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CHAPTER

16

Bacterial Diseases

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INTRODUCTION

Common marmosets are susceptible to a number of bacterial infections, which may be enzootic, causing sporadic but occasionally severe disease, or which may result in epizootics associated with more severe colony morbidity and mortality. The spectrum of these diseases often differs from those observed in macaque species, and veterinarians caring for common marmosets need to be aware of these unique susceptibilities. In formulating differential diagnoses for sick or diseased animals, it should be recognized that diseases once common in imported animals in the 1960s and 1970s are now rare. It is also important to recognize that housing and sanitation conditions can influence exposure to potentially pathogenic bacteria. In a zoological setting where mixed- or free-ranging exhibits are utilized, animals may be exposed to many more potential pathogens, than would be the case in animals raised in a barrier facility.

Additional work is needed to understand the role of bacteria in common marmoset health and disease. We have limited understanding of how the normal microbial flora of the gastrointestinal tract impacts marmoset health issues such as inflammatory bowel disease (IBD) or colonization by pathogenic organisms. Moreover, much of the current literature describing the clinical and epizoological features of bacterial infections in this species is dated, and laboratory animal scientists should be encouraged to study these conditions and publish their results in further detail.

GRAM-POSITIVE COCCI

Staphylococcus spp.

Members of the *Staphylococcus* genus are part of the normal microbial flora of the common marmoset mucosal surfaces [1]. *Staphylococcus albus* and *Staphylococcus faecalis* have been isolated from the throat, conjunctiva, vagina, penis, and rectum of normal marmosets in the absence of disease. *Staphylococcus aureus* was not detected in a survey of normal animals but was identified in a single animal with a purulent otitis externa. While *S. aureus* may be isolated from superficial or deep purulent infections in a variety of primate species, it is relatively uncommon in common marmosets. If identified, antimicrobial selection should be based on culture and sensitivity due to the presence of resistance to betalactam antibiotics. Therapy of 3–6 weeks may be required to eliminate established infections.

Streptococcus pneumoniae

Streptococcus pneumoniae is an important pathogen of nonhuman primates which may cause a rapidly progressive disease resulting from dissemination via the respiratory tract. The disease is characterized by involvement of the meninges, serosal surfaces, or joints and may result in rapid death with few clinical signs. Systemic manifestations may be preceded by mild upper or lower respiratory signs in severely affected or contact animals. The source of epizootics is likely symptomatic or asymptomatic human contacts. While the organism remains highly susceptible to penicillin G, the clinical response in animals with systemic disease is often poor. Treatment of contact animals may help control spread of infection. Previous reviews have suggested that *S. pneumoniae* be considered an important pathogen of callitrichids [2,3]; however, definitive reports of infection of common marmosets with *S. pneumoniae* are lacking and their susceptibility to infection and severe disease remains to be determined.

GRAM-POSITIVE RODS

Erysipelothrix rhusiopathiae

Etiology

Erysipelothrix rhusiopathiae is a Gram-positive, nonmotile bacillus, which is known to infect a variety of domestic and wild animals, and is well described in swine and turkeys in which it causes the disease erysipelas. Septicemia with *E. rhusiopathiae* (*insidiosa*) has been reported in cotton-top tamarins, red-bellied tamarins, and common marmosets [4].

Clinical Signs

In the reported epizootic of *E. rhusiopathiae*, common marmosets were found dead with no antecedent clinical signs.

Epizootiology

E. rhusiopathiae can be shed in the feces by a variety of wild and domestic animals where it may survive in the environment for up to 2 weeks, contaminating fomites, bedding, and food sources. Previous outbreaks in nonhuman primates have been linked to exposure to rodents, birds, and swine. In the reported outbreak observed in common marmosets, animals were housed in a barrier facility and the source of infection was unclear. Worker contact with swine at home was proposed as one potential source. Alternatively, tree branches and foliage used for enrichment may have been contaminated with bird droppings, thereby exposing animals in the barrier facility [4].

Pathology

Grossly at necropsy, hemorrhages may be noted in a number of organs, including the kidneys, adrenal glands, gastrointestinal tract, and brain [4]. These changes may be accompanied by splenomegaly and lymphadenopathy. Acute myocarditis, hepatitis, encephalitis, and enteritis involving the duodenum and jejunum may be recognized histologically.

Diagnosis

Definitive diagnosis requires culture and identification of *E. rhusiopathiae*. The organism can be recovered from blood or tissue, and use of enrichment broth or selective media with sodium azide decreases contamination and improves isolation [5]. Polymerase chain reaction (PCR) assays have also been described [6].

Differential Diagnosis

Other causes of bacterial septicemia and disseminated infection such as *Yersinia* spp., *Pseudomonas* spp., and *Klebsiella* spp. should be considered. *Yersinia* spp. and *Klebsiella* spp. should be visualized on histological evaluation of tissue. None of these organisms are associated with multifocal hemorrhage described with *E. rhusiopathiae* and bacterial culture, and identification would distinguish them. The extensive hemorrhages noted at necropsy are also suggestive of coagulopathy, and rodenticide toxicity should be considered in the differential diagnosis.

Prevention and Control

An epizootic of *E. rhusiopathiae* has been stopped by administration of a commercially available *E. rhusiopathiae* vaccine manufactured for swine to remaining animals [4]. *E. rhusiopathiae* should also be susceptible to penicillins and erythromycin, which could be considered in the treatment of clinically affected or contact animals. As *E. rhusiopathiae* may be carried by rodents, pest control measures should be evaluated.

Research Complications

E. rhusiopathiae is a known zoonotic bacterial infection producing the disease erysipeloid in humans. While described in common marmosets in a single report, risk for this potential infection in laboratory-reared animals appears low. Infection risk may be more of a concern in animals housed in zoological collections, particularly if exposed to other species, including rodents and birds.

Mycobacterium spp.

Mycobacterium spp. are small acid-fast bacilli that can be classified into three groups based on biology and disease causation: (1) *Mycobacterium tuberculosis* complex (MTC), (2) nontuberculous mycobacteria (NTM), and (3) leprosy bacilli. The former two groups may infect and cause disease in common marmosets. As a group, mycobacteria have a complex cell wall high in glycolipids, including mycolic acid that imparts unique staining characteristics and functions as an important virulence factor.

Nontuberculous Mycobacteria

Etiology

More than 170 distinct NTM species have been described, most not associated with human or animal disease [7]. The Runyon classification system for NTM was developed in 1959 and is based on growth rate and pigmentation. The slow-growing mycobacteria include Runyon groups I, II, and III, and the rapid-growing mycobacteria are in group IV. Runyon group I or photochromogens produce a yellow pigment on exposure to light and include *Mycobacterium kansasii, Mycobacterium marinum,* and *Mycobacterium simiae*.

Runyon group II or scotochromogens produce pigment regardless of light exposure and include *Mycobacterium gordonae* and *Mycobacterium scrofulaceum*. Runyon group III or nonchromogens fail to produce pigment and contain the species *Mycobacterium avium* and *Mycobacterium intracellulare*. Runyon group IV or rapid growers fail to produce pigment and contain *M. fortuitum*. More recent phylogenetic analysis of NTM 16S rRNA largely reflects the Runyon classification system [8].

Colonization of common marmosets by NTM has recently been described [9]. In this report, routine screening of marmosets by tuberculin skin testing using old mammalian tuberculin revealed that 3.6% of the colony had equivocal or positive reactions. *M. gordonae* was isolated from the fecal culture of a single animal. Subsequent PCR screening for *M. gordonae* sequences revealed that 66.7% of the tuberculin skin test (TST)-positive animals were colonized by *M. gordonae* compared with 16.7% of the TST-negative controls. Animals with positive TSTs were necropsied, and lymph nodes were cultured. *M. kansasii* was isolated from tissues with morphologic evidence of mycobacterial disease. The authors concluded that detection of *M. gordonae* may serve as an indicator of NTM colonization, whereas *M. kansasii* was associated with tissue pathology [9] (Fig. 16.1).

M. kansasii is a Runyon group I photochromogen and is considered one of the more pathogenic NTM. It may be isolated from immunologically normal human patients or in patients with underlying malignancies such as leukemia or solid tumors. It is most commonly isolated from patients with chronic lung disease but has also been observed to cause disseminated disease, tenosynovitis, and cutaneous infections [10].

FIGURE 16.1 Tuberculin skin test (TST) in common marmosets with atypical mycobacterial infection. Grade 5-positive TST demonstrating marked swelling and complete closure of the palpebral fissure.

Clinical Signs

Infected animals are often without clinical signs, and NTM infection is suspected based on equivocal or positive intradermal skin test results. Occasional animals may demonstrate weight loss and peripheral lymphadenopathy [9].

Epizootiology

NTM are found widely in environmental soil, vegetation, and water samples and form biofilms on surfaces that promote survival and persistence of the organism. These films occur commonly in water distribution systems and may serve as a source of infection through ingestion or inhalation. Recirculating hot water distribution systems are often used in buildings such as hospitals and animal facilities and may promote the formation of biofilms. They have been linked to the development of disseminated *M. avium* complex in both immunocompromised humans and rhesus macaques [11].

Because of their ubiquitous nature, exposure to NTM from the environment is nearly constant but disease is rare. Colonization and disease may be dependent on the level of exposure and on poorly defined host factors. In general, cases of NTM arise sporadically, and once an animal is infected, the organisms are not thought to be communicable and therefore do not pose a risk to contact animals or human handlers. One exception to this observation was documented in groups of simian immunodeficiency virus-infected rhesus macaques, in which horizontal transmission between animals occurred [12].

Pathology

At necropsy, multiple gastrointestinal lymph nodes may be enlarged. Less commonly tracheobronchial and/or peripheral lymph nodes may be involved. Histologically, there is granulomatous lymphadenitis with variable perinodal fibrosis. In these granulomas, there are central regions of caseous necrosis surrounded by multinucleated giant cells and variable numbers of epithelioid macrophages and lymphocytes. The remaining lymph nodes often appear hyperplastic. Ziehl–Neelsen stains reveal small numbers of acid-fast bacilli both within regions of caseous necrosis and within multinucleated giant cells. The findings are similar to those previously described for *M. kansasii* infection in squirrel monkeys [13] and differ from those seen with NTM in immunocompromised hosts. It is important to note that these morphological features appear similar or identical to MTC infections of nonhuman primates, and histopathology cannot be used to distinguish the two different infections (Fig. 16.2).

