5 and F41, and producing STa. Infection is typically restricted to the first 2–3 days of life, probably due to the loss of receptors for F5 in older calves.

ETEC must be differentiated from the other major causes of undifferentiated diarrhea in neonatal calves – *Bovine coronavirus* (BCoV), *Coronavirus*, *Rotavirus*, and *Cryptosporidium*, which typically dominate in calves older than a few days of age. However, ETEC is not uncommonly found in combination with BCoV or *Rotavirus* infection.

The gross findings in calves with enterotoxigenic colibacillosis are the nonspecific appearance of diarrhea and dehydration. The infection is differentiated in tissue sections from the other infectious causes of this syndrome by the *absence of severe villus atrophy* (Fig. 1.129) and by the presence of *bacteria on the surfaces of villi* in the distal small intestine. As in piglets, application of a variety of presumptive or specific tests for the presence of ETEC in the intestine confirms the diagnosis (Fig. 1.130).

Enterotoxigenic colibacillosis is a significant problem in **lambs** in some areas. The serotypes involved, pathogenesis, and diagnosis of the condition are similar to those in calves. Synergism with *Rotavirus* infection may occur.

There are several reports of ETEC isolated from **foals** with diarrhea. The organisms have pili, probably F41, and secrete LT or STa. However, their capacity to produce disease in foals is unproven. Diarrhea has not ensued in foals inoculated with F4-bearing *E. coli*,

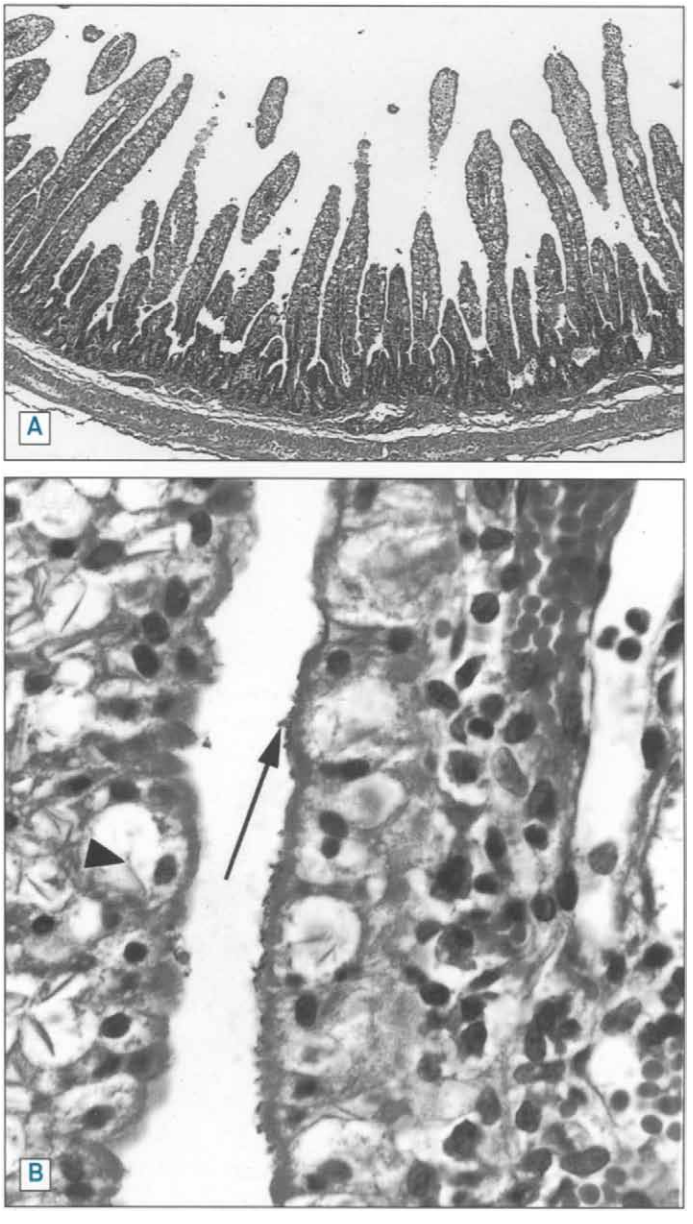


Figure 1.128 Enterotoxic colibacillosis in a piglet. **A.** Villi are tall and crypts are short, as is expected in a 2-3-day-old animal. **B.** Bacteria are present on surface of enterocytes (arrow). Cytoplasmic vacuoles containing eosinophilic spicules (arrowhead) are normal in the ileal mucosa of young piglets.

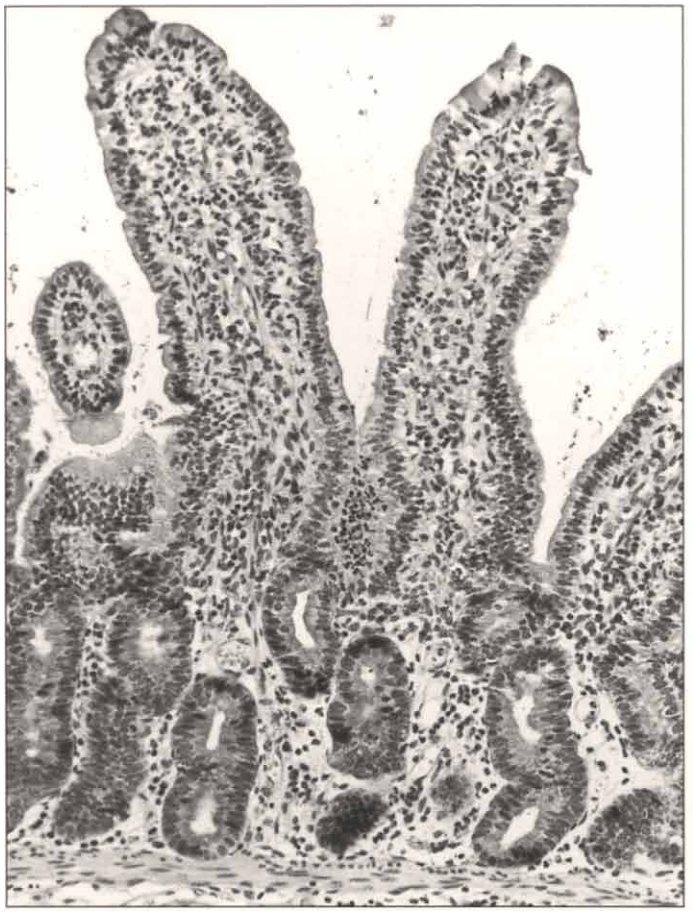


Figure 1.129 Enterotoxic colibacillosis in a calf. Mild neutrophil infiltrate in lamina propria and between base of villi. Atrophy of villi not evident, surface epithelium normal. (Courtesy of JJ Hadad, CL Gyles.)

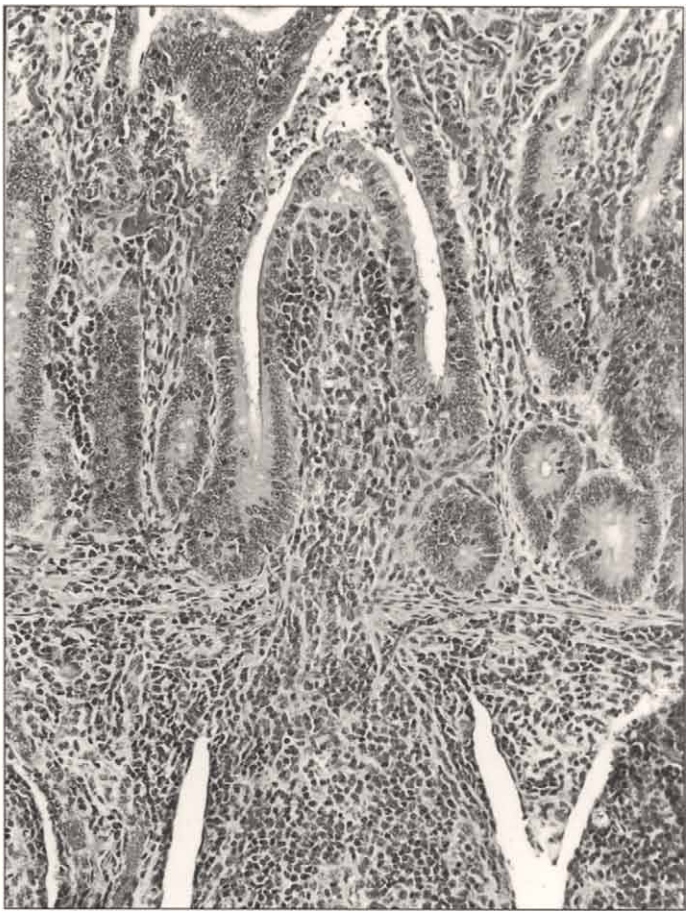


Figure 1.130 Enterotoxic colibacillosis in a calf. Neutrophil effusion into lumen over dome of Peyer's patch. (Courtesy of J Bellamy.)

despite the presence of F4 receptors on enterocytes, and it seems that ETEC has little significance in this species.

Strains of *E. coli* have been associated with diarrhea in neonates of **other species** of animals, especially young dogs, where they mainly produce STa. Generally the enterotoxigenicity and other attributes of virulence have not been well described in strains from other species.

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Enteropathogenic colibacillosis

Enteropathogenic *E. coli* (EPEC) are those that cause direct damage to the mucosa, through a *characteristic mechanism of attachment to, and effacement of, epithelium*. These **attaching-effacing *E. coli*** (AEEC) are more common in humans than in animals, where they are most important in pigs, dogs, and rabbits, though they have also been isolated from cats. Control of attaching-effacing activity resides in the **locus for enterocyte effacement** (LEE), a chromosomal pathogenicity island. EPEC have a complicated and sequential relationship with host cells. Long polar fimbriae may mediate initial bacterial interaction with the enterocyte. Secretion of bacterial proteins ensues, including **intimin**, which is an adhesin. A second protein, the **translocated intimin receptor**, is transported via a type III secretion system into the enterocyte cytoplasm, emerging on the cell membrane as the intimin receptor. In response to translocated EPEC proteins, the cell's cytoskeleton is reorganized, resulting in formation of cupped *pedestal-like structures* beneath the attached bacteria, and the subsequent loss of microvilli (Figs 1.119, 1.120, 1.131, and 1.132). Paracellular permeability increases as tight junctions between enterocytes loosen, and neutrophils migrate between cells into the lumen.

In animals and humans, some strains of AEEC are pathogenic despite failure to secrete enterotoxins or cytotoxins. A heavy layer of plump coccobacilli may be found over the luminal aspect of enterocytes on villi throughout the small intestine, and on the surface of the large intestine. *The degree of diarrhea seems related to the extent of bacterial colonization, which is most consistent in lower small intestine and large bowel.* Enterocytes to which bacteria are adherent round up or contract, and exfoliate from the mucosa singly or in clumps, resulting in mild to severe atrophy of villi in the small bowel, and attenuation of surface cells, or microerosions, in the large intestine. Fusion of villi may occur in small intestine, and goblet-cell numbers are depleted in both large and small bowel. There is moderate mucosal congestion, and local infiltration by neutrophils.

Diarrhea is presumably related to maldigestion and malabsorption of nutrients and electrolytes in small intestine, perhaps with the additive effect of increased mucosal permeability, overloading the colon, the absorptive ability of which is also compromised by damage to surface cells. *Microscopic diagnosis is based on recognition of bacteria on the mucosal surface.*

In **pigs**, EPEC belonging to serogroups O45 and O103, infecting small and large intestine, are responsible for some cases of postweaning diarrhea. In **dogs**, and occasionally in cats, EPEC have been associated with diarrhea, often as a component of co-infections with viral or protozoal agents. Microscopic lesions characteristic of AEEC are typically found in the jejunum and ileum, less commonly in the colon, and, in dogs, sometimes in the stomach.

A distinct subset of EPEC is the **Shiga toxin-producing *E. coli*** (STEC), also known as **enterohemorrhagic *E. coli*** (EHEC). In addition to their ability to attach and efface, these strains produce cytotoxic Shiga toxins (Stx1, and its homologue Stx2 with its variants, c, d, e, f). Shigatoxin 1 is structurally identical to the Shiga toxin produced by *Shigella dysenteriae,* which has a profound cytopathic effect. Due to their effect on Vero cells in culture, these *E. coli* are also referred to as **verotoxin-producing *E. coli*** (VTEC). Shiga toxins, encoded in the genome of bacteriophages, are composed of an A subunit that has enzymatic activity and a B subunit that binds the toxin to the glycolipid receptor globotriaosylceramide (Gb3) on the

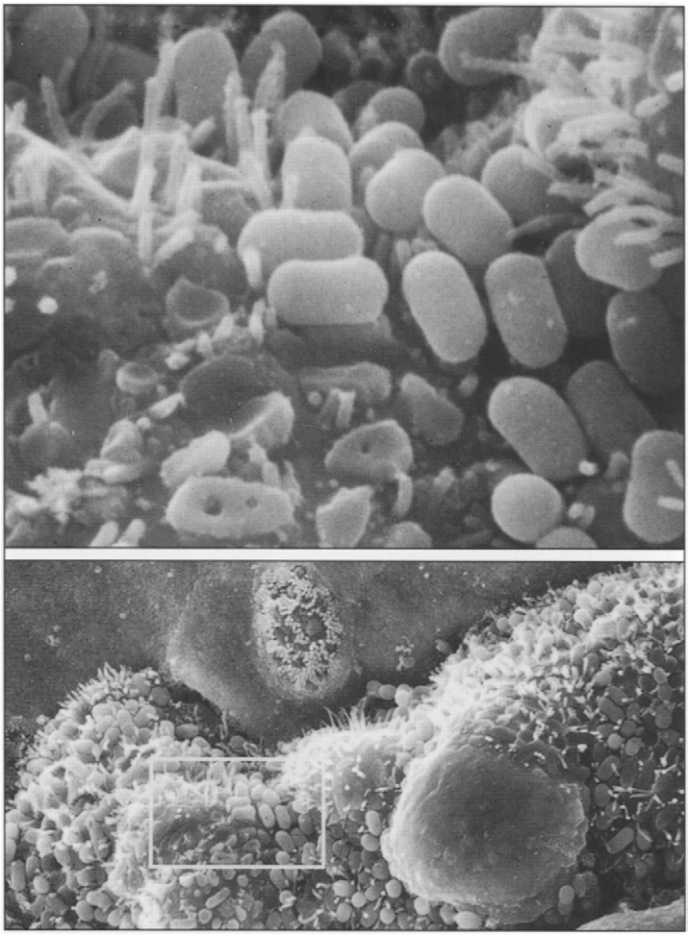

Figure 1.131 Scanning electron micrograph of the colon of a calf with **enterohemorrhagic *Escherichia coli* infection**. Lower: note irregularity of microvilli on cells infected by attaching-effacing *E. coli*, in comparison with microvilli on uninfected cells in background. Outline indicates field illustrated in upper photo. Adherent bacteria are on pedestals projecting from the surface of enterocytes. Occasional bacteria have been lost artifactually, exposing underlying "mushroom-like" pedestals. (Courtesy of M Schoonderwoerd, R Clarke.)

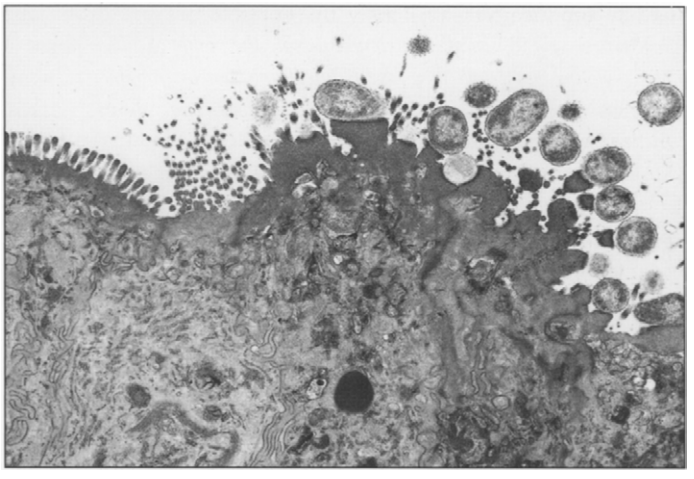

Figure 1.132 Transmission electron micrograph of enterocytes in the colon of a calf infected with enterohemorrhagic *E. coli*. **Enterocyte attaching-effacing *E. coli*** are on pedestals projecting from surface of infected cells. Microvilli are irregular and effaced on infected cells. Normal cell (left). (Reprinted with permission from Schoonderwoerd M, et al. Can J Vet Res 1988;52:484–487.)

cell surface. Once endocytosed and transferred via the Golgi apparatus to the rough endoplasmic reticulum, the toxin inhibits protein synthesis, which may be lethal to the target cell, and via separate mechanisms may induce apoptosis. The presence or absence of Gb3 on cell surfaces is a major determinant of the distribution of tissue susceptibility to Shiga toxins, which mainly affect intestinal epithelium and vascular endothelium. Some EHEC also produce hemolysins, which may assist survival in the gut by increasing iron availability. Acid tolerance may also promote colonization efficiency by enhancing survival in the stomach.

The EHEC strains produce disease predominantly in humans, although involvement of domestic animals has been highlighted in the public health arena due to the tendency for cattle and some other species to carry the organism asymptomatically, adherent to epithelium over lymphoid follicles in the rectal mucosa. The most widely recognized EHEC serotype is **O157:H7**, a major pathogen in humans, although over 200 other STEC serotypes have been identified. In addition to Shiga toxin production, virulence is attributable to attaching and effacing capability, encoded on the LEE.

In **calves under 4 weeks of age** (generally over 3 days of age, and most commonly in the second week of life), strains of EHEC (O5:NM, O8:H9, O26:H11, O103:H2, O111:NM, O111:H8, and O111:H11) have been associated with a syndrome of *erosive fibrinohemorrhagic enterocolitis*, with the development of dysentery. Fever is not characteristic, and animals may remain bright until the effects of dehydration and blood loss supervene. Death may occur within several days of onset of illness, but some cases will recover in 7–10 days.

At necropsy, gross lesions are usually confined to the spiral colon and rectum, though the ileum and cecum are occasionally involved with mild fibrinous or fibrinohemorrhagic enteritis/typhlitis. In the colon, changes vary from mild patchy congestion of the mucosa to marked mucosal reddening, with adherent mucus, necrotic debris, and blood; colonic contents are fluid and frequently blood-tinged (Fig. 1.133). There may be congestion of the margins of mucosal folds in the rectum, or overt fibrinohemorrhagic proctitis. Mesenteric lymph nodes are often enlarged, especially along the ileum, and occasionally there are lesions (arthritis, serositis) suggesting septicemia.

Microscopically, *in affected small intestine the profile of villi is ragged or markedly scalloped, and they are blunted, moderately atrophic, or fused.* Epithelial cells on villi in small bowel, and on the colonic surface, where lesions are most severe, are short, rounded up, and in some cases exfoliating singly or in small clumps, causing focal microerosions. Cells in some areas may be markedly attenuated. The microvillus border is indistinct, and covered by a heavy layer of prominent gram-negative coccobacilli (Fig. 1.134). Lesions in large bowel may extend down into glands, which may be dilated, lined by flattened epithelium, and filled with sloughed epithelium and leukocytes. In the small intestine, foci of bacterial adhesion may be patchy, on the sides of the upper third of villi, with extensive surrounding areas of normal epithelium. Crypts in areas of atrophic small intestine may be elongate, with numerous mitotic figures. In severely affected bowel, the mucosa and submucosa are congested, edematous, and occasional microvascular thrombi may be present. Sloughed enterocytes, erythrocytes, neutrophils, fibrin, and bacteria are in the lumen.

In **dogs**, STEC have been associated with dysentery, and in some dogs, hemolytic uremic syndrome and cutaneous edema and ulceration. In Greyhounds, the syndrome involving this triad has been termed *cutaneous and renal glomerular vasculopathy*, and has been attributed to consumption of beef contaminated with O157:H7 *E. coli*, and other STEC. Renal and cutaneous lesions are attributable to vascular damage caused by Shiga toxin (see Vol. 2, Urinary system; Vol. 1, Skin and appendages).

Figure 1.133 Fibrinohemorrhagic enteritis in the ileum of a calf with **enterohemorrhagic *E. coli* infection**. (Courtesy of M Schoonderwoerd, R Clarke.)

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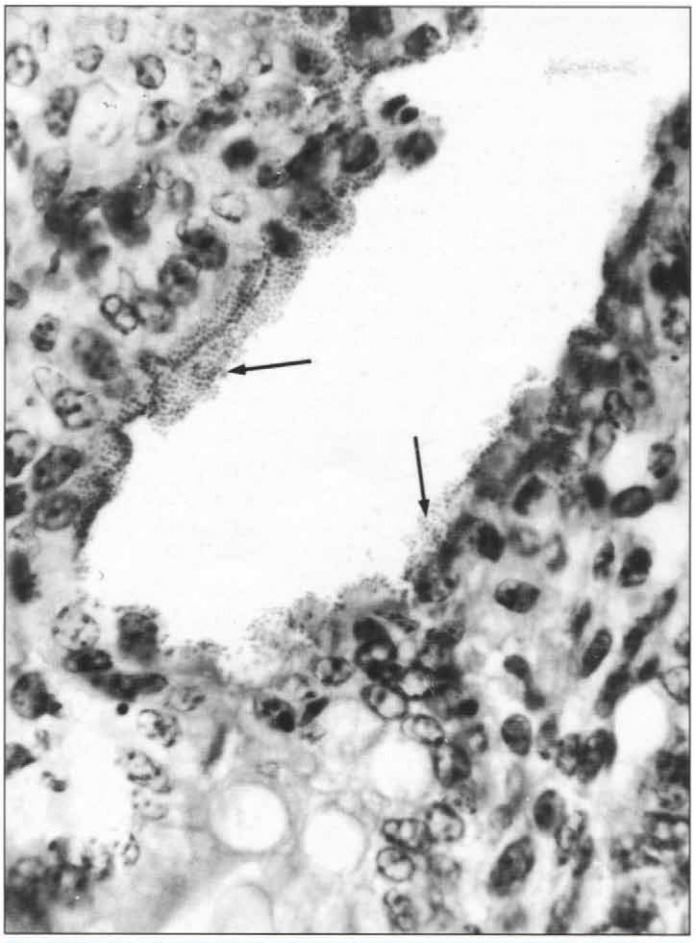

Figure 1.134 Adherent bacteria (arrows) on surface of enterocytes in the colon of a calf with **enterohemorrhagic *E. coli* infection**. (Courtesy of M Schoonderwoerd, R Clarke.)

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Edema disease and postweaning *E. coli* enteritis

Edema disease *is a distinct syndrome in pigs, characterized by sudden death, or the development of nervous signs, associated with enteric colonization by STEC*, especially serotypes O138, O139, and O141. The disease occurs most commonly in pigs within a few weeks after weaning, or after other change in feeding or management. It often occurs in association with outbreaks of postweaning *E. coli* enteritis. Rare reports exist of edema disease in suckling and mature animals. The disease may be sporadic or occur as an outbreak, usually affecting the best animals in a group, and mortality often approaches 100% of affected animals. Edema disease and postweaning *E. coli* enteritis have apparently declined in prevalence in parts of North America, perhaps with the use of concentrate rations based largely on soybeans and corn, rather than other grains.

Bacterial colonization of the gut is mediated by F18ab fimbriae. Susceptibility of pigs is genetic and related to the presence of receptors for the fimbriae. A Shiga toxin (Stx2e) producing vascular injury and edema has been incriminated in the pathogenesis of edema disease, and vaccination with Stx2e toxoid almost entirely prevents edema disease.

Some strains of *E. coli* that cause edema disease also produce secretory enterotoxin. Diarrhea is not a usual concomitant of edema disease in individual animals. Significant gross or microscopic lesions in the intestinal mucosa do not occur in edema disease, which appears to be a *classical enterotoxemia*, the active principle being absorbed from the gut and acting at a distant site. However, the means by which the toxin enters the circulation is unknown.

Experimentally, the target of Stx2e, like other Shiga toxins, is *vascular endothelium*, particularly of small arteries and arterioles. Preferentially affected organs include spinal cord, cerebellum, eyelid, and colon. However, a study to determine preferential binding

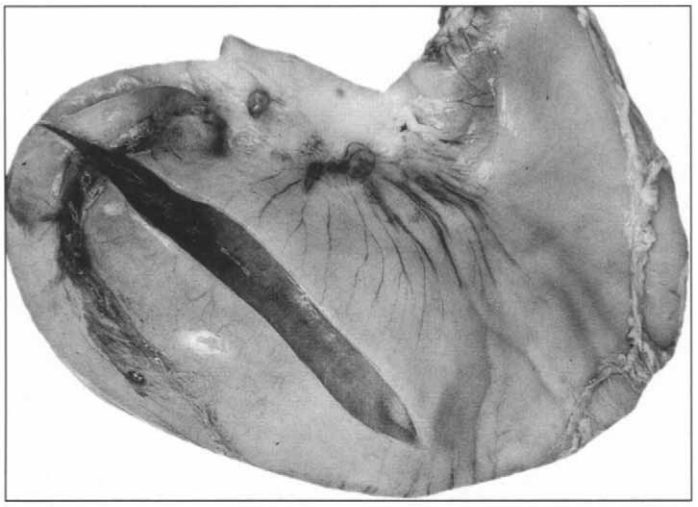

Figure 1.135 Edema of stomach wall in **edema disease** in a pig.

sites for Stx2e found receptors on a variety of tissues, not just the aforementioned. Stx2e causes *angiopathy*, which, in its early stages in experimental intoxication, is recognized by swelling of endothelial cells and mild intramural and perivascular hemorrhage. Pyknosis and karyorrhexis of smooth-muscle nuclei, often accompanied by fibrinoid degeneration or hyaline change in the tunica media, may be seen in subacute spontaneous cases. Proliferative mesenchymal elements are found in the tunica media and tunica adventitia in more advanced cases. However, inflammation is not at any stage a prominent component of the angiopathy, nor of the associated edema in most sites, and thrombosis of vessels is rarely encountered. Edema is probably due to vessel damage during the early stages of the angiopathy. The lesions are distinct from those expected with endotoxemia.

Swine with **edema disease** may die without premonitory signs. Others may have anorexia, or, more characteristically, show nervous signs, usually of less than a day's duration. An unsteady staggering gait, knuckling, ataxia, prostration and tremors, convulsions, and paddling occur. A hoarse squeal, the hoarseness attributed to laryngeal edema and dyspnea, may also be noted clinically.

At necropsy, **gross lesions** in acute deaths may be subtle or absent. Typically, *edema is variably present in one or more sites*. However, it may be mild and must be carefully sought, especially by "slipping" the suspected area over subjacent tissue. Subcutaneous edema may be present in the frontal area and over the snout, in the eyelids, and in the submandibular, ventral abdominal, and inguinal areas. Internally, there may be some hydropericardium, and serous pleural and peritoneal effusion, perhaps accompanied by mild or moderate pulmonary edema. More commonly, the serous surfaces merely appear glistening and wet. Edema of the mesocolon, of the submucosa of the cardiac glandular area of the stomach over the greater curvature, and of mesenteric lymph nodes is most consistently found. The gastric submucosal edema should be sought by carefully cutting through the muscularis to the submucosa. The edema fluid is clear, and slightly gelatinous (Fig. 1.135). It is rarely blood-tinged, and overt hemorrhage is usually not present in uncomplicated edema disease. The stomach is often full of feed, but the small intestine is relatively empty and the mucosa is grossly normal. The colon may contain somewhat inspissated feces.

In swine dying after a more prolonged clinical course, gross edema is often not present, though enlargement of mesenteric lymph nodes is present in a large proportion of cases. A few pigs may show foci of yellow malacia, usually bilaterally symmetrical, in the brainstem at various levels from basal ganglia to medulla.

Microscopically, edema in the sites of predilection mentioned above is the main lesion in swine dying acutely. It is generally devoid of much protein and contains few erythrocytes and inflammatory cells. A proportion of animals will also have meningeal edema and distended Virchow–Robin spaces in the brain. Vascular lesions may not be well developed in pigs dying suddenly. When present they usually consist of edema, hemorrhage, myocyte necrosis, and hyaline degeneration in the tunica media. Angiopathy is more consistently found in cases of longer standing. Affected vessels may be found in any tissue in the carcass. Brain edema and focal encephalomalacia in the brainstem are associated with the presence of lesions in cerebral vessels; necrosis may be a sequel to edema and ischemia. "Cerebrospinal angiopathy of swine" is probably a manifestation of edema disease.

A **diagnosis** of edema disease is based on nervous signs or sudden death in growing pigs, in association with typical gross and microscopic lesions, when they are present. In acute cases, heavy growth of hemolytic *E. coli* of one of the serotypes known to produce Stx2e is essential.

Edema disease must be differentiated from enteritis and endotoxemia due to *E. coli* in postweaning pigs; from mulberry heart disease in animals dying suddenly; and from salt poisoning, *Salmonella* meningoencephalitis, and other infectious encephalitides, in animals with nervous signs.

Postweaning *E. coli* enteritis (coliform enteritis of weaned pigs) typically occurs during the first week or two following weaning, or after some other change in feed or management. Postweaning diarrhea may be caused by classical enterotoxigenic F4 (K88) *E. coli*, but it is often associated with hemolytic *E. coli* of the same serotypes primarily implicated in edema disease, as well as serotype O149. The two diseases often occur in the same population of pigs, though usually affecting different animals. Typically, postweaning colibacillosis is a disease of high morbidity and variable mortality, with loss of condition in pigs suffering prolonged illness. Diarrhea is usually yellow and fluid, and stains the perineum. Deaths that occur may or may not follow a prior episode of diarrhea, and often appear to be related to endotoxemia.

In fatal cases, there may be blue-red discoloration of the skin and evidence of dehydration. Deep red *gastric venous infarcts* are present in almost all cases (Fig. 1.136). The small intestine is flaccid. The mucosa may be normal in color and the content creamy. In other animals the mucosa of the distal small intestine will be congested and the contents watery and perhaps blood-tinged or brown with flecks of yellow mucus or fibrin (Fig. 1.137). Cecal and colonic lesions are usually mild, but there may be some congestion and fibrinous exudate in the proximal large bowel. Mesenteric lymph nodes may be somewhat enlarged, congested, and juicy. Other organs are usually unremarkable grossly.

The **pathogenesis** of postweaning *E. coli* enteritis due to non-F4 *E. coli* is poorly understood, and the microscopic pathology is not well described. In swine with diarrhea, *E. coli* may be attached to the surface of villi by F18ac fimbriae. Atrophy of villi does not seem to be evident, and diarrhea is presumed to be mediated by enterotoxins. Mortality in animals with prolonged diarrhea and few gross intestinal

Figure 1.136 Deep red areas of venous infarction in the gastric mucosa in **postweaning colibacillosis** in a pig.

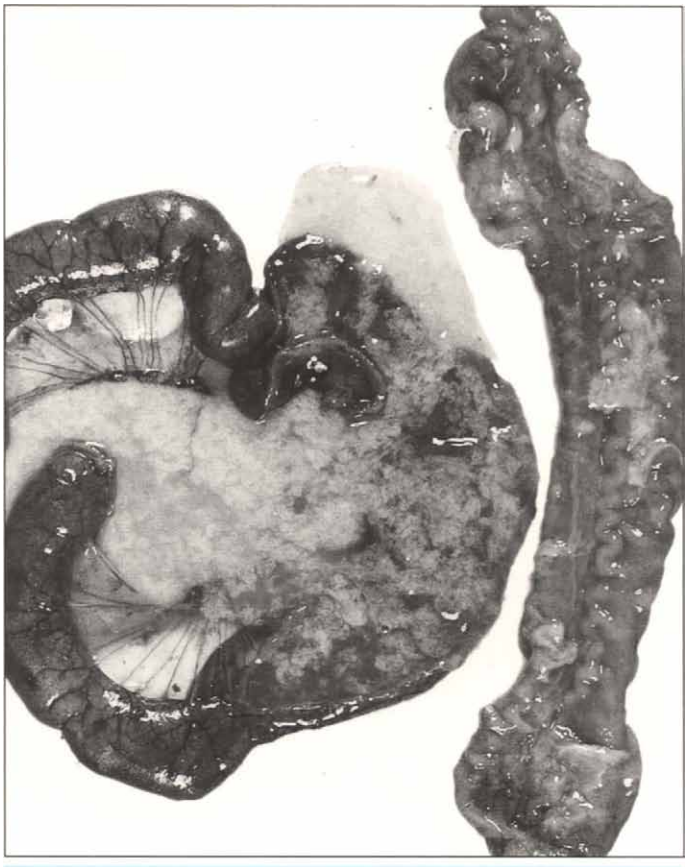

Figure 1.137 Acute catarrhal enteritis, with congested, flaccid small intestine in **postweaning colibacillosis** in a pig.

or extraintestinal lesions may be ascribed to dehydration. In animals dying of more acute disease, there is local microvascular thrombosis in sections of congested mucosa (Fig. 1.138), and the gross and microscopic lesions in other organs, especially those related to gastric mucosal and submucosal thrombosis and venous infarction, are suggestive of endotoxemia. Hemolytic *E. coli* of the implicated strains are consistently isolated in virtually pure culture from the lower small intestine and colon. However, they are present in the spleen and liver in only a few cases, suggesting terminal bacteremia.

The factors predisposing to the massive colonization of hemolytic *E. coli* are unclear. Loss of lactogenic immunity, a favorable environment for proliferation of bacterial strains with specific nutrient requirements, and promotion of epithelial colonization by the effects of antecedent *Rotavirus* infection, have been variously implicated.

A **diagnosis** of postweaning colibacillosis is suggested by the gross lesions in animals dying acutely or subacutely, and it is confirmed by culture and serotyping of associated strains of *E. coli*. The fatal disease must be differentiated from edema disease, proliferative hemorrhagic enteropathy, salmonellosis, and swine dysentery. Postweaning diarrhea due to uncomplicated *Rotavirus* infection, transmissible gastroenteritis, or associated with attaching-effacing O45:K "E65" *E. coli*, is usually nonfatal.

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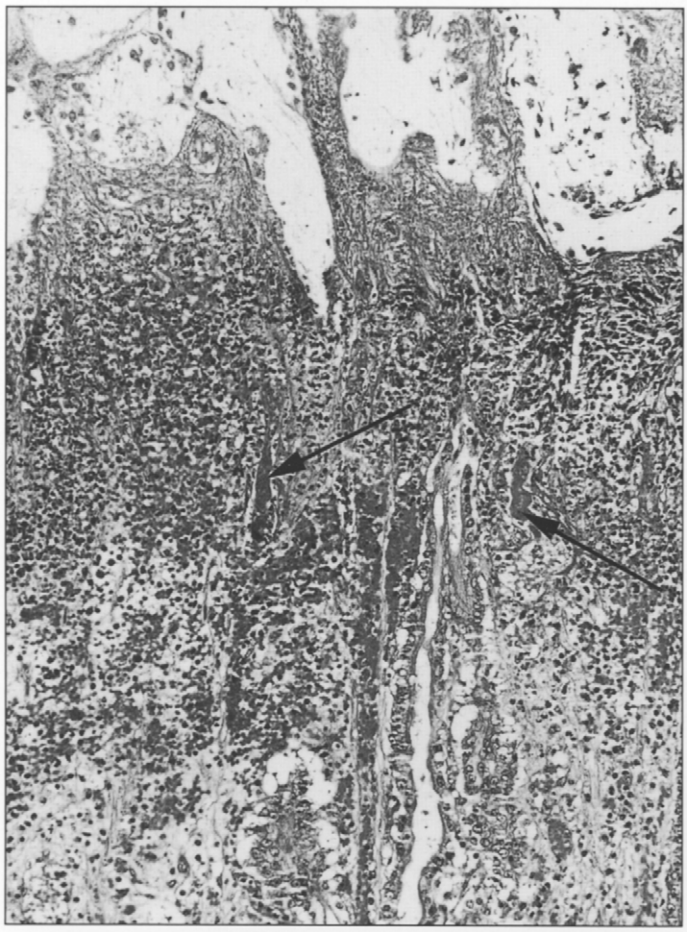

Figure 1.138 Thrombosis of venules (arrows) and necrosis of the superficial gastric mucosa in venous infarction in **postweaning colibacillosis** in a pig.

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Enteroinvasive *E. coli*

Strains of *E. coli* are recognized, infecting humans and certain other species, which have the capacity to invade or to be internalized by surface enterocytes of the small and large intestine, in which they multiply. In this sense they resemble *Shigella* in primates, and *Salmonella*. The enteroinvasiveness of *Shigella* and some strains of *E. coli* appears to be correlated with the presence of a high-molecular-weight plasmid coding for outer-membrane proteins involved in invasion. Multiplication of the organism within epithelial cells results in *local erosion and ulceration*, associated with acute inflammation in the mucosa.

Among domestic animals, *enteroinvasive colibacillosis has only been confirmed experimentally in neonatal swine*, using a strain of O101 *E. coli*. Spontaneous enteritis that appears to be due to enteroinvasive *E. coli* is rarely encountered in piglets up to weaning and in calves under 2 weeks of age. Diarrhea in experimentally infected piglets is described as gray-yellow, watery, and containing small clots. The gross findings may not be remarkable, or the intestine may appear congested in comparison with that in most diarrheic piglets. In spontaneous cases suspected of being due to enteroinvasive *E. coli*, the gastric fundus may also be congested, and this correlates with the presence of venous infarction visible microscopically. Experimental enteroinvasive colibacillosis in piglets causes villus atrophy that is comparable in severity to that induced by the common viruses of neonates. Enterocytes appear cuboidal or flattened and some are seen lysing. The lamina propria is edematous; capillaries are congested and infiltrated by neutrophils and other inflammatory cells. In spontaneous cases, thrombi may be evident in proprial capillaries and submucosal lymphatics. Neutrophils and tissue fluid effuse into the lumen between villi through epithelial discontinuities. Similar microthrombosis, proprial inflammation, enterocyte destruction, and effusion may be found in the cecum and colon. Intracellular organisms of O serogroup 101 were demonstrated by immunoperoxidase staining in the experimental study, but are not generally recognized in spontaneous cases suspected to be due to enteroinvasive *E. coli*. Edema and neutrophil accumulation in sinusoids of mesenteric lymph nodes are present. Experimental enteroinvasive colibacillosis in piglets has been associated with malabsorption and protein loss into the gut, presumably due to villus atrophy and effusive enteritis, respectively.

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Septicemic colibacillosis

Generalized systemic infection with *E. coli* occurs commonly in *calves*, and less commonly or sporadically, especially among young animals of the other domestic species. Predisposition to infection is a prerequisite for *E. coli* septicemia. This usually results from reduced transfer or absorption of maternal colostral immunoglobulin, or from intercurrent disease or debilitation. But certain strains of *E. coli*, especially O8, O9, O15, O26, O35, O45, O78, O86, O101, O117, and O137 in calves and lambs, and O115 in pigs and calves, are particularly associated with septicemia, and may possess characteristics that enhance their ability to invade and proliferate systemically in compromised animals.

Among factors conferring virulence upon these strains are plasmids coding for colicin V (Col V). Col V plasmids carry genes coding for aerobactin, a bacterial hydroxamate siderophore permitting survival in low-iron extracellular environments; outer-membrane proteins resisting bactericidal effects of serum, such as complement activation; and hydrophobic properties that impede phagocytosis, conferred by a capsule. Some produce cytolethal distending toxin, or fimbriae which impede phagocytosis. Endotoxin released by dying bacteria causes the vascular damage and shock associated with *E. coli* septicemia.

The portal of entry of *E. coli* causing septicemia probably varies somewhat. The navel in the neonate, the upper respiratory tract and possibly the tonsil, and the intestine are likely sites. In calves, adhesins such as P, F17, AfaE-VIII, and CS31A may promote enteric colonization and invasion. Enteritis is not a necessary, or even common, concomitant of colisepticemia in animals.

Colisepticemia is most commonly a disease of neonates, and may vary from peracute septicemia and endotoxemia resulting in sudden death, to subacute or chronic disease in which signs are related to sites of bacterial localization, especially in the meninges, joints, and eyes.

The lesions associated with colisepticemia in young animals of any species, especially calves, lambs, and foals, may vary from subtle to obvious. Mortality in hypogammaglobulinemic neonates may occur acutely with little in the way of abnormal gross findings. These may be limited to mildly congested or blue-red, slightly rubbery lungs, and a firm spleen, perhaps with evidence of omphalitis. *Microscopic changes* in the lungs include thickening of alveolar septa by mononuclear cells and neutrophils, and effusion of lightly fibrinous exudate and a few neutrophils into alveoli. There may be a corona of neutrophils around white pulp in the spleen, and neutrophils may be present in abnormal numbers in circulation in many organs, including lung and hepatic sinusoids. Kupffer cells may also be prominent in sinusoids in the liver. Fibrin thrombi may be evident in pulmonary capillaries, glomeruli, and hepatic sinusoids. Some calves will develop acute interstitial nephritis with foci of neutrophil accumulation, which with time evolve into "white-spotted kidney" in surviving animals.

More severe acute cases will show evidence of serosal hemorrhage, with perhaps some serosanguineous pericardial fluid. The lungs may be deep red-blue, rubbery, and fail to collapse. Interlobular septa may be slightly separated by edema, and froth or fluid may be present in the major airways. Meningeal vessels may be congested, and the meninges wet. The abomasum or stomach may have focal superficial ulcers, or more extensive deep red areas of venous infarction. There may be evidence of diarrhea and dehydration, with congestion of the small intestine. Microscopic lesions resemble those previously described, with more severe congestion, thrombosis, and edema in lungs, and perhaps other tissues. In cases not examined for some time after death, clumps of small bacilli may be seen in vessels throughout the body. The vascular permeability, thrombosis, and hemorrhage reflect endotoxemia and its sequelae.

Subacute cases may develop localized infection on serous surfaces, in the joints and meninges. Fibrinous peritonitis, pleuritis, and pericarditis, fibrinopurulent arthritis and meningitis, and hypopyon are commonly found, alone or in various combinations. Affected animals may have a history of lameness ascribable to arthritis, nervous signs due to meningitis, or general debilitation. Microscopic examination reveals the lesions already described in animals with active systemic disease, with the addition of extensive congestion and edema of inflamed serous surfaces, associated with an acute fibrinous inflammatory exudate.

In *lambs*, congestion and edema of the mucosa of turbinates and sinuses, perhaps with mucopurulent to hemorrhagic sinusitis, have been described. Fibrinous polyserositis and arthritis are sporadic manifestations of *E. coli* septicemia in growing or adult swine, and must be differentiated from the more significant *Haemophilus, Mycoplasma,* and streptococcal infections causing these lesions. Colisepticemia is a sporadic cause of mortality in litters of young puppies.

Diagnosis of colisepticemia is based on the isolation of *E. coli* in large numbers from more than one parenchymatous organ or other internal site, other than mesenteric lymph node (preferably liver, spleen, lung, or kidney), or from a site of serosal localization, in conjunction with compatible gross and/or microscopic lesions.

"**Watery mouth**," a syndrome characterized by drooling, depression, loss of appetite, and abomasal and abdominal distension, is associated with *E. coli* infection/bacteremia in lambs under 3 days of age in the UK. At necropsy, affected lambs are in poor condition. They may have unclotted milk and mucinous fluid in the distended abomasum; there is gas in the abomasum and intestine, and meconium retention is common. It is hypothesized that *E. coli* colonize the bowel, and in some manner cause loss of motility and functional obstruction. Fluid and gas accumulate in the abomasum. Bacteremia/septicemia is terminal.

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Salmonellosis

The **taxonomy of *Salmonella*** is confusing and has recently been modified, based on molecular genetic analysis. The genus *Salmonella* is now considered to be comprised of two species, ***S. bongori*** and ***S. enterica***. There are six subspecies of *S. enterica* (*enterica, salamae, arizonae, diarizonae, indica,* and *houtenae*) and many (>2200) antigenically distinct serotypes or serovars. About 60% of *Salmonella* serotypes belong to *S. enterica enterica*, and occur in birds and mammals. Members of *S. e. enterica* are the predominant cause of salmonellosis in humans and domestic animals, but fewer than 50 of these serotypes have been isolated from mammals or birds with any frequency worldwide. The remainder of *S. enterica* and *S. bongori* serotypes are found in ectothermic animals or in the environment. In conventional terminology, the serotypes have been treated as species, but in the new terminology, the names of serotypes are capitalized, but not italicized (e.g., *S. enterica* Typhimurium when first used, followed later by *S.* Typhimurium). They are usually named on the basis of the locality in which the serotype was first isolated or identified, or on their host association and the clinical syndrome they may produce. Identification of isolates at the subserotype level, by phage typing, plasmid profile analysis, or other molecular techniques, is desirable when there is evidence of zoonotic transmission, or when epidemiologic tracing is necessary.

The **clinical and pathologic syndromes of salmonellosis** typically vary from localized enterocolitis to septicemia; abortion may also occur, with or without obvious systemic disease. While

some serotypes are strongly host-adapted, others have a very wide host range. Highly host-adapted serotypes, such as *S.* Typhi (humans), *S.* Dublin (cattle), and *S.* Choleraesuis (swine), tend to produce severe systemic disease in adult, as well as juvenile animals, whereas serotypes with a broad host range, e.g., *S.* Typhimurium, tend to affect predominantly young animals in most species, and mainly cause enterocolitis, though septicemia may occur. There may be overlap between the two forms of disease, and if the animal survives, a carrier state of variable duration usually follows.

Asymptomatic ***Salmonella* carriage** may be common, depending on the species, and transmission can occur directly, or indirectly, by contamination of feed, water, or the environment from which the organism is ingested or inhaled. Stressors that compromise immune competence or disrupt the enteric bacterial ecosystem are often implicated in salmonellosis, and disease is usually more common and severe in young animals. The more common "*stressors*" associated with salmonellosis in domestic animals include transportation, starvation, changes in the ration, overcrowding, pregnancy, parturition, exertion, anesthesia, surgery, intercurrent disease, immunosuppressive drugs, and oral treatment with antibiotics and anthelmintics. Consequent changes in the anaerobic bacterial ecosystem that alter the volatile fatty acid composition of the enteric environment are permissive of *Salmonella* colonization.

There are many examples of enhanced susceptibility to salmonellosis associated with intercurrent disease. The best known is that between the *Classical swine fever virus* and *S.* Choleraesuis, an association so close as to have caused early pathologists to disregard the bacterium as a significant pathogen. The disease in adult cattle is usually sporadic, and often there are predisposing conditions, such as parturient paresis, ketosis, mastitis, and parasitic infestations. The stress of anesthesia and surgery may account in part for the serious outbreaks of salmonellosis that occur in hospitalized animals, especially horses, at veterinary schools.

The **pathogenesis of salmonellosis** may be divided into several stages: *entry* of the bacteria into the host and attainment of the primary site of infection, usually the enterocyte; attachment to the surface (*colonization*); and *invasion* of enterocytes.

For infection to take place, *Salmonella* must be present in sufficient numbers; generally a minimal infective oral dose of 10^7–10^9 organisms is needed to infect large domestic animals. After ingestion, the *Salmonella* must overcome nonspecific resistance factors, including the bactericidal effects of salivary enzymes, and the acid pH of the gastric environment. Mucus and lysozymes in the glycocalyx, peristalsis, and constant sloughing of enterocytes may interfere with attachment. Those organisms that survive the nonspecific resistance factors may colonize and invade enterocytes.

Invading *Salmonella* in some species enter the mucosa through M cells in the Peyer's patches, and host specificity of some *Salmonella* serotypes may be associated in part with specific receptor sites on these cells. *Salmonella* have been demonstrated in the Peyer's patches as early as 6 hours postinoculation. When bacteria invade through M cells, smaller numbers of *Salmonella* may enter through enterocytes in other areas of the small intestine. However, the M cell is not the main site of attachment in some circumstances, for instance in *S.* Typhimurium infections of calves and pigs.

In salmonellosis characterized primarily by enterocolitis, the organisms do not usually disseminate beyond the mucosa and the mesenteric lymph nodes, and the ensuing inflammation remains confined to the intestine. In those cases where bacteremia ensues, the organisms must be able to survive and replicate in macrophages and disseminate to other systemic sites, e.g., liver, lung, joints, meninges, or placenta and fetus.

The ability to attach, invade, and penetrate enterocytes is crucial to virulence, and the first step in the development of salmonellosis. A number of known virulence factors contribute to the pathogenesis of salmonellosis, including motility, pili, or fimbriae, effector proteins modifying the metabolism or causing death of host cells, and lipopolysaccharides. The information for such virulence attributes is often encoded in chromosomes in clusters of genes known as *Salmonella* pathogenicity islands (SPI).

Invasion of enterocytes, especially those in the ileum, occurs within 12 hours of oral infection. Ability to invade cells is dependent on a type III protein secretion system encoded in SPI-1. Protein targets of the secretion system are translocated into host cells, where they facilitate bacterial invasion by causing changes in the cytosol and ruffling of the cell membrane.

Motility, associated with the presence of flagella, is characteristic of many *Salmonella* serovars. Bacterial motility is generally not considered to be an important virulence determinant. However, it may enhance the movement of bacteria through the glycocalyx and facilitate attachment to specific receptor sites on enterocytes.

Fimbriae (pilus adhesins) encoded in chromosomes and on virulence plasmids are present on salmonellae, and they may play a role in colonization of the gut. Adherence of *Salmonella* to intestinal epithelial cells takes place in two stages. The first step is reversible, since the organisms can be easily washed off. Weak ionic and nonionic interactions between bacterial and host cell membrane surfaces are thought to be the binding forces responsible for this attachment. The second stage, referred to as "receptor-mediated endocytosis," is irreversible. It occurs after a lag period and it is characterized by degeneration of the microvilli on the epithelial cells, "ruffling" of the cell membrane, and macropinocytosis, resulting in the formation of membrane-bound vacuoles (endosomes) containing *Salmonella*.

The ultrastructural changes of *Salmonella* infection in the intestine were first described in experimental infections of guinea pigs. Large numbers of organisms are present in the lumen, on the surface of the brush border, and in enterocytes. There is an increase in the number of neutrophils in the gut lumen and within intercellular spaces, and some of these contain bacteria. Degeneration of microvilli, characterized by loss of filamentous cores, is associated with close adherence of bacteria. Other changes consist of elongation, swelling, budding and fusion of microvilli, and loss of the terminal web.

The organisms usually invade the cells through the brush border; however, they may also enter the mucosa through the intercellular junctional complex. In the cytoplasm, the bacteria are located within membrane-bound vacuoles, which may also contain remnants of microvilli and cytoplasmic debris. Most organisms remain intact and multiply during their transcellular migration in endosomes. Often, many bacteria are present in a single enterocyte during the early stages of infection, but cellular damage is mild and transient. The *Salmonella*–receptor complex dissociates as a result of the acidification of the endosomal content, allowing the receptor site to return to the apical plasma membrane and repeat the processes of endocytosis. After 24 hours, most bacteria are located within membrane-bound vacuoles in macrophages in the lamina propria. Many organisms are

evident in the lumina of crypts, but invasion of cryptal epithelial cells evidently does not take place.

The **lipopolysaccharide** (LPS) moiety of *Salmonella* with smooth cell walls consists of an *O-specific side chain, a core portion, and a lipid A portion*. Most *Salmonella* isolated from animals have smooth cell walls, which influences virulence in several ways. These strains are more invasive, and are more successful at avoiding phagocytosis, and lysis in phagolysosomes after invasion, than are "rough" counterparts with incomplete LPS. LPSs reduce the susceptibility of the organisms to the host's cationic proteins; they stimulate local prostaglandin synthesis; and they prevent the activation and deposition of complement on the bacterial surface.

The main function of LPS may be to facilitate survival in the intestinal mucosa and eventual entry into deeper tissues. The involvement of LPS in invasion apparently varies among *Salmonella* serotypes, since some strains of *S.* Typhimurium do not require intact LPS to invade epithelial cells in vitro. On the other hand, more host-specific *Salmonella* serotypes, such as *S.* Typhi and *S.* Choleraesuis, require intact LPS or O-side chains. The lipid A portion of LPS is responsible for the endotoxin-mediated effects of *Salmonella* infection that are seen in systemic disease. Septicemia (endotoxemia) typically causes fever, leukopenia, hemoconcentration, lactic acidosis, coagulopathies, hypotension, and death.

Diarrhea in salmonellosis is not mediated by enterotoxins such as those involved in cholera and *E. coli* infections. Rather, *effector proteins* associated with SPI-1 induce secretory diarrhea by blocking chloride channel closure, while others attract neutrophils, and induce apoptosis of enterocytes. Proteins encoded in SPI-5 also promote neutrophil recruitment and electrolyte secretion. Mucosal inflammation leads to the accumulation of a number of mediators, including prostaglandin E_2, capable of causing hypersecretion of chloride by enterocytes, and consequent passive osmotic movement of water into the lumen. Loss of enterocytes, dying as a sequel to *Salmonella* invasion and neutrophil-induced tissue injury, results in a reduction in absorptive surface area, and causes defects in mucosal integrity, through which the protein- and neutrophil-rich exudate leaking from permeable vessels effuses.

Thus diarrhea is an outcome of active secretion of electrolyte, malabsorption due to reduced mucosal surface area and enterocyte competence, and inflammatory exudation, which may contain sufficient fibrinogen to form a pseudomembrane over the affected surface. The volume of fluid originating in lesions in the small intestine may overwhelm the capacity of the colon to compensate; as often as not in salmonellosis, the large intestinal mucosa is also involved, further compounding the compromise to electrolyte and water homeostasis in the gut.

Thrombosis of mucosal venules is common in *Salmonella* enteritis, and may contribute to loss of mucosal viability. Such lesions may be due in part to the large amounts of **endotoxin** absorbed through the damaged mucosa, or released locally.

Enteritis in salmonellosis is thus characterized by fibrinous or fibrinohemorrhagic exudates over denuded small and large intestinal mucosae, directly mediated by the apoptosis and necrosis induced by invading bacteria, and by the necrotizing effects of local neutrophil activity and microvascular thrombosis.

The **systemic outcome of an infection with *Salmonella*** is determined by the genetic virulence determinants of the invading organism and the ensuing innate, humoral, and cell-mediated immune response of the host. *Salmonella* are considered to be *facultative intracellular pathogens*, and invading strains must have the ability to survive and replicate within macrophages in order to cause bacteremia or septicemia. This capacity is conferred by components coded in SPI-2, perhaps largely through inhibition of NADPH oxidase-mediated oxidative killing of *Salmonella* in cytoplasmic vacuoles, and in some species by factors encoded in SPI-2 and SPI-3. The virulence of several serotypes commonly associated with systemic infections in animals, including *S.* Typhimurium, *S.* Dublin, and *S.* Choleraesuis, is enhanced by intracellular survival in macrophages mediated by attributes encoded on virulence plasmids.

Salmonella taken up by resident macrophages elicit a *major immune response* in the host. There is considerable controversy about the roles played by cell-mediated and humoral immunity in the pathogenesis of salmonellosis, but *Salmonella* infection results in the release of cytokines by specifically stimulated T lymphocytes. They activate macrophages that phagocytose the organisms, and in such a circumstance, cell-mediated immunity is of paramount importance.

Once *Salmonella* bacteria have crossed the mucosa, they may enter the bloodstream via the lymphatics, perhaps carried in macrophages, and cause septicemia or transient bacteremia. Or they may remain indefinitely in the gut-associated lymphoid tissues and mesenteric lymph nodes. Increased susceptibility to salmonellosis in animals with intercurrent disease, or subjected to stress, may be related to relaxation of cell-mediated immunity to the organism. Septicemia may be of variable duration and severity but, as a rule, it is rapidly fatal in young animals. If, however, there is transient bacteremia, the organisms are removed by fixed macrophages, especially those of the spleen, liver, and bone marrow. They may continue to proliferate in such extravascular locations and subsequently may cause another bacteremic phase that may result in fatal septicemia or secondary localization in other tissues.

The carrier state is important in the epidemiology of the disease. Whether *Salmonella* can maintain themselves in the intestinal lumen is not clear; to some extent, at least, fecal shedding is likely to depend on intermittent seeding from the bile, or from macrophages in the lamina propria and gut-associated lymphoid tissue. The duration of the carrier state may be prolonged, or animals may rid themselves of the infection, probably by means of cell-mediated immunity. The carrier state is an unstable one, for it appears that if the carrier is subjected to some stress or debilitating disease it may succumb to disease; this often seems to occur in adult cattle. The carrier animal is a potential threat to any other animal that it contacts, either directly, or through the medium of its excreta, or byproducts such as bone or meat meal.

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Salmonellosis in swine

Many serotypes of *Salmonella* have been isolated from swine, and with poultry and cattle they form an *important reservoir of the organism*. The bacteria are carried in the lamina propria of the intestine, but also in the regional lymph nodes of the alimentary tract, so that carrier animals may not excrete the organism in the feces.

Three syndromes are associated with *Salmonella* infections in swine. (1) *Septicemic salmonellosis* is usually associated with the host-adapted *S.* Choleraesuis var. *kunzendorf*, although enteric lesions may be present with this serovar. Sporadic infections with *S.* Dublin have also been associated with septicemia in nursing pigs. (2) *Salmonella* Typhimurium most commonly causes *acute or chronic enterocolitis*, including necrotizing proctitis which may lead to rectal stricture. (3) *Salmonella* Typhisuis infection is characterized by *ulcerative enterocolitis*, as well as caseous tonsillitis and lymphadenitis.

***Salmonella* Choleraesuis** was once thought to be the cause of classical swine fever (formerly hog cholera) because gross lesions of septicemic salmonellosis and acute classical swine fever are similar. The latter disease is often complicated by *S.* Choleraesuis, the bacterium being recovered from 10–50% of pigs with classical swine fever.

The major clinical manifestations of *S.* Choleraesuis infection are *septicemia and enteritis*; they usually occur separately. Septicemia is more common. Oral inoculation of *S.* Choleraesuis initially results in septicemia and acute enterocolitis, followed in some cases by large necrotic and ulcerative lesions ("*button ulcers*") in the colonic mucosa. Enteritis is not necessarily chronic, or even clinically evident. Interstitial pneumonia and multifocal hepatic necrosis are the most consistent systemic lesions. In Europe, infection has also been associated with the development of fulminant fibrinous pneumonia. Immunohistochemical techniques reveal the preferential location of *S.* Choleraesuis in the colon and surface of ileal M cells in Peyer's patches. The invasive capability of this serovar is indicated by the presence of large numbers of organisms in proprial macrophages and regional lymph nodes.

Salmonellosis that is clinically septicemic is usually fatal. Death may occur quickly without observed illness, or after a course of a week or more. There is a high fever; characteristic but not pathognomonic blue discoloration of the skin, especially of the tail, snout, and ears (Fig. 1.139); caudal weakness; dyspnea that often leads to misdiagnosis of primary pneumonia; and sometimes terminal convulsions. Sows may abort during the septicemic phase of infection. Pigs recovered from this phase may have dry gangrene of the ears and tail, caudal paralysis, blindness, and diphtheritic enteritis. The *chronic or enteric form* may develop from the acute, but is usually insidious from the onset. It is characterized by loose yellow feces containing flakes of fibrin, progressive emaciation and debility, and eventual death. Some recover but fail to thrive, often partly owing to chronic bronchopneumonia.

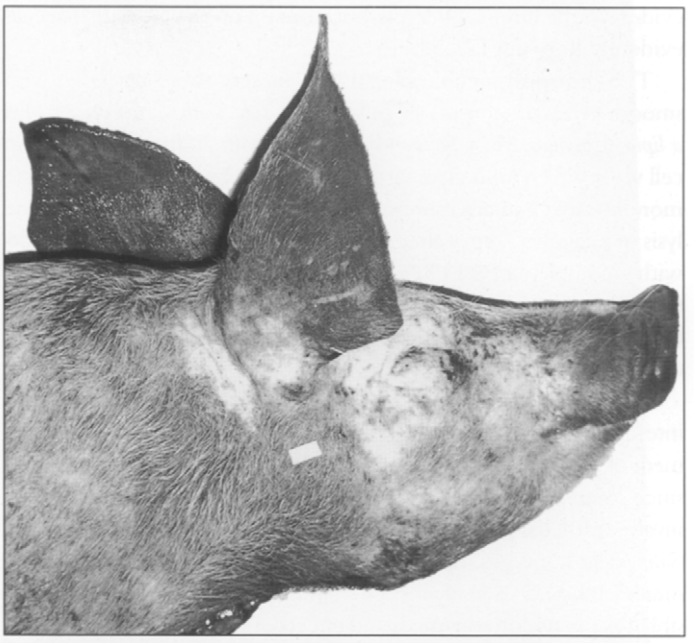

Figure 1.139 Note blue-red congestion of skin of ears, snout, and neck due to microvascular thrombosis caused by endotoxemia in **septicemic salmonellosis** in a pig.

At **autopsy**, there is blue or purple discoloration of the *skin*, which may be very intense about the head and ears. There may be superficial ischemic necrosis of the ears. Typically there are petechial hemorrhages in many organs and tissues. The lymph nodes are almost invariably hemorrhagic. The visceral nodes are more frequently and obviously involved than the peripheral ones, with the exception of those of the throat, which are usually hemorrhagic. The mesenteric lymph nodes are greatly enlarged, and they may be speckled with hemorrhages.

There may be hemorrhages, petechial or as small discrete blebs, on the *laryngeal mucosa* (Fig. 1.140). The *lungs* do not collapse because there is frothy fluid in the respiratory passages. They may be pale blue or purple. Beneath the visceral pleura there are small dark foci of hemorrhage. The lungs are wet and there is fluid in the interlobular tissue. The changes are best appreciated in the caudal lobes, because the cranial lobes are often the seat of acute lobular pneumonia. These pulmonary changes, attributable in part to endotoxin, account for the respiratory signs observed clinically. The *pneumonia* is interstitial due to endotoxemia and embolic organisms. The lobar cranioventral pneumonia may be due to ascending *Salmonella* alveolitis and bronchiolitis. Occasionally, the injury to the alveolar septa by *Salmonella* results

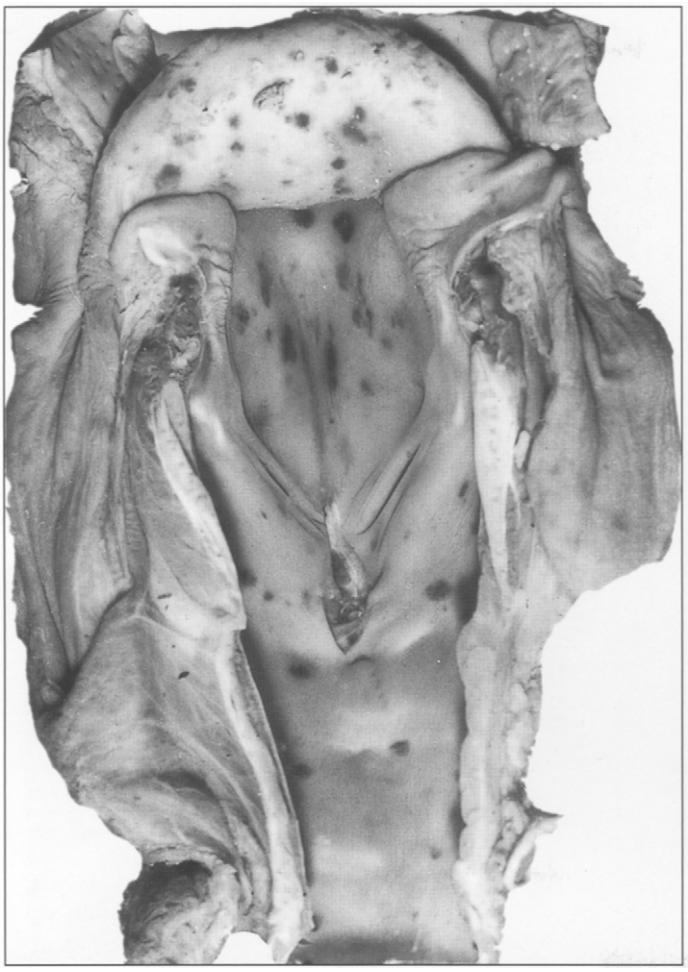

Figure 1.140 Laryngeal hemorrhages in **porcine salmonellosis**.

