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<Case Report>

Salmonella enterica subsp. *enterica* infections in eastern great egrets (*Ardea alba modesta*)

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Abstract : Five eastern great egrets with a history of ataxia, wry neck, and wet feathers were submitted to the Veterinary Diagnostic Center for pathologic examination. Slightly enlarged livers with diffuse white-grayish nodules were observed. Microscopically, the hepatic and lung parenchyma contained granulomatous lesions consisting of central necrosis. Some hearts showed myofiber necrosis with infiltration of histiocytes and heterophils. Partial *16SrRNA* and *gyrB* gene sequences of all isolates showed high similarities (99–100%) to those of *Salmonella* (*S.*) *enterica* subsp. *enterica*. Based on pathological and molecular biological results, *S. enterica* subsp. *enterica* systemic infections were diagnosed in eastern great egrets of Korea.

Keywords : Korea, *Salmonella*, egret, infection

Salmonella spp. is a group of gram-negative facultative anaerobic pathogenic bacteria that has been isolated from a variety of mammals and avian species. Salmonellosis outbreaks have been reported in various wild birds including gulls, ducks, and passerine birds [11]. Salmonellosis in wild birds has long been recognized as a potential health risk to humans and livestock [6, 10]. *Salmonella* (*S.*) *enterica* subsp. *enterica* infections appear as primary enteropathogens that cause gastroenteritis and serious systemic infections in young or emaciated birds [3].

The eastern great egret (*Ardea alba modesta*), a white heron in the genus *Ardea*, is a subspecies of the great egret (*Ardea alba*). There have been several reports about egrets infected by viruses, including eastern equine encephalitis virus [2], Newcastle disease virus [13], and influenza virus [15]. A previous report about cattle egrets in Texas showed 17 *S. enterica* subsp. *enterica* serotypes that were isolated in the digestive tracts, spleens, and livers of nestling egrets [7]. A previously published study suggests that *Salmonella* infection in herons is much more common than expected [5]. We report here the first cases of *S. enterica* subsp. *enterica* infections in eastern great egrets of Korea.

In August 2012, 13 dead eastern great egrets (12 juveniles and one adult) were found along with one live juvenile egret in a Dukjin-pond in the urban area of Jeonju City. The live egret presented with ataxia, wry neck, and wet feathers. The juvenile egret was euthanized due to poor prognosis and debilitated condition. Since 9 birds were severely decomposed, only five birds (four dead birds and one euthanized bird) were submitted to the Veterinary Diagnostic Center of Chonbuk National University for pathologic examination.

At postmortem examination, no ectoparasites or skin lesions were found. Grossly, the main lesions were slightly enlarged livers with multifocal white-grayish nodules, and there were no lesions in other organs (Fig. 1A). Swabs from blood, liver, and feces were cultured on blood agar plates (BAP) and tryptic soy agar (TSA) at 37°C for 24 h and subcultured to MacConkey agar. Single colonies from the liver and fecal cultures appeared white with no hemolysis on BAP and MacConkey agar. Colonies were separately re-cultured on TSA, and the isolates were stored at −70°C using Cryocare Bacteria Preservers (Key Scientific Products, USA). DNA was extracted from all isolates using a DNA mini kit (Qiagen, Germany). After the isolated pathogen was presumptively identified by partial *16SrRNA* sequencing, the primer sequences, PCR amplification, and *gyrB* gene sequencing were performed in accordance with a previous study [1]. DNA fragments were sequenced and analyzed by BLAST search and BLAST distance tree (National Center for Biotechnology Institute, USA).

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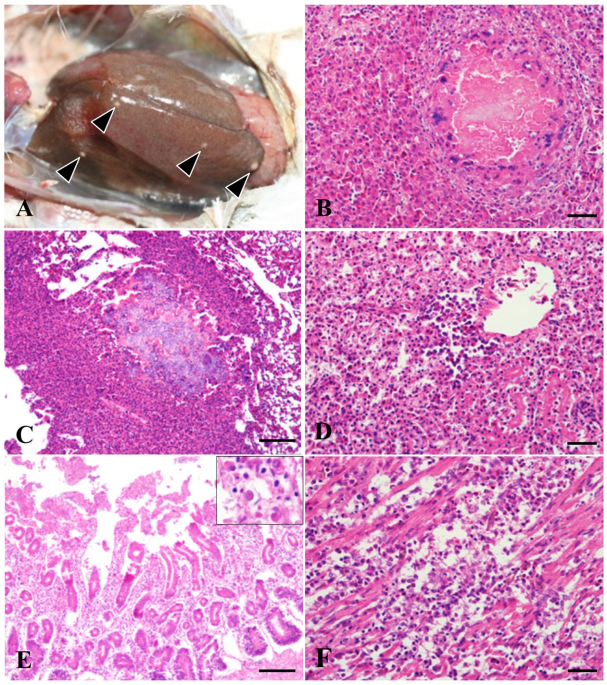

