NOTE *Wildlife Science*

Yersiniosis caused by *Yersinia pseudotuberculosis* in captive toucans (Ramphastidae) and a Japanese squirrel (*Sciurus lis*) in zoological gardens in Japan

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(Received 18 May 2015/Accepted 22 August 2015/Published online in J-STAGE 5 September 2015)

ABSTRACT. Two captive Keel-billed toucans and a Chestnut-mandibled toucan in another zoological garden died suddenly without any pre-existing symptoms, and three months later, a Japanese squirrel died of diarrhea. All these animals showed necrotic enteritis and multifocal necrosis in the liver and spleen with Gram negative bacilli. The bacilli showed strong positive immunolabeling for *Yersinia pseudotuberculosis* O4 in the Keel-billed toucans, *Y. pseudotuberculosis* O2 in the Chestnut-mandibled toucan and *Y. pseudotuberculosis* O1 in the Japanese squirrel, while *Y. pseudotuberculosis* 4b, 2b and 1b were respectively isolated from the lesions. To our knowledge, this might be the first reported case of fatal yersiniosis in a Japanese squirrel in the world as well as in toucans in Japan.
KEY WORDS: infectious disease, Japanese squirrel, toucan, *Yersinia pseudotuberculosis*, yersiniosis

doi: 10.1292/jvms.15-0298; *J. Vet. Med. Sci.* 78(2): 297–299, 2016

Yersinia pseudotuberculosis is a Gram-negative bacillus and an important zoonotic agent. Yersiniosis, which is caused by *Y. pseudotuberculosis*, occurs in a wide variety of animals and humans and has a global distribution [6, 10]. Infection with *Y. pseudotuberculosis* typically occurs via the oral route and manifests clinically as enteritis, mesenteric lymphadenitis and, in some cases, septicemia [11]. Yersiniosis can cause significant mass mortality among zoo animals. Nonhuman primates are highly susceptible to *Y. pseudotuberculosis*, and numerous cases have been reported [9]. Although rodents and birds, especially free-living species, are considered an important reservoir for *Y. pseudotuberculosis*, few cases of yersiniosis have been reported in these species [8, 10]. In this study, we report four cases of fatal yersiniosis caused by *Yersinia pseudotuberculosis* infection in captive toucans and a Japanese squirrel.

In May 2003, a captive female sub-adult Keel-billed toucan (*Ramphastos sulfuratus*) (No. 1) died suddenly for no apparent reason in a zoological garden in Japan. One week later, another male adult Keel-billed toucan (No. 2) housed in the same cage exhibited a moribund state and died. In January 2005, a captive female adult Chestnut-mandibled toucan (*Ramphastos swainsonii*) (No. 3) at another zoological garden exhibited a moribund state and died. Three months after the death of No. 3, a 2-year-old female Japanese squirrel (*Sciurus lis*) (No. 4) died of diarrhea in the same zoological garden. All toucans were housed for a long time in the same outdoor cages and showed good general condition. The Japanese squirrel died under quarantine in an isolated cage 8 days after it was transported from another zoological garden. The dead squirrel had low body weight (150 g) compared to other similar-aged squirrels (approximately 220 g). Complete necropsies were performed on the four animals, and their visceral organs were fixed in 10% phosphate-buffered formalin for histological and immunohistochemical analyses. The tissues were embedded in paraffin and sectioned at approximately 4 μm for hematoxylin and eosin (HE) and Gram staining. Immunohistochemical analysis was performed using a commercial rabbit anti-*Y. pseudotuberculosis* sera set (O1, O2, O3, O4, O5 and O6) (Denka-Seiken Co., Tokyo, Japan). Bacterial examinations of the livers from No. 1 and No. 2, a cloacal swab from No. 3 and liver, spleen and intestines from No. 4 were performed as previously described [9].

At necropsy, all three toucans and the Japanese squirrel had enlarged livers and spleens with multifocal fine white nodules. Many organs, including the spleen, liver and kidneys, were friable, and systemic venous congestion was observed in the three toucans. There were several fine white nodules in the kidney and fibrin deposition on the surface of the heart in No. 2. The colon and rectal contents of the Japanese squirrel had high moisture levels. Microscopically, the nodules in the liver and spleen in the three toucans were areas of focal necrosis containing large clusters of Gram negative bacilli (Fig. 1). The hepatic sinusoids in the three toucans were filled with numerous bacterial clusters. The intestine, especially the ileum, showed moderate to severe necrotic enteritis with clusters of Gram negative bacilli accompanied by lymphocytic infiltration. Additionally, Gram negative bacilli were seen in the heart, lung and kidney of all the toucans and the cerebrum, thyroid gland, testis and ovary of No. 1 and No. 2. Areas of hemosiderosis were scattered moderately in the hepatocytes

