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. Single colonies from the liver and fecal cultures were re-cultured on TSA, and the isolates were stored at −70°C using CryoCult (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).
An antibiotic susceptibility test (AST) was performed by the disc diffusion method using 9 different antibiotics (ampicillin, amoxicillin, cefazolin, cefotaxime, gentamicin, enrofloxacin, metronidazole, ceftriaxone, and cefotaxime) 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 16S rRNA and gyrB gene sequences revealed that the etiologic bacteria were Salmonella enterica subsp. enterica, as expected. Results showed 100% query coverage and high similarities (99–100%) with 16S rRNA (GeneBank No. CP002614.1) and gyrB (GeneBank No. CP006602.1) gene sequences of Salmonella 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 16S rRNA gene sequences. Therefore, gyrB gene sequence analysis is considered a more accurate method for identifying unknown bacteria than 16S rRNA 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 Salmonella enterica subsp. enterica.

In the current case, a group of the same species died in a limited area at the same time. Salmonella enterica subsp. enterica was the only infectious bacteria found 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 Salmonella 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].
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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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