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CHAPTER 6

Gastrointestinal Tract

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Gastrointestinal disorders account for approximately half of all equine medical problems.[1] Cytology can be an invaluable quick, inexpensive aid in the diagnosis of these disorders. Sample procurement has previously limited gastrointestinal cytology to the evaluation of thoracic and abdominal fluid (Chapters 8 and 9), necropsy specimens, and fecal material. Endoscopy, laparoscopy, and ultrasonography have made visualization and biopsying of gastrointestinal lesions physically and economically possible.[2-6] Therefore, antemortem sampling of the gastrointestinal tract has become not only feasible but also an integral part of complete diagnostic workups.

Definitive cytologic diagnoses can often be made for mass lesions that are neoplastic or inflammatory/infectious. Cytologic patterns such as hemorrhage, chronic inflammation, or necrosis, although not specific diagnoses, may provide helpful prognostic information or presumptive diagnoses. A negative cytologic examination, however, cannot rule out a neoplastic or inflammatory disease. Sample size, quality of specimen, effect of therapy on tissue reactions, representativeness of sample, propensity of biopsied cells to exfoliate, and cytologic differentiation of reactive cells versus neoplastic disorders are concerns with cytologic diagnoses. Cytologic interpretations must always be correlated to clinical, endoscopic, and ultrasonographic findings.

Sampling

Investigation of gastrointestinal disorders usually begins with rectal palpation after collection of a minimum database. If an abnormality such as abnormal masses, enlarged lymph nodes, thickened loops of bowel, or excessive abdominal fluid is found, ancillary diagnostic tools such as endoscopy, ultrasonography, radiography, or abdominocentesis can more specifically define the disorder. Endoscopic biopsies are usually small (1.8 to 2.3 mm) but sufficient for diagnostic purposes. Collection of excellent quality biopsies is challenging, especially with endoscopy and laparoscopy. Experience and teamwork are essential for the collection of good endoscopic or laparoscopic biopsies. Biopsy instrument slippage, incorrect angle placement, hemorrhage, risks of full-thickness biopsying, and iatrogenic perforation of the diseased gut with the endoscope are serious problems to manage. Bacterial contamination is also a real problem with endoscopic samples. Culture results must be compared to cytologic findings to reduce the risk of misinterpretation.

Endoscopy, laparoscopy, and ultrasonography have become the new standards to visualize and biopsy the equine gastrointestinal tract. Gastrointestinal biopsy, brushings, and lavage samples can be collected by endoscopy. Additionally, tissue biopsies can be obtained by laparoscopy, exploratory surgery, or percutaneous ultrasound guidance.

There are authoritative texts describing endoscopic and ultrasonographic techniques.[2-7] Briefly, with endoscopes that are 3 meters in length, the esophagus, stomach, and proximal duodenum of most adult horses can be examined. Restraint may be minimal (twitch or light sedation), depending on the horse. Fasting is not required for esophagoscopy if anesthesia is not used.

Esophagoscopy is commonly performed by nasopharyngeal placement. Advance the scope smoothly and observe the characteristic whitish esophageal mucosa of a collapsed esophagus. If esophageal placement is uncertain or tracheal rings observed, withdraw the scope and make another attempt. Never force the endoscope without knowing its course. Pharyngo-oral retroflexion is a serious risk to equipment, and esophageal perforation is a serious risk to the horse. Examine the stomach along with the esophagus because gastric lesions frequently accompany esophageal lesions. For adequate visualization of the stomach in the adult horse, withhold food for at least 12 hours and water for 6 hours if the horse can tolerate water deprivation.[6] To examine the duodenum, pass the endoscope along the greater curvature of the stomach and through the pylorus. This takes patience and experience. For colonic and rectal endoscopy, remove the feces either manually or, in small horses, using an enema. Pass a well-lubricated endoscope into the rectum. Air insufflation distends the esophagus or gastrointestinal tract for better mucosal visualization, especially for ulcers. Transrectal (5 MHz) and transabdominal (2.5 to 3.5 MHz) ultrasonography can be used to visualize and take a transcutaneous aspiration or punch biopsy of a mass.[8]

Once collected, biopsy specimens can be gently rolled on glass slides or imprinted for cytology before placing in 10% buffered formalin for histopathologic examination. Additionally, firm or particulate material can be scraped or squashed and aspirated material can be smeared like a blood smear. Suspected infectious lesions should be cultured and cytology can be valuable in selecting the appropriate culture media.

Collection of gastric fluid is relatively easily accomplished by passing the appropriate-sized polyethylene tubing through the biopsy channel of an endoscope and aspirating fluid from the area of the lesion or flushing the lesion with physiologic saline and then reaspirating the fluid. Direct, concentrated direct, and cytospin smears are recommended on all fluid specimens in addition to squash preparation of any particulate material. Rectal scraping is performed with a blunt instrument such as a chemical spatula after feces are removed from the rectum. Gentle but firm pressure is used to scrape the rectal mucosa deep enough to sample the lamina propria but not perforate the rectum.

Cytologic Features of Normal Tissues

Esophagus and Stomach

The esophagus and nonglandular anterior region of the stomach are lined by stratified squamous epithelium (Fig. 6-1). A keratinized thick outer stratum corneum overlies the deeper stratum granularis (flattened cells with shrunken nuclei and large basophilic staining cytoplasmic keratohyaline granules), stratum spinosum (flattened cuboid cells), and stratum basale (columnar cells).[1] Superficial epithelial cells are large cells with abundant pale blue homogeneous cytoplasm, angular cytoplasmic borders, and sometimes small, dense, round to oval nuclei. Bacteria can adhere to the surfaces of these epithelial cells, but inflammatory cells are lacking. Deeper samples may contain some basal epithelial cells, which are round with darker blue, slightly granular cytoplasm, larger ovoid, nuclei, and a higher nucleus to cytoplasm (N:C) ratio.

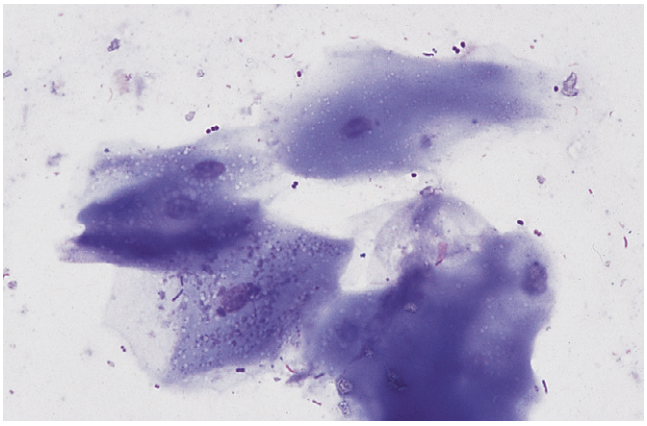

Fig. 6-1 Squamous epithelial cells scraped from stomach of horse.
Bacterial flora are seen on surfaces of some cells. (Wright-Giemsa stain)

Grossly, the margo plicatus forms a distinct boundary between the white-pink squamous region and the reddish glandular region of the equine stomach. The equine glandular stomach is lined by tall, periodic acid–Schiff–positive columnar epithelial cells that overlie deeper chief cells, parietal cells, mucous neck cells, and rare enteric endocrine cells.[1] Cytologic preparations from the glandular region of the stomach contain clusters and sheets of a uniform population of columnar epithelial cells. On low magnification the cells may have a honeycomb appearance (Fig. 6-2). Individualized cells exhibit a columnar shape with basal, round to oval nuclei, stippled chromatin pattern, and pale blue granular cytoplasm. Surface microvilli may give the apical margin a feathery appearance (Fig. 6-3).

