NOTE *Avian Pathology*

Riemerella anatipestifer infection in domestic ducks in Japan, 2014

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ABSTRACT. The outbreak of *Riemerella anatipestifer* (RA) infection has been confirmed in meat-type domestic ducks (*Anas platyrhynchos*) for the first time in 27 years in Japan. In January 2014, increased mortality in a 14- to 21-day-old duck flock was reported to veterinary officials by the owner. The affected ducks exhibited reduced movement, ataxia and dorsal recumbency with leg paddling. Pathological findings were typical for an RA infection. Fibrinous and heterophilic pericarditis, airsacculitis, perihepatitis, ventriculitis and meningitis were observed. The bacterial isolate from duck organs was identified as RA by PCR-based 16S ribosomal RNA sequencing.
KEY WORDS: duck, Japan, pathology, *Riemerella anatipestifer*

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Riemerella anatipestifer (RA) is a non-spore forming, non-motile, gram-negative rod [17]. RA has a complex classification history. Before the latest reclassification to the genus *Riemerell*a, this bacterium previously belonged to the genera *Pfeifferella*, *Pasteurella* or *Moraxella* [17, 20].

RA infection is a bacterial disease that primarily affects domestic ducks [5, 11, 17], and young ducks are more susceptible to the disease than adults [12, 17]. RA infection occurs as an acute or chronic form of the disease characterized by fibrinous pericarditis, perihepatitis, airsacculitis and meningitis [12, 17]. RA transmission can occur horizontally via the respiratory tract and skin wounds [17], whereas vertical transmission of RA via eggs is controversial [7, 8, 13]. Some studies documented that RA can be a part of the normal flora in the throat of some domestic and wild duck species [2, 18].

RA infection has been reported in the duck industry worldwide. However, to the best of our knowledge, an outbreak of RA infection has not been confirmed since 1987 in Japan [1, 19, 21]. In 2014, we encountered a disease outbreak by RA in domestic ducks with clinical symptoms. We report the pathological and bacteriological findings of the affected ducks and discuss the possible reason for the long absence of this disease in Japan.

The outbreak site was a duck farm in the Mie Prefecture that housed 4,000 ducks. The domestic ducks (*Anas platyrhynchos*) were a meat-type Cherry Valley breed that was not vaccinated against any pathogens. This farm has four separate open-sided poultry houses with a floor feeding system. Four groups of ducks, aged 1- to 9-day-old, 10- to 13-day-old, 14- to 21-day-old and 22- to 70-day-old, were housed separately until shipped for slaughter. Ducks were routinely treated with fluoroquinolone for 3 days when 1-day-old birds were introduced to the farm from the breeder. They were also treated a few times with amoxicillin from the age of 14- to 21-day-old. In January 2014, an increased mortality in the 14- to 21-day-old duck flock was reported to veterinary officials by the owner. The mortality rate of this month was 16%, 316 out of 2,020 birds. The owner explained that a similar increase in mortality had been noticed several times in this farm since 2009. The clinical signs of some ducks were reduced movement, ataxia and dorsal recumbency with leg paddling that was indicative of neurological involvement.

One 27-day-old duck (No. 1) and three 20-day-old ducks (Nos. 2–4) were sacrificed and sampled for routine histopathological and bacteriological examinations. The gross findings of the most severe case (No. 3) were pericarditis, perihepatitis and airsacculitis that were consistent with a typical RA infection in ducks. The surface of the heart and liver was covered by whitish, gelatinous and fibrinous exudates (Fig. 1). Other ducks (Nos. 1, 2 and 4) exhibited fibrinous pericarditis, but lacked perihepatitis.

Histologically, heterophilic and fibrinous inflammation was observed at the serosal surface of the heart (Nos. 1–4), liver (No. 3), air sac (Nos. 2 and 3) and intestine (No. 3). The most frequently affected organ was the heart. The epicardium was thick due to the edema, hemorrhage, abundant fibrinous exudates, and infiltration of heterophils and macrophages (Fig. 2). Fibrinous exudates were often surrounded by multinucleated giant cells. Lesions of the liver, air sac and intestine were essentially similar to those of the heart. Gram-negative stained bacterial colonies were rarely found in the fibrinous exudates (Fig. 3). Another characteristic lesion was heterophilic and fibrinous ventriculitis (Nos. 1–3) and meningitis (Nos. 1–3) in the brain (Fig. 4). Brain lesions were more severe in the brain stem, including the optic lobe, than