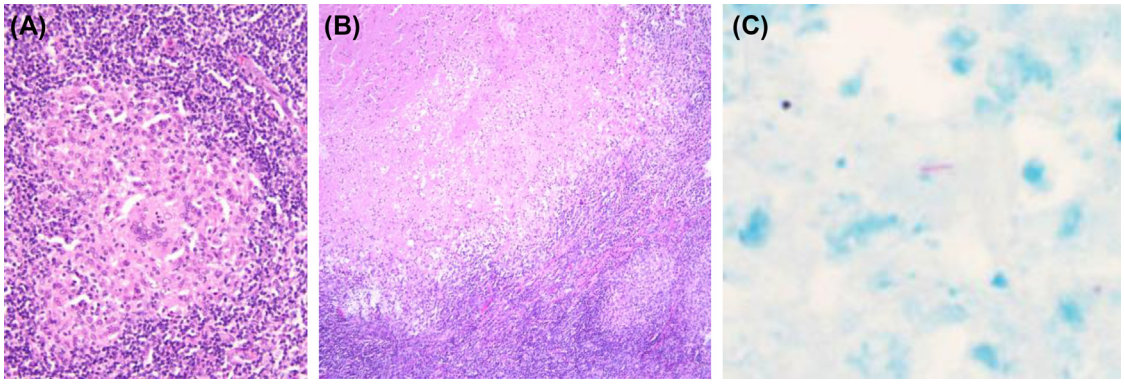

FIGURE 16.2 **Morphologic appearance of atypical mycobacterial infections in common marmosets.** (A) Multinucleated giant cell in the center of a noncaseating granuloma (lymph node, H&E stain, ×200). (B) Large region of caseous necrosis in mesenteric lymph node (lymph node, H&E stain, ×200). (C) Acid-fast bacillus in region of caseous necrosis (lymph node, Ziehl–Neelsen stain, ×500).

Diagnosis

PCR has been widely used to detect mycobacterial nucleic acid in environmental and patient samples and has a number of advantages, including speed and sensitivity. A variety of PCR assays have been described to distinguish MTC from NTM and to further speciate the latter [14,15]. Caution is advised in interpreting PCR results as false positive and negative results are possible. Moreover, the sensitivity of PCR to detect mycobacteria in nonhuman primate tissue or biological samples has not been rigorously assessed. Definitive diagnosis requires bacterial culture and isolation. Most atypical mycobacteria grow well and relatively rapid on Lowenstein–Jensen media.

Differential Diagnosis

NTM must be differentiated from MTC infections; this cannot reliably be done based on clinical and pathological findings. Because suspected cases are first identified by an equivocal or positive TST, other causes of TST reactivity should be considered such as previous administration of complete Freund's adjuvant or iatrogenic bacterial infection at the test site.

Prevention and Control

NTM are common environmental bacteria and can be found in a number of sources. It is likely that most marmosets are constantly exposed to NTM, but the vast majority do not become colonized or develop disease. Host factors that predispose animals to colonization and/or disease are unknown but might include host genetics, concurrent diseases, or alterations in the gastrointestinal microflora. As noted above, NTM form biofilms and can colonize water distribution systems in large facilities. If recurrent or persistent issues are observed, colony water sources should be assessed, and remedial actions to reduce water-borne mycobacterial content should be considered. In immunodeficient macaques, use of autoclaved water sources has been shown to decrease the incidence of NTM infections [16]. Treatment of nonhuman primates for NTM has not been attempted. Treatment of NTM in human patients is difficult, but amikacin has been shown to have the best activity for rapidly growing mycobacteria, and clarithromycin was the most active drug against *M. avium* and other slow growers [17]. *M. kansasii* also responds to rifampin-based therapies, but prolonged treatment may be necessary [18].

Research Complications

Atypical mycobacteria are largely noncommunicable from animal to animal and do not cause zoonotic disease in human handlers. As NTM must be distinguished from MTC infections, colonies should be quarantined until a definitive diagnosis is reached. While this may disrupt ongoing research and breeding programs, it is necessary to protect staff and colony health.

Mycobacterium tuberculosis *Complex*

Etiology

The MTC consists of *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium canettii*, *Mycobacterium microti*, and *M. tuberculosis* and contains known pathogens of humans and wild and domesticated species of mammals. They are obligate pathogens and the etiologic agents of tuberculosis, so named because of the nodular lesions observed in lungs, which are termed tubercles. These organisms can be visualized using acid-fast stains such as the Ziehl–Neelsen, Fite, and Kinyon stains, which fail to decolorize with weak acids in mycobacteria due to the organism's waxlike cell wall. If a Gram stain is performed, bacilli may be weakly Gram-positive or -negative. *M. tuberculosis* and *M. bovis* are the two most common species in the MTC to be reported in Old World primates and can cause sporadic or epizootic disease of high morbidity and mortality in these species. Infection

with other members of the MTC (*M. africanum*) is less commonly reported [19].

PCR surveys of neotropical primates kept as pets or in zoological collections have detected a high rate of *M. tuberculosis* in buccal swabs [20], and mycobacterial cultures of gastric lavage have demonstrated similar results [21]. However, reports of MTC-associated disease in neotropical primates are uncommon. There is a single case report documenting *M. tuberculosis* in a pet common marmoset with likely human-to-animal transmission [22]. In contrast, common marmosets are highly susceptible to experimental inoculation of *M. tuberculosis* by the aerosolized and intratracheal routes [23,24]. The reasons for this disparity are unclear, and efforts should be made to further document the natural history of MTC infection in common marmosets and neotropical primates in general.

Clinical Signs

In the case report, a pet common marmoset was presented for chronic weight loss, and an abdominal mass was palpated [22]. The animal died when a biopsy was attempted, and few additional clinical details were provided. Publications on experimental inoculation of marmosets provide additional insight into disease manifestations in this species [23–25]. Progressive weight loss is the most common clinical sign following experimental infection and is observed as early as 2 weeks following inoculation correlating with disease burden. Other clinical signs recognized less frequently included low-grade pyrexia, dehydration, anorexia, and tachypnea. As in other primate species, coughing is infrequent but may be observed.

Epizootiology

In Old World species of primates, infection most commonly originates from infected human handlers, although ungulates have been implicated in epizootics of *M. bovis.* Transmission in these species most often occurs by aerosolization, but infection has also been documented to occur by ingestion, direct contact, or contact with fomites. Less is known about the transmission in common marmosets, although these routes would all appear relevant. Experimental studies indicated that common marmosets are highly susceptible to infection by the aerosolized route, with as few as 1–12 colony-forming units capable of causing rapidly progressive and fatal disease [24].

Pathology

In the case report of naturally occurring tuberculosis in a pet common marmoset, only a limited necropsy was performed and analysis was restricted to an enlarged mesenteric lymph node, which appeared to be abscessed. Moderate numbers of acid-fast bacilli were visualized in the lymph node, and *M. tuberculosis* was cultured and identified [22].

Experimental studies provide additional details on the distribution and histological appearance of tuberculosis in this species [23–25]. Following inoculation with *M. tuberculosis* or *M. africanum*, marmosets developed progressive weight loss and were euthanized when body weight had decreased by 20% of baseline. Progression rates of clinical disease were dose and strain specific. At necropsy, a range of gross findings and histological features were identified similar to those described in primary human tuberculosis. Pulmonary lesions were most frequent and ranged from complete consolidation of lung lobes to the presence of well-defined nodules. These changes were accompanied by enlargement of the tracheal bronchiolar lymph nodes. Histologically both necrotizing and nonnecrotizing lesions were evident. Typical granulomas consisting of a central region of necrosis surrounded by a cuff of epithelioid macrophages admixed with neutrophils and an outer layer of lymphocytes and histiocytes were observed. Multinucleated giant cells and regions of mineralization characteristic of more chronic infections were not observed. Occasional granulomas appeared cavitated and contained semiliquid caseum. Regions of pulmonary consolidation consisted of large numbers of neutrophils which filled alveolar spaces and effaced the normal architecture, eventually leading to overt necrosis. Discrete granulomas were often lacking in regions of consolidation. Results of acid-fast stains were not described [23–25].

Strain-specific dissemination to the liver and spleen was observed in some animals and recognized at necropsy by hepatosplenomegaly and the formation of discrete miliary to nodular granulomas [23,24]. Histologically these comprise both necrotizing and nonnecrotizing granulomas. Less frequently granulomatous interstitial nephritis was observed.

Diagnosis

Animals are most likely to be first identified through a positive TST or because of chronic weight loss and/or respiratory signs. Definitive antemortem diagnosis of tuberculosis can be difficult in nonhuman primates, and the clinician should rely on a variety of modalities to assess animals. The TST is effective in assessing exposure to mycobacteria on a colony basis, but results can be difficult to interpret in an individual animal. The diagnostic sensitivity and specificity of TST have not been assessed in common marmosets. The diagnostic work up should include a complete review of the animals' housing, medical, and research records. Records from contact animals should also be evaluated to determine the TST status and whether unexpected weight loss has occurred. Thoracic and abdominal radiographs

may provide evidence of pulmonary lesions, lymphadenopathy, and/or hepatosplenomegaly. Buccal swabs, gastric lavage, and fecal samples can be utilized to assess for mycobacterial growth or nucleic acids.

Mycobacterial isolation and identification remains the gold standard for the diagnosis of tuberculosis but does have disadvantages. Isolation can be a prolonged process due to the slow-growing nature of MTC organisms and can take weeks to several months to complete. PCR offers the advantage of rapid results, which may be available in a matter of days rather than weeks, and a number of assays have now been developed and are commercially available. Some caution is advised in the interpretation of these results as both false-positive and -negative results are possible. As noted above, detection of environmental NTM sequences does not equate with disease causation and could potentially mask an underlying MTC infection. Moreover, the diagnostic sensitivity and specificity of the various PCR assays have not been rigorously assessed in fluid and tissue samples obtained from clinically affected marmosets. The relative sensitivity of PCR compared with culture in this setting is not defined.

Definitive diagnosis in the living animal may not be possible and euthanasia and necropsy may be required. While this can be a difficult decision, safety of staff and colony health are paramount. Necropsy should be performed with additional respiratory protection such as a fitted N95 respirator or powered air-purifying respirators. At a minimum the lung, spleen, liver, gastrointestinal tract, and tracheobronchial and mesenteric lymph nodes should be collected for formalin fixation, mycobacterial culture, and PCR.

Differential Diagnosis

Progressive weight loss is a common clinical finding in many chronic diseases affecting common marmosets. The clinical and experimental data indicate that mycobacterial infections should be considered in the differential diagnosis in animals presenting with this finding. In animals presenting with a positive TST, differentiating NTM from MTC infections can be difficult but is required due to the different implications for human safety and colony health.

