

tion is still the only method that can be used to demonstrate the presence of live viruses in field samples. Field strains must be obtained for further studies, e.g., antigenic relationships, pathogenesis, development of serologic tests, and production of vaccines.

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## An outbreak of *Klebsiella pneumoniae* infection in dogs with severe enteritis and septicemia

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*Klebsiella pneumoniae* is a facultatively anaerobic gram-negative bacterium. *Klebsiella* is a minor intestinal commensal organism that rarely causes enteric disease. This bacterium, however, is an opportunistic pathogen that has been implicated in cases of mastitis in cattle,<sup>3</sup> metritis in mares,<sup>4</sup> bacteremia in calves,<sup>6</sup> pneumonia and urinary tract infections in dogs, pneumonia and septicemia in foals,<sup>20</sup> and polyarthrititis in kids.<sup>2</sup> Additionally, *Klebsiella* can acquire resistance to multiple antibiotics and is an important cause of

nosocomial wound and urinary tract infections of hospitalized humans and animals.<sup>7</sup> This report documents a severe outbreak of *K. pneumoniae* enteritis in a kennel of Bordeaux mastiffs, resulting in septicemia and death.

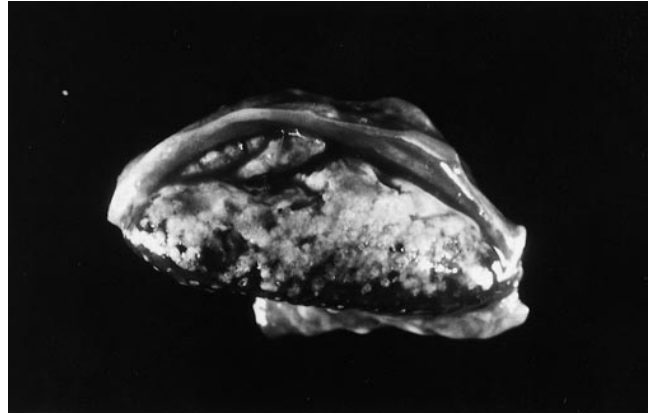
The outbreak began with symptoms of vomiting and diarrhea in a large breeding kennel containing 15 adult Bordeaux mastiffs. Seven pups were housed together in a pen separate from adults and were unaffected. All affected animals were housed in the same general area of the kennel. Adults were housed in clean individual pens with concrete flooring separated by chainlink fencing. No prior health problems had occurred in the kennel. All animals were current for vaccinations (distemper/canine infectious hepatitis/*Leptospira*/parvovirus/parainfluenza combination and rabies vaccines). During the outbreak, a total of 7 adult dogs had clinical signs, and 4 died. All affected animals showed the same symptoms: vomiting and watery or bloody diarrhea.

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**Figure 1.** Small intestine from Bordeaux mastiff with *Klebsiella pneumoniae* enteritis and sepsis. Note diffuse reddening with hemorrhage on the serosal surface. The mucosa is thickened and covered by fibrinonecrotic exudate.



**Figure 2.** Enlarged hemorrhagic tonsil from female Bordeaux mastiff with *Klebsiella pneumoniae* enteritis and sepsis. Note fibrinous exudate covering the surface.

Two animals were referred to the University of Georgia Teaching Hospital (1 for medical care and 1 for necropsy). One dog was a 2-year-old intact male Bordeaux who presented with a 4-day history of vomiting and a 2-day history of severe watery diarrhea. The dog's health had deteriorated despite treatment by the referring veterinarian with intravenous fluids and parenteral amoxicillin. Three other dogs from the same kennel were exhibiting similar clinical signs and were treated by the referring veterinarian with amoxicillin and subcutaneous fluids. At the time of referral of the male dog, an adult female dog that died at home after having watery diarrhea and vomiting for 2 days was submitted for necropsy (within 24 hours of its death).