Gastric fluid pH in the horse is variable. The horse appears to be a continuous, variable gastric hydrochloric acid secretor with intermittent periods of spontaneous alkalization.[9] Duodenogastric reflux, which is common in the horse, can contribute to this alkalization. Gastric fluid pH values are typically less than 2.0 during feed deprivation but can be greater than 6.0 after free access to timothy grass hay.[9] Cytologic examination

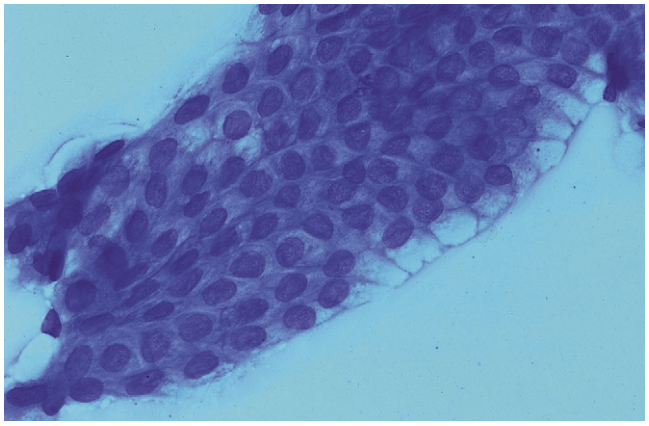

Fig. 6-2 Sheet of columnar epithelial cells from pyloric portion of stomach.
The uniform size and shape of the cells give this cluster a honeycomb appearance. (Wright's stain)

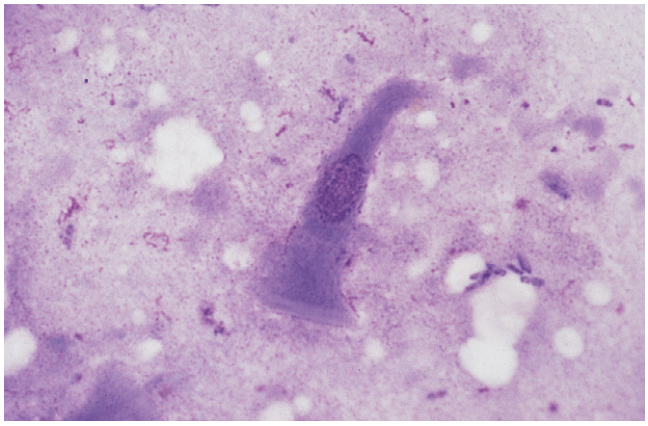

Fig. 6-3 Columnar epithelial cell imprinted from glandular region of stomach.
Cell has oval, basal nucleus and pale-staining microvillus apical border. (Wright-Giemsa stain)

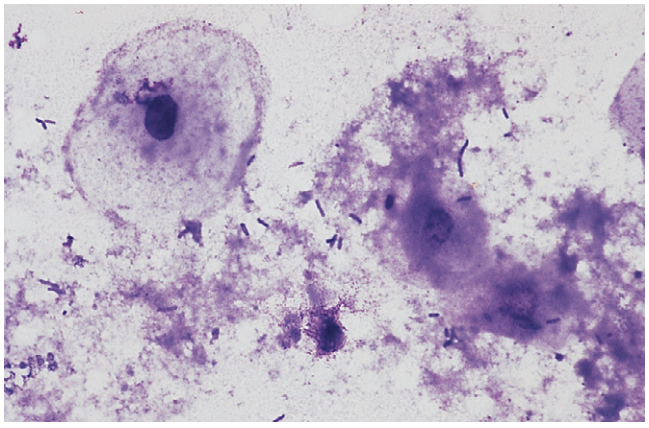

Fig. 6-4
Smear of gastric fluid from horse containing sloughed squamous epithelial cells, mixed population of bacteria, debris. (Wright-Giemsa stain)

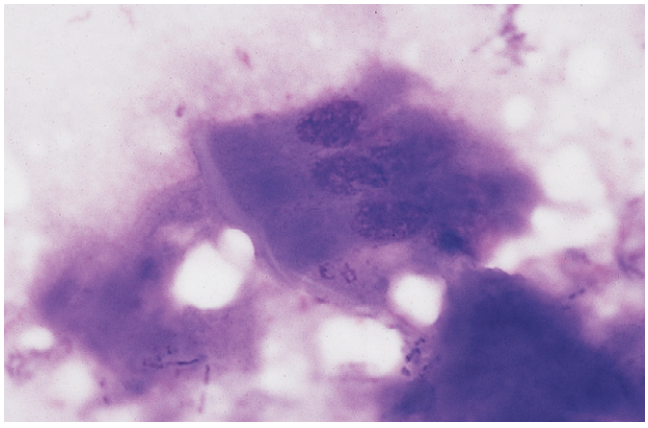

Fig. 6-5
Row of duodenal columnar epithelial cells with oval, basal nuclei and pale-staining microvillus apical "brush border" similar to gastric columnar epithelial cells. (Wright-Giemsa stain)

of gastric fluid contains exfoliated and degenerating squamous and columnar epithelial cells, mixed population of bacteria, and possibly plant material (Fig. 6-4).

Small and Large Intestine

Both small and large intestinal mucosal cells resemble stomach glandular epithelium (Fig. 6-5). Cells are columnar with pale blue, slightly granular cytoplasm, basal oval nuclei, and striated border composed of apical microvilli. Goblet cells, which have a vacuolated pale staining appearance and eosinophilic staining mucus, are more common in the colon and rectum (Fig. 6-6). Squamous epithelial cells are associated with the terminal rectum and anus. Endocrine cells, Paneth cells (pyramidal-shaped cells with prominent apical, spherical, acidophilic granules), and Brunner's gland cells (Alcian blue–positive submucosal serous-type intestinal glands) are present in the small intestine and granular cells are present in the colon, but these cells are infrequent and have not been cytologically described (Figs. 6-7 and 6-8).[1] A few lymphocytes are often seen in intestinal specimens from horses because of Peyer's patches and submucosal lymphoid tissue. Hemosiderin-laden macrophages have also been reported in the lamina propria of clinically normal horses.[10] Although neutrophils were not present in the