Prevention and Control

Proactive occupational health and safety and colony preventative health programs are critical for tuberculosis prevention and control [26]. Animals should be regularly examined on a quarterly basis; blood obtained for biobanking and 0.05 mL of old mammalian tuberculin should be administered with a 26-gauge needle in the upper eyelid. Purified protein derivative (PPD) used in the human TST is not appropriate. The animals are then visually examined at 24, 48, and 72 h for evidence of redness of swelling. The TST is scored as follows: grade 1, slight bruising of the eyelid; grade 2, erythema of the palpebrum without swelling; grade 3, variable degree of erythema, with minimal swelling; grade 4, obvious swelling, with drooping of the eyelid and erythema; and grade 5, marked swelling and/or necrosis of the eyelid [26]. A grade 3 reaction is considered equivocal, and grades 4 and 5 are considered positive. Due to the risk to colony and human health, in most cases a grade 4 or 5 will result in the euthanasia of the animal. A complete necropsy should be performed with collection of tissue for histopathology, mycobacterial culture, and PCR. While awaiting definitive diagnosis, contact animals should be placed under quarantine and the TST repeated once every 2 weeks until three negative tests are achieved in all remaining animals. Interpretation of equivocal or grade 3 reactions should be made with an assessment of the overall risk of tuberculosis in the individual animal and the colony as a whole. At a minimum, additional diagnostics such as blood work and radiographs should be completed, and the animal and its contacts should be retested by TST. If the risk is deemed high, because of clinical signs such as weight loss or potential recent contact with tuberculosis cases in other animals or humans, euthanasia and necropsy may be warranted.

As most cases of tuberculosis in nonhuman primates are acquired from human handlers, contact with infected individuals should be prevented. Facility staff with contact with nonhuman primates should be assessed for tuberculosis on an annual basis. In general, TST is performed with PPD, and individuals with positive reactions are referred for additional assessment. The QuantiFERON-TB test (manufactured by Cellestis Limited, Carnegie, Victoria, Australia) was approved in 2001 by the Food and Drug Administration as an aid for detecting latent *M. tuberculosis* infection. This in vitro diagnostic test measures a component of cell-mediated immune reactivity to *M. tuberculosis*. Quantification of interferon-gamma (IFN-gamma) released from sensitized lymphocytes is measured in whole blood incubated overnight with PPD from *M. tuberculosis* and control antigens [27,28]. Individuals with signs of upper or lower respiratory tract infections should not work with animals until cleared to do so. Personal protective equipment should be required of all staff while working with nonhuman primates. While a standard face mask does not provide respiratory protection, it does help contain aerosols generated by the wearer when coughing or sneezing. Protection from infected individuals may be more difficult or impossible in zoological settings in which animals are free ranging and may come in contact with visitors. These animals

may be at greater risk of contracting tuberculosis as indicated by recent surveys conducted in neotropical primates in Brazil [20,21].

Research Complications

M. tuberculosis is a known zoonotic disease, which has been transmitted from nonhuman primates to humans. Epizootics of *M. tuberculosis* also pose a threat to colony health and may require quarantine and a discontinuation of research efforts while diagnostic and control measures are implemented. Efforts at prevention are extremely important.

ANAEROBIC BACTERIA

Clostridium spp.

Clostridium perfringens—*Clostridial Myonecrosis*

Etiology

Clostridium perfringens (previously named *Clostridium welchii*) is a Gram-positive, rod-shaped, anaerobic, spore-forming pathogenic bacterium, which is found commonly in decaying vegetation and soils. *C. perfringens* isolates can produce more than 17 different bacterial exotoxins that may adversely affect the host. The alpha toxin is a metallophospholipase that causes activation of the arachidonic pathway and production of thromboxane A2 and platelet-activating factor. Coupled with the hemolytic activity of *C. perfringens* alpha toxin, the resulting increase in vascular permeability is responsible for the myonecrosis characteristic of *C. perfringens* in a variety of species. Several other species of *Clostridium* including *Clostridium histolyticum, Clostridium fallax, Clostridium novyi, Clostridium septicum*, and *Clostridium bifermentans* may result in clostridial myonecrosis in other species [29].

Clinical Signs

A single case of *C. perfringens* gas gangrene (clostridial myonecrosis) has been reported in the common marmoset [30]. The animal died acutely with only facial erythema reported the previous day. The animal had a history of diarrhea and weight lost several months prior to death but had recovered.

Epizootiology

Clostridial myonecrosis may be classified as traumatic, postoperative, or spontaneous. *C. perfringens* is commonly found in the environment and may contaminate wounds. In traumatic and postoperative clostridial myonecrosis wound factors such as low oxygen tension play a role in the establishment of tissue infection and in bacterial growth. In human patients, spontaneous clostridial myonecrosis is often associated with underlying malignancy, and it is proposed that initial clostridial colonization of the poorly oxygenated tumor microenvironment allows the infection to become established with subsequent dissemination. There was no evidence of trauma or malignancy in the reported common marmoset case, and it is thought that initial colonization and infection were in the gastrointestinal tract.

Pathology

At necropsy in the reported case, the abdomen was bloated and blood-tinged fluid was observed in the peritoneal cavity. Generalized subcutaneous hemorrhages were present, and the skeletal muscle appeared grayish-yellow and edematous. Histologically, small vacuoles accompanied by bacilli were observed in the skeletal muscle, liver, gastrointestinal tract, and lungs. In skeletal muscle, interstitial edema and foci of rhabdomyolysis and coagulative necrosis of myofibers were observed. Despite tissue damage minimal inflammatory infiltrates were observed.

Diagnosis

Isolation of *C. perfringens* and identification of alpha toxin activity should be coupled with histological examination of tissues demonstrating large numbers of Gram-positive bacilli, scant neutrophilic infiltrates, and the presence of fat globules that result from the breakdown of tissue.

Differential Diagnosis

Differential diagnosis should include death from other causes with postmortem overgrowth of enteric anaerobic bacteria and autolysis.

Prevention and Control

In other species with clostridial myonecrosis, aggressive treatment is required to prevent rapid progression and death. Wound management including debridement and drainage coupled with antibiotic therapy with penicillin G and clindamycin are used.

Clostridium perfringens—*Gastric Dilation*

Gastric dilation and death secondary to overgrowth of *C. perfringens* type A in a group of common marmosets have been described [31]. Shigellosis had been suspected in a colony of 180 marmosets, and animals in the affected housing unit were started on prophylactic treatment with a combination of parenteral gentamicin and oral furoxone administered in the drinking water. Animals subsequently developed signs of gastric distress and were started on gastrointestinal protectants and an anticholinergic antiemetic. Over the next 5 weeks, 29 of 59 animals were found in a moribund state and died with gastrointestinal dilation. *C. perfringens* type A was

cultured from gastric contents. It was proposed that antibiotic therapy altered the intestinal microbial flora, allowing clostridial overgrowth and promoting gas accumulation and dilation of the gastrointestinal tract [31].

Clostridium piliforme

Etiology

Clostridium piliforme (previously *Bacillus piliformis*) is a spore-forming, obligate intracellular pathogen and the etiologic agent of Tyzzer's disease. The organism appears filamentous in tissue and cannot be grown on cell-free media. It appears to stain Gram negative unless it is grown under hypoxic conditions in which case it will appear Gram positive as do other members of the *Clostridium* genus. *C. piliforme* can infect a number of species and most often causes disease in neonatal or young animals where it has been recognized in guinea pigs, mice, rats, rabbits, hamsters, foals, birds, cats, dogs, and nonhuman primates [32].

Clinical Signs

Tyzzer's disease has been described in common marmosets [33] and cotton-top tamarins [32] where it was recognized in neonatal animals. The disease is rapidly progressive, and neonatal animals are generally found dead with no antemortem clinical signs recognized.

Epizootiology

The organism is shed in the feces where spores may contaminate water, food, or bedding. Following ingestion, the organism may replicate in the lower gastrointestinal tract and seed the liver and systemic circulation through the portal vein. Rodents may represent natural reservoirs of infection. Once the environment becomes contaminated, the spores may survive for years and serve as a source of infection.

Pathology

C. piliforme infection is most often associated with multifocal hepatic necrosis, which may be recognized both grossly and microscopically. These foci are variable in size and contain karyorrhectic cellular debris and scant inflammatory cells. The organism can often be visualized in the cytoplasm of hepatocytes at the leading edge of necrosis as lightly basophilic filamentous bacteria. Silver or Giemsa stains may highlight the bacteria and are useful diagnostic aids. These morphological findings are diagnostic of Tyzzer's disease. In addition to hepatocellular necrosis, transmural enterocolitis of the lower gastrointestinal tract may be evident. This likely represents the site of bacterial colonization and entry but may be multifocal and missed. Typical organisms may be found in smooth muscle cells adjacent to regions of necrosis. The organism may further disseminate to the myocardium where it can cause myocarditis. Although unusual, involvement of the central nervous system was recognized in the common marmoset where necrosis and neutrophilic infiltration were observed [33] (Fig. 16.3).

Diagnosis

The morphologic findings are suitably distinct to be diagnostic. Recently immunohistochemistry using rabbit anti-*C. piliforme* sera has been described and can be used if routine stains are equivocal [34]. PCR has also been used to detect *C. piliforme* sequences in tissues, and serology can be used in surviving animals to demonstrate seroconversion.

Differential Diagnosis

The triad of intestinal, liver, and heart lesions in young animals is highly suggestive of Tyzzer's disease. Other causes of multifocal hepatic necrosis such as adenovirus, herpes virus simplex 1, and toxoplasmosis may be considered and would have distinct morphologic features on microscopic evaluation.

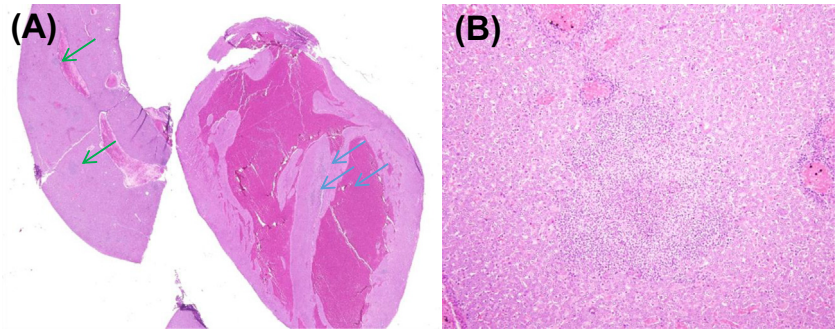

FIGURE 16.3 ***Clostridium piliforme.*** (A) Multifocal necrotizing myocarditis (*blue arrows*) and hepatitis (*green arrows*) (heart, H&E stain, ×4). (B) Focal necrotizing hepatitis (liver, H&E stain, ×100).

Prevention and Control

C. piliforme spores may contaminate the environment and be difficult to eliminate. Ethylene oxide or autoclaving of materials should be effective against the spores and surfaces may be decontaminated with 0.3% hypochlorite. As rodents may serve as a reservoir of infection, additional efforts at rodent control may be warranted.

GRAM-NEGATIVE BACTERIA

Bordetella bronchiseptica

Etiology

Bordetella bronchiseptica is a small, Gram-negative, rod-shaped bacterium and can cause infectious bronchitis in a variety of animal species and rarely humans. *B. bronchiseptica* is closely related to the human pathogen *Bordetella pertussis* to which the marmoset is also susceptible to experimental inoculation.