Upon admission, the male dog was recumbent, depressed, and dehydrated. The dog was tachycardic and tachypnic and had a rectal temperature of 103.8 F. Pale mucous membranes and a slow capillary refill time were noted. On abdominal palpation, fluid-filled intestines and a mild pain response were found. There was evidence of hematochezia on rectal exam.

Abnormalities found in complete blood count, biochemical profile, and urinalysis included thrombocytopenia, leukopenia, azotemia, hyponatremia, hypokalemia, hypochloridemia, and metabolic acidosis. These abnormalities were attributed to vomiting, diarrhea, and subsequent dehydration. Additionally, hypoglycemia, hypoalbuminemia, and elevated serum alkaline phosphatase activity were found and were attributed to severe sepsis when evaluated in conjunction with the leukopenia, fever, and thrombocytopenia. Gastrointestinal losses may have also contributed to the hypoalbuminemia. The urine was concentrated (specific gravity 1.048), and sediment abnormalities included mild proteinuria (2+) and bilirubin crystals. The dog also had a prolonged activated clotting time. A parvovirus antigen fecal test<sup>a</sup> result was negative, both at the referring veterinarian's office and again at the time of referral. Based on the history, physical examination, and laboratory abnormalities, septic shock with secondary disseminated intravascular coagulation (DIC) associated with an infectious enteric pathogen was suspected.

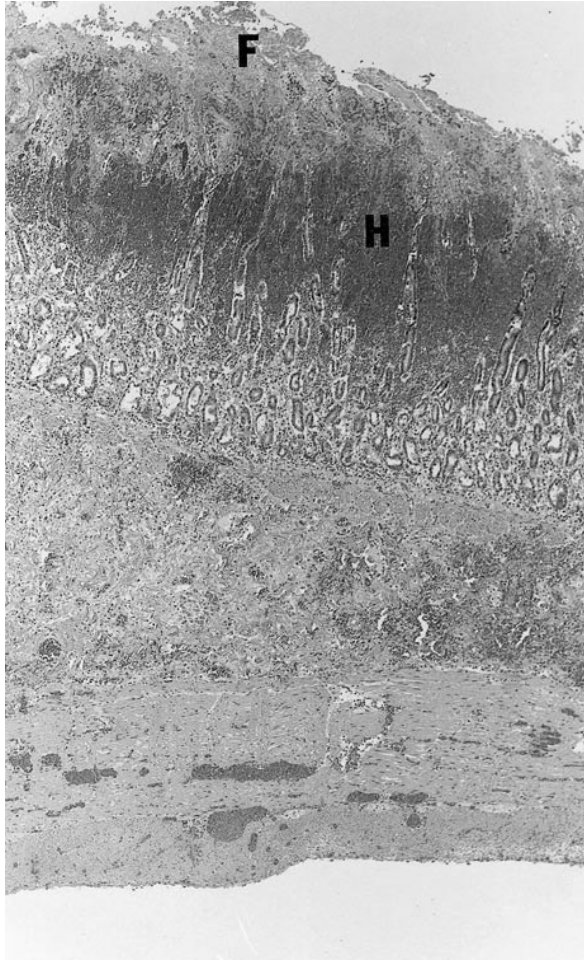
*Salmonella* and parvovirus were considered as potential

enteropathogens. The dog was treated with intravenous lactated Ringer's solution, initially as a rapid bolus and then at a constant rate of infusion. Intravenous enrofloxacin (5 mg/kg BID), clindamycin (10 mg/kg TID), and fresh frozen plasma incubated with heparin were initiated as treatment for septic shock and DIC. The dog died within 2 hours of admission.