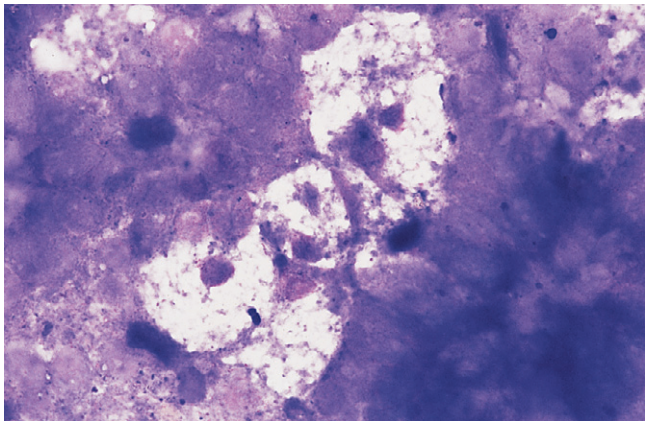

Fig. 6-6 Cluster of pale-staining goblet cells from colon of horse.
Note small central to eccentric nuclei and abundant clear to vacuolated cytoplasm. These cells are most prominent in the large intestine. (Wright-Giemsa stain)

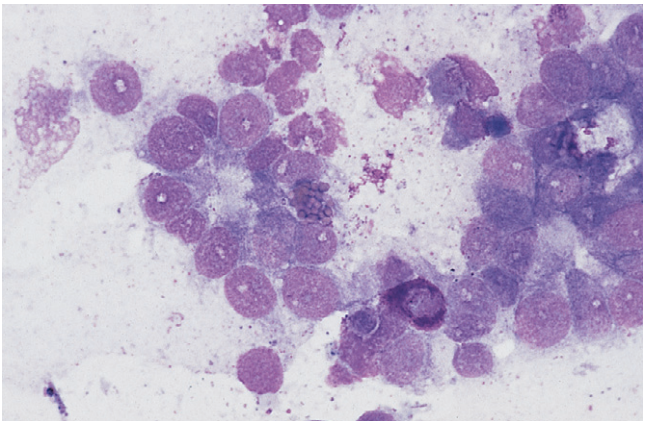

Fig. 6-7
Colonic scraping from healthy horse containing numerous clusters of epithelial cells in sheets or forming acinar-like structures. Mast cells are occasionally observed. (Wright-Giemsa stain)

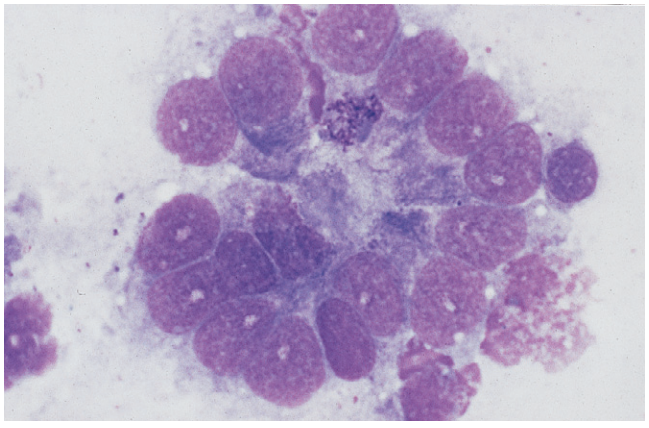

Fig. 6-8 Higher magnification of colonic epithelium.
A small granular cell with fine azurophilic granules is seen toward center of cell cluster. (Wright-Giemsa stain)

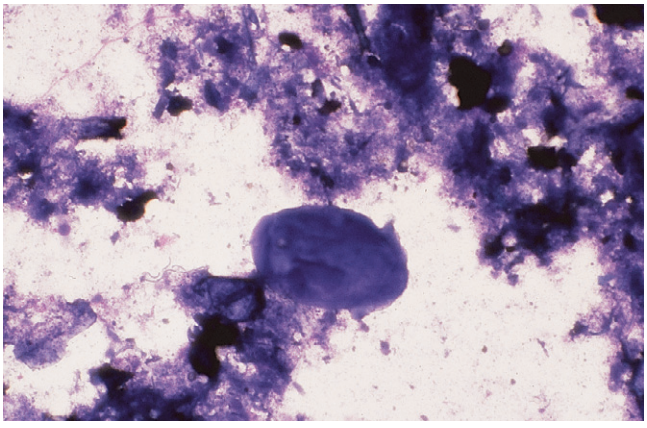

Fig. 6-9
Fluid from large intestine containing a large, dark-staining protozoa in center of field surrounded by plant material, a mixed population of bacteria, and cellular debris. (Wright-Giemsa stain)

surface epithelium of the equine rectum, scattered neutrophils and eosinophils have been reported in the rectal lamina propria of healthy horses.[11]

Intestinal fluid contains low numbers of squamous epithelial cells from the esophagus and stomach, a few columnar epithelial cells from the intestinal mucosa, a mixed population of bacteria, protozoa, fungal elements, food material, and rare to no inflammatory cells (Figs. 6-9 to 6-11). Minimal information is available on the microbial population inhabiting the gastrointestinal tract of the healthy horse. Proteolytic bacteria comprise a high proportion of culturable bacteria in the equine gastrointestinal tract.[12] Mean pH values reported for the equine duodenum, jejunum, ileum, and hindgut are 6.32, 7.10, 7.47, and 6.7, respectively.[12]

Cytologic Features of Abnormal Tissues

Neoplasia

Neoplasia of the gastrointestinal tract of horses is uncommon and usually occurs in older horses, except for lymphoma, which often occurs in younger horses (Table 6-1). General clinical signs associated with equine gastrointestinal neoplasia include weight loss, anorexia,

TABLE 6-1

Tumors of the Equine Gastrointestinal Tract

Esophagus	**Cecum**
Squamous cell carcinoma	Adenocarcinoma with/without osseous metaplasia
	Myxosarcoma
Stomach	**Colon**
Squamous cell carcinoma	Adenocarcinoma with/without osseous metaplasia
Adenocarcinoma	Lymphoma
Leiomyosarcoma	Lipoma
Leiomyoma	Lipomatosis
Gastric polyp	
Small intestine	**Rectum**
Lymphoma	Lymphoma
Adenocarcinoma	Lipoma
Leiomyoma	Leiomyosarcoma
Leiomyosarcoma	Polyps
Adenomatous polyposis	
Lipoma	
Carcinoid	

Data from Barker et al: in Jubb et al: *Pathology of Domestic Animals,* ed 4, Vol 2. San Diego, 1993, Academic Press, pp 33-317; East and Savage: Abdominal neoplasia (excluding urogenital tract). *Vet Clin North Am (Equine Pract)* 14:475-493, 1998; Orsini et al: Intestinal carcinoid in a mare: an etiologic consideration for chronic colic in horses. *JAVMA* 193:87-88, 1988; Patterson-Kane et al: Small intestinal adenomatous polyposis resulting in protein-losing enteropathy in a horse. *Vet Pathol* 37:82-85, 2000.