Clinical Signs

An epizootic of *B. bronchiseptica* has been reported in a colony of common marmosets resulting in pleuropneumonia and deaths [35]. A colony of 156 animals was initially established as specific pathogen free of *B. bronchiseptica*. Despite routine testing and precautions, an epizootic of *B. bronchiseptica* occurred, and the authors were able to demonstrate spread of the organism to 71 individual animals through evaluation of nasal swabs. The most common clinical sign was a purulent nasal discharge, which was observed in animals of all ages. Occasionally these signs were accompanied by an elevated body temperature and dyspnea when the animals were handled; however, affected marmosets never coughed. Sixteen animals died from *B. bronchiseptica* with the vast majority of lethally affected animals being under 1 year of age. In these animals, death was not preceded by premonitory clinical signs and animals were often found dead.

Epizootiology

In the described epizootic, in a specific pathogen-free colony *B. bronchiseptica* spread rapidly, resulting in upper respiratory infection in animals of all ages. While the organism appeared capable of colonizing animals of all ages, severe disease was largely limited to animals under 1 year of age.

Pathology

In animals subjected to necropsy, a purulent bronchopneumonia was observed, most frequently affecting the right cardiac and accessary lung lobes. In 6 of 16 cases the inflammatory process had extended to the pleura resulting in fibrinopurulent pleuritis, and in 2 cases extensive involvement of the pericardium was recognized. Microscopically, bronchi contained plugs of neutrophils admixed with macrophages, fibrin, and sloughed bronchiole epithelial cells. The inflammatory process extended into the surrounding alveolar spaces where edema and destruction of septa were recognized. Blood vessels in affected regions appeared edematous and contained infiltrating neutrophils. Laryngitis and rhinitis were observed in a minority of affected animals (Fig. 16.4).

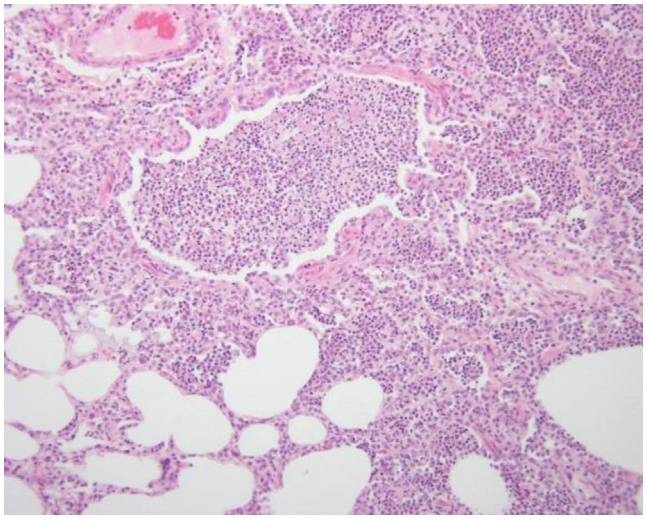

FIGURE 16.4 *Bordetella bronchiseptica*. Suppurative bronchopneumonia (lung, H&E stain, ×200).

Diagnosis

Definitive diagnosis requires bacterial isolation and identification. Antimicrobial profiling should be obtained on all isolates.

Differential Diagnosis

Other viral and bacterial causes of bronchopneumonia such as adenovirus and *Pasteurella* infection may cause similar clinical and/or pathologic findings. In other species, viral coinfection with viral respiratory pathogens such as coronavirus, influenza virus, and adenoviruses may exacerbate *B. bronchiseptica* and should be considered in recognized epizootics.

Prevention and Control

Antimicrobial susceptibility profiles have recently been published for *B. bronchiseptica* isolates from swine and companion animals [36]. *B. bronchiseptica* is generally resistant to macrolide antibiotics and cephalosporins and often carries β-lactamase resistance genes.

Canine isolates have been found to be sensitive to tetracycline, doxycycline, enrofloxacin, and amoxicillin/clavulanic acid [37].

Escherichia coli

Etiology

Six categories of diarrheagenic *Escherichia coli* are defined based on the underlying mechanism of disease pathogenesis, in vivo and in vitro growth characteristics, and the presence of specific genes encoding virulence factors. These include enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli*, enterotoxigenic *E. coli*, enterohemorrhagic *E. coli*, enteroinvasive *E. coli* (EIEC), and diffuse adherent *E. coli* [38]. EPEC is one of the most common bacterial causes of diarrhea in humans worldwide and is also known to infect a variety of other species including cattle, swine, rabbits, and nonhuman primates, including common marmosets and cottontop tamarins.

Studies over the past 30 years have revealed the molecular basis of EPEC pathogenesis and virulence. Typical EPEC carry a gene encoding the bundle-forming pilus (bfp), which is responsible for initial attachment of the bacteria to enterocytes. The organisms possess a type 3 secretion system (T3SS), which allows the direct insertion of more than 25 bacterial proteins into the target cell's cytoplasm. Intimin is an outer membrane protein encoded by the eaeA gene and is required for full virulence of the organism. Intimin binds to the translocated intimin receptor (Tir), which has been introduced into the target cell membrane by the T3SS. Tir–intimin interaction initiates full adherence of the organism to the target cell surface, and subsequent phosphorylation of intracytoplasmic Tir domains causes polymerization of actin. It is this effect on actin that results in effacement of the brush border and the formation of an attachment pedestal, both of which can be recognized ultrastructurally. The eventual induction of diarrhea correlates with these morphological changes in enterocytes and is caused by the destruction of the microvilli and tight junctions [39].

Sequencing of the *bfp*A and eae genes from isolates cultured from neotropical primates revealed, respectively, 98.4% and 99.2% identity to those observed in strains obtained from humans [43]. While zoonotic infection of human handlers has not been reported, the close genetic relationship between human and nonhuman primates suggests that care should be exercised when working with infected animals.

Recently, *E. coli* isolates encoding genes for colibactin and cytotoxic necrotizing factor (CNF), both cytotoxins causing DNA damage and cell cycle arrest, have been isolated from the feces of common marmosets [39a]. These *E. coli* strains have been associated with urogenital tract infections, septicemia, and meningitis in humans [40,41]. Their clinical relevance in marmosets requires further investigation. CNF-positive *E. coli* also have been isolated from macaques [42]. In a recent study, the prevalence of cytotoxins encoded by *pks* and *cnf* in *E. coli* cultured from rectal swabs of marmosets housed in three separate colonies was documented. Isolates of *E. coli* were identified and characterized biochemically. Specific PCR for *pks* and *cnf* gene amplification and phylogenetic group identification were performed on all *E. coli* strains. A total of 139 *E. coli* strains were isolated from 120 of the 138 marmosets (87%) in the three colonies. In Colony A, 31 strains of *E. coli* were isolated from 25 of the 31 animals; 55% of the animals had *pks*+ *E. coli*, and 52% had *cnf*+ *E. coli*. In Colony B, 33 strains of *E. coli* were isolated from 32 of the 33 animals, 6% of the animals had *pks*+ *E. coli*, and 9% had *cnf*+ *E. coli*. In Colony C, 75 strains of *E. coli* were isolated from 63 of the 74 animals, 43% of the animals had *pks*+ *E. coli*, and 35% had *cnf*+ *E. coli*. Both *pks*+ and *cnf*+ *E. coli* strains belonged mainly to phylogenetic group B2. Colibactin and CNF cytotoxic activities were confirmed using a HeLa cell cytotoxicity assay. The presence of *pks*+ or *cnf*+ *E. coli* did not correlate with health status. However, given the association of these cytotoxin-producing *E. coli* strains with clinical disease in humans, the colonization of marmosets with these cytotoxin-producing *E. coli* can potentially cause clinical and subclinical diseases that impact marmoset models [39a].

Clinical Signs

EPEC infection presents acutely in juvenile and adult common marmosets [43]. Animals may initially appear lethargic and anorexic and often appear hunched when observed in nest boxes or perches. As signs progress, animals may become anemic and hypotensive and may be found on the floor of large enclosures unable to return to their nest boxes. Diarrhea is voluminous and may appear watery, containing variable amounts of mucus and fresh blood. Feces may have the consistency of strawberry jam, and the animal's perineum may be stained with this material. Clinical disease may present in healthy robust animals who appeared normal 12–24 h previously. Despite the rapidity of disease progression and the severity of diarrhea and apparent blood loss, animals respond dramatically to antibiotics and fluid therapy. With treatment, overall morbidity is low, and animals return to full health within a few days. Failure to respond suggests an alternative diagnosis should be considered or that significant comorbidities may be present (Fig. 16.5).

FIGURE 16.5 Enteropathogenic *Escherichia coli* infection. Diarrhea and hematochezia in cage papers.

Epizootiology

The organism is transmitted by the fecal–oral route, and enzootic infections are likely common in most marmoset colonies. As described below, unless specialized diagnostic approaches are used, these infections may go unrecognized and undiagnosed. While enzootic infection is common, clinical signs and more severe disease occur sporadically in colonies. Factors that precipitate clinical disease are unknown. A recent survey demonstrated that 10.2% of clinically healthy marmosets were asymptomatic carriers of EPEC [44].

Pathology

Morphologic findings are characteristic of the attaching and effacing lesion. The colonic surface epithelium appears irregular with bacilli intimately associated with the apical cytoplasmic membrane and accompanied by varying degrees of colonic crypt hyperplasia. Surface epithelium variably has a "cobblestone" or "tombstone" appearance or, when accompanied by necrosis, a flattened or squamous morphology. There are often mild neutrophilic infiltrates and congestion of vessels within the mucosa. Individual epithelial cells with adherent bacilli often appear rounded and vacuolated. Distribution of bacilli is limited to the colonic surface epithelium and varies from diffuse to a locally extensive or focal pattern. Less frequently organisms are noted in the ileum and distal jejunum. In each of these instances, the colon was severely involved. In some cases multiple sections of colon needed to be evaluated to visualize the characteristic findings. Ultrastructurally there is effacement of normal microvillus architecture, and adherent bacilli are attached to the apical cytoplasmic membrane with pedestal formation and rearrangement of the underlying cytoskeleton. Experimental oral inoculation of common marmosets with the EPEC R811 strain produced acute hematochezia in high-dose animals and recapitulated microscopic findings observed following natural infection [44] (Fig. 16.6).