Fig. 1. Representative gross and microscopic lesions from egrets infected by *Salmonella*. (A) Multifocal distribution of white-grayish nodules (arrowheads) was observed in livers. (B) Necrotic foci surrounded by lymphocytes, liver. (C) Granulomatous lesions with central necrosis in parenchyma, lung. (D) Interstitial nephritis with infiltrations of mononuclear cells and heterophils, kidney. (E) Focal necrosis of villi and inflammation, (insert: mononuclear cell infiltration in lamina propria) small intestine. (F) Myofiber necrosis with infiltration of histiocytes and heterophils, heart. H&E stain. Scale bars 50 μm (C and E), =20 μm (B, D and F). 1,000× (insert in E).

An antibiotic susceptibility test (AST) was performed by the disc diffusion method using 9 different antibiotics (amikacin, gentamicin, enrofloxacin, ampicillin, amoxicillin/clavualnic acid, cefazolin, cefotazime, sulfamethoxazole/trimethoprime, and doxycycline) according to Clinical and Laboratory Standards Institute document M100-S17. Tissue samples from the heart, lung, liver, kidney, spleen, and intestine were collected, fixed in 10% neutral buffered formalin, and processed routinely for histopathology. Tissues sectioned at 6 μm were stained with hematoxylin and eosin (H&E) for histopathological examination.

Microscopically, there were granulomatous lesions in the hepatic and lung parenchyma consisting of central necrosis surrounded by lymphocytes (Fig. 1B and C). The kidneys were hyperemic and had interstitial nephritis with infiltrations of mononuclear cells and heterophils (Fig. 1D). Focal villi necrosis with mononuclear cell infiltration was also observed in the lamina propria of the small intestine (Fig. 1E). Two of the five hearts showed myofiber necrosis with infiltration of histiocytes and heterophils (Fig. 1F).

Bacterial cultures from blood and liver tissues showed growth of the suspected pathogens. A BLAST search using partial *16SrRNA* and *gyrB* gene sequences revealed that the etiologic bacteria were *S. enterica* subsp. *enterica*, as expected. Results showed 100% query coverage and high similarities (99–100%) with *16SrRNA* (GenBank No. CP002614.1) and *gyrB* (GenBank No. CP006602.1) gene sequences of *S. enterica* subsp. *enterica.* AST results indicated that the present isolates were susceptible to all antimicrobial drugs tested.

All submitted dead egrets showed systemic inflammation including necrotic foci and infiltration of heterophils and lymphocytes in the intestine, kidney, lung, and liver. Previous reports have proven these as characteristics of salmonellosis [5, 7, 8]. A previous report of *Salmonella* in cattle egrets showed a high incidence in young chicks with severe hepatitis characterized by necrotic cores with inflammatory cells [7]. This case also characteristically showed severe hepatitis with diffuse necrosis surrounded by mononuclear cells.

The *gyrB* gene encodes the subunit B protein of DNA gyrase (topoisomerase type II), which is an essential enzyme for DNA replication distributed universally among bacterial species. The *gyrB* gene sequences have been used widely in phylogenetic studies of *Salmonella, Shigella,* and *Escherichia coli* [1]. Previous studies of *gyrB* show that the *gyrB* gene is a suitable marker for investigating phylogenetic and taxonomic relationships at the species level. The rate of molecular evolution inferred from *gyrB* gene sequences is higher than that inferred from *16SrRNA* gene sequences. Therefore, *gyrB* gene sequence analysis is considered a more accurate method for identifying unknown bacteria than *16SrRNA* sequences [1, 14]. Although *gyrB* has limitations in identifying genes at a serotype level, it can reliably confirm genes to the subspecies level. Based on the use of *gyrB* to distinguish species, it is concluded that the gram-negative bacteria found in the eastern great egrets are *S. enterica* subsp. *enterica.*

In the current case, a group of the same species died in a limited area at the same time. *S. enterica* subsp. *enterica* was the only infectious bacteria found in common in these animals. Moreover, previous studies have indicated that salmonellosis results in high mortality in certain species of birds [9, 12]. Eastern great egrets might be vulnerable to *Salmonella* infection.