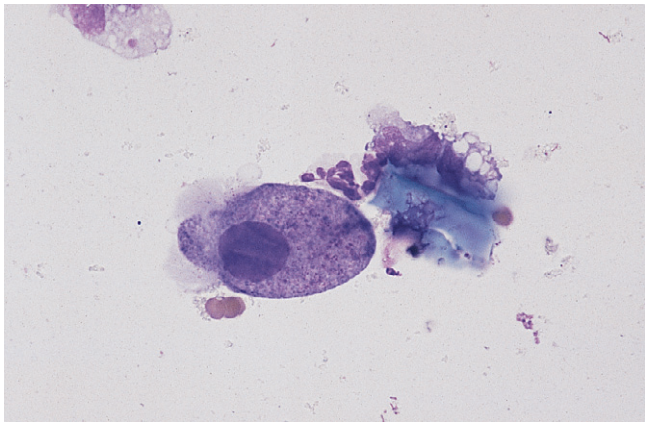

Fig. 6-10 Normal equine protozoa.
Large intestinal fluid illustrating the pleomorphic appearance of normal equine protozoa. Note large size of protozoa compared to red cells. Some plant material and cellular debris are adjacent to protozoa. (Wright-Giemsa stain)

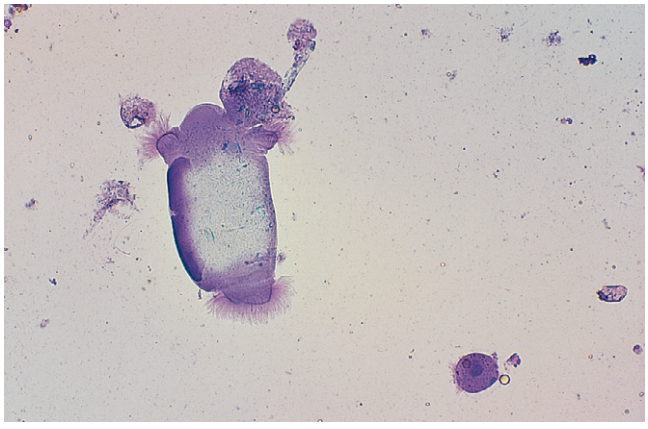

Fig. 6-11 Abdominal fluid from horse with chronic cecal impaction.
Two ciliated protozoa have marked difference in size. No evidence of intestinal rupture was found. (Wright-Giemsa stain)

lethargy, intermittent colic, intermittent fever, and variable fecal consistency. Clinical laboratory findings can include malabsorption (decreased glucose and d-xylose absorption), hypoalbuminemia, hypergammaglobulinemia, anemia (hemorrhage and chronic disease), and hypercalcemia (lymphoma and squamous-cell carcinoma). Peritoneal fluid frequently has an increased nucleated cell count (neutrophilic inflammation), increased protein, and, sometimes, exfoliated neoplastic cells. Microorganisms from the gut lumen may invade gastrointestinal neoplasms, resulting in abscessation and secondary septic peritonitis.

Lymphoma is the most common malignant neoplasm of the equine gastrointestinal tract.[8,13] It occurs most frequently in the small intestines and may be a primary alimentary lymphoma or a multicentric lymphoma that involves the intestines in addition to peripheral lymph

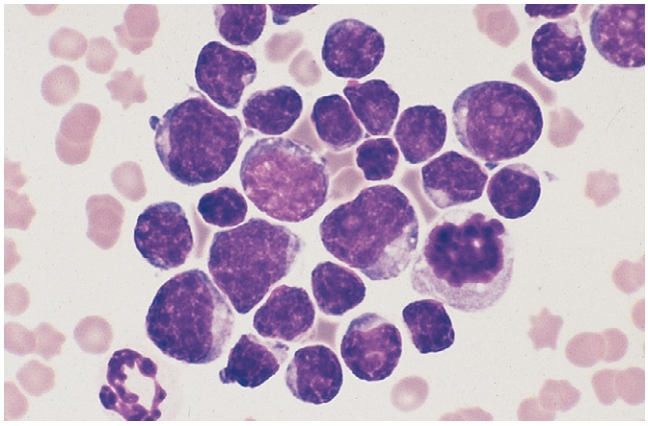

Fig. 6-12 Equine intestinal lymphoma.
Note mixed population of large, intermediate, and small lymphocytes with coarse chromatin pattern, indistinct nucleoli, and scant cytoplasm. (Wright-Giemsa stain)

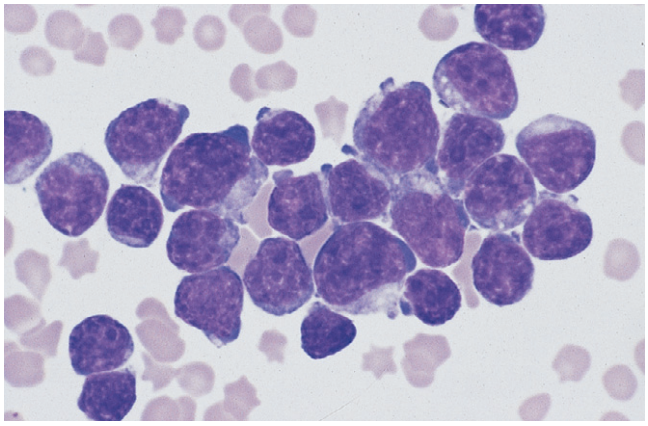

Fig. 6-13 Equine intestinal lymphoma.
Note pleomorphic population of large, immature lymphocytes with round to irregularly shaped nuclei, clumped chromatin pattern, one to four small nucleoli, and a scant to narrow rim of moderately basophilic, sometimes vacuolated cytoplasm. (Wright-Giemsa stain)

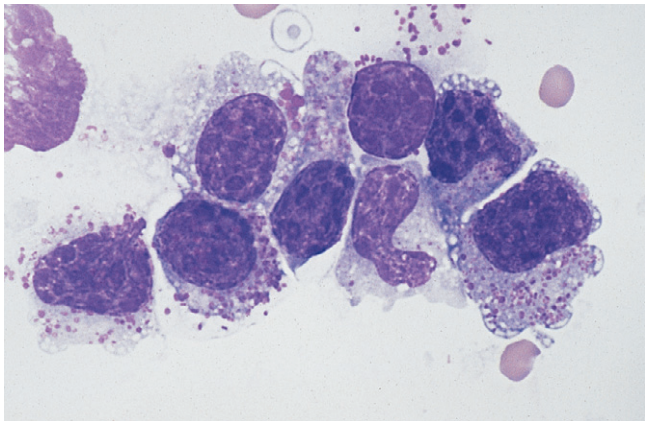

Fig. 6-14 Large granular lymphoma from intestine of horse with multicentric lymphoma.
Neoplastic cells are large cells with oval nuclei, clumped chromatin pattern, chromocenters, moderate amount of pale blue cytoplasm that contains cytoplasmic vacuoles, and numerous small to moderately large azurophilic granules. (Wright-Giemsa stain)