Diagnosis

The morphologic findings of the attaching and effacing lesion described above are pathognomonic for EPEC infection, and biopsy of the colonic mucosa may provide a rapid and reliable means of diagnosis. Bacterial isolation and identification may also be useful, but many clinical bacteriology laboratories do not routinely speciate lactose fermenters. Because nonpathogenic *E. coli* are common commensals of the lower gastrointestinal tract of marmosets, their isolation is not sufficient to diagnose EPEC infection. Additional phenotypic or genotypic characterization is required for definitive identification. Adhesion assays may demonstrate a focal adhesion pattern of EPEC organisms on intestinal epithelial cell lines. Alternatively PCR may be used to

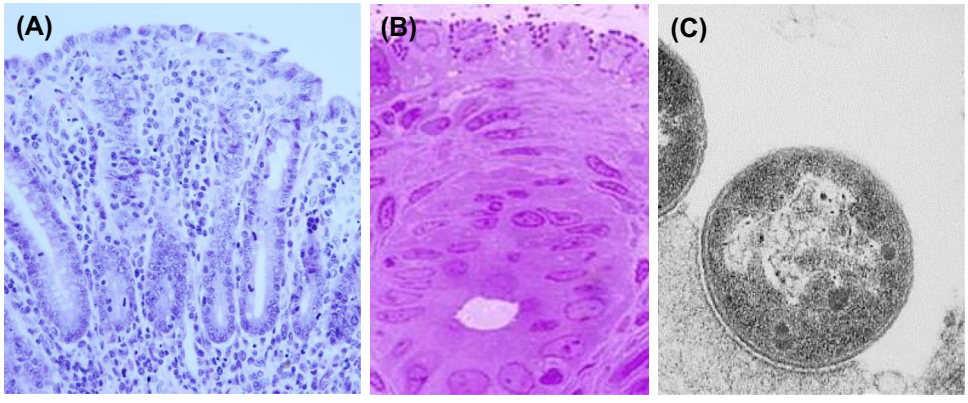

FIGURE 16.6 Morphologic appearance of enteropathogenic *Escherichia coli* infection. (A) Section of colon demonstrating a cobblestone appearance of the surface epithelium, marked hyperplasia within crypts, and minimal inflammatory cell infiltration (colon, H&E stain, 250×). (B) Surface epithelium appears rounded and vacuolated with numerous adherent rod-shaped bacilli (toluidine blue semithin section, colon, 500×). (C) Ultrastructural appearance of EPEC bacillus forming attachment pedestal (electron microscopy, colon).

detect EPEC-specific virulence genes and may be multiplexed to rapidly rule out other common enteric pathogens. Such kits are now commercially available for the diagnosis of human enteric infections but require specialized diagnostic equipment and have not been validated on nonhuman primate isolates.

Differential Diagnosis

EPEC is one of the most common enteric infections of common marmosets but must be distinguished from other enteric pathogens causing acute-onset diarrhea, including most commonly *Campylobacter* spp., *Klebsiella* spp., and *Yersinia* spp. While *Shigella* spp. and *Salmonella* spp. infections may produce similar clinical signs, these are now rare in marmosets raised in a laboratory setting. Adenovirus infection and cryptosporidiosis may be considered as nonbacterial etiologies. Due to the severity of clinical signs, empiric treatment may be required prior to definitive diagnosis, and fluoroquinolones provide adequate coverage against most of the common Gram-negative organisms causing acute enterocolitis in common marmosets.

Prevention and Control

EPEC infection in clinically affected animals responds rapidly to enrofloxacin and supportive therapy such as intravenous fluids and supplemental heat [26]. Diarrhea and clinical signs may resolve within a matter of hours. Previous infection does not prevent subsequent infections or disease months or years later. Whether this is due to the introduction of new strains or absence of an appropriate immune response is not known. The persistence of EPEC in colonies with only episodically recognized disease suggests that an asymptomatic carrier state may be common. Elimination of EPEC from colonies may be difficult due to the ubiquitous nature of *E. coli* commensals and the absence of a rapid diagnostic test.

Research Complications

Sequencing of the intimin gene from both New World primate (NWP) and Old World primate (OWP) isolates indicates a high degree of identity with human isolates, suggesting that primate EPECs may represent a potential zoonotic disease.

Francisella tularensis (Tularemia)

Etiology

Francisella tularensis, a Gram-negative, nonspore-forming, nonmotile, aerobic rod-shaped coccobacillus, is the causative agent of tularemia. Two distinct biovars are recognized: *Francisella tularensis tularensis* (type A), a highly virulent form and found only in North America, and a less virulent *Francisella tularensis holarctica* (type B), which is thought to be endemic throughout the Northern Hemisphere.

F. tularensis can infect a variety of mammals, including wild lagomorphs and rodents, which may serve as important reservoirs. Due to its virulence, ability to spread by the aerosolized route, and highly infectious nature, *F. tularensis* has been designated a select agent by the US government. Colloquially named "rabbit fever" in man, tularemia may take different forms, including ulceroglandular, oculoglandular, typhoidal/oropharyngeal, and pneumonic manifestations. A common marmoset model of pneumonic tularemia has been developed to examine the pathogenesis and antimicrobial therapy [45].

Clinical Signs

Apathy and hyperthermia or hypothermia may initially be observed. However, the disease is rapidly progressive and animals are often found moribund or dead. A survey of 55 contact animals from a German epizootic did not reveal seroreactivity, suggesting that tularemia had been fatal in all animals that had contracted the disease [46].

Epizootiology

Epizootic and sporadic cases of tularemia in common marmosets have been described in Europe with isolation of type B biovar (*F. tularensis holarctica*). In both instances, animals were housed outdoors in either a semifree-living [46] or caged habitat [47]. Increased numbers of ixodid ticks and dead rodents had been observed in the facility prior to the onset of the German epizootic. Rodents were trapped, and PCR identified *F. tularensis* nucleic acid in *Sorex araneus*, *Apodemus flavicollis*, and *Microtus agrestis* rodents [46]. It is postulated that rodent contamination of food and water sources may have served as the initial source of infection. Once within a colony, the organism may be rapidly spread from animal to animal.

Pathology

At necropsy, multifocal small (<1 mm) white foci may be visible in the liver, spleen, lymph nodes, and kidneys. Histologically, these correspond to foci of acute coagulative necrosis surrounded and infiltrated by variable numbers of neutrophils, macrophages, and lymphocytes. Large intralesional bacterial colonies as observed with yersiniosis are absent. In animals that survive longer, the inflammatory infiltrate may be more granulomatous in nature. Lymph nodes appear hyperplastic and infiltrated by large numbers of neutrophils. Foci of overt lymph node necrosis may be evident. In many cases, a hemorrhagic to necrotizing enteritis may be evident and accompanied by necrotizing

lymphadenitis of the submandibular lymph nodes, suggesting an oral route of entry similar to the typhoidal/oropharyngeal form observed in man.

Diagnosis

Several assays proved valuable in the diagnosis of *F. tularensis* in the German epizootic, including bacterial isolation and characterization, antigen detection in the spleen or liver by competitive ELISA, PCR of the *tul4*, *iglC*, and *ISFtu2* genes, and serology [46]. For bacterial isolation 50 μL aliquots of homogenated spleen and liver were plated on cysteine heart agar supplemented with 9% sheep's blood. When plates with antibiotics were used to suppress growth of contaminating bacteria, complete agreement between PCR, competitive ELISA, and culture was observed [46].

Differential Diagnosis

Differential diagnosis should include other rapidly progressive epizootic diseases such as callitrichid hepatitis, *Yersinia enterocolitica*, and *Klebsiella pneumoniae*. In particular, *Y. enterocolitica* can produce similar clinical and gross pathological findings. Histopathologic analysis of tissue samples is helpful in distinguishing these entities and allows a presumptive diagnosis of tularemia.

Prevention and Control

Treatment of affected animals has not been attempted due to the rapidity and severity of the disease course. In human patients, administration of streptomycin, gentamicin, doxycycline, or ciprofloxacin for 10–21 days has been used successfully to control infection and might be considered in nonhuman primates. Levofloxcin 14.5 mg/kg twice daily orally administered 24 h following *F. tularensis* aerosolized challenge protected all common marmosets [48]. Wild animal reservoirs and insect vectors play an important role in epizootics. In the German epizootic, rodent control measures, including the use of clap traps and elimination of rodent habitat, were used to reduce rodent populations. These efforts were coupled with increased hygiene and credited with stemming the epizootic event [46].

Research Complications

Tularemia is a known zoonotic disease that may be transmitted to human handlers by a variety of routes including exposure to open skin wounds and inhalation. One animal handler from the German epizootic was found to be positive by immunoblot and ELISA but had not demonstrated clinical signs [46]. If tularemia is suspected, additional personal protective equipment may be implemented to include N95 and/or powered air-purifying respirators.

Klebsiella pneumoniae

Etiology

K. pneumoniae is a Gram-negative, encapsulated, nonmotile, facultative anaerobic, rod-shaped bacterium, which appears as a mucoid lactose fermenter on MacConkey agar. Lipopolysaccharide and capsular polysaccharide are two of the most important virulence factors of *K. pneumoniae*, promoting invasion and sepsis [49]. A hypermucoviscosity phenotype is increasingly cited as a virulence marker in septic humans and NHPs [50,51]. *Klebsiella* spp. are viewed as opportunistic pathogens and in a hospital setting often infect individuals with concurrent medical issues. Plasmid-mediated antimicrobial resistance is widespread in *K. pneumoniae* isolates making treatment difficult.

Clinical Signs

Clinical signs may be nonspecific such as diarrhea, depression, and anorexia. Animals often lack clinical signs and may be found unexpectedly dead. Cases may occur sporadically over a long period or may cluster as an epizootic. While *Klebsiella* spp. may affect debilitated animals as an opportunistic infection, it may also cause disease in young healthy animals without preexisting conditions.

Epizootiology

K. pneumoniae can be found and isolated from water sources, and these strains possess similar virulence factors such as serum resistance and capsular polysaccharides, pili, and siderophores as found in isolates from clinically affected human patients [49]. The environment may become contaminated with *K. pneumoniae* and serve as a source of infection to colony animals. Fomites and human handlers may spread the infection from the source in the environment to colony animals. *K. pneumoniae* may be nearly ubiquitous in these environments, and host factors that allow marmosets to become colonized and develop disease remain unknown.

Pathology

In animals that die unexpectedly, gross findings at necropsy may be minimal but may be associated with green-yellowish fluid in the gastrointestinal tract. In such animals, large numbers of small Gram-negative encapsulated bacilli may be found disseminated throughout small blood vessels, consistent with fulminant septicemia. The inflammatory reaction to the bacteria may be minimal. In cases in which animals have survived longer, a fibrinopurulent serositis and/or interstitial pneumonia may be evident. The presence of fetid liquid gastrointestinal contents suggests that this may represent the site of bacterial colonization and entry,

when interstitial pneumonitis is present. *K. pneumoniae* can also cause a bronchopneumonia similar to that observed with *B. bronchiseptica*. *Klebsiella* spp. can be associated with acute-onset meningitis in several species of NHPs, including rhesus, owl monkeys, and lemurs [50].

Diagnosis

The microscopic findings of myriads of small encapsulated bacilli in blood vessels with scant inflammatory reaction are characteristic. Bacterial isolation and identification is diagnostic. As antibiotic resistance is common, antimicrobial sensitivity profiles should be requested on all isolates. Isolates obtained from animals at necropsy subjected to antibiotic sensitivity assays aid in the empiric choice of antimicrobial therapy in future cases.

