Salmonella enterica serotype Choleraesuis infection in weaned pigs: a first clinicopathological case report from Korea

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Abstract

Salmonella enterica serotype Choleraesuis causes swine paratyphoid, with clinical findings of enterocolitis and septicemia. However, the clinicopathological features of S. Choleraesuis infections in pigs have not been reported in Korea. We describe the pathological findings of two weaned pigs with S. Choleraesuis infections, presenting with diarrhea, cough, and sudden death. Pathological examination indicated severe necrotic colitis in pig 1 and septicemic lesions in pig 2. Multidrug-resistant S. Choleraesuis was isolated from the pigs’ lungs and intestinal contents. Further research is required for the surveillance of S. Choleraesuis infections in pigs and the virulence estimation in the S. Choleraesuis isolates.

Keywords: enterocolitis; pathology; pigs; Salmonella Choleraesuis; septicemia
tions in weaned pigs in Korea.

In August 2020, a farrow-to-finish pig farm in Yangsan, Republic of Korea, breeding 2,000 pigs, reported lethargy and sudden death of weaned pigs (40 days old) that exhibited mucoid diarrhea and cough. Twenty-five percent (100/400) of the weaned pigs exhibited clinical signs, and 20 pigs died (mortality rate, 5%). Two weaned pigs were referred to the Animal and Plant Quarantine Agency for necropsy and differential diagnosis.

Both pigs exhibited non-collapsed lungs with consolidation. Pig 1 had severe hyperemic mucosa in the colon, with a yellow, fibrinous membrane and pasty contents (Fig. 1A). Pig 2 had consolidation in the cranioventral lobes and multifocal ecchymoses in the dorsocaudal lobes of the lungs (Fig. 1B). Furthermore, the mesenteric lymph nodes were enlarged and congested (Fig. 1C).

After necropsy, representative tissues, including the brain, lungs, liver, spleen, kidneys, small intestines, large intestines, and lymph nodes, were fixed in 10% neutral buffered formalin for 24 hours. The fixed tissues were processed according to a previous study [9], and 2-µm sections were stained with hematoxylin and eosin. Histopathologically, both pigs exhibited severe bronchointerstitial pneumonia (Fig. 2A). In pig 1, severe necrosis of the intestinal epithelial cells and cryptic dilation were observed, and submucosal infiltration of macrophages and lymphocytes were also detected in the colon (Fig. 2B). In pig 2, moderate perivascular infiltration of mononuclear cells with bacterial colonies and multifocal gliosis were observed in the cerebrum (Fig. 2C and D). Lesions corresponding to hemorrhages, lympholysis, and bacterial colonization were observed in the spleen and mesenteric lymph nodes of pig 2 (Fig. 2E-G). In addition, coagulative necrosis and bacterial colonies were observed in the liver of pig 2 (Fig. 2H).

The lungs and small and large intestinal contents with their gross lesions were aseptically collected and cultured onto sheep blood agar (Asan Pharmaceutical Co. Ltd., Korea) and MacConkey agar (Becton; Dickinson and Company, USA) under 5% CO₂ at 37°C for 24 hours. The identification of S. Cholerae-suis was confirmed using the AccuPower Salmonella spp. 3-plex polymerase chain reaction (PCR) Kit (Bioneer Corporation, Korea), according to the manufacturer's instructions. For the detection of major porcine viruses, including the porcine reproductive and respiratory syndrome virus (PRRSV), the classical swine fever virus (CSFV), the swine influenza virus (SIV), the porcine circovirus type 2 (PCV2), the transmissible gastroenteritis virus (TGEV), the porcine epidemic diarrhea virus (PEDV), and rotaviruses, we used the VDx PRRSV HPMP RT-PCR, CSFV 5’NCR RT-PCR, SIV RT-PCR, and PCV2 qPCR kits (Median Diagnostics Inc., Korea), the LiliF TGEV/PEDV RT-PCR kit (iNtRON Biotechnology Inc., Korea), and the PO-BGEN Rotavirus (A, B, C) detection kit (POSTBIO Inc., Korea), according to the manufacturer's instructions. In addition, serotyping procedures were performed as previously described [6]. Antimicrobial susceptibility testing was performed with the disc diffusion method according to a previous study [1]. The following discs (Oxoid Ltd., UK) were used: ampicillin (10 µg), cefotiofur (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), and tetracycline (30 µg). Escherichia coli ATCC 25922 was used as a control strain.