Necropsy of this male dog and the female dog were performed at the Athens Diagnostic Laboratory, Athens, GA. At necropsy, both dogs had similar lesions, which were more severe in the female dog (that was dead on arrival). In both dogs, the serosal surfaces of the small intestine were covered by ecchymotic hemorrhages, and the small intestinal contents were bloody. In addition, the entire small intestine of the female dog was intensely reddened and the mucosal surfaces were covered by fibrinonecrotic exudate (Fig. 1). The male dog had ecchymotic hemorrhages on the epicardium of the heart, and the female dog had hemorrhages in the skin of the ventrum and generalized hemorrhagic lymphadenopathy. The female dog had greatly enlarged tonsils that were hemorrhagic and covered by fibrinous exudate (Fig. 2). The liver, lung, and kidneys were congested, the bone marrow was red in both dogs, and the gall bladders were distended with bile.

Fresh tissues were submitted for bacteriologic culture and other representative tissues were fixed in 10% formalin for 24 hours and then processed and embedded in paraffin for histopathology and immunohistochemistry. Paraffin-embedded tissues were sectioned at 3  $\mu$ m and stained with hematoxylin and eosin (HE) and by the Lillie-Twort Gram stain method.

Microscopically, the small intestine of the male dog had extensive areas of superficial mucosal necrosis that contained a mixture of multiple types of gram-positive and gram-negative rod-shaped bacteria. Many intestinal glands were mildly dilated and lined by hyperplastic or attenuated epithelium; gram-negative bacterial rods that appeared encapsulated were present in some crypts. In the female dog, small intestinal changes were more severe, with multifocal to coalescing mucosal to transmural hemorrhage (Fig. 3). Superficially, the mucosa was necrotic and covered by a lay-



**Figure 3.** Small intestine from female Bordeaux mastiff with *Klebsiella pneumoniae* enteritis. There is severe diffuse necrosis of the superficial mucosa, with associated fibrinous exudation (F) and hemorrhage (H). Note the dilated crypts lined by irregularly attenuated epithelium and the hemorrhage in the submucosa and tunica muscularis. HE.

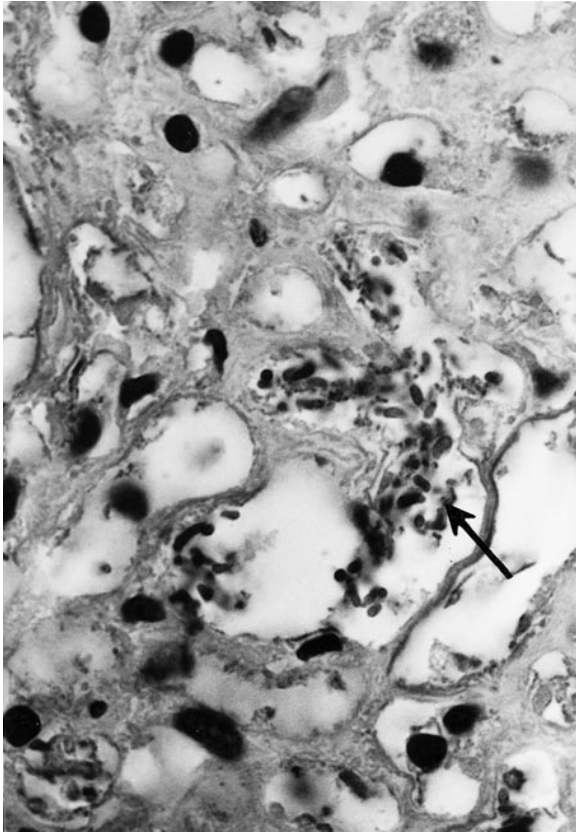
er of fibrin containing numerous bacteria that were a mixture of gram-negative rods and gram-positive rods and cocci. Multifocally, gram-negative rods that appeared to have a capsule infiltrated deep into the mucosa. Intestinal crypts were dilated and lined by attenuated and regenerating epithelium. Mucosal vessels were thrombosed. Multiple fibrin thrombi and gram-negative rods were present in small renal and glomerular vessels in both dogs. The lungs of both dogs were congested and edematous and had multiple foci of hemorrhagic necrosis with associated intravascular gram-negative bacterial rods. In the female dog, the tonsils had severe superficial necrosis with severe fibrin exudation and numerous gram-negative bacterial rods. The larynx and pharynx of this dog were severely ulcerated with thrombosis and severe edema, fibrinous exudation, and numerous gram-negative rods in the underlying soft tissues. In both dogs, gram-negative rods were found in blood vessels in various organs, including brain, spleen, liver, bone marrow, adrenal gland, mesenteric lymph nodes, stomach, and pancreas. Bone marrow was extremely congested.