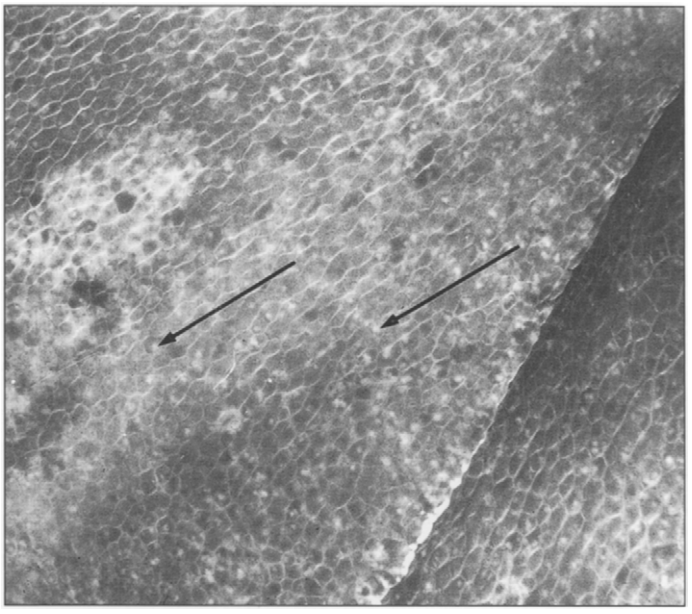

Figure 1.141 Paratyphoid nodules (arrows) in liver, in *Salmonella* septicemia in a pig.

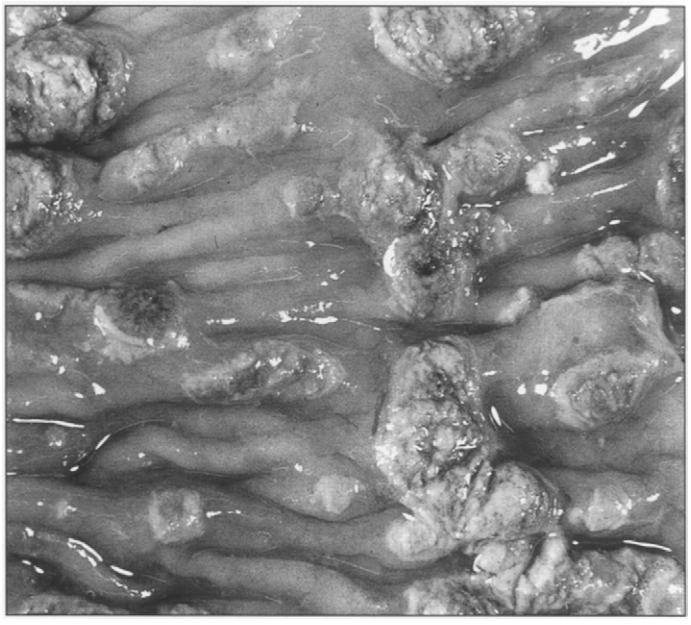

Figure 1.142 Button ulcers in colon in porcine salmonellosis.

in extensive fibrinous pneumonia of the caudal lobes. The cardiac serosae often bear petechiae, and in some more virulent infections there is fibrinohemorrhagic pericarditis with scant fluid exudation.

The *spleen is enlarged*, deep blue, firm with sharp edges; little blood oozes from the cut surface. There may be petechiae on the capsule, but the marginal infarcts of classical swine fever are not present. Other causes of splenomegaly, such as erysipelas, other septicemias, and African swine fever, must be differentiated.

The *liver* is usually congested, and focal hemorrhages may be visible in the capsule. In some cases the hemorrhages are very large, involving up to half of the central area in a lobule. They may be scattered at random throughout the liver or grouped, often at the edge of a lobe. In some, there are tiny yellow foci of necrosis, referred to as "*paratyphoid nodules*" (Fig. 1.141). Pinpoint hemorrhages are consistently present in the renal cortex. There may be only a few in each kidney or they may be so numerous as to cause the "turkey egg" appearance.

The *stomach* shows the intense red-black color of the severe congestion and venous infarction common to endotoxemia in pigs. If the animal survives a week or more, the superficial necrotic layer of the affected gastric mucosa sloughs. There may be no lesions in the *intestine*. There may be catarrhal enteritis or, more frequently, the enteritis is hemorrhagic, increasing in severity lower in the tract and terminating in a hemorrhagic ileitis. The mucosae of the colon and cecum may be normal but, if the course is prolonged, there is hyperemia, fibrinohemorrhagic inflammation, or button ulcers (Fig. 1.142).

Petechial hemorrhages may occur in the *meninges and brain*, but there is no gross inflammation. Localization sometimes occurs in *synovial membranes*, producing polysynovitis and sometimes **polyarthritis**. It is more usual to have an increase in the volume of fluid with red velvety hypertrophy of the synovial villi. The gross features described are usually not all present in any one case.

The **histologic changes** that occur in internal organs in acute disease are mainly associated with *endothelial damage due to endotoxin*, and *focal localization of bacteria*. The discoloration of the skin is initially due to intense dilation, congestion, and thrombosis of capillaries

and venules in the dermal papillae. There is activation and necrosis of the endothelial cells in affected vessels. The renal lesions vary but principally affect the glomeruli. In some there is diffuse glomerulitis, and this is associated with mild nephrosis and hyaline casts. In others, the glomerulitis is exudative and hemorrhagic and in these a great many capillary loops contain hyaline thrombi. Embolic bacterial colonies are occasionally seen in the glomerular and intertubular capillaries. Fibrin thrombi may also be found in the afferent arterioles and interlobular arteries.

The pulmonary lesions are also characterized by thrombosis and vasculitis and a largely mononuclear cellular response in alveolar septa. There is flooding of the alveoli by edema fluid and moderate numbers of alveolar macrophages. This is the usual histologic picture; the extremes are acute fibrinous inflammation or a few scattered parenchymal hemorrhages.

In the liver, the *paratyphoid nodules* may be found in all transitional stages from foci of nonspecific necrosis to reactive granulomas. Typically there are few neutrophils, and whether the nodules are necrotic or reactive depends on their duration. The initial change is focal coagulative necrosis. About the margin, the macrophages accumulate and form small histiocytic granulomas which expand and displace the surrounding parenchymal cords.

In the spleen there are some scattered hemorrhages, but the overall histologic impression is of increased histiocytes with a scattering of neutrophils. The follicles are small and rather inactive. Very small foci of necrosis, containing many bacteria, may be sparse or relatively numerous, and these develop a reactive macrophage response and form the typical paratyphoid nodules.

Meningoencephalomyelitis occurs in a proportion of cases of septicemic salmonellosis. The lesion is fundamentally a vasculitis. There may be petechiae in the meninges but, microscopically, there is infiltration of large mononuclear cells in the pia-arachnoid and concentrated about the veins. The organism is relatively fastidious and cultures from postmortem samples may not be uniformly positive.

***Salmonella* Typhimurium** infection in swine produces a syndrome that differs from *S.* Choleraesuis in a number of ways. Clinically, the disease occurs in *feeder pigs* and is characterized by fever, inanition, and yellow watery diarrhea that may contain blood and mucus, especially in the later stages. The diarrhea may be chronic and intermittent. There is high morbidity but low mortality. Most pigs recover but may remain *carriers* for variable periods of time, and some may develop rectal stricture. The organism persists in tonsils, lower intestinal tract, submandibular, and ileocolic lymph nodes.

The pathogenesis and morphology of the enteric lesions differ from those described for *S.* Choleraesuis enteritis. The lesions with *S.* Typhimurium infection are mainly confined to the colon, cecum, and rectum, with minor involvement of the distal small intestine. There is acute enterocolitis with formation of a pseudodiphtheritic membrane on the mucosal surface (Fig. 1.143). Button ulcers are not associated with this or other nonhost-adapted serovars. Systemic dissemination and septicemia are rare.

Rectal stricture is thought to be a *sequel in most cases to ulcerative proctitis of ischemic origin,* caused by *S.* Typhimurium. It is characterized clinically by marked progressive distension of the abdomen, loss of appetite, emaciation, and soft feces. At autopsy, there is marked dilation of the colon, which is caused by narrowing of the rectum, 1–10 cm cranial to the anus (Fig. 1.144). The stricture is usually <1.0 cm in diameter and varies in length from 0.5 to 20 cm. There is marked fibrous thickening of the rectal wall, which may contain microabscesses. The dilation of the colon, proximal to the stricture, may consist of a well-demarcated widened area several centimeters long and wide. The colonic mucosa in this area is usually

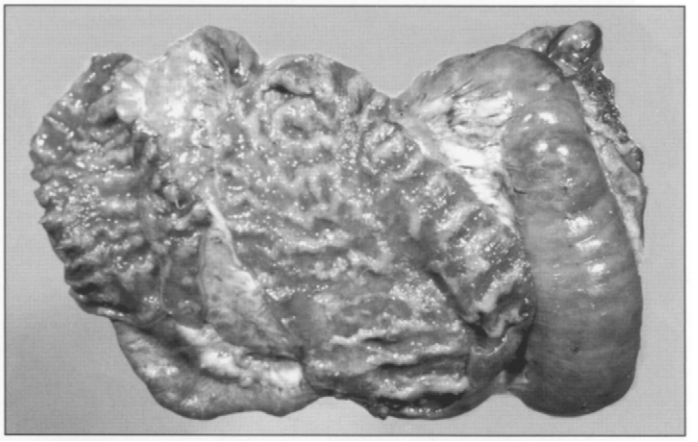

Figure 1.143 Fibrinous colitis in a pig infected with *Salmonella* Typhimurium.

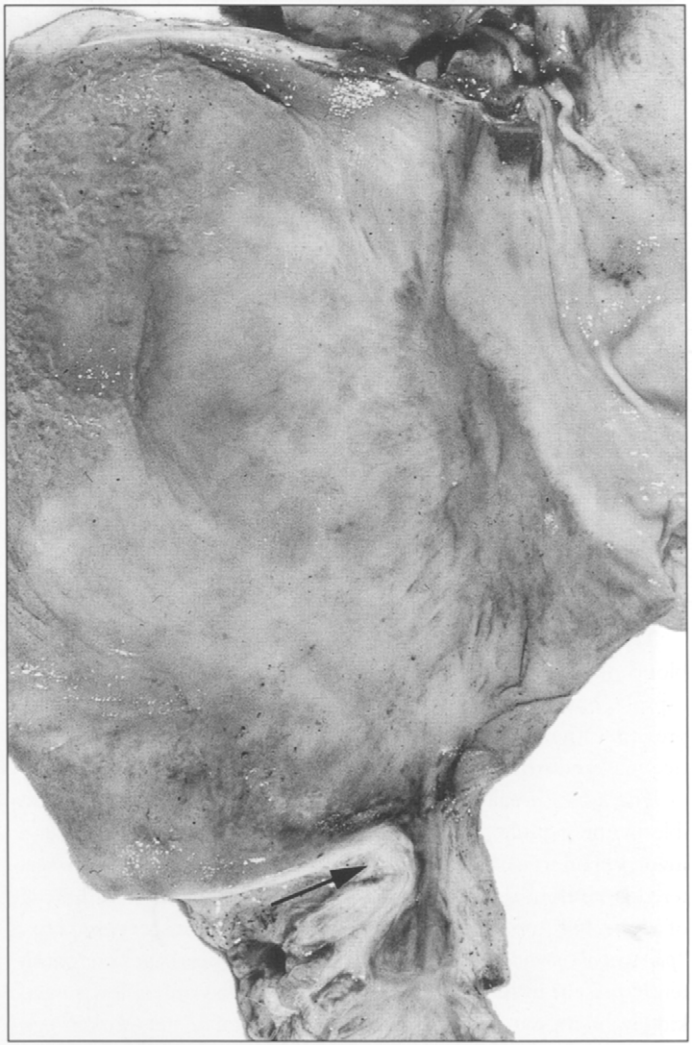

Figure 1.144 Rectal stricture in porcine salmonellosis. Opened colon is massively dilated proximal to stricture in rectum (arrow).

ulcerated and may be covered by fibrinous exudate. In some cases, there is more gradual but sometimes massive dilation of the entire colon and cecum, which are full of digesta, with ulceration of the mucosa just proximal to the stricture. The mucosa is always excessively corrugated; this is mainly the result of marked thickening of the internal muscularis. Anastomoses of the small intestine and/or colon to the dilated portion of the descending colon may occur. Localized chronic peritonitis is often associated with the dilated segments of the colon.

The stricture is located in an area of rectum that has a relatively poor blood supply, namely the junction of the circulatory fields of the caudal mesenteric and pudendal arteries. Ulcerative proctitis is consistently found in swine with typhlocolitis due to *S.*Typhimurium infection. Granulation of such lesions probably leads to cicatrization and stricture. The location, the persistent nature of this lesion in some pigs, and its limited capacity to heal are probably related to the restricted blood supply of the affected area.

***Salmonella* Typhisuis** infection is an *uncommon* condition in pigs. The disease, called *paratyphoid* in Europe, is now known to cause disease in pigs in the Americas and Asia. It is a progressive disease of 2–4-month-old pigs that is clinically characterized by intermittent diarrhea, emaciation, and frequently, massive enlargement of the neck region, the latter associated with caseous palatine tonsillitis, cervical lymphadenitis, and parotid sialoadenitis. There is also circular or button-like, to confluent, ulceration of the mucosa of the ileum, cecum, colon, and rectum. Other less frequent findings are caseous lymphadenitis of the mesenteric lymph nodes, interstitial pneumonia, hepatitis, and pericarditis.

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Salmonellosis in horses

The most common serovar in horses in most areas is ***S.* Typhimurium**, and its prevalence is increasing, especially that of multidrug-resistant definitive phage type 104 (DT104). Other serovars are usually associated with sporadic cases of disease. *Many horses are Salmonella carriers, and when they are stressed, diarrhea follows.* Abortion of pregnant mares has been associated with *S.* Abortusequi. *Salmonella* Infantum has been associated with disseminated infection localizing in a variety of tissues, including muscle, in a horse.

Treatment with antibiotics, especially orally, increases the risk of salmonellosis. Resistance to certain antibiotics is associated with the presence of *resistance* (*R*) *plasmids* that may be transferred to other bacteria, of the same or different species, by conjugation or transduction. Antibiotic-resistant *Salmonella* may not respond to treatment, and antimicrobial therapy may increase the potential for infection and disease, due to the suppression of the normal flora. Antibiotic-resistant strains have been associated with outbreaks of salmonellosis at veterinary teaching hospitals.

Salmonellosis in horses may be manifested clinically as *peracute* (usually septicemic), *acute*, and *chronic* forms, and as an *asymptomatic carrier state*.

The septicemic form occurs most commonly in foals 1–6 months of age. These animals are usually with their dams at pasture and predisposing factors are unclear. The infection in foals tends to be fatal. Affected animals are lethargic and develop severe diarrhea, often with characteristic green color, which may contain casts and blood. They are febrile and waste rapidly, to die in 2–3 days. Some survive for a week or more and these may develop signs of pneumonia, osteitis, polyarthritis, and meningoencephalitis.

The primarily enteric forms of the disease are more likely to occur in older horses. Most of the predisposing factors mentioned earlier apply to horses. *Salmonellosis is an occupational hazard of horses*, since most are exposed to long periods of transport, and to exertion due to overwork or excessive training.

Clinically, the acute disease is characterized by diarrhea and fever for a period of 1–2 weeks, followed by recovery or death. The chronic form persists for weeks or months. Affected horses pass soft, unformed manure that resembles cow feces. They lose their appetite, with subsequent progressive loss of weight and condition. In later stages, they become dehydrated and emaciated.

The gross lesions are those of enteritis and/or septicemia; the former are most consistently found at autopsy. As a rule, the longer the course, the lower in the intestine does one find the most severe lesions.

Acute septicemic cases show small hemorrhages on the serous or mucosal membranes. The visceral lymph nodes are always enlarged, juicy, and often hemorrhagic. Marked pulmonary congestion and edema, and renal cortical pallor and medullary congestion may occur. The main lesions are in the stomach and intestines. In **peracute or septicemic cases**, there is intense hyperemia of the gastric mucosa, probably venous infarction, with some edema and

scattered hemorrhage. The small intestine may be congested with a mucous or hemorrhagic exudate. In **acute cases**, there is diffuse and intense fibrinohemorrhagic inflammation of the cecum and colon overshadowing any lesions in the upper intestine, and leading rapidly to superficial necrosis of the mucosa and a gray-red pseudomembrane (Fig. 1.145A). In **chronic salmonellosis**, enteric lesions may be few or subtle. Some animals have extensive or patchy fibrinous or ulcerative lesions of the cecum and colon. In others, raised circumscribed lesions about 2–3 cm in diameter may be evident, with a gelatinous submucosa and ulcerated mucosa. Some such lesions are more fibrinous, and resemble button ulcers (Fig. 1.145B).

Histological alterations of significance are usually limited to the intestine. However, in septicemic animals lesions typical of endotoxemia are present in lung, liver, kidney, spleen, and adrenal. There may be acute ileocecocolic lymphadenitis, and inflammation in sites of localization, such as growth plates in long bones, and the meninges. Depending on the duration of the enteritis, hemorrhage, necrosis, or diphtheresis may predominate, but the infiltrating leukocytes are largely mononuclear. The superficial coagulative necrosis of the mucosa may extend over large areas. A layer of fibrinocellular exudate may cover the necrotic mucosa. Fibrin thrombi are frequently present in the capillaries or venules of the lamina propria (Fig. 1.145C). There is usually marked congestion of submucosal vessels, which is accompanied by considerable edema.

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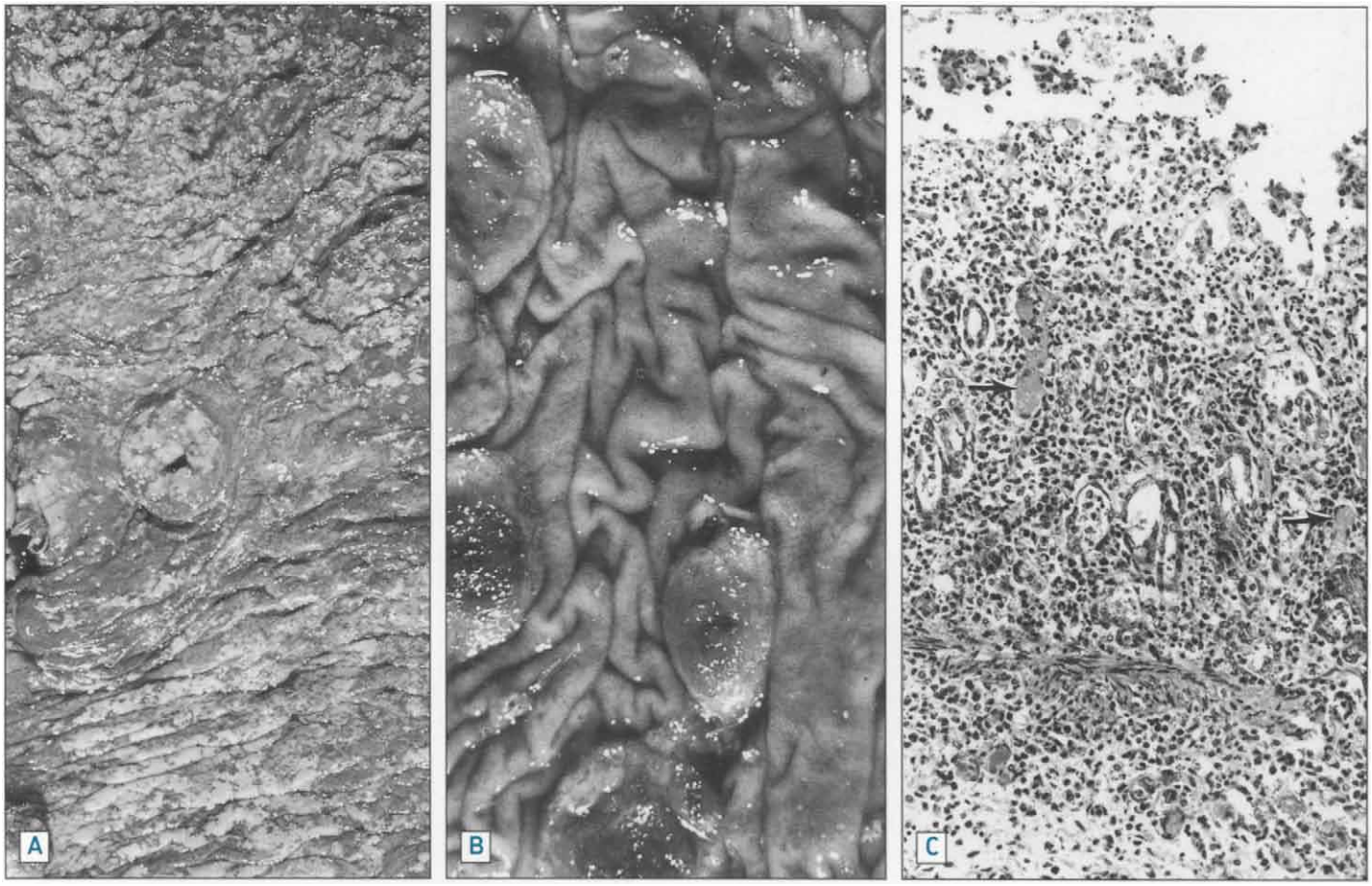

Figure 1.145 Salmonellosis in a foal, *Salmonella* Typhimurium. **A.** Focal and coalescent ulceration and diphtheresis involving ileocecal valve and mucosa. **B.** Nodular ulcerative lesions in colon in chronic salmonellosis. **C.** Superficial necrosis and effusion from colonic mucosa; several thrombosed vessels are in the propria (arrows).

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Salmonellosis in cattle

The serotypes usually incriminated are ***S.* Typhimurium** and ***S.* Dublin**; both are distributed worldwide. Wherever *S.* Dublin is found, it tends to be adapted to cattle and to occur in epizootics, whereas other serotypes usually cause more sporadic disease.

It is unusual to find salmonellosis in **calves** less than a week of age, in contrast to enteric and septicemic colibacillosis, which usually affect very young animals. *In calves, salmonellosis is a febrile disease typified by dejection, dehydration, and usually diarrhea.* Diarrhea is not always present, but when it is, the feces are yellow or gray, and have a very unpleasant odor. In older calves, there may be blood and mucus in the feces. In less acute cases, there may be delayed evidence of localization in the lung and synovial structures. Morbidity and mortality may be considerable, especially in calves that are confined, such as in vealer operations. Experimental infections in calves indicate that survival is inversely related to the numbers of *Salmonella* in the inoculum, and directly to the age of the calves.

The general appearance at **autopsy** of a calf with salmonellosis may resemble one with septicemic colibacillosis. However, *enlargement of mesenteric lymph nodes and gross enteric lesions* are generally observed in salmonellosis. There is moderately severe gastrointestinal inflammation, acute swelling, and hemorrhage of the visceral lymph nodes, and some petechiation of serous membranes. The enteritis may be catarrhal, but sometimes it is hemorrhagic or more commonly causes exudation of yellow fibrin (Figs 1.146 and 1.147). The mucosa overlying the lymphoid tissues may become necrotic and slough. In animals with fibrinous enteritis, the bowel wall is somewhat turgid and the serosa may have a ground-glass appearance. There is often diffuse, but perhaps mild, fibrinous peritonitis.

The intestinal lesions are usually most severe in the ileum, especially during the early stages of the disease. With time the jejunum and colon become involved but the duodenum remains relatively normal. The regional distribution of the lesions may, in part, be related to differences in the level of bacterial colonization of the mucosa. Twelve hours after oral infection of calves with *S.* Typhimurium, the numbers of bacteria are generally lower in the abomasum and duodenum than in the lower intestinal tract, while they are relatively constant from the jejunum through to the rectum.

The early **microscopic lesions** in the small intestine consist of a thin layer of fibrinocellular exudate on the surface of short and blunt villi (Fig. 1.148). This is followed by extensive necrosis and ulceration of the mucosa, with fibrin and neutrophils exuding from the ulcerated areas into the lumen (Figs 1.149 and 1.150). The lamina propria may be moderately infiltrated by mononuclear inflammatory cells. Fibrin thrombi are often evident in proprial capillaries and venules. There is also marked submucosal edema and the centers of lymphoid follicles in the Peyer's patches are completely involuted. Similar erosion, ulceration, and fibrinous effusion occur in the proximal large bowel.

Scanning electron microscopy of small intestine shows large numbers of bacteria on a tattered mucosal surface. Clusters of enterocytes slough off short villi (Fig. 1.151). Strands of fibrin emerge from the mucosal defects and cover the mucosa. Ultrastructurally, the lesions are similar to those described originally in guinea pigs, except that there is more damage to epithelium in calves experimentally infected with *S.* Typhimurium.

Characteristic changes usually occur in the *liver and spleen*, but may be absent in peracute septicemic cases. There is often fibrinous cholecystitis. In acute cases, the spleen is enlarged and pulpy as a result of congestion, but this is soon replaced by acute splenitis, present as miliary, tiny foci of necrosis or as reactive nodules. The liver is often pale with many minute *paratyphoid nodules*. In the spleen, macrophage reaction is sometimes diffuse. "Paratyphoid" granulomas may also be found microscopically in the kidney, lymph nodes, and bone marrow. These probably represent a cell-mediated immune response to embolic bacteria. In those animals that survive the acute phase of the disease, the inflammatory changes in lymphoid tissues progress

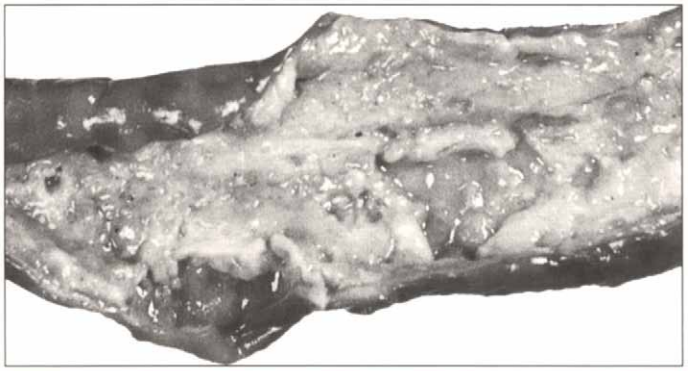

Figure 1.146 Diphtheritic enteritis caused by *Salmonella* Typhimurium infection in a calf.

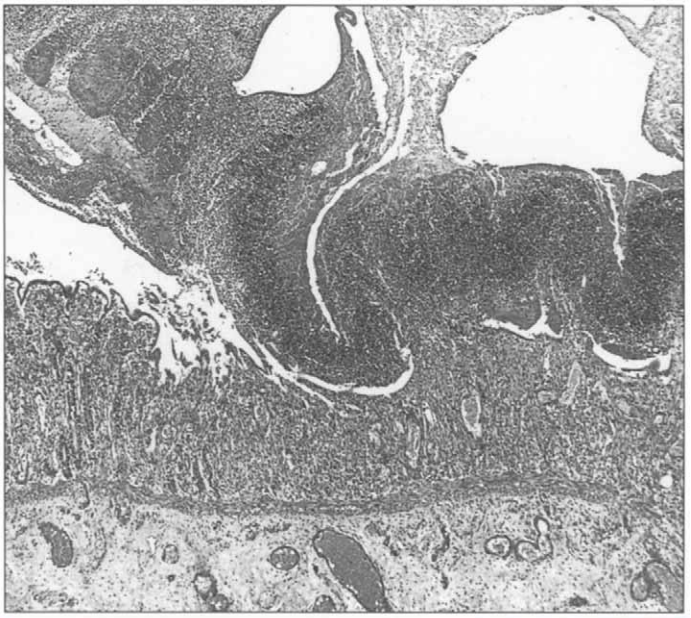

Figure 1.147 Diphtheritic membrane on the surface of the ileum in bovine salmonellosis. Exudate arises from eroded mucosa in which crypts of Lieberkühn are sparse or absent.

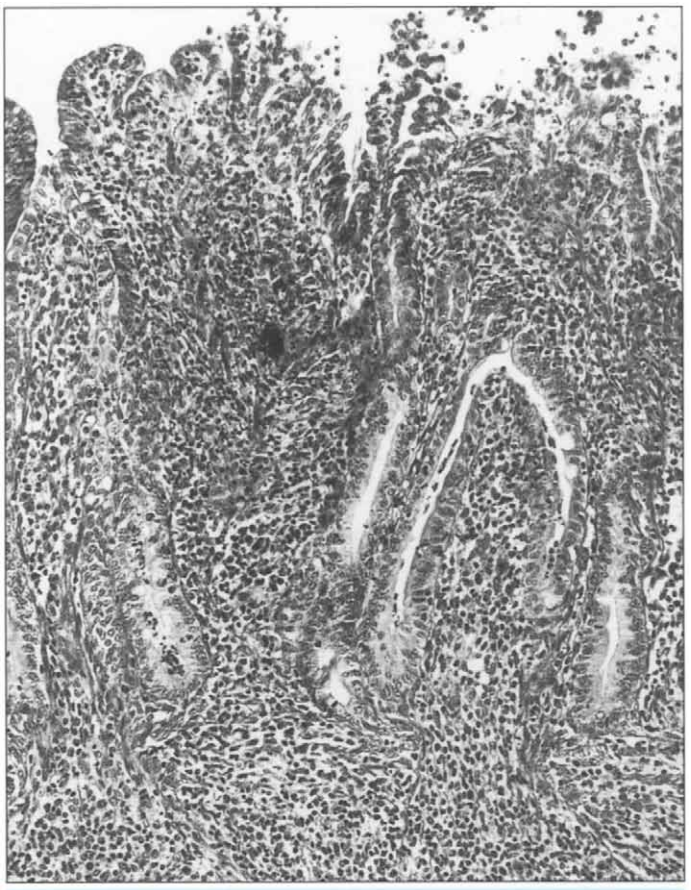

Figure 1.148 Atrophy of villi, exfoliation of surface epithelium, and effusion of neutrophils in ileum of a calf, 12 hours after inoculation with **S. Typhimurium**. (Courtesy of R Clarke, CL Gyles.)

Figure 1.149 Atrophy of villi, erosion, and effusion of neutrophils and fibrin into the lumen of the small intestine of a calf. Some thrombosis of proprial vessels 36 hours after inoculation with **S. Typhimurium**. (Courtesy of R Clarke, CL Gyles.)

to an immunologic response, characterized by a diffuse reaction of medium-sized and large lymphocytes in the follicles, and plasma cells in the sinusoids. There may be marked cortical atrophy of the thymus. In calves with acute septicemia, pulmonary congestion and edema are visible at necropsy, with interstitial thickening of pulmonary alveolar septa by mononuclear cells in tissue section. There may be thrombosis of septal capillaries, and some effusion of edema fluid and macrophages into alveolar spaces.

In subacute salmonellosis of calves, there may be cranial bronchopneumonia, usually with adhesions and abscessation. Purulent exudate is in synovial cavities, and the organism is recoverable in pure culture from such affected joints and tendon sheaths. It may be mixed with *Arcanobacterium pyogenes* and *Pasteurella* in the lungs.

Salmonellosis in **adult cattle** may occur in *outbreaks* as it does in calves, but more often it is sporadic, and it may cause *chronic diarrhea and loss of condition*. The source of infection is usually the carrier animal. Other sources, such as feed containing protein of animal origin, or bone meal, should be considered when the disease is caused by an uncommon serovar. *Abortions* are most common with *S.* Dublin, but may occur with any serovar. In some herds, this may be the only clinical evidence of infection, although other animals often excrete the offending serovar in the feces. The *carrier state* of *S.* Dublin infection in adult cattle may persist for years, sometimes for life, in contrast to infections with other serovars, which rarely persist for more than 18 months. Dairy cows may persistently shed *Salmonella*, especially *S.* Dublin, in milk and cause infections in humans who drink raw milk. The morbid changes in adult cattle correspond to those in calves except that there is more pleural hemorrhage and the enteritis may be more hemorrhagic and fibrinous. The histologic changes in the liver and other organs are the same as those seen in calves.

Of serious concern for both cattle and humans is the emergence of a new multidrug-resistant strain of *S.* Typhimurium. This new strain, known as *Salmonella* Typhimurium definitive phage type 104 (DT104), was first seen in the UK in the late 1980s. It has now spread worldwide and in some parts of the USA is the leading cause of bovine salmonellosis. The potential for this strain to emerge as a major foodborne pathogen has caused great consternation among both agricultural and public health communities.

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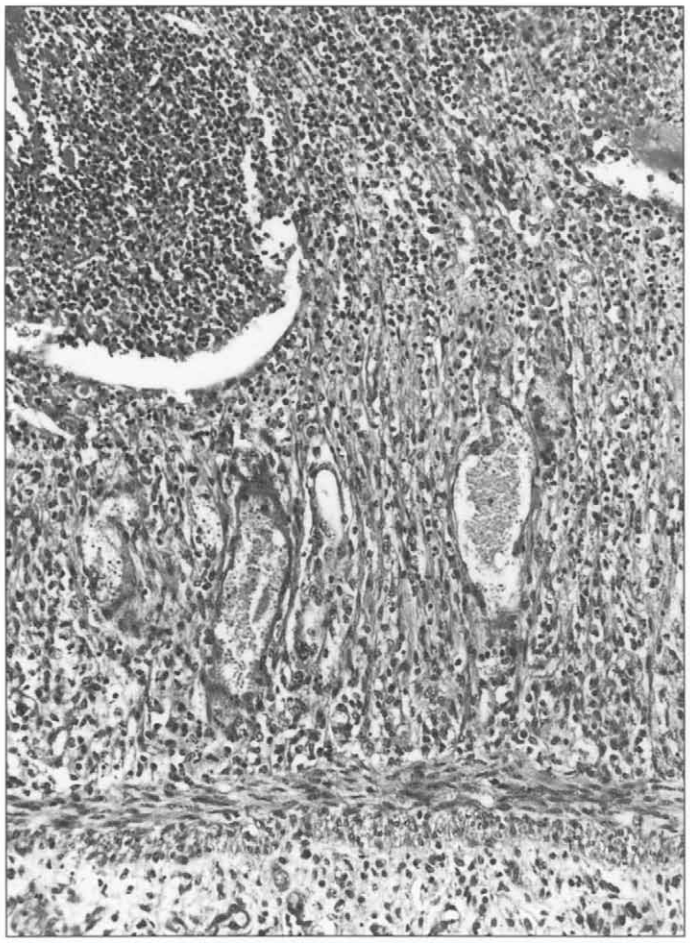

Figure 1.150 Eroded ileal mucosa, largely devoid of crypts of Lieberkühn, fibrin, and neutrophils in lumen, forming diphtheritic membrane, in a calf with **salmonellosis**.

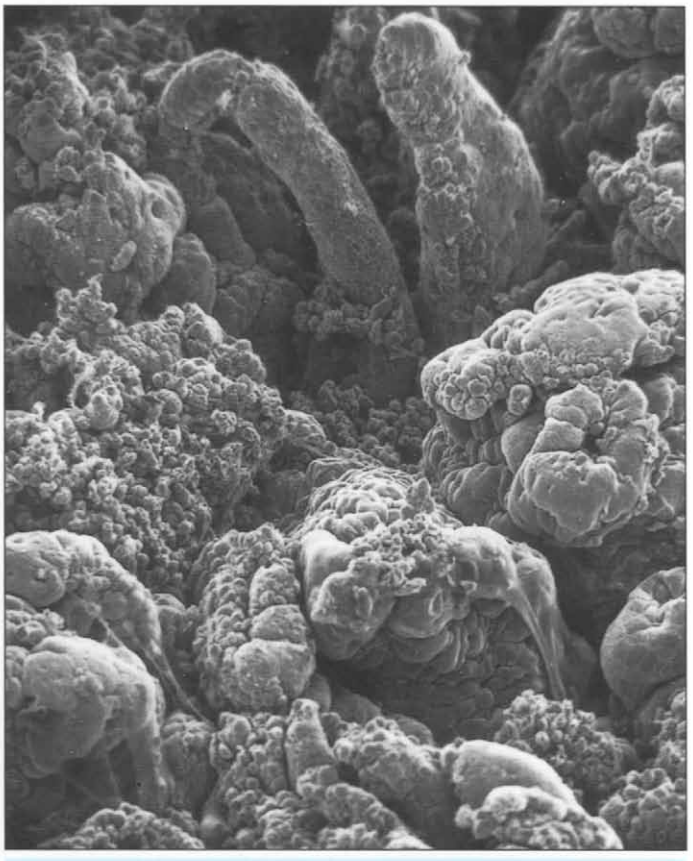

Figure 1.151 Scanning electron micrograph of the ileum of a calf 12 hours postinoculation with **S. Typhimurium**. Villi are atrophic, and rounded cells are exfoliated from surface. (Courtesy of R Clarke, CL Gyles.)

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Salmonellosis in sheep

As well as abortion caused by *S.* Abortus-ovis, abortion and neonatal death may follow infection of pregnant ewes by any species of *Salmonella*. While the prevalence of *S.* Abortus-ovis in the UK seems to be waning, *S.* Montevideo, on the other hand, has been associated with abortions in several flocks in the British Isles.

Salmonellosis is not a common disease in sheep, but outbreaks are always severe and may cause very heavy losses. Predisposing influences are necessary, and these are usually provided by circumstances that enforce congregation. Deprivation of food and water for 2–3 days may be sufficient and, coupled with fatigue, is the usual predisposing factor when sheep are transported or confined in holding yards. Deaths usually continue for 7–10 days after debilitating circumstances have been remedied.

The serovars usually found in sheep are ***S.* Typhimurium**, ***S.* Arizonae**, and ***S.* Enteritidis**. *Salmonella* Dublin is increasing in prevalence in the UK and the midwestern states of the USA. Experimental inoculation of sheep with *S.* Arizonae produces infection but rarely disease. Under natural conditions this host-adapted organism is frequently considered to be an infection secondary to

some other disease, or an incidental finding in apparently healthy animals. Most serovars produce the same sort of disease, which closely resembles that seen in cattle both clinically and at autopsy. The major findings are fibrinohemorrhagic enteritis, and septicemia.

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Salmonellosis in carnivores

Salmonella can often be recovered from apparently healthy dogs and cats. However, primary disease rarely occurs. *Salmonella* has been recovered in high frequency from normal sled dogs lacking clinical disease. Raw meat fed to dogs has been shown to have a high incidence of *Salmonella* contamination. In dogs and cats, nosocomial infections are sometimes associated with hospitalization and antibiotic therapy. In dogs, salmonellosis may be secondary to canine distemper. It can cause bronchopneumonia, acute hemorrhagic gastroenteritis, swelling of the spleen and mesenteric lymph nodes, serosal hemorrhages, and foci of necrosis in the liver and other organs. Septicemia in puppies has been associated with *S.* Dublin infection. Salmonellosis has been reported in dogs with lymphosarcoma, shortly after the initiation of chemotherapy. The immunosuppressive effect of the treatment, or depressed cell-mediated immunity, probably predisposes to the development of disease.

Various serovars have been isolated from cats and most of these appear to cause subclinical infections. However, salmonellosis may be a problem in catteries and hospitals, affecting animals that are subjected to stressful conditions. Immunosuppression associated with *Feline leukemia virus, Feline immunodeficiency virus, Salmonella*-contaminated panleukopenia vaccine, or other intercurrent diseases is thought to predispose to salmonellosis in cats. *S.* Typhimurium is most commonly associated with such outbreaks. The disease is characterized by gastroenteritis and septicemia or a more chronic, nonspecific febrile illness, with neutrophilia and left shift. Conjunctivitis and abortions have also been associated with *Salmonella* infection in cats.

Because of their close association with humans, especially children and the aged, dogs and cats that are carriers are a potential source of zoonotic infection.

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Yersiniosis

Yersinia enterocolitica and *Y. pseudotuberculosis* are gram-negative organisms that, in domestic animals, cause *enterocolitis, mesenteric lymphadenitis*, and, less commonly, *septicemia*. There are sporadic reports of placentitis with abortion or perinatal mortality, epididymitis-orchitis, and mastitis in sheep and goats, and of abortion and pneumonia in cattle. *Y. pestis*, the cause of plague in animals and humans, is not considered here.

The *epidemiology* of yersiniosis is complex and poorly understood. These organisms may be shed in the feces by asymptomatic animals in the herd or flock, and by other species, such as rodents and birds, in the environment. The organisms can survive and grow in the environment at low temperatures, and in cool weather environmental contamination by *Yersinia* spp. may be considerable, resulting in significant oral challenge. Disease may in part be due to compromise of cell-mediated immunity, permitting establishment of invading organisms, or recrudescence of latent infection. Often, outbreaks occur under stressful circumstances, such as poor weather, flooding, after transport, during the breeding season, or in animals on a poor plane of nutrition.

Subsequent to ingestion, *the organisms invade through the intestinal epithelium or M cells* overlying Peyer's patches and attain the lamina propria or submucosal lymphoid follicles. Enormous recruitment of neutrophils and ensuing destruction of cytoarchitecture of the Peyer's patch and overlying epithelium result in formation of suppurative foci in place of follicles in Peyer's patches, and microabscesses in the lamina propria of the small or large intestine if invasion occurs elsewhere. *Yersinia* disseminates via lymphatics and hepatic portal venous drainage to mesenteric lymph nodes or to liver and the systemic circulation.

A number of *virulence factors* are recognized; all are related to the ability to invade cells or to evade the inflammatory response. These include expression of an adhesin termed invasin, which binds to beta-1 integrins expressed on the surface of intestinal M cells, promoting uptake of *Yersinia* by these and normally nonphagocytic cells, and causing epithelial cells to produce cytokines chemotactic for inflammatory phagocytes. A plasmid-encoded type III secretion system translocates effector proteins termed "yops" from bacteria into host cells. These enable invasive *Yersinia* to remain extracellular, evading phagocytosis and killing by neutrophils and macrophages by a variety of mechanisms. Although *Yersinia* reside extracellularly as microcolonies in suppurative foci in the lamina propria and lymph nodes, at least some appear to reside intracellularly, since a T-cell-mediated immune response is required to clear infection. Giant cells wall off foci of infection in subacute to chronic lesions, hence the specific name *pseudotuberculosis*.

Disease may be gradual in onset, subtle and chronic, producing a syndrome of diarrhea and ill-thrift, in cattle, sheep, and goats. Mild diarrhea with low mortality has been reported in Australia in weaned pigs with *Y. pseudotuberculosis*. More fulminant disease, characterized by severe, sometimes hemorrhagic, diarrhea, systemic infection, and prostration, may occur in cattle, some species of deer, especially chital and red deer, water buffalo, and exotic ungulates. Yersiniosis is an apparently uncommon cause of diarrhea, and occasionally fatal enterocolitis, mesenteric lymphadenitis, and systemic infection in carnivores. *Yersinia* also causes sporadic pneumonia and septicemia in foals.

Yersiniosis has been described worldwide, as a cause of disease in sheep, cattle, goats, deer, and pigs. The lesions of *Y. pseudotuberculosis* and *Y. enterocolitica* cannot be differentiated reliably grossly or microscopically.

In all species, **gross lesions** in clinically subacute to chronic yersiniosis may be mild. They are usually limited to abnormally fluid intestinal content, with congestion, edema, roughening, and perhaps small foci of pallor, focal hemorrhages, erosion, or mild ulceration and fibrin effusion. Raised nodules up to 5 mm in diameter, with depressed centers, may be evident in affected large bowel. Mesenteric lymph nodes are enlarged, congested, and edematous, perhaps with foci of necrosis. There may be mild fibrinous cholecystitis, and pale foci of necrosis scattered in the liver.

The infection is characterized **histologically** by *masses of gram-negative coccobacilli forming microcolonies*, in the lamina propria of villi and around the necks of crypts in the distal half of the small intestine, in Peyer's patches, and in the superficial mucosa of the large intestine. Intense local infiltrates of inflammatory cells, predominantly neutrophils, accumulate to form *microabscesses* up to about 300 μm in diameter around the bacteria, and effuse into the lumen through microerosions on the mucosal surface or in crypts. Small crypt abscesses may be present. In small intestine, there may be moderate atrophy of villi and hyperplasia of crypts, associated with increased infiltrates of chronic inflammatory cells. Microabscesses, or pyogranulomas surrounded by macrophages or giant cells, and sometimes containing bacterial microcolonies, may be present in the subcapsular and medullary sinuses of mesenteric lymph nodes.

In fulminant *Yersinia* infection in all species, there is *fibrinous or fibrinohemorrhagic enterocolitis*, with heavy local mucosal colonization by masses of coccobacilli, and marked neutrophil infiltration. Peyer's patches may be particularly involved, with grossly visible foci or confluent masses of caseous necrotic debris, as may be found in draining mesenteric lymph nodes, which are enlarged. There may be serosal hemorrhages on the gut, fibrinous peritonitis, and pleuritis, and foci of necrosis may also be present in the liver, lungs, and occasionally other parenchymatous organs; the characteristic microcolonies of coccobacilli are usually evident in them.

Caseous mesenteric lymphadenitis, with mature pyogranulomas containing microcolonies of bacteria, surrounded by neutrophils and giant cells, may occasionally be found as an incidental lesion, or in animals with *Yersinia* abscesses in other organs.

Yersiniosis is **diagnosed** in tissue section by finding characteristic microcolonies of coccobacilli in microabscesses (Fig. 1.152), and is confirmed by bacterial isolation. Microscopic lesions may not be detected in the intestinal mucosa of some clinically affected animals from which isolates are made, perhaps because lesions are patchy. Since *Yersinia* spp. are psychrophiles, cold enrichment and culture at temperatures below 37°C are used in their isolation.

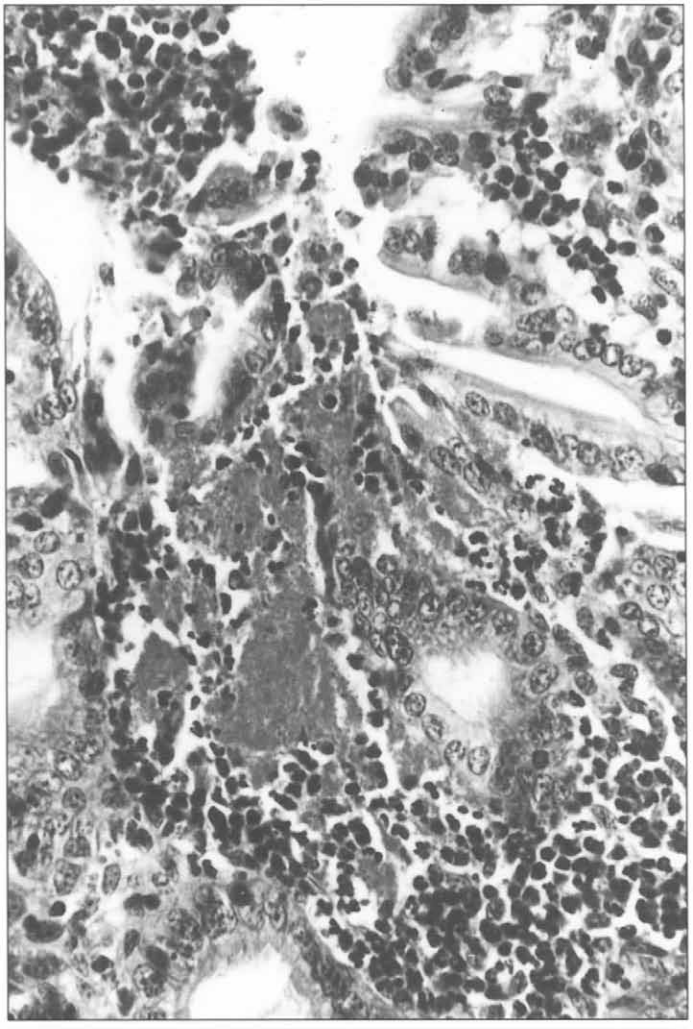

Figure 1.152 Microabscess around colony of ***Yersinia*** in lamina propria of the colon in a sheep. Note effusion of neutrophils through mucosal microerosion into lumen. (Courtesy of R Mason, Tasmanian Dept Primary Industry.)

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Lawsonia intracellularis infection

Lawsonia intracellularis infects a variety of species, including swine, horses, donkeys, deer, hamsters, guinea pigs, mice, rabbits, foxes, dogs, ferrets, several species of nonhuman primates, ostrich, and emus. In most, it causes a *characteristic proliferative lesion of cryptal epithelium in distal small intestine and/or large bowel*, associated with a diarrheal syndrome, ill-thrift, and, in emus, rectal prolapse.

L. intracellularis is a gram-negative, curved or S-shaped rod, that is microaerophilic and nonflagellated. It is an *obligate intracellular organism*; hence, cultivation requires the use of tissue culture.

Lawsonia is prevalent in swine worldwide, and it is in this species that infection is most important. Once infected, pigs shed the organism for weeks.

Formerly referred to as *intestinal adenomatosis complex*, and characterized by a number of syndromes (*porcine intestinal adenomatosis, necrotic enteritis, regional ileitis, and proliferative hemorrhagic enteropathy*), the conditions in swine now known to be caused by *L. intracellularis* are lumped as **porcine proliferative enteropathy** (PPE). The multiplicity of names reflects the fact that the etiology of these syndromes eluded definition for decades. PPE was associated with *Campylobacter mucosalis* or *C. hyointestinalis*, until it was finally reproduced by a newly discovered bacterium, "ileal symbiont intracellularis," the taxonomy of which was formalized as *Lawsonia intracellularis* in 1995. Although proliferative enteropathy due to *L. intracellularis is primarily a disease of swine*, it has been reported uncommonly in a number of other species, the most important among domestic animals being the *horse*, in which it mainly affects animals 3 months to 2 years of age.

Disease occurs most commonly in feeder pigs. However, piglets as young as 3 weeks of age, and adults, may have lesions of PPE. Clinical effects may vary from subtle subclinical disease with a mild decrease in growth rate to diarrhea and unthriftiness. Animals with extensive lesions may have anorexia, intermittent or persistent diarrhea, and severe weight loss. Death may follow a period of diarrhea and progressive cachexia, or it may occasionally occur as a result of perforation of an ulcerated intestine, or through peracute hemorrhage. Mortality may be very high.

The **pathogenesis** is dependent on undefined interactions with other bacteria in the gut, because gnotobiotic pigs inoculated with *L. intracellularis* fail to develop disease, whereas conventional pigs are quite susceptible. The pathogenicity of *L. intracellularis* is related to its active uptake by epithelial cells, in which they replicate, and cause to become hyperplastic. It has been theorized that it disrupts intestinal cell differentiation, but specific virulence attributes of *L. intracellularis* are poorly understood, as are molecular details of the agent–host cell interaction. In PPE, infection of cells lining mucosal glands may occur initially in the vicinity of Peyer's patches and mucosal lymphoid aggregates in the ileocecocolic region. In some experimental studies, lesions were first seen here, and in mildly affected spontaneous cases, lesions sometimes seem associated with these structures preferentially. Later, cells in cecal and colonic crypts become infected. *L. intracellularis* organisms internalized within membrane-bound vesicles are released into the apical cytoplasm of glandular epithelium where they lie free and replicate. Cell division is required for bacterial replication, which may explain its tissue tropism. Bacteria are passed on to daughter epithelial cells and exit via extrusion from the cytoplasm of enterocytes on villi or between crypt openings.

Infected epithelium is transformed to a population of highly mitotic cells, and goblet cells disappear. Glands are lined by crowded, dysplastic pseudostratified columnar epithelium, with basophilic cytoplasm (Fig. 1.153A). Nuclei may be open and vesicular with prominent nucleoli, or laterally compressed. The cytologic and biochemical characteristics of these cells suggest that they are poorly differentiated. Infected glands become elongate, dilated, and branching, causing thickening of the mucosa. Isolated plaques of affected mucosa may project above adjacent tissue. The use of the term "*adenomatosis*" to describe such a change is obvious. Hypertrophic glands sometimes protrude into lymphoid tissue in the submucosa. Occasionally, microscopic foci of adenomatous epithelium may be found in submucosal lymphatics or the regional lymph node.

Villi in infected small intestine undergo progressive atrophy, so that they may be entirely absent in well-established lesions. However, the proliferative lesion in the crypts seems primary, and not a hyperplastic response to increased epithelial exfoliation. Adenomatous areas merge sharply with adjacent normal mucosa. Masses of *L. intracellularis* are readily recognized in silver-stained tissue sections, as *curved rods*, infecting especially the *apical cytoplasm of cells in adenomatous glands* (Fig. 1.153B). These organisms have also been identified ultrastructurally in degenerate cells and macrophages in the lamina propria. Proprial and submucosal inflammation in areas of uncomplicated adenomatosis is not marked.

In the least complicated forms of the disease, **lesions** *are always found in the terminal portion of the ileum*, extending proximally from the ileocecal-colic orifice for usually less than a meter, though sometimes they can be found more cranially. In a proportion of cases, they occur in the cecum and proximal third of the spiral colon, but not without ileal involvement. In mild cases, which are likely to be subclinical, only a few ridge or plaque-like thickened areas project above the remainder of the mucosa. However, more typical widespread lesions cause the thickened mucosa to form irregular longitudinal or transverse folds or ridges. The surface may be intact, but commonly, small foci of fibrin exudation or necrosis may be evident.

Thickening of the adenomatous mucosa, and perhaps some edema of the submucosa, is reflected in accentuation of the normal reticular pattern on the serosa of the ileum (Fig. 1.154A). This results in a *cerebriform or gyrate pattern of projections and depressions on the serosal aspect of the intestine which is readily recognized, and virtually pathognomonic for this condition.* Mucosal lesions in the large intestine often form thickened plaque-like or almost polypoid masses, which may be confluent in

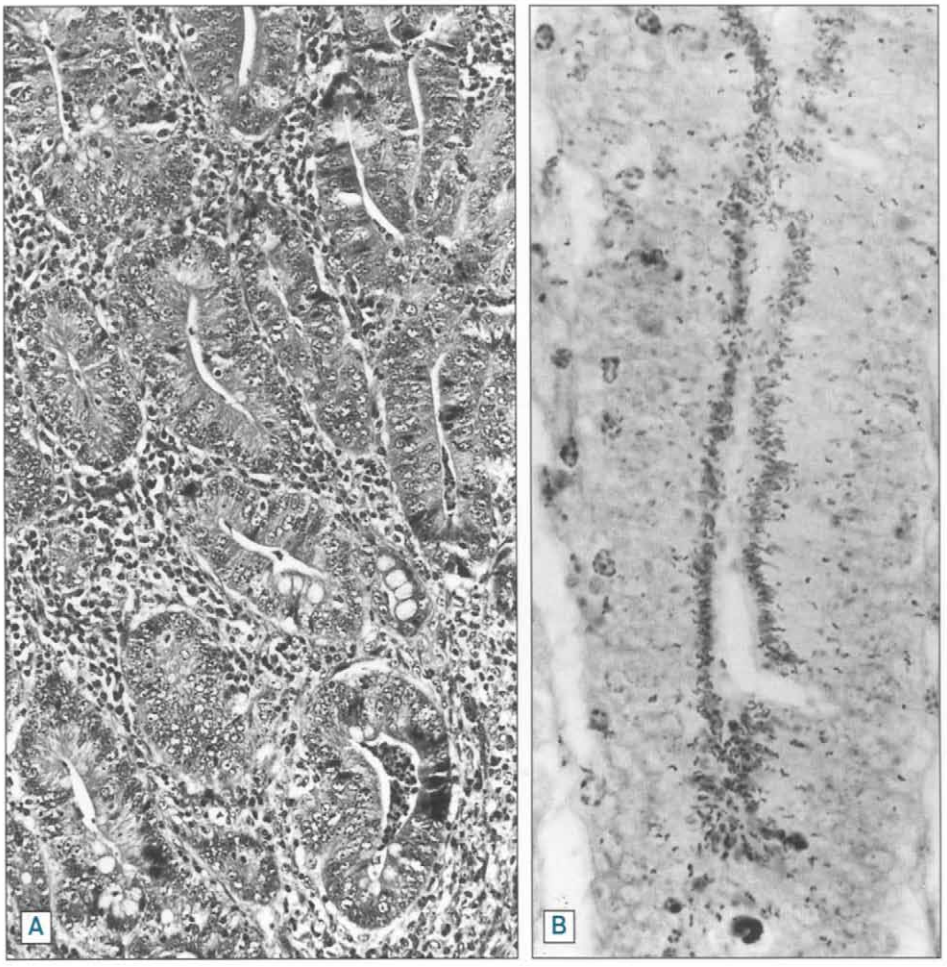

Figure 1.153 Porcine proliferative enteropathy. A. Adenomatous change in glands in the lamina propria. **B.** Masses of silver-stained ***Lawsonia* organisms** in cytoplasm of hyperplastic epithelium lining intestinal glands.

some areas, and are often eroded, with fibrin exudation (Fig. 1.154B). Serosal folds may be evident on extensively affected large intestine. The ileocolic lymph nodes are enlarged and hyperplastic.

Coagulative necrosis of adenomatous mucosa commonly occurs and is referred to as **necrotic enteritis**. It may be partly the result of pathogenic anaerobic large-bowel flora, colonizing the affected terminal ileum and large intestine. There may be effusion of fibrin from superficial lesions, and a pseudodiphtheritic membrane or luminal fibrin cast may be present. Caseous yellow-brown or blood-tinged necrotic mucosa may be found focally or widely in the distal ileum and proximal large intestine. The cerebriform pattern of serosal folding is evident in such cases (Fig. 1.155). While necrotic enteritis may be a sequel to other enterocolitides in swine, *adenomatosis is the most common primary lesion*.

Microscopically, coagulative necrosis of the mucosa may be focal and superficial, with local effusion of neutrophils and fibrin into the lumen, and an acute inflammatory infiltrate at the margin of the necrotic tissue. Frequently, necrosis extends to involve most the thickness of the mucosa, sometimes penetrating to the submucosa. A few islands of viable adenomatous crypts or glands may be left deep among the necrotic debris. Masses of bacteria, presumably fecal anaerobes, are found superficially in the necrotic tissue. With time, granulation tissue develops in ulcerated areas. Tissue in the upper ileum at the proximal margin of the zone of mucosal necrosis should be examined for adenomatosis since, in severe cases of necrotic enteritis, no remnants of such mucosa may persist elsewhere.

As the distal ileum experiences bouts of proliferation, necrosis, and ulceration, such granulation of ulcerated gut may result in progressive stricture of the lumen. This is the syndrome of PPE known as **regional ileitis**. There is often hypertrophy of the external muscle layer. Idiopathic ileal muscular hypertrophy also occurs in swine, apparently independent of antecedent PPE.

Acute or subacute intestinal hemorrhage and anemia occur in PPE, and were considered a distinct syndrome, **proliferative hemorrhagic enteropathy**, within the porcine intestinal adenomatosis complex. Animals may exsanguinate so quickly as to die without passing blood. Others pass dark-red feces for several days. This syndrome is more common in young adults, rather than growing pigs. It is usually sporadic, or of relatively low morbidity, but up to half the clinically recognized cases may die.

Animals dead of this *massive intestinal hemorrhage* are pale, and the perianal area may be smeared with blood. The typical cerebriform pattern is evident on the external surface of the distal ileum, which is thickened and turgid (Fig. 1.156A). Fluid blood or a loose or firm fibrin and clotted blood may be present in the ileum (Fig. 1.156B), and the contents of the cecum and colon may contain dark bloody digesta and feces. The mucosa of the affected ileum usually resembles that in uncomplicated PPE, and overt points of hemorrhage or ulceration are rarely discernible grossly. Rather the animals appear to suffer widespread diapedesis from the mucosa.

In tissue sections from animals dead with the hemorrhagic syndrome, there are extensive erosion and necrosis of proliferative

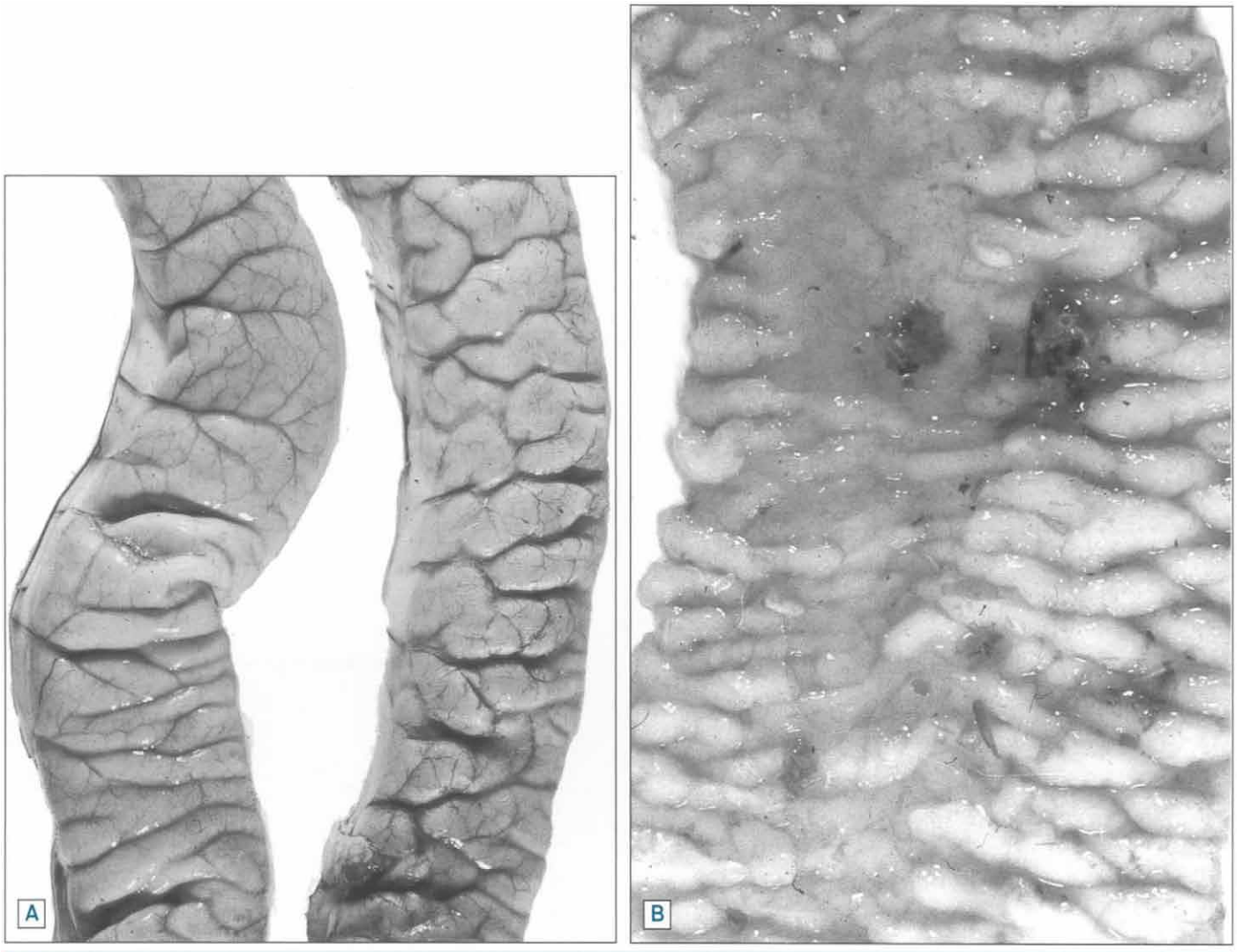

Figure 1.154 Porcine proliferative enteropathy. A. Exaggerated **reticular pattern of folds** on serosal aspect of the ileum. **B.** Raised nodular or **ridge-like areas of thickened mucosa** in the ileum, resulting from hypertrophy of glands.

epithelium in the superficial mucosa (Fig. 1.157). An acute inflammatory infiltrate is present in the upper lamina propria, small vessels are thrombosed, and heavy effusion of neutrophils on to the mucosal surface and into lumina of glands is evident. Fibrin and hemorrhage emanating from superficial mucosal vessels are in the intestinal lumen. More extensive coagulative necrosis of the mucosa is occasionally associated with this hemorrhagic clinical entity of PPE.

In **other species**, the lesions and pathogenesis of **proliferative enteritis** seem similar to those in PPE. In all species, including swine, *diarrhea is probably related to loss of functional mucosal surface area in distal small intestine and large bowel, while ill-thrift or wasting syndromes are attributable to protein-losing enteropathy*. Clinical signs and lesions associated with hypoproteinemia predominate in young horses, and diarrhea is an inconsistent finding, perhaps related to the large absorptive capacity of the equine colon. In horses, adenomatosis may involve a considerable amount of the small intestine.

