Three 6-week-old pigs were submitted alive for necropsy to the Animal Disease Diagnostic Laboratory at Purdue University. The pigs were from a farm that operated a multisite production system, with a farrowing site and a wean-to-finish site. Among the 4,400 pigs at the wean-to-finish site, there was an outbreak of illness with an estimated morbidity rate of 20% and estimated mortality rate of 8%. Pigs at the wean-to-finish site had been born within 2.5 weeks of each other and had been weaned at 3 weeks of age. The 3 pigs submitted (all 6 weeks old) for euthanasia and necropsy were from the wean-to-finish site and had a 1-month history of diarrhea, wasting, and a rough coat. All pigs at the wean-to-finish site had been treated with gentamicin in the water supply for 3 days followed by neomycin in the water supply for 9 nonconsecutive days (5 days of treatment, followed by an interruption of indeterminate duration, followed by 4 days of treatment). Sick pigs had been injected IM with ceftiofur.

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Histopathologic and Additional Test Findings

Specimens of major organs and tissues were collected, fixed in neutral-buffered 10% formalin, routinely processed, embedded in paraffin, sectioned, stained with H&E stain, and examined by light microscopy. Enteric lesions in all 3 pigs were confined to the spiral colon. The colon contained multiple mucosal erosions and ulcers that extended into the submucosa (Figure 2). The defects were partially covered by a thick exudate of fibrin admixed with cellular debris, neutrophils, degenerated leukocytes, hemorrhage, and rod-shaped bacteria measuring approximately 1 by 3 μm. The remaining lamina propria in affected areas was expanded by a mixed population of inflammatory cells (predominantly lymphocytes, plasma cells, and neutrophils with fewer macrophages and eosinophils). The apparently normal, eroded, and ulcerated areas all contained variable numbers of protozoal organisms consistent with flagellates. Protozoal organisms were ovoid (approx 100 μm in length by 50 μm in width) with a kidney-shaped macronucleus and spherical micronucleus (consistent with *Balantidium coli* trophozoites); other organisms were spherical and 40 μm wide with a thick wall containing a kidney-shaped macronucleus (consistent with *B. coli* cysts). Multi-focally, there was dilation of crypts filled with cellular debris and degenerated leukocytes. Herniated cystic crypts that had replaced depleted submucosal lymphoid aggregates were scattered throughout the colon; the herniated cystic crypts corresponded to the suberosal white nodules observed grossly. Diffusely, crypt epithelial cells had greater than normal cytoplasmic basophilia and vesicular nuclei, and cysts had lost goblet cells and a mitotic index of 2 to 4 mitotic figures/crypt (indicative of regeneration). The lymph nodes in all 3 pigs had marked cortical hyperplasia characterized by enlarged lymphoid follicles with prominent germinal centers (ie, lymphoid hyperplasia). No histopathologic lesions were observed in the cecum. Gram staining revealed numerous gram-negative rod-shaped bacteria both individually and in colonies in the colonic lumen. Warthin-Starry stain revealed numerous rod-shaped bacteria in the colonic lumen.

Pooled samples of spiral colon from these pigs were submitted on the day of necropsy for multiplex real-time PCR assays for *Salmonella* spp, *Lawsonia intracellularis*, *Brachyspira hyodysenteriae*, and *Brachyspira pilosicoli*. The tissues were positive for *Salmonella* spp and negative for the other 3 pathogens. A group B *Salmonella* sp was subsequently isolated from sections of spiral colon submitted to the bacteriology laboratory at the Indiana Animal Disease Diagnostic Laboratory; this isolate was speciated as *Salmonella enterica* serotype Typhimurium var 5– at the National Veterinary Services Laboratories. Porcine circovirus type 2 antigens were not detected in sections of spiral colon by immunohistochemical analysis.

Morphologic Diagnosis and Case Summary

Morphologic diagnosis and case summary: necroulcerative and fibrinosuppurative colitis with intralesional bacteria (specifically *S. enterica* serotype Typhimurium var 5–) and myriad intralesional protozoans consistent with *B. coli* in pigs.

Discussion

The main differential diagnoses for the gross and histopathologic lesions observed in the case described in the present report were enteric salmonellosis, swine dysentery (infection with *Brachyspira hyodysenteriae* or the provisionally designated *Brachyspira hampsonii*), and porcine proliferative enteritis (infection with *Lawsonia intracellularis*).1,2 Pigs with swine dysentery typically remain alert and have mucoid bloody diarrhea. Lesions in the intestinal tract associated with swine dysentery are also diffuse, shallow, and restricted to the large intestine, with occasional mild lymph node enlargement. Silver staining of large intestine tissue sections reveals large numbers of spirochetes along surface epithelial damage and within the lumen of crypts.1 Distinguishing characteristics of porcine proliferative enteritis are more severe ileal involvement with mild lesions that are generally confined to the proximal portion of the colon, variable lymph node enlargement, and a markedly hyperplastic mucosa underlying the necrotic membrane. Small curved rods free in the apical cytoplasm of hyperplastic epithelial cells are usually visible with Warthin-Starry staining.1 Other diseases that can cause diarrhea in weaned pigs include infection with rotavirus, coronavirus, *Escherichia coli*, *Trichuris suis*, or *Isospora suis*.1

For the pigs of the present report, clinical history, gross lesions, histologic findings, results of histochemical staining, positive results of bacterial culture for *S. enterica* serotype Typhimurium var 5– (previously *Salmonella* Typhimurium var Copenhagen), and a positive real-
time PCR assay for *Salmonella* spp were consistent with a diagnosis of enteric salmonellosis. The identification of *B. coli* in sections of diseased colon from these pigs was of no clinical importance; *B. coli* is a commensal organism that commonly proliferates during episodes of colonic disease and is rarely a primary pathogen.