Immunohistochemical staining for *Klebsiella* was performed by placing 3- $\mu$ m tissue sections on silane-coated slides and staining them using a commercially available immunoperoxidase kit.<sup>b</sup> Sections were deparaffinized, hydrated, treated with 0.05% protease<sup>c</sup> for 2.5 minutes at 37 C, blocked for 10 minutes at room temperature with horse serum, and incubated for 2 hours with a rabbit polyclonal antiserum (1:10) raised against the *Klebsiella* K5 capsular polysaccharide.<sup>d</sup> Sections were rinsed in phosphate-buffered saline (PBS) with 0.25% Brij 35 solution (PBS-Brij) and incubated with biotinylated horse anti-rabbit antibody (10 minutes at room temperature). After rinsing with PBS-Brij and blocking with 3% hydrogen peroxide for 5 minutes, streptavidin-peroxidase conjugate was applied (5 minutes at room temperature). Slides were then washed with PBS-Brij and incubated with diaminobenzidine tetrahydrochloride (DAB) substrate until color change was noted on the positive control. Sections were counterstained with methyl green. The positive control was obtained by embedding a pure culture of the outbreak-associated *K. pneumoniae* in an agar pellet, fixing the pellet for 24 hours in 10% formalin, and then routinely embedding it in paraffin. A separate negative control slide for each tissue section received PBS with 3% bovine serum albumin in place of the primary antibody.

Immunohistochemical staining for the *K. pneumoniae* K5 antigen demonstrated positively stained rods within the necrotic mucosa and vessels of the intestinal wall in the female dog. Rod-shaped bacteria positive for the *K. pneumoniae* K5 antigen were also observed in the larynx, pharynx, adrenal gland, and lung of the female dog and in the intestine, kidney (Fig. 4), and spleen of the male dog.

Several tests were performed on fresh tissues, including aerobic and anaerobic culture as well as fluorescent antibody testing. *Salmonella* and *clostridium perfringens* cultures were negative after 7 days. *Yersinia* and *Campylobacter* cultures were not requested. Fluorescent antibody detection for canine parvovirus, canine coronavirus, and *Leptospira* antigen were negative. Intestinal contents were also negative for verocytotoxins of *Escherichia coli*.<sup>10</sup>

Aerobic cultures were plated on blood agar thioglutinate and incubated at 37 C. *Enterococcus* was isolated from lymph node, liver, and lung of the male dog, and *E. coli*, *Pseudomonas*, and *Enterobacter* were isolated from intestine of the female dog. *Klebsiella pneumoniae* was isolated from intestine, lung, and kidney in the male dog and from liver, lymph node, lung, and kidney of the female dog. Five strains of *K. pneumoniae* isolated from lung, kidney, and intestine of the male dog and from lung and kidney of the female dog were submitted for identification and K-typing to the International *Escherichia* and *Klebsiella* Reference Center (WHO), Copenhagen, Denmark (IEKRC). Strains were identified according to *Bergey's Manual of Determinative Bacteriology*.<sup>9</sup> Utilization testing was only done with histamine as previously described.<sup>13</sup> K-typing was done by counter-current immunoelectrophoresis using a modification of a previously described method.<sup>17</sup> An extract was used as antigen instead of a whole cell suspension.<sup>16</sup> The extract procedure was modified by heated only once for 1 hour at 100 C before centrifugation. All strains were investigated by the classical