A presumptive **diagnosis** of PPE in swine, and of proliferative enteritis in other species, can be based on typical gross and histologic lesions, accompanied by Warthin–Starry staining to visualize the intracellular bacteria. Confirmation can be obtained by immunohistochemistry using a specific antibody for *L. intracellularis* or by specific polymerase chain reaction.

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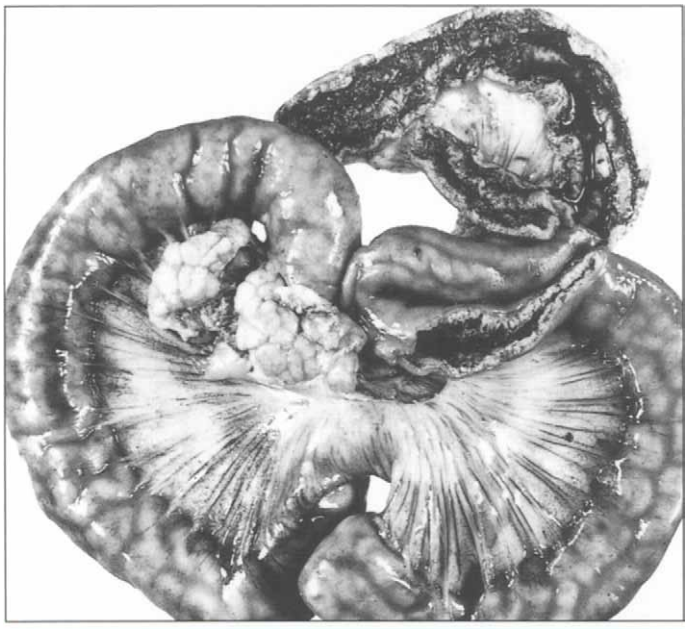
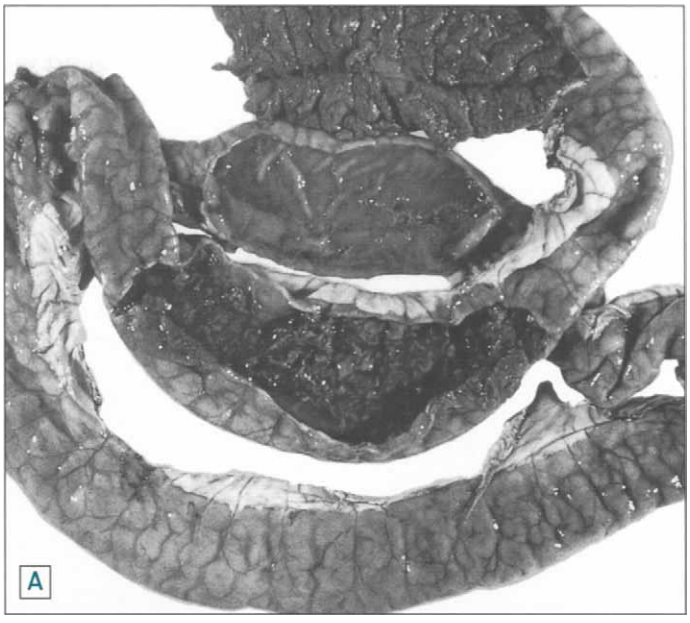


Figure 1.155 Necrotic enteritis, thick ileal wall, enlarged lymph nodes, and opaque mesentery, in **porcine proliferative enteropathy**.



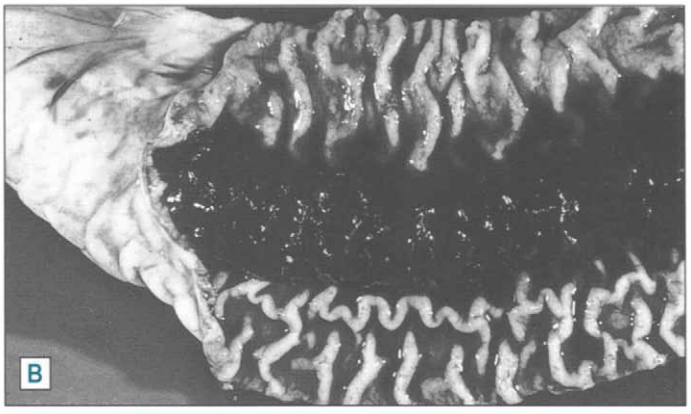


Figure 1.156 Proliferative hemorrhagic enteropathy form of porcine proliferative enteropathy. **A.** Folded necrotic mucosa, and fibrinohemorrhagic exudate in terminal ileum. **B.** Hemorrhage and blood clot on the nodular folded mucosa of the terminal ileum.

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Enteritis associated with Campylobacter spp.

Campylobacter jejuni and ***C. coli*** are common causes of diarrhea in humans, and *C. jejuni*, at least, is also capable of causing enteritis in animals. *C. jejuni* penetrates the surface mucus layer and adheres to the epithelium. Virulent strains enter cells by directed endocytosis caused by the products of a type III secretion system translocated from the bacteria into the cytoplasm of the cell. They often attain the lamina propria, inhabiting inflammatory cells there, from which organisms ultimately reach the systemic circulation. Many human infections are acquired by drinking raw milk, or from other animal foodstuffs, especially poultry products. *Chickens* are common asymptomatic shedders of *C. jejuni*; *C. coli* comes more commonly from *swine*.

C. jejuni has been associated with diarrhea characterized by the presence of blood and mucus in some dogs, despite the fact that it can often be isolated from a high proportion of asymptomatic animals. It has also been isolated from dogs with parvoviral enteritis and other viral infections; pre-existing infections may predispose to the development of pathologic effects of *C. jejuni*. The role of *Campylobacter* as a significant primary pathogen in dogs has been demonstrated in a few cases. These usually involve communal housing situations and often young dogs. *C. jejuni* can be implicated as the cause of lymphoplasmacytic inflammation centered on ileum and colon by ruling out other known etiologies, and demonstrating the organism in large numbers in association with the lesion. *Mild to moderate enteritis and colitis* has been described in naturally infected dogs, while in

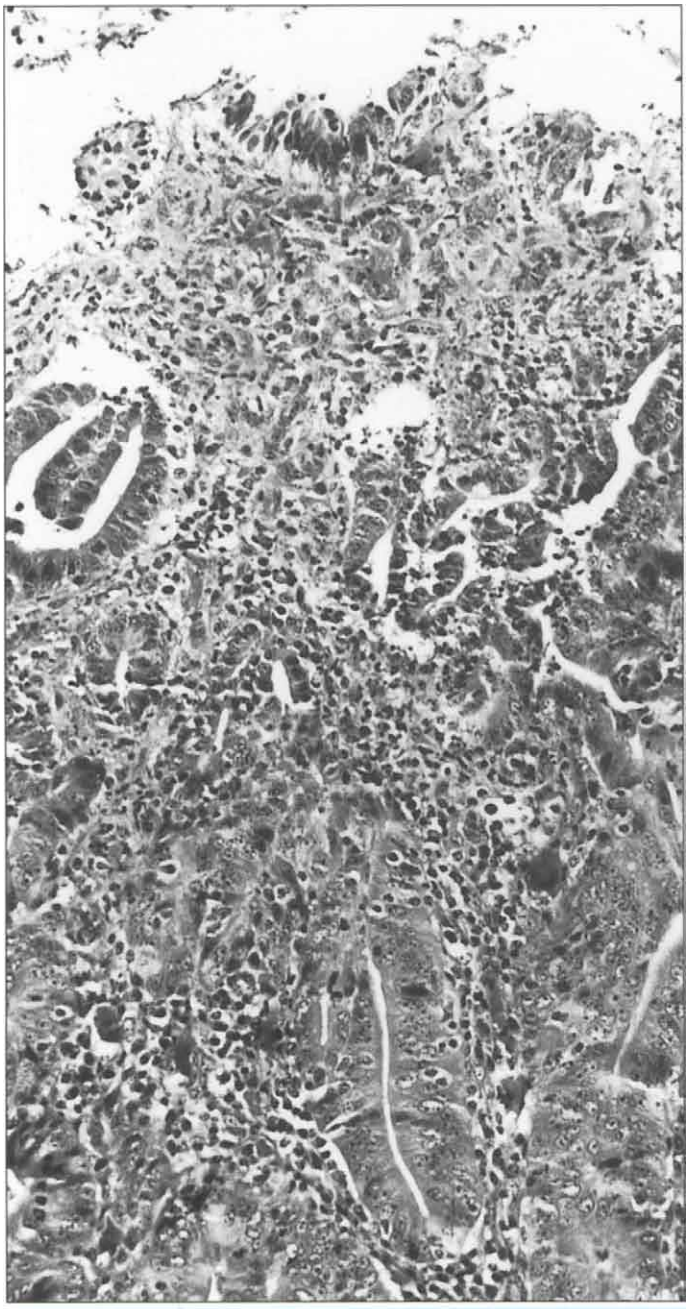

Figure 1.157 Proliferative hemorrhagic enteropathy, with superficial necrosis of mucosa associated with thrombosis of small vessels, hemorrhage, and effusion of fibrin and neutrophils, in porcine intestinal adenomatosis complex.

experimentally infected gnotobiotic and conventional dogs, lesions are limited to mild mucosal colitis. Erosive colitis has been associated with *C. jejuni* infection in mink, and was reproduced experimentally. Cats are merely asymptomatic carriers of *C. jejuni*.

"Weaner colitis" in sheep is a diarrhea of high morbidity and low mortality, reported from southeastern Australia, and associated with an unidentified *Campylobacter* species, not *C. jejuni*. At necropsy, there is abnormally fluid colonic content, but mucosal lesions are not seen; chronically affected animals may have edema and loss of body condition suggestive of enteric protein loss. Microscopically, there is erosive typhlocolitis. Goblet cells are depleted in number, and multifocal erosion, or occasionally, ulceration, of the mucosa is evident. Consistently, a layer of bacteria is present covering surface epithelial cells and in crypts. The disease has been reproduced by inoculation of the thermophilic catalase-negative *Campylobacter*-like organism, which is isolated from spontaneous cases on modified mycoplasma agar base medium.

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Spirochetal colitis in swine

Swine dysentery, caused by ***Brachyspira*** (formerly *Serpulina, Treponema*) ***hyodysenteriae***, is a well-recognized production-limiting disease of swine worldwide, characterized by *large-bowel diarrhea* with mucus, blood, or fibrin in the feces. It is now recognized that at least one other spirochete, ***B. pilosicoli***, can cause colitis in feeder swine, in a disease referred to as *porcine colonic spirochetosis.*

Swine dysentery occurs as a highly infectious disease, mainly of weaned pigs. As long ago as 1924, swine dysentery was known to be experimentally transmissible by dosing young pigs with colonic contents from affected pigs. *B. hyodysenteriae* is a gram-negative, anaerobic but oxygen-tolerant, *spirochete*, 6–9 μm long and 0.4 μm in diameter. It produces strong beta hemolysis on blood agar plates. The organism is motile, moving in serpentine fashion; it is loosely coiled and has 7–13 periplasmic flagella. Originally thought to have a very narrow host range, *B. hyodysenteriae* has also been associated with necrotizing typhlocolitis in rheas.

Swine dysentery can be reproduced by feeding pure cultures of *B. hyodysenteriae* to specific-pathogen-free and conventionally reared swine. Experimental reproduction of the disease in gnotobiotic pigs requires the presence of anaerobic bacteria indigenous to the normal colon, along with *B. hyodysenteriae*. There is apparently a *synergistic action between the spirochete and other anaerobes*, mainly *Bacteroides* and *Fusobacterium*. Several studies have documented the importance of a diet low in fiber and high in rapidly fermentable carbohydrates for the successful establishment and pathogenicity of *B. hyodysenteriae*.

The **pathogenesis** of swine dysentery is still incompletely understood. The only virulence factor consistently associated with

B. hyodysenteriae is the production of hemolysin, and how or whether it contributes to disease is unclear. Other potential virulence attributes await definitive correlation with severity of disease.

B. hyodysenteriae colonizes the mucus on the mucosal surface, in the lumen of colonic glands, and in goblet cells. Lesions, including exfoliation of surface epithelium, are associated with the presence of *large numbers of spirochetes and other anaerobic bacteria on the mucosa.* By in situ hybridization, large numbers of *B. hyodysenteriae* can be seen colonizing the mucus layer, the surface epithelium, and the crypts. Their association with epithelium appears random. Attachment of spirochetes to host cells has not been observed in vivo, and invasion is not essential for epithelial loss to occur. When spirochetes invade surface epithelial cells, they appear to do so through lateral membranes, and do not attach to and penetrate the luminal membrane. *Brachyspira* do not usually invade beyond the epithelial cells. The factors responsible for local necrosis or exfoliation of superficial epithelium in *B. hyodysenteriae* infection are unknown. However, the suite of associated lesions – hyperplasia of the proliferative compartment in crypts; goblet-cell hyperplasia; premature exfoliation of surface epithelium between crypt openings; and an associated mixed mucosal inflammatory cell infiltrate – is reminiscent of the changes in cell-mediated villus atrophy in the small intestine, raising the possibility that the lesion is at least in part immune-medicated.

The result is *mucosal colitis*, characterized by superficial erosion, with hyperplasia of cells in colonic glands, hypersecretion of mucus, and a mixed inflammatory infiltrate in the lamina propria. Thrombosis of capillaries and venules in the superficial areas of the colonic mucosa and in the gastric fundic mucosa (gastric venous infarction) is probably due to absorption, through the damaged mucosa, of endotoxin released by gram-negative bacteria.

The diarrhea in swine dysentery is due to *malabsorption of fluids and electrolytes in the colon.* This presumably results from damage to the superficial colonic epithelium. The normal colon of the pig has tremendous absorptive capacity. Interference with colonic absorption results in severe diarrhea and dehydration. Active fluid secretion by the colon, associated with bacterial enterotoxins, does not occur in swine dysentery. Fluid and electrolyte transport are normal in the small intestine.

There is usually an introduction of pigs, presumably *carriers*, into a herd prior to an outbreak. Once established in a herd, the infection tends to remain *enzootic*, and although treatment can effect a rapid clinical amelioration, it may not be curative, and relapses at greater or lesser intervals can occur. Apparently infection is not followed by a substantial immunity, although individual carrier pigs are resistant to further challenge with *B. hyodysenteriae* after recovery from disease. The morbidity may reach 90% and mortality 30%.

The disease occurs in pigs of all ages over ~2–3 weeks old, but particularly in pigs 8–14 weeks of age. Once initiated, it spreads rapidly by pen contact. The disease is initially febrile, but, with the onset of diarrhea, fever tends to subside. The initial diarrheic feces are thin, semisolid, and without blood or mucus; it is usually only after 1–2 days of diarrhea that blood and mucus appear in the feces. Some pigs die peracutely without showing diarrhea, and many that show diarrhea do not have dysentery, but pass feces which contain much mucus.

Grossly, pigs that die of swine dysentery are usually gaunt with a contracted abdomen; the eyes are sunken; and there may be blue discoloration of the abdominal skin. Associated lesions may include pericardial serous effusion, and intense congestion of the gastric mucosa due to venous infarction. The intestinal lesions, especially in young pigs dying acutely, and those that have been treated, can be easily overlooked because the mucosal colitis may be mild, patchy, and often more catarrhal than fibrinous.

In typical cases, dehydration gives a semiopaque ground-glass appearance to the serosa, and the wall of the cecum and colon is thickened. The colonic content in these cases is usually scant, and porridge-like dirty gray to red-brown and greasy in appearance. The mucosa, with patchy foci of light fibrin exudation, has the velvety thickening of catarrhal secretion (Fig. 1.158A). The most severe lesions approach those of salmonellosis in the extent and severity of fibrin effusion. The production of mucus in swine dysentery becomes copious in many chronic cases due to *remarkable goblet-cell hyperplasia.*

The earliest **microscopic lesions** are characterized by discrete areas of epithelial erosion on the superficial mucosa. Thin layers of fibrinocellular exudate cover the eroded areas. In more advanced cases, these areas become more diffuse but remain merely erosive, and exudation is more copious (Fig. 1.158B). There may be minor bleeding from small vessels in eroded mucosa. Fibrin thrombi are evident in the capillaries and venules of the superficial lamina propria. There is usually some edema of the lamina propria, submucosa, and serosa. Initially mucus is expelled from the basilar portions of the crypts (Fig. 1.159A). In concert with the increased turnover of epithelial cells associated with the superficial erosion, there is hyperplasia of cells deeper in the glands. The crypts are elongated, lined by proliferative basophilic epithelial cells that have large nuclei, and few differentiated goblet cells (Fig. 1.159B). Often, crypts subsequently become dilated and contain necrotic debris. Others have marked goblet-cell hyperplasia, and copious mucus production.

Porcine intestinal spirochetosis is caused by ***Brachyspira*** (***Serpulina***) ***pilosicoli*** (formerly *Anguillina coli*), which differs from *B. hyodysenteriae* in that it is only weakly beta-hemolytic and is capable of hippurate hydrolysis. *B. pilosicoli* has a wide host range, having been isolated from a number of other species of animals with lesions of intestinal spirochetosis, and it may be zoonotic.

Porcine intestinal spirochetosis has been seen in most major swine-producing areas of the world. The disease is characterized by *generally transient watery to mucoid diarrhea without blood.* Depression of weight gain is a significant clinical finding. Grossly, there may be mesocolonic edema, and the large intestine has abundant watery contents, with variable degrees of mucosal erosion. In more severe cases there may be a diphtheritic membrane.

Virulence attributes of *B. pilosicoli* are poorly defined, but motility and chemotaxis for mucus may be important. The organism is capable of polar attachment by one end of the organism to the apical membrane of enterocytes, resulting in displacement or effacement of microvilli. Histologically, spirochetes can often be seen as a dark fringe or "false brush border" on the luminal surface early in infection (Fig. 1.160). The organism can invade paracellularly, especially at the extrusion zone between colonic crypt units, attaining the lamina propria, where it may be phagocytosed by macrophages; ultimately, in some cases, it reaches draining lymph nodes. A mixed inflammatory infiltrate, including lymphocytes and macrophages, is present in the lamina propria between crypts. Later in infection, when epithelial attachment is rare, the organism persists in crypt lumens and mucus in goblet cells, and goblet-cell hyperplasia is evident, along with inflammatory cells and cellular debris in gland

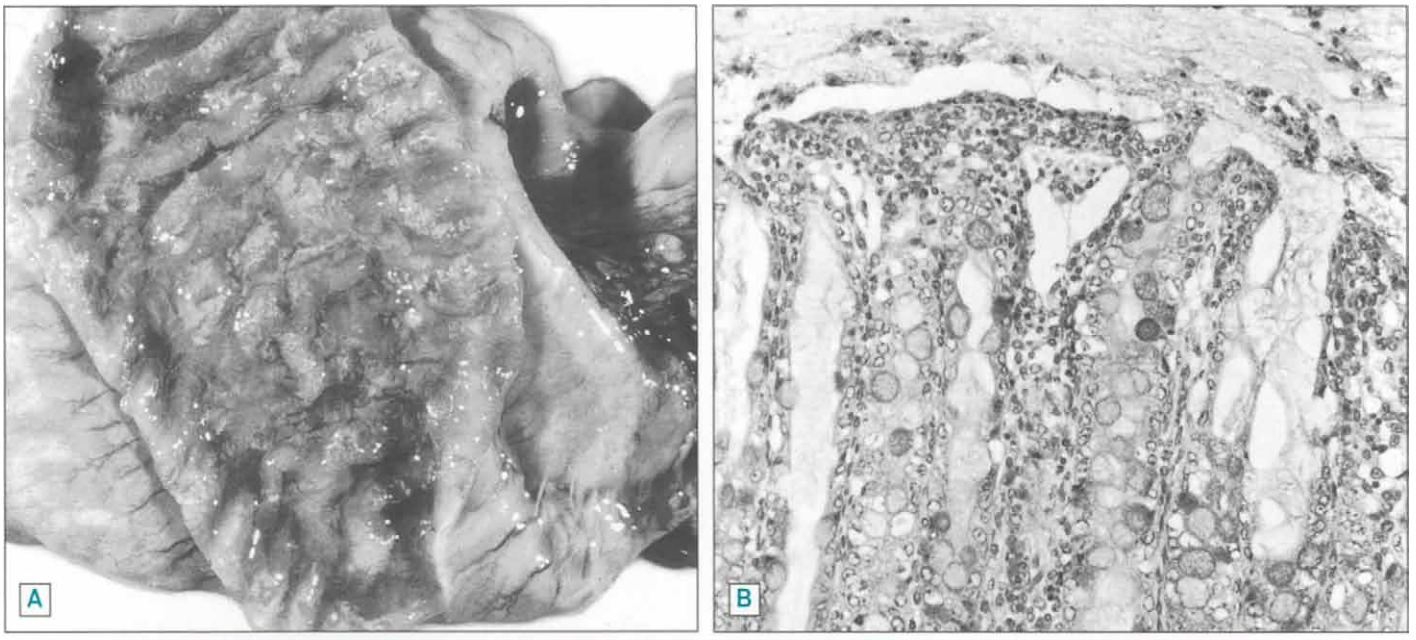

Figure 1.158 Swine dysentery. A. Patchy fibrinocatarrhal exudate on the colonic mucosa. **B.** Flattened and exfoliating epithelium on mucosal surface, and edema of superficial lamina propria. Mucus in glands and on surface, mixed with neutrophils and exfoliated epithelium.

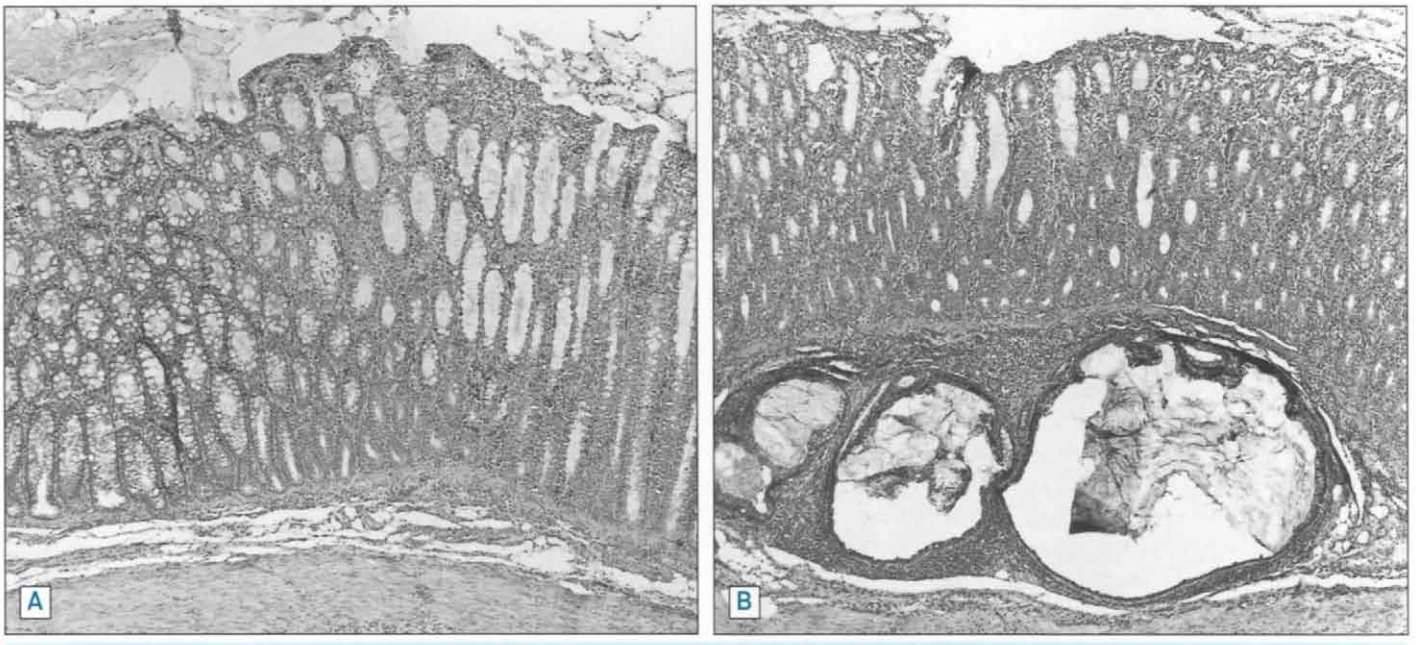

Figure 1.159 Swine dysentery. A. Hyperplastic glands with few goblet cells adjacent to mucosa with normal density of goblet cells. Copious mucus on surface. **B.** Hyperplastic glandular lining virtually devoid of goblet cells; "colitis cystica profunda," or herniation of mucous glands into submucosal lymphoid tissue.

lumens. The diarrhea and ill-thrift characteristic of infection may be related to loss of absorptive function, secondary to disruption of the brush border of enterocytes, and increased exfoliation of poorly differentiated cells at intercrypt extrusion zones, perhaps with enteric loss of plasma protein.

In **dogs, colonic spirochetosis** with mucosal colitis has also been described, in association with *B. pilosicoli* and perhaps with other *Brachyspira* spp., but a causal relationship has not been established experimentally.

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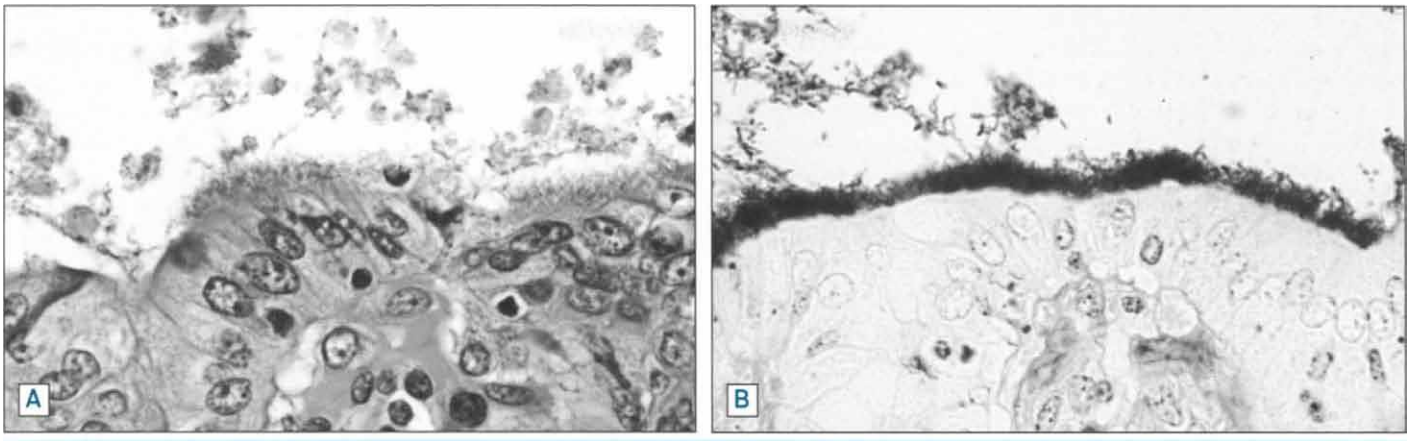

Figure 1.160 Porcine intestinal spirochetosis. A. Fringe of ***Brachyspira pilosicoli*** embedded in the apical border of colonic surface epithelial cells. Remnants of exfoliated epithelium are in the lumen (HE). **B.** *Brachyspira pilosicoli* demonstrated with Warthin-Starry stain.

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Diseases associated with enteric clostridial infections

Most of the important enteric clostridial diseases occur in herbivores and are caused by one of the five toxigenic types of ***Clostridium perfringens***. Enteritis in dogs is associated with *C. perfringens* and ***C. difficile***, and the latter agent is implicated in fibrinous enteritis, especially in horses, neonatal pigs, and dogs. ***C. piliforme*** (formerly *Bacillus piliformis*) causes Tyzzer's disease, characterized by enteritis and colitis, usually with multifocal necrotic hepatitis and myocarditis, in many animal species. ***C. chauvoei*** may affect the tongue and the smooth muscle of the lower alimentary tract, causing blackleg-like myositis (see Vol. 1, Muscle and tendon), while ***C. septicum*** causes clostridial abomasitis (braxy) in sheep and calves, discussed in the section on Stomach and abosmasum, above. ***C. botulinum*** causes toxicoinfectious botulism in horses, and by ingestion of toxin, botulism in cattle (see Vol. 1, Nervous system).

Table 1.2 Relationships among the five types of *Clostridium perfringens* A–E and the four major exotoxins (alpha, beta, epsilon, and iota)

	Toxin			
C. perfringens	Alpha	Beta	Epsilon	Iota
Type A	++	–	–	–
B	+	++	+	–
C	+	++	–	–
D	+	–	++	–
E	+	–	–	++

++ = significant amount of toxin, + = small amount, – = none detected.

There are five types of *C. perfringens*, designated *A–E*, which are differentiated on the basis of their production of the *four major antigenic exotoxins*, which, along with an enterotoxin, are the most significant virulence attributes of *C. perfringens* in the gut. *The major exotoxins are alpha, beta, epsilon, and iota*; the relationships among the five types and the four toxins are illustrated in Table 1.2.

Eight minor toxins are produced by *C. perfringens*, and some of these may be useful in identification of types and in division of types A, B, and C into varieties. Genetic evaluation of clostridial species may ultimately also provide more specific classification. There is not always a clear distinction among the different types of *C. perfringens*. Some strains lose their ability to produce one or more of their toxins when stored or cultured, and this complicates the identification of isolates and the assessment of their significance in disease outbreaks.

The **alpha toxin** is a lecithinase that acts on cell membranes, producing hemolysis or necrosis of cells. The **beta toxin** is a pore-forming toxin that induces a variety of neurologic effects; it appears to have a paralyzing effect on the intestine. The **epsilon toxin** is

produced during active growth, as an inactive prototoxin that is activated by enzymic digestion. In culture, the appropriate enzymes (the minor toxins kappa and lambda) may be produced by the organism; in the intestine, trypsin is an effective activator. The **iota toxin** is also elaborated as a prototoxin and activated by proteolytic enzymes either in culture (lambda toxin) or in the intestine; it increases capillary permeability. **Kappa toxin** is a collagenase, and **lambda** a nonspecific proteinase. An additional toxin, **beta$_2$**, which, despite its name, is not related to beta toxin, has been implicated in enteric disease in swine and in typhlocolitis in horses caused by *C. perfringens* types A and C. Its pathogenicity is uncertain, but it may at least act as a virulence marker. Other minor toxins include **mu**, a hyaluronidase, and **delta**, a hemolysin.

Clostridial diseases of the intestine are often called **enterotoxemias**. Disease produced by *C. perfringens* type D, whose epsilon exotoxin is elaborated in the intestine but exerts its important effects on distant organs such as brain and kidney, is a true enterotoxemia. The hemolytic disease attributed to type A is also an enterotoxemia, but *in general the other types produce local intestinal lesions*. The production by some types of *C. perfringens*, especially type A, of an **enterotoxin**, distinct from the classical exotoxins, is potentially confusing. This enterotoxin is only elaborated by sporulating cells and is released upon lysis of vegetative cells. It is produced by all strains, but is not effective in type E. This enterotoxin is not involved in the pathogenesis of enterotoxemia ("*pulpy kidney disease*") caused by type D. Most significant in food poisoning by type A strains in humans, it has also been associated with antibiotic treatment-related diarrhea and infantile diarrhea. It is activated by proteolysis and alters plasma membrane permeability of the mammalian cell.

The **pathogenesis** of enteric infection with *C. perfringens* and *C. difficile* requires a *change in the enteric microenvironment* favorable to massive expansion of luminal populations of clostridia. Such changes may include a change in feed, abnormally nutrient-rich digesta, antibiotic therapy, altered pancreatic exocrine function or trypsin inhibitors, reduced motility, and primary infections with agents such as *Canine parvovirus*, or coccidia in piglets and chickens. These clostridia produce disease in thee general ways: (1) *local necrotizing effects of toxin* on the mucosa, causing hemorrhagic, fibrinous, or necrotic enteritis; (2) *secretory effects of locally acting enterotoxin*, causing diarrhea and minor mucosal lesions; or (3) *systemic absorption of (entero) toxin*, with effects at sites distant from the gut.

Alpha toxin produced by *C. perfringens* type A and beta toxin produced by types B and C probably account for the *severe mucosal necrosis and/or hemorrhage* evident in these infections. Bacteria alone are not pathogenic; exotoxins are required to induce disease. Beta toxin is trypsin-labile, and circumstances such as low enzyme levels in young animals, very high levels of toxin, or trypsin inhibitors could be important. Sows' colostrum contains a trypsin inhibitor, but it is not known if colostrum in other species possesses this factor. "Pig bel," a necrotizing jejunitis of humans in New Guinea that is probably caused by the beta toxin, has been causally related to consumption of heat-stable trypsin inhibitors in sweet potatoes. Naturally occurring protease inhibitors in soybeans appear to have a similar effect in guinea pigs. Intraduodenal inoculation of *C. perfringens* type C in combination with soybean flour produces acute fatal hemorrhagic enterotoxemia in lambs.

C. difficile produces two exotoxins, A, which is an enterotoxin, and B, which is a cytotoxin. Tissue damage is probably due to the effects of both toxins, which glycosylate and inactivate Ras GTPases, disabling signaling pathways in the cell. As well, they glycosylate Rho, which regulates the actin cytoskeleton; it condenses, tight junctions open, cells round up, and undergo apoptosis. They also cause release of proinflammatory mediators, attracting neutrophils, and activate secretion stimulated by the enteric nervous system. Hence, disease is characterized by fluid intestinal content, with patchy areas of colonic epithelial necrosis, through which neutrophils exude into the lumen, producing a so-called "volcano" lesion. Although not all isolates of *C. difficile* are toxigenic, over 20 toxinotypes or ribotypes have been described, based on sequence variations in the genes for the A and B toxin molecules, while 14 serotypes are recognized. Molecular epidemiologic investigation may permit associations of toxinotype with virulence.

C. piliforme, the cause of Tyzzer's disease, as an obligate intracellular agent infects epithelial cells, causing necrotizing and inflammatory lesions. The pathogenesis of Tyzzer's disease is not clearly dependent on toxin production, though some strains do produce cytotoxic proteins.

Diagnosis of disease due to the toxin-producing clostridia is dependent on demonstration of toxin or enterotoxin in gut content or feces of affected animals, by the most specific test available. While the presence of large numbers of a particular type of *C. perfringens* is suggestive of involvement with disease, this organism is commonly present in the gut in a variety of circumstances, where it cannot be implicated as an etiologic agent.

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Clostridium perfringens type A

C. perfringens type A is the *most common of the five types* and is the only one associated with the microflora of both soil and intestinal tract; it may be part of the normal intestinal microflora of domestic livestock species. Its major toxin is the *alpha toxin*; it also produces beta$_2$ toxin and the enterotoxin.

This is one of several clostridia that produce *gas gangrene* in humans and animals. The production of gas gangrene in wound and puerperal infections is probably a composite effect of the major and minor toxins elaborated by the organism. The necrotizing and hemolytic activity of the alpha toxin is assisted by the collagenase and hyaluronidase that disrupt connective tissues and permit the infection to spread. It is also associated with *alimentary syndromes* in domestic animals, including enteritis in foals; enterocolitis in horses; necrotizing enterocolitis

in neonatal piglets; enterotoxemia and hemorrhagic enteritis in lambs and neonatal calves; diarrhea, and hemorrhagic enteritis in dogs; as well as necrotic enteritis in chickens. Type A has been suspected of causing disease in cattle but proof is often lacking. Calves inoculated intraruminally with *C. perfringens* type A developed anorexia, depression, bloat, and diarrhea and some of these calves died. Lesions included variable degrees of abomasitis and abomasal ulcers, discussed more fully with gastritis.

In **foals** infected with *C. perfringens* type A, lesions have been more localized to small intestine, the mucosa of which was dark purple. Microscopic lesions were confined to the gut, mainly the small intestine, and were characterized by marked diffuse necrosis of the mucosa. The remnants of necrotic villi were covered by large numbers of gram-positive rods consistent with clostridia. There was also marked hyperemia and hemorrhage of the lamina propria, submucosa and subserosa without a significant leukocytic reaction. Intravenous inoculation of *C. perfringens* type A in ponies resulted in acute colic and hemorrhagic gastroenterocolitis. The syndrome of adult equine clostridial colitis is covered below, in the section on Other clostridial diseases.

C. perfringens type A has been associated with white scours in suckling **pigs** and diarrhea in feeder pigs, although this association is not commonly recognized. The pigs had necrotizing enterocolitis, villus atrophy, and serositis, and enterotoxigenic strains may be associated with the syndrome.

A very rare disease of **calves** and **lambs** characterized by *acute intravascular hemolysis* is also associated with type A infections. Affected animals may be found dead or moribund, and jaundice and hemoglobinuria may be evident clinically. At autopsy, icterus, anemia and other changes of severe, acute, intravascular hemolysis are prominent. Severe diarrhea may occur in calves but enteric lesions are likely to be obscured by rapid autolysis. This hemolytic disease must be distinguished from other causes of acute intravascular hemolysis such as leptospirosis, bacillary hemoglobinuria caused by *C. novyi* type D (*haemolyticum*), and chronic copper poisoning. Presumably the hemolytic effect of the exotoxin is responsible for the intravascular hemolysis. Given that type A strains are commonly found in the intestines of ruminants, and that alpha toxin given intravenously is destroyed rapidly, it is apparent that there must be complex pathogenetic requirements for the development of this disease. The pathogenesis may be somewhat analogous to that of enterotoxemia caused by type D, which is discussed below.

Strains of *C. perfringens* type A, some determined to be enterotoxin-secreting, have been associated with diarrhea, sometimes bloody, in dogs. **Hemorrhagic canine gastroenteritis** (canine gastrointestinal hemorrhage syndrome) is a sporadic, peracute, hemorrhagic gastroenteritis, associated in some cases with *C. perfringens* type A, though in other cases the type involved has not been identified. Dogs with the peracute hemorrhagic disease are often found dead lying in a pool of bloody excreta; sometimes hemorrhagic diarrhea is noted prior to death. Autopsy reveals hemorrhagic enteritis and colitis (Fig. 1.161), and sometimes hemorrhagic gastritis is present. Colonic lesions tend to be more severe. Microscopically there is hemorrhagic necrosis of the gastrointestinal mucosa, which extends from the luminal surface into the mucosa. Numerous clostridia may line the necrotic intestinal structures or be distributed through the detritus, but they do not invade the intact tissue (Fig. 1.162). Recurrent diarrhea, sometimes bloody, has been associated with enterotoxin-secreting type A strains. Multiple serotypes of clostridia have been associated with nosocomial, usually nonfatal, cases of diarrhea in dogs. *C. perfringens* enterotoxin has been demonstrated twice as frequently in hospitalized dogs with diarrhea compared to controls without diarrhea.

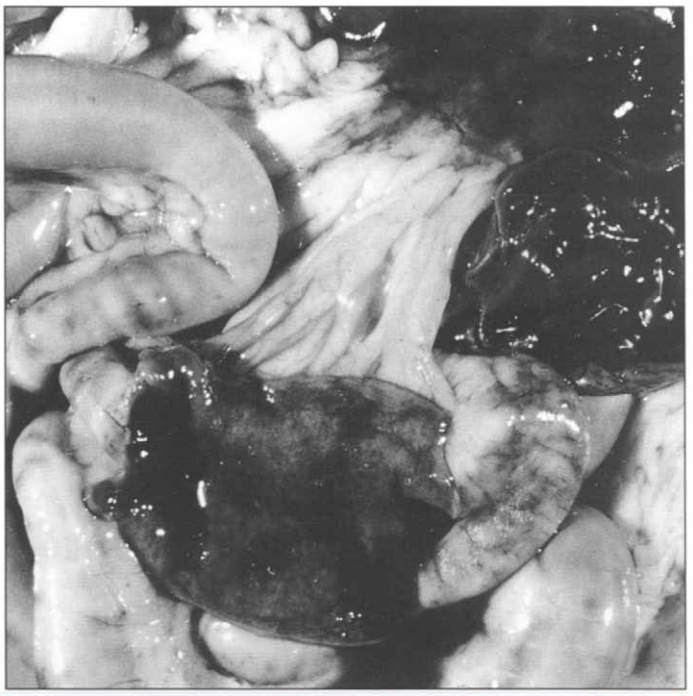

Figure 1.161 Hemorrhagic enteritis in a dog with **canine gastrointestinal hemorrhage syndrome**, associated with *Clostridium perfringens*.

Hemorrhagic bowel syndrome has been described in mainly *dairy cattle* in the USA. Affected cattle become acutely ill, with bloating, gut stasis, and melena. The lesions relate to necrohemorrhagic enteritis with extensive intraluminal hemorrhages and the lumen may contain large amounts of clotted blood (Fig. 1.163). The condition is associated with very high mortality. *C. perfringens* is present in large numbers in the feces from affected animals, and there may be an association with *C. perfringens* type A, though whether there is a causal relationship is uncertain.

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Figure 1.162 Coagulative necrosis and hemorrhage in the superficial mucosa of the colon in **canine gastrointestinal hemorrhage syndrome**. The mucosal surface is highlighted by a dark rim of clostridia.

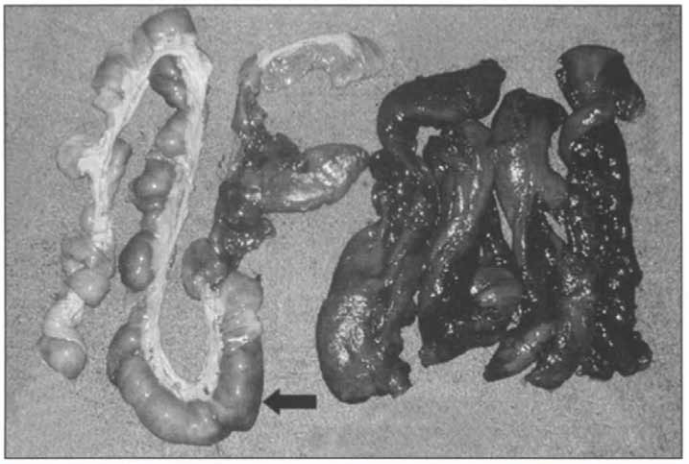

Figure 1.163 Bovine hemorrhagic bowel syndrome, associated with *Clostridium perfringens* type A. Unopened loop of jejunum containing clotted blood (arrow), and opened intestine with blood clots (right).

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Clostridium perfringens type B

Clostridium perfringens type B is reported from Europe, South Africa, and the Middle East, but not from North America and Australasia. It causes "*lamb dysentery*," usually in lambs up to ~10–14 days of age, dysentery in calves of approximately the same age, and dysentery in foals within the first few days of life.

In **lambs**, death may occur without premonitory signs, but there is usually abdominal pain, especially when animals are forced to rise, and passage of semifluid dark feces mixed or coated with blood. The abdomen is often tympanitic. A more chronic form in older lambs, which among other diseases is known as "pine" in England, is characterized by unthriftiness and depression, reluctance to suckle, and a peculiar stretching when the animal rises; such cases are reputed to respond well to specific antiserum.

Typical lesions are usually present, although in exceptional peracute cases they may be indistinct. The first impression on opening the abdominal cavity is that there is mesenteric torsion, a not uncommon accident in young lambs. The characteristic lesion is *extensive hemorrhagic enteritis*. The peritoneal cavity often contains a small amount of serous or blood-stained fluid.

In cases with more severe and deeply penetrating mucosal ulcerations, there may be overlying peritonitis with red fibrin strands on the local mesentery and intestinal adhesions. On the mucosal surface, they are irregular but well defined by a sharp margin and rim of intense hyperemia, and they contain a yellow necrotic deposit; they may coalesce to form extensive areas of necrosis. Usually the intestinal contents are blood-stained and may appear to be composed of pure blood, but in lambs that live for 3–4 days, there may be little or no hemorrhage evident. Histologically the wall of the intestine is hemorrhagic, and the areas of necrosis extend deeply into the mucous membrane, in some cases penetrating to the external muscle layers and serosa. There are large numbers of typical bacilli in the necrotic tissue, but few inflammatory cells.

The lesions present in other organs are those of *severe toxemia*. The liver is usually pale and friable, but may be congested. The spleen is normal or slightly enlarged and pulpy. The kidneys may be enlarged, edematous, pale, and soft from toxic degeneration. The pericardial sac contains abundant clear gelatinous fluid, the myocardium is pale and soft, and hemorrhages beneath the serous membranes of the heart are almost constant. The lungs are often slightly congested and very edematous.

The disease in **calves** caused by type B *C. perfringens* closely resembles that in lambs, usually affecting sucklings less than 10 days of age, with a course of 2–4 days characterized by prostration and dysentery. Older calves up to 10 weeks of age are sometimes affected. It appears that calves are more likely to recover, albeit slowly, than are lambs. The intestinal lesion is acute hemorrhagic enteritis with extensive mucosal necrosis and patchy diphtheritic membrane formations, especially in the ileum.

Although both "lamb dysentery" and "hemorrhagic enterotoxemia" occur in pastured animals, they are most serious in animals that are confined.

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Clostridium perfringens type C

Clostridium perfringens type C is present worldwide, and causes disease in adult sheep and goats, feeder cattle, and in neonatal lambs, calves, foals, and pigs.

In adult **sheep**, *C. perfringens* type C causes "*struck*," a disease of pastured animals that has a mortality rate of 5–15% in some areas. The disease in adult **goats** is probably similar in most respects to that in sheep. Death usually occurs suddenly with terminal convulsive episodes, but some animals, with infections not so peracute, stand in a straining position that probably indicates acute abdominal pain. In adult sheep, diarrhea or convulsions do not occur.

At necropsy, the peritoneal cavity contains up to 3 liters of clear, pale-yellow fluid that clots on exposure to air and that becomes stained with hemoglobin if necropsy is delayed. The small intestine is intensely hyperemic, either in patches or along most of its length, and in the zones of hyperemia there may be ulcers that may attain several centimeters in size. Ulcers are usually present, mostly in the jejunum, and are surrounded by a zone of hyperemia with a deep red base, although in some the necrotic material is dark green and adherent. The large intestine is normal. The primary intestinal lesion is superficial mucosal necrosis that advances more deeply, with a peripheral leukocytic reaction, congestion, and hemorrhage. The organisms are limited to the necrotic tissue.

The disease in feedlot **cattle** is similar to "struck." Animals are found either dead or moribund, and congestion and hemorrhage of the gastrointestinal tract are prominent. The jejunal and ileal content are bloody with fibrin clots and necrotic debris. Excessive straw-colored pleural and pericardial fluid and petechiation of epicardium and endocardium are present. Autolysis and postmortem bloat occur rapidly, and differentiation from ruminal tympany and other clostridial diseases is necessary.

The diseases caused by type C in **lambs**, **calves**, **pigs**, and **foals** are very similar and will be discussed together. Affected animals are usually young sucklings, which contract the disease within the first few days of life, often within the first 12 hours if they have been confined. Foals and most clinically affected lambs die, but in calves and pigs, subacute disease may occur in which there is diarrhea and unthriftiness.

Often, affected animals are found dead. Sick lambs may shiver, show abdominal pain, abdominal distension, dysentery, and prostration, and die in 12 hours or less. Sick calves show abdominal pain, some show diarrhea of sudden onset, and death is preceded by spasmodic convulsions.

Similar lesions occur at autopsy in all species, but may be less severe in lambs. In lambs, the intestinal changes vary from catarrh to acute hemorrhagic enteritis with mucosal necrosis that, like lamb dysentery, suggests strangulation. *The most prominent changes occur in the jejunum and ileum*, the lumen of which may contain *free blood*, which forms a clotted cast in fresh cadavers. Sometimes there is merely acute hyperemia of a segment of jejunum with edema of the wall, scant creamy intestinal content, and a few small ulcerations of the mucosa. The peritoneal cavity contains a small quantity of serous blood-stained fluid, and the local mesentery and peritoneum are often mildly inflamed, hyperemic, and bear red strands of fibrin. The mesenteric nodes are enlarged, wet, and congested. There is usually excess pericardial fluid and pulmonary interstitial edema. Ecchymoses on the serous membranes are nearly constant, and in a few cadavers all tissues, but especially the meninges and brain, are liberally sprinkled with small hemorrhages. These are sites of *bacterial embolism*, due to a massive terminal bacteremia by *C. perfringens*.

C. perfringens type C causes *hemorrhagic enteritis* of suckling **piglets** in many parts of the world. Rarely, epizootics occur in 2–4-week-old pigs and in weaned pigs. The disease occurs as epizootics in affected herds and regions, and may then remain enzootic. Poor hygienic conditions, overcrowding, and antibiotic treatment are thought to be predisposing factors in some outbreaks. Affected animals pass blood-stained feces in the terminal stages and there is marked hyperemia of the anus just prior to death. The predominant lesions occur in the small intestine, especially the jejunum, but the cecum and spiral colon are often involved, and occasionally lesions are confined to the large intestine. Lesions are similar in all areas, and in acute cases consist of *intestinal and mesenteric hyperemia, extensive necrosis of the intestinal mucosa, and blood-staining of the contents* (Fig. 1.164). There may be emphysema of the intestinal wall, which becomes fragile. Mesenteric lymph nodes are red, and sanguineous peritoneal and pleural fluid is present. Fibrinous intestinal adhesions may develop.

Microscopically, the necrotic process extends deeply and sometimes penetrates the muscularis mucosae. Numerous typical bacilli line up along the margin of involved villi and inhabit the necrotic tissue. Older pigs may not show intestinal hemorrhage but do have mucosal necrosis, and peritoneal and pericardial effusion.

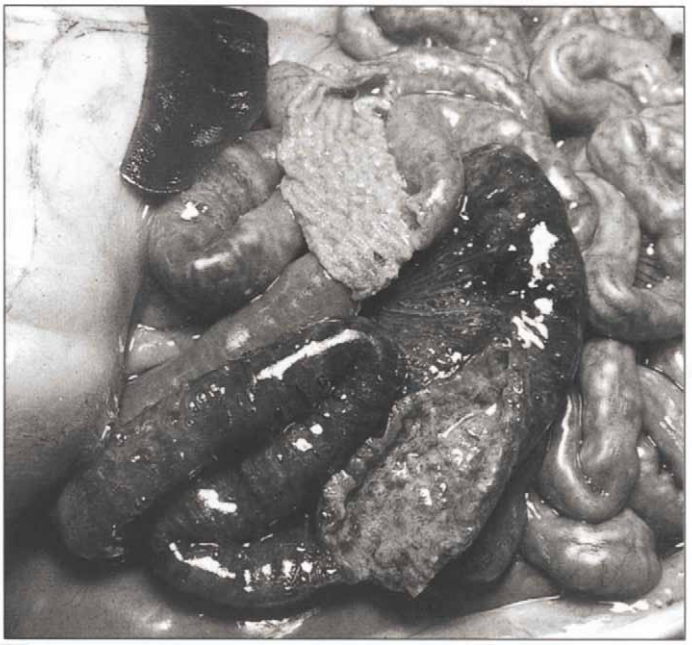

Figure 1.164 Necrotizing enteritis in a piglet, caused by ***Clostridium perfringens* type C** infection. (Courtesy of M. Bergeland.)

The disease due to *C. perfringens* type C in **foals** has been reported from the USA, Canada, and Australia. It usually occurs in foals <4 days of age. Typical clinical signs include weakness, yellow to brown watery diarrhea or dysentery, colic, and dehydration. Affected foals usually die in <24 hours. Macroscopic lesions are those of *acute hemorrhagic necrotizing enteritis*, usually in the distal two-thirds of the small intestine, although in some cases most of the small and large intestine may be affected. The microscopic lesions are similar to those described previously in the other species. In foals, the lesions must be differentiated from those seen with other clostridia.

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Clostridium perfringens type D

Enterotoxemia (*"pulpy kidney" disease, "overeating" disease*) caused by the toxins of *Clostridium perfringens* type D is an important disease of *sheep and goats* with a worldwide distribution. It occurs occasionally in calves, and very rarely in horses. *Focal symmetrical encephalomalacia* (FSE) of sheep is caused by the epsilon toxin of type D. Epsilon toxin binds to receptors on endothelial cells, especially in the brain and renal tubular epithelial cells, resulting in changes described below.

In most **lambs** and **calves** with type D enterotoxemia, the course is peracute and the animal is found dead. Lambs and calves may die in a few minutes in convulsions, and calves often bawl as from severe pain. Animals that survive longer may show drooling, rapid breathing, hyperesthesia, straining, opisthotonos, and terminal coma or convulsions. In adult sheep, in which the clinical course may be several days, diarrhea with the passage of dark semifluid feces is common. In sheep, subacute cases may occur and be followed by recovery. In some such cases, neurological signs may develop. These include blindness, ataxia, head pressing, and caudal paraparesis; the lesions of FSE are present in the brains of such cases. On other occasions these lesions are not preceded by signs of enterotoxemia. In **goats**, signs of enterotoxemia similar to those in sheep and lambs may be seen, but chronic enterotoxemia characterized by abdominal distension and pain, depression, and dark-green diarrhea may persist for several days to weeks. Nervous signs do not occur.

Lambs fed large amounts of grain or concentrate are highly susceptible – thus the synonym "overeating disease." The manner in which overeating leads to clostridial enterotoxemia is complex. Cultures of *C. perfringens* type D given orally are largely destroyed in the rumen and abomasum. The few organisms that reach the intestine proliferate rapidly and produce toxin, but when the numbers are no longer reinforced by escapees from the stomachs, they are rapidly cleared from the intestine by peristalsis.

The critical factor is almost certainly the *presence of starch in the small intestine*, providing a suitable substrate for these saccharolytic bacteria, and they proliferate to immense numbers – perhaps more than 10^9 organisms per gram of intestinal contents – and produce correspondingly large amounts of toxin. When the animal is suddenly provided with excessive quantities of food, or food of a different type, there is a delay before the ruminal flora can adapt. In this period, undigested or partially digested food may escape into the intestine, and if starch is there, as it is with overeating on grain, *C. perfringens* type D is likely to take advantage of it. The epsilon prototoxin is activated by digestive enzymes, especially the combination of trypsin and chymotrypsin.

A high concentration of epsilon toxin facilitates its own absorption from the intestine, probably in part by increasing the permeability of the mucosa. Necrosis of epithelium and moderate atrophy of villi are evident in some animals with type D enterotoxemia, and it is reasonable to assume that epithelial damage precedes the facilitated absorption.

Probably the disease develops in the same way in the calf as in the sheep. The virtual confinement of the disease to calves that are overfed suggests that this is the case. The acute disease in goats likely has a similar pathogenesis but the chronic disease, with lesions confined to the intestine, appears to be caused by local effects of type D toxins.

In lambs dead of acute enterotoxemia, the carcass is usually well nourished. In those with a course of 1–2 days, there is often evidence of a dark scour about the rump. Putrefactive changes occur rapidly. Often there is excessive straw-colored pericardial fluid that clots on exposure to air and congestion and edema of the lungs that may be severe enough to produce froth in all the respiratory passages; and hemorrhage beneath the endocardium of the left ventricle. There may be hemorrhages beneath other serous membranes (Fig. 1.165) such as the epicardium, and blotchy hemorrhages beneath the parietal peritoneum are characteristic. Sometimes the liver is congested and the spleen enlarged and pulpy. There is no gastrointestinal inflammation visible at necropsy. Short lengths of the small intestine are distended with gas, and are hyperemic.

Epsilon toxin binds to receptors on distal renal tubular epithelial cells and causes selective degeneration of distal tubules in a variety of species. In experimental cases and natural cases in lambs examined immediately after death, there may be medullary congestion, hemorrhage, and tubular degeneration (Fig. 1.166). However, rapid autolysis or delayed necropsy can also produce "pulpy kidney." The renal "lesions" can be useful diagnostic aids, as are the hyperglycemia and glucosuria associated with the toxemia, if they can be detected.

In adult sheep, the lesions are the same as those in lambs, but are more consistent and more advanced, with the exception of renal autolysis, which occurs less rapidly, and less commonly progresses to the stage of "pulpiness."

Brain lesions occur in lambs with subacute enterotoxemia and these are sufficiently consistent to be of diagnostic significance. They

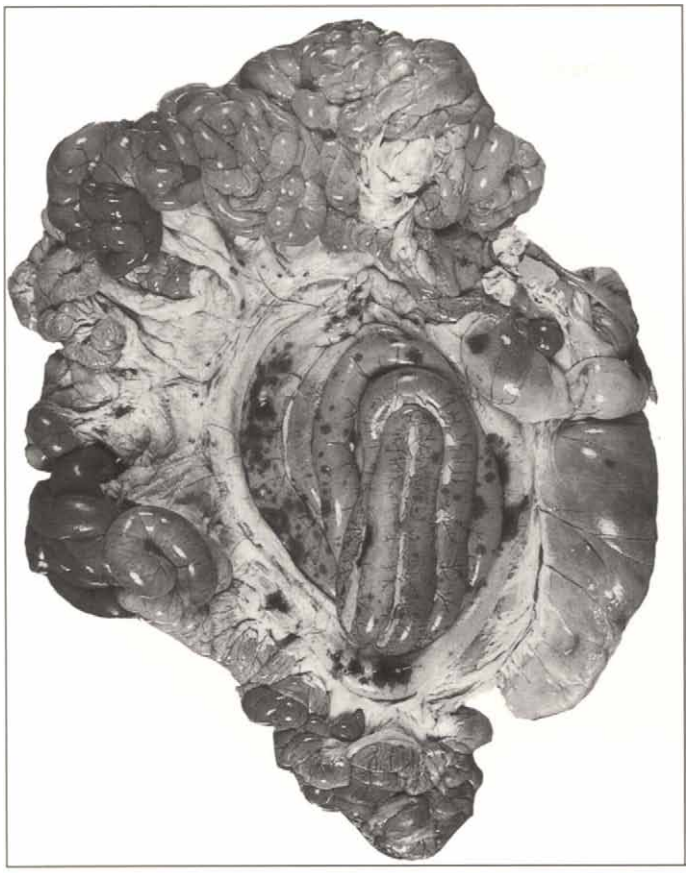

Figure 1.165 Peritoneal hemorrhages in a sheep with ***Clostridium perfringens* type D** enterotoxemia.

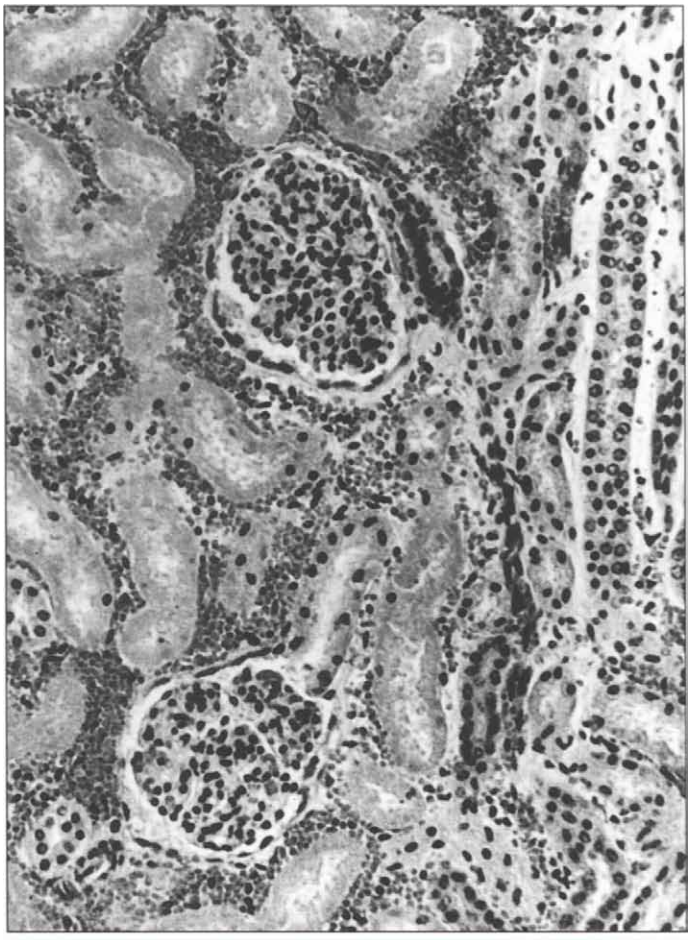

Figure 1.166 Nephrosis and intertubular hemorrhage in a sheep with ***Clostridium perfringens* type D** enterotoxemia.

develop in two patterns; each is bilaterally symmetric, hence the term **focal symmetrical encephalomalacia**. The commonest pattern involves the basal ganglia, internal capsule, dorsolateral thalamus, and substantia nigra; there are some minor variations of the pattern, but the lesions are always of the same type (Fig. 1.167). The second pattern affects the white matter of the frontal gyri, sparing only the communicating U fibers. The lesion begins with edema and the leakage of plasma and then red cells from the venules and capillaries in the affected areas. The altered permeability of the vessels is diffuse throughout the brain, sparing only heavily myelinated tracts such as the optic tracts and corpus callosum. This is well demonstrated by vital staining with trypan blue. The least change visible by light microscopy is the accumulation of protein droplets around small venules. Electron microscopically, *severe damage to vascular endothelium* is apparent and there is swelling of protoplasmic astrocytes. The foot processes around blood vessels and the processes around neurons are most severely swollen. Edema and hemorrhage lead to malacia in the affected areas.

Lesions in calves dying of enterotoxemia caused by *C. perfringens* type D may resemble those in lambs. Affected calves are usually 1–3 months of age. Splenic swelling is more common in calves than in lambs, and rapid autolysis of the kidney is not a prominent finding, as it is in lambs. However, subcapsular congestion and hemorrhage occur (Fig. 1.168), and sometimes a black clot of blood up to 1.0 cm thick forms around the kidney.

The *histologic changes* in enterotoxemia include, in addition to the brain lesions described above: mild degeneration and necrosis of the epithelium of the proximal convoluted tubules with edema, congestion, and interstitial hemorrhage in the renal cortex, and congestion of the medulla (these are autolytic changes but are useful diagnostically); superficial desquamation in the intestine with congestion, and numerous typical bacilli in the contents; congestion and hemorrhage of the spleen with disruption of reticulum; subepicardial hemorrhage and degeneration in the Purkinje network; and proteinaceous edema fluid in the lungs. All of these changes are secondary to endothelial damage produced by the epsilon toxin.

Type D enterotoxemia may be seen in both adult **goats** and kids. Four forms of the disease are recognized in goats: *peracute, acute, chronic, and subclinical*. The peracute disease is similar to that seen in lambs, and usually manifests as sudden deaths. The acute form is characterized by diarrhea and severe abdominal discomfort. Affected animals either recover or die within 2–4 days after the onset of clinical signs. The chronic form of the disease may last for a few days or weeks. Weight loss and diarrhea are its main clinical features. The principal macroscopic lesions in the acute and chronic disease are mild to severe hyperemia of the mucosa, especially of the distal small intestine, cecum, and spiral colon. The affected areas may be covered by a thin layer of fibrin. The intestinal contents are olive-green to red and mucoid. The mesenteric lymph nodes are enlarged and edematous. Hydropericardium, pulmonary edema, and "pulpy" kidneys may be seen, but are inconsistent, and brain lesions are absent.

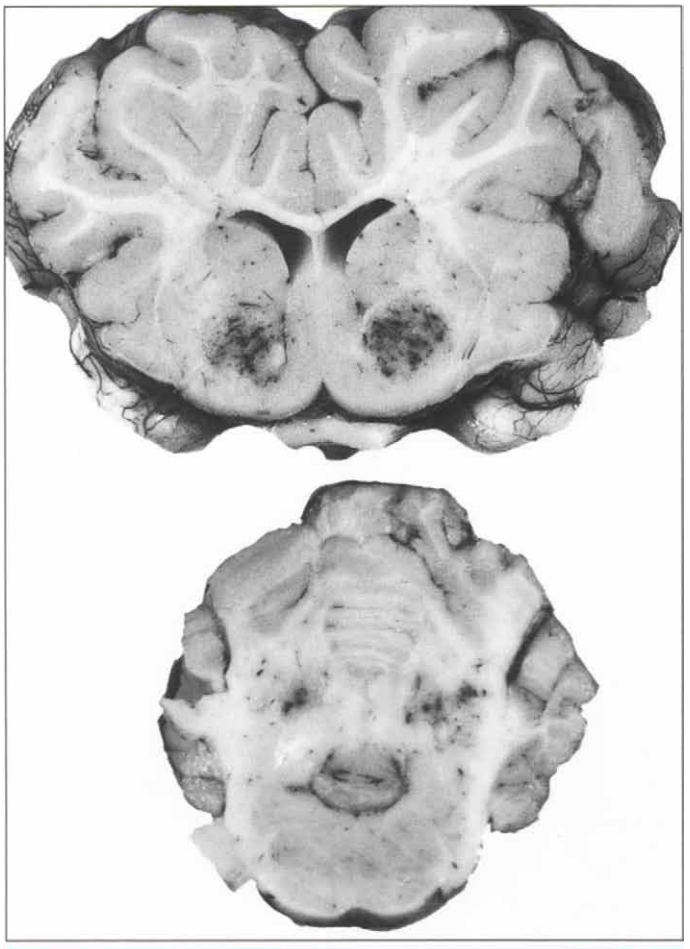

Figure 1.167 Focal symmetric encephalomalacia; hemorrhages and softening in internal capsules and cerebellar white matter in a sheep with *Clostridium perfringens* type D enterotoxemia.

