**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

*Salmonella enterica* serotype Choleraesuis is a facultative intracellular pathogen that causes swine paratyphoid, with clinical manifestations of enterocolitis and septicemia [1]. Infection with the septicemic *S.* Choleraesuis is considered a differential diagnosis for pigs with African swine fever virus (ASFV), since it shows similar clinical signs and lesions [2].

In the 1950s and 1960s, *S.* Choleraesuis was the predominant serotype in the global pig industry [1]. However, it is at present rare in the European Union and Australia [1,3]. As *S.* Choleraesuis has diminished, *Salmonella* Typhimurium has become the most frequently isolated serotype [4]. Despite its low prevalence in pigs, *S.* Choleraesuis is becoming more prevalent in wild boars from Europe, which are suspected to be carriers [1,5]. Furthermore, antimicrobial resistance to *S.* Choleraesuis has been reported in many countries [1,2,5]. To control the occurrence of salmonellosis in pigs, antimicrobial administration to the affected animals is necessary, and antimicrobial susceptibility testing is required for the selection of empirical antimicrobials [5].

In Korea, there are many studies on salmonellosis in pigs; however, most are focused on *S.* Typhimurium, the most common serotype in Korea [6,7]. Although two isolates of *S.* Choleraesuis were isolated from diarrheic pigs in Korea in 2004, information on *S.* Choleraesuis in Korea is limited [8]. Herein, we describe the clinical signs, pathological lesions, and laboratory results of *S.* Choleraesuis infec-
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 Mac-Conkey agar (Becton; Dickinson and Company, USA) under 5% CO\(_2\) 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 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), ceftiofur (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-

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**Fig. 1.** Gross findings of *Salmonella enterica* serotype Choleraesuis infections in pigs. (A) Pig 1. Colon. Hyperemic colonic mucosa (asterisk) with yellow, fibrinous membrane. (B) Pig 2. Lungs. Non-collapsed lungs with consolidated cranioventral lobes and multifocal ecchymoses (black arrows). (C) Pig 2. Mesenteric lymph nodes. Enlarged and congested mesenteric lymph nodes (black arrows).
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.
Choleraesuis isolates in pigs needs to be elucidated through experiments. The diagnosis, and the pathogenic significance of these microbes (PRRSV, S. suis) in pigs on the farm in this study (5%), and systemic pathological lesions among pigs on the farm. The susceptibility of pigs to Choleraesuis infection. S. Choleraesuis isolates also exhibited resistance to the three antimicrobials most frequently used in pigs in Korea (ampicillin, gentamicin, and tetracycline) [13]. Further antimicrobial characterization studies with minimum inhibitory concentrations for a variety of antimicrobials is required to elucidate the antimicrobial resistance pattern of the S. Choleraesuis isolates.

African swine fever has been reported in domestic and wild pigs in Korea since September 2019 and remains a substantial threat to the pig industry [14]. Septicemic salmonellosis causes specific gross lesions, such as splenomegaly and congestive swelling in the lymph nodes, similar to ASFV infection [2]. 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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Pathological features of *Salmonella* Choleraesuis infections in weaned pigs

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