**Figure 4.** Paraffin-embedded kidney from male Bordeaux mastiff. Note the bacterial rods staining positive for the *Klebsiella pneumoniae* K5 capsular antigen in a glomerular vessel (arrow). *Klebsiella pneumoniae* K5 capsular antigen stain, streptavidin–peroxidase immunohistochemical technique. DAB chromagen and methyl green counterstain.

quelling reaction.<sup>5</sup> O-typing<sup>8</sup> was done by an inhibition enzyme-linked immunosorbent assay.<sup>9</sup>

Using these procedures, the *Klebsiella* were determined to be *K. pneumoniae* serotype O1:K5. The isolates were negative for the virulence factors VT1 (verotoxin-1), VT2, east-1, EHEC, EIHEC, ipaH, EaggEC, DA, BFP, EAF, eae, LT, Stp, and STh using DNA probes.<sup>1,18</sup> These DNA probes are used in the diagnosis of diarrheagenic *E. coli* in humans<sup>14</sup> (Table 1).

In an attempt to document *Klebsiella* infection in the surviving affected dogs, serum samples from 10 dogs from the breeding kennel were submitted to the IEKRC for testing of antibodies against the capsular (K5) and somatic (O1) antigen of the outbreak strain. The blood samples were drawn 4 weeks after the outbreak occurred. Three dogs had exhibited gastrointestinal signs similar to those of the 2 dogs of this report. The remaining 7 dogs had no clinical signs according to the kennel owner; they had never been seen by a veterinarian.

Determination of antibody titer against the O-antigen was done by classical test tube agglutination using an autoclaved broth culture (120 C, 1 hour) of the O1 prototype strain (Friedländer 204) as antigen. A titer of 1:320 was considered positive. None of the 10 serum samples showed an antibody

**Table 1.** Culture and immunohistochemistry (IHC) results for 2 Bordeaux mastiffs with severe enteritis.

Dog	Bacterial culture	IHC*
Female		
Intestine	<i>E. coli</i> , <i>Pseudomonas</i> , <i>Enterobacter</i>	+
Liver	<i>Klebsiella</i>	ND
Lung	<i>Klebsiella</i>	+
Kidney	<i>Klebsiella</i>	ND
Lymph node	<i>Klebsiella</i>	+
Male		
Intestine	<i>Klebsiella</i>	+
Liver	<i>Enterococcus</i>	ND
Lung	<i>Klebsiella</i> , <i>Enterococcus</i>	ND
Kidney	<i>Klebsiella</i>	+
Lymph node	<i>Enterococcus</i>	ND

\*ND = not done.

response against the capsular (K5) antigen, but 6 dogs had high antibody titers against somatic (O1) antigen of the strain associated with the outbreak (2 dogs had titers of 1:2,560, 4 dogs had titers of 1:5,120). Two dogs had low titers (1:320, 1:640), and 2 dogs had negative titers (1:80) against the somatic (O1) antigen. Of the 10 dogs tested, 3 exhibited clinical signs. The titers for these symptomatic dogs were 1:5,120, 1:5,120, and 1:80.

Several surviving dogs that did not manifest obvious clinical signs had high O-antigen titers, suggesting that infection was widespread throughout the kennel. Titer levels found in the normal dog population are unknown. However, the titer levels found in these dogs were equal to those found in rabbits after undergoing an intensive immunization course. The degree of titer elevation suggests infection rather than normal gastrointestinal tract colonization. The surviving dogs that were tested may have been able to mount a sufficient antibody response to resist infection. One dog that had exhibited severe gastroenteritis and fever had a negative antibody titer.

The fact that these dogs did not show an antibody response against the K5 antigen is not surprising because the particular capsule type that was isolated in these cases has been shown to illicit a very poor antibody response, termed immunologic paralysis, in other species.<sup>11</sup> A similar phenomenon may occur in dogs and could explain why none of the 10 dogs had (measurable) antibodies to the K5 capsular antigen and why 1 dog was unable to mount an antibody response to the O1 antigen of the outbreak strain. The inability to mount an antibody response would have predisposed this dog to development of clinical signs.