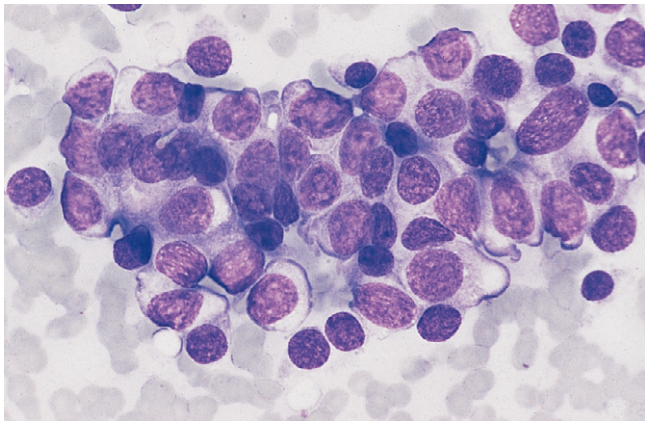

Fig. 6-15 Granular lymphoma.
Horse with granular lymphoma having lymphocytes with round to oval nuclei, coarsely stippled chromatin, and moderate amounts of pale blue cytoplasm that contains very small, eosinophilic cytoplasmic granules. Cytoplasmic granules were not obvious on histologic specimens. Small lymphocytes are interspersed among the granular lymphocytes. (Wright-Giemsa stain)

nodes and/or the thoracic cavity. Alimentary lymphoma is associated with local to diffuse thickening of the gut wall and marked enlargement of the mesenteric lymph nodes.[14] Neoplastic infiltrates can extend from the lamina propria and submucosa to the serosal surface. The lymphocyte population can vary from sheets of lymphoblasts or granular lymphocytes to a mixed population of large and small lymphocytes (Figs. 6-12 to 6-15).[14-18] Cytoplasmic granules in granular lymphoma cells are readily seen on cytology but may be poorly visible on histopathology.[15] Plasmacytoid cells and plasma cells can be abundant, and giant cells can occasionally be seen in the gut wall and lymph node of horses with lymphoma.[13,14]

A recent study of equine malignant lymphomas found that 77% (24/31) were high-grade tumors composed of large, atypical cells of B-cell origin.[16] The only intestinal lymphoma in this study was classified as a multicentric T-cell lymphoma, and all lymphomas with thymic masses were of T-cell origin. Forty-six percent (11/24) of the B-cell lymphomas were T-cell–rich,

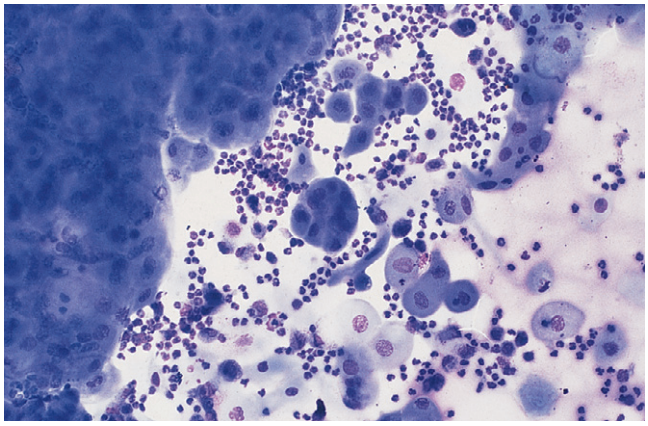

Fig. 6-16 Gastric squamous-cell carcinoma.
Fine-needle aspiration biopsy from gastric squamous-cell carcinoma from horse. Cells exfoliating in large sheets tend to be more uniform than the cells that are in small clusters or individualized. Cells vary from round to spindloid with very basophilic to pale basophilic cytoplasm. Neutrophils are numerous. (Wright-Giemsa stain)

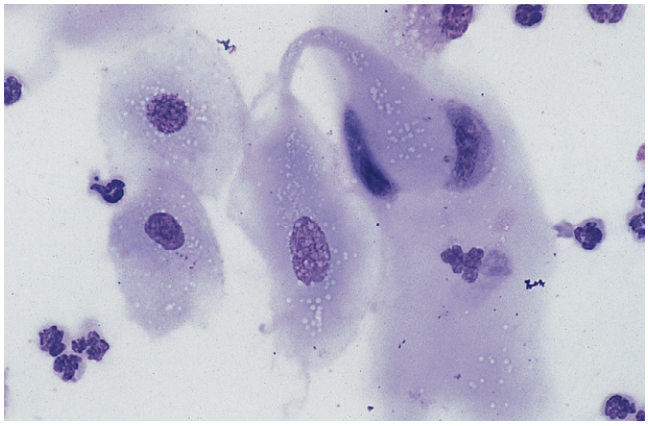

Fig. 6-17 Gastric squamous-cell carcinoma.
Higher magnification of cells from an equine gastric squamous-cell carcinoma illustrates two cells with cytoplasmic tails. (Wright-Giemsa stain)

large B-cell lymphomas that contained a mixed lymphoid infiltrate of nonneoplastic, normal-appearing T-cells and large atypical B-cells with large, irregularly shaped nuclei, coarse chromatin, and atypical mitotic figures. Lymphocyte markers are necessary to differentiate mixed small- and large-cell lymphoma from T-cell–rich, large B-cell lymphoma. A mixed lymphoma composed of both small and large neoplastic B-cells has occurred in the stomach of a horse.[17] Additionally, a B-cell lymphoma was diagnosed in a horse with Sezary-like cells in the peripheral blood, cutaneous nodules, heart, abdominal cavity, and ventral colon.[18] Paraneoplastic hypereosinophilia, although uncommon, has been reported in a horse with intestinal lymphoma.[19]

Squamous-cell carcinoma, which is the second most common malignant neoplasm of horses, is the most common tumor of the equine esophagus and stomach.[13,20,21] Cytologically, squamous-cell carcinoma has been classified into three groups[20]:

1. Well-differentiated (>50% well-differentiated squamous cells called flakes and up to 30% round or oval squamous cells)
2. Moderately differentiated (>50% round or oval squamous cells and lesser numbers of flakes)
3. Poorly differentiated (round or oval pleomorphic cells with rare flakes)

Neoplastic cells frequently occur in thick cell clusters or sheets (Fig. 6-16). Thinner areas or individualized cells must be scrutinized to evaluate cellular details (Figs. 6-17 to 6-19). Marked variation in cell size, nuclear size, nucleolar size and shape, and number of perinuclear or large cytoplasmic vacuoles may

