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# **Pathology in Practice**

In collaboration with the American College of Veterinary Pathologists

### History

A 45-day-old female Canary Black Pig (an autochthonous endangered breed) was submitted to the Faculty of Veterinary Medicine of the University of Las Palmas de Gran Canaria, Spain, for postmortem examination. The animal came from a farm with 28 breeders (25 sows and 3 boars), located on the southeast side of the Gran Canaria Island. The pig had been found outside the farm wandering near rubbish dumps; 2 days later, it exhibited lethargy, dehydration, hypothermia, and weight loss along with vomiting and mucopurulent nasal discharge. The animal died within a day after the clinical signs were noticed. No other pigs showed any clinical signs.

## **Clinical and Gross Findings**

The gross examination revealed severe lesions in the cecum, large intestine, liver, and palatine tonsils. From the serosal aspect of the spiral colon, multiple well-demarcated white nodules with occasional hemorrhage were observed (Figure 1). These areas corresponded to multifocal to coalescing, well-demarcated foci of mucosal necrosis (button ulcers), covered with fibrinosuppurative (diphtheritic) membranes. In the liver, a locally extensive 5-cm-wide area of necrosis was observed on the visceral surface of the caudate lobe. On cut surface, the necrotic lesion extended deep into the parenchyma. The palatine tonsils had focal and bilateral areas of necrosis and congestion. Other gross findings were enlarged and congested mesenteric lymph nodes, serous abdominal effusion, and gas-distended small intestine.

#### Formulate differential diagnoses, then continue reading.

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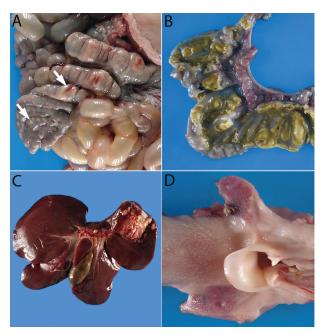
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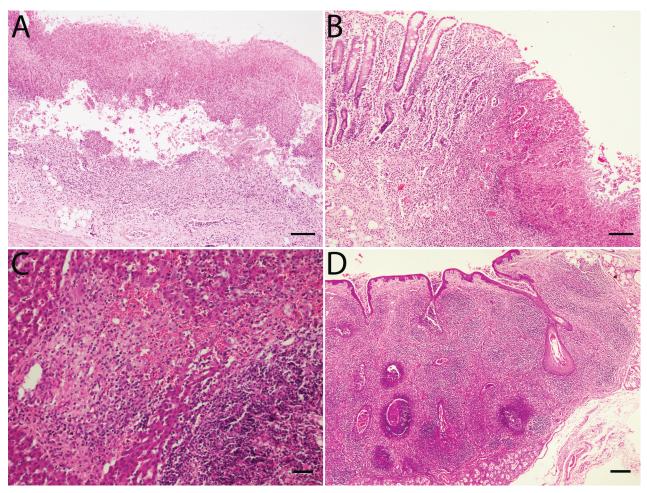
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**Figure I**—Postmortem images of the colon (A and B), liver (C), and palatine tonsils (D) of a 45-day-old female Canary Black Pig that had died a day after developing lethargy, dehydration, hypothermia, weight loss, vomiting, and mucopurulent nasal discharge. A—Evident are multifocal, well-demarcated, pale tan to yellow nodules (arrows) with occasional hemorrhage on the serosal surface. B—There are multifocal to coalescing areas of mucosal necrosis (button ulcers) covered with yellow fibrinonecrotic debris. C—The caudate lobe of the liver has a locally extensive area of necrosis. D—There are bilateral areas of focal necrosis of the palatine tonsils.

# **Histopathologic Findings**

Samples from the gastrointestinal tract, liver, kidneys, spleen, mesenteric lymph nodes, palatine tonsils, heart, lung, brain, and urinary bladder were fixed in neutral-buffered 10% formalin, embedded in paraffin-wax, sectioned at 4-µm slice thickness, and then stained with H&E stain and Gram stain for histologic examination. Microscopically, numerous, well-demarcated areas of necrosis affecting both the mucosa and the submucosa of the cecum and spiral colon were observed. These areas were covered with fibrin, admixed with large numbers of neutrophils and cellular debris. The surrounding tissue was hyperemic, congested, and infiltrated by macrophages, lymphocytes, and neutrophils; occasional thrombi were noted **(Figure 2)**. The liver



**Figure 2**—Photomicrographs of the colon (A and B), liver (C), and palatine tonsils (D) of the pig described in Figure I. A—The colon has necrotic mucosa overlain by a thick diphtheritic membrane composed of fibrin, neutrophils, and necrotic cellular debris. H&E stain; bar = 100  $\mu$ m. B—Full-thickness necrosis of the mucosa with mixed inflammatory infiltrates is evident. H&E stain; bar = 100  $\mu$ m. C—An extensive area of liver necrosis is surrounded by neutrophils, macrophages, and lymphocytes. H&E stain; bar = 40  $\mu$ m. D—A section of palatine tonsil shows multifocal areas of necrosis with cellular debris and neutrophils. The lumens of the crypts are expanded by cellular and karyorrhectic debris. H&E stain; bar = 200  $\mu$ m.