In an attempt to isolate the source of the *Klebsiella* infection, dog food from the kennel was submitted for bacterial culture. This culture was negative; however, the owner had been unable to supply the same bag of food as was used during the outbreak. Thus, the food could not be ruled out as a source of the infection.

Enteropathogenic bacteria can produce intestinal disease by several mechanisms: invading and damaging intestinal epithelium, producing enterotoxins that stimulate intestinal secretion, producing cytotoxins, and/or attaching and effac-

ing microvilli.<sup>12</sup> Potential enteropathogens include *Campylobacter jejuni*, *Clostridium perfringens*, *E. coli*, and *Salmonella* spp.; however, these organisms are implicated in less than 4% of acute diarrhea cases in dogs.<sup>15</sup>

*Klebsiella pneumoniae* is a commensal organism in dogs and cats; however, its importance in causing diarrhea is unknown. One report documented the isolation of this organism from 2 dogs with diarrhea.<sup>15</sup> Some strains of *K. pneumoniae* have been shown to produce an enterotoxin, similar to the heat-stable enterotoxins of *E. coli*, which stimulate secretion of fluid by mediating activation of the guanyl cyclase-cyclic guanosine monophosphate mechanism. In the rat, *Klebsiella* enterotoxin produces net secretion of water, sodium, and chloride in both jejunal and ileal segments. At high doses of enterotoxin, morphologic changes occur in the mucosa.<sup>11</sup> With these characteristics, *Klebsiella* could be implicated as an enteropathogen.

This outbreak of gastrointestinal disease involving 7 adult animals (46% morbidity, 57% mortality) from a breeding kennel was most likely the result of primary intestinal infection with *K. pneumoniae* serotype O1:K5 and subsequent sepsis. This particular capsular type (K5) has been of pathogenic significance in genital infections in mares<sup>1</sup> and in bovine mastitis.<sup>3</sup> In both necropsied dogs, the gastrointestinal tract appeared to be the primary site of infection. Both dogs exhibited clinical signs associated with gastrointestinal disturbances (i.e., vomiting and bloody diarrhea) in the initial phase of illness, and the intestinal lesions appeared to be the most significant and advanced of the lesions observed at necropsy. There was no evidence of a viral pathogen in either dog by commercial parvovirus tests<sup>a</sup> or by fluorescent antibody testing at necropsy. Although *K. pneumoniae* was not isolated from the intestinal tract of the female dog, there were bacteria morphologically compatible with *Klebsiella* associated with the intestinal lesions, and immunohistochemical staining demonstrated that these bacteria reacted with the *Klebsiella* K5 antiserum. Several other organisms were isolated from the intestines of the female dog and may have competed with the *Klebsiella*, making isolation of this organism more difficult. Additionally, 3 days of antimicrobial therapy may have affected culture results. The presence of the organisms detected by histopathology and immunohistochemistry suggests intestinal infection with *Klebsiella* in spite of a negative bacterial culture.

For a pathogen to cause diarrhea, it must be ingested in sufficient quantity to survive the protective mechanisms of the host.<sup>12</sup> These affected dogs were overwhelmed by large numbers of bacteria, were unable to mount a sufficient immune response to the bacterial infection, or were affected by a combination of these factors. These dogs had had no health problems prior to this incident; thus, it is unlikely they suffered from any type of immunodeficiency. However, *K. pneumoniae* does have a capsule that may prevent binding of antibody or complement factors, thus enabling the organisms to avoid detection by neutrophils,<sup>19</sup> even in immunocompetent hosts. The capsule hinders phagocytosis, allowing the bacteria to multiply and spread. Once intestinal mucosa is compromised, bacteremia can occur, resulting in severe sepsis as seen in these dogs.