Differential Diagnosis

K. pneumoniae should be distinguished from other Gram-negative bacteria such as *E. coli* and *Pseudomonas* spp., which may produce septicemia. If bronchopneumonia is present, *B. bronchiseptica* should also be considered.

Prevention and Control

Treatment of affected animals may be difficult due to the rapidity of disease progression and extensive antimicrobial resistance in *K. pneumoniae*. In the absence of historical antimicrobial resistance data of resident animals, systemic enrofloxacin may be initiated in suspected cases and provides adequate coverage for other Gram-negative bacteria. Careful assessment of contact animals is warranted, and prophylactic treatment may be considered. While *K. pneumoniae* may be difficult to eliminate from the environment, improved levels of sanitation and hygiene may be useful in reducing exposure. A prophylactic autogenous *Klebsiella* vaccine has reportedly been used in a common marmoset colony [3].

Pseudomonas simiae

Pseudomonas simiae is a novel Gram-negative bacillus isolated from a neonatal white-headed marmoset (*Callithrix geoffroyi*) and its dam [52]. The offspring had a bronchointerstitial pneumonia with Gram-negative rods visualized within alveolar spaces, and the dam had glomerulonephritis with uremic pneumonitis. A bacterial isolate was found to belong to the *Pseudomonas fluorescens* intragenic cluster and to be phylogenetically and biochemically distinct from other species.

Salmonella spp.

Salmonella are Gram-negative motile bacilli with peritrichous flagella and fall into two species: *Salmonella enterica* and *Salmonella bongori*. *S. bongori* is restricted to cold-blooded animals, whereas *S. enterica* may infect a variety of warm-blooded animals. The latter is divided into six subspecies and more than 2500 serotypes. Salmonellosis has been described in a number of neotropical primate species, including the common marmoset [53]. Serovars isolated from common marmosets have included *typhimurium, anatum, seftenberg, newington, oranienburg,* and *habana* [3]. Few clinical details are provided and cases were documented in marmosets recently imported from South America in the 1960s and 1970s. Enteritis induced by EPEC and *Campylobacter* spp. should be considered far more common [53]. Clinical disease is likely exacerbated from comorbidities, stress of capture, and shipment. Histologically, enterocolitis with congestion, hemorrhage, and edema was observed and could be accompanied by focal necrosis in the gastrointestinal tract, spleen, and liver. In human patients with noninvasive salmonellosis, symptoms generally resolve in 5–7 days with supportive therapy [54]. Systemic antibiotics are reserved for individuals with invasive disease (enteric fever). Diagnosis of invasive disease can be problematic and relies on clinical findings as well as blood cultures and isolation of the organism. As the natural history of salmonellosis is poorly described in neotropical primates, criteria for initiation of antimicrobial therapy have not been established. In severe cases, enrofloxacin or amoxicillin may be considered empirically until culture and sensitivity results are available and should be used in conjunction with supportive therapy and isolation of affected animals.

Shigella—Bacillary Dysentery

Etiology

Shigella spp. are Gram-negative, facultative anaerobic, nonspore-forming, nonmotile, rod-shaped bacteria closely related to *E. coli* from which they have evolved. *Shigella* spp. are the etiologic agent of bacillary dysentery, a severe and often hemorrhagic infection of the large intestine that may affect humans as well as a number of Old and New World primates. *Shigella* spp. are classified into four serogroups *Shigella dysenteriae* (type A), *Shigella flexneri* (type B), *Shigella boydii* (type C), and *Shigella sonnei* (type D) and must be differentiated from EIEC with which they share a number of characteristics.

Clinical Signs

Shigellosis has rarely been reported in common marmosets [3,55] but can produce signs of bacillary dysentery, including bloody diarrhea, dehydration, anorexia, and depression, which can rapidly progress to death. Animals may initially appear hunched with dried blood adhered to the perineum.

Epizootiology

Shigella spp. are regarded as highly infectious by the fecal–oral route, with fewer than 100 colony-forming units required to produce infection in humans. In nonhuman primates, epizootics may occur when asymptomatic carrier animals are introduced into a naive colony or when animals are inadvertently exposed to waste from infected humans. Once animals are infected, *Shigella* spp. pose a significant zoonotic risk to human handlers.

Pathology

At necropsy, gross findings are typical of typhlocolitis with multifocal punctate ulcers and hemorrhages observed on the cecal and colonic mucosal surfaces and mesenteric lymph nodes. The large intestine may be dilated and contain fresh blood. Occasionally, microhemorrhages may be visible through the serosal surface. Microscopically, mononuclear and neutrophilic infiltrates can be observed in the colon and cecum and accompanied by edema.

Diagnosis

Bacterial culture and isolation can provide definitive diagnosis, but care is required to ensure that samples are collected and handled properly as *Shigella* spp. do not survive well outside of the host. Cary-Blair or Amies and Stuart's transport media is recommended, and cultures should be initiated as soon as possible. Multiplex PCR assays have also been developed and clinically validated to detect common human enteric pathogens in stool, including *Shigella* spp. isogroups. These offer the advantage of detecting other pathogens; however, these assays do not allow assessment of antimicrobial sensitivities achievable with *Shigella* isolates.

Differential Diagnosis

Shigellosis has rarely been recognized in neotropical primates, suggesting there may be some inherent resistance compared with their Old World counterparts who are highly susceptible. EPEC infection is far more common in *Callithrix jacchus* and produces a similar clinical syndrome of hemorrhagic diarrhea. Microscopically EPEC infection is characterized by an attaching and effacing lesion, whereas shigellosis demonstrates erosion and extensive neutrophilic infiltrates. Bacterial culture and isolation and molecular assays will also differentiate EPEC from *Shigella* spp. Initial treatment and control measures are similar for both pathogenic species.

Prevention and Control

In clinically affected animals, treatment should be directed at correcting dehydration and electrolyte imbalances along with initiation of antimicrobial therapy. Both enrofloxacin and trimethoprim–sulfamethoxazole are empiric first-line choices. Antimicrobial resistance has been increasing in recent years, and microbial antibiotic sensitivity should be assessed in any isolates obtained. If an epizootic is recognized, affected and exposed animals should be quarantined from the remainder of the colony. The use of quarantine-dedicated staff and equipment as well as disinfectant foot baths should be considered. Prophylactic treatment of nonaffected contact animals has been of benefit in controlling epizootics in Old World primates.

Strict separation of marmosets from other primates known to harbor *Shigella* and from infectious human waste should prevent exposure and infection. Given the rarity of shigellosis in marmosets, it is unlikely that newly introduced animals pose a significant risk for this infection. However, routine quarantine and health assessment of all new arrivals is warranted and particular attention directed to minimize personnel exposure to *Shigella*, given the documented zoonotic risk of *Shigella* infection in NHPs [56].

Yersinia enterocolitica

Etiology

Yersinia are Gram-negative facultative anaerobes that have peritrichous flagella. Two species have been associated with disease in nonhuman primates (*Yersinia pseudotuberculosis* and *Y. enterocolitica*). In common marmosets, disease (yersiniosis) has been associated with *Y. enterocolitica* [57,58]; however, in other callitrichids *Y. pseudotuberculosis* has produced similar clinical and pathological findings [57].

Clinical Signs

Animals may be found dead without displaying clinical signs. In others cases, animals may develop diarrhea with or without hematochezia and constitutional signs such as anorexia and lethargy.

Epizootiology

Yersinia species are a diverse group of Gram-negative organisms and can be found widely in soils and decaying vegetation. Outbreaks in nonhuman primates often follow a seasonal pattern and increase in the wetter months. Rodents may become infected with *Yersinia* spp. and asymptomatically shed the organism in feces. These animals may represent an important reservoir and initiate epizootics when contaminating food sources or when hunted and eaten by primates.

Pathology

Yersinia infections in nonhuman primates generally present with the gross pathological triad of (1) hepatic or splenic necrosis or abscess formation; (2) mesenteric lymphadenopathy; and (3) ulcerative enterocolitis. It is believed that the gastrointestinal tract represents the point of entry, with subsequent septicemia and seeding of the liver, lymph nodes, and spleen. Grossly micropyogranulomas are recognized as multiple small (1–3 mm) white foci within the liver and spleen and histologically are characterized by necrosuppurative hepatitis, splenitis, and lymphadenitis. An ulcerative enterocolitis, usually visible grossly and microscopically, is characterized by submucosal edema, marked hyperemia, and the presence of discrete masses of bacteria within the mucosa and submucosa. Occasionally, *Yersinia* may disseminate and be associated with pneumonitis, meningitis, and pleuritis. The presence of large colonies of Gram-negative organisms within regions of necrosis is highly suggestive of the diagnosis (Fig. 16.7).

Diagnosis

A presumptive diagnosis may be made based on the characteristic gross and microscopic findings. Definitive diagnosis requires bacterial culture and isolation and allows serotyping of isolates to assist in epizoological investigations.

Differential Diagnosis

Other bacterial gastrointestinal infections such as EPEC and *K. pneumoniae* may produce similar clinical findings. In particular, *F. tularensis*, the cause of tularemia, may produce similar clinical and pathological findings. However, the gross and microscopic findings of yersiniosis should enable a rapid presumptive diagnosis.

Prevention and Control

In clinically affected animals, supportive therapy such as supplemental heat, intravenous fluids, and vitamins may be helpful. Intramuscular treatment of affected and contact animals with enrofloxacin 5 mg/kg once a day for 7–10 days appeared to have some benefit in mildly affected animals [57]. However, in severe cases, animals failed to respond to antimicrobial and supportive therapy.

Control measures should be directed at preventing transmission between exposed and nonexposed animals and reducing potential wild animal reservoirs [58]. Affected and contact animals should be segregated from the remaining colony if possible. Dedicated equipment, personal protective equipment, and disinfectant foot baths should be used.