Salmonella-suspected colonies were isolated from the large intestinal contents (pig 1), lungs (pig 2), and small intestinal contents (pig 2). Based on the serotyping procedures, all Salmonella spp. isolates belonged to the C1 group, serovar 6,7,c: 1,5, and were confirmed as S. Choleraesuis via PCR (Fig. 3). In ad-
Fig. 2. Histopathological findings of *Salmonella enterica* serotype Choleraesuis infections in pigs. (A) Pig 1, lung: infiltration of inflammatory cells, including pulmonary alveolar macrophages with fibrin (translucent arrow and inset) in the alveolar spaces and numerous neutrophils (black arrows) in the bronchioles. H&E, scale bar: 100 µm; inset scale bar: 20 µm. (B) Pig 1, colon: necrotic intestinal epithelium (translucent arrows) and cryptic dilation with inflammatory cell debris (black arrows), and submucosal infiltration of mononuclear cells (asterisk). H&E, scale bar: 200 µm; inset scale bar: 20 µm. (C) Pig 2, cerebrum: moderate perivascular cuffing with mononuclear cells (black arrows) and intravascular bacterial colonies (asterisk). H&E, scale bar: 50 µm. (D) Pig 2, cerebrum: microglial nodules. H&E, scale bar: 50 µm. (E) Pig 2, spleen: severe hemorrhages (asterisk) and small lymphoid follicles (white arrows). H&E, scale bar: 200 µm. (F) Pig 2, spleen: splenic lympholysis (white arrows) with bacterial colonies (green translucent arrow). H&E, scale bar: 20 µm. (G) Pig 2, mesenteric lymph node: moderate hemorrhages and lymphoid depletion (asterisk). Inset: necrotic lymphocytes (black arrows) and bacterial colonies (blue translucent arrow). H&E, scale bar: 100 µm; inset scale bar: 20 µm. (H) Pig 2, liver: coagulative necrosis with necrotic hepatocytes (black arrows) and bacterial colonies (green translucent arrow). H&E, scale bar: 20 µm.

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PRRSV was detected in all the lung samples of both pigs. The isolates from lungs and intestines exhibited resistance to multiple antimicrobials (ampicillin, gentamicin, and tetracycline) according to the results of the antimicrobial susceptibility test. However, the only gross lesions found in the current study were enlarged and congested mesenteric lymph nodes. On the other hand, all the pigs on the farm were administered antimicrobials (colistin sulfate) by a veterinary practitioner after the onset of the clinical signs. These treatment attempts reportedly result in milder disease in cases of S. Choleraesuis infection [2].

Recently, the occurrences and characteristics of S. Choleraesuis in wild boars have been reported in Europe, suggesting the necessity of S. Choleraesuis surveillance in wildlife [1,5]. To the best of our knowledge, there is limited information on S. Choleraesuis isolates in Korea, and this is the first clinicopathological report of such infections in domestic pigs in Korea. Future surveillance investigations of S. Choleraesuis infections in domestic and wild pigs are required, and vaccines need to be considered to prevent a severe outbreak of this pathogen in Korea.

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Fig. 3. Multiplex polymerase chain reaction assay revealing the positive identification of *Salmonella enterica* serotype Choleraesuis isolates from the colonic samples. Lanes: M, 1-kb marker; ST, *Salmonella Typhimurium* positive control (843 and 307 bp); SC, *Salmonella enterica* serotype Choleraesuis-positive control (843 and 409 bp); pig 1 and pig 2 DNA from the isolates; N, negative control.
References