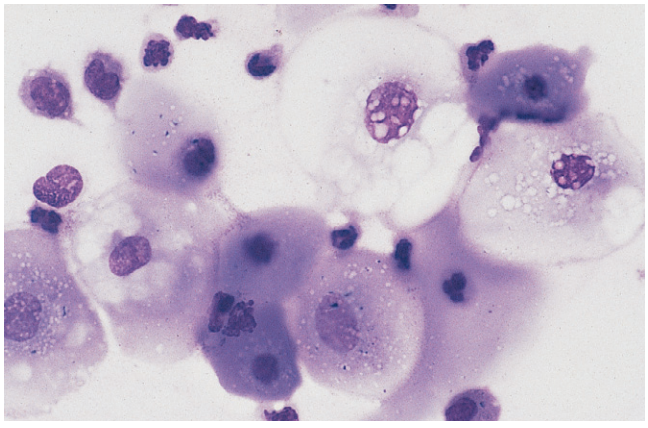

Fig. 6-18 Gastric squamous-cell carcinoma.
Higher magnification of cells from an equine gastric squamous-cell carcinoma illustrating anisocytosis, anisokaryosis, and variability in cytoplasmic vacuolization and keratinization. (Wright-Giemsa stain)

be present. Cells from well-differentiated squamous-cell carcinoma are large polyhedral to spindle-shaped squamous cells with low nuclear to cytoplasmic ratios and abundant pale blue cytoplasm that forms angular borders. Cells may also appear dendritic, round, elongated, or caudate and have several colorless, refractile, or minute deep pink to purple cytoplasmic granules or a diffuse pink- to reddish-tinged cytoplasm. The nucleus is small, oval to round to irregular, with coarse chromatin. Irregularly shaped chromocenters and nucleoli, presence of cytoplasmic rings (dyskeratosis),

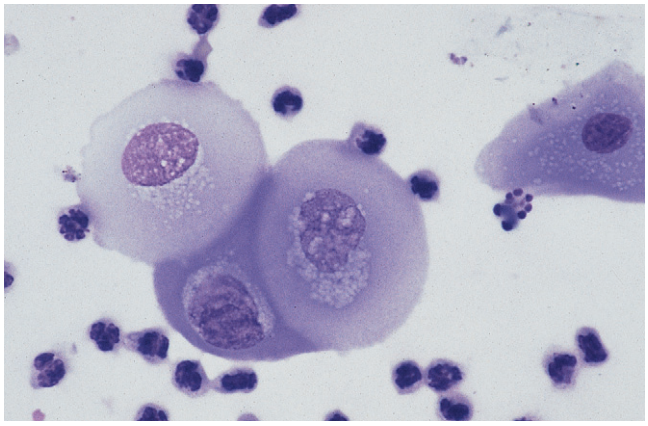

Fig. 6-19 Gastric squamous-cell carcinoma.
Note cluster of three large immature squamous cells with oval nuclei, coarsely stippled chromatin, indistinct nucleoli, and fine perinuclear cytoplasmic vacuolization that is a dyskeratotic feature of squamous neoplasia. (Wright-Giemsa stain)

hyperchromasia, anisokaryosis, anisocytosis, caudate cells with extremely long cytoplasmic processes (tadpole cells), and intracytoplasmic migration of cells (emperipolesis) are features of dysplasia and neoplasia. Papanicolaou's stain is the best stain to evaluate keratinization, dyskeratotic changes, and cytologic features of thick cellular clusters. Cytoplasmic orangeophilia correlates to cellular keratinization. An extensive inflammatory reaction is frequently associated with squamous-cell carcinomas, especially with superficial ulceration.

Cells from well-differentiated squamous-cell carcinoma look like normal squamous epithelium, and cells from poorly differentiated squamous-cell carcinomas may be difficult to distinguish from reactive/dysplastic mesothelial cells or cells from poorly differentiated adenocarcinomas. Therefore, for the best cytologic interpretation, other causes for the presence of squamous cells in the sample must be ruled out. Two conditions that might be associated with squamous cells are ruptured gastric ulcers (possible dysplasia and neutrophilic inflammation) and inadvertent aspiration of amniotic fluid from a pregnant mare. Esophageal-gastric ulcers usually occur in foals or young horses whereas squamous-cell carcinomas usually occur in older horses (6 to 18 years of age).[21] Endoscopy and ultrasonography are helpful in confirming a cytologic opinion. Squamous-cell carcinomas have a propensity to seed organ surfaces and metastasize extensively throughout the pleural and peritoneal cavities, similar to mesothelioma, resulting in pleural and peritoneal fluids containing carcinoma cells. Most horses with gastric squamous-cell carcinoma have abundant peritoneal fluid that is characterized by a neutrophilic inflammation, hemosiderophages, and erythrophagia.[21] Sediment concentration and cytospin preparations increase the probability of finding squamous epithelium and evidence of neoplastic cytologic features in these samples.

Adenocarcinomas of the equine intestinal tract are rare.[13,22,23] Most frequently they are reported in the large intestine as solitary, nodular masses with low metastatic potential but a high tendency to undergo osseous metaplasia.[23] Tumor cells are round to columnar, with moderate to abundant amphophilic to basophilic cytoplasm and large nuclei with prominent nucleoli. Occasional signet ring cells, extracellular eosinophilic mucin, and rare acinar formation may be observed.[22] Histologically, these tumor cells vary from well-differentiated columnar cells forming glandular structures that contain goblet cells to anaplastic cells forming clusters or irregular glandular structures.[22,23]

Adenomatous polyposis was reported in a young, adult quarter horse gelding that presented with protein-losing enteropathy, extensive subcutaneous ventral edema, and severe hypoproteinemia.[24] Numerous polypoid, papillary, and glandular masses comprised of pseudostratified tall columnar cells with interspersed goblet cells were found in the small intestines.

Carcinoid tumors arising from the endocrine or paracrine cells in the mucosal lining of the stomach and intestine are rare in horses.[25] These endocrine cells are uniform, round to oval cells with oval, vesicular nuclei that lack prominent nucleoli but have abundant finely granular eosinophilic cytoplasm that is often stripped from the cell, leaving a pale eosinophilic background and naked nuclei. Special stains for argentaffin and argyrophilic properties or electron microscopy to identify secretory cytoplasmic granules are necessary to confirm the diagnosis.