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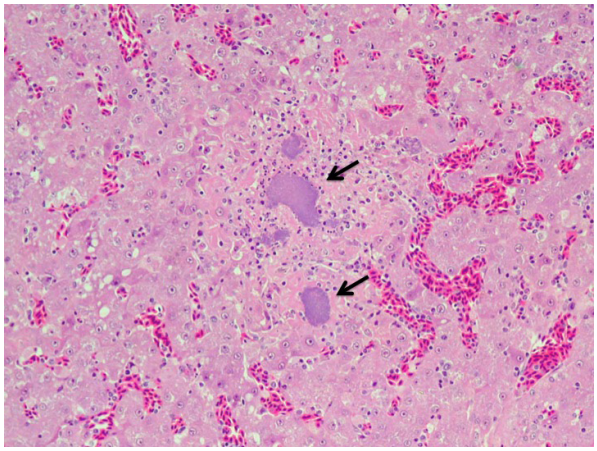

Fig. 1. The liver of No. 2. Note the areas of focal necrosis containing central bacterial clusters (arrows). Hematoxylin and eosin (HE) stain.

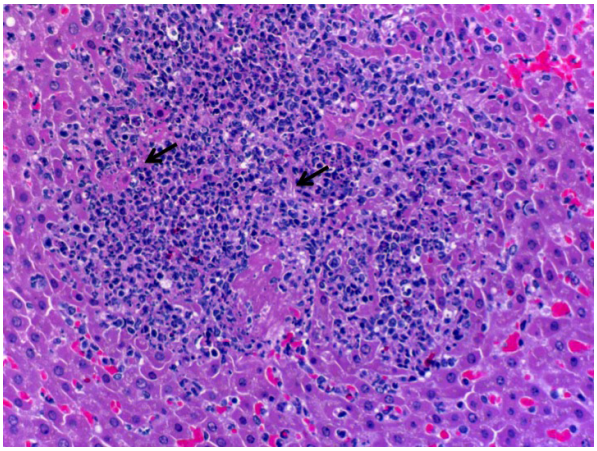

Fig. 2. The liver of No. 4. Focal necrosis with neutrophilic infiltration. Note the greater neutrophilic infiltration and nuclear debris and unclear bacterial clusters than those observed in the toucans (arrows). HE stain.

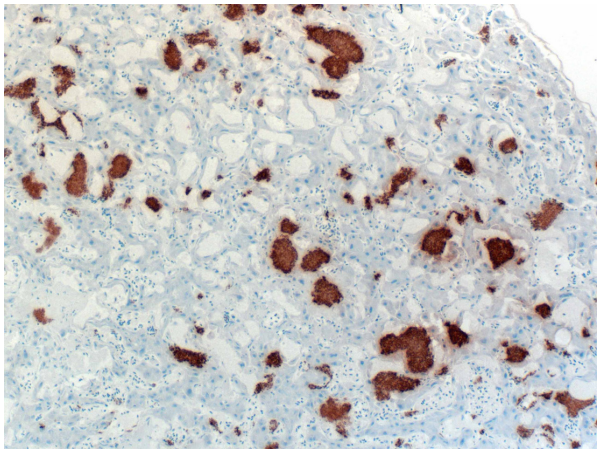

Fig. 3. The liver of No. 3. Strongly positive to immunoperoxidase staining for anti-*Y. pseudotuberculosis* O2 sera.

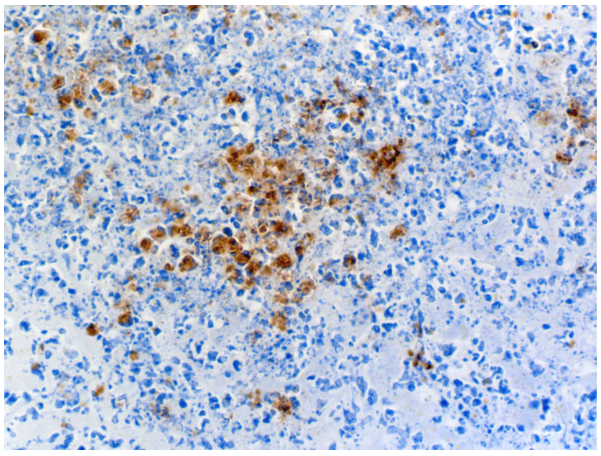

Fig. 4. The liver of No. 4. Strongly positive to immunoperoxidase staining for anti-*Y. pseudotuberculosis* O1 sera.