The source of the infection in this disease outbreak re-

mains unknown. Possibilities include the food (which was a nonpreserved meat product), the environment, or human contact. The environment seems an unlikely source because the kennel was extremely clean and well managed. Wood shavings have been implicated in *Klebsiella* infections in horses and livestock,<sup>3</sup> but these dogs were housed in dry concrete runs. The owner of the kennel was a human health-care provider. The owner may have introduced the infection, but neither the owner nor the family members had any clinical signs; therefore, this source also seems unlikely. The food is the most likely source, possibly as the result of improper preparation or storage. The fact that *Klebsiella* was not cultured from the food tested does not negate the possibility of potential food contamination; the owner was unable to provide the same lot of food that was in use when the outbreak began.

Although the source of infection was undetermined, necropsy results support an outbreak of *K. pneumoniae* serotype O1:K5 enteritis and septicemia, with high morbidity and mortality. Based on the predominance of the gastrointestinal symptoms, the multiple dogs affected, the dietary history, and the necropsy findings, the systemic *Klebsiella* infection most likely originated within the gastrointestinal tract via bacterial replication and caused the death of these animals.

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## ***Listeria monocytogenes* septicemia in a Thoroughbred foal**

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**Abstract.** *Listeria monocytogenes* septicemia was diagnosed in a 6-day-old Thoroughbred foal. Primary clinical signs included fever, depression, diarrhea, and respiratory distress. Hematologic abnormalities included leukopenia, neutropenia, degenerative left shift, and hyperfibrinogenemia. Clinical chemistry and blood gas abnormalities included metabolic acidosis, hypoxemia, hypocapnia, hypoglycemia, and hyponatremia. Despite aggressive therapeutic intervention and intensive care, the foal died within 12 hours of admission. A postmortem examination was performed, and the primary gross lesion was bilaterally severe, focally extensive bronchopneumonia. Histopathology revealed severe subacute multifocal suppurative bronchopneumonia with necrotizing vasculitis and intralesional coccobacilli. Cultures of blood collected at admission and immediately prior to death were positive for *L. monocytogenes*, as were cultures obtained from lung and liver at necropsy. Immunohistochemical examination of formalin-fixed tissues revealed abundant intra- and extracellular *L. monocytogenes* antigen within the lung and intravascularly in multiple organs.

*Listeria monocytogenes* is rarely reported as a cause of septicemia in equine neonates; only 3 case reports have appeared in the veterinary literature.<sup>1,8,14</sup> One of the reported cases was in a 4-week-old foal with concurrent combined immunodeficiency, and a second case involved a 3-week-old foal with a 2-week clinical history. Here, we describe the clinical course, laboratory findings, microbiology results, and pathologic findings in a 6-day-old Thoroughbred foal.

A 6-day-old Thoroughbred filly foal was examined because of a 3-day period of diarrhea and depression. The filly had been born postmature at 5 weeks past the expected parturition date, based on last known breeding date. Parturition was reportedly normal, and the placenta was reportedly in-

tact and of normal appearance. Diarrhea had been treated with an intestinal protectant<sup>a</sup> and had reportedly resolved, but depression continued unabated. The filly was mildly anemic prior to referral (packed cell volume ≈ 20%) and was treated with an oral iron supplement source, which the farm manager reported the foal aspirated.

At presentation, the foal was standing and able to walk into the stall. General appearance was thin and unthrifty, and attitude was dull and depressed. Rectal temperature of 101.4 F and heart rate of 90 beats/minute were within normal limits. The filly was severely tachypneic at 80 breaths/minute and moderately dyspneic. Clinically, the foal was approximately 8% dehydrated. Generalized harsh lung sounds were ausculted, and a red sticky fluid, similar in appearance to the iron supplement, was present in and around both nares. There was a generalized decrease in borborygmi and evidence of moderate colic. Dried diarrhetic feces were present on the perineum. The umbilical cord remnant was dry and palpably within normal limits for a 6-day-old foal. Abdom-

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