Leiomyomas and *leiomyosarcomas* are smooth muscle tumors of the intestine in old horses. These tumors may occur in the small intestine, form encapsulated masses on the serosal surface, or cause pedunculated tumors protruding into the lumen of the rectum.[13] The cytologic appearance is similar to that of smooth muscle tumors in other sites. Cells are individualizing spindle-shaped cells with oval to elongated, cigar-shaped nuclei, lacy chromatin pattern, one or two indistinct nucleoli, and moderate amounts of pale blue cytoplasm. Cells become more pleomorphic and less spindle-shaped with malignant transformation. Cytologic differentials for well-differentiated mesenchymal cells from the gastrointestinal tract of horses include intestinal fibrosis, disseminated peritoneal leiomyomatosis, omental fibrosarcoma, and fibroplasia secondary to inflammation.[26,27] Intestinal fibrosis is characterized by diffuse thickening of the small intestine, arteriole sclerosis, capillary endothelial-cell hypertrophy, and hypertrophy and degeneration of smooth muscle nuclei. Disseminated peritoneal leiomyomatosis is a rare nonmalignant,

multicentric proliferation of smooth muscle tissue that develops in the abdomen of females.[26,27]

Pedunculated lipomas associated with the intestinal mesenteric adipose tissue are well documented but infrequent causes of intestinal obstruction in aged horses.[28] Neither these tumors nor lipomatosis (uncommon benign infiltrative form of lipoma) can be definitively diagnosed by cytology.[8]

Inflammatory Lesions

Equine inflammatory gastrointestinal disorders are common.[13] Results of rectal biopsies in 105 horses revealed pathologic changes in 60 (57%).[11] Proctitis was the most frequent diagnosis and was classified as simple (neutrophilic inflammation), chronic (lymphocytes and plasma cells), or chronic suppurative (neutrophils, lymphocytes, and plasma cells). Proctitis in this study was often nonspecific and best reflected disease in the proximal gut rather than a morphologic entity.[11] However, even with their limitations, rectal biopsies and histopathology (and likely cytologic biopsies) are a useful adjunct for evaluating intestinal diseases in the horse. Neutrophilic inflammation and neutrophils on fecal smears have been observed with bacterial diseases such as salmonellosis.

Bacterial Enterocolitis: Bacterial gastrointestinal infections can be deadly, especially in the foal or the immunocompromised horse. Culture and sensitivity are always recommended to make a specific diagnosis and choose the best therapeutic approach. However, cytology can be helpful in quickly confirming a change in gastrointestinal flora and tentatively identifying the type of bacteria or the etiologic agent so that the appropriate therapy and diagnostics can be undertaken. Romanowsky stain is preferred over Gram's stain for the initial screening of fecal cytologic smears. With Romanowsky stains, bacteria (except negatively staining *Mycobacterium* spp.) appear dark blue, and morphologic differences such as rod or coccoid shape are readily distinguishable. Also, and very importantly, cellular morphology of inflammatory cells and epithelial cells is superior with Romanowsky stains.

The inflammatory reaction of bacterial infections not only varies with the specific bacterial agent but also with the duration of the bacterial infection. For example, *Rhodococcus (Corynebacterium) equi*, a gram-positive coccobacillus that causes enterocolitis in foals, initially causes a neutrophilic inflammation that progresses to a chronic inflammatory process with macrophages containing intracellular coccoid organisms and multinucleated giant cells infiltrating into the lamina propria. Equine salmonellosis can clinically manifest itself as a septicemic, acute, chronic, or asymptomatic carrier stage with differing cellular changes that may reflect the stage of the disease. *Clostridium* spp. are potential causes of diarrhea, especially in the foal. Although *Campylobacter jejuni* and enterotoxigenic *E. coli* have been isolated from foals with diarrhea, their significance is unclear.[13]

Equine monocytic ehrlichiosis *(E. risticii)* causes Potomac horse fever, a condition characterized by diarrhea, leukopenia, fever, and depression. Lesions in the intestines include hyperemia, congestion, hemorrhage, ulcers, and superficial necrosis. *Ehrlichia* spp. are obligate intracellular bacterial pathogens that replicate within the phagosomes of host cells. *E. risticii*, unlike *E. equi*, has not been identified on peripheral blood smears. The organisms are also not evident in hematoxylin and eosin–stained tissue but appear as small clusters of 10 to 15 fine brown dots (less than 1 μ in diameter) in epithelial cells and macrophages with modified Steiner silver stain.[13]

Proliferative enteritis characterized by small intestinal thickening, crypt epithelial-cell hyperplasia, curved intracellular bacteria, and severe enteritis has been described in several foals. *Lawsonia intracellularis* was identified as the etiologic agent.[29]

Chronic Inflammatory Bowel Disease: Chronic inflammatory bowel disease is characterized by focal or diffuse infiltration of leukocytes into the intestinal wall. Several equine diseases are in this group and include equine granulomatous gastroenteritis, eosinophilic gastroenteritis, intestinal tuberculosis, histoplasmosis, and lymphoproliferative disorders.[30-34] Cytologic features of granulomatous inflammation include macrophages, epithelioid cells, multinucleated giant cells, lymphocytes, and plasma cells. Specimens having granulomatous inflammatory cells should be scrutinized for fungal hyphae (Plates 5B and 5C), *Histoplasma* organisms (Plate 4C), *Mycobacterium* (Plates 4A and 4B), and parasitic larva.

Granulomatous gastroenteritis: Although granulomatous gastroenteritis and eosinophilic gastroenteritis are considered to be two distinct syndromes, there are some similarities, such as enteric as well as nonenteric granuloma formation.[32] Equine granulomatous enteritis is a focal to multisystemic granulomatous disease primarily affecting the small intestine.[35] Granulomas are composed of macrophages, lymphocytes, epithelioid cells and giant cells. Young horses appear to be at higher risk. Although the etiology of equine granulomatous gastroenteritis is unknown, an immunologic mechanism and an association with aluminum have been postulated.[31,35,36]

Eosinophilic gastroenteritis: Equine chronic eosinophilic gastroenteritis has been described as part of a distinct multisystemic epitheliotropic syndrome associated with eosinophilic dermatitis and eosinophilic granulomatous pancreatitis.[13,33,35] Diffuse or focal inflammatory infiltrates occur in the esophagus, stomach, small and large intestine, and mesenteric lymph nodes. The gut wall is infiltrated by eosinophils, mast cells, macrophages,

TABLE 6-2

Inflammatory Disorders of the Equine Gastrointestinal Tract

Infectious disorders

Parasites

Stomach *Gasterophilus* spp., *Habronema* spp., *Draschia megastoma*, *Trichostrongylus axei*

Small intestine *Strongyloides westeri, Parascaris equorum, Anoplocephala magna, Eimeria leuckarti, Cryptosporidia*

Large intestine larval Cyanthostomiasis (encysted small strongyle larvae), small strongyles (*Strongylus vulgaris, Strongylus edentatus, Strongylus equinus), Triodontophorus* spp., *Anoplocephala perfoliata, Oxyuris equi, Probstymayria vivipara, Tritrichomonas equi, Giardia, Cryptosporidia*

Bacteria (nongranulomatous)

Small intestine *Clostridium perfringens, Clostridium welchii, Lawsonia intracellularis*

Large intestine *Salmonella* spp., *Clostridium perfringens, Clostridium welchii, Ehrlichia risticii*

Bacteria (granulomatous)

Small intestine *Mycobacterium avium, Mycobacterium paratuberculosis, Rhodococcus equi*

Fungus/yeast

Candida, Histoplasma, Pythium, Aspergillus

Viruses

Small intestine rotavirus, coronavirus, adenovirus, equine herpesvirus-1 (adult horses)