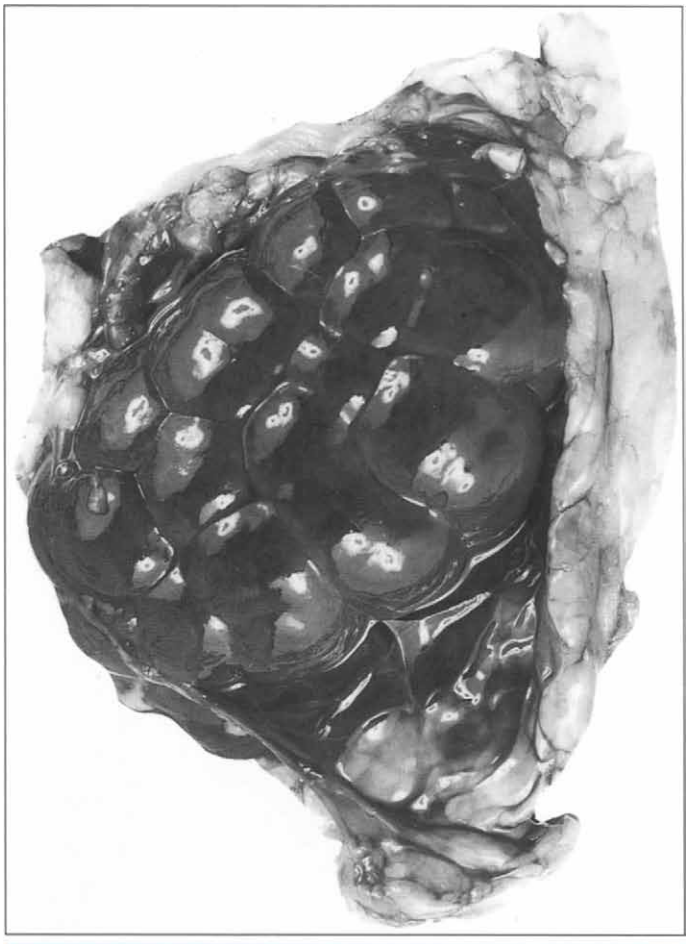

Figure 1.168 Renal cortical hemorrhage in a calf with *Clostridium perfringens* type D enterotoxemia.

In natural cases, the microscopic lesions in the small intestine vary from a mild pleocellular leukocytic reaction in the lamina propria to a mild fibrinous, or, rarely, hemorrhagic enteritis. In the latter, the tips of the villi are necrotic, eroded, and covered by fibrinocellular exudates or blood. Villus atrophy may follow. The leukocytic reaction in the lamina propria extends into the edematous submucosa. The proprial and submucosal vessels are congested. Essentially similar lesions occur in the large intestine, bearing in mind the anatomic differences. There is lymphocytolysis in the centers of the lymphoid follicles of the mesenteric lymph nodes. Lesions in the lungs and kidneys may be similar to, but are less consistent than, those seen in lambs. Cerebral edema may be present, but FSE has not been reported in goats with enterotoxemia, except after experimentally induced disease.

Experimental intraduodenal inoculation of whole cultures of *C. perfringens* type D in kids mainly results in colitis of the spiral colon. The reasons for the different manifestations of enterotoxemia in sheep and goats are unknown.

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Clostridium perfringens type E

Clostridium perfringens type E can cause intestinal disease in calves. Calves die acutely and have a congested ulcerated abomasum, and

Figure 1.169 Marked edema of the mesocolon in a neonatal piglet infected with ***Clostridium difficile***. (Courtesy of AP Loretti.)

hemorrhagic enteritis that occurs segmentally along the small intestine. Mesenteric nodes are enlarged and red, and pericardial effusion and serosal hemorrhages may be present.

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Other clostridial diseases

Clostridium difficile is a gram-positive to gram-variable inhabitant of the soil and gut. This organism causes pseudomembranous colitis in humans, often, but not necessarily, with a history of antibiotic treatment. Its role in enteric disease of animals is just emerging, and perhaps with the exception of swine, evidence in most species is associative, rather than conclusively causal. Definition of its diagnostic significance is dependent on identification of the A and B toxins in gut content or feces by tests with high sensitivity and specificity.

C. difficile is increasingly being recognized as a cause of diarrhea due to *fibrinous colitis* in **neonatal pigs**, under about a week of age, and the disease has been reproduced experimentally using pure cultures of the organism. Piglets have diarrhea, dyspnea, scrotal edema, and mild abdominal distension. Hydrothorax and characteristic, though not pathognomonic, *edema of the mesocolon* (Fig. 1.169) are evident grossly, usually in association with patchy to extensive fibrinous typhlocolitis, with yellow pasty to fluid content and feces. Microscopically, there is exfoliation and attenuation of surface enterocytes, and patchy focal erosions on the colonic mucosa, through which fibrin and neutrophils exude, producing the "volcano" lesions. Occasionally deeper necrosis of the mucosa and colonic wall may occur.

C. difficile and its cytotoxin have been demonstrated in feces of **dogs** with diarrhea. Descriptions of lesions are not available, however, since there are no necropsies or biopsies reported. However, the organism is frequently shed in feces of normal puppies and their bitches, which makes its significance difficult to interpret.

In **horses**, *C. difficile* may be incriminated in diarrheal syndromes, including *"proximal enteritis"* and *hemorrhagic enteritis* in foals, and *colitis* in horses of all ages. Diarrhea and colic have been reproduced in foals using inocula of *C. difficile* spores and vegetative cells.

Equine intestinal clostridiosis is still undergoing definition; many cases probably fall into the syndrome formerly described as "colitis X," the etiology of which has been undefined. The syndrome is one of *severe acute-to-peracute colitis*, and it is probably related to dysbacteriosis and clostridial overgrowth, instigated by antibiotic therapy, stress, or changes in feeding regimens.

The clostridia most commonly incriminated in this syndrome include $beta_2$ toxin-secreting *C. perfringens* type A and *C. difficile*. There is a positive association between the disease and the presence of *C. perfringens* enterotoxin and/or *C. difficile* toxin A. Lesions are usually confined to the large bowel. There is extensive hemorrhage and edema in the mucosa, submucosa, and mesocolon. Microscopically, full-thickness necrosis of the mucosa is associated with thrombosis of small vessels; variable numbers of bacilli may be visible in section, but the lesion is not readily differentiated from other forms of severe acute colitis, for instance salmonellosis.

Tyzzer's disease is caused by the obligate intracellular bacterium ***Clostridium piliforme***. It is an uncommon affliction of many species of mammals, among them horses, cats, and dogs, though there seem to be bacterial strain differences that determine host susceptibility. Affected animals are often very young, or appear to be immunocompromised in some way.

While the lesions are not limited to the intestinal tract, animals become infected initially through the epithelium of the ileum, cecum, and colon, where lesions may vary from subtle catarrhal to fibrinohemorrhagic. Bacilli can be demonstrated using silver stains in enterocytes, often forming characteristic "pick-up-sticks" arrays. Ultimately, in most cases they disseminate elsewhere in the body, especially to liver and myocardium, where they cause acute-to-subacute necrotic lesions. The disease is discussed more fully in Vol. 2, Liver and biliary system.

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Paratuberculosis (Johne's disease)

Johne's disease is caused by ***Mycobacterium avium* subsp. *paratuberculosis*** infection. The etiologic agent of Johne's disease has been reduced to subspecies status within *M. avium* on the basis of the high (>90%) DNA homology among typical paratuberculosis strains and type strains of *M. a. avium*. The Johne's disease agent, which in culture is slow-growing, and dependent on mycobactin as a source of iron, possesses some unique cultural and biochemical traits. Genetically, a distinct difference from *M. a. avium* is the presence of the insertion sequence, **IS900**, of which *M. a. paratuberculosis* has 15–20 copies per organism.

The virulence attributes of *M. a. paratuberculosis* are poorly understood, but presumably reside in *resistance to killing in macrophages*, through inhibition of the conversion of phagosomes to phagolysosomes. The organisms proliferate in cytoplasmic vacuoles, and transmit to adjacent macrophages, expanding the population of infected cells and recruiting elements of the humoral and cell-mediated arms of the immune system to the site. Diffuse foci of granulomatous inflammation accumulate, reflected in a distinct profile of local cytokine expression. Infected macrophages traffic via lymphatics to the draining lymph nodes and via the portal venous drainage to the liver, ultimately gaining the central circulation.

The disease is most common in *domestic ruminants*, but infections by *M. a. paratuberculosis* can also be produced in pigs, and spontaneous disease occurs in a number of free-ranging and captive wild ruminants, camelids, and, rarely, in equids and captive primates. Numerous species of wild mammals, including lagomorphs, rodents, and carnivores, and several species of wild birds, are naturally infected, though not necessarily diseased. The infection can be transmitted experimentally to mice, hamsters, guinea pigs, rabbits, and macaques. A debate exists as to the role of *M. a. paratuberculosis* in the genesis of *Crohn's disease in humans*, a chronic granulomatous enteritis. *M. a. paratuberculosis* has been found in many of these cases, as detected by polymerase chain reaction of reactive fragments, but it is still undecided whether the association is causal or coincidental.

The **epidemiology and pathogenesis** of Johne's disease are best understood in cattle, and are assumed to be similar in sheep and goats. Infection is systemic, and organisms may be present in milk, semen, and urine, and may cross the placenta. However, exposure is mainly by ingestion of organisms shed in the feces. This may explain higher prevalence in dairy as opposed to beef animals. Susceptibility to infection is greatest in the first 30 days of life, although clinical disease does not usually develop in cattle until 2–5 years of age. This long incubation period has been termed "the iceberg effect" because, in any infected herd, although few animals may be showing clinical signs of Johne's disease, a much greater number are silently infected. Adults may become infected, but are less likely to develop the disease, and often recover from the infection. In addition, evidence is accumulating that the epidemiology is influenced by the soil and pasture type, presumably because these factors can affect the survival and proliferation of organisms in the environment.

Bacteria are taken up by M cells of the dome epithelium over lymphoid follicles and transported to macrophages in Peyer's patches. The immaturity of macrophages in younger animals, combined with the greater volume of organized intestinal lymphoid tissue, may help to explain the age susceptibility of infection. *The major lesions of Johne's disease are usually confined to the ileum, large intestine, and draining lymph nodes.* However, the infection is generalized, because in both clinical and subclinical cases, the organism can be cultured from a variety of parenchymatous organs and widely distributed lymph nodes. In fulminating infections, there is *bacteremia*, in blood or in infected phagocytes.

The incubation period of Johne's disease is protracted and irregular. Some carriers, in which bacteria persist in the mucosa and draining lymph nodes, may be infected for life without showing signs. The relationship between immune events and stages of the disease is speculative. Cell-mediated immunity clearly plays a role in the development of mucosal lesions and the onset of clinical disease. Exacerbations of clinical disease are often associated with parturition, a low nutritional plane, heavy milk yield, and intercurrent disease.

The pathogenesis of Johne's disease is related to the *granulomatous immunoinflammatory response in the lamina propria in the small intestine*, and the associated villus atrophy that develops. Malabsorption in the ileum, and filtration secretion from inflamed mucosa, overloads the capacity of the colon to resorb electrolyte and fluid; the function of the colon itself may be compromised by mycobacterial infection. There is malabsorption of amino acids, and enteric loss of plasma proteins, causing reduced productive efficiency, and when negative nitrogen balance occurs, a decline in body condition, and ultimate emaciation. Hypoproteinemia, when it develops, will further promote filtration secretion.

Clinically affected cattle are usually 2 years of age or older. The typical manifestation of Johne's disease is *profuse diarrhea passed effortlessly*. Clinical signs may be intermittent, with long intervening periods of remission. *Emaciation is progressive and ultimately fatal*, but the appetite is often retained and animals remain bright until the terminal stages.

Grossly, advanced cases of Johne's disease have marked loss of muscle mass and serous atrophy of fat depots, intermandibular edema, and fluid effusion in the body cavities. Plaques of intimal fibrosis and mineralization may be evident in the thoracic aorta. Specific gross lesions occur in the intestine and regional lymph nodes. The *mesenteric nodes, particularly the ileocecal, are always enlarged*, sometimes remarkably so, pale, and edematous, especially in the medulla. *Lymphangitis* is common, and the lymphatic vessels can often be traced as thickened cords from the intestinal serosa through the mesentery to the mesenteric nodes (Fig. 1.170). Often lymphangitis is the only recognizable gross change, and is specific enough to justify a presumptive diagnosis of Johne's disease at necropsy. The intestinal serosa often has a slight granular and diffusely opaque appearance because of subserosal edema and cellularity.

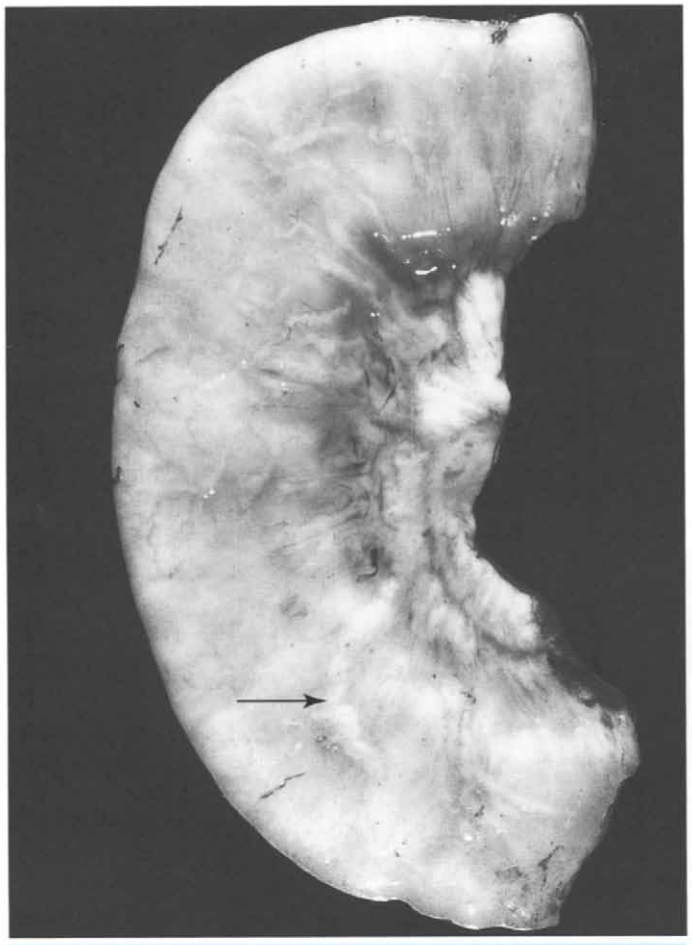

Figure 1.170 Serosal edema and **lymphangitis** (arrow) in a sheep with Johne's disease.

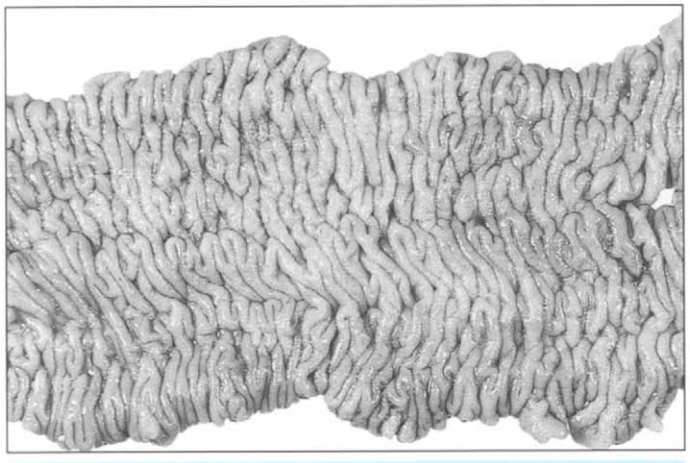

Figure 1.171 Thickened mucosal folds in the jejunum of a cow with **Johne's disease**.

Mucosal lesions may occur from the duodenum to the rectum; they may be segmental or continuous. They are usually best developed in the *lower ileum* (Fig. 1.171), and in the upper large intestine. The ileocecal valve is frequently described as enlarged, and this area is considered by some to be the earliest and most consistently affected, but abnormalities in this vicinity may not be notable.

The classical intestinal change is *diffuse thickening of the mucosa*, which is folded into transverse rugae, the crests of which may be congested. When well developed, the mucosal folds cannot be smoothed out by stretching. This lesion is due to accumulation of chronic inflammatory cells and edema in the mucosa and submucosa.

When gross lesions are well developed, the characteristic **microscopic change**, *transmural granulomatous enteritis*, is obvious. But in cattle in which gross changes are minimal or absent, the microscopic abnormalities are more subtle. In these the lamina propria is diffusely infiltrated with lymphocytes and plasma cells, and a large number of eosinophils. There may be very few macrophages (Fig. 1.172A), and the most characteristic change is an infiltrate of lymphocytes and plasma cells in the submucosa, and associated with the submucosal and mesenteric lymphatics.

In more clear-cut cases, villi are moderately to markedly atrophic, and macrophages are focally or diffusely distributed, in the villi, or deeper in the lamina propria, as part of an increased chronic inflammatory cell infiltrate. Giant cells may be present. The inflammatory infiltrate may abnormally separate and displace crypts, which are elongate, with hyperplastic epithelium. Crypts may be distended with mucus and exfoliated cells, probably due to compression and obstruction of their mouths by edema and inflammatory cells (Fig. 1.172B). *Masses of epithelioid macrophages may accumulate in the submucosa.* Foci of necrosis may occur within these aggregates of macrophages (Fig. 1.173), but in cattle, caseation and mineralization are extremely rare.

Lymphangitis is one of the most consistent changes. Initially the lymphatics are surrounded by lymphocytes and plasma cells and many contain plugs of epithelioid cells in the lumen. Granulomas may form in the wall and project into the lumen. These nodules may undergo some central necrosis.

Granulomatous lymphadenitis occurs in mesenteric lymph nodes in advanced cases. In the early stages, there is histiocytosis of the subcapsular sinus. Ultimately, nodular or diffuse infiltrates of epithelioid macrophages and giant cells may replace much of the cortex, and infiltrate the medullary sinusoids.

Of the other organs and tissues from which the bacilli may be isolated in cattle, lesions have only been described in the liver, hepatic lymph nodes, and, very rarely, the kidney and lungs. These are characteristically *focal granulomas*. They are most common in the liver, where foci of epithelioid cells and lymphocytes are found in the triads and scattered throughout the parenchyma. These lesions usually contain *demonstrable bacilli*, if macrophages are evident.

In sheep and goats, Johne's disease mainly occurs in adults and is characterized by *chronic wasting*; there may be breaks in the wool in sheep, and submandibular edema due to hypoproteinemia. The feces are often normal; they may be soft and unpelleted, but overt diarrhea is unusual, except intermittently in the terminal stages, perhaps because of the innately greater efficiency of electrolyte and fluid absorption in the colon of these species. In **farmed deer**, Johne's disease is clinically similar, but there are reports of disease in animals well under a year old.

In sheep, goats, and deer, enteric gross lesions are often mild, with little obvious thickening, and no transverse ridges; they are easily missed at necropsy. In sheep and goats, the bowel is occasionally quite remarkably thickened, and the lymphatics may be knotted as well as corded, the knots being focal granulomatous accumulations of epithelioid cells and lymphocytes, with relatively few organisms. Others may have an intestinal inflammatory infiltrate that is more lepromatous in

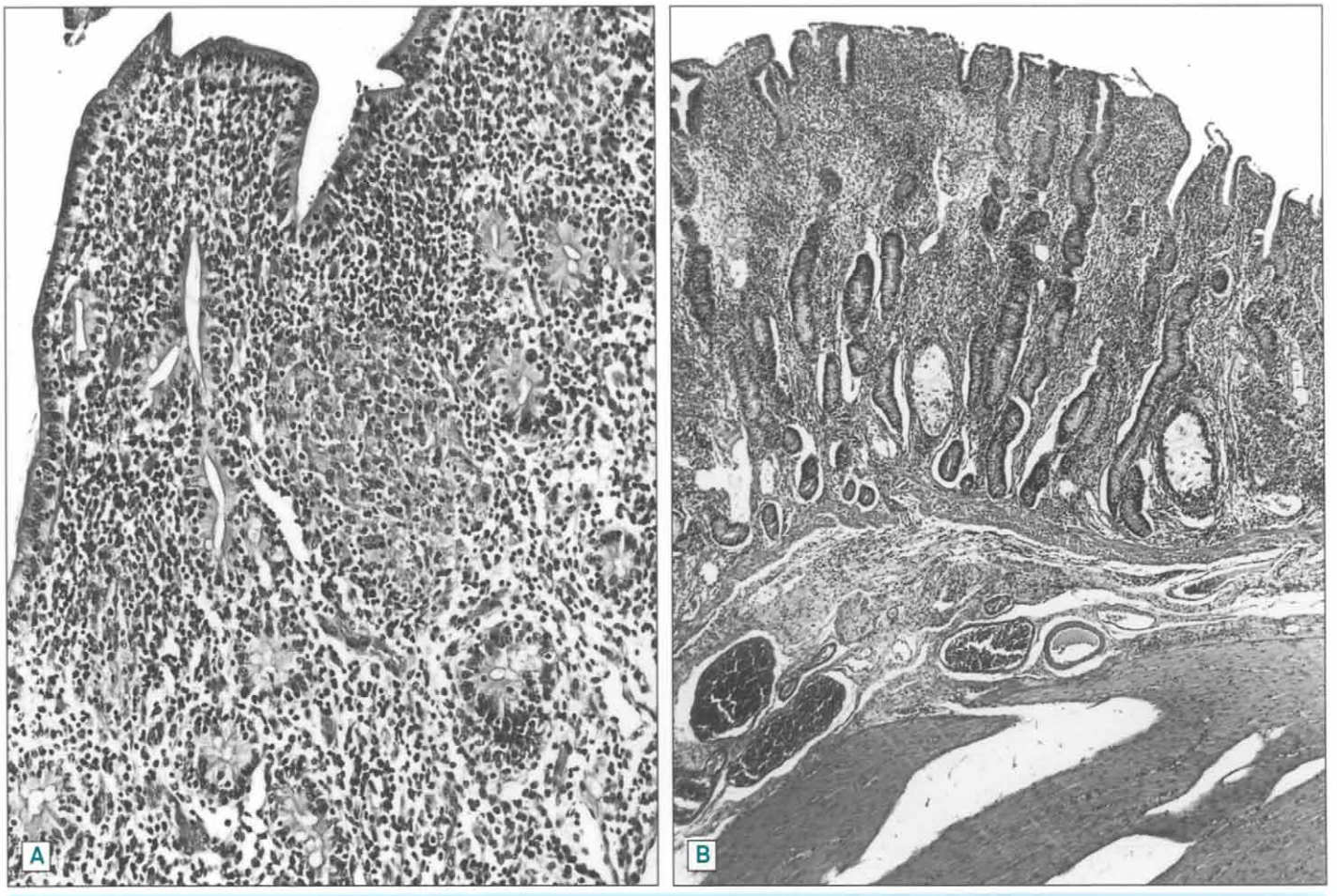

Figure 1.172 Johne's disease in a cow. **A.** Aggregate of **macrophages** in hypercellular lamina propria. **B.** Blunt **atrophic ileal villi**, and hyperplastic, and occasionally cystic, crypts. Edema of lamina propria, submucosa, and muscularis. Note heavy **inflammatory infiltrate** in lamina propria.

nature, with abundant organisms. In small ruminants, there may be lymphadenopathy affecting internal lymph nodes in particular, and variable lesions in the walls of blood vessels, meninges, liver, and spleen, all related to irregular lymphogranulomatous inflammatory foci. This latter lepromatous appearance is associated with negative intradermal skin test responses to johnin.

Goats, and some sheep, develop foci of tubercle-like caseation, often with mineralization, in the mucosa, submucosa, serosa, and lymphatics. Some may be grossly visible as white foci 1–4 mm in diameter, with modest surrounding fibrosis. Tubercles in the mucosa and lymph nodes of goats and deer may also mineralize (Fig. 1.174), and be large enough to replace much of the node. In sheep infected with the pigmented strain of the organism, the mucosa and lymph nodes may be orange. Scattered lymph nodes elsewhere in the body, and liver, lung, spleen, and other organs, may contain focal granulomatous lesions in sheep and goats. Some may mineralize. Lesions resembling those of lepromatous leprosy, with lymphocytic neuritis, have been described in the intestine of sheep, and axonal degeneration of the brachial and sciatic nerves has been reported in goats. Amyloidosis involving glomeruli, and occasionally other tissues, has also been reported in goats.

The organism is usually readily demonstrable in macrophages and giant cells in the lesions when appropriately stained by acid-fast techniques. However, in some clinical cases, especially the paucibacillary form in sheep, an extensive search may have to be made for individual macrophages or giant cells bearing a few acid-fast bacilli. Johne's disease in sheep, and especially in goats and deer, may resemble tuberculosis, on account of caseation and mineralization in granulomatous foci, and for such cases, and in any other situation where there is uncertainty about the acid-fast organism involved, positive identification of the etiologic agent is necessary. Antibodies with well-defined specificity for *M. a. paratuberculosis* have allowed the development of immunohistochemical tests, but these are not helpful when there are very few organisms. Polymerase chain reaction techniques are useful for confirming the diagnosis in individual cases. For isolation, the ileocecal lymph node, and affected segments of gut, are candidate sites for culture to confirm a diagnosis. Isolation may be difficult to accomplish, especially from sheep.

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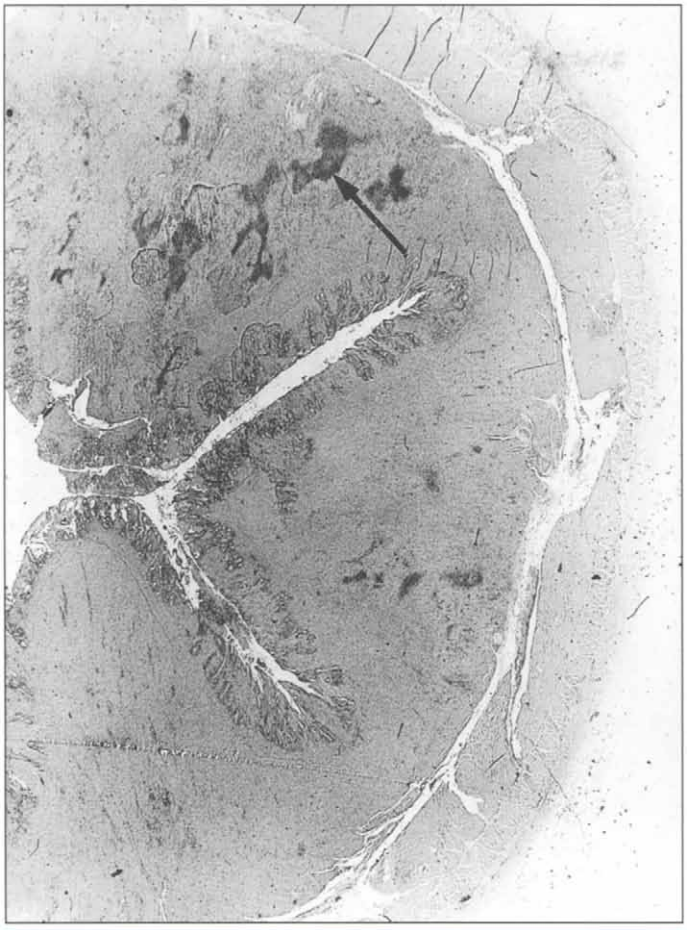

Figure 1.173 Hemisection of ileum showing **diffuse infiltration of cells** and small areas of **necrosis** (arrow) in **Johne's disease**.

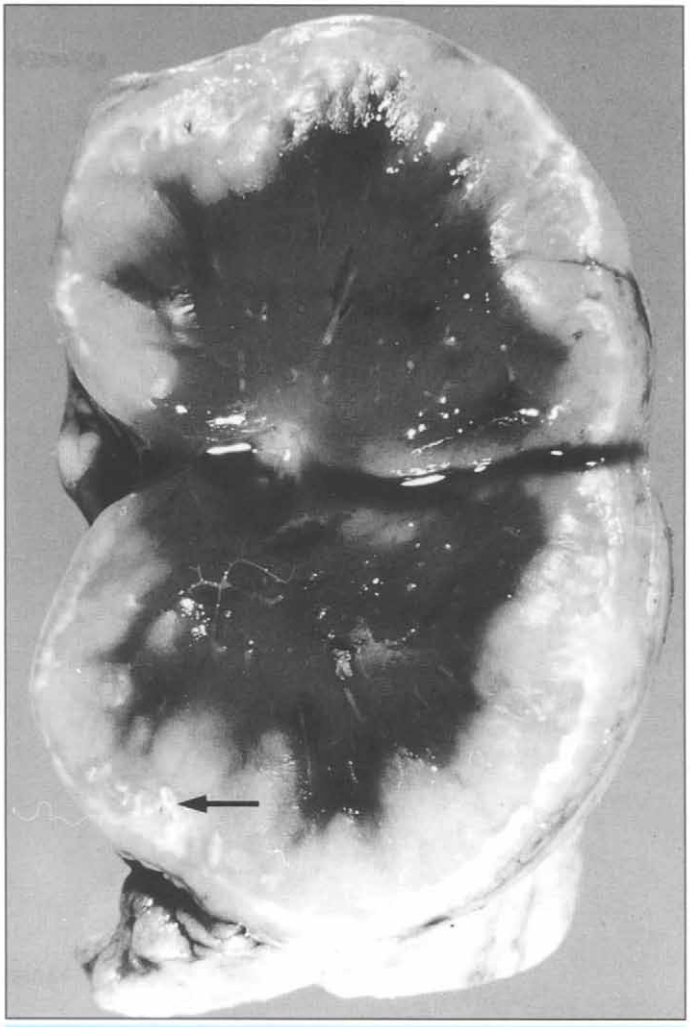

Figure 1.174 Ileocecal lymph node in a goat with **Johne's disease**. **Hyperplasia of lymphocytes** in the cortex. White foci of **mineralized caseous necrosis** in cortex (arrow).

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Rhodococcus equi enterocolitis of foals

Rhodococcus equi is an intracellular pathogen found in soil and as part of the normal intestinal flora of horses. There are both virulent and avirulent forms, with various *virulence factors* proposed, including capsular polysaccharide, cholesterol oxidase, cell wall mycolic acids, and a plasmid-encoded surface-expressed protein VapA. On farms where disease due to *R. equi* is endemic, there is a much higher proportion of virulent forms. Virulence factors may not be necessary for production of disease in an immunocompromised host. *R. equi* is usually associated with *suppurative bronchopneumonia of foals*. About half the pneumonic foals also have *ulcerative colitis*, and in some foals intestinal lesions alone occur.

The development of intestinal lesions appears to be dose-related, in that reproduction of the disease requires repeated oral infection. In natural disease, continual exposure to bacteria in swallowed respiratory exudate is probably an important source of infection in those animals with pneumonia.

Gross lesions may occur throughout the small and large intestines, but are usually most severe over Peyer's patches in small intestine, and in the cecum, large colon and related lymph nodes (Fig. 1.175A). Mucosal lesions consist of irregular ulcers up to 1–2 cm in diameter, often covered by purulent or necrotic debris (Fig. 1.175B). Edema of the wall of the gut may be severe. Mesenteric or colonic lymph nodes are often massively enlarged by edema and by caseous or purulent foci that may obliterate the structure of the node. Occasionally, massively enlarged abscessed lymph nodes are found without evidence of concurrent enteritis.

Microscopically, infection seems to occur by penetration of the specialized epithelium over Peyer's patches or intestinal lymphoid follicles. An initial neutrophilic response occurs and erosions of the epithelium develop subsequently. Macrophages and neutrophils accumulate in the lamina propria. The macrophages contain aggregates of *R. equi* but do not destroy them. Later, necrosis of lymphoid follicles occurs, and deep ulcers develop that contain masses of neutrophils, macrophages, and multinuclear giant cells. Pyogranulomatous lymphangitis and mesenteric lymphadenitis characterize the chronic enteric disease.

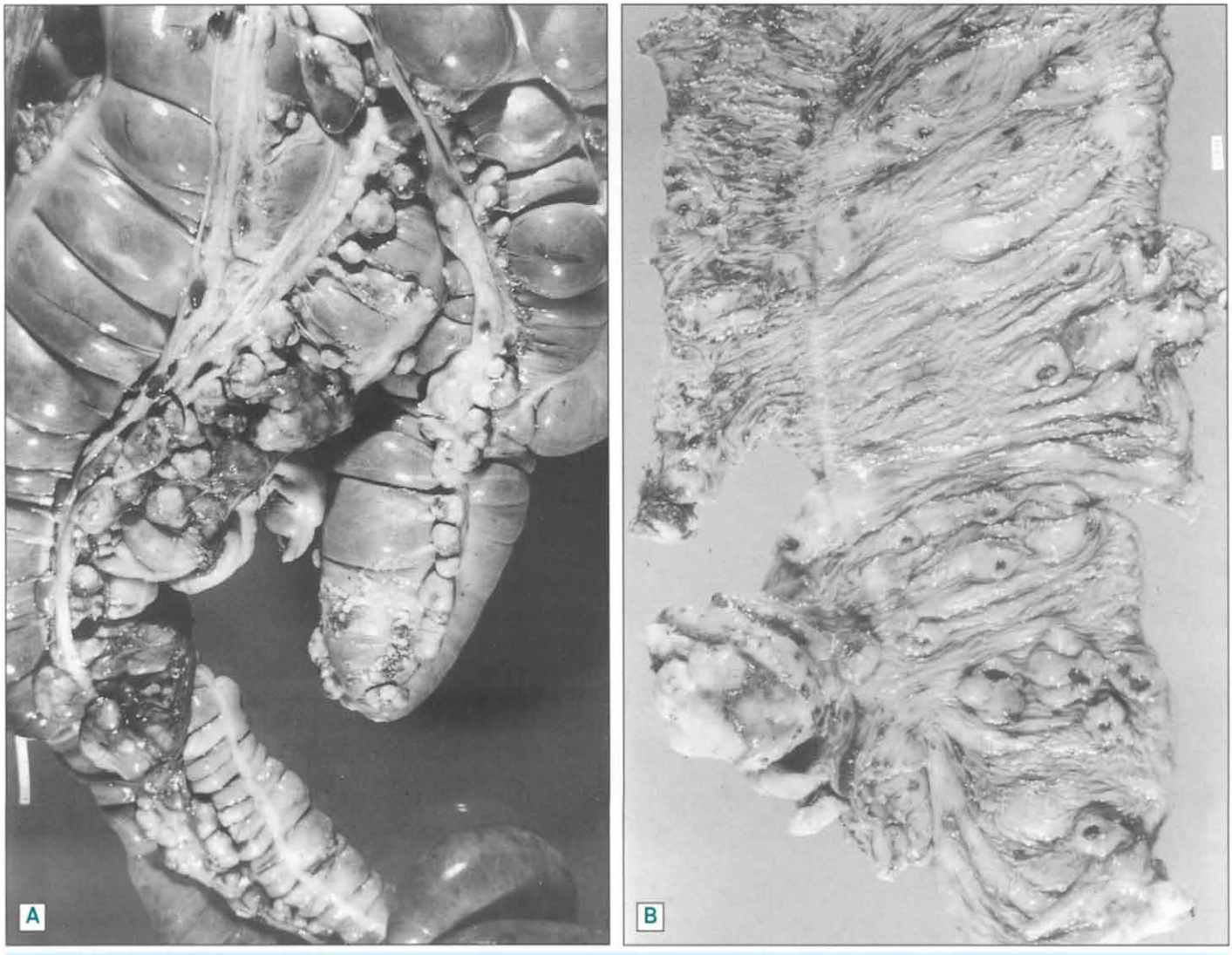

Figure 1.175 *Rhodococcus equi* infection in a foal. **A.** Enlarged suppurative cecal and colic lymph nodes. **B.** Craterous ulcerated lesions on colonic mucosa.

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Enterococcus sp. enteritis

Small gram-positive cocci identified as *Enterococcus durans*, found in the environment and the gut of various species, have occasionally been associated with diarrhea in suckling pigs, puppies, foals, calves, and suckling rats. The organisms from rats have been described, on genetic grounds, as *E. ratti*, whereas those from piglets have been described as *E. villorum* or *E. porcini*, which may be synonyms.

These bacteria adhere to the microvillus surface of enterocytes by fine filamentous pili. In tissue section, they form a layer of small cocci crowded on the entire surface of epithelial cells, from the tips to the base of villi. There may be mild-to-moderate villus atrophy and some desquamating enterocytes. Malabsorption associated with reduced brush border enzyme activity may explain diarrhea. Although in spontaneous cases in piglets, *Enterococcus* is frequently associated with other pathogens, the organism isolated from foals produced diarrhea when inoculated alone into gnotobiotic pigs.

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Bacteroides fragilis-associated diarrhea

Bacteroides fragilis is a nonspore-forming obligate anaerobe that is part of the normal enteric flora. Some enterotoxin-secreting strains have been associated with diarrhea in piglets, calves, lambs, foals, and humans. This enterotoxin is a *protease*, and probably damages the zonula adherens at the tight junction between enterocytes. Enterotoxigenic strains or cell-free culture filtrates cause secretion in ligated lamb or calf intestinal loops, and bacterial inocula cause diarrhea when administered orally to gnotobiotic piglets.

Bacteria do not adhere to the surface. Surface enterocytes round up and exfoliate, with villus attenuation and crypt elongation and hyperplasia. Infiltration of neutrophils is common. Damage may be seen in both small and large intestines. Ultrastructurally, affected cells lose their intercellular interdigitations, microvilli are shortened or absent, and the terminal web is disrupted.

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Anaerobiospirillum ileocolitis

In **cats**, ileocolitis has been associated with small spiral gram-negative bacteria, probably of the genus ***Anaerobiospirillum***. Cats may be asymptomatic, lethargic, and anorexic, or have vomiting and diarrhea. Microscopically, exfoliated epithelial cells and neutrophils are in dilated crypts in the ileum and colon, and bacteria stained with silver can be found in the lumen of crypts, in goblet cells, and sometimes in the lamina propria. Septicemia may occur, and renal failure has been associated.

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Enteritis due to Chlamydiaceae

Members of the family Chlamydiaceae are *obligate intracellular parasites*. Two genera are recognized on the basis of molecular genetics: *Chlamydia* and *Chlamydophila*. Among the three species in the former genus, only ***Chlamydia suis*** in pigs is associated with enteritis, while only one of the six species in the latter genus, ***Chlamydophila pecorum***, is associated with enteritis, in calves. Other syndromes associated with Chlamydiaceae in domestic animals include respiratory disease, polyarthritis, conjunctivitis, abortion, and encephalomyelitis, discussed in appropriate chapters elsewhere in these volumes.

The intestinal tract is the natural habitat for *C. pecorum*. Most infections are probably inapparent, but the intestine may be an important portal of entry in the development of systemic infections leading to hepatitis, arthritis, encephalitis, and pneumonia in ruminants. Enteritis may accompany or presage these diseases, and occasionally *C. pecorum* causes severe enteric disease in calves.

Following oral infection, *C. pecorum* infects mainly the enterocytes on the tips of ileal villi. These cells are in the G_1 phase of the cell cycle, which is required by *Chlamydophila* for multiplication. *C. pecorum* also infects other cells, including goblet cells, enterochromaffin cells, and macrophages, and the latter cells may transport the organisms systemically prior to being destroyed by them.

C. pecorum adsorbs to the brush border of enterocytes and enters the cell by pinocytosis. Following multiplication of organisms in the supranuclear region, the cells degenerate. *Chlamydophila* are released into the gut lumen, and into the lamina propria, where they infect endothelial cells of lacteals, whence they are released and become systemic.

Gastrointestinal disease caused by *Chlamydophila* is usually a problem of **calves** less than 10 days old, but it may affect older calves, and can produce recurrent diarrhea. Watery diarrhea, dehydration, and death are often accompanied by lesions, though not necessarily signs, of hepatitis, interstitial pneumonia, and arthritis. Gross lesions may occur in the abomasum and throughout the intestinal tract but are most consistent and severe in the *terminal ileum*. Mucosal edema, congestion, and petechiae, sometimes with ulceration, are usually observed. Serosal hemorrhages and focal peritonitis may occur. Histologically, *Chlamydophila* in inclusions may be demonstrable with Giemsa, Macchiavello, or immunoperoxidase staining. Central lacteals and capillaries are dilated, and neutrophils and monocytes infiltrate the lamina propria. Occasionally, granulomatous inflammation occurs in the intestinal submucosa and extends into the mesentery and to the serosa, producing the peritonitis observed grossly. Crypts in the small and large intestine may be dilated, lined by flattened epithelium, and contain inflammatory exudate.

C. suis has been recognized in the intestinal mucosa of **swine**, with approximately equal frequency in diarrheic and nondiarrheic animals. After experimental inoculation of *C. suis* into gnotobiotic piglets, there was necrosis and exfoliation of enterocytes on the apical half of villi, resulting in mild-to-severe villus atrophy in the distal jejunum and ileum, with moderate diarrhea. Lymphangitis and perilymphangitis were also evident in affected gut. In weanling pigs, similar lesions were induced, but no diarrhea.

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Potomac horse fever

This condition, also described as *equine monocytic ehrlichiosis* and *equine ehrlichial colitis*, was first defined clinically in 1979, and is characterized by fever, leukopenia, depression, loss of appetite, and diarrhea. It is caused by ***Neorickettsia*** (formerly *Ehrlichia*) ***risticii***, a member of the order Rickettsiales, which are *obligate intracellular bacterial pathogens*. *Neorickettsia* spp. replicate within the phagosome in the host cell, and use trematodes as hosts. Potomac horse fever typically occurs in the summer. It was first described in the Potomac river valley, and is associated with other river valleys in the northeastern USA. The condition is not commonly diagnosed as a cause of death elsewhere, though antibody has been reported from horses in many parts of North America, and in Europe. *N. risticii* in trematode metacercariae found in aquatic insects are probably ingested accidentally while drinking. The definitive hosts of trematode reservoirs are insectivorous bats, which themselves become infected with *N. risticii*.

The disease may be highly variable. Many infected horses seem not to get sick. Others develop severe colic, subcutaneous edema, laminitis, and shock: mortality can be up to 30% in untreated cases. Abortions of pregnant mares have been attributed to *N. risticii* infection.

The incubation period in experimental infections is about 9–14 days, and diarrhea begins 1–3 days after the onset of fever. Not all experimentally infected animals develop disease.

At **necropsy** of spontaneous cases, small vesicles are reported in the oral cavity, and epicardial hemorrhages and pulmonary congestion and hemorrhage, compatible with endotoxemia, are described. These are not reported in experimental cases; nor is laminitis. The lesions of the gastrointestinal tract are the most significant, in both spontaneous and experimental cases. In some animals there may be focal or more extensive erosions in the gastric mucosa, perhaps with some overlying fibrinous exudate. Lesions in the small intestine are generally limited to segmental areas of mucosal congestion or hyperemia, with occasional focal ulcers or hemorrhage, and are much less consistent and severe than those in the *cecum and colon*. The content of the large bowel is abnormally fluid, and may have a brown or red-brown color, and foul odor. In the cecum and colon, there may be patches of hyperemia 5–10 cm in diameter, aggregates of small ulcers a few millimeters in diameter, and petechial hemorrhages. Sometimes the mucosa of the entire cecum is widely hyperemic. Ulcers and petechial hemorrhage are more severe and consistent in the right dorsal colon. The small colon is usually unaffected grossly.

Microscopic lesions are most consistent in the large intestine; similar changes may be evident in small bowel. In areas of gross hyperemia, there is marked congestion and superficial hemorrhage in the mucosa. Associated with these lesions are superficial epithelial necrosis, erosion, and fibrin effusion. The mucosal surface is denuded, or perhaps covered by fibrinocellular exudate, and epithelium in the upper half of crypts is attenuated. Deeper parts of crypts are dilated and may contain necrotic epithelium and inflammatory cells. An abnormally intense mixed inflammatory cell population is in the lamina propria, and sometimes, the submucosa. Lymphoid tissue in the gut, mesenteric lymph nodes, and spleen is moderately involuted, compatible with the effects of the stress of systemic illness.

Organisms are not evident in hematoxylin and eosin-stained tissue. They are visible, in colon, and less consistently, in cecum, small colon, and small intestine, with *modified Steiner silver stain*. They appear as small clusters of 10–15 fine brown dots, under 1 μm in diameter, in the apical cytoplasm of epithelial cells deep in crypts, or as more numerous, smaller black structures distributed in the cytoplasm of macrophages in the periglandular lamina propria, or in a few glandular epithelial cells. Ultrastructurally, small dense elementary bodies may be found, alone or in small clusters in vacuoles in the cytoplasm of macrophages, mast cells, and crypt epithelium, or as the morula – aggregates of larger, more open organisms, in the same locations. *N. risticii* can be identified in feces or peripheral blood buffy coat by polymerase chain reaction, providing a more sensitive and specific means of diagnosis.

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Mycotic diseases of the gastrointestinal tract

Intestinal phycomycosis and aspergillosis

Mycotic invasion of the wall of the gastrointestinal tract is a common sequel to many diseases and lesions affecting the mucosa, and it may be the precursor to systemic infection. It is generally agreed that one or more of heavy fungal challenge, disruption of the normal flora, a primary local lesion, or lowered host resistance, is required for establishment of mycotic disease in the gut. Spores are probably carried across the mucosa by macrophages in the normal course of events, and only if phagocyte function is compromised, permitting germination, will they establish in the deeper tissues or become disseminated. Neutrophil function seems important in prevention of establishment of mycoses, whereas T-cell-mediated macrophage activity is involved in resolution of lesions.

Organisms associated with alimentary tract mycoses are *Aspergillus*; zygomycetes of the family Mucoraceae, including the genera *Absidia*, *Mucor*, and *Rhizopus*; the aquatic oomycete *Pythium*; and entomophthoracetes such as *Basidiobolus* and *Conidiobolus*. *Candida* spp. may also invade the wall of the alimentary canal; that is considered separately, below, as are enteric manifestations of histoplasmosis.

Aspergillus has relatively uniform narrow (3–6 μm) hyphae with relatively numerous septa; it typically displays acute angled dichotomous branching. **Mucoraceous fungi** are characterized in their invasive mycelial form by broad (6–25 μm), coarse irregular hyphae, with infrequent septation and random branching, sometimes surrounded by an eosinophilic sleeve in tissue sections. ***Pythium* spp.** have relatively narrow (up to 9–10 μm), thick-walled hyphae, with almost parallel walls and occasional septa; they branch at about right angles. In **entomophthoromycosis**, hyphae may vary from 5 to 25 μm in diameter, with thin irregularly parallel walls, infrequent septa, and rare random branching. They are characteristically surrounded by a wide sheath or sleeve of eosinophilic material in tissue section.

Lesions occur anywhere in the gastrointestinal tract, including the forestomachs of ruminants, and in the mesenteric lymph nodes. Clinical signs may be specifically related to the location of lesions (vomition, bloody diarrhea), or be nonspecific (malaise, weight loss), or be absent. Three types of lesion are produced: (1) *hemorrhagic and infarctive*; (2) *caseating*; *and* (3) *granulomatous*.

Mucoraceous fungi and ***Aspergillus*** typically cause *hemorrhagic and infarctive lesions*. Cases in cattle are seen following grain overload, mastitis, "downer cow syndrome," parturition, and subsequent to neonatal infectious bovine rhinotracheitis infection. Mucoraceous fungi frequently complicate Peyer's patch necrosis in cattle with mucosal disease, and are seen along the margins of the abomasal folds in calves with bacterial septicemia, producing mycotic abomasitis. These fungi can be found at any level of the gastrointestinal tract; however, omasum followed by rumen and reticulum are the most common sites. *Aspergillus* tends to be most common in the abomasum. These fungi have a propensity to invade mucosal and submucosal veins, producing *thrombosis and venous infarction*. Characteristically there is mucosal to full-thickness necrosis of the wall, which grossly is edematous and red-black due to venous stasis and hemorrhage. Often there is a relatively mild inflammatory response to the fungi. Spread to the liver and more distant organs via the portal and systemic circulations is not uncommon.

Mycotic ileitis and colitis in cats may occasionally be a sequel to panleukopenia. Intestinal lesions caused by the fungi (often *Aspergillus*) may be hemorrhagic and necrotizing with a prominent cellular response, but sometimes are small, localized, and difficult to find. In the latter cases, lesions of panleukopenia in the intestine and multifocal mycotic emboli in the lung suggest the pathogenesis. The lung appears to be the favored site of dissemination in cats. Mycotic enteritis with dissemination is a rare sequel to *Canine parvovirus 2* enteritis.

The gastrointestinal tract is probably a common portal of entry for many sporadic, disseminated zygomycoses, and aspergillosis, in animals. The presence of fungal hyphae in mesenteric lymph node granulomas of many clinically normal cattle indicates that, contrary to general impressions, *invasion by these agents across the intestinal mucosa does not lead invariably to systemic disease*. Fungi may produce a localized granulomatous lesion in specialized lymphoid tissue of the Peyer's patch or may be carried to the regional lymph node, while the mucosal lesion, if any, heals. In the lymph nodes, a granulomatous response, with giant cells that contain hyphal fragments, often develops; asteroid bodies may form around *Aspergillus* spp. Usually the granulomatous lesions produce little or moderate enlargement of lymph nodes, but sometimes a massive, caseating lymphadenitis results, with adhesions to adjacent structures.

Pythiosis (*oomycosis*), caused by the aquatic oomycete *Pythium insidiosum*, occurs most commonly in tropical and subtropical areas, but on occasion can be found in more temperate climates. It is best recognized as a cause of *cutaneous lesions in horses* (see Vol. 1, Skin and appendages). However, it causes enteric disease in dogs, and, less commonly, horses. There is segmental thickening and ulceration of the stomach and small intestine, with transmural granulomatous inflammation, and sometimes granulomatous peritonitis, with adhesions of the omentum. Obstruction may occur. Lymphovascular channels on the intestinal serosa are thickened, and the mesenteric lymph nodes are greatly enlarged and frequently embedded in a granulomatous mass. Small firm or caseous yellow foci, known as "*leeches*" or "*kunkers*," may be embedded in the firm fibrotic reactive tissue. Segments of bowel may be infarcted. Granulomatous inflammation is evident in the mucosa, and especially the submucosa; it extends along lymphatics transmurally. Granulomas, with a local mixed inflammatory infiltrate, often including eosinophils, may have liquefactive or caseous necrotic centers. Characteristic hyphae are difficult to see with hematoxylin and eosin; they are best exposed, in the areas of necrosis or centers of granulomas, by *silver stains*. Similar lesions are reported in horses with intestinal obstruction.

Entomophthoromycosis involving the gastrointestinal tract has been reported in a few dogs. Ulcerative stomatitis, gastritis, and enteritis are reported, with induration of involved tissues by a granulomatous inflammatory reaction containing the typical hyphae. In horses, entomophthoromycosis due to *Conidiobolus* may involve the lips and pharynx, as well as the nostrils and nasal mucosa.

Definitive **diagnosis** of mycotic lesions requires culture, which may be difficult, and identification of the isolate, which is frequently a specialist activity. If possible, granulomatous lesions of the gut should always be cultured for fungi as well as bacteria, in order to increase the frequency of specific diagnosis. Mycotic lymphadenitis must be differentiated from a mycobacterial, actinomycotic, or nocardial lesion. A presumptive diagnosis may be based on morphologic characteristics of organisms in tissue sections. Immunochemical procedures using specific antibody may add to the confidence of a morphological diagnosis.

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Candidiasis

Candida spp. are normal inhabitants of the alimentary tract of animals, existing as *budding yeasts* in association with mucosal surfaces. When there are changes in the mucosae, particularly squamous mucosae, or in the mucosal flora, the yeasts may become invasive; *branching, filamentous pseudohyphae and hyphae largely replace the yeast forms*. Only a few of the almost 200 *Candida* species cause candidiasis; in animals the most important are ***Candida albicans*** and ***C. tropicalis***.

Changes in the mucosal flora usually result from antimicrobial therapy that reduces the numbers of anaerobic bacteria and allows proliferation of *Candida* spp. Environmental and social stress, and treatment with anticancer and anti-inflammatory agents, may also predispose to candidiasis.

Candida spp., especially *C. albicans*, are capable of adhesion to epithelium, and this is important in virulence. Hypha formation is favored by carbohydrates such as sucrose, or polysaccharides that are less readily fermentable than glucose. Glucose is necessary for keratinolysis by the fungus. An endotoxin released during reproduction and death of *Candida* organisms may cause local damage, permit deeper penetration into squamous epithelium, and perhaps promote systemic dissemination.

Candida spp. are occasionally opportunistic invaders of mucosal lesions anywhere in the alimentary tract, but other fungi are more likely to take advantage of this kind of lesion, particularly in older animals. *Candidiasis is mainly a disease of keratinized epithelium in young animals, especially pigs, calves, and foals*. Accumulation of keratin due to anorexia probably contributes to the extensiveness of lesions in all species by increasing the substrate available to the fungus. Systemic infection appears to be associated with activation of virulence factor genes of *Candida* that facilitate dissemination and colonization. Adequate myeloperoxidase activity of leukocytes is important in prevention of dissemination of *Candida* spp. Foci of necrosis with a mainly neutrophilic infiltrate, and containing masses of proliferating organisms, are found in organs such as the spleen, liver, kidney, and heart.

In **pigs**, *Candida* spp. often invade the parakeratotic material that accumulates on the *gastric squamous mucosa*. Apparently these infections are innocuous. "*Thrush*" is candidiasis of the oral cavity; it is occasionally seen in young pigs, especially those raised on artificial diets, or in pigs with intercurrent disease. Lesions may be confined to the tongue, hard palate, or pharynx, but often involve the esophagus and gastric squamous mucosa as well. Rarely the glandular stomach is involved. Grossly the lesions are yellow-white, smooth, or wrinkled plaques more or less covering the mucosa. Histologically, the epithelium is spongy and contains yeasts and abundant hyphae and pockets of neutrophils and bacteria beneath the cornified layer. Congestion of vessels and a few inflammatory cells are present in the mucosal propria. Desquamation of the epithelium may produce small ulcers.

In **calves**, candidiasis occurs following prolonged antimicrobial therapy and in association with rumen putrefaction. Lesions are most often seen in the *ventral sac of the rumen*, but may involve the omasum and reticulum and occasionally the abomasum. Grossly the lesions resemble those of "thrush" in pigs, but the keratin layer tends to be thicker, less diffuse, and light gray. In the omasum, the leaves may be stuck together by the mass of fungus-riddled keratin. *C. glabrata* in the abomasum has been implicated as a cause of diarrhea in calves, especially during winter months. Disseminated candidiasis occurs more often in calves than in pigs, probably because of the relatively prolonged survival of calves with alimentary lesions. Candidiasis in calves must be differentiated from alimentary herpesviral infections.

Gastroesophageal candidiasis in **foals** involves the squamous epithelium and is associated with *ulceration adjacent to the margo plicatus*. Colic and anorexia are seen, and are probably related to the development of the ulcers, which may perforate, causing peritonitis.

In tissues, the presence of oval or round blastospores about 3–6 μm in diameter, which may be budding, mixed with pseudohyphae comprised of chains of elongate yeast-like cells, or with tubular septate hyphae, permits a provisional identification of *Candida* spp. Silver or periodic acid–Schiff stain enhances the organisms in section.

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Intestinal histoplasmosis

Histoplasma capsulatum is a *soil organism* of worldwide distribution. The disease, histoplasmosis, is endemic in certain areas, such as the Mississippi and Ohio river valleys of the USA, and in parts of southern Ontario and the Ottawa–St. Lawrence river valleys in Canada. There are sporadic cases elsewhere. It is important in humans and dogs, and occasionally occurs in other species. Infection generally occurs via *inhalation of spores*, and if lesions occur, they are usually confined to the lungs. Dissemination, with hepatic, splenic, and sometimes gastrointestinal lesions, develops in some dogs. It is associated with heavy exposure, young age, and perhaps some degree of host immunocompromise. Infection can also be produced by *ingestion*, and the rare examples of disease confined to the gastrointestinal tract may develop in this manner. Intestinal infection by ingestion of infected sputum is also possible.

Disseminated histoplasmosis is a disease predominantly of young dogs that are usually presented with weight loss, generalized lymphadenopathy, and often diarrhea with blood, and tenesmus. *Intestinal histoplasmosis* is reported as part of disseminated disease in cats, and as an isolated lesion in a horse.

At postmortem there may be hemorrhagic enteritis involving small and large intestine, or granulomatous thickening of the mucosa and intestinal wall with ulceration (Fig. 1.176), or no apparent lesions. Mesenteric lymph nodes are often markedly enlarged. Histologically, lesions, characteristically *transmural granulomatous inflammation*, may occur in stomach and small or large intestine. The nonulcerated areas of the mucosa contain focal to diffuse infiltrations of macrophages laden with *H. capsulatum* organisms within cytoplasmic vacuoles. The mucosa may be grossly thickened by the infiltrate, causing necrosis and ulceration. The cellular reaction may extend through the muscularis to the serosa. Macrophages filled with organisms are particularly prominent in the lymphoid tissue of the gut and the mesenteric nodes. *Periodic acid–Schiff stain* highlights the organisms in tissues. Microscopic diagnosis of gastrointestinal histoplasmosis is not difficult, but grossly the disease must be distinguished from intestinal lymphoma and in the colon, from colitis of other types. Histoplasmosis is discussed in detail in Vol. 3, Hematopoietic system.

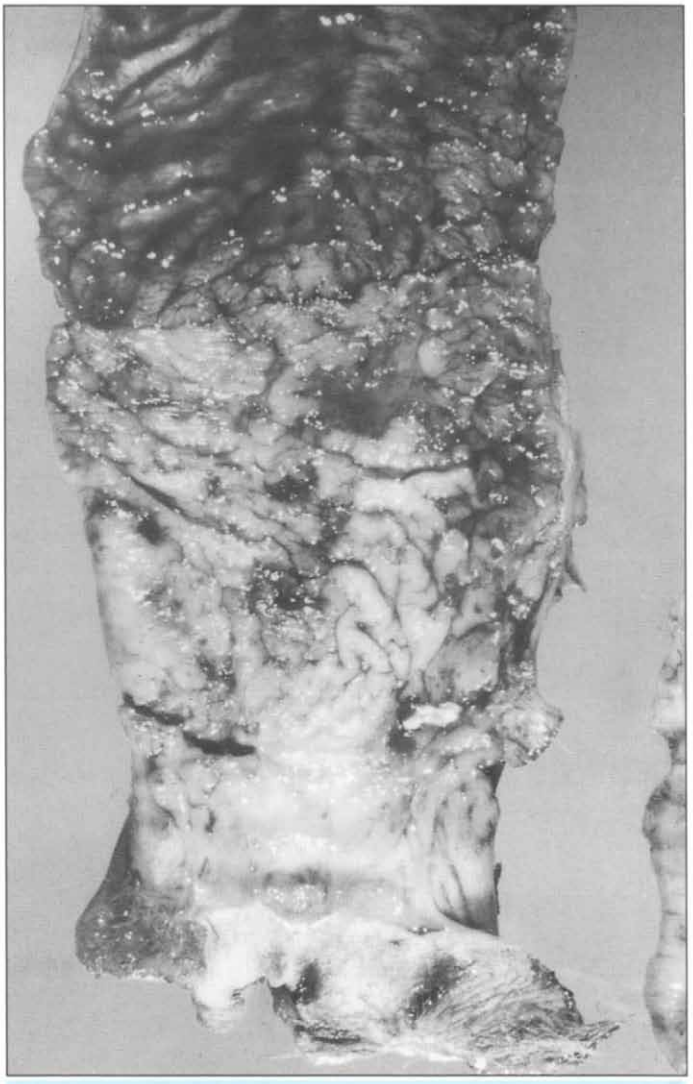

Figure 1.176 Ulcerative colitis in a dog with **histoplasmosis**.

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Protothecal enterocolitis

Prototheca spp. are *colorless algae* closely related to *Chlorella*. They are ubiquitous in raw and treated sewage and in water, and are found in feces, plant sap, and slime flux of trees. Two species, ***P. zopfii*** and ***P. wickerhamii*** cause disease in animals with both occurring occasionally in the same animal.

Lesions caused by *Prototheca* spp. include *cutaneous* infections of cats and human, *mastitis* in cows, and *disseminated* infections in cattle and dogs. The intestine and the eye are the most commonly involved sites in protothecosis of dogs.

Factors predisposing to the development of intestinal protothecosis are poorly understood. Skin infections are thought to result from traumatic inoculation, and it is possible that in the alimentary tract *Prototheca* is an opportunistic invader of existing mucosal lesions. Cattle, horses, and wild pigs pass *Prototheca* in the feces without apparent clinical disease. The chronicity of the disease and the mild host response are not consistent with a virulent infection. In dogs, Collies

seem overrepresented among the cases reported, suggesting breed-related susceptibility or immune compromise.

Chronic, intractable, bloody diarrhea, or passage of blood-stained feces, is a frequent presenting sign, with progressive weight loss. *Hemorrhagic and ulcerative colitis* is a prominent enteric lesion, but changes may also develop in the small intestine. Mesenteric lymph nodes may be enlarged.

The *mild host response to infection* is characteristic of protothecosis; usually only a few lymphocytes and monocytes are present. In early lesions, the extracellular organisms are scattered in the lamina propria, but later they fill the lamina propria and are often packed in cords in the connective tissue of the submucosa. Lacteals are distended, while lymphatics and the sinuses of draining lymph nodes are filled with organisms.

Prototheca in tissue sections range from 5 μm spheres to 9 × 12 μm ovoids, with a refractile capsule, and are positive with *periodic acid–Schiff and silver stains*. The presence of endosporulation with formation of 2–20 sporangiospores within a single sporangium characterizes *Prototheca* spp. and *Chlorella* spp. *Chlorella* contain periodic acid–Schiff-positive cytoplasmic starch granules that are PAS-negative following diastase digestion; *Prototheca* do not contain these granules. Differentiation of the genera by a fluorescent antibody test using formalin-fixed material is also possible.

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Gastrointestinal helminthosis

The diagnosis of disease due to gastrointestinal helminths must be made with knowledge of their pathogenic potential and the mechanisms by which it is expressed. *Parasites are much more common than the diseases they cause*, and "**helminthiasis**," *the state of infection*, must be clearly differentiated from "**helminthosis**," *the state of disease*.