A *Y. pseudotuberculosis* vaccine (Pseudovac) has been developed and used in common marmosets and tamarins to control seasonal epizootics of yersiniosis observed at the Biomedical Research Center in Rijswijk, the Netherlands [57]. This vaccine contains different serotypes of formalin-killed *Y. pseudotuberculosis* and was developed at the Department of Veterinary Pathology, section Zoo and Exotic Animals, Utrecht University. A dose of 0.25 mL was administered subcutaneously to all colony animals over 7 weeks of age and boosted at 3 weeks. The vaccine has been repeated annually thereafter in the fall months, and no adverse effects have been observed. While a formal efficacy study was

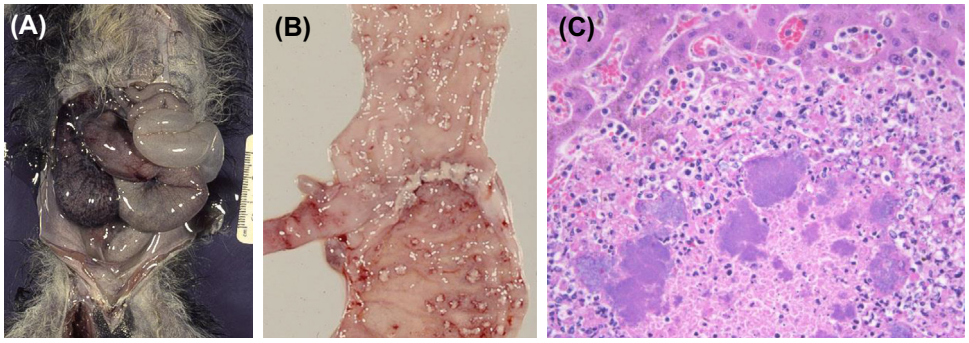

FIGURE 16.7 Morphologic appearance of *Yersinia enterocolitica* infection. (A) Dilatation of the large intestine and cecum. (B) Multifocal necrotizing typhlocolitis. (C) Necrotizing hepatitis (liver, H&E stain, 200×).

not conducted, the investigators conclude that the vaccine was essential in their *Yersinia* spp. control program as no further periods of high mortality were observed [57].

CURVED, SPIRAL, AND FUSIFORM-SHAPED GRAM-NEGATIVE RODS

Campylobacter spp.

Campylobacter is a genus of Gram-negative, curve-shaped bacteria and a frequent cause of foodborne disease in humans. *Campylobacter jejuni* and *Campylobacter coli* are the two most common species and can inhabit the gastrointestinal tract of a variety of warm-blooded animals. Campylobacters can be isolated from rectal swabs of the majority of normal macaques where they are most often not associated with disease. In callitrichids, *Campylobacter* spp. are not considered part of the normal flora and when present are usually associated with disease [26,59]. Histologically infection is associated with neutrophilic colitis with the formation of crypt abscesses. In juvenile cotton-top tamarins, *C. coli* was more frequently associated with diarrhea in juvenile nursery animals, whereas *C. jejuni* was more commonly observed in clinically affected adult animals [59]. Both are susceptible to macrolide and fluoroquinolone antimicrobials, which can effectively eliminate the organisms from infected individuals. *Campylobacter* spp. isolation from rectal swabs of callitrichids should be treated as potential pathogens and steps should be taken to eradicate the organism from the colony. Screening of all colony animals by fecal culture followed by treatment of affected individuals may eliminate infection.

Helicobacter spp.

Etiology

A novel *Helicobacter* species, "*Helicobacter callitrichis*," was isolated from the feces of clinically normal, common marmosets [60]. In addition, an unclassified *Helicobacter* sp. was identified in the stomachs of three species of marmosets, based on immunohistochemistry of paraffin-imbedded tissues using a polyclonal *Helicobacter pylori* antibody [61]. A novel *Helicobacter* species, *Helicobacter jaachi* sp. nov. [62], shared many biochemical properties with those of "*H. callitrichis*," with the exception that *H. jaachi* was urease-positive and alkaline phosphate hydrolysis (PO_4)-negative, whereas "*H. callitrichis*" is urease-negative and PO_4-positive. By 16S rRNA analysis, these two *Helicobacter* spp. are 4% different, which warrants separate species designations [61].

Clinical Signs

In addition to the inflammatory lesions found at necropsy, the colony from which *H. jaachi* was isolated was noted to have clinical gastrointestinal disease as evidenced by sporadic cases of diarrhea and chronic weight loss [63]. The finding of IBD, sometimes in conjunction with colon adenocarcinoma or hepatobiliary inflammatory changes, is reminiscent of findings reported in other nonhuman primates (NHPs) infected with *Helicobacter* spp [64–68]. A novel *Helicobacter* sp., *Helicobacter saguini*, was identified in cotton-top tamarins diagnosed with progressive colitis mimicking features of ulcerative colitis in humans [63,69]. The diarrheic marmosets reflected only the clinical state of the animal at the time of sampling. The importance of *H. jaachi* in eliciting clinical disease needs further study.

Epizootiology

Enterohepatic helicobacters have evolved to colonize and inhabit the mucus of the intestinal crypts of a variety of animals, including humans [70]. *Helicobacter* species that colonize the lower bowel and biliary tract of nonhuman primates have been routinely isolated from cases with chronic inflammation of the lower bowel [60,64–69]. Purpose-bred common marmosets from domestic sources have been reported to have a high incidence of spontaneous IBD and sporadic cholecystitis and cholangiohepatitis. Inflammatory infiltrates increase in incidence and severity with age. Thirty-nine marmosets screened for *Helicobacter* spp. by culture and PCR were frequently detected to be colonized; 48.7% were positive by *Helicobacter* genus-specific PCR, whereas 28.2% of the marmosets were positive for a novel species, *H. jaachi* sp. nov., by culture [62]. *Helicobacter* spp. were also isolated from two liver samples and one gallbladder sample. *Helicobacter* spp. were detected in 36% of the gallbladder samples by PCR, which may be related to the finding that about 10% of the marmosets had cholecystitis or biliary inflammation. Animals aged 6 years and older had higher *Helicobacter*-positive prevalence rates than animals younger than 5 years (39% by culture and 57% by PCR vs. 0% by culture and 27% by PCR). *Helicobacter* species were isolated from animals ingesting a high-fat diet, from primates treated with chemical compounds, and from animals without diarrhea [62].

Pathology

Numerous lesions were noted in this population of marmosets screened for *Helicobacter* spp., and these findings recapitulated the spectrum of survey pathology previously reported [62]. These changes included glomerular and interstitial nephritis, amyloidosis, increased extramedullary hematopoiesis, and metabolic

bone disease. In addition, in the recent study, lesions of the hepatobiliary and gastrointestinal systems were recognized and characterized by prominent inflammatory changes in a relatively high percentage of animals [62]. In the liver, the inflammatory lesions consisted of lymphoid infiltrates in hepatic portal regions as well as mild-to-moderate infiltrates in the mucosa and submucosal tissues of large bile ducts and gallbladder. Occasional animals had mild-to-moderate cholangiohepatitis, and several marmosets had cholecystitis with transmural lymphocytic infiltration of the gallbladder. Lymphoid infiltrates increased in incidence and severity with age [62]. Enteric pathology consisted of a continuous spectrum of IBD changes ranging from relatively minimal focal to segmental lymphoid infiltrates in the lamina propria to severe lymphoid cell accumulations with effacement of the normal bowel architecture in both large and small intestines. Severe accumulations of chronic inflammatory cells found in the lamina propria were associated with changes in villous and crypt architecture, with intraepithelial inflammatory cell accumulations. It is not known what role, if any, *H. jaachi* plays in the development of these lesions [62] (Fig. 16.8–16.10).

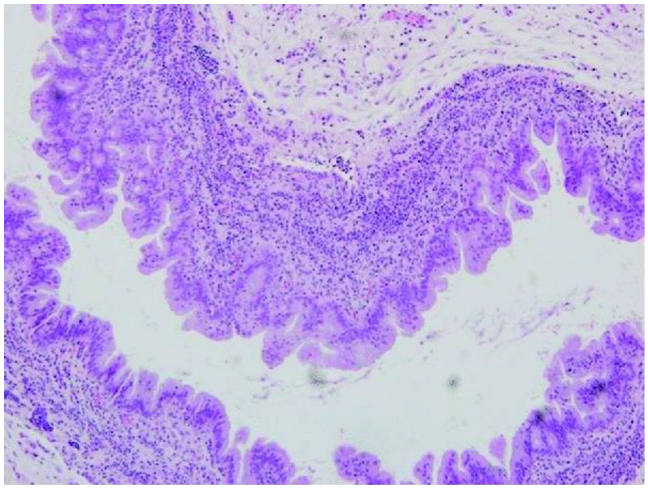

FIGURE 16.9 Chronic cholecystitis characterized by diffuse submucosal infiltrate of lymphoid cells in the wall of the gallbladder (gallbladder, H&E stain, × 200). *Reprinted with permission from Shen Z, Feng Y, Sheh A, Everitt J, Bertram F, Paster BJ, Fox JG. Isolation and characterization of a novel Helicobacter species,* Helicobacter jaachi *sp. nov., from common marmosets* (Callithrix jaachus*). J Med Microbiol 2015;64: 1063–73.*

Diagnosis

H. jaachi sp. nov. are fastidious, microaerophilic, motile bacteria, which are catalase-, urease-, and oxidase-positive and have fusiform morphology, with periplasmic fibers and multiple bipolar, sheathed flagella. This novel *Helicobacter* species is closely related by 16S rRNA analysis to "*H. saguini*" (97%), a species isolated from cotton-top tamarins. Its 16S rRNA is 96% similar to "*H. callitrichis*," isolated previously from the feces of common marmosets. The bacterium is Gram-negative and nonsporulating. The organism grows slowly and

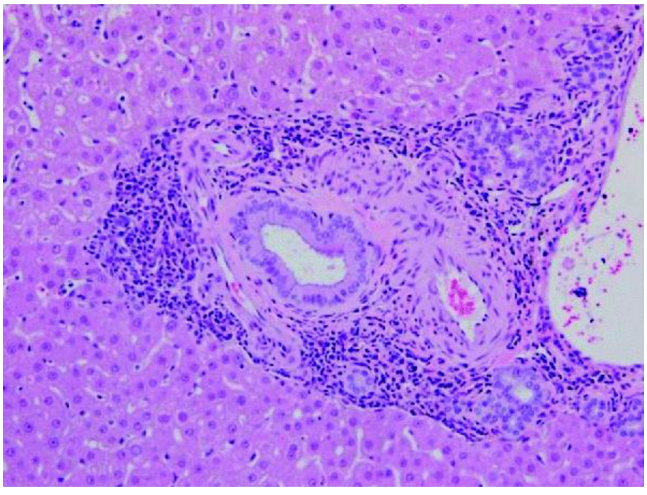

FIGURE 16.8 Lymphohistiocytic inflammatory infiltrate in periportal region of liver from a marmoset with chronic cholangiohepatitis. Inflammatory cells circumscribe the portal triad including the bile duct (liver, H&E stain, × 400). *Reprinted with permission from Shen Z, Feng Y, Sheh A, Everitt J, Bertram F, Paster BJ, Fox JG. Isolation and characterization of a novel Helicobacter species,* Helicobacter jaachi *sp. nov., from common marmosets* (Callithrix jaachus*). J Med Microbiol 2015;64: 1063–73.*

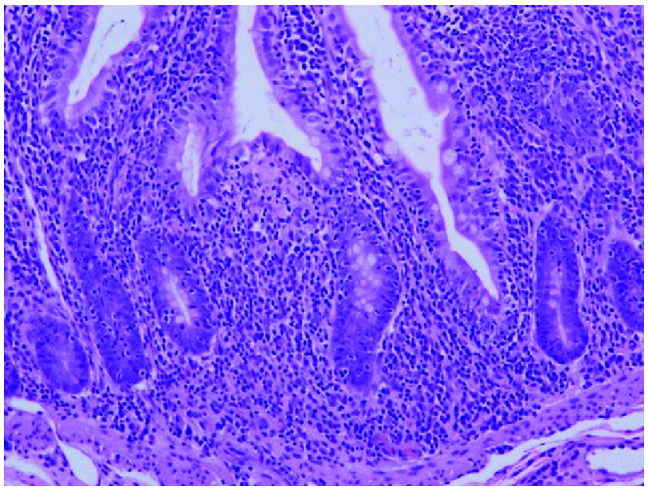

FIGURE 16.10 Section of jejunum from a marmoset with inflammatory bowel disease characterized by loss of crypts and effacement of normal villous structure by severe diffuse lymphohistiocytic infiltrate in the lamina propria (jejunum, H&E stain, × 200). *Reprinted with permission from Shen Z, Feng Y, Sheh A, Everitt J, Bertram F, Paster BJ, Fox JG. Isolation and characterization of a novel Helicobacter species,* Helicobacter jaachi *sp. nov., from common marmosets* (Callithrix jaachus*). J Med Microbiol 2015;64:1063–73.*

appears on solid agar as a spreading film on the surface. The bacterium grows at 37–42°C, but not at 25°C, under microaerobic conditions, but not aerobically or anaerobically.