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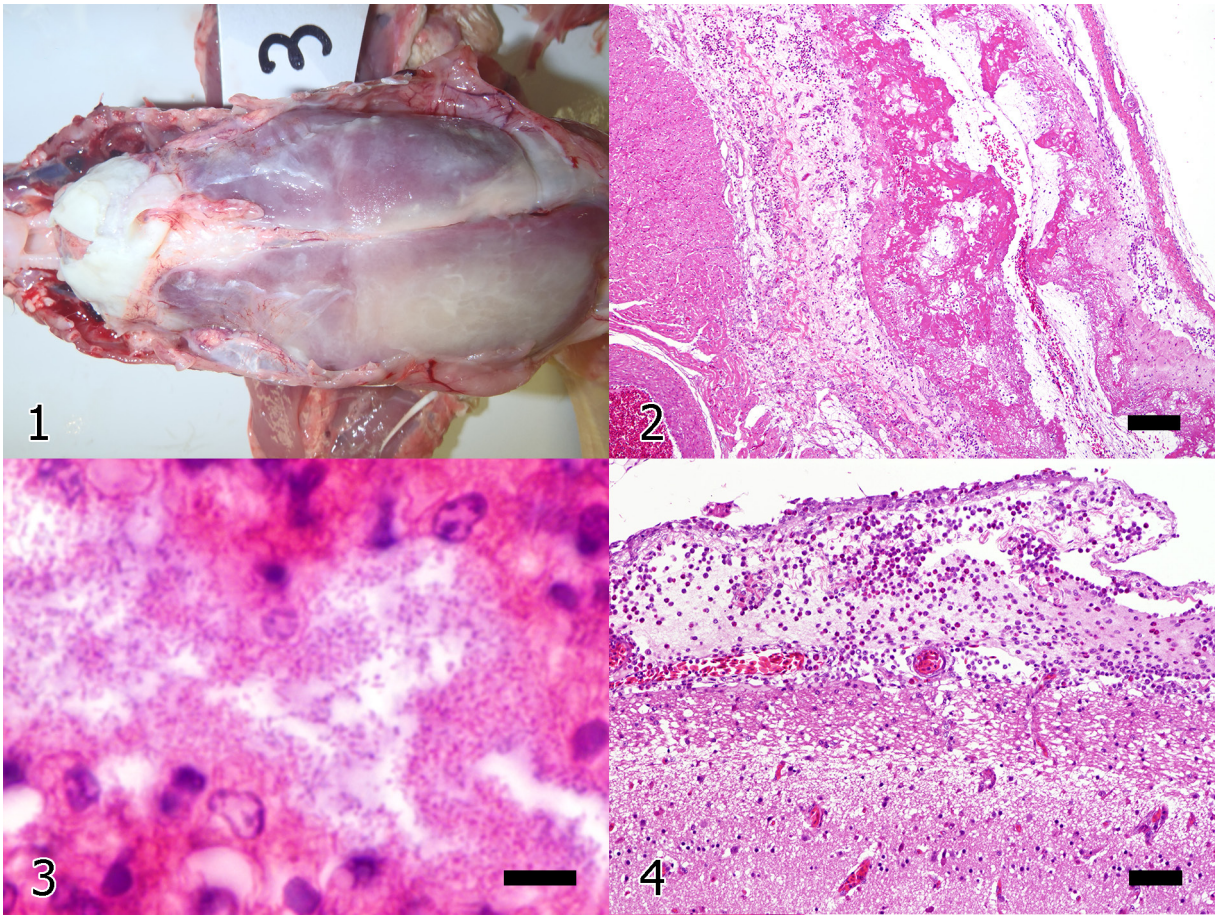

Fig. 1. Heart and liver, duck (No.3). The surface of the heart and liver was covered by whitish, gelatinous and fibrinous exudates.
Fig. 2. Heart, duck (No.1). The thickened epicardium with fibrinous exudates, edema and inflammatory cells. Hematoxylin and eosin stain. Bar=110 μm.
Fig. 3. Body cavity, duck (No.3). Colony of rod-shaped bacterium embedded in the fibrinous exudates in the body cavity. Hematoxylin and eosin stain. Bar=10 μm.
Fig. 4. Brain, duck (No.3). Heterophilic meningitis with edema at the optic lobe of the brain. Hematoxylin and eosin stain. Bar=50 μm.

in the cerebrum, cerebellum and spinal cord. Heterophilic and granulomatous bronchopneumonia was prominent in the most severe case (No. 3). Focal to massive hepatocellular necrosis (No. 1) and increased numbers of macrophages in the spleen with occasional fibrin thrombi (No. 2) were rare findings.