Gastrointestinal helminths fall into *five categories*, according to pathogenesis of disease:

1. The first group **resides free in the lumen of the intestine**, competing with the host for nutrients in the gut content. They are generally of low pathogenicity, except for rare massive infections, and are not likely to be lethal, except by obstruction. Some of these worms, in sufficient numbers, may cause subclinical disease such as inefficient growth, or clinical disease in the form of ill-thrift; others are essentially nonpathogenic. The ascarids, adult small strongyles (cyathostomes) of horses, and tapeworms such as *Moniezia* and *Taenia* spp., fall into this group, as may *Physaloptera* in the stomach of carnivores.
2. A second group of helminths, all nematodes, primarily cause **blood loss**. These worms feed on the mucosa, causing bleeding, or they actively suck blood. Anemia, hypoproteinemia, and their sequelae cause production loss, clinical disease, and death. *Haemonchus* in the abomasum, and in the intestine the hookworms of carnivores and ruminants, the large strongyles of horses, and *Oesophagostomum radiatum* in cattle are the main examples.
3. The third group, composed of nematodes and some flukes, mainly causes **protein-losing gastroenteropathy**, usually associated with inappetence and diarrhea. In the abomasum, *Ostertagia* and *Trichostrongylus axei* cause mucous metaplasia and hyperplasia of gastric glands, achlorhydria, and diarrhea. In the small intestine, *Cooperia*, *Nematodirus*, *Strongyloides*, *Trichostrongylus*, and larval paramphistomes in sheep and cattle cause villus atrophy. This may cause malabsorption of nutrients, electrolytes, and water. But probably more important is the loss of endogenous protein into the gut, mainly related to chronic mucosal inflammation. In heavy infestations with *Trichuris* spp., erosion results in loss of absorptive function, and effusion of tissue fluids, or, in severe cases, hemorrhagic exudate.
4. The fourth group causes **physical trauma to the intestinal wall** by burrowing into or inciting inflammatory foci in the submucosa or deeper layers. In the stomach, various species of spirurids embed in the mucosa, or establish in cystic spaces in the submucosa. In the intestine, acanthocephala cause local ulceration by their thorny hold-fast organ; larval stages of equine cyathostomes and *Oesophagostomum* spp. become encapsulated in the submucosa. Protein loss may occur from ulcerated areas, or when larvae emerge from the submucosa. The potential exists for perforation of the stomach or bowel, or for complications due to sepsis of submucosal nodules. Adhesion of inflamed serosal surfaces associated with nodules or perforations may impair motility.
5. Finally, some intestinal helminths, among them a few in the categories above, have **effects at sites distant from the gut**. This is usually the result of migrating larval stages of the worm, either in definitive or intermediate hosts. Larval *Habronema*, ascarids, hookworms, and equine strongyles may cause lesions in a variety of extraintestinal sites in the definitive host. Larval ascarids and taeniid metacestodes may cause lesions or signs due to migration in nonenteric locations in accidental or intermediate hosts.

A **diagnosis of helminthosis** should be reserved for cases where, ideally, three criteria are met: (1) the helminth is present, in numbers consistent with disease; (2) the lesions (if any) typically caused by the agent, are evident; and (3) there is a syndrome compatible with the pathogenic mechanisms known to be associated with the worm.

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Abomasal and gastric helminthosis

Ostertagiosis

A complex of related genera and species of trichostrongylid nematodes, including *Ostertagia*, parasitize the *abomasum of ruminants*. The nomenclature of these worms is in a state of flux; for the sake of simplicity, the disease that they cause will be termed ostertagiosis. *Ostertagiosis is probably the most important parasitism in grazing sheep and cattle in temperate climatic zones throughout the world*. It causes subclinical loss in production, and clinical disease characterized by diarrhea, wasting, and, in many cases, death. ***Ostertagia ostertagi*** and the associated *O. lyrata* infect cattle. Sheep and goats are infected by ***Ostertagia*** (*Teladorsagia*) ***circumcincta***. Some cross-infection by these genera occurs between sheep and cattle, but is of minor significance. Other species of *Ostertagia* and related genera, including *Marshallagia*, *Spiculopteragia*, and *Camelostrongylus*, infect wild ruminants, including farmed deer; some may also parasitize the abomasum of cattle, sheep, and goats. Their behavior in general resembles that of *Ostertagia* and *Teladorsagia*.

The life cycle is direct. Third-stage larvae exsheath in the rumen and enter glands in the abomasum, where they undergo two molts. Normally, early fifth-stage larvae emerge to mature on the mucosal surface, beginning 8–12 days after infection in *O. circumcincta* infections in sheep, and about 17–21 days after *O. ostertagi* infection in cattle. However, a proportion of larvae ingested may persist in glands in a hypobiotic state at the early fourth stage, only to resume development and emerge at a future time, perhaps many months hence. The prepatent period is about 3 weeks.

During the course of larval development, the normal architecture of the gastric mucosa is altered by *interstitial inflammation*, and *mucous metaplasia and hyperplasia of the epithelium lining glands*. In sheep infected with *O. circumcincta*, mucous metaplasia and hyperplasia occur in infected and surrounding glands early in infection, reaching a peak about the time of emergence of larvae on to the mucosal surface. In cattle with *O. ostertagi* (Fig. 1.177A), only glands infected with larvae undergo significant mucous change until about the time larvae leave the glands for the surface of the mucosa (Fig. 1.177B). Mucous change then becomes more widespread, involving uninfected glands in the vicinity of those that contained larvae.

In both species, affected glands are lined by mucous neck cells that proliferate, displacing parietal cells. Affected areas of mucosa thicken. In infected glands, in many cases the lining is flattened adjacent to worms (Fig. 1.177C), but is composed of tall columnar mucous cells elsewhere in the gland. The undifferentiated mucous cells lining uninfected glands also eventually differentiate into tall columnar mucous cells. If infection is not heavy, lesions are limited to a radius of a few millimeters around infected or previously infected glands. These form raised nodular pale areas in the mucosa, often with a slightly depressed center. Confluence of these lesions in heavily infected animals leads to the development of *widespread areas of irregularly thickened mucosa with a convoluted surface pattern*, likened to "*Morocco leather*" (Fig. 1.178).

Mucous metaplasia and hyperplasia are accompanied by a mixed population of inflammatory cells in the lamina propria. Lymphocytes, plasma cells, eosinophils, and a few neutrophils are present between glands in the infected abomasum, and globule leukocytes are common in gland epithelium. There may be edema of the lamina propria associated with permeability of proprial vessels. Lymphoid response in local lymph nodes has been characterized as primarily B-cell-oriented, which is a surprising reaction to a nematode parasite.

Mucosal lesions lead to achlorhydria, elevation of plasma pepsinogen levels, and local vascular permeability with loss of plasma protein. Widespread replacement of parietal cells by mucous neck cells results in progressive and massive decline in hydrogen ion secretion, with severe cases having a pH of up to 7 or more. This increased abomasal pH results in elevated levels of gastrin in the circulation. The permeability of the mucosa is also increased, which is reflected in backdiffusion of pepsinogen from the lumen of glands to the propria, and ultimately to the circulation. Intercellular junctions between poorly differentiated mucous neck cells are also permeable to plasma protein in tissue fluids, emanating from the leaky small vessels in the inflamed lamina propria. Significant loss of protein occurs into the lumen of the abomasum.

The cardinal signs of ostertagiosis in sheep and cattle are *loss of appetite, diarrhea, and wasting*. Plasma protein loss into the gastrointestinal tract, in combination with reduced feed intake, seems largely responsible for the weight loss and hypoproteinemia that occur in clinical ostertagiosis, and for loss in productive efficiency that occurs in subclinical disease.

Clinical ostertagiosis occurs under two sets of circumstances. The first, **"type I" disease**, is seen in lambs or calves at pasture during or shortly after a period of high availability of *infective larvae*. It is due to the direct development, from ingested larvae, of large

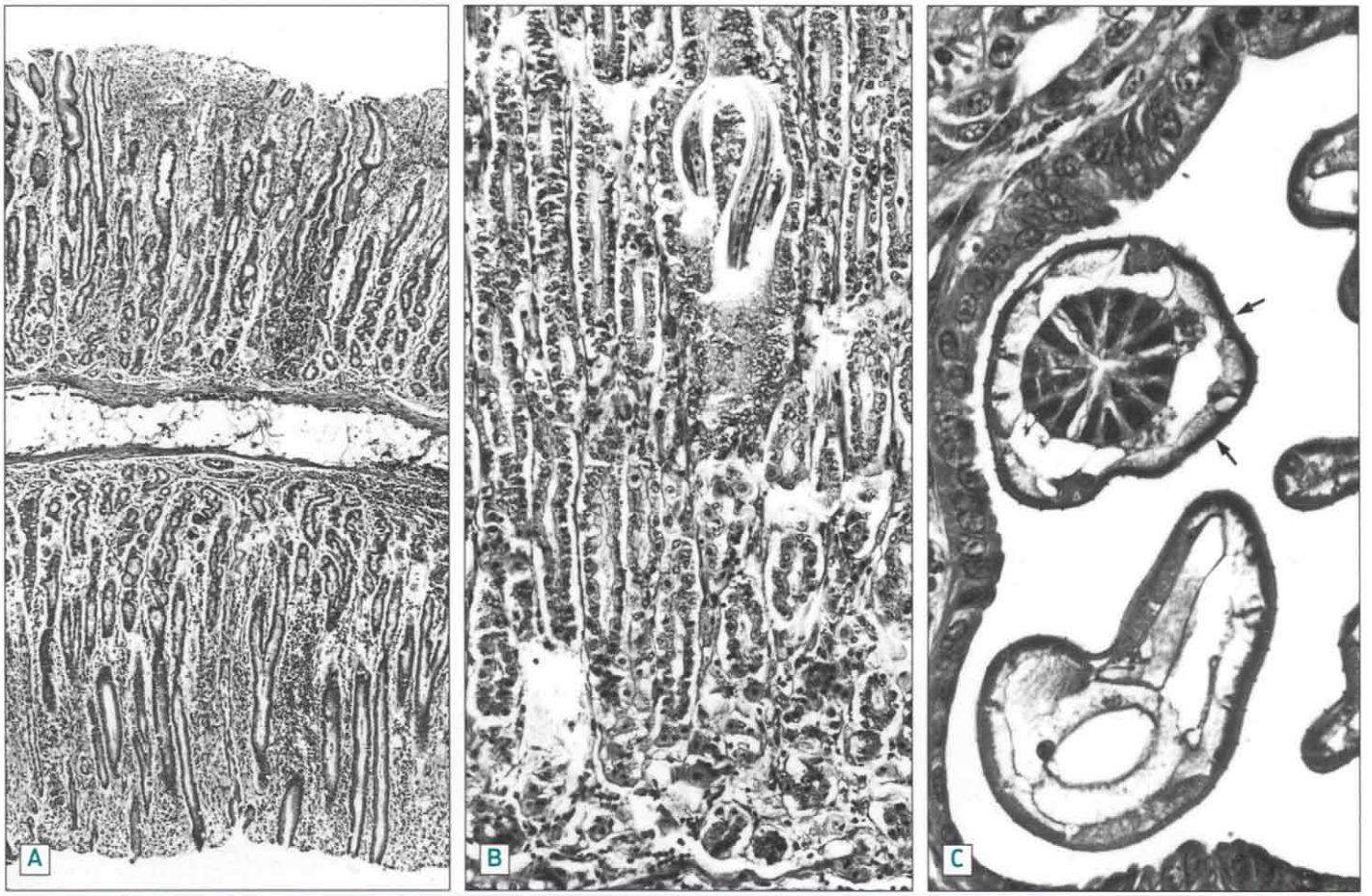

Figure 1.177 Ostertagiosis in the bovine abomasum. **A.** Mucous metaplasia and hyperplasia thickening fundic mucosa on abomasal fold. Moderate inflammatory infiltrate in propria between glands. **B.** Mucous metaplasia and hyperplasia deep in fundic mucosa. Larva in section in gland. **C.** *Ostertagia* in dilated gland lined by cuboidal mucous neck cells. Longitudinal cuticular ridges are visible as fine projections on the nematode (arrows).

numbers of adult worms, over a relatively short period of time. In contrast, **"type II" disease** is due to the synchronous maturation and emergence of large numbers of *hypobiotic larvae* from the mucosa, and it occurs when intake of larvae is likely low or nonexistent. It may occur in yearlings during the winter in the northern hemisphere, or during the dry summer period in Mediterranean climates. Heifers about the time of parturition may succumb, and this syndrome is also occasionally seen in animals experiencing environmental stress of any type.

The **diagnosis** of ostertagiosis is indicated at autopsy by an *abnormally elevated abomasal pH* (*>4.5*), in association with *typical gross lesions* on the mucosa. The adult worms are brown and thread-like, up to 1.5 cm long, but very difficult to see on the mucosa with the unaided eye. *Abomasal contents and washings* should be quantitatively examined for the presence of emergent or adult *Ostertagia* and other nematodes. A *portion of the mucosa should be digested* to permit recovery and quantitation of pre-emergent stages. Significant worm burdens in sheep are in the range of 10 000–50 000 or more. In cattle >40 000–50 000 adult worms may be present, and in outbreaks of type II disease hundreds of thousands of hypobiotic larvae are often detected in the abomasal mucosa. Typically, there is widespread mucous metaplasia and hyperplasia in dilated glands in sections of abomasum. *Ostertagia* are recognized in sections, on the mucosal surface or in glands, by the presence of prominent longitudinal cuticular ridges (synlophe) that project from the surface of worms cut transversely. In some cases, the worm burden may have been lost through attrition or recent treatment, and the diagnosis must be presumptive, based on the characteristic mucosal lesions.

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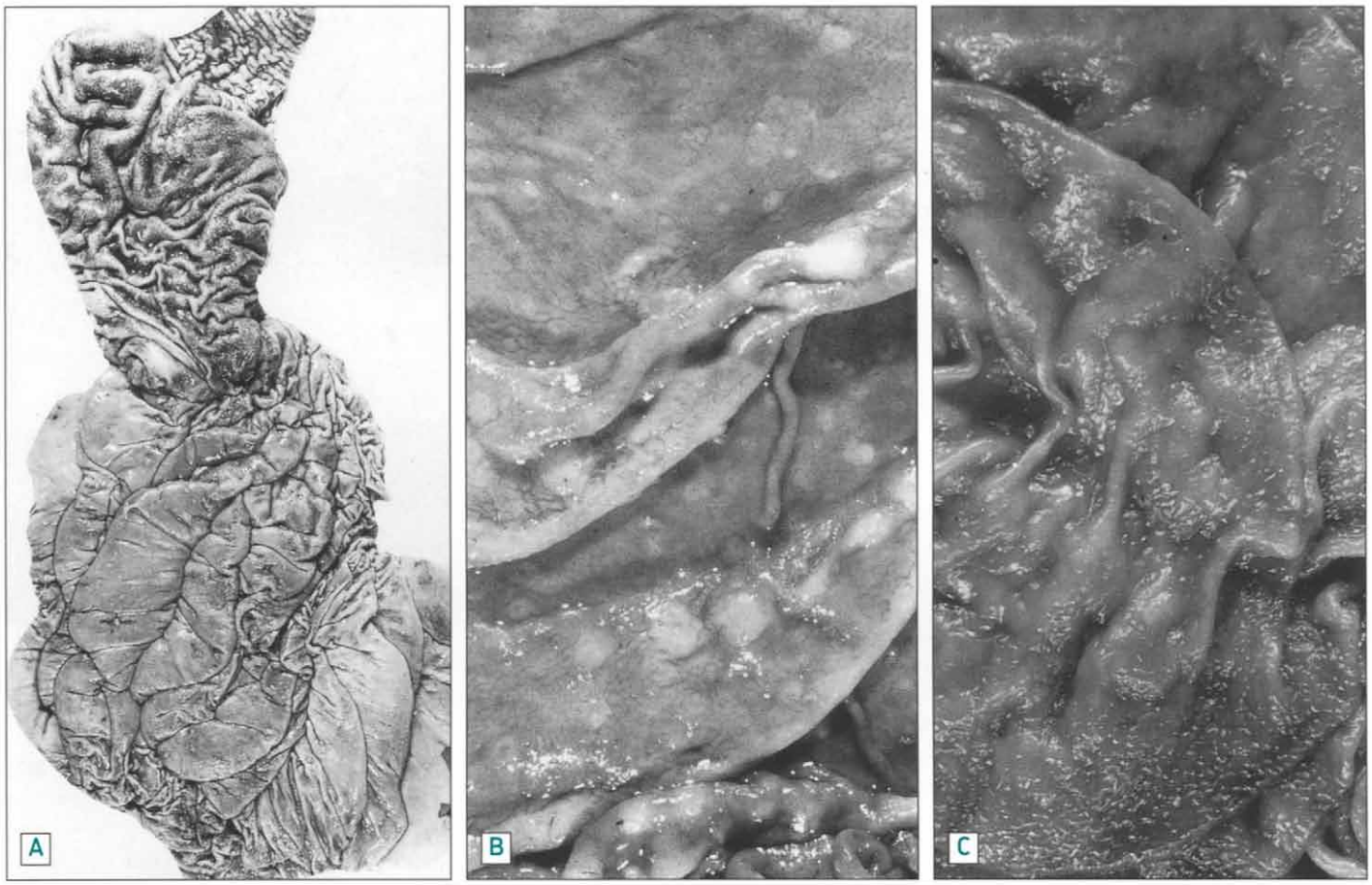

Figure 1.178 Ostertagiosis in abomasum. **A.** Acute edematous gastritis in a cow. **B.** Individual nodules and some confluent lesions in a sheep. **C.** Confluent thickening of hyperplastic glandular mucosa in a sheep.

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Hemonchosis

Hemonchosis is a common and severe disease in some parts of the world. *Haemonchus* species require a period of minimum warmth and moisture for larval development on pasture. As a result, they tend to be *most important in tropical or temperate climates with hot wet summers*. ***Haemonchus contortus*** infects mainly sheep and goats, whereas ***H. placei*** occurs mainly in cattle. Though *H. contortus* and *H. placei* will infect the heterologous host, the host–parasite relationship appears to be less well adapted, and the species do appear to be genetically distinct. ***Mecistocirrus digitatus*** causes disease very similar to hemonchosis in cattle and sheep in Southeast Asia and Central America.

By exploitation of hypobiosis or retardation of larvae, populations of *H. contortus* are able to persist in the abomasum of the host through periods of climatic adversity, such as excessive cold or dryness. Disease can be expected in animals, especially females, experiencing the synchronous "*spring rise*" or periparturient development and maturation of previously hypobiotic larvae, and in young animals heavily stocked at pasture during periods of optimal larval development and availability.

Haemonchus, commonly called the *large stomach worm, or barber pole worm*, is ~2 cm long. Females give the species its common name by their red color, against which the white ovaries and uterus stand out. The male is a little shorter and uniform deep red. These worms are equipped with a buccal tooth or lancet, and *fourth-stage and adult worms suck blood*. Ingested third-stage larvae enter glands in the abomasum, where they molt to the fourth stage and persist as hypobiotic larvae, or from which they emerge as late fourth-stage larvae to continue development in the lumen. The prepatent period for *H. contortus* in sheep is ~15 days; for *H. placei* in cattle is ~26–28 days; and for *M. digitatus* is ~61–79 days.

Figure 1.179 Severe **submandibular edema** ("bottle-jaw"), and **anemia**, manifest as conjunctival pallor, in ***Haemonchus contortus* infection** in a sheep.

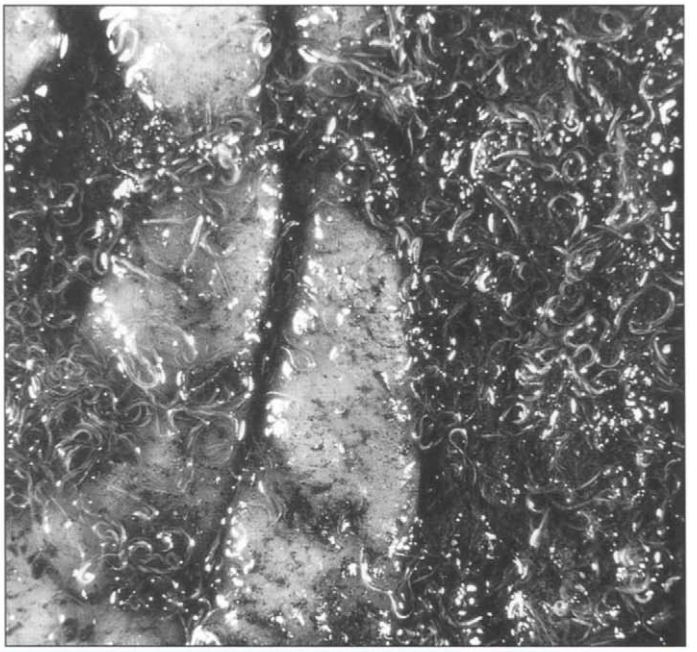

Figure 1.180 ***Haemonchus contortus*** in acid-hematin-tinged abomasal content in a sheep.

Hemonchosis may occur as *peracute or acute disease*, resulting from the maturation or intake of large numbers of larvae. It may cause more insidious *chronic disease*, if worm burdens are lower. The pathogenicity of *Haemonchus* infection, whatever its manifestation, is the result of blood-sucking activity, which causes anemia and hypoproteinemia.

Individual *Haemonchus* worms in sheep cause the loss of about 0.05 mL of blood per day. Of the order of a tenth to a quarter of the erythrocyte volume may be lost per day by heavily infected lambs; the plasma loss is concomitant and may be several hundreds of milliliters. The potential for the *rapid onset of profound anemia and hypoproteinemia* in heavily infected animals is obvious. Such animals succumb quickly, some even before the maturation of the worm burden. Less heavily infected animals may be able to withstand the anemia and hypoproteinemia for a period of time. They compensate by expanding erythropoiesis two- to threefold, and increasing hepatic synthesis of plasma protein. However, they are unable to compensate adequately for the enteric iron loss, despite intestinal reabsorption of a proportion of the excess, and they ultimately succumb some weeks later to *iron-loss anemia*, when iron reserves are depleted. Low-level infections may contribute to subclinical loss of production or ill-thrift through chronic enteric protein and iron loss. Low protein rations compound the effect of infection.

The clinical syndrome may vary somewhat. Some animals are found dead, without the owner observing illness. Others lack exercise tolerance, fall when driven, or are reluctant to stand or move, so weak are they from anemia. Edema of dependent portions, especially the submandibular area or head in grazing animals, is often observed (Fig. 1.179). In primary hemonchosis, there is no diarrhea; diarrhea may occur if intercurrent infection with large numbers of other gastrointestinal helminths occurs.

The **postmortem** appearance of animals with hemonchosis is dominated by the extreme pallor of anemia, apparent on the conjunctiva and throughout the internal tissues. The liver is pale and friable. There is usually edema of subcutaneous tissues and mesenteries, with hydrothorax, hydropericardium and ascites, reflecting the severe hypoproteinemia. The abomasal content is usually fluid, and dark red-brown, due to the presence of blood (Fig. 1.180). The abomasal rugae may be edematous due to hypoproteinemia, and focal areas of hemorrhage are evident over the surface. In animals that are not decomposing, the worms will be evident to the naked eye: if alive, writhing on the mucosal surface; if dead, less obvious and free in the content.

In clinically affected sheep and goats, usually about 1000–12 000 worms are found. The severity of the disease is a function of the number of worms on one hand, and to some extent, the size of the animal on the other. In lambs, 2000–3000 worms is a heavy burden, while in adult sheep and goats, 8000–10 000 are associated with fatal infection.

A high egg count is usually found on fecal flotation, since *Haemonchus* is a prolific egg-layer. However, in peracute prepatent infections, no eggs will be present. In recently treated animals, no worms may be present, and the diagnosis may have to be presumptive. On the other hand, treated animals returned to contaminated pasture may succumb to reinfection within 2–3 weeks. A serologic test targeting a somatic antigen has been developed.

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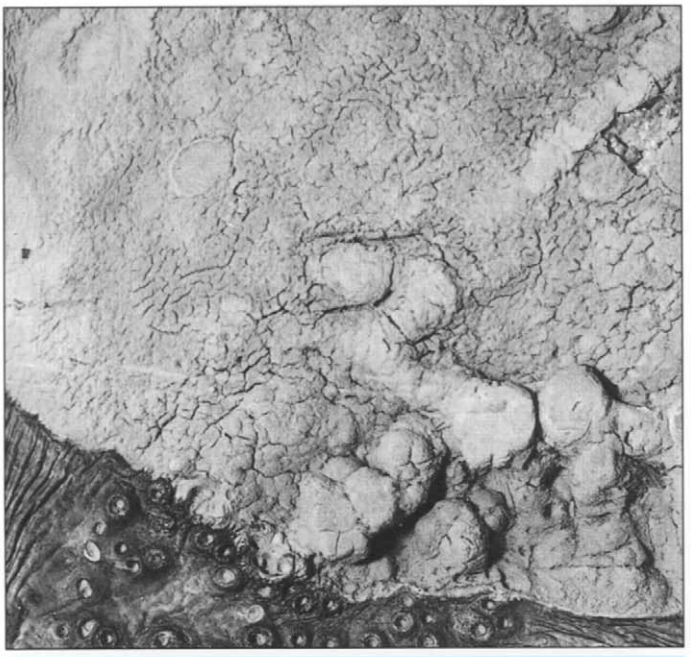

Figure 1.181 Hypertrophic gastritis in the fundic mucosa of a horse with **trichostrongylosis**. (Courtesy of NO Christensen and Skand Vet.)

Trichostrongylus axei infection

Trichostrongylus axei infects the *abomasum of cattle, sheep, and goats*, and the *stomach of horses*. It has a direct life cycle: third-stage infective larvae enter tunnels in the epithelium of the foveolae and neck of gastric glands in both fundic and pyloric areas. The worms live throughout their life *partly embedded in intraepithelial tunnels* at about this level of the mucosa. They molt to the fourth stage about a week after being ingested and to the fifth stage by ~2 weeks after infection. The prepatent period is ~3 weeks in calves and sheep, and about 25 days in horses.

Infections with *T. axei* are usually part of a *mixed gastrointestinal helminthosis*. However, in all hosts this species alone is capable of inducing disease, if present in sufficient numbers. After a period of several weeks, *mucous metaplasia and hyperplasia are seen in glands in infected areas of the mucosa*. In severely affected animals, flattening of surface epithelium with desquamation, or erosion of the mucosa, develops, accompanied by effusion of neutrophils, eosinophils, and tissue fluid. Fibroplasia may occur in the superficial propria in eroded areas.

In light infestations there may be no changes visible in the abomasum other than congestion of the mucosa. The gross lesions present in heavy *T. axei* infections reflect the hypertrophy of glands, and superficial erosion. *Circular or irregular raised white plaques of thickened infected mucosa* are present, often with a thick layer of mucus. Erosions or shallow ulcers may be present. In severe infections the entire mucosa appears edematous and congested.

Infection in horses is uncommon and is usually related to sharing pasture with sheep or cattle. In chronically infected horses, white raised plaques (Fig. 1.181) or nodular areas of mucosa are present, covered by tenacious mucus and surrounded by a zone of congestion. Mucosal lesions may be confluent in heavily infected animals, and erosions and superficial ulceration may be encountered. Infection may extend into the proximal duodenum, where polypoid masses of hypertrophic glandular mucosa are occasionally observed. Plasma pepsinogen levels may be elevated.

Achlorhydria develops in heavily infected sheep and cattle, associated with *diarrhea*, particularly in the latter species. Dehydration may prove severe in scouring calves. Plasma pepsinogen and gastrin levels increase, and hypoproteinemia and *wasting* occur. This suggests that the mucous metaplasia in the glands is associated with increased permeability and that plasma protein loss occurs into the gastrointestinal tract.

Though *T. axei* is not common as a primary cause of disease in any species, it should be sought at autopsy of animals with signs of wasting and perhaps diarrhea. The typical gross lesions in the stomach are distinctive in horses. In ruminants, they must be differentiated from those due to *Ostertagia*, with which animals may be intercurrently infected. The worms are very fine, and gastric washes or digestion are required to recover them quantitatively. The *distinctive intraepithelial location* of *T. axei* in section differentiates it from other nematodes inhabiting the abomasum of ruminants and the stomach of horses.

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Gastric parasitism in horses

The commonest parasites of the equine stomach are *larvae of bot flies* of the genus ***Gasterophilus***. Though they are not helminths, it is convenient to consider them here. There are six species of the genus, the common ones being ***G. intestinalis***, ***G. nasalis***, and ***G. haemorrhoidalis***, and the uncommon ones being *G. pecorum*, *G. nigricornis*, and *G. inermis*. The ova are deposited on the ends of the coat hairs in the face, intermandibular region, or on the lower body and legs. The eggs hatch spontaneously, or when stimulated by licking. The first-stage larvae penetrate the oral mucosa, molt, emerge, and migrate down the alimentary canal.

Gasterophilus intestinalis usually wander about in tunnels in the superficial mucosa of the cheeks, tongue, or gums for 3–4 weeks before moving to periodontal pockets containing purulent exudate in the gingival sulcus on the lingual aspect of molars, especially in the upper arcade. Here they molt before moving on to the base of the tongue, and to the stomach. This is the most common species, and in the stomach it attaches itself to the squamous mucosa of the cardia to complete its subsequent molts. ***G. nasalis*** first invade the gums, where they may be associated with pockets of purulent exudate in the interdental spaces, then pass to the stomach and settle on the pyloric mucosa and in the first ampulla of the duodenum. Members of any of these species may occasionally be found attached to the pharynx and esophagus but, except for *G. pecorum* that congregates in the pharynx

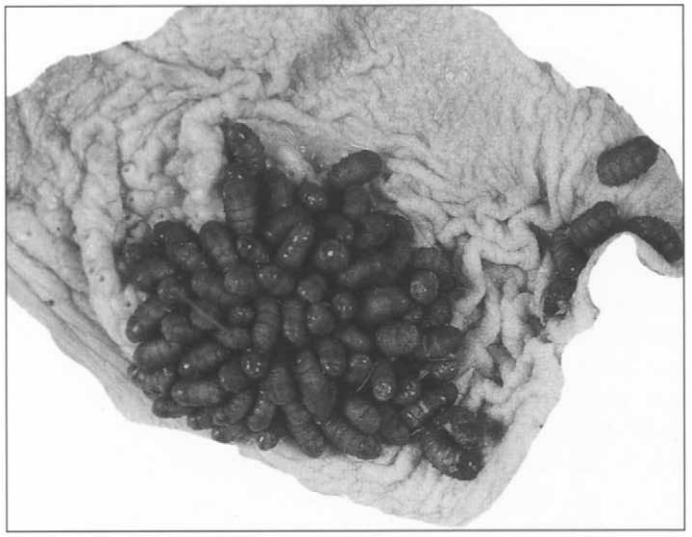

Figure 1.182 ***Gasterophilus*** **larvae** on gastric mucosa of a horse.

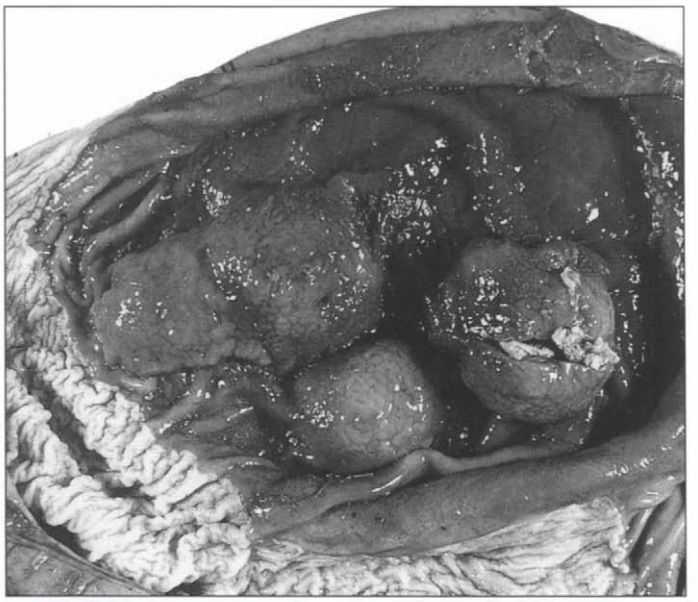

Figure 1.183 Nodules containing ***Draschia megastoma*** in the submucosa of glandular mucosa, near margo plicatus. Purulent content in sectioned nodule.

and causes pharyngitis, these preliminary migrations are uneventful for the host. In the summer following the deposition of the ova, the larvae leave the stomach and pass out in the feces to pupate. Those of *G. pecorum* and *G. haemorrhoidalis* may attach themselves for a short while to the wall of the rectum.

It is generally assumed that the larvae of *Gasterophilus* have little effect on their host. The larvae fasten themselves to the mucosa by chitinous oral hooks and they bore into the mucosa (Fig. 1.182). They apparently subsist on blood, exudate, and detritus, producing focal erosions and ulcerations at the point of contact. These defects in the cardia are surrounded by a narrow rim of hyperplastic squamous epithelium. Usually the number of epithelial defects exceeds the number of larvae, suggesting that they move about on the mucosa.

Severe infestations produce a dense pock-marked appearance of the pars esophagea, with chronic inflammatory thickening. Ulcers may occur in the glandular mucosa and, rarely, a large proportion of the affected pyloric mucosa may be lost. Healing occurs when the larvae migrate on, but may be complicated by secondary bacterial infection. Histologically, the ulcers penetrate the submucosa, which is chronically inflamed. The deep layers of eroded epithelium and the epithelial margins of ulcers in the squamous mucosa become hyperplastic and develop rete pegs. There seems to be no relationship between bot infestations and the development of gastric ulcers in the pars esophagea.

The **spirurid nematodes *Draschia megastoma*, *Habronema majus***, and ***H. muscae*** are also parasitic in the stomach of horses. The adult worms are 1–2 cm in length. The latter two species lie on the mucosal surface and are probably insignificant except possibly for a few erosions and mild gastritis. *D. megastoma* burrows into the submucosa to produce large tumor-like nodules (Fig. 1.183).

H. majus mainly uses *Stomoxys calcitrans* as its intermediate host and the other two species use various muscid flies. The *Habronema* larvae in the feces are swallowed by maggots of the appropriate intermediate host and persist through pupation and maturation of the fly. They leave the host fly via the proboscis when it seeks moisture, for instance, on the lips. Larvae deposited on or in cutaneous wounds, or in the eye, invade the skin or conjunctiva and provoke an intense local reaction, which becomes granulomatous and densely infiltrated with eosinophils (see Vol. 1, Skin and appendages). Occasionally *Draschia* and *Habronema* larvae may be found in the brain, or in the lungs, where they may become encapsulated and mineralize.

The only one of concern in the stomach is *D. megastoma*, which burrows into the submucosa of the fundus, usually within a few centimeters of the margo plicatus. Within the submucosa, the worms provoke a surrounding granulomatous reaction that contains them in a central core of necrotic and cellular detritus, with abundant eosinophils. These lesions form protrusions up to ~5 cm in diameter, with a small fistulous opening to the lumen. The nodules generally produce no clinical disturbance, though they have been considered to lead rarely to abscessation, adhesions of the stomach to the spleen, or perforation when infected with pyogenic bacteria.

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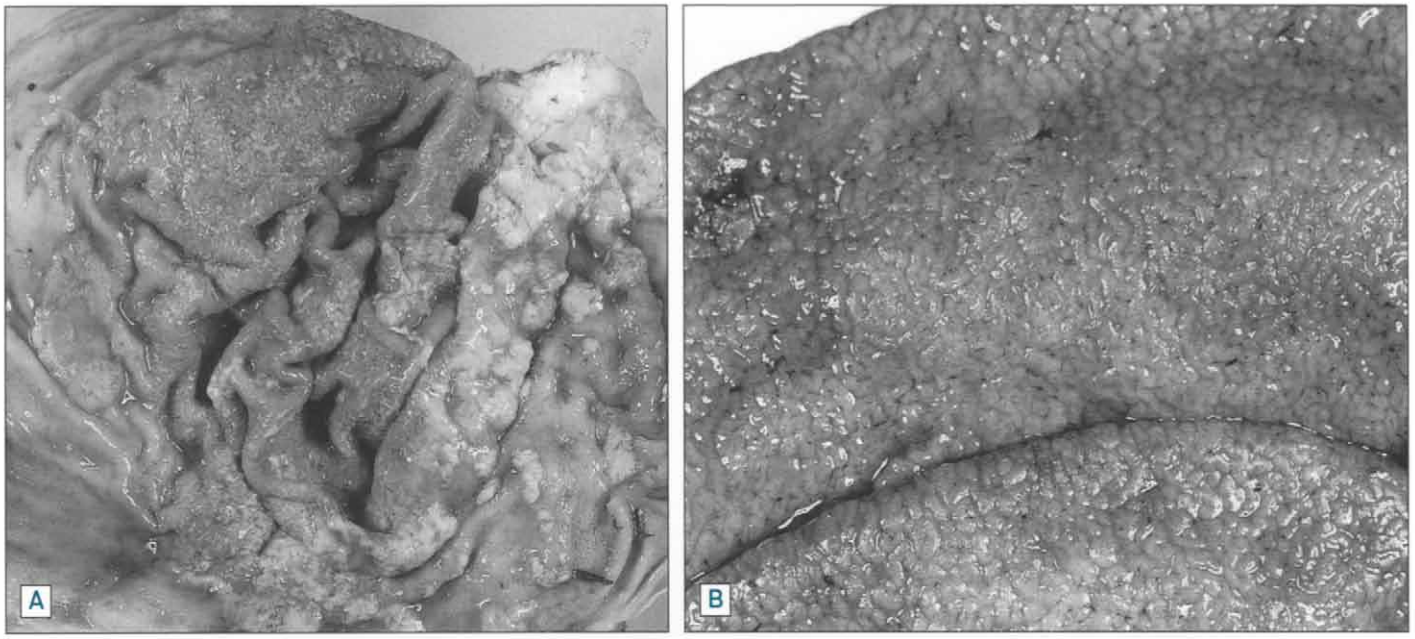
Gastric parasitism in swine

Gastric parasitism is not of great clinical or pathologic importance in swine and is rare in pigs reared in modern total confinement systems.



Ascaris suum, normally inhabiting the small intestine, may migrate or reflux to the stomach after death. *Hyostrongylus rubidus* is probably the most significant parasite of the stomach of swine; it and the various spirurids are more common in pigs allowed to forage. *Ollulanus tricuspis* is reported in pigs. It is more commonly encountered in cats, and is discussed with gastric parasitism in dogs and cats.

Hyostrongylus rubidus is a trichostrongylid nematode with a typical life cycle. Third-stage larvae enter glands in the stomach, especially in the fundic region, where they develop and molt twice. Pre-adult and adult worms emerge on to the gastric mucosa ~18–20 days after ingestion. The lesions produced by *Hyostrongylus* resemble those caused by *Ostertagia* in ruminants. There is *mucous metaplasia and hyperplasia of the lining of infected and neighboring glands*, and dilation of infected glands. The lamina propria in infected mucosa is edematous and infiltrated by lymphocytes, plasma cells, and eosinophils, and lymphoid follicles develop deep in the mucosa. Neutrophils and eosinophils may transmigrate the epithelium into dilated glands, the lining of which may become quite attenuated. There may be extensive erosions.

During the course of development of the worms, the mucous metaplasia and hyperplasia cause the formation of pale nodules in the vicinity of infected glands (Fig. 1.184A). In heavy infections these may become confluent, causing the development of an irregularly thickened convoluted mucosa (Fig. 1.184B), most notable in the fundic area and along the lesser curvature. Adult worms are fine, red, and thread-like in the gastric mucus; they are difficult to see with the naked eye.

Experimental infections of moderate degree do not produce obvious clinical signs or loss of production. However, loss of plasma protein has been documented in heavy *Hyostrongylus* infections. Inappetence, diarrhea, and reduced weight gains and feed efficiency also occur in these circumstances. In the field, hyostrongylosis is mainly associated with the "thin-sow syndrome," in which it seems probable that it may interact with nutritional and metabolic factors.

Spirurid nematodes parasitizing the porcine stomach include *Physocephalus sexalatus*, *Ascarops strongylina*, *A. dentate*, and *Simondsia paradoxa*. *Physocephalus* and *Ascarops* utilize dung beetles as intermediate hosts. ***Ascarops*** and ***Physocephalus*** are common in many parts of the world in swine with access to grazing. Large numbers of worms are required to cause ill-thrift. Worms in affected pigs may be free in the lumen or partly embedded in the mucosa, which may be congested and edematous, or eroded and ulcerated with fibrinous exudate on the surface. There may be chronic interstitial inflammation and fibrosis in the mucosa. ***Simondsia*** is found in swine in Europe, Asia, and Australia. The caudal portion of the female worm is globular, and is embedded in palpable nodules up to 6–8 mm in diameter in the gastric mucosa. *Gnathostoma doloresi* causes gastric ulcers and granulomas in pigs in eastern Asia. *G. hispidum* may cause lesions in the liver, and submucosal nodules in the gastric wall of pigs, similar to those produced by *G. spinigerum* in carnivores.

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Gastric parasitism in dogs and cats

Parasites are uncommonly encountered in the stomach of dogs and cats at necropsy and most are incidental findings, or postmortem migrants from the intestine.

Gnathostoma spinigerum occurs in the stomach of dogs and cats, and of a variety of nondomestic carnivores. It is more common in areas with warm climates. The life cycle of this spirurid nematode involves *Cyclops* as an aquatic invertebrate intermediate host, and a variety of fish, amphibian, or reptiles as second intermediate hosts. Ingested third-stage larvae may migrate in the liver, leaving tracks of necrotic debris, which eventually heal by fibrosis. In heavy infections, lesions associated with larval migration may be found elsewhere in the abdominal and pleural cavities, and in the skin. Adults are found in groups of up to 10 in nodules in the gastric submucosa. Nodules are up to ~5 cm in diameter, and open into the gastric lumen. Portions of nematodes may protrude through this opening. The worms lie in a pool of blood-tinged purulent exudate in the lumen of the nodule, the wall of which is comprised of granulation tissue and reactive fibrous stroma. Focal granulomas may center on nematode ova trapped in the connective tissue. Infection with *Gnathostoma* has been considered significant. Illness and death may be associated with disturbance of motility, chronic vomition, and occasional rupture of verminous nodules on to the gastric serosa, leading to peritonitis.

A number of species of ***Physaloptera***, including *P. praeputialis* (cat), *P. rara* (dogs and wild Canidae and Felidae) and *P. canis* (dog) are found in the stomach of dogs and cats. These nematodes utilize arthropod intermediate hosts and probably some vertebrate transport hosts. The adult worms, which may be mistaken for small ascarids, are found in the stomach, where they may be free in the lumen. More commonly they are attached as individuals or in small clusters to the gastric mucosa. Ulcers may be formed, and the cranial end of the worm may be embedded in the submucosa. Hyaline periodic acid–Schiff-positive material surrounds the cranial end of some worms, perhaps anchoring them in the tissue. These nematodes are not highly pathogenic, though heavy burdens may have the potential to cause significant gastric damage and chronic vomition.

Cylicospirura felineus and members of the genus ***Cyathospirura*** may be found in the stomach of domestic and wild felids. *Cylicospirura* are usually found in the submucosal nodules, similar to those formed by *Gnathostoma*, while *Cyathospirura* is usually found free in the lumen, or sometimes associated with *Cylicospirura* in gastric nodules. The pathogenicity of these species is poorly defined, but is likely low.

Ollulanus tricuspis is a small trichostrongyle, ~1 mm long, which inhabits the stomach of cats and swine. It is viviparous, and third-stage larvae developing in the uterus of the female are transmitted in vomitus. As a result, infection is usually not detected by fecal examination, and infection with this species may go unnoticed. In some parts of the world it is common, particularly in cat colonies and cats that roam. Clinical signs and gross lesions due to *O. tricuspis* are uncommon. *Vomition, anorexia, and weight loss* are the signs most frequently associated with infection. The worms lie beneath the mucus on the surface of the stomach, or partly in gastric glands. Infection is associated with increased numbers of lymphoid follicles deep in the gastric mucosa, increased interstitial connective tissue in the mucosa, and numerous globule leukocytes in the gastric epithelium. Heavy infection results in mucous metaplasia and hyperplasia of gastric glands, causing the surface of the stomach to be thrown into thickened convoluted folds, grossly resembling idiopathic hypertrophic gastritis of dogs. Gastric glands are often separated by the heavy reactive fibrous stroma in the mucosa. In gastric biopsies, this suite of microscopic changes in the mucosa should be recognized as characteristic of *Ollulanus* infection, even if worms are not present. *Ollulanus* are characterized in section by the numerous longitudinal cuticular ridges (synlophe) recognized as projections on the surface of sectioned worms.

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Intestinal helminth infections

Strongyloides infection

Strongyloides spp. parasitize all species of domestic animals considered here. Ruminants are infected by *S. papillosus*; horses by *S. westeri*; swine mainly by *S. ransomi*; dogs by *S. stercoralis*; and cats by *S. felis*, *S. planiceps* (= *S. catti*), and *S. stercoralis* in the small intestine, and by *S. tumefaciens* in the colon. The parasitic worms are parthenogenetic females, which produce larvae capable of direct infection of the host, or of developing into a free-living generation of males and females. The offspring of the free-living generation then adopt a parasitic existence.

Infection by third-stage larvae takes place by skin penetration, or to a lesser extent by ingestion and probably subsequent penetration of the gastrointestinal mucosa. Larvae attain the bloodstream, and in young animals, break out into pulmonary alveoli. They migrate to the large airways, whence they are carried up the mucociliary escalator to be swallowed, and establish in the small intestine.

Typically infecting the proximal small intestine of all species, *Strongyloides larvae establish in tunnels in the epithelium about the base of villi or in upper crypts*, and they persist in that location (Fig. 1.185). Adult worms are small, only 2–6 mm long, depending on species. In sufficient numbers, they cause villus atrophy, associated with a mixed, but mainly mononuclear inflammatory cell infiltrate into the lamina propria. Cryptal epithelium is hyperplastic. Villi are stumpy or there is subtotal villus atrophy. Surface epithelium is usually low columnar to cuboidal, with an indistinct brush border; it may be squamous, or in some cases, eroded. The nematodes are usually found in tunnels in the surface epithelium, not beneath the basal lamina. Embryonated or larvating ova may be retained in epithelial tunnels, and help to distinguish this nematode in tissue section from *Trichostrongylus*, in hosts in which both species occur.

Strongyloides ransomi is responsible for *diarrhea in suckling piglets* in some parts of the world. Minor local hemorrhage occurs as larvae migrate through the lungs, and thickening of alveolar septa is reported, associated with scattered aggregates of lymphocytes and plasma cells. In the duodenum, villus atrophy is associated with local malabsorption

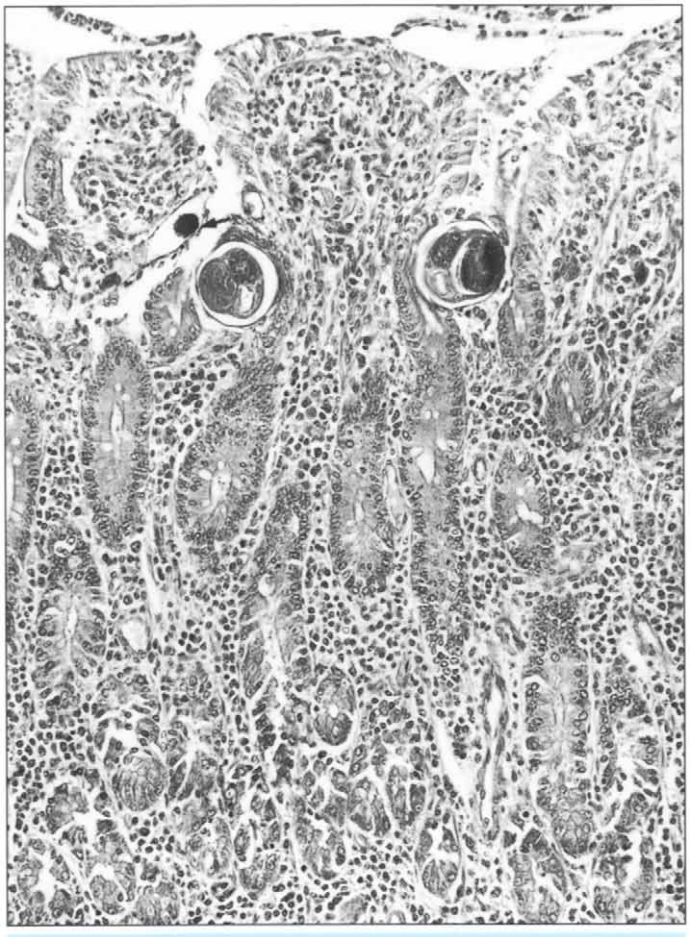

Figure 1.185 ***Strongyloides westeri*** in tunnels at base of a moderately atrophic villus in the intestine of a foal with diarrhea. An ovum is in a tunnel on an adjacent villus (arrow).

of amino acids, and with protein loss into the gut. Diarrhea occurs, presumably the result of malabsorption. Debilitation is the product of anorexia, protein loss into the gut, and nutrient malabsorption. Specific gross lesions other than those associated with diarrhea may be absent. Moderate to severe clinical disease in 3-month-old pigs is associated with 20 000–70 000 worms, most in the cranial 30–40% of the small intestine. Worms are evident in mucosal scrapings at autopsy.

S. westeri commonly infects *foals*. It is associated with diarrhea, occasionally fatal, in some animals under 4–5 months of age (Fig. 1.185). It is claimed that skin lesions by larval penetration may permit entry of *Rhodococcus equi*, which causes lymphadenitis. Millions of larvae are necessary to cause fatal infections experimentally.

S. papillosus may cause diarrhea and, in occasional overwhelming infections, death, in *suckling ruminants*, or young animals being artificially reared. There is also a syndrome of sudden death due to cardiac failure that is associated with heavy infections of *S. papillosus*, the pathogenesis of which is unclear.

S. stercoralis infections are most commonly fatal in *puppies* up to 2–3 months old, often from kennel environments. Affected dogs are wasted and dehydrated with evidence of diarrhea, perhaps blood-tinged, but the intestine may only be congested or unremarkable at autopsy. Severe villus atrophy and heavy mononuclear interstitial infiltrates are evident in the duodenum of affected dogs. Occasionally larvae may be present in granulomas in the lamina propria and submucosa. Focal interstitial pneumonia may be associated with pulmonary migration. There are numerous reports of *S. stercoralis* infecting humans, especially in situations of immunocompromise.

S. felis may cause mild focal granulomatous or eosinophilic interstitial pneumonia in response to lung migration of larvae. In some cases there is local adenomatous hyperplasia of the crypts of Lieberkühn in the vicinity of worms in the small intestine, but no more general lesions. Diarrhea is uncommon.

S. tumefaciens in cats has rarely been associated with chronic diarrhea. It differs from the other species discussed above, in being associated with the formation in the colon of submucosal nodules of proliferative glands, in which the worms are found. It is uncertain whether this is a specific lesion induced by infection, or merely herniation of infected colonic glands into space left by involution of a submucosal lymphoid follicle.

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Intestinal trichostrongylosis

Members of the genus *Trichostrongylus* parasitize the *proximal small intestine of ruminants* the world over. They cause significant subclinical inefficiency in production, or clinical disease characterized by *diarrhea, ill-thrift and, in some cases, death*. The most important species infecting sheep and goats are ***T. colubriformis***, ***T. vitrinus***, and ***T. rugatus***; others include *T. longispicularis*, *T. falculatus*, *T. capricola*, and *T. probolurus*. *T. colubriformis* and *T. longispicularis* also parasitize cattle. Though some *T. axei* may be found in the duodenum of cattle and sheep, this species is primarily parasitic in the abomasum.

Trichostrongylosis is most important in zones with a cool climate at some time of the year, but without extreme winters. It is a very important problem in many sheep-grazing areas of New Zealand, Australia, South Africa, South America, and the UK.

The life cycle is direct. Ingested third-stage larvae exsheath in the acid abomasal environment and establish preferentially in the proximal 5–6 meters of the small intestine in sheep. A small proportion of the population settles in the abomasal antral mucosa near the pylorus. The larvae in the intestine enter tunnels above the basal lamina, between enterocytes, mainly at the base of villi, and they persist throughout their life at least partially embedded in the epithelium. Usually, infecting larvae all develop over ~2 weeks into adult worms, with a prepatent period of ~16–18 days.

Experimental infections of all *Trichostrongylus* species studied indicate that the lesions and pathogenesis of disease caused by them are similar, though *T. vitrinus* seems more pathogenic than *T. colubriformis* and *T. rugatus*. Villus atrophy occurs in areas of intestine heavily populated by the worms, and the severity of the lesion within individual animals is correlated with the local density of the worms. The mechanism by which the atrophy occurs has not been investigated. However, in experimental infections, hyperplasia of cryptal epithelium seems to precede the onset of villus atrophy, which may be T-cell mediated.

The established lesion is characterized microscopically by *villus atrophy* that may vary considerably in severity (Fig. 1.186), in association with elongate, dilated, often straight crypts containing many mitotic cells. Goblet cells may be numerous in crypts in some infected animals. In animals with subtotal villus atrophy, the surface epithelium may vary from tall columnar relatively normal-appearing cells, to more domed or cuboidal epithelium lacking a well-defined brush border. Ultrastructurally, such epithelium appears poorly differentiated, containing numerous polyribosomes in the cytoplasm, and with stumpy, sparse, and irregularly oriented microvilli. In animals with subtotal villus atrophy, exfoliating rounded enterocytes are seen, as are focal and probably transient "leaks" of neutrophils and tissue fluids through the epithelial surface. In animals with more severe atrophy, the surface epithelium is flattened between the openings of crypts, and erosions of the mucosa may be evident, from which inflammatory cells and tissue fluid effuse.

The lamina propria in the affected area of intestine is populated by a moderately heavy mixed inflammatory cell population. Lymphocytes and plasma cells are prominent between crypts, with an admixture of eosinophils. Globule leukocytes may be present in the epithelium of crypts and occasionally villi, but this is often not marked in severely affected mucosa. The presence of globule leukocytes is associated with a positive immune response. In the superficial lamina propria, neutrophils often accumulate beneath the epithelium, and in areas of erosion or previous erosion there may be a thin transversely oriented layer of connective tissue. Abnormal permeability of the endothelium of capillaries and venules in heavily infected mucosa has

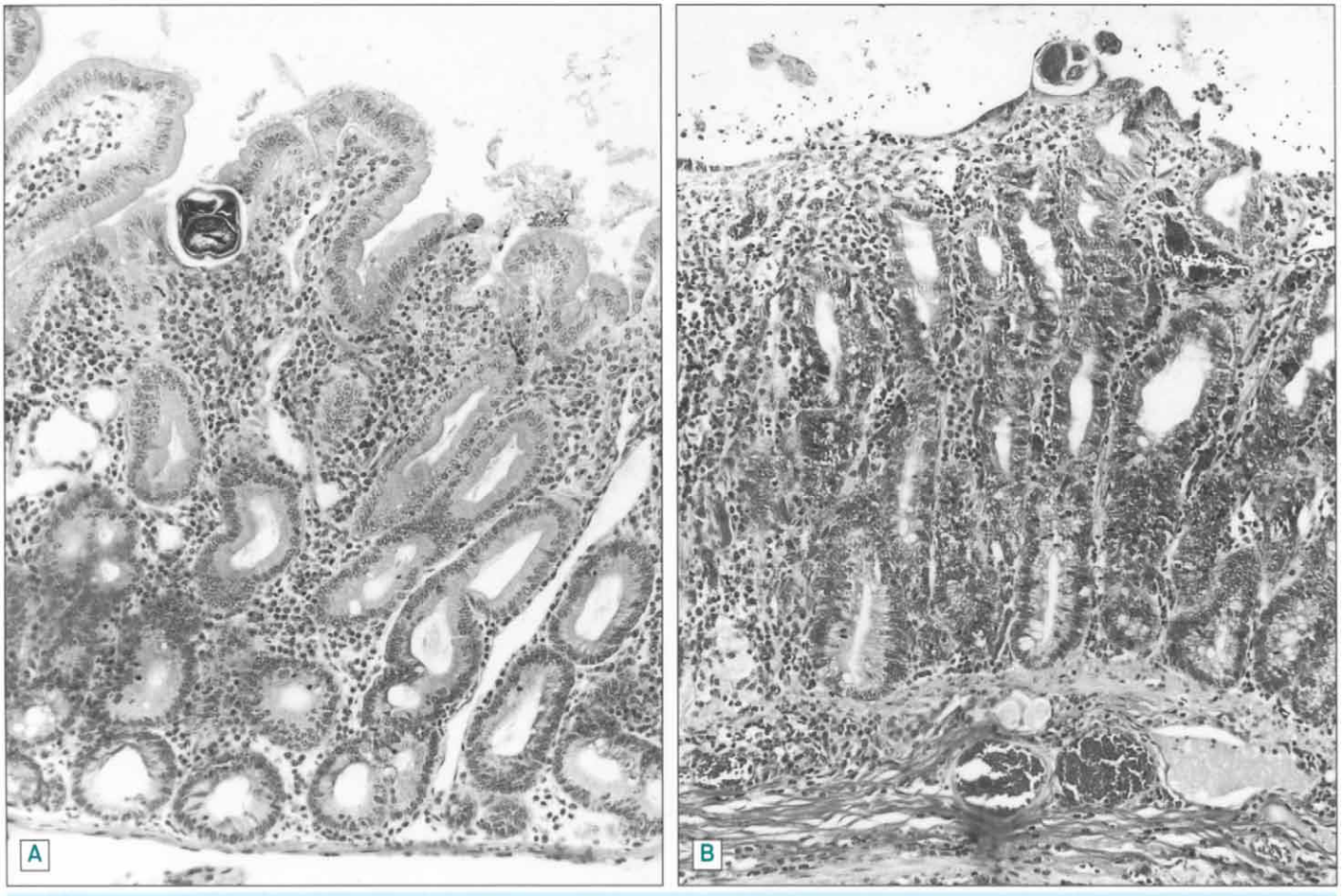

Figure 1.186 Intestinal trichostrongylosis in a sheep. **A.** Subtotal villus atrophy, caused by ***Trichostrongylus colubriformis***. Exfoliation of enterocytes, focal effusion of tissue fluid, dilated crypts, and mononuclear cells in lamina propria. **B.** Severe villus atrophy. Surface epithelium is attenuated or eroded, and crypts are hyperplastic. Nematode in tunnel in surface epithelium.

been demonstrated, and edema of the lamina propria may be evident in these areas.

The disease is marked clinically by depression, inappetence, which may be mild or profound, and by diarrhea and wasting. The cause of the inappetence is unclear. The pathogenesis of the diarrhea is uncertain. It is associated with the period when inappetence and gastric dysfunction occur. Though local malabsorption of nutrients, and presumably electrolytes and water, occurs in the duodenum, it seems unlikely that the absorptive capacity of the remaining small intestine and large bowel would be overwhelmed.

Weight loss or reduced productive efficiency is not related to nutrient malabsorption, since net absorption of nutrients over the length of the small intestine does not seem to be severely affected. Rather, the interaction of reduced feed consumption with increased loss of endogenous nitrogen into the gut seems to be responsible. There is considerable effusion of plasma protein into the intestine of infected animals, and this, coupled with exfoliation of epithelium that appears to be turning over at an increased rate, is the source of *protein loss*. In trichostrongylosis, compensation for increased catabolism of plasma protein and mucosal epithelial protein is at the expense of anabolic processes elsewhere in the body. Wool and muscle growth are hindered. Osteoporosis may occur.

Animals succumbing to trichostrongylosis are usually cachectic and dehydrated. Dark-green scoured feces will be on the skin or wool of the escutcheon or breech. There may be serous atrophy of internal fat depots, and marked atrophy of skeletal muscle. The subcutis is tacky. There may be edema of the mesentery and perhaps serous effusion into the body cavities, associated with hypoproteinemia, if dehydration is not severe. Mesenteric lymph nodes are enlarged and wet. The intestines are flaccid and the small bowel contains thin fluid green content, which in the duodenum may appear somewhat mucoid. The large intestine may contain similar fluid or pasty green, foul-smelling feces.

The mucosa of the duodenum in the freshly killed animal may be glistening and pink, but in spontaneous mortalities with superimposed postmortem change, it will be unremarkable. The proximal third of the small intestine (~5–7 meters) contains the bulk of the population of *Trichostrongylus*. A worm count on the small bowel usually reveals 15 000–80 000 *Trichostrongylus* in severe clinical infections. Subclinical or mild disease may be associated with fewer worms. The *diagnosis* is based on recovery of substantial populations of *Trichostrongylus* spp. in association with the clinicopathologic syndrome and villus atrophy. Mixed infections with other genera are common.

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Nematodirus and *Cooperia* infection

Nematodirus species infect the *proximal third of the small intestine of ruminants*. The most important species are *N. helvetianus*, which infects cattle; *N. spathiger*, *N. filicollis*, and *N. abnormalis*, which infect sheep, goats, and cattle; and *N. battus*, a parasite mainly of sheep, which will cause disease in calves.

The life cycle is direct. However, hatching of infective larvae of *N. battus* and *N. filicollis* from the egg is delayed. Eggs of *N. battus* deposited on the ground in one year hatch the next spring, following a period of conditioning by cold over winter. The epizootiologic pattern is that of infection of a susceptible lamb crop during one year by larvae produced by the previous year's lambs. Under these conditions, with many infective larvae, parasitic enteritis dominated by *Nematodirus* may occur. The larvae of *N. spathiger* and *N. helvetinus* are not delayed in hatching, and their epizootiologic pattern resembles that of *Trichostrongylus* spp. in grazing animals. They often form part of a mixed population of worms in parasitic gastroenteritis in grazing lambs and calves. The disease may occur in yarded calves as well as animals at pasture.

Third-stage larvae enter the deeper layers of the mucosa, perhaps entering crypts. Larvae emerge at the fourth or fifth stage to take up residence coiled among the villi, with their caudal ends protruding toward the lumen. They do not normally penetrate the epithelium.

The presence of large numbers of some *Nematodirus* species is associated with the development of villus atrophy, which is usually moderate in comparison with that induced by *Strongyloides* or *Trichostrongylus*. Villi are stumpy, bifurcate, perhaps fused, or ridge-like surface alterations that may replace the normal villus structures. Crypts may appear elongate and dilated.

Surface enterocytes may be domed, with loss of the prominent brush border, and irregular nuclear polarity. Ultrastructurally, such cells appear poorly differentiated and have irregular, deformed microvilli. Biochemical studies reveal reduction in levels of mucosal alkaline phosphatase and disaccharidases, which correlate with the severity of diarrhea in affected sheep.

The pathogenesis of the villus atrophy has not been determined, but it may be related to the development of an immune response to the nematodes in the lumen. A moderate mixed inflammatory

response with substantial numbers of lymphocytes, plasma cells, and eosinophils is evident in the lamina propria. The presence of such an infiltrate, and moderate shortening of villi associated with poorly differentiated surface enterocytes, is consistent with the postulated induction of villus atrophy by cell-mediated immune activity in the lamina propria.

Lambs and calves with nematodirosis develop *severe dark-green diarrhea*, which stains the escutcheon or the breech of lambs. Affected animals may become inappetent, scour, and waste for several weeks before recovering, or they may die acutely. Disease is presumably mainly related to malabsorption and loss of appetite. At necropsy, other than the changes associated with dehydration and cachexia, findings are limited to fluid mucoid content in the upper small intestine, and soft or fluid feces in the colon. The mucosa of the duodenum is usually unremarkable or perhaps hyperemic with excess mucus on the surface. Clinical disease is associated with populations of ~10000–50000 or more *Nematodirus*.

Cooperia infect the *upper small intestine of ruminants*. The important species include *C. curticei*, mainly in sheep and goats, and *C. pectinata*, *C. punctata*, and *C. oncophora*, mainly in cattle. The latter is regarded as the least pathogenic of the three. Though both sheep and cattle may suffer from mixed burdens of helminths containing or dominated by populations of *Cooperia*, this species seems to be more significant in cattle, especially in cool temperate regions.

Cooperia has a typical trichostrongylid life cycle, but larvae do have the capacity to undergo hypobiosis to carry the population through periods of regular climatic adversity. The normal prepatent period is ~16–20 days. Like *Nematodirus*, *Cooperia* do not tunnel in the epithelium, but rather brace or coil themselves among villi to maintain their place in the intestine. In light infections, the worms are concentrated in the proximal third of the small intestine. Heavier infections, perhaps because they are associated with villus atrophy and therefore loss of the substrate against which to brace, are more evenly distributed down the intestine.

Heavy burdens of *Cooperia* in calves, >70000–80000 nematodes, may be associated with *inappetence, reduced weight gain, or weight loss and diarrhea*, with protein-losing enteropathy in experimental infections. The associated atrophy of villi is concomitant with reductions in the brush-border enzymes. The syndrome is typical of intestinal helminthosis and the diagnosis is confirmed by finding large numbers of the fine, coiled *Cooperia* in the small intestine.