Differential Diagnosis

Helicobacter spp. must be distinguished from *Campylobacter* spp., which are also microaerophilic and also colonize the gastrointestinal tract of marmosets.

Prevention and Control

No data exist regarding prevention and control of *Helicobacter* spp. in marmosets. However, triple antibiotic treatment (clarithromycin, amoxicillin, and metronidazole) has successfully eradicated *Helicobacter hepaticus* from endemically infected mouse colonies [71].

Research Complications

Several enteropathogenic *Helicobacter* species (EHS) have zoonotic potential. For example, other EHS isolated from animals and birds, such as *Helicobacter bilis* and *Helicobacter pullorum*, have been identified in patients with cholecystitis and biliary neoplasia [72–74]. Recent studies in humans highlight the ability of enteric helicobacters to translocate across the intestinal epithelia in humans with bacteremia, osteomyelitis, and myositis [75–77]; translocation to the liver is also noted with *H. hepaticus* during the development of hepatitis and hepatocellular carcinoma in A/JCr and B6C3F1 mice [78–80]. The fact that these organisms are routinely cultured from the intestine and biliary tract infers that they may cause inflammation in these tissues and complicate interpretations of studies using marmosets.

Leptospira spp.—Leptospirosis

Etiology

Leptospirosis is a bacterial infection caused by members of the *Leptospira* genus. Over 200 serotypes of *Leptospira* are recognized and may be saprophytic or pathogenic. Morphologically they are thin spiral bacteria 6–20 μm in length and survive in moist or wet environments. Common marmosets are susceptible to experimental infection with pathogenic *Leptospira* serovars, and leptospirosis has been described in Wied's marmosets (*Callithrix kuhlii*). Clinical disease has also been reported in other neotropical primates, including squirrel monkeys (*Saimiri sciureus*) and white-lipped tamarins (*Saguinus labiatus*) [81,82]. Serological surveys of neotropical primates suggest that nonclinical infection with the *icterohemorrhagiae* serogroup may be common in these species, including common marmosets [83].

Clinical Signs

Leptospirosis of two Wied's marmosets has been described in a zoological collection [84]. Both adult animals presented with weight loss and jaundice and succumbed to disease despite antimicrobial and supportive therapy. Complete blood counts on presentation of the first case revealed neutrophilic leukocytosis with a left shift and a regenerative anemia. Serum chemistries demonstrated markedly elevated total and direct bilirubin consistent with intravascular hemolysis. Clinical coagulopathy, a common finding in Weil's disease (icteric leptospirosis) of humans and reported in previous nonhuman primate cases, was not observed. The animal died 17 days into the course of treatment, and necropsy revealed diffuse severe proximal tubular nephrosis in which spirochetes could be demonstrated by Steiner's silver stain. The second case, presented on the same day and while initially recovering with antimicrobial treatment, presented 3 months later with progressive weight loss and jaundice. Histological examination of tissues obtained at necropsy revealed lymphoplasmacytic infiltrates in a number of organs, including the gastrointestinal tract and kidney. Seroconversion to *Leptospira borgpetersenii* serovar *Ballum* was demonstrated in the second case.

Common marmosets are also susceptible to experimental infection with *Leptospira interrogans* serovar *Copenhageni* and develop a severe pulmonary form of leptospirosis (SPFL) [85]. SPFL is recognized in human patients as a rapidly progressive and often fatal form of infection characterized by extensive pulmonary hemorrhage. Marmosets were inoculated by the intraperitoneal route with a clinical isolate obtained from a human patient with SPFL. Animals developed weight loss, dehydration, and jaundice within 6–12 days postinoculation. Serum chemistry results indicated hyperbilirubinemia, increased aminotransferase, creatinine, and blood urea nitrogen. Pathology revealed hemorrhage within the pulmonary parenchyma, and pathological changes were noted in the liver, kidneys, and skeletal muscle. The study was conducted as a time course experiment, so it was not possible to determine if severely affected animals would have recovered.

Epizootiology

Rodents, dogs, swine, and cattle may serve as reservoirs of *Leptospira* spp. and may excrete spirochetes in the urine and contaminate water, food, or bedding. The organisms may survive in stagnant water for several weeks but are susceptible to desiccation. Transmission may then occur through direct oronasal contact, ingestion of infected tissue, or bite wounds. Once a new host is infected, the organism may begin to replicate in the kidneys where it can be shed in large numbers in

the urine. Specific serovars may be carried by particular host species and their identification can help in understanding the source of infection in epizootics. The cases in Wied's marmosets were associated with *L. borgpetersenii* serovar *Ballum*, a serovar commonly found in mice [84,86].

Pathology

Necrosis of the proximal convoluted tubules accompanied by variable lymphoplasmacytic infiltrates have been observed with leptospirosis in marmosets. Silver stains, such as Steiner's stain, can be used to visualize the elongated tightly coiled spirochetes within the affected tubules. Additional lesions were observed in the lung, liver, and skeletal muscle of common marmosets with experimentally induced SPFL. In these animals, the alveolar spaces in the lung were filled with erythrocytes and proteinaceous fluid in a multifocal to coalescing pattern. The alveolar septa were thickened by infiltrating macrophages, neutrophils, and lymphocytes. Multifocal myofiber necrosis with interstitial edema and hemorrhage was observed in the skeletal muscle. In the liver, multifocal hepatic necrosis with occasional Councilman bodies was evident. These changes were accompanied by mild interstitial edema, Kupffer cell activation, and foci of parenchymal hemorrhage. Hepatocellular dissociation which may be seen in other species with icteric leptospirosis was not a predominant finding.

Diagnosis

The combination of jaundice, hemolytic anemia, and renal disease is highly suggestive of leptospirosis. Antemortem diagnosis may be achieved by demonstrating seroconversion or detecting the organism in biological samples by bacterial culture/isolation or PCR [87]. Earlier, during the course of infection (<10 days), spirochetes may be visualized in blood by dark-field microscopy and cultured from blood on semisolid media such as Fletcher's Ellinghausen–McCullough–Johnson–Harris media. In later phases of infection, bacteremia may not be evident, but dark-field examination of urine and urine culture may detect the organism. Isolation and serotyping are useful in defining the epizootiology of infection and implementing control programs.

Differential Diagnosis

Jaundice and weight loss appear to be common clinical findings in marmosets naturally infected with *Leptospira* spp. and following experimental inoculation. Callitrichid hepatitis caused by lymphochoriomeningitis virus can also result in these clinical signs and should be considered. Small-intestinal adenocarcinoma can be associated with weight loss and may obstruct the bile duct resulting in jaundice [88]. In more chronic cases, leptospirosis may be associated with lymphoplasmacytic infiltrates and nephrosis. Other causes of renal failure and nephritis such as glomerulonephritis and pyelonephritis may produce similar clinical findings. Silver stains can be used to detect spirochetes in formalin-fixed, paraffin-embedded sections.

Prevention and Control

Identification of serovars responsible for initiating epizootics can provide important information regarding the source of infection. Wild rodents are an important reservoir of leptospirosis and have been linked to infection in a variety of species including marmosets. Rodent control programs should be intensified if this source is suspected. Vaccines can be highly effective in preventing leptospirosis but have not been utilized in nonhuman primates. Vaccination may be considered if repeated epizootics are observed or if the disease becomes enzootic. As immunity is primarily serovar specific, data concerning outbreak-associated isolates would be required to design an effective vaccine program.

Leptospirosis is sensitive to intravenous penicillin which may be attempted in severe acute cases in conjunction with supportive therapy such as intravenous fluids. Penicillins are less effective at eliminating chronic renal infection, and generally doxycycline is administered to animals in the later stage of disease to eliminate this phase of infection.

Research Complications

Leptospirosis is a zoonotic disease and may pose a risk to human contacts. Adequate personal protective equipment to prevent contact with skin and mucous membranes should be provided [86].

ATYPICAL BACTERIA

Ehrlichia canis

Ehrlichia, small Gram-negative coccoid organisms that are obligate intracellular pathogens, are the etiologic agent of ehrlichiosis. *Ehrlichia canis* has recently been detected in free-living primates of the genus callithrix by PCR [89]. Phylogenetic analysis revealed the organism to be closely related to those previously observed in domestic dogs and wild cats. *Ehrlichia* infection in the marmoset has not been associated with clinical disease. In other species, *Ehrlichia* spp. commonly causes fever, lethargy, lymphadenopathy, and splenomegaly. Thrombocytopenia and leukopenias may be present, and morulae may be visible in white bloods during acute infection.

Mycoplasmas and Ureaplasmas

Mycoplasmas are small bacteria that lack a cell wall and are the smallest free-living organism. The lack of a cell wall imparts resistance to antimicrobials such as the beta-lactams, which target the cell wall for activity. *Mycoplasma salivarium* can be isolated from marmosets as part of the normal bacterial flora of the throat and nasal pharynx; its presence, however, has not been associated with disease. Callitrichids have been used as animal models of *Mycoplasma genitalium* (*C. jacchus*) and *Mycoplasma pneumoniae* (*S. labiatus*) infection [90,91]. Marmosets inoculated with a *M. genitalium* isolate, originally recovered from a human patient with nongonococcal urethritis, developed prolonged infections with evidence of salpingitis and antibody-specific responses [90].

Ureaplasmas are commonly isolated from the common marmoset upper respiratory tract and rarely from the genital tract but have not been associated with disease [1,92]. Treatment with minocycline in the drinking water eliminated ureaplasmas, and animals were resistant to reinfection unless in close contact with infected conspecifics. It is believed that marmosets initially acquire infection within 24 h of birth.

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