Bacteria were isolated in pure culture from the brain, heart, liver and spleen of two ducks (Nos. 2 and 3). The kidney of a duck (No.2) was also positive for isolation. Isolates grew well on sheep blood agar plates under an aerobic environment after 48 hr of incubation, but did not grow on MacConkey agar plates. Isolates were non-motile, non-hemolytic and non-glycolytic gram-negative rods. Isolates were positive for catalase and oxidase, and they did not utilize citrate.

The isolate was first identified as *Moraxella* spp. based on the results of the bacterial identification kit API 20 NE (bioMérieux S.A., Lyon, France), in which the identification of RA is not included. To genetically identify the isolate, total DNA was extracted from the bacterial colonies, and PCR was performed for bacterial 16S ribosomal RNA [4]. The genetic sequence of the PCR product revealed that isolates were 99.9% identical (1,455/1,456 bp) to RA type strain ATCC11845, also known as DSM15868 (GenBank accession no. CP002346.1).

Two of the RA isolates from ducks (Nos. 2 and 3) were tested for their susceptibility to antibacterial drugs. Both isolates were susceptible to amoxicillin (AMPC) and trimethoprim–sulfamethoxazole (ST) and resistant to ampicillin (ABPC), benzylpenicillin (PCG), cloxacillin (MCIPC), enrofloxacin (ERFX), kanamycin (KM) and norfloxacin (NFLX). Susceptibility to oxytetracycline (OTC) was low or resistant depending on the isolate.

RA infection is an important disease that can cause severe economic losses to the duck industry [12, 17]. Characteristic

pathological lesions, such as pericarditis and perihepatitis, observed in the present study were highly suggestive of an RA infection. However, a definitive diagnosis of RA infection requires isolation and identification of RA from ducks suspected to be infected [9, 12, 17]. Other bacterial infections, such as colibacillosis, may cause lesions similar to those seen in RA-infected ducks [12]. Differential diagnoses also include salmonellosis, pasteurellosis, streptococcosis and *Coenonia anatina* infection [17].

There are difficulties associated with the identification of RA [9, 16]. RA is characterized by the absence of species-specific biochemical properties [9]. Conventional bacterial identification kits, which are used in laboratory practices worldwide, may not identify RA. Application of the API 20 NE kit, which aims to identify non-fastidious, non-enteric gram-negative rods, was not suitable for the identification of the RA isolate in the present study and could have led to a misdiagnosis had we relied on the result from this kit alone. Genetic sequencing of bacterial 16S ribosomal RNA and matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry are currently considered valid for the identification of RA [3, 8, 16, 22]. In the present study, isolates from diseased ducks were identified as RA by almost a full length of 16S ribosomal RNA sequencing of the isolate with a 99.9% similarity to the RA type strain.

The long absence of an RA infection in Japan may have been because of the low number of duck inspections. It is unclear to what extent RA is invading in Japan at this moment. The pathway of RA transmission was not identified in this outbreak. However, because the owner had observed symptoms that were similar to the present case since 2009, RA may have persisted in this farm for a long period until it was detected in 2014. Eradicating RA from duck flocks can be difficult, and repeated infections in the farm are possible [22]. Some RA strains can produce a biofilm, which may involve resistance to antibacterial drugs and a longer persistence in the environment [10]. In the past outbreak in Japan, antibacterial drug treatment alone was not effective to eliminate RA from the farm [14]. Improved management for ducks including the renovation of the farm has led to the successful elimination of RA [14].

Serotyping of the isolated RA was not performed, because of a lack of antiserum against various RA serotypes. At least 21 serotypes have been reported for RA, although there is some confusion regarding its classification [15–17]. Serotyping RA can be useful for epidemiological analyses and a vaccination strategy [6, 15, 16]. To date, RA vaccination has not been applied in Japan. Hygiene management against introduction of RA to duck farms would be essential to prevent the disease.

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