had multifocal, randomly distributed paratyphoid nodules, characterized by foci of lytic and coagulative hepatocellular necrosis, surrounded by numerous neutrophils, macrophages, and lymphocytes, with hemorrhage and fibrin. Portal vessel walls were occasionally infiltrated by moderate numbers of neutrophils and macrophages, with rare lymphocytes (vasculitis). The lumina were rarely occluded by fibrin thrombi. In the palatine tonsils, there were extensive areas of lymphoid necrosis and degenerate neutrophils; the crypts contained karyorrhectic cellular debris. In the spleen and mesenteric lymph nodes, there was congestion, microthrombosis, and multifocal small areas of neutrophilic infiltration. Mild lymphoid depletion was also noted in lymph nodes. The lung had multifocal bronchointerstitial pneumonia, with thickening of the alveolar septa by edema, moderate numbers of macrophages, and lymphocytes. The alveolar lumina were filled with edema, fibrin, and small numbers of macrophages,

sloughed epithelial cells, and cellular debris. The lumen of bronchi and bronchioles often contained mucus and degenerate neutrophils. Multifocal microthrombi and acute hemorrhage were seen in the brain neuroparenchyma and leptomeninges. Gram stain highlighted intralesional gram-positive and, to a lesser extent, gram-negative rod-shaped bacteria in the large intestine and palatine tonsils and small to moderate numbers of gram-negative bacteria in the liver.

# **Clinicopathologic Findings**

Bacterial analyses including typing (reference laboratory, Algete-Madrid, Spain) identified *Salmonella enterica* subspecies enterica serotype Enteritidis 9,12:g.m. from samples of large intestine, liver, and mesenteric lymph nodes. All the other animals from the same farm tested serologically negative for *Salmonella*.

### Morphologic Diagnosis and Case Summary

Morphologic diagnosis: Necrotizing, fibrinosuppurative (diphtheritic), multifocal, severe, subacute typhlocolitis, with mixed bacteria and thrombosis. Necrotizing, neutrophilic and lymphohistiocytic, multifocal random, moderate hepatitis, with bacteria, vasculitis, and thrombosis. Necrotizing, neutrophilic, locally extensive, bilateral, moderate tonsillitis, with bacteria. Bronchointerstitial, lymphohistiocytic and neutrophilic, multifocal, moderate pneumonia.

Case Summary: disseminated salmonellosis by *Salmonella enterica* serovar Enteritidis in a Canary Black Pig.

#### Comments

Salmonellosis is one of the main causes of enteric disease in humans worldwide. Poultry and pigs are the main reservoirs of the zoonotic strains of *Salmonella enterica*.<sup>1</sup> In general, avian infections are associated with serovar Enteritidis, whereas porcine infections are usually caused by serovar Typhimurium or serovar Derby.<sup>1</sup> *Salmonella enterica* serovar Enteritidis (*S* Enteritidis) is not adapted to a specific animal host; therefore, different species may be infected. Sporadic cases of *S* Enteritidis have been reported in healthy fattening pigs<sup>2,3</sup> and in wild boars with no clinical signs<sup>4</sup> in various European countries. To the authors' knowledge, the present report was the first to describe salmonellosis in a Canary Black Pig caused by *Salmonella enterica* serovar Enteritidis.

Depending on the host's susceptibility and the serotype of *Salmonella* involved, the infection course may vary. Route of infection is usually oral; multiplication of bacteria occurs in the intestinal lumen, and it is followed by adhesion and invasion of the intestinal mucosa (enterolytic form). The acute coagulative necrosis of the intestinal wall is attributed to bacterial toxins as well as enzymes and chemical mediators released during the inflammatory process.<sup>5,6</sup> Mucosal macrophages are thought to be responsible for spreading the bacterium to secondary sites of infection, such as the liver and the lymph nodes, and systemically (septicemic form).<sup>5,6</sup>

The histopathologic lesions observed in the present case were similar to those observed in the septicemic form associated with *S* Choleraesuis infection,<sup>7</sup> as different organs were involved. This contrasted with what was previously reported in pygmy hogs during an outbreak of *S* Enteritidis, where only intestinal lesions were described.<sup>8</sup>

Transmission can occur directly after contact with carriers or indirectly after exposure to water, feed, or litter contaminated by birds, rodents, or other carriers.<sup>6,9</sup> Various predisposing factors have been associated with salmonellosis, such as transportation, starvation, changes in rations, overcrowded environment, exertion, concurrent diseases, and oral treatment with antimicrobials and anthelmintics.<sup>9</sup> Although none of those were identified in this specific case, individual susceptibility and the age of the animal could have been considered predisposing factors, as disease is usually more common and severe in young individuals.

In an experimental study<sup>1</sup> with pigs inoculated with S Enteritidis, the authors concluded that the pathogen was more invasive than S Typhimurium. That could explain the acute course of the clinical signs in our case.

In the present case, it was possible that contaminated material at the reported small rubbish dump was the source of infection. The fact that all the other animals at the farm tested negative for *Salmonella* supported this hypothesis. Housing of pigs in facilities other than total confinement is a factor associated with infection.<sup>6</sup>

In summary, we described the pathological findings in a case of salmonellosis by S Enteritidis in a Canary Black Pig. The reasons why these findings are of importance are as follows: (1) the increasing interest of the European Union in determining the exact prevalence of Salmonella spp in the porcine industry to design adequate control strategies; (2) the higher probability of infections occurring in this breed due to the fact that breeding is carried out in small farms; and (3) the importance of infectious diseases research in an indigenous endangered breed of valuable genetic potential. Canary Black Pigs are exclusive to the Canary Islands archipelago and were officially recognized after being included in the Official Register of Spanish Livestock Breeds published in the Real Decreto (Royal Decree) 1682/1997.10

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