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Hookworm infection

Members of the Ancylostomatidae infect dogs, cats, ruminants, and swine. Hookworms of the genus *Globocephalus* appear to be of little significance in swine. In dogs, *Ancylostoma caninum*, *A. braziliense*, and *A. ceylanicum* occur. The former is most common in tropical, subtropical, and warm temperate zones of Africa, Australia, Asia, and North America, where adequate humidity for larval development occurs. *A. braziliense* occurs in dogs and cats in the tropics and subtropics, while *A. ceylanicum* is found in both species in Sri Lanka and Southeast Asia. *Uncinaria stenocephala* occurs in dogs in cool temperate regions of Europe and North America. *A. tubaeformae* only occurs in the cat.

***Ancylostoma* spp.** are capable of infecting the host by four routes: (1) *orally*, with direct development to adult worms in the intestine; (2) by *skin penetration*, resulting in movement through the bloodstream to the lungs, and thence via the trachea to the pharynx and gut; (3) by the *lactogenic* transmission of third-stage larvae mobilized from dormancy in the skeletal muscle of parturient bitches; and (4) in occasional instances, by prenatal *transplacental* transmission of mobilized larvae. The latter route of transmission does not apparently occur in *A. braziliense* infection. Some larvae of *A. caninum* may become arrested at the third stage in the intestine, to resume development at a later time.

Ancylostoma spp. all usually *inhabit the small intestine*, where they move about the surface, several times a day attaching to feed, then moving on. They penetrate deeply into the mucosa, sometimes to, or through, the muscularis mucosae, taking a plug of tissue into the large buccal capsule. Tissue is lacerated by prominent teeth, and anticoagulant is released, permitting persistent blood flow. Blood-sucking activity begins when larvae enter the adult stage, in *A. caninum* ~8 days after infection. Blood loss is maximal while worms are attaining maturity between 12 and 16 days after infection, and then again during the peak period of egg production after ~3.5 weeks of infection. The prepatent period for *A. caninum* is ~15 days.

Ancylostomosis is the result of persistent blood loss, resulting in anemia and hypoproteinemia. There is considerable variation in the blood-sucking activity, and therefore the pathogenicity, of the members of the genus. *A. caninum* consumes in the range of 0.01–0.2 mL of blood per worm per day. Pups several months old with populations of the order of 300–400 worms may lose 10–30% of their blood volume per day, depending on body weight. *A. tubaeformae* in cats is a significant blood sucker. Experimentally, about 200 worms may cause anemia, weight loss, and mortality in 1.5 kg cats.

The anemia in ancylostomosis is at first normochromic and normocytic. Small size, poor iron reserves, and the low level of iron in bitch's milk make suckling pups with ancylostomosis susceptible to

rapid development of the microcytic hypochromic anemia characteristic of iron deficiency.

Acute fatal ancylostomosis occurs most commonly in pups only 2–3 weeks of age, infected via the bitch's milk. Heavy infections acquired by this route may result in death from anemia and hypoproteinemia within a few days of initiation of blood-sucking activity, and before eggs are present in the feces. Anemia may also lead to mortality of pups after a course of longer duration. Percutaneous infection results in disease in older dogs held in runs or kennels under conditions of moisture and temperature conducive to larval development on the ground. Dermatitis due to larval penetration may be observed between the toes or on ventral contact surfaces of the body. Ancylostomosis in older dogs is usually typified by anemia, lack of exercise tolerance, weakness, and emaciation. Feces may be diarrheic, dark red or black, and are often mucoid. The major effect of ancylostomosis is due to increased loss of erythrocytes, iron, and plasma protein.

Animals dying of ancylostomosis are characteristically *extremely pale*. There is often glistening edema of subcutaneous tissues and mesenteries, and serous effusion into body cavities, attributable to hypoproteinemia. In chronic infections, cachexia may be evident. If recent exposure to heavy percutaneous infection has occurred, there may be dermatitis, and numerous focal hemorrhages scattered in the pulmonary parenchyma, reflecting disruption of vessels by larvae breaking out into alveoli. The intestinal content throughout the entire length is mucoid and deep red, from the erythrocytes voided into it by the worms (Fig. 1.187). The latter are visible, ~1–1.5 cm long, translucent, gray or red, depending on when they last consumed blood, dispersed over the mucosa, sometimes into the large intestine. They are often attached to the mucosa, and pinpoint red sites of recent feeding activity may be scattered over the intestinal surface. In a young pup, as few as 20–50 worms may be present in fatal infections, and they may be sufficiently sparsely scattered as to be overlooked, if not sought.

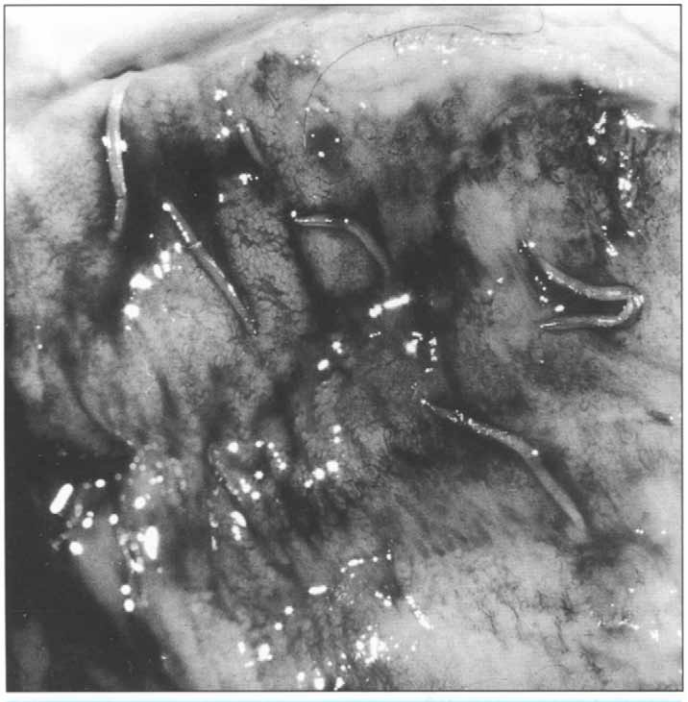

Figure 1.187 Robust ***Ancylostoma caninum*** nematodes attached to mucosa of the small intestine of a pup that died from exsanguination by hookworms. Gut content is bloody.

Uncinaria stenocephala mainly infects by the oral route; percutaneous infection is not efficient, though dermatitis may result; and prenatal and lactogenic transmission appear not to occur. This species sucks little blood and is much less pathogenic than *A. caninum*. However, heavy infections with this species, usually arising in contaminated communal kennel environments, may cause clinical disease and occasional mortality in pups. *Nonspecific lethargy, inappetence, and ill-thrift* are signs of infection, perhaps with diarrhea; anemia does not occur. Disease is associated with burdens of over ~1000 worms. The intestinal mucosa appears thickened, with scattered focal hemorrhages at sites of attachment.

The presence of large numbers of worms is associated with moderate atrophy and thickening of villi. The surface epithelium is irregular. Focal aggregates of mononuclear cells and some neutrophils are in the vicinity of the cranial end of worms embedded deep in the mucosa, a plug of tissue within their buccal cavity. Disease due to *U. stenocephala* may be related to villus atrophy, with protein loss into the gut, and perhaps malabsorption. Hypoproteinemia may be evident, but anemia does not occur. A similar syndrome may be associated with heavy infections of *A. braziliense*, in which hypoproteinemia also occurs.

All of these hookworms, but most especially *A. braziliense,* are associated with the development of cutaneous larva migrans in humans, as larvae can penetrate intact skin. *A. caninum* has been shown to cause eosinophilic enteritis in humans as well.

The **hookworms of ruminants** include: in cattle, *Bunostomum phlebotomum*, and in India and Indonesia, *Agriostomum vryburgi*; in sheep, *B. trigonocephalum* and, in India and Southeast Asia, Africa, and South America, *Gaigeria pachyscelis*. The life cycle of these nematodes is typical of hookworms. *Bunostomum* third-stage larvae infect by the oral or percutaneous routes, while *Gaigeria* only infect across the skin. Eggs and larval stages on the ground are extremely susceptible to desiccation, and hookworm disease in ruminants is most common in tropical or subtropical areas during wet seasons. However, stabled animals in cooler temperate areas may suffer disease resulting from larvae invading the skin from contaminated bedding. Following skin penetration, the usual pattern is seen, with migration of larvae to the lungs, where they molt to the fourth stage, and subsequently pass up the trachea to the digestive tract. Larvae taken in by ingestion spend some time in the deep mucosa of the intestine before emerging to mature in the lumen of the small intestine. The prepatent period of *Bunostomum* is long, ~7–8 weeks. *Gaigeria* larvae migrate via the lungs, and worms begin to lay eggs ~10 weeks after infection.

Both *Bunostomum* and *Gaigeria* cause *hemorrhagic anemia and hypoproteinemia*, especially in animals under a year of age. These species often occur with mixed gastrointestinal helminth burdens, and their effects are at least additive to those of the other worms. As few as 20–30 *Gaigeria* will cause anemia and hypoproteinemia in lambs and kids, though several times that number may be more usual in fatal cases. The size of the animal, the status of its iron reserves, and the plane of nutrition, especially the level of protein, likely influence the pathogenicity of these species.

At autopsy, the lesions expected in anemia and hypoproteinemia are evident. *Bunostomum* are often found in the lower half of the small

intestine, while *Gaigeria* tends to be concentrated high in the duodenum. Blood and bite marks may be evident on the mucosa in the infected areas of intestine, but hemorrhage may be occult. The relatively low numbers of worms associated with disease, and their peculiar distribution, dictate that the entire gut be examined and flushed, and a careful search be made for these species in suspect cases.

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Oesophagostomum and *Chabertia* infection

Members of the genus ***Oesophagostomum*** infect *sheep, cattle, and swine*. They form *inflammatory nodules in the wall of the intestine*, incited by histotropic larval stages. *Ill-thrift, diarrhea*, and, in cattle, *anemia*, can be induced by adult populations in the lumen of the colon.

In **sheep**, two species, *O. columbianum* and *O. venulosum*, are probably most significant; the former is considerably more pathogenic and is particularly important in warm temperate to tropical areas. Third-stage *O. columbianum* penetrate deeply into the lamina propria, or sometimes to the submucosa, mainly in the small intestine, where they normally spend ~1 week. They molt, emerge, and mature in the colon. However, a proportion of fourth-stage larvae enter a second histotropic phase in nodules in the colonic submucosa. Adult worms in the colon may be pathogenic for lambs. Burdens of only a few hundred *O. columbianum* are associated with anorexia, mucoid feces or diarrhea, and ill-thrift.

At necropsy of animals with clinical oesophagostomosis, the carcass is emaciated, the mesenteric lymph nodes are enlarged, and the colonic mucosa is thickened, congested, and covered by a layer of mucus in which the worms are scattered. There is hyperplasia of goblet cells, and the lamina propria contains a heavy mixed inflammatory infiltrate with eosinophils and many immune-active cells. Globule leukocytes are in the epithelium of glands. Nodules caused by histotropic fourth-stage larvae, mainly in the large intestine, are ~0.5–1 cm in diameter and are comprised of a central caseous or mineralized core surrounded by a thin fibrous encapsulating stroma. Microscopically, the nematode (or its remnants) is present among a mass of necrotic debris in which eosinophils are prominent. Giant cells and macrophages may surround the necrotic material. Similar nodules may be found in liver, lungs, mesentery, and mesenteric lymph nodes. Those in the deeper layers of the gut project from the serosal surface, hence the name "*pimply gut.*" They may cause adhesion to adjacent loops of gut or to other organs, and rarely may incite intussusception or peritonitis. However, in most cases, nodules are incidental findings at necropsy. *O. venulosum* is a much less significant parasite. It seldom causes significant nodule formation; when it does, the nodules are small and mainly in the cecum and colon.

In **cattle**, two species, *O. radiatum* and *O. venulosum*, occur, the former being the significant parasite. The life cycle is similar to that of *O. columbianum*. The disease caused by *O. radiatum* is characterized by *loss of appetite, reduced productive efficiency, anemia, hypoproteinemia, and diarrhea*. Anemia results from hemorrhage at sites of larval emergence, and from mucosal erosions and discontinuities in the gland lining, associated with maturing and adult populations of worms. Considerable exudation of tissue fluids and plasma protein from colonic lesions, in addition to that due to hemorrhage, contributes to the hypoproteinemia and gastrointestinal protein loss. Reduced growth, or loss in condition, is mainly the product of the interaction between protein effusion into the gut and inappetence. Diarrhea presumably results in part from loss of colonic absorptive capacity.

Oesophagostomosis may be fatal in calves. Animals may be pale from anemia, and edematous from hypoproteinemia. Cases of some duration will be cachectic. Colonic lymph nodes are enlarged. The mucosa of the colon is grossly thickened and folded by edema and increased mixed inflammatory cell infiltrates, including many immune-active cells, in the lamina propria. Colonic submucosal lymphoid follicles are large and active. Pathogenic worm burdens in calves are in the range of ~1000–10 000 *O. radiatum*. Although repeated exposure to infective larvae may result in the accumulation of large numbers of fourth-stage worms in nodules, formation of nodules has little pathogenic significance in cattle.

In **swine**, *O. dentatum*, *O. quadrispinulatum*, and several other species occur in the large intestine; the two mentioned are most widespread. The life cycle is typical of the genus. Third-stage larvae enter the wall of the cecum and colon, where they encyst and molt to the fourth stage, emerging ~1 week later to mature in the lumen. There is some indication that inhibitors of eicosanoid metabolism (indomethacin, acetylsalicylic acid) will suppress migration of these third-stage larvae. The larvae initially lie about the level of the base of the mucosa. They incite a reaction which causes local loss of the muscularis mucosae, so that the nodule formed involves both mucosa and submucosa, and the larvae ultimately reside in the submucosa. The nodules are grossly ~1–20 mm in diameter, umbilicate, and may contain yellow or black cheesy exudate in the center. Once the larvae molt and begin to move to the lumen, an intense influx of eosinophils and neutrophils occurs into the nodules, and a focus of necrotic debris and fibrin lies over the evacuated nodule.

Mucosal and submucosal edema causes thickening of the wall of the large bowel, and contraction of the cecum. Mucosal crypts elongate with increasing worm burdens. Lesions resolve following emergence of larvae.

Oesophagostomosis in swine is a *mild, usually subclinical disease*. Occasional diarrhea, depression in weight gain, and inefficiency of feed conversion may occur, especially during the period of emergence of larvae and maturation of worms in the lumen of the large intestine. Burdens of ~3000–20 000 adult worms are associated with subclinical disease experimentally. The nematodes are ~1–2 cm long, white, and are present in mucus on the surface of the gut, or in luminal content. Massive repeated challenge will cause severe typhlocolitis, but this seems to be purely an experimental phenomenon.

Chabertia ovina, a robust worm ~1–2 cm long, inhabits the *colon of sheep, goats, and cattle*. It is particularly a problem in cooler climatic zones, mainly in sheep. Phylogenetic analysis based on ribosomal DNA sequence data indicates that *C. ovina* is clustered within the Oesophagostominae. The life cycle of *Chabertia* resembles that of *Oesophagostomum*; third-stage larvae encyst in the wall of the small intestine, then emerge to mature in the cecum and colon. Disease in sheep is associated with the presence of mature worms in the colon. Feces are soft, mucoid, and perhaps blood-flecked, and ill-thrift may occur. The adults penetrate to the muscularis mucosae and take a plug of mucosa into the buccal capsule; minor hemorrhage may be related to physical trauma to the mucosa. More significant is loss of plasma protein from the mucosa, associated with numerous focal sites of trauma, and with widespread areas of mononuclear infiltration in the mucosa and submucosa. There is also hyperplasia of goblet cells. Grossly the lesions are characterized by edema of all layers of the wall of infected parts of the colon, and enlargement of colonic lymph nodes. Worms are generally concentrated in the proximal portion of the spiral colon, and the area that they inhabit may have numerous hemorrhagic foci corresponding to sites of former attachment. Pathogenic burdens may be as few as 150 worms and the species must be sought in its usual site of predilection or be missed.

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Equine strongylosis

Members of the family Strongylidae are common nematode parasites of the cecum and colon in horses, usually present as mixed infections. The subfamily Strongylinae, or **large strongyles**, includes the important genus *Strongylus* and the less significant genera *Triodontophorus*, *Oesophagodontus*, and *Craterostomum*. Members of this group are *plug feeders or blood suckers*, and *Strongylus* spp. undergo extensive extraintestinal migrations. The subfamily Cyathostominae, or **small strongyles**, includes eight genera of nematodes. Adults of this group *feed mainly on intestinal contents*, and are of little pathogenic significance. However, emergence of *histotropic larval stages* from the gut wall may cause disease.

Large strongyles

Strongylus vulgaris is relatively common, and has been considered the *most significant nematode parasite in horses*. However, infection levels have considerably decreased with the advent of improved anthelmintics. Larval forms cause *endoarteritis* in the mesenteric circulation, resulting in colic and infarction of the large bowel, while the adults cause *anemia and ill-thrift*. Infective third-stage larvae, ingested from pasture, penetrate the mucosa of the small and large intestine, and molt to the fourth stage. They enter the lumina of small arterioles, up which they migrate, on or under the intima, to reach the cranial mesenteric artery within 3 weeks. Three or 4 months later, after molting in that location to the fifth stage, the immature adults return down the mesenteric arteries to the wall of the cecum or colon, where they encapsulate in the subserosa, forming nodules ~5–8 mm in diameter. Returning larvae in nodules are surrounded by necrotic debris, neutrophils, some eosinophils, and macrophages, and the adjacent arteriole may be thrombosed. They eventually break into the lumen of the large bowel, especially cecum and right ventral colon, where they mature in another 1–2 months, ~6–7 months after infection. Some larvae may become trapped and encapsulated in arterioles in the mesentery on their way back to the gut, and remain there to die eventually.

Endoarteritis associated with migration and establishment of larvae in the cranial mesenteric artery and its branches is discussed in Vol. 3, Cardiovascular system, as are the consequences of aberrant migration in the aorta and other arteries. Syndromes associated with aberrant migration include *cerebrospinal nematodiasis* and *aortic-iliac thrombosis*. Lesions of the cranial mesenteric artery and of the cecal and colic arteries may lead to *colic* as a result of reduced perfusion or thromboembolism, or perhaps due to impingement upon autonomic ganglia in the vicinity of the arterial root at the aorta. Though many older horses are infected with adult worms, or have arterial lesions, the complications of colic and infarction caused by this parasite are most common in young horses. An *acute syndrome*, characterized by pyrexia, anorexia, depression and weight loss, diarrhea or constipation, colic, and infarction of intestine occurs in foals infected with large numbers of larvae, but not often in animals previously exposed to infection.

S. edentatus is also common and has a life cycle characterized by extensive larval migration. Third-stage larvae enter the intestinal wall and pass in the portal system to the liver where they incite inflammatory foci. Here they molt to the fourth stage and, ~30 days after infection, begin migrating through the hepatic parenchyma. The foci of inflammatory reaction in the liver are probably related to antigens

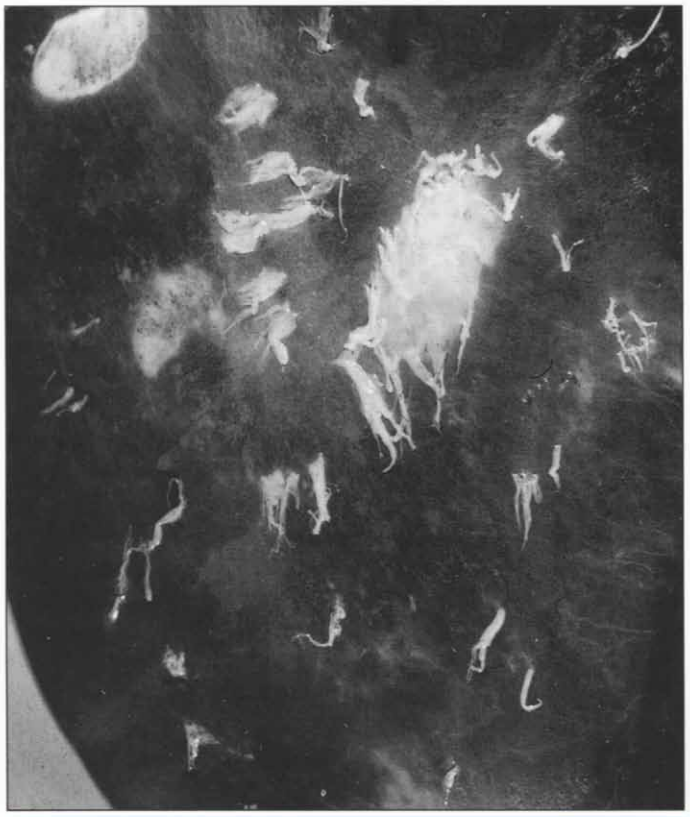

Figure 1.188 Fibrous tags and capsular scars, associated with migration of larval ***Strongylus edentatus***, on the diaphragmatic surface of a horse liver.

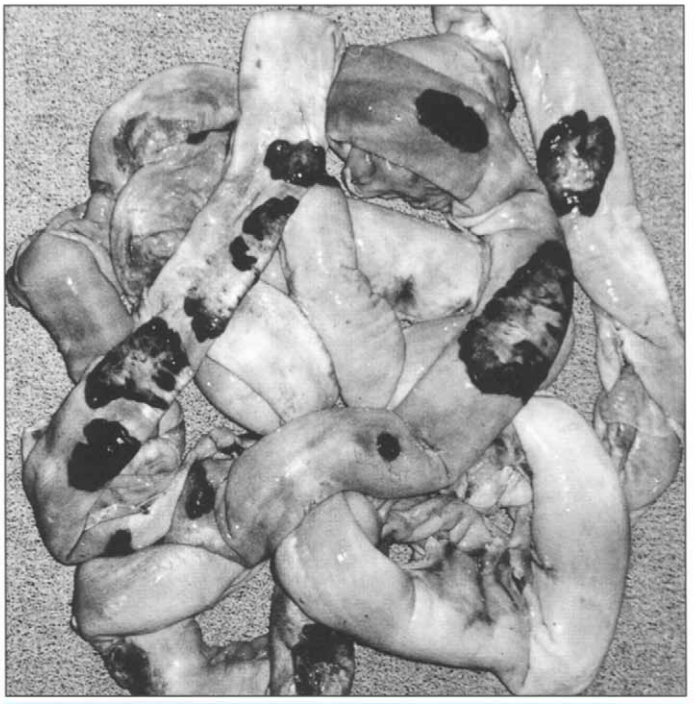

Figure 1.189 Hemomelasma ilei, large subserosal plaques of resolving hemorrhage on the small intestine of a horse, associated with damage caused by migrating larvae of **large strongyles**.

released by migrating and trapped larvae. They consist of a core of necrotic eosinophils, with a surrounding fibrous capsule, a mixture of neutrophils, eosinophils, and mononuclear cells, or recent necrotic foci or tracks infiltrated by neutrophils and a few eosinophils.

By 8–10 weeks after infection, larvae are migrating from the liver via the hepatic ligaments. Hemorrhagic tracks may be produced in the hepatic parenchyma. *Parenchymal scars and tags of fibrous tissue on the hepatic capsule*, especially the diaphragmatic surface (Fig. 1.188), commonly found at autopsy, are the legacy of migrating *S. edentatus*. Those migrating in the hepatorenal ligament enter the retroperitoneal tissue of the flank, where they may be encountered, often associated with local hemorrhage. Larvae in aberrant locations in the omentum, hepatic ligaments, and diaphragm may become encapsulated in eosinophilic granulomas and destroyed. Omental adhesions may also be a sequel to aberrant larval migration. In the flank, larvae persist for several months, molting to the fifth stage before returning from the right flank via the cecal ligament to the cecum and origin of the colon. Here they form nodules and edematous or hemorrhagic plaques in the wall of the gut, eventually perforating to the lumen, where they mature and begin to lay eggs ~10–12 months after infection. Lesions associated with the larval migration of *S. edentatus* are usually incidental findings at autopsy.

S. equinus is less prevalent and abundant than the other two members of the genus. Exsheathed third-stage larvae penetrate to the deeper layers of the wall of the ileum, cecum, and colon, molt to the fourth stage and produce hemorrhagic subserosal nodules, before moving to the liver through the peritoneal cavity. They migrate in the hepatic parenchyma for 6–7 weeks, then leave the liver, probably via the hepatic ligaments, to the pancreas and peritoneal cavity, where they molt to the fifth stage ~4 months after infection. They regain the lumen of the cecum and especially right ventral colon, by an unknown route, probably by direct penetration from the peritoneal cavity or pancreas. Pancreatic damage is usually mild and is mainly manifested by slight periductal infiltration of eosinophils.

Hemomelasma ilei is the term applied to slightly elevated *subserosal hemorrhagic plaques*, up to 1–2 × 3–4 cm in size, usually found along the antimesenteric border of the distal small intestine, or rarely on the large bowel (Fig. 1.189). They are associated with trauma by migrating larvae of *S. edentatus* in particular, but may be caused by larvae of any of the *Strongylus* spp. These lesions are comprised of edema, hemorrhage, and a mixed population of leukocytes, with macrophages ingesting erythrocytes prominent in evolving lesions. Occasionally a fragment of nematode or cuticle, or a migratory track, may be found in section. The lesion may be an incidental finding, and is probably a rare cause of clinical signs of colic.

Adults of all species in the Strongylinae are plug feeders and blood suckers. In sufficient numbers they may cause ill-thrift and anemia, as the result of active hematophagia and blood loss from recent sites of feeding activity. Increased albumin catabolism causing accelerated turnover of the plasma pool, and reduced red cell survival, have been demonstrated in horses infested with relatively low numbers (<100) of *S. vulgaris*. *Triodontophorus tenuicollis*, the most important species of that genus, tends to attach to the mucosa in clusters, usually in the right dorsal colon, causing local congestion and ulceration. *Triodontophorus* may be associated with significant blood loss.

Cyathostomins The **small strongyles**, or cyathostomins, are *essentially nonpathogenic as adults*, despite the fact that tens or many hundreds of thousands may be in the content of the large bowel.

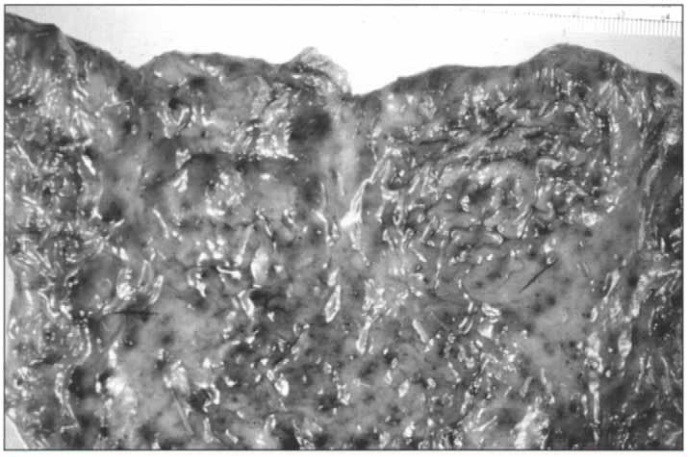

Figure 1.190 Equine colonic mucosa studded with 2–5 mm diameter nodules formed by **larval cyathostomins**, some of which are emerging, others of which have been released by incising nodules. Larvae are red due to nematode, not host, hemoglobin.

The larval stages migrate into the deep mucosa or submucosa of the large bowel (mainly cecum and ventral colon) to molt and develop, before emerging to the lumen to molt again and mature. Adults are mainly found in the dorsal and ventral colon. The third- or fourth-stage larvae may undergo hypobiosis or retarded development, persisting in nodules in the gut wall, only to mature sporadically, or perhaps more synchronously, as the adult population in the lumen turns over or is lost. Emergence of larvae causes rupture of the muscularis mucosae and intense local eosinophilia and edema, followed by infiltration of neutrophils and macrophages.

Mucosal nodules are up to only a few millimeters in diameter, slightly raised red or black, and may be umbilicate. Incision reveals a small translucent gray or red larval nematode. In heavy infections, the mucosa of the cecum and colon may be diffusely pocked by such nodules.

Disease attributable to larval cyathostomins usually occurs in heavily infected horses at the time of turnover of the adult population. It is due to *emergence of large numbers of hypobiotic larvae* over a short period, somewhat analogous to type II ostertagiosis in cattle. This occurs in the late winter, spring, and early summer in northern temperate climates. Cyathostominosis is a disease of horses over a year of age, and little resistance is apparent to repeated infection. Animals develop a syndrome characterized by *diarrhea, ill-thrift, and hypoalbuminemia*. In animals examined at this time, numerous nodules, containing immature cyathostomes or recently ruptured, are present in the mucosa of the cecum and colon (Fig. 1.190). The mucosa and submucosa are edematous and the mucosa congested and many recently emerged fourth-stage or early fifth-stage larvae may be in the gut content. Microscopically, there are numerous encysted cyathostomin larvae within the mucosa.

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Ascarid infection

Members of the family Ascarididae are *common and important parasites of swine, horses, dogs, cats, water buffalo, and, to a lesser extent, cattle*. They do not normally occur in sheep and goats. Their importance is related to incidental and sometimes significant lesions caused by *larvae during migration* in the tissues of definitive and accidental hosts, and to the effects of *adult worms* in the small intestine of the definitive host.

Ascaris suum is a large parasite, usually found in the *upper half of the small intestine of swine*; females measure up to 40 cm long. The life cycle is direct. Infective larvae, present in the resistant egg, are released in the intestine and penetrate the mucosa to be carried in the portal blood to the liver. They then pass to the lungs in the blood, and break out of capillaries into alveoli. Third-stage larvae may be found in liver and lung 3–5 days after infection. Larvae move up the respiratory tree to the pharynx, where they are swallowed, arriving in the intestine about a week after infection. Worms mature in the intestine, and begin to lay eggs ~2 months after infection. Small doses of eggs more commonly give rise to patent infections than do large doses. This probably results from excessive loss of migrating larvae due to resistance incited by the antigenic mass of the heavier infections.

Larval migration induces lesions in the liver and lungs (Fig. 1.191). Infections heavy enough to cause clinical signs are rare in swine reared under conditions of good hygiene and husbandry. However, respiratory signs characterized by dyspnea (commonly termed "thumps") may occur in piglets if large numbers of larvae migrate through the lungs. Gross lesions in pigs, associated with pulmonary migration of ascarids, are largely limited to numerous focal hemorrhages scattered over and through the pulmonary parenchyma.

Microscopically there is eosinophilic bronchiolitis. Bronchioles are surrounded by macrophages and eosinophils, and the bronchiolar mucosa is thrown into small folds, the epithelium frequently disorganized or perhaps eroded. The bronchiolar wall is infiltrated by

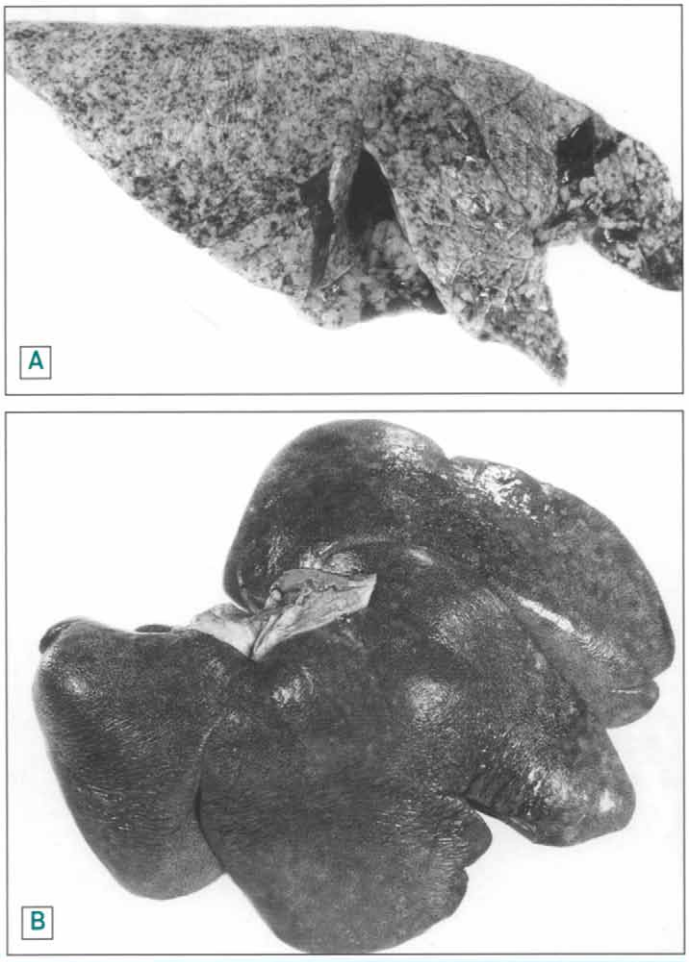

Figure 1.191 Effects of migrating ***Ascaris suum*** larvae in a pig. **A.** Multifocal parenchymal hemorrhages in the lung. **B.** Interstitial hepatitis.

eosinophils that are also present, with necrotic debris, in the lumen. There may also be an eosinophilic and granulomatous vasculitis.

Larvae are usually readily found in section. They may be present in alveoli, alveolar ducts, bronchioles, or bronchi, perhaps surrounded by eosinophils. In more chronic cases, larvae in tissue are in eosinophilic granulomas. The worms may be dead in cases of some standing, and are only recognized as an eosinophilic remnant or some bits of cuticle. Like all larval ascarids of mammals, *A. suum* in the lungs have *lateral alae visible in section.*

Lesions in the liver due to migrating *A. suum*, though not causing clinical disease, do result in considerable economic loss from condemnation at meat inspection. At first exposure to larvae, the lesions are related to mechanical damage caused by the worms, subsequent repair, and hypersensitivity reactions to excretory and secretory products of the larvae. Initially, hemorrhagic tracks are present near portal areas and throughout lobules. They are visible through the capsule as pinpoint red areas, perhaps slightly depressed and surrounded by a narrow pale zone. These lesions collapse and heal by fibrosis, causing scarring that involves most intensely the adjacent portal tracts. However, fibrosis extends diffusely through more distant tracts, emphasizing lobular outlines. There is a heavy eosinophil infiltrate in fibrotic septa, which becomes most obvious beginning ~10–14 days after infection. Granulomatous foci containing giant cells, macrophages, and eosinophils may center on the remnants of larvae trapped and destroyed in the liver.

The inflammatory infiltrates in livers of animals exposed to larval ascarids may become severe and diffuse, and this is reflected in the gross appearance of the liver, which has extensive "*milk spots*," and prominent definition of lobules. The liver is firm, and heavy scars may become confluent, obliterating some lobules and extending out to exaggerate interlobular septa throughout the liver. Where pigs are raised intensively, it is now rare to encounter extreme fibrosis of the liver associated with ascarid migration.

The pathogenicity of adult ascarids in the intestine is poorly defined. Heavy infections may obstruct the gut, being visible as rope-like masses throughout the intestinal wall. Ascarids may occasionally pass to the stomach and be vomited or migrate up the pancreatic or bile ducts. Rarely, intestinal perforation occurs. The presence of ~80–100 worms in 3-month-old swine fed low protein rations may depress feed intake and the efficiency of feed conversion. *A. lumbricoides* in humans interferes with carbohydrate, fat, and protein absorption, and *A. suum* probably has a similar influence. The effects of infection seem to be most apparent in animals on diets marginal in energy, and in quantity and quality of protein.

A. suum also infects animals other than swine. In sheep, and occasionally cattle, immature ascarids may be found in the intestine. Dyspnea and coughing associated with eosinophilic pneumonia, and focal eosinophilic hepatitis, may occur in lambs exposed to *A. suis*; mortality may occur, rarely. Liver lesions in lambs are usually too small to be significant at meat inspection.

In **calves** exposed to yards contaminated by pig feces containing *Ascaris* eggs, *severe acute interstitial pneumonia* may occur. Signs of dyspnea, tachypnea, coughing, and increased expiratory effort are usually first seen ~7–10 days after exposure, when large numbers of larvae are present in the lungs. Deaths may ensue over the following few days, and the lungs are moderately consolidated, light pink to deep red, with alveolar and interstitial emphysema and interlobular edema. Microscopically, there is thickening of alveolar septa, and effusion of fibrin, proteinaceous edema fluid, and macrophages into alveoli. Hemorrhage into alveoli may also occur. Larvae are present in alveoli and bronchioles and provoke acute bronchiolitis. Neutrophils are found around larvae in bronchioles; eosinophils may be present but are not prominent in animals dying acutely. In addition to being usually observed readily in tissue sections, larvae may be recovered from the airways by washing with saline, or from minced lung in saline or digestion fluid, by use of a Baermann apparatus. Tens of thousands to millions of larvae may be present in the lungs of fatal cases.

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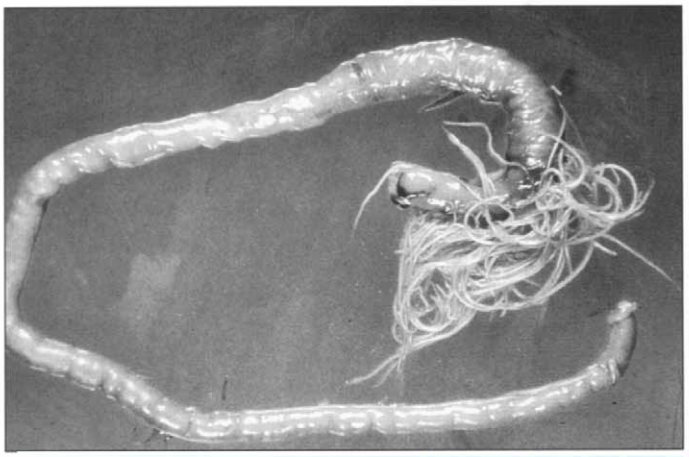

Figure 1.192 A heavy burden of ***Parascaris equorum*** impacted in and obstructing small intestine in a foal.

Stephenson LS, et al. *Ascaris suum*: nutrient absorption, growth, and intestinal pathology in young pigs experimentally infected with 15-day-old larvae. Exp Parasitol 1980;49:15–25.

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Parascaris equorum is the *ascarid of horses*. It is widespread and common in young horses; it may contribute to ill-thrift and occasionally causes death by obstruction. *P. equorum* is a large nematode, females being up to 50 cm long. The life cycle resembles that of *A. suum*. Similarly, *hepatic and pulmonary lesions are associated with larval migration*, and coughing may occur at the time larvae are in the lungs, if infections are heavy. The prepatent period is ~10–15 weeks. The lesions in the lungs of foals with migrating *Parascaris* larvae, ~2 weeks after infection, are like those described in swine with *Ascaris*. Animals with resolving pulmonary lesions develop subpleural nodular accumulations of lymphocytes up to 1 cm in diameter, and there may be lymphocytic cuffing of pulmonary vessels.

It is possible to establish heavy infections of *P. equorum* in the intestines of foals a few months old, but not in yearlings, where larvae appear to be killed during hepatopulmonary migration. However, in heavily infected foals, many worms are lost from the intestine prior to patency, suggesting the possibility of an effect of crowding on the population of growing worms. A heavy burden of ascarids in the intestine may reduce weight gains in growing foals. Inappetence occurs but increased plasma protein catabolism or loss into the gut does not. Reduced weight gain may be due to decreased protein intake. Ascarid infection may reduce rate of intestinal transit. Heavy burdens can be associated with obstruction, intussusception or, rarely, perforation of the intestine (Fig. 1.192).

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The **ascarids of small animals** are *Toxascaris leonina*, infecting both cats and dogs, and *Toxocara canis* and *T. cati*, infecting the dog and cat respectively. All occur in the *small intestine*, mainly in young animals.

Toxascaris leonina has a life cycle that may be direct, but can involve a paratenic host. In the definitive host, larvae ingested in infective ova enter the wall of the gut, where they remain for several weeks, molting to the fourth stage and emerging to the intestinal lumen to molt again and mature. The prepatent period is ~10–11 weeks.

Toxocara canis has a complex life cycle. Puppies may be infected by ingestion of larvated ova, in which case larvae follow the pathway of hepatopulmonary movement in the bloodstream, and tracheal migration to the pharynx and gut, though some reach other tissues in the circulation. In older dogs most larvae ingested in eggs are disseminated in the circulation to various tissues, where they encyst, rather than undergoing development and tracheal migration. In the pregnant bitch, these larvae are mobilized, crossing the placenta to infect the fetus after day 42 of gestation. In the fetus, they remain in the liver, passing to the lungs after birth. Transmammary transmission of mobilized second-stage larvae also occurs, infecting the neonate via the colostrum. In a wide variety of species, infective larvae are disseminated hematogenously to many organs, where they settle, mainly in muscle. In some abnormal hosts, including humans, a syndrome termed "*visceral larva migrans*" is described, characterized by eosinophilia, general malaise, and perhaps signs related to granulomatous reactions to larvae in the eye, liver, lungs, and brain. Larvae in paratenic hosts eaten by young dogs undergo tracheal migration before maturing in the gut.

T. cati may infect cats directly from the larvated egg, via paratenic hosts, or, in kittens, by the transmammary route from the postparturient queen. Prenatal infection apparently does not occur. Larvae hatching from eggs migrate via the liver, lungs, and trachea, while those taken in from milk or prey do not. Following tracheal migration or ingestion in milk or prey, larvae enter the gastric wall, while fourth-stage larvae are found in the gastric contents, and the wall and lumen of the small intestine. *T. cati* will cause visceral larva migrans in humans, and if ingested as immature worms, will mature in the intestine.

Focal hemorrhages may be found in the lungs of puppies with migrating *T. canis* larvae. Larval *T. canis* are occasionally found in or associated with eosinophilic granulomas in the tissues of pups and older dogs. Inflammatory foci are most commonly seen grossly in the *kidney*, as white elevated spots 1–2 mm in diameter in the cortex beneath the capsule. They may be encountered in section in any organ, and are comprised of a small focus of macrophages, lymphocytes, and plasma cells, with a few eosinophils, and possibly containing a larva. Larvae may be destroyed in such foci, which heal by scarring. Considering the large numbers of larvae that must move through the tissues of dogs, and in many cases be sequestered there, relatively few are encountered incidentally, free or encapsulated in granulomas. Granulomas incited by *T. canis* larvae may be found in

the *eye* on ophthalmoscopic examination, and retinitis, postinflammatory retinopathy, and blindness associated with *T. canis* larvae confirm that *ocular larva migrans* is a significant entity in dogs. Occult lesions similar to those occurring in dogs may be found in the tissues of cats infected with *T. canis*, though ocular larva migrans has not been recognized.

T. cati developing in the mucosa of the stomach and intestine may provoke a mild granulomatous response comprised of lymphocytes and a few macrophages about the coiled larva. Larvae free of such a response are also found in the mucosa and submucosa.

Heavy infections of ascarids in puppies and kittens, usually those reared in unhygienic communal environments, may result in *ill-thrift*. The most significant effects are those caused in the stomach and intestine by maturing *T. canis* in young puppies infected prenatally. The animals may develop weakness, lethargy, and vomition that is occasionally fatal. At autopsy the animal appears poorly grown for its age, pot-bellied, and cachectic, and masses of maturing worms are present in the intestine (Fig. 1.193) and perhaps stomach. Sometimes up to 20% of the body weight of young puppies may be accounted for by the worm burden. *T. cati* may be associated with clinical disease, but usually not death, in kittens up to several months of age. Medial hypertrophy of the pulmonary artery in cats has been associated with *T. cati* and *T. canis* infection; whether it is causal is unclear. Disease is rarely attributed to *T. leonina*.

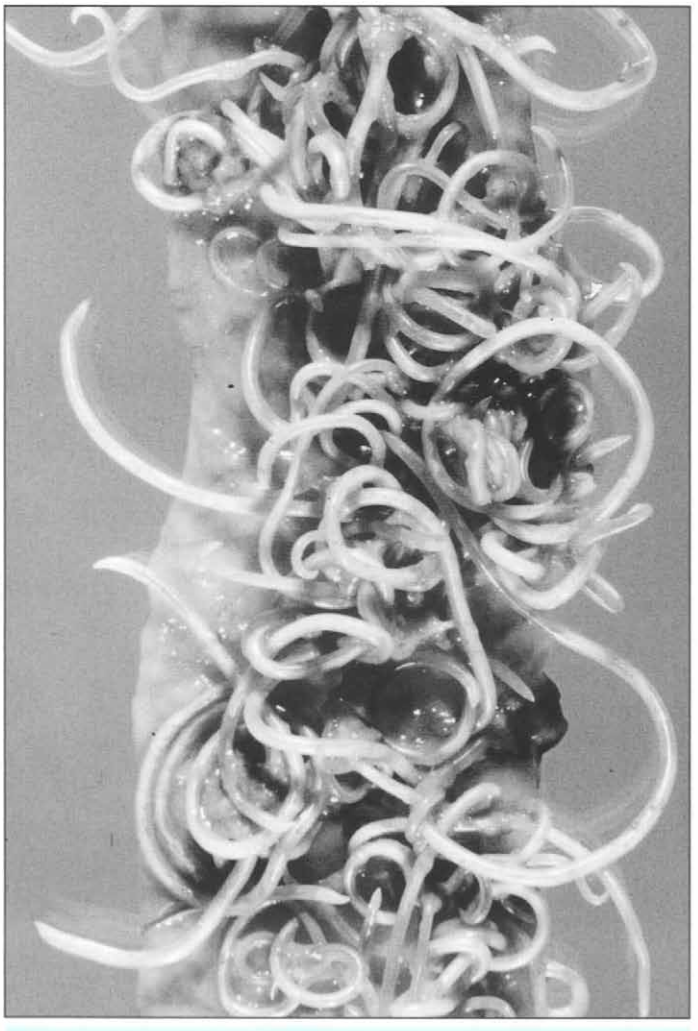

Figure 1.193 Tangled mass of ***Toxocara canis*** in the small intestine of a pup.

Mature *T. cati* are up to ~10 cm long; *T. canis* are up to ~18 cm long. In freshly dead animals, they are often coiled like a spiraled spring. They may maintain their place in the intestine by bracing against the gut wall in this way. The mechanism by which adults of these ascarids in the intestinal lumen impair growth has not been investigated. Ascarids occasionally enter the bile or pancreatic ducts, and many perforate those structures or the intestine.

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Toxocara (*Neoascaris*) ***vitulorum*** infects the *small intestine of young calves of domestic cattle*, mainly in the tropics and subtropics, and it is especially significant in water buffalo.

The life cycle involves transmammary transmission of third-stage larvae mobilized from the tissues of the dam within a few days of parturition. The larvae attain the liver of the calf and undergo tracheal migration. Patency occurs within ~1 month of birth, but worms are expelled within a short time, and by ~2–3 months of age, none are present.

Signs of infection include foul-smelling *diarrhea and ill-thrift*. Immature and mature worms both contribute to the signs. Heavily infected calves may die in an emaciated state, with burdens of up to 400–500 worms as much as 30 cm long in the intestine. Occasionally, migration up the bile duct or perforation of the gut may occur.

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Trichuris infection

Trichuris species, the **whipworms**, are so called because of their long thin cranial end and shorter stouter caudal portion. They inhabit the *cecum*, and occasionally the *colon*, of all the domestic animals considered here, except the horse. The host–parasite relationships

include: in dogs – *T. vulpis*; in cats – *T. campanula* and *T. serrata*; in swine – *T. suis*; in sheep and goats – *T. ovis, T. globulosa, T. skrjabini*; in cattle – *T. discolor* and, less commonly, *T. ovis* and *T. globulosa*.

The life cycle is direct. Larvated ova are resistant to climatic insult, and persist in contaminated environments for several years. Ingestion of larvated eggs leads to release of third-stage larvae, which enter the mucosa of the proximal small intestine for up to 7–10 days, before returning to the lumen and passing on to the cecum, where they establish their adult existence. The prepatent period varies from ~6–7 weeks in the case of *T. suis* to 11–12 weeks for *T. vulpis*. In rare instances, disease may occur during the prepatent period, in which case ova will not be in the feces.

In all species, the cranial end of the worm is embedded at least partially in tunnels within the surface epithelium (Fig. 1.194), but not normally breaching the basal lamina. Light infections apparently cause little morphologic alteration in the mucosa and no disease. While *Trichuris* ingest blood, disease associated with them is not usually related to this activity. A protease produced by *T. globulosa* has been shown to promote degradation of mucosal tissue.

Heavy infection with *Trichuris* is associated with *severe and often hemorrhagic typhlitis or typhlocolitis in all species*. In the **dog**, large populations of worms overflow their normal habitat and infect the mucosa of the ascending, and often more distal, colon, sometimes extending to the rectum. The signs are chronic diarrhea or dysentery, perhaps with some weight loss. The blood and foul odor of the feces are due to hemorrhage and effusion of tissue fluid from the eroded mucosal surface. The mucosa is thickened, red, and edematous. The colonic content is fluid or porridge-like, and brown, tinged pink, or red. Masses of tangled worms are visible on the mucosa (Fig. 1.195). Microscopically the mucosa is widely eroded, or mildly ulcerated, and effusion of inflammatory exudate and blood is evident. Glandular epithelium is hyperplastic. Occasionally, *T. vulpis* infection may be associated with local or regional transmural lesions, with granulomatous foci and fibroplasia in deeper layers of the mucosa. Sometimes ova or worms are in these aberrant locations.

Trichuris suis infection in **swine**, if heavy enough, may cause *mucohemorrhagic typhlocolitis* that is associated with anorexia, diarrhea, or dysentery, dehydration, ill-thrift and, in some cases, death. The disease is most common in animals exposed to dirt yards contaminated with infective *Trichuris* ova. The lesion is one of mucosal colitis, resembling that described in the dog. There is thickening of the mucosa with mucus hypersecretion from hypertrophic glands, coupled with erosion of, and effusion from, the mucosal surface. Lesions are more severe in swine with conventional gut flora than in those reared germfree, or free of known enteric pathogens. *T. suis* may suppress mucosal immunity to resident bacteria.

The large bowel in swine with *Trichuris* is thickened and congested, possibly with focal hemorrhages. The surface is glistening with mucus, perhaps with some fibrin exudation. The gross appearance may resemble that found in swine dysentery and the microscopic lesions are similar. However, closer examination reveals the presence

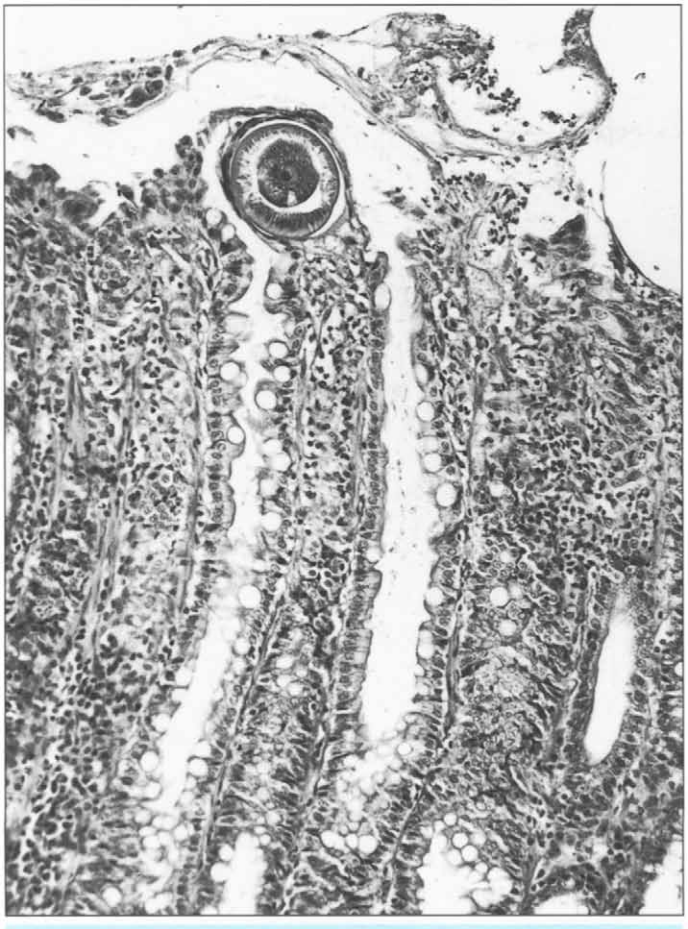

Figure 1.194 Mild erosive colitis in a dog with ***Trichuris vulpis*** infection. Cranial end of nematode is in tunnel in surface epithelium. There is exfoliation of epithelium, and effusion of neutrophils and fibrin from the surface.

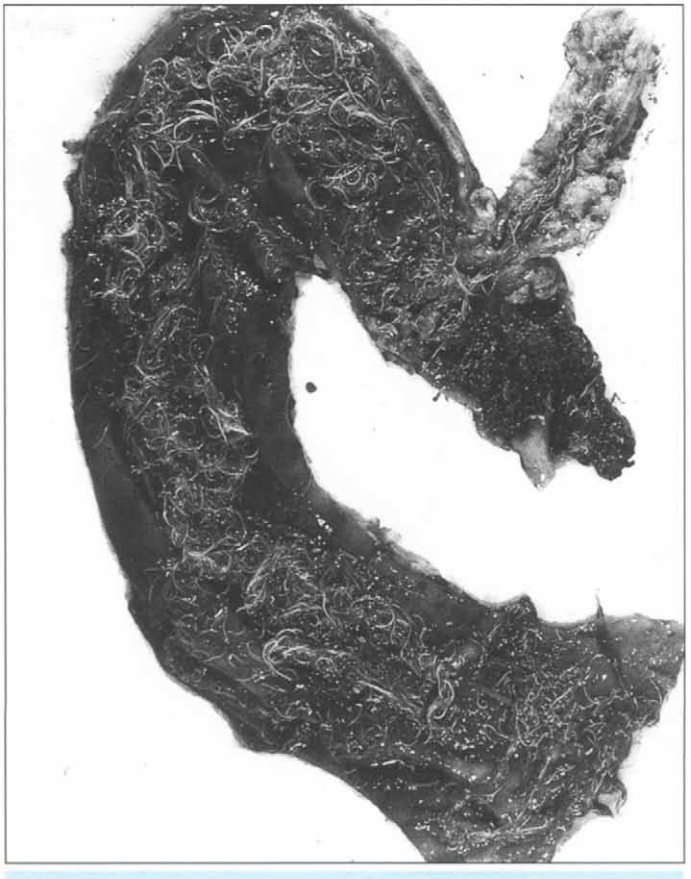

Figure 1.195 Hemorrhagic typhlocolitis in a dog caused by ***Trichuris vulpis***.

of the nematodes over the mucosa. Usually the thicker caudal end of the worms is noted. They may resemble at first glance *Oesophagostomum*, and only on more careful observation is the elongate thread-like cranial end seen.

The signs of the disease appear to be referable to loss of colonic absorptive function, and probably partly due to effusion of protein into the lumen. Erythrocyte loss is a minor component of the pathogenesis.

Trichurosis in **sheep** and **cattle** resembles that described in swine. The disease usually occurs in animals that are concentrated in areas contaminated by ova, and, in one reported case, immunocompromise due to persistent *Bovine viral diarrhea virus* may have predisposed. Affected animals develop chronic diarrhea with brown feces or dysentery, and loss of condition. At autopsy, the lesions are those of cachexia and hypoproteinemia, associated with mucohemorrhagic typhlitis or typhlocolitis.

A **diagnosis** of trichurosis in all species is usually readily made at autopsy. The worms have a characteristic morphology and are usually easily seen on the inflamed mucosal surface. In section, the thin cranial end of the nematodes, embedded in tunnels in the surface epithelium, contains the stichosome esophagus typical of members of the Trichuroidea, and a single bacillary band. The ova may be seen in the body of worms, in the gut lumen, or occasionally in tissue. They are barrel-shaped, have a thick wall and plugs at both poles of the egg. *Capillaria* spp. and their ova may be similar in tissue section, but are not expected in the cecum and colon.

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Cestode infection

Adult tapeworms inhabit the gastrointestinal tract, or the ducts of the liver and pancreas, where they are generally of minor significance. They are flattened, segmented colonies of sequentially maturing hermaphroditic reproductive units, or *proglottids*, forming an elongate strobila a few millimeters to many meters long. The Eucestoda are attached to the host by a specialized hold-fast organ, or *scolex*, which usually has four suckers, and perhaps a rostellum, sometimes armed with hooks. The Cotyloda may have elongate muscular grooves (bothridia) on the scolex. Cestodes lack an alimentary tract and absorb nutrients through the specialized absorptive surface or tegument of the proglottids.

Carnivores may be infected by tapeworms that use certain prey species as intermediate hosts. Metacestodes, or larvae, of members of the Taeniidae use as intermediate hosts some species of domestic animals and, accidentally, humans. They may cause disease, result in economic loss due to condemnation of tissues or organs at meat inspection, or have zoonotic significance.

Adult cestodes in tissue section are flattened, with internal organs in a loose parenchymatous matrix, often contain calcareous corpuscles in the outer region, and lack tubular digestive structures. They are segmented and the scolex may be encountered at the cranial end, attached to the intestine.

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Ruminants In ruminants, the more common and widely distributed intestinal tapeworms are *Moniezia expansa*, *M. benedeni*, and *Thysaniezia* (*Helictometra*) *giardi*. *Stilesia globipunctata* is found in the small intestine of sheep and goats in Europe, Asia, and Africa, whereas *S. hepatica* occurs in the bile ducts of ruminants in Africa and Asia. *Thysanosoma actinioides* occurs in the small intestine, pancreatic and bile ducts of ruminants in North and South America. *Avitellina* spp. occur in the small intestine of sheep and other ruminants in parts of Europe and Asia. The intermediate hosts of these tapeworms are oribatid mites or psocids (book lice).

Heavy infestations of the small intestine by *Moniezia, Thysaniezia*, and *Avitellina* are associated by some with diarrhea and ill-thrift in young lambs and calves. However, the evidence suggests that *Moniezia* is harmless; no effect on production or clinical signs can confidently be assigned to it.

The scolex of *Stilesia globipunctata* may be embedded in mucosal nodules 6–10 mm in diameter in the upper small intestine, with the thread-like strobila streaming into the lumen. There is a chronic inflammatory reaction around the scolex, which is deep in the mucosa, plugs of tissue being grasped by the suckers. Glands in the vicinity are hyperplastic, causing the nodules. The presence of up to 100 of these nodules has been associated with wasting, edema, and diarrhea.

Stilesia hepatica and *Thysanosoma actinioides* may cause mild fibrosis and ectasia of the bile ducts. Worms are often concentrated in the segmented sac-like dilations in the duct. These worms cause economic loss through condemnation of infected livers at meat inspection, and in areas where infection is common, this cost may be very significant.

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Horses The cestodes found in horses are *Anoplocephala perfoliata*, which colonizes the proximal cecum, especially at the ileocecal junction, and *A. magna* and *Paranoplocephala mamillana* in the small intestine and occasionally the stomach. The latter worm is small, under 5 cm in length, and is rarely associated with disease or lesions. *A. magna* tends to live in the lower small intestine, where it can attain a length of up to 80 cm, and a width of 2.5 cm. All use oribatid mites as intermediate hosts.

Anoplocephala perfoliata is more commonly associated with lesions, and occasionally with mortality. The risk of spasmodic colic increases with infection intensity. In areas of concentrated mucosal attachment by clusters of up to several hundred of this stumpy species, especially at the ileocecal orifice, erosion and ulceration of the mucosa occur. The depressed surface is often covered by fibrinous exudate, perhaps with some hemorrhage, or a local verrucous granulating mass may project into the lumen. *Ileal impaction* has been strongly associated with *A. perfoliata*. Partial obstruction of the ileocecal orifice may occur rarely, but no clear relationship is established between infection with *A. perfoliata* and the development of ileal muscular hypertrophy. *Ileocecal and cecocecal intussusception*, and, occasionally, perforation of the intestine or cecal rupture, have also been associated with infection by this tapeworm.

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Carnivores Dogs may be parasitized by ***Diphyllobothrium*** spp., as may be humans, cats, swine, and many other fish-eating mammals. The adults can be large, attaining lengths of up to 12–15 meters in humans, though those in animals tend to be shorter. The worm is ~2 cm across, and marked centrally by the uterus containing dark eggs, which are operculate. Intermediate stages occur in copepods and fish. The adult worm matures in the intestine of piscivorous mammals. Infection by *Diphyllobothrium* spp. is rarely, if ever, associated with clinical disease in animals.

Spirometra species are, like *Diphyllobothrium*, members of the subclass Cotyloda, and their life cycle is similar. The taxonomy of the genus is difficult, but among recognized species are *S. mansonoides*, infecting dogs, cats, and raccoons in North and South America, *S. mansoni* in dogs and cats in East Asia and South America, and *S. erinacei* found in cats and dogs in Australia and the Far East. Prospective hosts must have the opportunity for predation, since they are infected by the *plerocercoid* or "*sparganum*" found in the body cavity of the second intermediate host, usually an amphibian or reptile, or in another transport host.

Spargana can also occur in carnivores, swine, or even humans, if the procercoid in the first intermediate host, the *Cyclops*, is ingested, usually while drinking. Spargana are white, ribbon-like, but otherwise structureless worms up to several centimeters long. They may be found free or encysted in a thin, noninflammatory fibrous capsule, in the peritoneal cavity and intermuscular or subcutaneous tissue. A chronic inflammatory reaction may occur about dead spargana. The adult worms are nonpathogenic. Plerocercoids are of significance in humans, where they migrate mainly in subcutaneous tissues.

Mesocestoides spp. occasionally infect dogs, as well as other mammals and some birds, in North America, Europe, Asia, and Africa. These members of the subclass Eucestoda have a life cycle involving an insect or mite, and a vertebrate as second intermediate host. In the latter, infective *tetrathyridia*, ~1–2 cm long, flat, narrow, and bearing an invaginated scolex with four suckers, are found in the body cavities, liver, and lung. Tetrathyridia have the capacity for asexual multiplication, resulting in massive infections of intermediate hosts such as amphibia and reptiles, as well as dogs, cats, and other mammals. In the intestine of definitive hosts, *Mesocestoides* adults may also replicate asexually, and heavy infections may occur as a result of this, or from the consumption of large numbers of tetrathyridia in an intermediate host. Animals infected with intestinal *Mesocestoides* may develop diarrhea. Tetrathyridia replicating in the intestine of the dog may also penetrate the gut wall, and proliferate in the peritoneal cavity.

Tetrathyridia in the abdominal cavity of dogs and cats may cause peritoneal effusion (*parasitic ascites*), perhaps with the development of pyogranulomatous peritonitis and adhesions. Tetrathyridia 1–2 mm in diameter are scattered in the thick creamy exudate, along with similarly-sized "white bodies," comprised of necrotic parasite and host cellular debris. Mild infections may be discovered incidentally at necropsy. *Mesocestoides* infection of the abdominal cavity must be differentiated from peritoneal infections by cysticerci of several *Taenia* spp., which occur very rarely in carnivores.

Dipylidium caninum occurs in the dog, cat, fox, and, occasionally, children. It is ubiquitous. The narrow worms, up to 0.5 meters long, have distinctive cucumber-seed-like segments, and are often encountered incidentally in the small intestine at autopsy. They are of no pathologic significance. Cysticercoids develop in fleas and perhaps in the dog louse *Trichodectes canis*. Infection in the normal definitive hosts, or in accidental ones such as humans, is by ingestion of fleas containing cysticercoids.

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Taeniid tapeworms

Taeniid cestodes are the most important tapeworms in domestic animals, not because of the effects of the adult worm in the carnivorous definitive host, but rather due to the *metacestodes*, or larval forms, in intermediate hosts. Single oncospheres hatch from the egg in the upper small intestine, penetrate the epithelium, and are carried in the portal blood to the liver. Some species of metacestodes migrate in the liver, eventually to enter the peritoneal cavity. Others persist to develop in the liver, while still others pass on to the heart, lungs, and systemic circulation, establishing in muscle or a variety of other sites and tissues. Metacestodes may occasionally be found in organs other than the site of predilection.

Taeniid metacestodes assume four basic forms. The **cysticercus** is a fluid-filled, thin-walled, but muscular cyst, into which the scolex and neck of a single larval tapeworm are invaginated. The **strobilicercus** is a modification of this theme; late in larval development the scolex evaginates and is connected to the terminal bladder by a segmented strobila, so that it resembles a tapeworm, several centimeters long. The **coenurus** is a single or loculated fluid-filled cyst, in which up to several hundred nodular invaginated scolices are present in clusters on the inner wall. Each scolex is capable of developing into a single adult cestode in the intestine of the definitive host. The **hydatid cyst** is a uni- or multilocular structure, on the inner germinal membrane of which brood capsules develop. Within the brood capsules, invaginated protoscolices form. Brood capsules may float free in the cyst fluid, where they are termed "*hydatid sand*." Internal daughter cysts can develop. Release of brood capsules or protoscolices into tissues, as a result of rupture of the hydatid cyst, may lead to development of new cysts. The alveolar hydatid cyst proliferates by budding externally.