Enteric salmonellosis develops most commonly in pigs after weaning to approximately 4 months of age. It is most often caused by infection with *Salmonella Typhimurium* (group B salmonellae) and less frequently *Salmonella enterica* serotype Choleraesuis (group C, salmonellae) or *Salmonella enterica* serotype Heidelberg (group B salmonellae). Initial clinical signs of enteric salmonellosis include watery yellow diarrhea of 3 to 7 days’ duration, although the diarrhea can wax and wane for several weeks. Affected pigs are commonly febrile, anorexic, and dehydrated. The mortality rate is low, but the morbidity rate is high, with many pigs remaining as subclinical carriers and intermittent shedders for at least 5 months.1,3

Pathological changes induced by *Salmonella* organisms differ with the serotype responsible for infection and disease. Serotypes of *Salmonella* can be broken down into 3 broad classes: ubiquitous serotypes that induce acute but self-limiting enteritis in a broad range of hosts; host-specific serotypes associated with severe systemic disease, which may not involve diarrhea, in healthy adults of a single species; and host-restricted serotypes primarily associated with systemic disease in a single host.4 In pigs, *Salmonella Typhimurium* represents a ubiquitous serotype, whereas *Salmonella Choleraesuis* represents a host-specific serotype. Results of serotyping provide clinicians with an expectation of the gross and histopathologic lesions likely to develop in infected individuals of a particular species. Serotyping of salmonellae is done by direct slide agglutination or tube agglutination by use of various antisera on pure colonies.5

Fecal-oral transmission is the most likely mode of transmission for virulent salmonellae. Experimentally, 107 organisms/g of intestinal content are required to cause lesions associated with enteric salmonellosis.1 Alterations to the intestinal environment as a result of administration of antimicrobials or cold-induced alterations in motility can decrease the number of organisms required to induce disease.1

In pigs, the severity of disease caused by salmonellae varies by serotype, bacterial virulence, natural and acquired host resistance, route of infection, and quantity of infective dose.1,6 Once salmonellae have become established, they cause intestinal pathological changes via a variety of mechanisms. Major mechanisms of enterocyte damage include toxic effects of certain outer membrane proteins as well as lipid A associated with lipopolysaccharide, microvascular thrombosis (probably in response to locally produced endotoxin), and chemical products of mucosal inflammation.1

Pigs with enteric salmonellosis can harbor this bacterium subclinically in their tonsils, intestinal tract, and gut-associated lymphoid tissue, where it can serve as a source of reinfection or spread to other pigs. Results of a recent study7 in young pigs indicated that increased serum cortisol concentration after 24 hours of feed withdrawal or IM injections of dexamethasone increased the number of *Salmonella Typhimurium* in the intestines. This highlights the role that stress, mediated through cortisol, has in recrudescence of enteric salmonellosis.1

Gross lesions that typify enteric salmonellosis in pigs include focal to diffuse necrotizing colitis or typhlitis with rare involvement of the distal portion of the small intestine. Necrotizing typhlitis, colitis, and enteritis appear as an adherent gray-yellow (pseudomembranous) membrane on the mucosal surface. Lesions are mainly confined to the intestinal tract with rare systemic dissemination and septicemia. Marked lymphadenopathy is also a typical gross finding of both the mesenteric and inguinal lymph nodes.1,3 Histopathologic lesions in the pigs for the present report were typical for enteric salmonellosis. Although not observed in these pigs, the liver may contain paratyphoid nodules; however, this is much more common in cases of septicemic salmonellosis. Paratyphoid nodules are clusters of histiocytes amid foci of acute coagulative hepatocellular necrosis.1

Antimicrobial treatment for enteric salmonellosis in pigs is controversial. The evidence for treatment is based on results of studies designed to test the prophylactic efficacy of drugs and not their therapeutic efficacy. In trials designed specifically to test therapeutic efficacy of drugs, antimicrobials were considered to have little merit.1 However, antimicrobials decrease the extent of transmission and diminish the severity of disease in pigs that become infected after beginning a course of antimicrobials. Antimicrobials are ideally chosen on the basis of results of microbial culture and antimicrobial susceptibility testing of the organism isolated during the outbreak. Antimicrobial use is also controversial because of the concern regarding increased antimicrobial resistance in salmonellae that may contaminate the food supply. Salmonellae are most commonly resistant to streptomycin, sulfisoxazole, and tetracycline, which have a long history of use in livestock and animals.8 Evidence for increasing resistance of salmonellae is supported by the decreasing pansusceptibility among pigs to antimicrobials (38.1% in 2000 and 20.4% in 2006) and increasing proportion of isolates that are resistant to ≥3 antimicrobials (32.8% in 2000 and 57.7% in 2006).8

Besides antimicrobials, successful treatment of enteric salmonellosis in pigs relies heavily on husbandry practices such as removal and isolation of sick animals, regimented pen cleaning, frequent cleaning of water bows, restriction of animal and staff movement, decreasing crowding and stress, and increasing pig comfort.1

References