Because salmonellosis is a zoonotic disease, *Salmonella* infection in wild birds is a potential health risk to the public. Accumulating evidence has shown that *S. enterica* subsp. *enterica* infections in wild birds disseminate bacteria and cause salmonellosis in livestock, dogs, cats, and humans [6, 10, 11]. When *Salmonella* spreads to a farm or any place containing livestock, it can persist to cause lasting economic losses. In the current case, dead egrets were found in a pond of an urban area, which could have caused infection in humans or pet animals. Despite the latent danger to humans, there are not many cases or studies regarding salmonellosis in wild birds in Korea. Wild birds may function as a critical reservoir for the transmission of salmonellae to humans [4],

so it is necessary to investigate salmonellosis in wild birds from an epidemiological point of view.

Based on pathological and molecular biological results, *S. enterica* subsp. *enterica* systemic infections were diagnosed in eastern great egrets. To our knowledge, the present case is the first report that describes fatal salmonellosis in eastern great egrets in Korea. For reasons mentioned earlier, this case implies the importance of studying salmonellosis in wild birds in Korea.

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References

1. **Fukushima M, Kakinuma K, Kawaguchi R.** Phylogenetic analysis of *Salmonella*, *Shigella* and *Escherichia coli* strains on the basis of the *gyrB* gene sequence. J Clin Microbiol 2002, **40**, 2779-2785.
2. **Gottdenker NL, Howerth EW, Mead DG.** Natural infection of a great egret (*Casmerodius albus*) with eastern equine encephalitis virus. J Wildl Dis 2003, **39**, 702-706.
3. **Hébrard M, Kröger C, Srikumar S, Colgan A, Händler K, Hinton JC.** sRNAs and the virulence of *Salmonella enterica* serovar Typhimurium. RNA Biol 2012, **9**, 437-445.
4. **Kapperud G, Stenwig H, Lassen J.** Epidemiology of *Salmonella* Typhimurium O:4-12 infection in Norway: evidence of transmission from an avian wildlife reservoir. Am J Epidemiol 1998, **147**, 774-782.
5. **Locke LN, Ohlendorf HM, Shillinger RB, Jareed T.** Salmonellosis in a captive heron colony. J Wildl Dis 1974, **10**, 143-145.
6. **MacDonald JW, Bell JC.** Salmonellosis in horses and wild birds. Vet Rec 1980, **107**, 46-47.
7. **Phalen DN, Drew ML, Simpson B, Roset K, Dubose K, Mora M.** *Salmonella enterica* subsp. *enterica* in cattle egret (*Bubulcus ibis*) chicks from central Texas: prevalence, serotypes, pathogenicity, and epizootic potential. J Wildl Dis 2010, **46**, 379-389.
8. **Piccirillo A, Mazzariol S, Caliari D, Menandro ML.** *Salmonella* Typhimurium phage type DT160 infection in two Moluccan cockatoos (*Cacatua moluccensis*): clinical presentation and pathology. Avian Dis 2010, **54**, 131-135.
9. **Prescott JF, Hunter DB, Campbell GD.** Hygiene at winter bird feeders in a southwestern Ontario city. Can Vet J 2000, **41**, 695-698.
10. **Tauni MA, Österlund A.** Outbreak of *Salmonella typhimurium* in cats and humans associated with infection in wild birds. J Small Anim Pract 2000, **41**, 339-341.
11. **Tizard I.** Salmonellosis in wild birds. Semin Avian Exot Pet Med 2004, **13**, 50-66.
12. **Wilson JE, MacDonald JW.** *Salmonella* infections in wild birds. Br Vet J 1967, **123**, 212-218.
13. **Xie Z, Xie L, Chen A, Liu J, Pang Y, Deng X, Xie Z, Fan Q.** Complete genome sequence analysis of a Newcastle disease virus isolated from a wild egret. J Virol 2012, **86**, 13854-13855.
14. **Yamamoto S, Harayama S.** PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. Appl Environ Microbiol 1995, **61**, 1104-1109.
15. **Zhao G, Zhong L, Lu X, Hu J, Gu X, Kai Y, Song Q, Sun Q, Liu J, Peng D, Wang X, Liu X, Liu X.** Characterisation of a highly pathogenic H5N1 clade 2.3.2 influenza virus isolated from swans in Shanghai, China. Virus Genes 2012, **44**, 55-62.