Noninfectious disorders

Equine granulomatous enteritis
Equine eosinophilic gastroenteritis
Equine basophilic gastroenteritis
Lymphocytic/plasmacytic gastroenteritis
Chemical gastroenteritis blister beetle poisoning
Uremic gastritis
Amyloid

lymphocytes, and plasma cells. Peripheral eosinophilia is not observed, but diarrhea and hypoalbuminemia occur. The cause is unknown but immune-mediated etiology is likely.[13] Marked eosinophilic infiltration of the rectum may be of low diagnostic significance unless accompanied by pathologic lesions of equine eosinophilic gastroenteritis. Eosinophils were common finding in rectal biopsies from healthy horses.[11]

Basophilic enterocolitis: Basophilic enterocolitis, which may be a variant of eosinophilic gastroenteritis, has been described in a horse. Basophils were prominent in the inflammatory infiltrate of the ileum, cecum, and colon along with lymphocytes, plasma cells, macrophages, and eosinophils.[13,37]

Lymphocytic-plasmacytic enteritis: The etiopathogenesis of lymphocytic-plasmacytic enteritis is unknown, but it causes a protein-losing enteropathy and hypoalbuminemia.[13,33,34] Normal fecal consistency indicates that the protein-losing enteropathy primarily involves the small intestine and that the major portion of the large intestine is still functional. Therefore, small intestinal biopsies are more likely to be diagnostic than more readily obtainable rectal biopsies. Increased numbers of well-differentiated lymphocytes and plasma cells

infiltrate the lamina propria. In the horse and dog, submucosal or transmural lymphocytic-plasmacytic infiltrates may signal a precursor to lymphoma.[13] Also, a differential for lymphocytic and eosinophilic infiltrates must include parasitic invasion.[13]

Viral Enteritis: Adenovirus, coronavirus, and rotavirus cause diarrhea in foals, and equine herpesvirus-1 can cause a necrotizing enterocolitis in the adult horse.[13] Unfortunately, cytology is not very helpful for diagnosing equine viral gastrointestinal diseases. Serology, molecular testing, viral cultures, and/or electron microscopy are necessary to document a viral etiology.

Fungus/Yeast: See granulomatous inflammation. Fungi and *Histoplasma* can cause gastroenteritis/enteritis in horses. *Candida* spp., budding yeast that are normal mucosal flora of the alimentary tract, can become opportunistic invaders with changes in the microenvironment. Branching, filamentous pseudohyphae and hyphae can replace the yeast forms. Gastroesophageal candidiasis occurs most frequently in foals and is associated with ulceration of the squamous epithelium.[13]

Parasitic Agents: Equine gastrointestinal parasites are listed in Table 6-2.[13,38] Encysted small *Strongylus* larvae (cyanthostomes) have become the principal parasitic pathogen of horses.[39,40] These encysted larvae, unlike small and large strongyles, are resistant to most modern anthelmintics. These small *Strongylus* larvae enter the large intestinal mucosa where they may arrest for years and accumulate to massive numbers. Clinical disease is associated with the simultaneous emergence of these encysted larvae. Risk factors for cyathostomosis are young horses, early spring, and recent anthelmintic treatment.[39] Acute-onset diarrhea and acute weight loss are the most common signs of this syndrome. Diagnosis is facilitated by finding excysted larvae on a rectal sleeve following rectal examination. Other laboratory findings may include peripheral neutrophilia and hypoalbuminemia.[39, 40]

Equine cestodiasis (*Anoplocephala perfoliata* infections) also appear to be increasing.[39,41] Infections have been associated with colic, intestinal perforations, and intussusceptions at the ileocecocolic junction. Development of a serologic assay for *A. perfoliata* has enhanced diagnosis. Difficulties with fecal flotation relate to resistance of cestode eggs to float and passage of intact tapeworm segments without the release of eggs.[39]

Ciliated protozoa are abundant in the large intestine of the horse. Little is know about their function but many are bizarre nonpathogenic organisms (see Figs. 6-9 to 6-11). Chronic diarrhea has been associated with the presence of many fecal flagellates, especially *Tritrichomonas*; however, the pathogenicity is uncertain.[38]

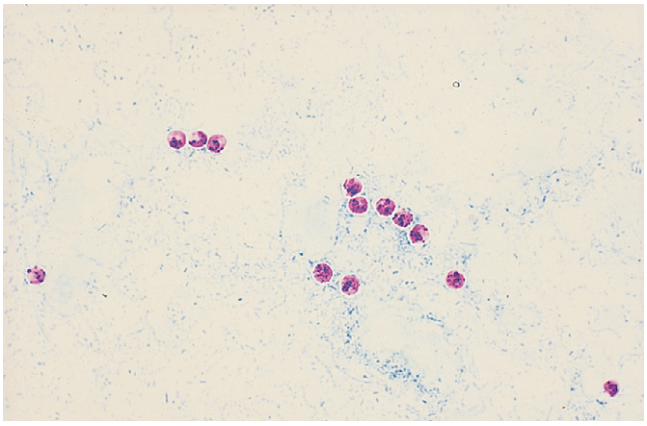

Fig. 6-20 Fecal smear with numerous small red staining *Cryptosporidia*.
Oocysts are slightly smaller than erythrocytes. (Acid-fast type stain)

Ciliated protozoa can invade the intestinal tract, but they can be postmortem invaders of colonic mucosa in enteric and nonenteric disorders.[13,42] Another protozoan, *Eimeria leuckarti*, may be frequently found in foal feces, but its pathogenicity is also questionable.[13,38]

The interpretation of infections with *Giardia* and *Cryptosporidia* are not straightforward, but newer more sensitive and specific tests such as immunofluorescent tests and fecal enzyme-linked immunosorbent assays are available to diagnose *Cryptosporidium* and *Giardia*.[43] In foals, infection rates with either *Cryptosporidium* or *Giardia* can be high (about 15% to 35%), even in foals without clinical signs (Fig. 6-20).[43]

Other Causes: Amyloid-associated gastroenteropathy is rare but when present is associated with malabsorption and enteric protein loss.[44] On cytologic preparations, amyloid appears as a homogenous pink extracellular staining matrix. Cytology is of limited help in diagnosing blister beetle poisoning or uremic gastritis/gastroenteritis, which are associated with nonspecific hemorrhagic lesions.[45]

Cytologic Features of Fecal Smears

Direct fecal smears can be valuable in horses with enteric disease. Normally, fecal smears contain large numbers of a mixed bacterial population, mucus, plant material, a few epithelial cells, and a lack of inflammatory cells. Feces in sick horses should be examined for occult blood, sand, clostridial organisms, *Salmonella* organisms, giardial cysts, cryptosporidial oocysts, ciliated protozoa, parasitic ova, parasitic larva, and WBCs. Ciliated protozoa should be present in normal fresh feces. Absence or massive numbers of ciliate protozoa

indicate severe alternations of colonic flora. Fecal WBCs indicate an active inflammatory disease. If eosinophils are numerous, further investigations for eosinophilic gastroenteritis should be undertaken. Direct smears can be valuable in addition to flotation tests to detect parasites such as trophozoites or cestode eggs that are destroyed or do not levitate in flotation fluids.

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