Taenia taeniaeformis infects the intestine of domestic cats and some wild felids, and the strobilicercus, ***Cysticercus fasciolaris***, is found in the liver of small rodents. Formation of fibrosarcomas has been associated with chronic inflammation due to *C. fasciolaris*. The adults are up to 60 cm long, have no neck, and caudal segments are somewhat bell-shaped, so that this species is readily differentiated from the other cestodes found in the feline small intestine. Usually, only a few worms are present in the cat, and they are of no consequence.

T. pisiformis is common in the small intestine in dogs and some wild canids, which prey on rabbits and hares. ***C. pisiformis*** migrates in the liver of the intermediate host, causing hemorrhagic tracks that are infiltrated by a mixed inflammatory reaction, and ultimately heal by scarring. The pea-size cysticerci encyst in a thin, noninflammatory fibrous capsule on the mesentery or omentum, or on the ligaments of the bladder. Occasionally, cysticerci persist beneath the hepatic capsule. Burdens of up to 20–30 worms, sometimes more, may be present in the intestine of the dog.

T. hydatigena infects the dog, and the metacestode, ***C. tenuicollis***, the long-necked bladder worm, or false hydatid, is found in the peritoneal cavity of sheep, cattle, and swine, and occasionally other species. Immature cysticerci in the liver migrate through the parenchyma for several weeks as they develop, before emerging to encyst on the peritoneum anywhere in the abdominal cavity. The immature cysticerci are less than 1 cm long, ovoid, and translucent. They cause tortuous hemorrhagic tracks similar to those produced by immature liver flukes, and if large numbers are present, they may cause a syndrome of depression and icterus identical to acute fascioliasis.

Heavily infected livers, with 4000–5000 actively migrating cysticerci, are mottled due to the subcapsular and parenchymal hemorrhagic foci and tracks. Cysticerci up to 6–8 mm long may be present beneath or breaching the capsule by about 3 weeks after infection. Rarely, animals may exsanguinate into the abdominal cavity, or the hepatic necrosis may predispose to the development of black disease or bacillary hemoglobinuria.

Cysticerci trapped in the liver may persist in a fibrous capsule, or be destroyed in a cystic eosinophilic granuloma that may mineralize; this is common on the diaphragmatic surface where the falciform ligament is attached. Usually, the intensity of infection is low, and a few, but occasionally scores of cysticerci – delicate translucent fluctuant fluid-filled cysts up to 5 cm or more in diameter – are contained in individual thin, noninflammatory fibrous capsules scattered on the peritoneal serosa. When a cyst degenerates, it is destroyed by a granulomatous reaction and the fibrotic mass may mineralize. Hepatic migration by *C. tenuicollis* may, at any stage, cause condemnation of lamb and pig liver at meat inspection.

T. ovis infects the intestine of the dog, while the metacestode, ***C. ovis***, is in the muscle of sheep, where it causes cysticercosis, or "*sheep measles.*" Cysticercosis of muscle caused by *C. ovis*; by *C. bovis* in cattle; and by *C. cellulosae* in swine and other species, including dogs, is considered in Vol. 1, Muscle and tendon. The adult stages of the latter two cysticerci, *T. saginata* and *T. solium* respectively, occur in the small intestine of humans.

T. multiceps occurs in the intestine of dogs and wild canids but the metacestode, ***Coenurus cerebralis***, develops in the brain and spinal cord of sheep and other ungulates, and, rarely, in humans. In the goat, coenuri may also occur in other organs, beneath the skin and intramuscularly. The migration of small metacestodes in the central nervous system may cause tortuous red or yellow-gray tracks in the brain due to traumatic hemorrhage and malacia, and nervous signs or death may occur at this stage. More commonly, signs of central nervous system disease, termed "sturdy" or "gid," do not develop until coenuri, up to 4–5 cm in diameter, have developed more fully, 4–8 months after infection. Cysts may be present at any level and depth in the brain and spinal cord, and projecting into the cerebral ventricles, but they are most common near the surface of the parietal cortex in the cerebrum. They cause increased intracranial pressure, hydrocephalus, necrosis of adjacent brain, and sometimes lysis, perhaps extending to perforation, of the overlying cranial bone. Coenuri developing in the spinal cord may cause paresis or paralysis.

T. serialis infects dogs and foxes throughout the world. The larval coenurus is found in the subcutaneous and intermuscular connective tissue of lagomorphs. Cerebral coenurosis has been reported in cats.

Histologically, cysticerci and coenuri are recognized as generally cystic structures with an eosinophilic outer layer or tegument, which may appear fibrillar or almost ciliate on the outermost surface. Beneath the tegumental cells a less cellular area, which may contain calcareous corpuscles, gives way to a web-like, lightly cellular matrix, and the central open fluid-filled portion of the cyst. No internal organs are seen. Muscular inverted scolices, with suckers, and (in all but *C. bovis*) hooks on the rostellum, may be encountered in favorable sections, extending into the center of the metacestode. Size and shape of hooks may assist in a specific diagnosis, if they are fully developed. Immature migrating metacestodes lack organized scolices. Other sources should be consulted for details on the taxonomy and specific identification of adult and larval taeniid tapeworms.

Echinococcus spp. tapeworms occur in the small intestine of a number of species of carnivores, predominantly canids. In enzootic areas, the distinctive metacestodes, or *hydatid cysts*, are commonly found in normal or accidental intermediate hosts. Humans may accidentally become infected with the metacestode, and echinococcosis or hydatidosis is a significant public health problem where carnivores shedding *Echinococcus* eggs come in close contact with humans.

The species are *E. granulosus, E. multilocularis, E. oligarthus*, and *E. vogeli*. The latter two involve sylvatic cycles in Central and South America, with felids and canids as definitive hosts respectively, and rodents as intermediate hosts in which polycystic hydatidosis occurs; *E. vogeli* may infect humans. The other two species may use domestic animals as definitive hosts, and will be considered further here.

E. granulosus uses the dog and some other canids as the definitive host. The most widespread strain or genotype uses a *sheep–dog cycle*, and has been disseminated wherever there is pastoral husbandry of sheep. It is significant as a potential zoonosis in many parts of Eurasia and the Mediterranean region, some parts of the UK, North America, South America, continental Australia, and Africa. Eradication has been accomplished, or virtually so, in Iceland, New Zealand, and Tasmania. Other cycles affecting domestic animals include horse–; cattle–; camel–; pig–; water buffalo–; goat–, and human–dog. Sylvatic cycles include: in Eurasia and North America, cervid–wolf; in Argentina, hare–fox; in Sri Lanka, deer–jackal; in Australia, macropod–dingo. Not all cycles represent different genotypes.

In the small intestine of the definitive host, protoscolices evaginate and establish between villi and in the crypts of Lieberkühn. The scolex distends the crypt and the epithelium is gripped by the suckers and occasionally eroded, but there is little or no inflammatory response. The worms that develop are short, usually less than 6–7 mm long. They commonly have only 3–5 proglottids, the caudal gravid one making up almost half the length of the worm. Burdens of *E. granulosus* are often heavy, no doubt due to the large numbers of protoscolices ingested at a meal containing one or more hydatid cysts. The heavily infected intestine is carpeted by the tiny white blunt projections, partially obscured between the villi; the worms may resemble lymphangiectasia. Enteric signs are not normally encountered in dogs with intestinal hydatid tapeworms.

Penetration of oncospheres released from eggs in the intestine of the intermediate host takes them into the subepithelial capillaries, or perhaps the lacteal. The majority probably migrate via the liver, some carrying on to the lungs and general circulation. However, those gaining the lacteal may bypass the liver, entering the vena cava with the lymph, and either are filtered out in the pulmonary circulation or are disseminated. *Hydatid cysts occur most commonly in the liver and lung*, with some strain and host species variation in the relative prevalence in these organs. In sheep they may be more common in lungs, while in cattle and horses, the liver is the usual site of establishment. Less commonly in domestic animals, the brain, heart, bone, and subcutaneous tissue may be sites of development of hydatid cysts. A single cyst, or up to several hundreds, may be present, displacing tissue in infected organs. Disease is rarely attributed to hydatidosis in animals, even in those heavily infected. However, strategic location of one or more cysts may lead to heart failure, bloat or central nervous signs. Condemnation of infected organs at meat inspection causes economic loss.

Hydatid cysts are usually spherical, turgid, and fluid-filled. They usually measure 5–10 cm in diameter in domestic animals; rarely, cysts in animals may be larger, but in humans hydatid cysts can become huge. On the other hand, some fertile cysts in equine livers may be as small as 2–3 mm across. The lining of fertile cysts is studded with small granular *brood capsules*, which contain protoscolices; and "hydatid sand," comprised of free brood capsules and protoscolices, is in the fluid. The lining of sterile cysts is smooth. Though the potential exists for development of internal daughter cysts, and rare exogenous budding by herniated cysts, most hydatid cysts in domestic animals are unilocular. However, they may be irregular or distorted in shape due to the variable resistance of parenchyma and portal tracts or bronchi and by the profiles of bone or other resistant tissues.

Microscopically, immature hydatid cysts are surrounded by an infiltrate of mixed inflammatory cells, including giant cells and eosinophils. As they develop, a layer of granulation tissue, which may contain round cells and eosinophils, invests the cyst, and this evolves so that the inner portion of the fibrous capsule is comprised of mature collagenous connective tissue that is relatively acellular. Within this, and in close apposition, is the acellular lamellar hyaline outer layer of the hydatid cyst wall, comprised of a polysaccharide–protein complex, which, with time, may become hundreds of micrometers thick. The cyst is lined by the thin syncytial germinal layer from which the brood capsules form on fine pedicles. If the cyst is ruptured and protoscolices are released into tissue, secondary cysts may form from them.

Hydatid cysts may degenerate. The inner structures collapse, and the mass becomes caseous and may mineralize. Degenerate hydatid cysts grossly may resemble tuberculous lesions or metastatic squamous cell carcinoma.

E. multilocularis has a holarctic distribution, the adults occurring mainly in foxes, and the metacestodes in small rodents, especially voles and lemmings. Dogs and cats may also become infected with the worms in enzootic areas. Though the parasite is principally arctic, the cycle is found in the northern prairie area of North America and in eastern and central Europe, and is moving progressively southward. The mature cestodes in the intestine are similar to, but smaller than, *E. granulosus*. In the intermediate host, the metacestode mainly infects the liver, forming a cystic structure with internal brood capsules and protoscolices; but it is capable of external budding. As a result, racemose proliferative masses of metacestodes infiltrate infected livers. They may metastasize via the bloodstream to the lungs or bone, or implant in the peritoneal cavity. The inflammatory reaction to alveolar hydatids is comprised of macrophages, perhaps giant cells, lymphocytes, and plasma cells in an encapsulating fibrous stroma. The metacestodes are rarely found in domestic animals, but may infect humans who ingest eggs shed by infected carnivores.

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Intestinal fluke infection

Trematode infections of the intestine of domestic animals are, on the whole, uncommon. Dogs and cats in many parts of the world may be infected with *Alaria* spp., the second intermediate hosts for which are frogs or other amphibia. *Heterophyes heterophyes, Metagonimus yokagawi, Echinochasmus perfoliatus*, and *Phagicola longa* may infect dogs and cats fed fish that contain metacercariae. The former two occur in the Mediterranean area and the Far East; the latter in Eurasia. *Cryptocotyle* spp., most commonly parasitic in piscivorous birds, may also be found in dogs, cats, and mink fed infected marine fish.

Enteritis is attributed to ***Alaria, Echinochasmus,* and *Cryptocotyle***. The flukes attach to the mucosa by suckers, and perhaps cause their effects by local irritation, erosion, and ulceration that large numbers of them may induce. Vomiting and ill-thrift have been associated with infection. The production of excessive intestinal mucus and hemorrhagic enteritis have been associated with intestinal fluke infection in small animals. The flukes involved are small, less than 4–5 mm long, and must be sought carefully at autopsy.

Nanophyetus salmincola occurs in the small intestine of dogs, cats, and humans, and in various fish-eating wild mammals and birds in the northwestern USA; Vancouver Island, Canada; and eastern Siberia. Its distribution is determined by that of the snails that are the first intermediate hosts. The second intermediate hosts are fish, especially members of the family Salmonidae. The adult flukes inhabit the small intestine, where they penetrate and attach to the mucosa. Large numbers may cause mucoid or hemorrhagic enteritis. *N. salmincola* transmits *Neorickettsia helminthoeca*, which causes "*salmon poisoning disease*," to canids consuming raw salmon infected with metacercariae. The disease is thought to be restricted to North America. However, similar organisms have been recognized in dogs with lesions compatible with salmon poisoning disease in southern Brazil.

Salmon poisoning disease has an incubation period of about 5–7 days, and is characterized clinically by pyrexia, anorexia, depression, weakness, and weight loss. There may be serous nasal discharge and mucopurulent conjunctivitis. Diarrhea with tenesmus develops; feces are scant yellow and mucoid or watery, often with some blood. The condition is usually fatal; if untreated, only 5–10% of infected dogs recover. They are immune to reinfection.

At necropsy, lesions are most consistently found in the lymphoid tissues. There is *generalized enlargement of lymph nodes*, especially in the abdominal cavity. Enlarged tonsils are everted from their fossae. The thymus is often increased in size in young dogs and the spleen may be swollen and congested. Peyer's patches and other intestinal lymphoid aggregates are elevated above the mucosal surface, and there may be petechial hemorrhages on the mucosa. Lymphoid tissue near the ileocecocolic valve may ulcerate and bleed. Ileocolic intussusception occurs in many cases. The liver of foxes becomes friable, and may rupture, causing hemorrhage into the peritoneal cavity. Focal hemorrhages may be seen in the mucosa of the bladder, and subpleural hemorrhages up to 2 cm in diameter usually occur.

Microscopic changes in lymph nodes include depletion of lymphocytes, focal necrosis with neutrophilic infiltrates, and increased numbers of histiocytes in the cortex and medulla. Similar changes may occur in the thymus, and splenic follicles may undergo necrosis. *Elementary bodies* of the *Neorickettsia* may be demonstrated in the cytoplasm of macrophages in lymphoid tissue, and in other visceral organs, by use of Giemsa or Macchiavello stains, or immunohistochemistry. In the small intestine, the flukes may be present embedded deep in the mucosa, though usually little reaction to them is present.

Lesions of the central nervous system occur in most cases. Leptomeninges may be somewhat opaque, but the lesions are best recognized microscopically. They are composed of macrophage accumulations in the leptomeninges and Virchow–Robin spaces, and focal gliosis in the parenchyma. Meningeal reaction is perhaps most consistent over the cerebellum, and is comprised of mild or moderate perivascular or more diffuse accumulations of histiocytes. Similar cells may cuff small and medium-sized vessels throughout the parenchyma. Focal gliosis is relatively sparsely distributed but seems most common in the brainstem. Elementary bodies are also demonstrable in macrophages in the central nervous system, and the diagnosis is usually made on the basis of this finding in macrophages in lymphoid tissue and/or brain. The organisms can be isolated and grown on primary canine monocyte cultures and in several other cell culture systems, but this is not a routine procedure.

Paramphistome infections in ruminants may cause significant intestinal disease. The adults, of the genera *Paramphistomum, Cotylophoron, Calicophoron, Ceylonocotyle, Gastrothylax, Fischoederius,* and *Carmyerius*, occur in the forestomachs of ruminants in various areas around the world. Infection is most common in warm-temperate to tropical areas. In the rumen, the red pear-shaped adult flukes, with their characteristic cranial and caudal suckers, are considered innocuous, though some papillae may become atrophic and slough (see Fig. 1.36).

When ingested, metacercariae encysted on herbage give rise to immature flukes that inhabit the duodenum, where massive infections may cause severe enteritis. In cattle, water buffalo, and American bison, the species incriminated in disease include *P. cervi*,

P. microbothrium, P. explanatum, Calicophoron calicophorum, and various species of *Cotylophoron, Gastrothylax*, and *Fischoederius*. In sheep and goats, *P. microbothrium, P. ichikawai, P. cervi, P. explanatum, G. crumenifer, Cotylophoron cotylophorum*, and *F. cobboldi* have been associated with disease. The species involved vary with the host and geographic area.

After ~3–5 weeks in the small intestine, the worms normally migrate forward, through the abomasum, to establish and mature in the reticulorumen. However, if massive infection occurs, growth in the small intestine is retarded, and flukes may persist for months in the duodenum, prolonging the course of disease.

Calves and lambs with intestinal paramphistomosis are depressed and inappetent. Fetid *diarrhea* usually develops within several weeks of infection, and may contain immature flukes. Hypoproteinemia is reflected in submandibular edema in some animals and anemia is reported to occur occasionally. Morbidity and mortality can be substantial, and survivors may suffer considerable loss in condition.

The carcass may be in good or cachectic condition, depending on the duration of the disease, and there may be edema of subcutaneous tissues, abomasal folds, and mesentery, and fluid in the body cavities, due to hypoproteinemia. The proximal small intestine appears congested externally, and immature paramphistomes, deeply penetrating the intestinal wall, may be visible through the serosa. Occasionally, they will perforate the gut and be found free in the abdominal cavity. The mucosal surface of the duodenum is edematous, thickened, corrugated, and covered with mucus. Many immature pink or brown paramphistomes, a few millimeters long, are scattered over the surface and embedded in the mucosa. Most larval paramphistomes are in the first 3 meters of small intestine. In advanced infections, some may be present migrating forward on the abomasal mucosa, or already in the forestomachs.

In section, small larval paramphistomes are found deep in the lamina propria, occasionally in the submucosa, and sometimes in Brunner's glands. Larger immature forms are attached to the surface of the mucosa by a plug of tissue taken into the acetabulum. There is atrophy of villi, elongation of crypts, and possibly erosion or ulceration of the mucosa in heavily infected areas. Heavy infections are associated with fibroplasia. Protein loss into the gut, coupled with loss of appetite, seems to be the most important pathophysiologic consequence.

The other fluke occurring in the intestine of ruminants is *Skjrabinotrema ovis*, associated with catarrhal enteritis in sheep in Eurasia.

In **swine**, the paramphistomes *Gastrodiscoides* and *Gastrodiscus* may be found in the colon, where they are of little significance. *Fasciolopsis buski* and *Artyfechinosomum malayanum* may infect the small intestine of swine, as well as humans. They are of little importance in pigs other than as reservoirs for human infection.

In **horses** in Africa and India, the paramphistomes *Gastrodiscus aegyptiacus* and *Pseudodiscus colinsi* occur in the large bowel. Larvae of the former species have been associated with severe colitis in horses, but they are generally nonpathogenic.

Intestinal schistosomiasis, due mainly to *Schistosoma* spp. in ruminants, and *Heterobilharzia* in dogs, may cause *protein-losing enteropathy*, perhaps associated with granulomatous enteritis in response to deposition of ova in mucosal venules (see Vol. 3, Cardiovascular system). Treatment may precipitate hepatic lesions consisting of granuloma formation around dead parasites as well as periportal fibrosis.

Flukes in tissue section are generally somewhat flattened or globose, with a loose mesenchymal parenchyma in which the internal structures are suspended. The cuticle is eosinophilic, and may be spiny. Muscular oral and acetabular suckers and pharynx may be encountered in sections. Ceca are usually present, and elements of the male and female reproductive systems in these typically hermaphroditic adult worms (excepting the schistosomes) may be seen. The uterus may contain ova with a tan-yellow or brown shell, perhaps with an operculum, and ova are often seen in the intestinal lumen or in tissue. The developing miracidium may be present in ova. Schistosomes are recognized by their intravascular location and sexual dimorphism, the leaf-like male perhaps enveloping the slender cylindrical female within the gynecophoric canal, in section.

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Acanthocephalan infections

The Acanthocephala are a phylum of parasitic animals that have an elongate sac-like body, no internal alimentary canal, and use as the hold-fast a spiny protrusible proboscis. The life cycle typically

involves obligate development in an intermediate host, usually an arthropod, and perhaps the utilization of a paratenic host to facilitate transmission. The acanthocephala of concern in domestic animals are in the genera *Macracanthorhynchus* and *Oncicola*.

Macracanthorhynchus hirudinaceus is the *thorny-headed worm of swine*, infecting the small intestine. The life cycle involves dung beetles or other Scarabaeidae, and foraging or rooting swine are prone to infection. Males are ~10 cm long, and the females up to 30–40 cm long, slightly pink, curved, and tapering caudally. The proboscis has ~6 rows of hooks, and is used to penetrate deeply into the intestinal wall. It incites a local granulomatous nodule called a "*strawberry mark*," with a purulent focus about the embedded proboscis. The proboscis may penetrate the muscularis, and the nodules around the proboscis, ~1 cm in diameter, may be visible on the serosal surface of the gut as gray or yellow suppurative foci, surrounded by a halo of hyperemic tissue. They occasionally perforate, causing peritonitis. Severely infected pigs may suffer ill-thrift and perhaps anemia, probably related partly to the potential for plasma protein loss and hemorrhage from numerous focal ulcerative lesions.

M. catalinum and *M. ingens* are smaller but similar thorny-headed worms that inhabit the intestine of a variety of wild carnivores, and occasionally the dog.

Oncicola canis occurs in the small intestine of wild carnivores, and occasionally the dog and cat. It rarely causes disease. Intermediate hosts are presumably arthropods, with insectivorous vertebrates acting as paratenic hosts. Up to several hundred worms, 0.5–1.5 cm long and dark gray, may infest the small intestine; usually infections are light. The proboscis is embedded to the subserosal level, and a focal nodular lesion develops about it.

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Protistan infections

Coccidiosis

The coccidia are members of the protistan phylum Apicomplexa, intracellular parasites characterized at some stage of the life cycle by a typical "apical complex" of organelles at one end of the organism. Members of the subclass Coccidiasina, which will be considered together under coccidiosis, all have a similar basic life cycle. It begins with infection of a cell, often, but not always, in the intestinal mucosa, by a **sporozoite** released from a **sporocyst** in the lumen of the gut. One or more cycles of asexual division, termed **schizogony or merogony**, follow, and the **merozoites** produced infect other cells, forming another generation of **meronts**, or transforming to sexual stages, termed **gamonts**. Gamonts subsequently develop into nonmotile female **macrogametes**, and motile male forms or **microgametes**. A nonmotile zygote produced by union of micro- and macrogametes forms an **oocyst**. **Sporogony**, production of sporocysts containing infectious sporozoites within the oocyst, may occur in the host, or, more commonly, after the resistant oocysts are passed in feces.

Members of the genus *Eimeria* and *Isospora* are **homoxenous**, with sexual and asexual development taking place in a single host. *Cystoisospora* (former *Isospora* spp. in carnivores) and the genera *Toxoplasma, Sarcocystis, Hammondia, Besnoitia, Frenkelia, Neospora*, and *Caryospora* are all **heteroxenous**, in which case asexual stages occur in an intermediate host. The heteroxenous genera exploit natural prey–predator relationships. In general, sexual development takes place in the intestinal mucosa of a predator, while at least one generation of asexual replication, often several, occurs in the tissues of one or more species of prey.

The *endogenous stages of coccidia are all intracellular*, except, temporarily, the merozoite and microgamete. Mature developmental stages are usually readily recognized; immature forms may not be easily identifiable. **Trophozoites**, small undifferentiated, rounded, basophilic forms with a single nucleus, usually within a parasitophorous vacuole in the host cell, are found at three stages of the life cycle. They occur following invasion by the infective sporozoite, prior to merogony; following invasion by a merozoite, prior to a subsequent generation of merogony; and following invasion by a merozoite, prior to differentiation into a recognizable gamont. Developing meronts are multinucleate. Merogony may involve "**endopolygeny**," multiple fission or apparent "budding" of merozoites from the periphery of the meront or from infoldings of it. A single residual body, surrounded by slightly curved, fusiform, or banana-shaped uninucleate merozoites, or many spherical clusters of merozoites with a central residuum, may be present. A second form of replication, termed "**endodyogeny**," occurs in meronts of many of the heteroxenous coccidia. Two daughter organisms develop within a mother organism, which is destroyed when they are released. The location of a meront, and the number of merozoites it contains, vary with the species and the generation of merogony. A very few, or up to tens or hundreds of thousands of merozoites may be released from a single meront.

Microgamonts mature in two steps. The first involves enlargement of the gamont and proliferation of nuclei. During the second phase, the microgametes differentiate about the periphery of the gamont, which may become deeply folded or fissured by invaginations. Immature microgametocytes during these stages may resemble developing schizonts. However, *fully differentiated microgametes* differ from merozoites in being small, densely basophilic, and comma-shaped, with 2–3 flagella. They may be present in swirling masses, perhaps with some residual bodies, in mature microgametocytes. Macrogametes, the female stage, have a large nucleus with a prominent nucleolus, and with time they usually enlarge to contain refractile eosinophilic "plastic granules" or wall-forming bodies, which give rise to the layers of the oocyst wall. *Mature macrogametes* typically have prominent wall-forming bodies, and contain clear or periodic acid–Schiff-positive amylopectin granules, and a large nucleus and nucleolus.

Fertilization by the microgamete leads to development of the zygote, and subsequent formation of the oocyst wall. The contained *sporont* is spherical, with nucleus and nucleolus, and amylopectin granules in the cytoplasm. Sporulation usually occurs outside the host, but in *Sarcocystis* and *Frenkelia* it occurs in the tissue of the definitive host; in *Caryospora*, sporulated oocysts develop in tissues of the prey host. Sporozoites are enclosed within sporocysts, which in turn are contained by the oocyst wall. Oocysts of most coccidia, or sporocysts of *Sarcocystis* and *Frenkelia*, are passed in the feces.

Coccidia of domestic animals are relatively host-, organ-, and tissue-specific. Asexual stages of *Toxoplasma* and *Neospora* are the

obvious exception to this generalization. Species of *Eimeria, Isospora*, and *Cystoisospora* rarely occur in more than one genus of definitive host. Similar coccidia occurring in related genera of hosts, when tested, usually prove incapable of cross-infection.

The economic cost of coccidiosis in the food–animal species is considerable, in terms of mortality, morbidity, subclinical disease, and the cost of prevention and treatment. It is even more so in chickens. In dogs and cats, coccidiosis is a minor problem.

Virulence reflects a number of factors. Among these are the location and type of cell infected by various stages of the organism, the function of infected cells, and the degree of host reaction stimulated by infection.

The effects of infection on the host cell are several, and vary somewhat with the infecting species. Infected cells may be functionally compromised. They may hypertrophy; nuclei may enlarge or a considerable amount of cytoplasm may be displaced; and the outer membrane of infected cells may be highly modified, perhaps to facilitate metabolic exchange. The intercellular relationships may be affected. The rate of movement of infected epithelial cells up villi is altered.

Immunoinflammatory reactions may be incited by coccidial infection. In experimental systems, resistance to coccidial infection is thymus-dependent, and is largely mediated by T-cell-promoted intracellular killing directed mainly against asexual stages in the life cycle.

In mammals, acute inflammatory reactions in intestinal coccidiosis are most commonly associated with heavy infection and destruction of cells by the sexual stages and oocysts, rather than in response to asexual stages. In toxoplasmosis and neosporosis, necrosis and focal acute or chronic inflammatory reactions may be incited by actively replicating asexual stages in many organs. A syndrome characterized by hemorrhage occurs in some species infected with asexual stages of *Sarcocystis*, about the time that merogony occurs in vascular endothelium.

The effects of *intestinal coccidiosis* in mammals vary with the host–parasite system. They are mainly related to malabsorption induced by *villus atrophy*, or to anemia, hypoproteinemia, and dehydration due to exudative enteritis and colitis caused by *epithelial erosion and ulceration*. A *neurotoxin* has been associated with the development of nervous disorders in cattle with coccidiosis. Many species of coccidia appear to have little pathogenic effect under normal circumstances.

Coccidiosis is typically a disease of *intensively managed animals*. It is especially important in naive young animals exposed to a high level of infection. This is predisposed by high contamination rates associated with crowding, yarding, or high stocking rates on pasture. A damp substrate promotes oocyst sporulation and survival, and practices such as feeding on the ground, or the natural propensity of young animals to nibble or perhaps indulge in coprophagy, may promote infection. Although infections may not proceed to patency, chronic ingestion of oocysts may cause an intestinal immune response, villus atrophy, and perhaps ill-thrift, in some situations. Immune reactions may only halt development of, but not kill, endogenous asexual stages. Epizootiologic evidence suggests that under some circumstances there may be relaxation of resistance and resumption of development of the organisms, ultimately expressed in disease. This seems the likely explanation for outbreaks of bovine coccidiosis occurring during midwinter in freezing climates, or in post-parturient stabled dairy cattle.

Coccidiosis caused by members of the genera *Eimeria* and *Isospora* in the various species will be considered further here. The heteroxenous organisms, including *Cystoisospora, Toxoplasma, Neospora*, and *Sarcocystis*, will be considered subsequently, as will *Cryptosporidium*.

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Coccidiosis in cattle

Over a dozen species of *Eimeria* parasitize cattle; of these, *Eimeria zuernii* and *E. bovis* are potentially highly pathogenic, while several others, notably *E. ellipsoidalis, E. alabamensis*, and *E. auburnensis*, may cause diarrhea, but probably not death. *Coccidial infection is common*, and it usually comprises several species. Almost half the calves and yearlings in confinement operations shed oocysts, with calves shedding high numbers, while a much smaller proportion of cows shed low numbers of oocysts.

Disease occurs mainly in calves or weaned feeder cattle under ~1 year of age, when one or both of the potentially pathogenic species produce heavy infection. It may occur in animals at pasture or on range, concentrated at water holes, but is most common in animals in feedlots or yards where the level of sanitation is not high. The stress of shipping, cold weather, or intercurrent disease may be associated with outbreaks, which can occur in midwinter when oocyst transmission is expected to be poor. *Bovine parvovirus* infections have been associated with outbreaks of coccidiosis in a dry environment in northern Australia. Reactivation of latent schizonts in tissue may explain coccidiosis in stressed animals, or at a time when transmission is unlikely.

Coccidiosis is characterized by *diarrhea that may progress to dysentery* with mucus, and tenesmus, perhaps causing rectal prolapse. Animals dehydrate, and become hyponatremic and perhaps anemic. Morbidity may be high, but mortality is usually low. The duration of severe disease is ~3–10 days, after which most cases recover, since infection is essentially self-limiting. Some animals develop concurrent nervous signs, including tremors, nystagmus, opisthotonos, and convulsions, and many of these die within a few days.

The signs in bovine coccidiosis due to *E. zuernii* and *E. bovis* occur when the epithelium in the glands of the cecum and colon is infected by second-generation schizonts and gametocytes. In heavily infected animals, disease, and perhaps death, can occur before many oocysts are passed in the feces. The life cycles of both agents are similar, two schizogonous generations preceding gametogony. The first-generation schizont of ***E. bovis*** infects hypertrophic endothelial cells in lacteals on the upper part of villi in the *lower small intestine*, several meters proximal to the ileocecal valve. These schizonts may be large,

up to ~300 μm in diameter, and are visible to the naked eye as *pinpoint white nodular foci in the mucosa*. They contain tens of thousands of merozoites, but are invested by only a narrow rim of mononuclear inflammatory cells, unless they degenerate, when a marked local mixed reaction develops, including neutrophils and macrophages. Merozoites released from these schizonts ~14–18 days after infection enter cells deep in cecal and colonic glands. In heavy infections, crypts of Lieberkühn in the terminal ileum may also be infected. Here they produce small second-generation schizonts, which in turn release merozoites, infecting other cells in the gland. Gametogony may begin as early as 15 days after infection, and oocyst production peaks ~19–21 days after infection.

The first-generation schizonts of ***E. zuernii*** may be about the same size as those of *E. bovis*. However, they are most common in the *terminal meter of the ileum* and are located in the lamina propria below the crypt–villus junction, often deep near the muscularis mucosae, rather than in the endothelium of the lacteal. Hence, they are not so readily visible grossly as those of *E. bovis*. The second-generation schizonts and gamonts of *E. zuernii* also occur in glands of the cecum and colon, but not the terminal ileum. The merozoites tend to be somewhat longer (up to 15 μm) and schizonts more numerous and of greater diameter (~14 μm) than those of *E. bovis*. The timing of the development of *E. zuernii* infection is similar to that of *E. bovis*. First-generation schizonts of *E. bovis* occasionally reach the mesenteric lymph node, where they may mature, with no significance.

Animals dying of coccidiosis have fecal staining of the hindquarters, and may be somewhat cachectic and anemic. The gross enteric lesions in severe cases are those of *fibrinohemorrhagic typhlocolitis*, which may extend to the rectum; if *E. bovis* is involved, the terminal ileum may be affected (Fig. 1.196) and perhaps a few schizonts will be visible in ileal villi. The contents of the large bowel are usually abnormally fluid, and may vary from brown to black to overtly bloody, possibly with flecks of mucus or fibrin. The mucosa is edematous, with exaggerated longitudinal and perhaps transverse folds, which may be congested. Submucosal edema is also marked. Fibrin strands or a patchy diphtheritic membrane may be present on the mucosa (Fig. 1.197), and fibrin casts can form. In milder cases, lesions are limited to congestion and edema of the mucosa.

In animals dying at the peak of infection, *virtually all cells lining cecal and colonic glands in many areas are infected by small schizonts, gamonts, or developing oocysts*. Cells infected by *E. bovis* tend to dissociate and project into the lumen of the gland. As cells are disrupted and oocysts are released into the lumen of glands, the remaining glandular epithelium becomes extremely attenuated, or the gland collapses (Fig. 1.198A).

Concurrently, the surface epithelium becomes squamous, or the *mucosa is eroded*, and effusion of fibrin, neutrophils, and hemorrhage occurs from dilated, congested superficial vessels. Oocysts released into the lumen of the colon may be seen in the exudate (Fig. 1.198B). At the same time, the mucosa begins to collapse, and the lamina propria is infiltrated by neutrophils, eosinophils, lymphocytes, macrophages, and plasma cells (Fig. 1.198C). Oocysts trapped in denuded glands in the collapsed mucosa may be surrounded by small giant cells.

If destruction is widespread, and the animal survives sufficiently long, the *mucosa may ulcerate* to the level of the muscularis mucosae, and begin to granulate. In areas where the lesion is patchy, glands that have been relatively spared may become lined with hyperplastic

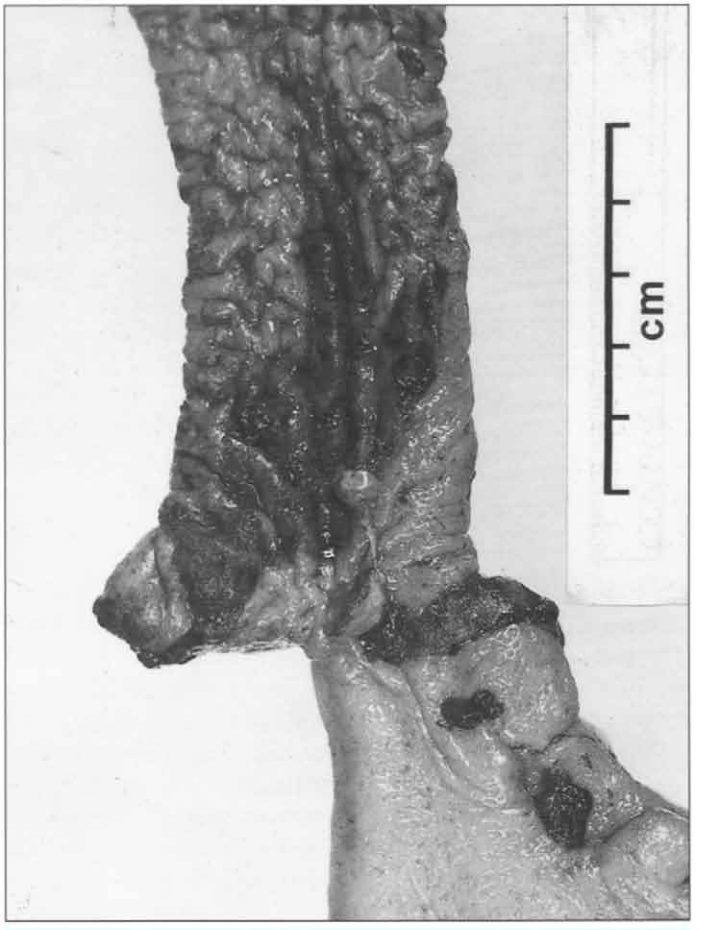

Figure 1.196 Acute enteritis, with mucosal thickening, large and minute ulcerations, and hemorrhages, in **bovine coccidiosis**.

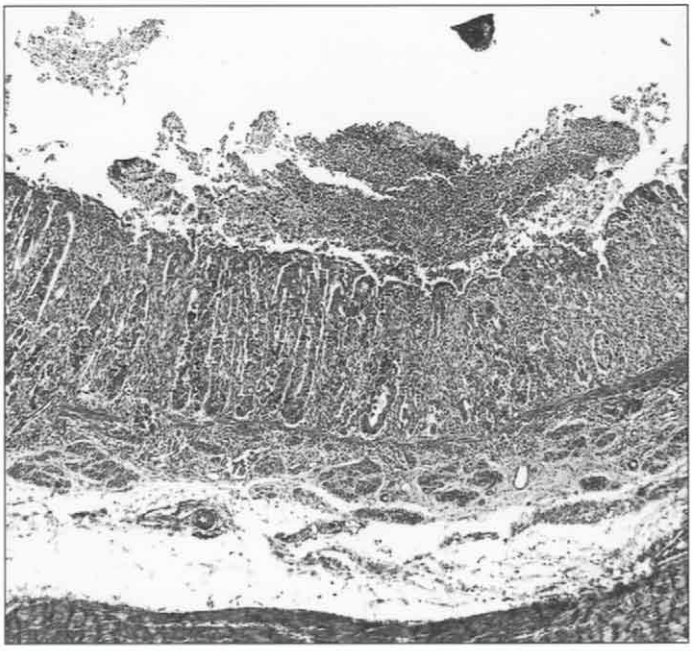

Figure 1.197 Damaged colonic glands and inflammatory exudate, in **bovine coccidiosis**.

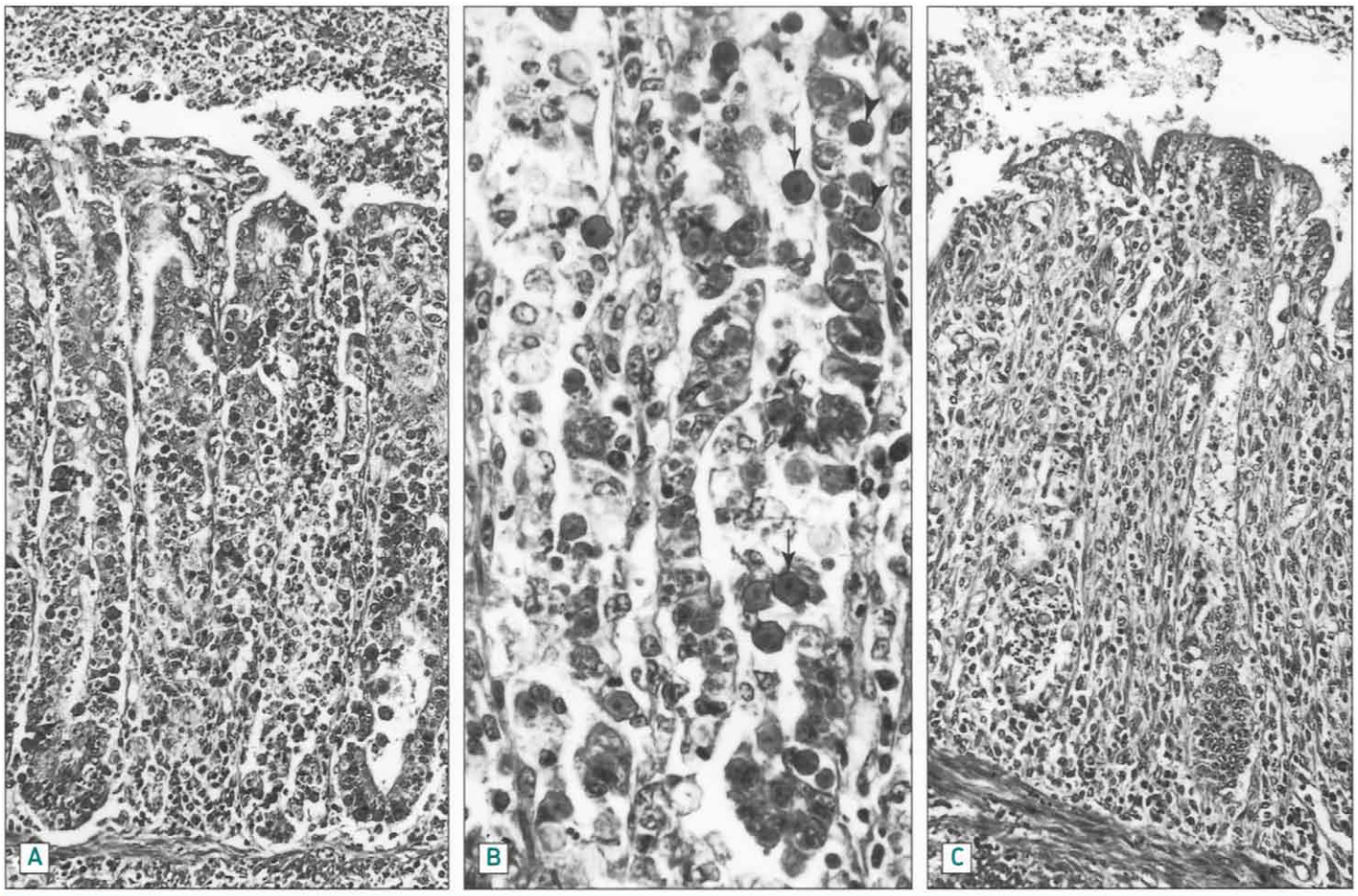

Figure 1.198 Bovine coccidiosis. A. Heavy infection and destruction of colonic glands by gamonts. Exudate covers mucosa. **B.** Destruction of colonic glands by developing gamonts (arrowheads). Oocysts are in the lumen of some glands (arrows). **C.** Destruction of colonic glands. Only a few glands remain in the mucosa.

epithelium, making an attempt to regenerate the mucosa. Flattened epithelial cells spread from these glands across the denuded surface, beneath the diphtheritic exudate. A few crenated oocysts in small giant cells in the stromal remnants of the mucosa may be all the evidence of coccidiosis found in lesions in animals surviving for 7–10 days.

Malabsorption due to mucosal damage in the cecum and colon, and inflammatory effusion and hemorrhage, explain the enteric signs of coccidiosis. The nervous signs in bovine coccidiosis are not associated with recognized lesions in the brain; they have been related to a neurotoxin found in the blood of affected animals.

The gross lesions of coccidiosis in cattle must be differentiated from those in salmonellosis, bovine viral diarrhea, rinderpest, malignant catarrhal fever, and bovine adenoviral infection, all of which may cause typhlocolitis. Coccidiosis can often be simply confirmed at autopsy by finding large numbers of developing stages in mucosal scrapings. Oocysts of *E. bovis* are ovoid, smooth, and ~28 × 21 μm; those of *E. zuernii* are subspherical to ovoid, smooth and ~18 × 15 μm.

While other coccidia are unlikely to be the primary cause of diarrhea or death in cattle, several have distinctive endogenous stages that may be recognized in tissue section. ***Eimeria auburnensis*** has a giant first-generation schizont that may be confused with those of *E. bovis* and *E. zuernii*. However, they are present usually 6–12 meters cranial to the ileocecal valve and form in the epithelium deep in crypts of Lieberkühn, though this may not be apparent due to plane of section, or following their migration into the lamina propria. Second-generation schizonts and gamonts of *E. auburnensis* develop in the lamina propria in the ileum, small schizonts in villi, and gamonts in the deeper lamina propria. Microgametocytes may be several hundred micrometers across. Oocysts are ~38 × 23 μm.

The other bovine coccidium with gamonts apparently developing in the lamina propria is ***E. bukidnonensis***. Oocysts of this species are large, ~48 × 35 μm and thick-walled, with a micropyle, and have been found in the lamina propria. ***E. alabamensis*** develops in vacuoles within the nucleus of epithelial cells in small intestine and, in heavy infections, the large bowel. Both schizonts and gamonts may be found together within the same nucleus. Gamonts of ***E. kosti*** have been described in the epithelium deep in the abomasal glands. None of these organisms is particularly pathogenic.

Eimeria bareillyi is associated with clinical coccidiosis in water buffalo calves. The serosal vessels in the distal half of the small intestine are congested, and the lumen of the lower small bowel contains creamy or yellow fluid content in which some mucus, fibrin, or blood may be present. Focal to coalescent pale raised plaques or polypoid masses may be present on the mucosa, or the surface may appear granular and necrotic, with petechial hemorrhages. The gross changes are caused by hypertrophy of crypts and villi, upon which virtually every

cell is infected with developing gamonts or oocysts. *E. bareillyi* will not cross-transmit to domestic cattle, though *E. ellipsoidalis* and *E. zuernii* of bubaline origin will. *E. zuernii* is pathogenic in water buffalo.

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Coccidiosis in sheep and goats

Coccidial infection is universal in sheep and goats, and coccidiosis can be a significant problem in the young of both species. The etiology of coccidiosis in these species is complicated by the morphologic similarity of the coccidia infecting sheep and goats. Assumptions on the potential for cross-infection of coccidia between sheep and goats, and of the species found in each host, have been revised as new taxonomic and biologic information has come to light.

At present, about a dozen species of coccidia are found in each of sheep and goats. Of these, three (*Eimeria pallida, E. caprovina, E. punctata*) may occur in both sheep and goats, though the validity of *E. punctata* as a species is questioned. Eight species pairs of *Eimeria* occur, in which the coccidia look and behave similarly in sheep and goats, but do not cross-infect. Listing the sheep-adapted species of each pair first, these are: *E. ahsata–E. christenseni*; *E. ovinoidalis–E. ninakohlyakimovae*; *E. bakuensis* (=*ovina*)*–E. arloingi*; *E. granulosa–E. jolchijevi*; *E. crandallis–E. hirci*; *E. faurei–E. apsheronica*; *E. parva–E. alijevi*; *E. intricata–E. kocharli*. Two species are unique to sheep, *E. weybridgensis* (formerly *E. arloingi* "B"), and *E. marsica*; in goats, one species, *E. caprina*, is unique. In addition, giant schizonts of an unknown coccidian, termed *Globidium gilruthi*, are seen incidentally as pinpoint white foci in the abomasum of sheep and goats. The taxonomic confusion has been carried over into descriptions of the natural or experimental disease, since many infections were of mixed species, resulted from inocula of poorly defined species of coccidia, or occurred under circumstances where the oocysts associated were not described. However, while the taxonomic picture has changed, the syndromes associated with coccidiosis in sheep and goats have not.

Coccidiosis in these species is a *disease of young animals*. Under conditions of intensive pastoral husbandry or confinement, lambs and kids are exposed to oocysts of many species of coccidia within the first few days of life. Weaned lambs, presumably exposed to only light infections while at range, are also prone to coccidiosis when brought into feedlots. In young suckled animals and those in feedlots, exposed to large numbers of oocysts, signs may occur before oocysts are passed. Suckling lambs, ~5–8 weeks old, reared at pasture at relatively heavy stocking rates, may also develop signs, and occasionally die. Under these conditions, the disease needs to be differentiated from gastrointestinal helminthosis, which may be concurrent.

Outbreaks of coccidiosis in confined lambs and kids are usually acute and characterized by moderate morbidity and low mortality; there is green or yellow watery *diarrhea*, occasionally with blood or mucus. Yarded and grazing animals may also suffer weight loss, or subclinical ill-thrift. Signs are usually associated with lesions in the lower small intestine, caused by *E. ahsata* and *E. bakuensis* in lambs, and their analogues in goats, *E. christenseni* and *E. arloingi*, or with typhlocolitis, caused by *E. ovinoidalis* in sheep, and *E. ninakohlyakimovae* in goats. Some pathogenicity is also ascribed to *E. faurei*, *E. intricata*, *E. parva*, and *E. crandallis* in sheep, and presumably to their analogues in goats. Infections may be mixed, and gross and microscopic lesions may reflect this.

E. ovinoidalis in sheep and ***E. ninakohlyakimovae*** in goats presumably have similar endogenous development. In the sheep, giant schizonts up to 300 μm in diameter develop in cells deep in the lamina propria, in the terminal ileum. They release merozoites that enter epithelium in the glands of the cecum and colon, and perhaps distal ileum. Here small second-generation schizonts evolve, and other cells in glands in the same area subsequently become infected by the gametocytes.

These species are considered highly pathogenic and *E. ovinoidalis* is often associated with disease in feedlot lambs. Lesions other than those related to diarrhea, dehydration, and hypoproteinemia are limited to the terminal ileum, and especially the cecum and proximal colon, and are associated with second-generation schizogony and gametogony. Affected areas of gut are edematous and thickened. The most significant microscopic lesions are those in the cecum and colon, which resemble those in cattle due to *E. bovis* and *E. zuernii*. ***E. caprina*** in goats also seems to have pathogenic potential. Like *E. ninakohlyakimovae*, it causes typhlocolitis; the small intestine is not involved.

E. christenseni and *E. arloingi* in goats and their analogues, *E. ahsata* and *E. bakuensis* in sheep, are also associated with serious disease. They seem to have somewhat similar developmental cycles and lesions, though interpretation of the literature is clouded by confusion among these species. Many cases of coccidiosis in lambs attributed to *E. bakuensis* (as *E. arloingi*) may in fact have been due to *E. ahsata*, since the unsporulated oocysts, though of differing sizes, can be confused.

E. christenseni has a developmental cycle that involves giant schizonts up to nearly 300 μm across in the endothelium of the lacteal in villi in the middle small intestine. In heavy infections, every cell in a number of contiguous crypt–villus units may be infected. Though there may be an acute local reaction around

ruptured primary schizonts, clinical disease is associated with the subsequent stages of development, diarrhea occurring during the late prepatent and patent periods. Affected intestine may be congested and edematous. Numerous pale white or yellow foci from a few millimeters to up to a centimeter in diameter, often visible from the serosa, are present as slightly raised plaques on the mucosa of the small bowel. These foci are areas of intense infection of cryptal and villus epithelium by gamonts and developing oocysts, and have been dubbed "*oocyst patches.*" There may be some hemorrhage into the intestine but the feces are rarely bloody.

E. arloingi undergoes a development similar to that of *E. christenseni* (Fig. 1.199) and causes similar gross and microscopic lesions in goats, with minor differences. First-generation schizonts are most numerous in the lacteals of villi in the lower jejunum, gamonts are mainly above the host cell nucleus, and the associated grossly visible plaques in the mucosa (Fig. 1.200) may tend to be more distal in the small intestine, and occasionally involve the large bowel. ***E. ahsata*** and ***E. bakuensis*** in sheep are similar.

Nodular polypoid structures, sometimes pedunculate, and about 0.3–1.5 cm in diameter, are encountered in the small intestinal mucosa of sheep and goats, usually as an incidental finding. These masses are comprised of hypertrophic crypt–villus units, in which virtually every epithelial cell is infected by mainly gametocytic stages of coccidia, which, in sheep, are probably *E. bakuensis* and *E. ahsahta*. Adjacent mucosa appears normal and is uninfected. The term "pseudoadenomatous" has been used to describe these polypoid lesions, and the oocyst patches or plaques discussed above in coccidia-infected sheep and goats (Fig. 1.200B). The infected epithelial cells appear somewhat hypertrophic, with eosinophilic cytoplasm and prominent brush borders. Often these coccidia-infected cells do not slough rapidly postmortem, in contrast to their uninfected fellows.

Why masses of infected cells apparently persist in chronically infected animals without clinical disease is unclear. However, the plaques and polyps may be the result of mitogenic stimuli from progamonts, the immature stages in crypt epithelium, which appear to divide by binary fission in synchrony with the infected host cell.

Coccidiosis may also cause ill-thrift and diarrhea in suckling or weanling lambs 5–6 weeks old heavily stocked on pasture. In the UK, ***E. crandallis***, which develops largely in the ileum, and ***E. ovinoidalis*** are mainly associated with this syndrome. ***E. weybridgensis*** (*E. arloingi* "B"), which infects most of the length of the small intestine, may also contribute. The only gross lesion in affected lambs is congestion and thickening of the mucosa of the lower small intestine.

Under some circumstances, probably sudden exposure to large doses of oocysts, ***E. crandallis***, at least, causes *villus atrophy* in infected areas of intestine. Giant first-generation schizonts develop in crypt cells that after infection migrate into the lamina propria. As the infection progresses, villi become stumpy or disappear, and in small bowel

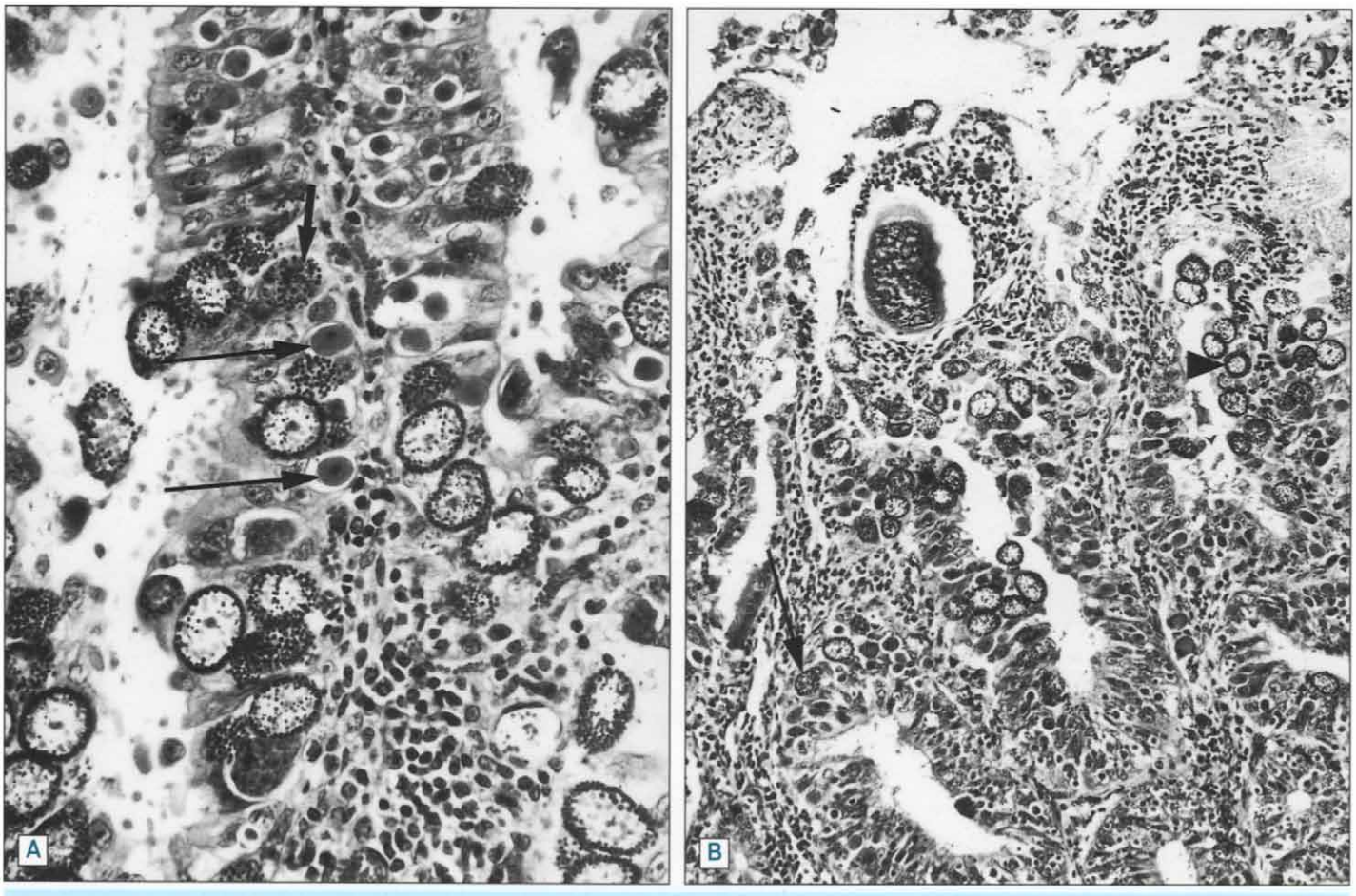

Figure 1.199 Coccidiosis (*Eimeria arloingi*) in a goat. **A.** Undifferentiated gamonts (long arrows), macrogametocytes (short arrow), and microgametocytes infect epithelial cells. **B.** Large schizont in lacteal in the Ileum. Gamonts (arrow) and developing oocysts (arrowhead) in epithelium of crypts and villi.

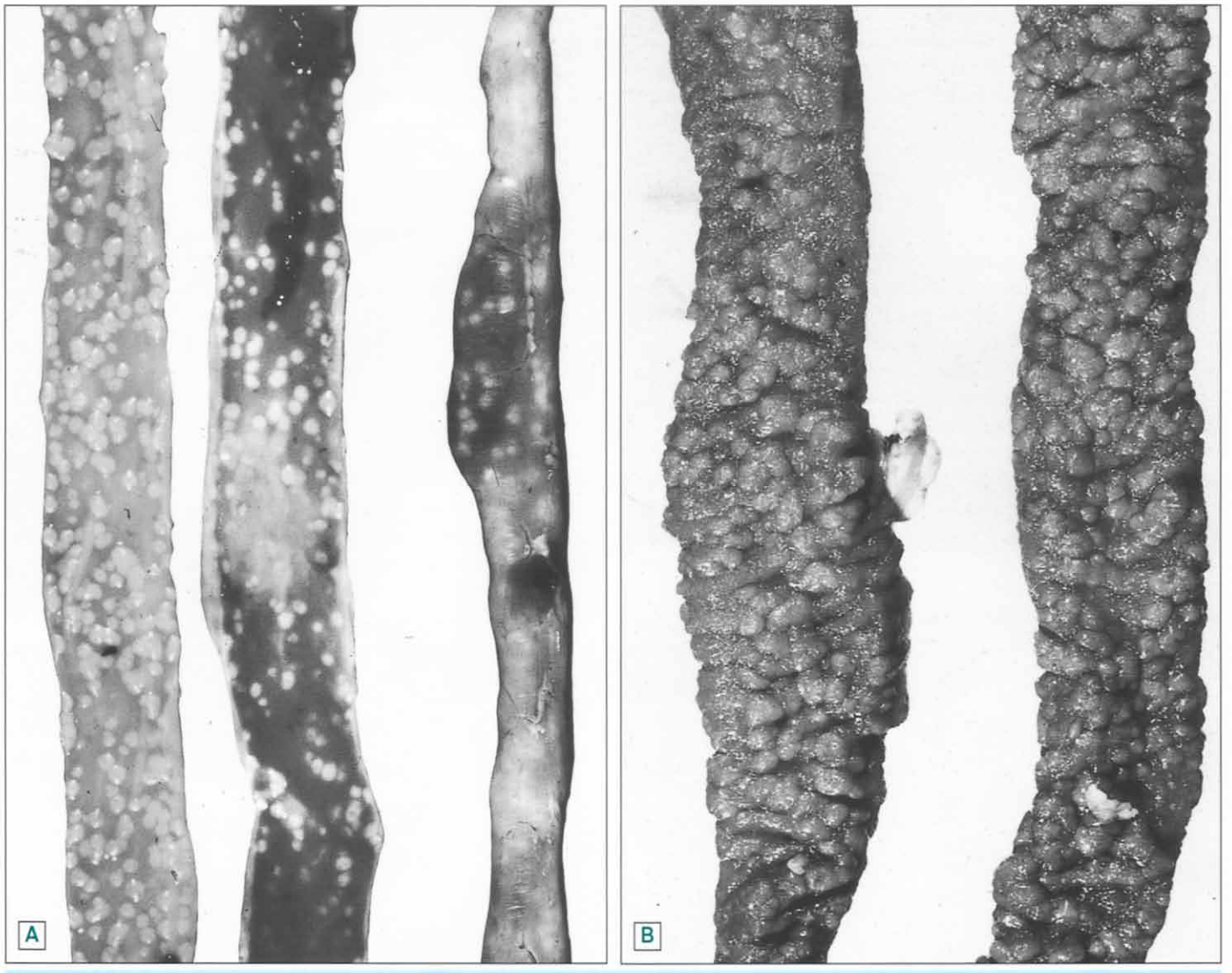

Figure 1.200 Coccidiosis (*Eimeria arloingi*) in a goat. **A.** White nodules on mucosa are visible from serosa (right). Hemorrhage in lumen. (Courtesy of PA Taylor.) **B.** Chronic coccidiosis showing mucosal hypertrophy.

and cecum, crypts are straight, hypertrophic, and contain proliferative epithelium. Asexual or, more commonly, sexual stages of coccidia are present in epithelium on the surface of the mucosa. In hyperplastic crypts, epithelial cells are infected by progamonts, which seem to be dividing in synchrony with host cells. Masses of macrophages may invest and invade the base of infected crypts, and apoptosis of infected and uninfected cells may occur, resulting in attenuation of surviving crypt epithelium. In heavy infections, there may also be thickening of the cecal mucosa by hyperplastic coccidia-infected cells. Occasionally, areas of small intestine and cecum, in which there has been severe damage to crypts, may become eroded.

Such lesions, if widespread, may cause malabsorption or perhaps protein-losing enteropathy. It is unclear whether atrophy of villi is the result of excess loss of epithelium directly due to the effects of coccidial infection, or whether it is mediated by an immune response.

E. apsheronica in the goat has minor pathogenic potential. Giant schizonts develop in the lamina propria of villi throughout the small intestine and in the cecum: second-generation schizonts are in the epithelium on villi in the small intestine, and in the cecum, but not the colon. Gametocytes have the same distribution. Pale foci in the mucosa, where gametocytes are concentrated, and focal areas of erosion and hemorrhage may occur in heavily infected animals.

Large schizonts are often encountered incidentally in submucosal lymphatics, or in the subcortical or medullary sinusoids of mesenteric lymph nodes in sheep and goats. Sometimes they may be visible grossly in these locations as pinpoint white foci. Occasionally, coccidial gametocytes or oocysts may also develop in intestinal lymphoid aggregates and mesenteric lymph nodes, where they may provoke a mild granulomatous reaction. Stages in lymph nodes probably result from establishment of sporozoites or primary merozoites swept from the lacteal into the lymphatic drainage early in infection. Development in such sites is not uncommon, but aberrant and likely dead-end. The species involved appear mainly to be those considered above, with a giant primary schizont developing in the lacteal.

In coccidiosis, *oocysts are usually numerous in feces*, but this is neither constant in, nor necessarily indicative of, disease. Mucosal

scrapings or tissue sections of mucosa containing large numbers of asexual and gametogenous coccidial forms, in association with diarrhea, and perhaps some hemorrhage into the intestine, support the diagnosis, in the absence of other syndromes such as gastrointestinal helminthosis.

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Coccidia in horses

The only coccidium of horses reported with any frequency is ***Eimeria leuckarti***, which is found in horses and donkeys the world over. In one survey of foals in Germany, it was found in 100%. It may also occur in older animals. Its reputation for pathogenicity rests largely on the *distinctive large gamonts* found by pathologists in the lamina propria of the small intestine in animals dead of obscure enteric disease. However, implication of *E. leuckarti* in the disease process is rarely, if ever, convincing, and it is encountered incidentally in the intestine of horses dead of other clearly defined conditions. Furthermore, heavy experimental inoculations, producing many gamonts in the gut and heavy oocyst passage, have failed to elicit clinical signs.

The stages present in the lamina propria of villi are giant microgametocytes and macrogametes, developing in markedly hypertrophic host cells, probably of epithelial origin (Fig. 1.201). The microgametocytes are up to ~250 μm in diameter, and when mature they

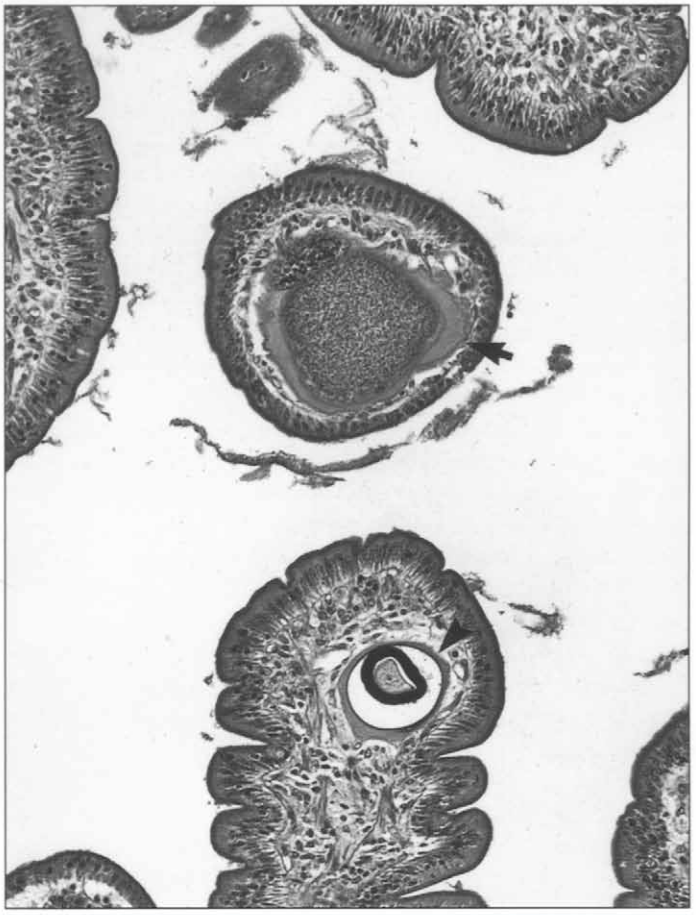

Figure 1.201 Microgametocyte (arrow) and developing oocyst (arrowhead) of ***Eimeria leuckarti*** in lamina propria, in a horse.

contain swirling masses of microgametes. Immature microgametocytes very much resemble some of the giant schizonts of other species of coccidia, and have frequently been referred to as such; this stimulated the application of the term *Globidium* to the organism. However, the only schizont containing merozoites that has been recognized in horses was very small (12.5 μm in diameter), and in the epithelium of the ileum. The macrogametes have distinctive large eosinophilic or Schiff-positive granules that may be individual or confluent. The host cells are markedly hypertrophic with a fibrillar periphery, and the enlarged nucleus forms a crescent along one side of the parasitophorous vacuole. There is no inflammatory response to the gamonts, and only a mild reaction to degenerate stages in the lamina propria.

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Coccidiosis in swine

At least 8–10 species of *Eimeria* are thought to occur in swine, along with a single species of *Isospora*. The latter, ***Isospora suis***, is the most important; it causes *porcine neonatal coccidiosis*, a disease of piglets from ~5–6 days to ~2–3 weeks of age. This disease is recognized in the USA, Canada, the UK, and western Europe; it also occurs in Australia, and probably wherever swine are reared intensively. The condition is most severe in herds where continuous farrowing and total confinement are practiced, and some laboratories report a prevalence of 10–50% among scouring baby pigs. Rapid sporulation (12 hours) and short prepatent period (5 days) promote rapid build-up of infection in a farrowing house.

Porcine neonatal coccidiosis has high morbidity, and usually low but variable mortality. It causes yellow watery diarrhea, dehydration, loss of condition, and death, or at least a temporary check in growth. Some animals may runt severely. Illness usually begins at ~7–10 days of age. Piglets continue to nurse, but may vomit clotted milk. At autopsy, many piglets have the typical appearance of undifferentiated neonatal diarrhea, with no specific gross findings in the gastrointestinal tract other than fluid yellow content. However, the intestine in some animals with coccidiosis may look turgid, rather than flaccid, and in a minority of animals a fibrinous or fibrinonecrotic exudate is present in the lower portion of the small intestine. Occasionally, casts will form.

I. suis replicates in the epithelium on the *distal third of villi* (Fig. 1.202), mainly in the *jejunum and ileum*, though infected cells may be found in the duodenum and colon in a few animals. Piglets usually become infected within the first day or two of life, perhaps by ingestion of the sow's feces. Merogony occurs in vacuoles in the cytoplasm, usually beneath the nucleus of the host cell. Infection of host cells is maximal 4–5 days after infection, and by 5 days, gametogony is evident. Thick- and thin-walled sporulated oocysts have been observed in feces of infected pigs, but either form may cause disease. The onset of lesions and clinical signs corresponds with this period of heavy infection of cells, which undergo lysis. Villi may become markedly atrophic. The surface epithelium that remains is cuboidal to squamous, and infected epithelial cells may be seen degenerating or exfoliating (Fig. 1.203). Erosions may develop at the tips of villi (Fig. 1.204). In the remnant of the villus, neutrophil infiltration, a moderate increase in round cells and eosinophilic proteinaceous material, perhaps collagen, may be present in the lamina propria. Effusion of neutrophils and fibrin from the eroded tips of villi contributes to the fibrinonecrotic membrane seen in some animals, and ulceration can occur. Gram-positive bacilli are often present in the exudate. In animals surviving for a few days, the cryptal epithelium may be markedly hyperplastic.

The severity of the lesions is a function of the size of the inoculum and the age of the pigs. Heavier inocula, within limits, produce more cellular damage and villus atrophy; fibrinonecrotic enteritis indicates ingestion of a large dose of oocysts. However, severe lesions may not be associated with heavy shedding of oocysts, since relatively few gamonts are able to develop in the reduced population of epithelial cells remaining on villi. The severity of lesions and signs is much greater in piglets a few days old in comparison with those 2 weeks of age. This partly relates to the lower rate of replication of epithelium in the crypts of young piglets, and therefore the development of more severe villus atrophy. The smaller size of young piglets also makes them more susceptible to the effects of malabsorption and diarrhea. Animals previously exposed to *I. suis* have relatively strong resistance to challenge.

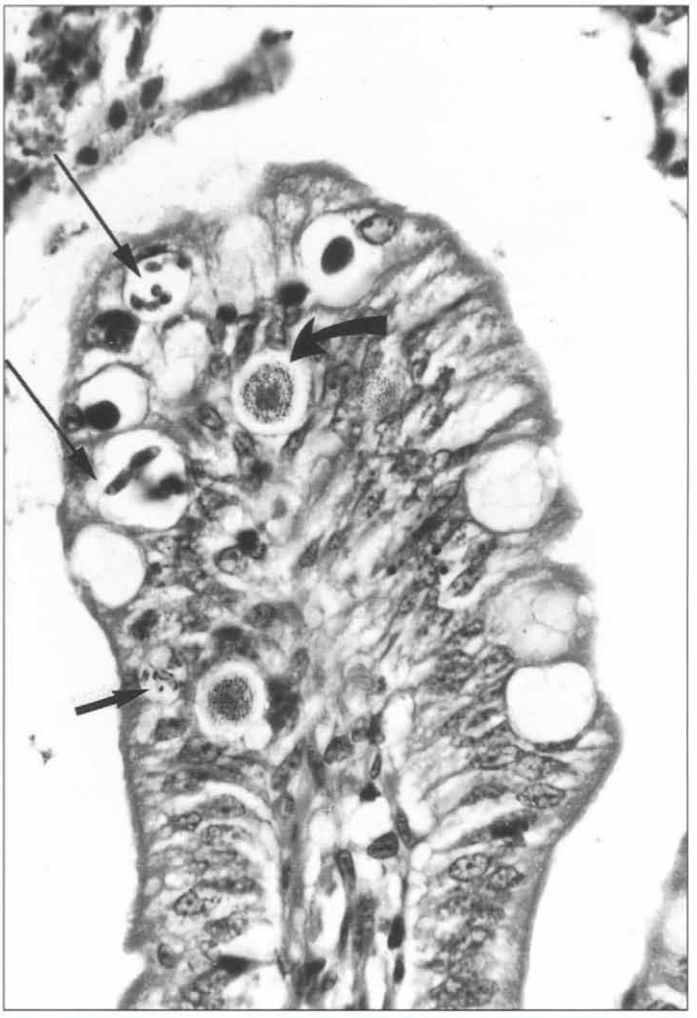

Figure 1.202 Meronts containing merozoites (long arrows), developing oocysts (curved arrow), and a microgametocyte (short arrow) in epithelial cells, in a pig with ***Isospora suis*** infection.

A **diagnosis** of coccidiosis must be considered in scouring neonatal piglets, and is strongly suggested by the presence of *fibrinonecrotic enteritis in the distal small bowel*. Atrophy of villi may be recognized at autopsy using a hand lens or stereomicroscope, or in tissue section. Asexual or sexual stages may be found in smears of mucosal scrapings. The distinctive binucleate type I meronts and pairs of large (12–18 μm in smears, 8–13 μm in sections) type I merozoites may be found in jejunal mucosa in the early phase of diarrheal disease. Multinucleate type II meronts and numerous small type II merozoites are the predominant stage during the clinical phase of disease. In section, these form clusters of 2–16 organisms like bunches of bananas, perhaps with a small residual body, in the parasitophorous vacuole in the enterocyte.

Macro- and microgamonts are present in moderate numbers by day 5 of infection, and a few oocysts may also be seen. Microgametocytes are ~9–16 μm in diameter, and are multinucleate. Oocysts in tissue sections are oval, ~15 × 12 μm, while those

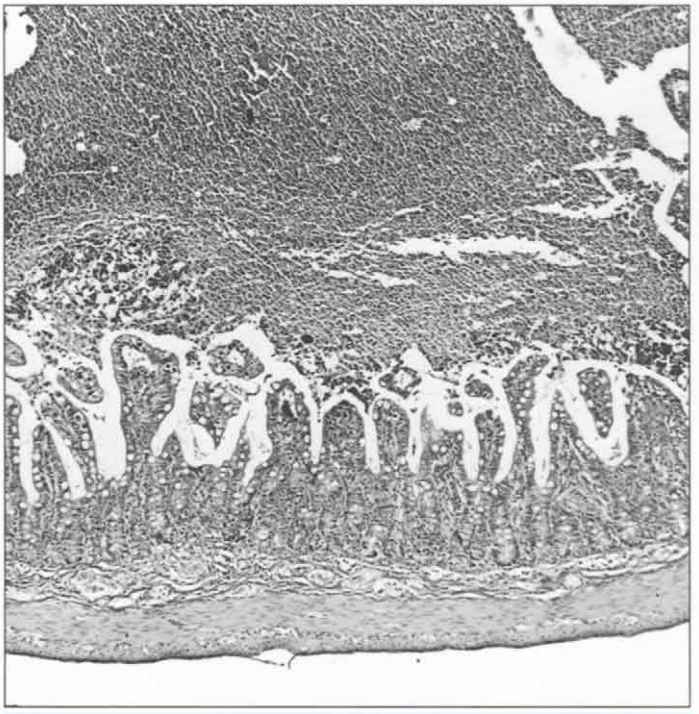

Figure 1.203 Blunting and atrophy of villi in a pig with ***Isospora suis*** infection. Lumen contains massive numbers of exfoliated epithelial cells, inflammatory cells, and coccidial stages, which appear grossly as exudate.

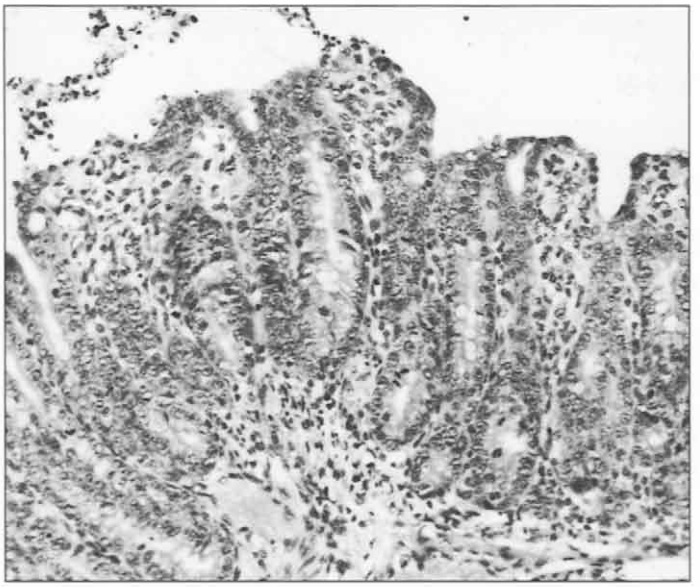

Figure 1.204 ***Isospora suis*** infection. Surface epithelium is severely attenuated, and there is erosion and effusion at tips of villi.

in smears are ~18 × 16 μm. Coccidial stages may be difficult to find in animals that have been ill for several days. Oocysts may not be found in feces, because the infection is not yet patent, the patent period has passed, or the lesions are very severe, reducing the number of oocysts produced.

Coccidiosis in older swine is due to several *Eimeria* species, and is uncommon but speciation can be accomplished. It typically occurs in animals with access to yards or pasture contaminated with oocysts. Weaners and growing pigs are affected. The species considered potentially pathogenic include ***E. scabra***, ***E. debliecki***, and ***E. spinosa***. It is difficult to produce disease in experimentally inoculated pigs; *E. scabra* is probably the most pathogenic. Coccidiosis in swine due to *Eimeria* spp. is usually sporadic, or affects a few pigs in a group. Typically it causes diarrhea of a few days' duration, loss of appetite, and perhaps transient ill-thrift, or, in severe cases, emaciation. Occasionally animals die.

Lesions are usually limited to the lower small intestine, which may be congested or hemorrhagic, though overt blood is rarely found in the feces. Large numbers of schizonts, gamonts, and developing oocysts are in epithelial cells on villi and sometimes in crypts. Atrophy of villi, or erosion and local hemorrhage or inflammatory effusion may be evident, the lamina propria is edematous, and desquamated epithelium and oocysts are in the lumen of the gut. Rarely, heavily infected animals may have lesions in the large intestine. The species involved are diagnosed on the basis of the morphology of oocysts in feces or mucosal scrapings.

Coccidial gamonts and oocysts of a species resembling *E. debliecki* have been found infecting epithelium on the papilliform mucosa of cystic bile ducts in porcine liver. This is probably an aberrant site of development.

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Heteroxenous apicomplexan protists

Cystoisospora, Toxoplasma, Neospora, Hammondia, Sarcocystis, Besnoitia, and ***Frenkelia*** comprise this group of protists. All of these heteroxenous members of the Apicomplexa are known to *use carnivores as definitive hosts*, and have one or more generations of merogony in the tissues of various species of prey. *Frenkelia*, which some would place in the genus *Sarcocystis*, as far as is known utilizes only raptorial birds as definitive hosts, and small rodents as intermediate hosts. It will not be considered further.

Coccidiosis in dogs and cats

Although several species of *Eimeria* have been reported from dogs and cats, their status as genuine parasites of these hosts is in doubt. The significant coccidia of dogs and cats are members of the genus *Cystoisospora*, considered here, and of the genera *Toxoplasma*, *Sarcocystis*, *Hammondia*, *Besnoitia*, and *Neospora*, dealt with subsequently. *Caryospora* spp. may occasionally produce dermal coccidiosis in immunosuppressed dogs.

***Cystoisospora* spp.** are characterized by oocysts that are passed unsporulated in feces, and which, when sporulated, have two sporocysts lacking a Stieda body, each with four sporozoites. Following ingestion of sporulated oocysts of heteroxenous species, transport hosts, usually prey species such as mice and other small rodents, but sometimes other hosts, are infected by large sporozoite-like "hypnozoites" in phagocytic cells in lymph nodes and other tissues. These, when ingested by the predator, resume development in the intestine, and lead to asexual and sexual development in the definitive host. Heteroxenous passage is not obligatory, and sporulated oocysts are also directly infective to the definitive host.

In **dogs**, four species of *Cystoisospora* are recognized. Meronts of ***C. canis*** develop in the subepithelial lamina propria of the villi in the distal small intestine and, to a lesser extent, in large bowel. Gamonts occur beneath and within the epithelium of the ileum and large intestine, and the oocyst is the largest among *Cystoisospora* spp. of dogs, being ~38 × 30 μm. The other three are members of the "*C. ohioensis* complex." ***C. ohioensis*** develops exclusively in epithelial cells, mainly in the distal portions of villi along the length of the small bowel, especially in the ileum, and occasionally in the large bowel. It may be the most pathogenic species in dogs. The oocysts of *C. burrowsi*, *C. ohioensis*, and *C. neorivolta* are similar. Original literature should be consulted for details that will permit differentiation of these species in tissue. Endogenous stages of ***C. burrowsi*** occur in epithelial cells, and in the lamina propria of the tips of villi in the distal two-thirds of the small intestine. ***C. neorivolta*** mainly develops in proprial cells beneath the epithelium in the tips of villi in the distal half of the small intestine, and rarely in the cecum and colon. Occasional stages may be in the epithelium. *C. canis* and *C. ohioensis* are known to be heteroxenous. Meronts of an unknown coccidian, probably a *Cystoisospora* sp., have been found in the intrahepatic bile ducts of a dog, associated with severe suppurative cholangiohepatitis.

In **cats**, two *Cystoisospora* spp. occur. Meronts and gamonts of ***C. felis*** develop in epithelium of villi in the small intestine, and occasionally in epithelium in the large bowel. The oocyst is large, ~43 × 33 μm. ***C. rivolta*** also develops in epithelium on villi and in crypts and glands in the small and large intestine. Oocysts are ovoid, ~25 × 23 μm. Subepithelial schizonts and gamonts of an unknown coccidian, possibly a *Cystoisospora* species, have been associated with fatal enteritis in a cat.

Coccidiosis in the dog and cat is largely a clinical entity, *usually nonfatal*. The lesions of coccidiosis in small animals are poorly defined, and care must be taken not to ascribe disease to these organisms simply on the basis of the presence of endogenous stages in the mucosa of animals dead of enteric disease. Rotavirus and coronavirus might be expected to produce similar signs. However genuine cases of fatal coccidiosis do occur, though few are recorded in the literature. Affected animals are young, and usually from environments such as pet shops, animal shelters, or kennels in which standards of sanitation may not be high. There is a history of diarrhea of several days' duration, and the animal is dehydrated. Other than mild hyperemia of the mucosa and excessively fluid content of the small intestine and colon, gross lesions in the gut may not be evident. Microscopically, there may be moderate atrophy of villi, with attenuation of surface enterocytes, and perhaps effusion of acute inflammatory exudate from the tips of some eroded villi. Asexual and sexual stages of coccidia will be evident in moderate to large numbers in the epithelium or lamina propria of villi. In some cases, the large bowel may be infected, with exfoliation of surface epithelium, and accumulation of necrotic debris in some dilated glands.

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Toxoplasmosis

Toxoplasma gondii uses members of the Felidae as definitive hosts. It is optionally heteroxenous; cats may be infected directly by ingestion of oocysts, but probably most commonly by ingestion of asexual stages in the tissues of prey species. Cats excrete oocysts 3–10 days after ingesting *bradyzoites*, approximately 13 days after ingesting tachyzoites, and 18 days after ingesting oocysts. The latter occurs with very poor efficiency. Intermediate hosts are infected by oocysts shed in the feces of cats, or by a variety of other routes considered below. Five stages of asexual development are recognized in the intestinal epithelium of cats infected with tissue cysts from intermediate hosts. The gametocytes also develop in epithelium on villi, especially in the *ileum*. In heavy infections, exfoliation of infected epithelium from villi is associated with the development of *villus atrophy*, and occasional spontaneous cases of diarrhea in kittens seem to be caused by *Toxoplasma*-induced atrophy of villi and malabsorption.

In intermediate hosts, and in cats, extraintestinal asexual development occurs in a variety of organs and tissues. Rapidly dividing forms (*tachyzoites*) may by endodyogeny proliferate in cells in many sites for an indefinite number of generations, and are the stage associated with acute toxoplasmosis in cats and other species. Eventually, tachyzoites induce the formation of a cyst wall in a host cell, and divide slowly, forming bradyzoites, which reside in quiescent tissue cysts.

T. gondii, with *Neospora caninum*, is unique among protists in its ability to parasitize a wide range of hosts and tissues. *It is one of the most ubiquitous of organisms*; experimentally, essentially all homeothermic animals can be infected, and natural infections occur in birds, nonhuman primates, rodents, insectivores, herbivores, and carnivores, including domestic species and humans. Serologic surveys indicate that infection is widespread in most species of domestic animals; however, except for abortions in sheep and goats, overt disease is sporadic and rare.

Transmission may occur by a number of different routes. The shedding of oocysts in the feces of cats and wild Felidae has been mentioned earlier. Transplacental infection occurs commonly in sheep and goats and sporadically in swine and humans. Carnivorous animals and humans may become infected by ingesting oocysts from cats, or more commonly from cysts containing bradyzoites in tissues of infected animals, implying that the cycle of infection can be maintained by means of facultative homoxenous transmission, without a definitive host.

Systemic toxoplasmosis occurs most often in young animals, especially immunologically immature neonates and in immunocompromised hosts. Low levels of γ-interferon and the associated inability to activate macrophages are predisposing factors for systemic toxoplasmosis. In dogs, canine distemper, ehrlichiosis, and lymphosarcoma are commonly concomitant with toxoplasmosis. The infection in juveniles may be acquired pre- or postnatally. After ingestion, *Toxoplasma* organisms penetrate the intestinal mucosa. In cats, the enterointestinal cycle and systemic infection occur almost simultaneously. In other animals the tachyzoites are the first stage of infection, after invasion of the lamina propria by sporozoites released from the oocyst, or by bradyzoites released from the tissue cyst digested from food in the intestine.

Dissemination of *Toxoplasma* occurs in lymphocytes, macrophages, granulocytes, and as free forms in plasma. From the intestine the organism may follow two routes. It may spread via the lymphocytes to the regional nodes and from there in the lymph to the bloodstream, or it may pass in the portal circulation to the liver and from there to the systemic circulation. Further dissemination occurs to a wide variety of organs. Tachyzoites actively invade or are phagocytosed by host cells and are surrounded in a parasitophorous vacuole formed of host cell membrane. Tachyzoites proliferate, destroying the host cell, and cell-to-cell transmission may occur within infected organs.

Focal necrosis is common, and appears to be directly related to the rapid replication of tachyzoites. The outcome of infection is determined by a number of factors, including the number and strain of *Toxoplasma* in the infecting dose, and the species, age, and immune status of the host. Lesions in visceral organs are usually evident within 1–2 weeks after oral infection. Variable numbers of tachyzoites are usually found in the vicinity of the necrotic areas.

Specific immunity develops within a few days after infection; the cell-mediated arm is most significant in toxoplasmosis, mediated in large part by interleukin-12. This reduces the severity of infection but usually does not terminate it. Immune animals develop a *chronic or dormant form* of *Toxoplasma* infection that is characterized by the formation of *cysts*, containing bradyzoites. These are mainly located in the brain, skeletal muscle, and myocardium. Cysts may form as early as 1–2 weeks after infection and they may persist for months, possibly years. Intracellular encystment protects the bradyzoites from both cellular and humoral immune mechanisms. *Inflammation is usually not associated with cysts.* When the level of resistance drops below a critical level, for example, due to treatment with immunosuppressive drugs, intercurrent disease, or other factors that depress immunity, particularly decreased levels of γ-interferon, *a chronic infection may become reactivated.* The cysts rupture and cause severe local inflammation. Released bradyzoites rarely survive to infect other cells.

The **clinical signs** of toxoplasmosis vary considerably, depending on the organs affected. The most consistent signs reported are fever, lethargy, anorexia, ocular and nasal discharges, and respiratory distress. Neurological signs include incoordination, circling, tremors, opisthotonos, convulsions, and paresis. Paresis is often associated with radiculitis and myositis. In the dog, signs may coexist with those of canine distemper and are not sufficiently distinctive to allow ready differentiation.

Systemic toxoplasmosis has been reported in most species of domestic animals. The hallmarks are *interstitial pneumonia, focal hepatic necrosis, lymphadenitis, myocarditis, and nonsuppurative meningoencephalitis.* Pulmonary lesions are probably most consistently found, followed by central nervous system lesions. The lesions in the various organs are morphologically similar in most species, varying mainly in degree.

Macroscopic lesions in the lung vary from irregular gray foci of necrosis on the pleural surface to hemorrhagic pneumonia with confluent involvement of the ventral portions. Careful examination of the liver usually reveals either areas of focal necrosis or irregular mottling, and edema of the gallbladder. The spleen is enlarged, as are lymph nodes, which are wet and often red. Pleural, pericardial, and peritoneal effusions occur irregularly. Pale areas may be evident in the myocardium and skeletal muscle. Occasionally, the pancreas is the most severely affected organ, in which case an acute hemorrhagic reaction may involve the entire organ. Yellow, small, superficial intestinal ulcers with a hyperemic border have been reported in piglets. Large pale areas of necrosis may be present in the renal cortices, mainly in goats and kittens.

Microscopically, the early pulmonary lesions are characterized by *diffuse interstitial pneumonia*; the alveolar septa are thickened by a predominantly mononuclear inflammatory cell reaction with a few neutrophils and eosinophils. Macrophages and fibrinous exudate fill the alveoli. Foci of necrosis involving the alveolar septa, bronchiolar epithelial cells, and blood vessels are scattered throughout the lobules. These lesions are soon followed by regenerative changes that are characterized by hyperplasia and hypertrophy of alveolar lining cells, mainly type II pneumocytes: so-called *epithelialization of alveoli*. In some areas this may be so marked as to give the affected areas an adenomatous appearance. Tachyzoites are usually evident in alveolar macrophages and may also be found in bronchiolar epithelial cells and the walls of blood vessels.

In the *liver*, irregular foci of coagulative necrosis are scattered at random throughout the lobules. There is usually little evidence of inflammation associated with the necrotic areas. Variable numbers of tachyzoites may be present in hepatocytes and Kupffer cells, usually at the periphery of the lesions, but often at some distance. If the

pancreas is involved, there is extensive peripancreatic fat necrosis, with areas of coagulative necrosis in parenchyma. Numerous tachyzoites are usually evident in both ductal and acinar cells.

Lesions in *lymph nodes* are often associated with infection in the corresponding organ. They are characterized by irregular areas of coagulative necrosis, mainly in the cortex. A moderate inflammatory reaction may be evident at the periphery of the necrotic areas. There may be necrosis and depletion of lymphocytes in the follicles. In more chronic cases, the changes are those of nonspecific hyperplasia of lymphoid cells in cortical and paracortical areas, with a large macrophage population in the medullary sinusoids. Tachyzoites may be seen in phagocytic cells in sinusoids. Similar lesions may occur in the spleen. Necrotic areas are mainly located in the red pulp in this organ.

In the *heart and skeletal muscle*, foci of necrosis and mononuclear cell inflammation may be part of toxoplasmosis. There is often some difficulty in distinguishing between tachyzoites and mineralization of mitochondria in myocytes but, at some distance from areas of acute reaction, inert cysts can usually be identified in healthy fibers.

Brain lesions may vary in appearance. In the most fulminating cases cerebral lesions may be relatively inconspicuous. They consist of nonsuppurative meningoencephalitis with multifocal areas of necrosis and often malacia. There is swelling of endothelial cells, necrosis of vessel walls, and vasculitis. There may be marked perivascular edema and hyperplasia of perithelial cells. Tachyzoites and occasionally cysts may be found in vessel walls and in necrotic areas in both gray and white matter at all levels of the brain. If survival is prolonged, residual cerebral lesions consist of *microglial nodules* along with more extensive hyperplasia of perithelial cells and perivascular fibrosis that tends to make the vessels very obvious. At this stage tachyzoites are rare, and cysts 30 μm in diameter with a wall of amorphous acidophilic material ~0.5 μm thick, located in areas away from the lesions, may be the only form seen. Spinal cord lesions resemble those seen in the brain.

Systemic toxoplasmosis is reported from *cats*, but is certainly seen less commonly in this species than in some others. Lesions are similar to those described for other species. Chronic granulomatous toxoplasmosis may involve the intestine in older cats and produce annular areas of thickening. The mucosa overlying the granulomas may be ulcerated.

The finding of tachyzoites and/or cysts in association with areas of coagulative necrosis in one or more organs is highly suggestive of toxoplasmosis. Often the *Toxoplasma* organisms are difficult to distinguish within the necrotic foci, and immunohistochemical techniques have proved very useful in highlighting their presence. Serological tests are of limited value in the diagnosis of disease associated with *T. gondii* infection.

The placental and fetal lesions associated with *Toxoplasma* infection and abortion are described in Vol. 3, Female genital system, and ocular lesions in Vol. 1, Eye and ear.

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Neosporosis

Neospora caninum causes disease in *dogs and ruminants* over much of the world. *Dogs and coyotes are definitive hosts*; cattle, water buffalo, and white-tailed deer can act as intermediate hosts. *Neospora* has been associated with systemic and central nervous system disease in dogs, and with abortion and central nervous system disease in neonatal ruminants. Tachyzoites undergoing endodyogeny, and cysts containing bradyzoites, are found in the tissues of affected animals. Transplacental transmission occurs in ruminants and dogs. Infection may be maintained in lines of cattle in this manner, while some subclinically infected bitches have given birth to successive litters of pups that became affected within the first few months of life. Transmission may also occur by ingestion of infected tissue, as in toxoplasmosis.

Dogs of all ages may be affected, but disease seems most characteristic as *encephalomyelitis, polyradiculoneuritis, and polymyositis* in puppies over ~5 weeks of age, and perhaps involving several animals in a litter. Ascending paralysis, muscle contraction causing hyperextension of the limbs, cervical weakness, and dysphagia may progress to death, or animals will stabilize with caudal paralysis. In adult dogs, there are signs of widespread involvement of the central nervous system, and disseminated disease may be evident, with polymyositis, myocarditis, and dermatitis associated with parasite infection. Though disease may be precipitated or exacerbated by glucocorticoid administration, *Neospora* is regarded as a *primary pathogen*. Circulating levels of γ-interferon may be related to immune resistance.

In acute systemic infections there may be hepatic enlargement with coalescing areas of pallor, related to widespread necrosis of hepatocytes; streaky pallor of muscles due to myonecrosis, mineralization, and nonsuppurative myositis; and pulmonary congestion and edema, due to subacute alveolitis. Tachyzoites are common in affected tissues.

Nonsuppurative encephalomyelitis is associated with the presence of tachyzoites and tissue cysts in neurons and neuropil; the

degree of necrosis, gliosis, neovascularization, and demyelination presumably depends to some extent on the duration of the lesion. Retinitis is also reported in association with *Neospora*, as is pyogranulomatous ulcerative dermatitis, occasionally.

In **ruminant abortion**, *Neospora* may be associated with *necrotizing placentitis*, and with *myositis* and *nonsuppurative encephalomyelitis* of the fetus after about 90 days' gestation. While a well-documented etiologic agent of abortion in cattle, the extent of natural *Neospora*-induced abortions in sheep and goats is unknown. Experimentally, sheep and pigs experience placental infection and necrotizing encephalitis in the fetuses. *N. caninum* has also been reported from a case of equine protozoal myelitis.

Tachyzoites are approximately ovoid, ~5–7 μm long, and are found in small groups or large clusters, free in the cytoplasm or in parasitophorous vacuoles in many types of cells throughout the body. Tissue cysts are found in only the brain and spinal cord. They are spherical or slightly elongate, up to ~110 μm in greatest dimension. The cyst wall is ~1–4 μm thick, usually greater than the width of the bradyzoites, which are slender (1.5 × 7 μm), slightly curved, with an obvious nucleus; they stain weakly periodic acid–Schiff-positive.

Neospora must be distinguished from *Toxoplasma* in all species, and from *Sarcocystis* in aborted fetuses. *Neospora* tachyzoites resemble those of *Toxoplasma* in tissue section. Ultrastructurally, *Neospora* tachyzoites have over 11 rhoptries, whereas there are few in *Toxoplasma*. *Toxoplasma* is always found in a membrane-bound vacuole in the cytoplasm; *Neospora* tachyzoites are often not within a parasitophorous vacuole. *Neospora* tissue cysts are relatively uncommonly encountered, especially in acute cases. They are distinguished from *Toxoplasma* by the thicker wall (thinner than 0.5 μm in *Toxoplasma*). Perhaps the most common method of distinction is by immunohistochemistry using specific antibodies, while polymerase chain reaction is also useful. *Sarcocystis* meronts divide by endopolygony in endothelium in domestic animals; they are not in a parasitophorous vacuole; and merozoites lack rhoptries. Sarcocysts in muscle cells are within a parasitophorous vacuole; they have a distinct wall; and they are usually subdivided internally by septa.

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Hammondia infection

Hammondia spp. are obligatorily heteroxenous organisms, with the cat (***H. hammondi***) and dog (***H. heydorni***) as definitive hosts. They have also been known as *Toxoplasma hammondi* and *Isospora bahiensis*, respectively. *Toxoplasma*-like oocysts are shed in the feces of the definitive host and are infectious to intermediate hosts, mammals and birds (*H. hammondi*), and ruminants (*H. heydorni*). Here, bradyzoites develop in cysts in striated muscle, which are infective when ingested by the carnivore. Disease is not associated with infection of intermediate hosts; diarrhea may occur in heavily infected dogs.

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Sarcocystis infection

Sarcocystis is comprised of about 200 obligatorily heteroxenous species. Inconspicuous sexual stages occur in the epithelium at the tips of villi in the small intestine, and oocysts sporulate in the subepithelial lamina propria, producing two sporocysts within a thin oocyst wall. Sporocysts containing four sporozoites are shed in feces. These are infective to intermediate hosts, in which three generations of merogony occur in vascular endothelium, and a final cyst containing first *metrocytes* (mother cells), which produce *merozoites* (bradyzoites), is formed in myocytes and occasionally other cells. Ingestion of tissue cysts containing bradyzoites initiates gametogony in the definitive host. There is apparently no resistance to the development of gamonts, and no disease is associated with them in the definitive host.

Many species of *Sarcocystis* are recognized, based on prey–predator cycles. Sporogony of a given species usually occurs in only one or a few genera of carnivores. The number of species capable of acting as intermediate hosts may be narrow or wide, depending on the species of *Sarcocystis*.

Sarcocystis cysts in ovine and, occasionally, bovine muscle may be grossly visible, causing losses at meat inspection. *Eosinophilic myositis*

can result from granulomas due to *Sarcocystis* infection of muscle. However, studies have shown that unaffected muscle often harbors more of these organisms than the inflamed portions. *Sarcocystis* infection in cattle (***S. cruzi***), sheep (***S. tenella***), and swine (***S. miescheriana***), and, experimentally, in goats (***S. capracanis***), may cause acute fatal disease characterized by anemia and widespread hemorrhage, which is associated with clotting disorders. As the disease progresses, cattle may develop inappetence, weight loss, reduced milk yield, hyperexcitability, hair loss, and in some animals, nervous signs. Illthrift associated with *Sarcocystis* infection may also occur in other species. Both syndromes are initiated during the endothelial phase of the infection. As well, abortion occurs during this phase in some species. Abortion associated with the acute disease is the result of the systemic illness, and the fetus is usually not infected. However, in cattle, some abortions, seen in otherwise clinically normal animals, are associated with meronts of *Sarcocystis* in the placenta and in vascular endothelium of the fetus, especially in the brain, and with nonsuppurative encephalitis. Encephalitis is occasionally associated with *Sarcocystis* infection in sheep, and in horses ***S. neurona*** is the cause of protozoal myeloencephalitis. Details of these syndromes are discussed in Vol. 1, Muscle and tendon; Vol. 3, Female genital system; and Vol. 1, Nervous system.

A *Sarcocystis*-like agent has also been implicated in mortality of Rottweiler dogs with hepatitis, encephalitis, and dermatitis.

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Besnoitia infection

Besnoitia spp. are also obligatorily heteroxenous, but the definitive host for many species has not been identified. Some stages of merogony and gametogony occur in the intestine of the only definitive host yet known, the cat, where they are not known to be pathogenic. Oocysts are shed unsporulated, and resemble those of *Toxoplasma* and *Hammondia* when sporulated. Meronts in the intermediate host develop in mesenchymal cells, probably fibroblasts, which become massively hypertrophic, forming cysts containing many clusters of merozoites (bradyzoites) in the host cell cytoplasm. Among domestic animals, cysts of *B. besnoiti* may assume some significance in the skin of cattle and goats (see The Skin and Appendages, Volume 1, Chapter 5), and *Besnoitia* cysts have been reported in association with laryngeal polyps in a horse.

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Cryptosporidiosis

Cryptosporidium is a small apicomplexan protist, found on the surface of epithelium in the gastrointestinal, biliary, and respiratory tracts of mammals, birds, reptiles, and fish. Respiratory infection is most significant in birds, and disease in mammals is generally enteric.

Although its taxonomic position in relation to other apicomplexan protists, and its species nomenclature, are in a state of flux, on molecular genetic grounds about 15 morphologically similar species of *Cryptosporidium* are recognized. Five of these occur in domestic animals: (1) ***C. parvum***, small, and initially described in the mouse intestine, but parasitic in cattle, other ruminants, and humans; (2) ***C. andersoni*** in cattle; (3) ***C. suis*** in pigs; (4) ***C. felis*** in cats; and (5) ***C. canis*** in dogs. *C. parvum* is zoonotic, as are *C. suis*, *C. felis*, and *C. canis* to a lesser extent, and disease in humans has been associated with contamination of water sources, food and milk products, as well as close contact with infected animals. However, humans also have a primate-adapted species – ***C. hominis***, and it is probably responsible for the majority of outbreaks of cryptosporidiosis not associated with direct animal contact.

All three stages of the *Cryptosporidium* life cycle – merogony, gametogony, and sporogony – occur extracytoplasmically in a vacuole within the apical region of epithelial cells, protruding above the cell surface (Fig. 1.205). The prepatent period of *C. parvum* in calves is ~7 days and infections usually persist for weeks or, if the animal is immunocompromised, perhaps months. Type I merozoites recycle in a new host cell to produce another meront generation, while type II merozoites differentiate to gamonts. Thin-walled oocysts excyst in the gut of the same host, resulting in autoinfection by the sporozoites released, while thick-walled oocysts are excreted to the external environment and are responsible for transmission to another host. Multiple generations of merogony, and autoinfection by excystment

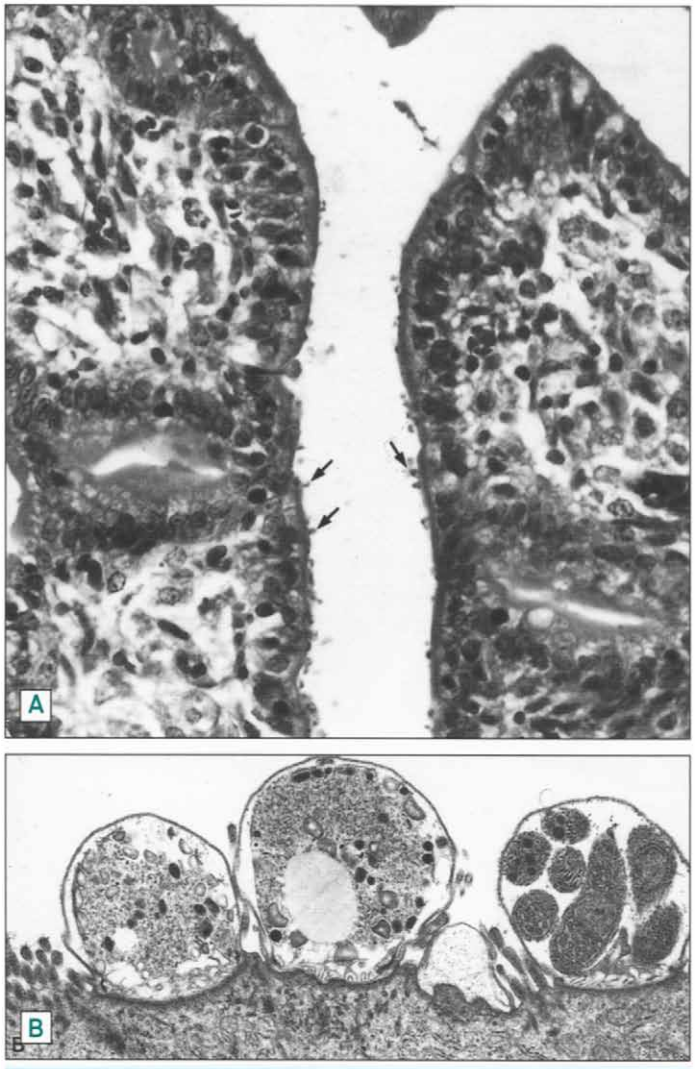

Figure 1.205 Cryptosporidia. A. Appearing as minute basophilic dots (arrows) on the brush border of enterocytes in the small intestine of a foal. **B.** Attached to apex of enterocytes in small intestine. Two macrogametes and a schizont containing merozoites. (Transmission electron micrograph courtesy of S. Tzipori.)

of thin-walled oocysts, result in a large biotic potential and promote heavy colonization of the gut.

The organisms are enclosed within a parasitophorous vacuole formed by apposition of two unit membranes of the host cell, probably caused by inversion of a microvillus by the infecting sporozoite or merozoite. A specialized "feeder" organelle is present at the attachment zone in the base of the vacuole, between the infecting organism and the cytoplasm of the host cell. Developmental stages are small, in most cases ~2–6 μm in diameter. Undifferentiated meronts and gamonts are recognized as small basophilic trophozoites. Mature schizonts contain small falciform merozoites. Macrogamonts are ~5 μm in diameter, and contain small granules. Oocysts in tissue sections are often collapsed into a crescent shape. The various stages may be recognized in wax- or plastic-embedded sections under the light microscope, but are best studied with the electron microscope. Oocysts containing four sporozoites may be demonstrated by fecal flotation, or in fecal smears stained with Giemsa, by a modified Ziehl–Neelsen technique, or with Auramine O or fluorescein-labeled antibody and examined with ultraviolet light.

Cryptosporidia are found in many circumstances, and in some species infection appears to be asymptomatic. Neonates are particularly susceptible to intestinal infections, and this is especially so among ruminants (calves, lambs, kids, red deer calves) infected with *C. parvum*. Diarrhea, anorexia, and depression in calves usually occur between ~1 and 4 weeks of age, and in lambs about 5–14 days old. However, naive calves up to 3 months of age are susceptible to infection and may develop diarrhea.

Cryptosporidial infections are mainly eliminated by cell-mediated immune phenomena, but the humoral arm also contributes. Immunosuppression is contributory to, but not essential for, the development of disease. Heavy infections are reported in Arabian foals with combined immunodeficiency, and cryptosporidiosis has occurred in cats with *Feline leukemia virus* infection, and in dogs with canine distemper. In immunocompromised individuals, organisms may be present at any level of the gastrointestinal tract, from esophagus to colon. Liver, gallbladder, pancreas, and their ducts may also be involved, as may the respiratory tract. Cryptosporidia frequently occur concurrently with enterotoxigenic *Escherichia coli*, rotaviral, or coronaviral infection in neonatal ruminants, but can be primary pathogens.

In all species, *intestinal cryptosporidiosis is associated with villus atrophy of variable severity*, characterized by blunting and some fusion of villi, and by hypertrophy of crypts of Lieberkühn (Fig. 1.206). Surface epithelium is usually cuboidal, rounded, or low columnar, and sometimes exfoliating or forming irregular projections at tips of villi. *Large numbers of cryptosporidia are usually visible in the microvillus border of cells on the villi* (Fig. 1.205A), and not in crypts of Lieberkühn, although occasionally, the reverse is true. Organisms typically are most heavily distributed in the distal half of the small intestine, especially in ileum, though occasionally cryptosporidia may occur in the cecum and colon. Mild proprial infiltrates of neutrophils and mixed mononuclear cells are present, probably attracted by proinflammatory cytokines released from infected epithelium. An increase in intraepithelial T lymphocytes has been documented in the intestine of infected calves.

Diarrhea in cryptosporidiosis is mainly attributable to malabsorption associated with villus atrophy and a population of immature enterocytes; and perhaps to the occupation of a large proportion of the surface area of absorptive cells by the organisms. Release of inflammatory mediators, principally prostaglandins, may stimulate mucosal secretion, and an increase in epithelial cell permeability to macromolecules has been demonstrated in vitro.

Cryptosporidium parvum is most significant in **calves**, as a cause of undifferentiated neonatal diarrhea, in which it must be differentiated particularly from coronaviral and rotaviral infection. Frequently it is concurrent with other agents causing this syndrome; it tends to be most prevalent in animals ~2 weeks old. A similar situation occurs in lambs, though disease does not appear to be as common, or well recognized, in that species. It is a sporadic or minor cause of sometimes fatal diarrhea in other species of ruminants.

Though cryptosporidiosis can be induced experimentally in **piglets**, it is a very rare cause of spontaneous disease in swine. It is only occasionally associated with disease of carnivores, and then often in probably immunocompromised animals. Though infection of **foals** is not uncommon, *Cryptosporidium* has been associated with disease mainly in animals with combined immunodeficiency,

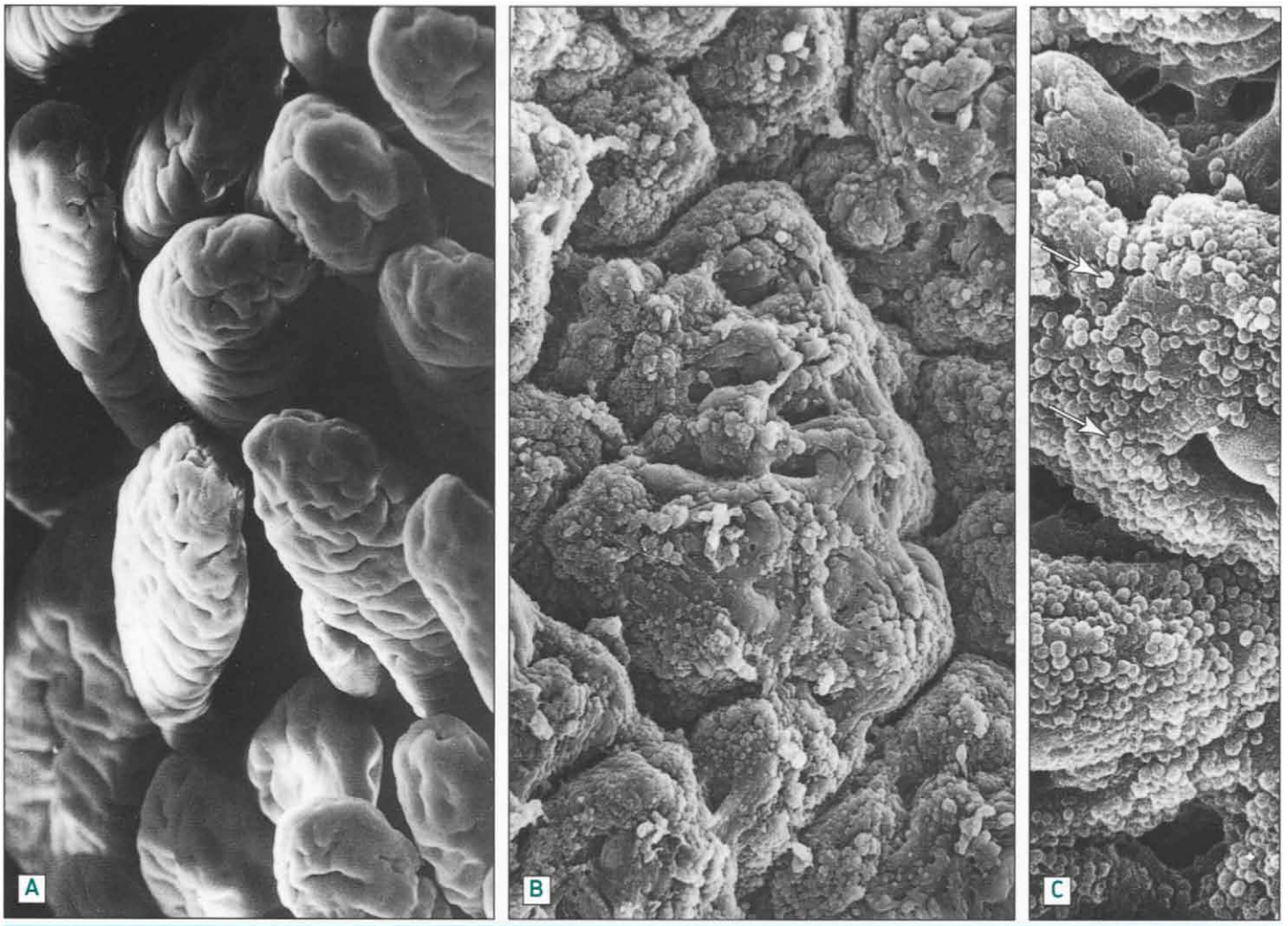

Figure 1.206 Cryptosporidia: scanning electron micrographs. **A.** Normal villi. **B.** Villus atrophy associated with cryptosporidiosis. Cryptosporidia are visible as minute spheres on mucosal surface. **C.** Detail of (**B**) showing cryptosporidia (arrows). (Courtesy of S. Tzipori.)

complicated by adenoviral infection. Disease has not occurred in successful experimental infections in foals, and the role of cryptosporidia in the etiology of neonatal diarrhea in foals is poorly defined, although occasional outbreaks attributable to *C. parvum* may occur.

The diagnosis is based on the presence of large numbers of cryptosporidia in sections of freshly fixed lower small intestine, preferably in association with villus atrophy. Examination of smears of ileal mucosa stained with Giemsa may allow a rapid diagnosis, or permit a diagnosis on tissue from an animal dead for some hours.

C. andersoni, in the *abomasum of weaned calves and older cattle*, is not associated with diarrhea, but plasma pepsinogen levels rise, and weight gains of some growing animals may be adversely affected. There is mucous metaplasia/hyperplasia in the fundic glands, which are dilated, with attenuation of the lining epithelium, on which cryptosporidia are numerous. Infections have been associated with decreased milk production.

Rarely, cryptosporidia are seen in gastric biopsies from **cats**, sometimes associated with mild gastritis. There is some indication that concurrent infection with *Helicobacter felis* will precipitate disease due to cryptosporidia.

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Amebiasis

Entamoeba histolytica is the cause of amebiasis in humans, nonhuman primates, and, rarely, in other species, including dogs and cattle; cats are susceptible to experimental infection. Infection in dogs is sporadic, probably acquired by exposure to cysts in feces from infected humans. Dogs tend not to pass encysted amebae; hence it has been suggested that they present little public health hazard, and are unlikely to support spread from dog to dog. However, under some circumstances, cysts may be shed, and fecal material containing motile trophozoites has been used to transmit infection orally to other dogs.

Amebae are usually nonpathogenic inhabitants of the lumen of the large bowel, but sometimes they cause colitis. The diet and immune status of the host, and virulence attributes of various strains of the organism, seem to influence pathogenicity. Adhesion to mucus by specific lectins, enzymatic degradation of mucus, and lectin-mediated adherence of amebae to host epithelium, are essential steps leading to tissue damage. Cysteine proteinases produced by *E. histolytica* contribute to epithelial cell damage and to induction of inflammation, both of which are involved in initiation of mucosal lesions. Cytolysis is induced by in-contact amebae.

Amebiasis in dogs is associated with diarrheic or mucoid feces, perhaps with some blood, or with dysentery. *Erosive mucosal colitis or ulcerative colitis* occurs in dogs with amebiasis, and disease seems more common or severe in animals with concomitant *Trichuris* or *Ancylostoma* infection.

Early lesions in human amebiasis seem to be diffuse acute mucosal colitis, with focal erosions or ulcerations. Amebae, though scarce, may be found in mucus on the colonic surface, but are most numerous in the fibrinocellular exudate over erosions or superficial ulcers. Ulcers advance as an area of necrosis and predominantly neutrophilic infiltrate, causing loss of glands, and extending for the full depth of the mucosa. Initial lesions consist of tiny erosions of the surface epithelium that evolve to deeper, more extensive ulcers. Established ulcerative amebic colitis classically has a flask-shaped ulcer, the narrow neck through the mucosa, and the broad base in the submucosa. There amebae, and necrosis, expand laterally, apparently less constrained by the architecture of the tissue. Intestinal perforations and intramural abscesses may form.

Amebae may be present, commonly in small clusters, in necrotic debris or in adjacent viable tissue, frequently not involved in an inflammatory reaction. Amebae in tissue, often surrounded by a clear halo, may be spherical or irregular, with extended pseudopodia, and are ~6–50 μm in diameter. The nucleus has a central dense karyosome and peripheral chromatin clumps. The cytoplasm may appear foamy, can contain remnants of erythrocytes in phagolysosomes, and contains glycogen, which makes the cytoplasm periodic acid–Schiff-positive. The lesions of established amebiasis in the colon of dogs resemble those in humans; the early lesions may as well.

Although dissemination of amebae, with abscessation in other organs, especially liver, lung, and brain, is a relatively common complication in humans, it seems rare in dogs. One such case occurred in an animal with canine distemper.

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Giardia and other flagellates

Giardia spp. are flagellate protists that inhabit the small intestine of a wide range of vertebrates. The taxonomy of the genus is difficult. It appears that four or five morphologically distinct "species" exist, each with a relatively wide host range within amphibians, birds, rodents, and other mammals. ***Giardia duodenalis*** (= *G. lamblia*) infection is common in humans, and it also occurs in a wide array of mammals. Molecular genetic investigations have identified at least seven genotypes within *G. duodenalis*, several of which (assemblages A, B) infect humans. Hoofed animals are infected by assemblages A and E, the former being potentially zoonotic, dogs by assemblage C, and cats by assemblage F. *Giardia* infection has been associated with disease, with various degrees of credibility, in most of these hosts.

Giardia trophozoites are pyriform in outline, ~10–20 μm long by 5–15 μm wide and 2–4 μm thick, and convex on the dorsal surface. The concave ventral surface is modified by the presence of a

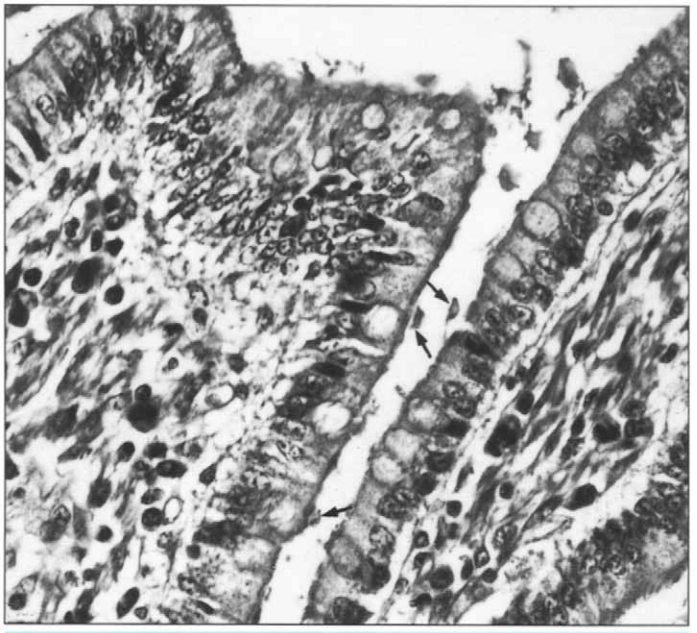

Figure 1.207 ***Giardia lamblia*** (arrows) applied to brush border of enterocytes on villi of a dog.

disk that functions in attachment. Nutrient absorption seems to occur through the dorsal surface. A pair of nuclei, two axonemes, two medial bodies, and four pairs of flagella are present, and the organisms *multiply by binary fission* in the gut lumen. They apply their ventral aspect to the microvillus surface of enterocytes (Fig. 1.207), usually between villi, in folds on the villus surface, or occasionally in crypts of Lieberkühn. *Giardia* has been demonstrated in the mucosa, but this is an unusual and probably aberrant location. Relatively resistant oval cysts are passed in the feces, and transmission is by the fecal–oral route.

The significance of *Giardia* as a pathogen in humans and other species has been controversial, since *asymptomatic infection is the rule*. However, under some circumstances *Giardia* may cause disease. How the host–parasite relationship is modified, and the pathogenesis of the disease, are still unclear.

In *young dogs and cats*, in which giardiosis is most important, though still uncommon, the main sign is intermittent or chronic *diarrhea*, which may persist for several months. The stool is soft, pale, mucoid, and greasy. Though appetite is not usually impaired, there may be a reduced growth rate or weight loss, suggesting malabsorption. A poor hair coat is attributed to deficiency of fat-soluble vitamins. Among animals other than dogs and cats, *Giardia* seems most convincingly to be associated with *enteric signs in neonatal calves*, which may pass soft mucoid feces, and have a reduced growth rate.

Gastrointestinal dysfunction has not been extensively documented in domestic animals. However, in a variety of experimental systems, deficiencies in microvillus-associated digestive enzymes, electrolyte and glucose malabsorption, and microvillus shortening or injury have been documented, though not consistently. Some humans with *Giardia* infection have malabsorption of D-xylose and vitamin B_{12}, with steatorrhea and hypocarotinemia. Excess fecal fat has been found in infected cats, but was not demonstrated in experimentally infected rats. Also, D-xylose malabsorption was not demonstrated in a dog with giardiosis.

Selective deficiencies in some brush border enzymes occur in humans with giardiosis, and in *Giardia*-infected calves. Possibly these are related to the direct effects of *Giardia* on microvilli, which may be deformed adjacent to adherent organisms, or diffusely shortened. *Giardia* may also inhibit the activity of pancreatic lipase, causing fat malabsorption. However, bacterial overgrowth of the small intestine may occur with *Giardia* infection, and associated bile salt deconjugation could explain steatorrhea in giardiosis.

Several mechanisms have been proposed to explain these findings. Although villus atrophy may occur in humans with giardiosis, this mainly occurs in a subgroup of patients with hypogammaglobulinemia. Marked histologic abnormality is not found in many cases of giardiosis in humans, and this also seems to be true for dogs, cats, and calves. In experimental murine giardiosis, infection is associated with hypertrophy of crypts and increased production of cells, combined with an increased rate of movement of enterocytes along villi, with an increased crypt:villus ratio. Deficiencies in brush border form and function may be attributable to incomplete differentiation of enterocytes in this circumstance, or perhaps to damage mediated by mucosal T cells. Intraepithelial lymphocytes are common in infected intestine, and altered epithelial kinetics may be related to cell-mediated immune reactions in the mucosa. Atrophy of villi has been associated with restoration of cell-mediated immune competence in *Giardia*-infected athymic mice, reinforcing the notion that immune phenomena may be involved in the pathogenesis of giardiosis.

Giardiosis is usually *diagnosed clinically* on the basis of typical cysts in fecal flotations, or trophozoites in intestinal aspirates or fecal smears, coupled with remission of clinical signs following therapy, and an inability to identify other potential causes of the signs. A diagnosis is sometimes based on findings in biopsies of small intestine, or at autopsy.

In all species, morphologic changes in the mucosa are not well defined in spontaneous cases of giardiosis. The mucosa may appear normal but there may be equivocal blunting of villi, perhaps associated with a moderate infiltrate of mononuclear cells into the core of the villus, or a heavy population of intraepithelial lymphocytes. *Giardia* should be sought in animals with malabsorption syndromes. They lie between villi, and are usually evident as crescent shapes, applied by their concave surface to the brush border of epithelial cells. In favorable sections through the level of the nuclei, they may appear to have a pair of "eyes." Trophozoites oriented along the plane of section may look as they do in smears, the paired nuclei giving the organism a "face-like" appearance. An abnormal number of bacteria, suggestive of overgrowth, may be present in the mucus and content in the vicinity, in symptomatic animals. A diagnosis of giardiosis should always be reserved for those cases in which no other explanation for the syndrome can be identified. Giardiosis has been associated with colitis in dogs but the association is not clearly causal.

Trichomonads, small flagellate protists that reproduce by binary fission, and are transmitted directly between hosts, are sometimes encountered in the feces of horses, cattle, dogs, and cats with diarrhea. Only in the latter species is there a causal association with disease.

Tritrichomonas foetus is associated with *persistent large-bowel diarrhea*, refractory to treatment, in **cats** under a year of age, and the syndrome has been reproduced experimentally. The microscopic lesions are typical of chronic mucosal colitis, most severe in areas colonized by the organisms. The diagnosis is confirmed in section by detection

of pyriform or crescent-shaped organisms, about 5 × 7 μm in size, with a faint nucleus and eosinophilic cytoplasm, applied to the surface epithelium, or in the lumen of colonic glands, usually in large numbers. However, the organisms are present in only a little over half the sections examined from infected cats, and multiple samples may be necessary in order to have a high probability of detecting them. In some cases, trichomonads appear to disrupt the epithelium, attaining the subepithelial lamina propria around crypts, or they are associated with ulceration and foci of necrosis and pyogranulomatous inflammation that are distributed transmurally in affected areas of colon, and in draining lymph nodes.

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Balantidium

Balantidium is a large oval protist ~50–60 μm or more long, and ~25–45 μm wide, with a macronucleus and micronucleus, and covered by many cilia arrayed in rows. ***B. coli*** occurs in the large bowel of swine, humans, and nonhuman primates. It is very common in pigs, and many infected humans live in close contact with swine. It has also been reported from several dogs with access to swine yards, as a complication of trichurosis.

Balantidium is normally present as a *commensal* in the lumen of the cecum and colon, but is capable of *opportunistic invasion of tissues injured by other diseases*. On rare occasions, it may be a primary pathogen. In swine, where the organisms are most commonly encountered by veterinary pathologists, *Balantidium* may be found at the leading edge of the crateriform necrotizing or ulcerative lesions of the large intestine that develop secondary to intestinal adenomatosis (Fig. 1.208), swine dysentery, or perhaps salmonellosis. *Balantidium* is recognized in tissue by large size, ovoid shape, the dense curved or kidney-shaped macronucleus, and the presence of cilia (which may be accentuated by silver stains) in rows on the surface.

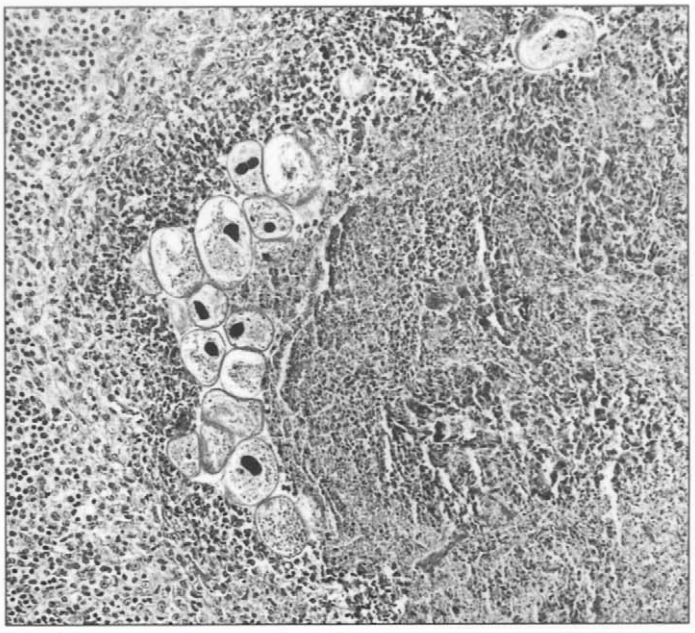

Figure 1.208 ***Balantidium coli*** in ulcerated colon of pig with intestinal adenomatosis and necrotic enteritis.

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PERITONEUM AND RETROPERITONEUM

General considerations

The peritoneum lines the abdominal cavity. Peritoneal diseases are commonly secondary to processes in the organs covered by peritoneum or arising in the retroperitoneum. The peritoneal cavity is incompletely divided into compartments by the mesentery, omentum, and ligaments covered by the peritoneum. Its surface area is greater than that of the skin. *The normal peritoneum is a smooth, shiny membrane that is semipermeable to the movement of water and small solute molecules. There is normally just enough fluid present in the cavity to keep it moist.* Peritoneal fluid is clear and watery, but in neonatal pigs and lambs, fluid may contain strands of mucinous coagulum lying on the intact serosal surfaces of abdominal viscera.

Normal peritoneal fluid is in osmotic equilibrium with plasma, but does not contain fibrinogen or other high-molecular-weight proteins, and generally does not clot, except in pigs. The peritoneal cavity has a dynamic circulation driven by intestinal motility and respiratory excursions, which can disperse fluid (and particles) completely throughout the abdominal and pelvic cavities within minutes to hours.

During organogenesis, the coelomic cavity develops and becomes partitioned into pleural, pericardial, and peritoneal cavities. The