and around the necrotic lesions of the spleen and intestines of No. 2. In the Japanese squirrel, necrotic lesions, including clusters of Gram negative bacilli in the liver, spleen, tonsillae and intestine, were observed, similar to those in the toucans. However, compared to the toucans, the squirrel showed greater neutrophilic infiltration and nuclear debris and smaller bacterial clusters in the liver and spleen (Fig. 2). Other findings in the Japanese squirrel were nematode infection in the intestinal crypt of the small intestine and *Eimeria* sp. infection in the intestinal epithelium of the colon and rectum. The Gram negative bacilli seen in various tissues showed strong immunolabeling for *Y. pseudotuberculosis* O4 in No. 1 and No. 2, *Y. pseudotuberculosis* O2 in No. 3 (Fig. 3) and *Y. pseudotuberculosis* O1 in Japanese squirrel No. 4 (Fig. 4). In the bacterial examinations, *Y. pseudotuberculosis* 4b was isolated from the livers of No. 1 and No. 2, *Y. pseudotuberculosis* 2b was isolated from the cloacal swab of No. 3, and *Y. pseudotuberculosis* 1b was isolated from the liver, spleen and intestine of No. 4.

All three toucans and the Japanese squirrel had enlarged, friable livers and spleens with multifocal white nodules. Microscopically, these lesions contained numerous bacterial clusters composed of Gram-negative bacilli with necrotic lesions. These lesions are typical of yersiniosis caused by *Y. pseudotuberculosis* and have been previously reported in various animal species [1, 15]. The Gram negative bacilli in the lesions showed strong immunolabeling for *Y. pseudotuberculosis* O4 in No. 1 and No. 2, *Y. pseudotuberculosis* O2 in No. 3 and *Y. pseudotuberculosis* O1 in No. 4, and *Y. pseudotuberculosis* serotypes 4b, 2b or 1b were isolated from the lesions, respectively. On the basis of these findings, it was concluded that the cause of death was yersiniosis induced by *Y. pseudotuberculosis* serotype 4b in No. 1 and No. 2, *Y. pseudotuberculosis* serotype 2b in No. 3 and *Y. pseudotuberculosis* serotype 1b in No. 4. Although the lesions did not differ by bacterial serotype or species in the avian cases, those in the Japanese squirrel showed greater neutrophilic infiltration and nuclear debris and smaller bacterial clusters.

Y. pseudotuberculosis has often been isolated from healthy birds and rodents, and free-living birds and rodents are considered important carriers [8, 10]. Although sporadic cases of

fatal yersiniosis have occurred in captive or wild birds in Europe and the United States [7, 13], cases of avian yersiniosis are extremely rare in Japan, and there are only few reports of isolation from healthy wild birds [5, 12]. In Europe and the United States, serotypes 1, 2 or 3 are generally isolated from birds, whereas serotype 4b is unknown in these countries [6, 8]. On the other hand, serotype 4b is a common serotype among humans and other animals in Japan [6]. The serotype 4b in pathological lesions of No. 1 and No. 2 was not very different from serotype 2b in lesions of No. 3 in this report or from other serotypes observed in cases of avian yersiniosis in previous studies. [4]. However, in contrast to the birds in previous reports, No. 1 and No. 2 with serotype 4b in this report showed bacterial colonies in multiple organs, including the cerebrum, thyroid gland, testis and ovary. Generally, stress factors, such as cold environment, poor feeding, immunosuppressive conditions and transport, are contributing factors to precipitating yersiniosis [11]. In the present cases, all three toucans had good nutritive conditions and died suddenly without any symptoms. The bacterial clusters in these animals were much larger with minimal inflammatory cellular reaction compared with those observed in mammalian yersiniosis. These findings suggest that death occurs suddenly with no clinical signs because of rapid bacterial growth before an inflammatory response can occur. The findings also suggest that toucans have a high sensitivity to *Y. pseudotuberculosis*. Free-living carrier mice were considered as the common source of infection, since the birds died within 1 week of each other, were housed in the same outdoor cage and showed presence of the same serotype 4b. Similarly, toucan No. 3 was considered to have acquired the infection from free-living carrier mice via the oral route, because necrotic lesions with bacilli were observed mainly in the intestine, liver and spleen. On the other hand, Japanese squirrel No. 4 was highly suspected of having carried *Y. pseudotuberculosis* before transportation. Nematode and coccidial infections are believed to be caused by inadequate development (low body weight), and transportation stress may be strongly associated with the occurrence of yersiniosis. The presence of nematodes and *Eimeria* sp. in the intestine could have increased the severity of *Y. pseudotuberculosis* infection in the Japanese squirrel.

In human patients, a relationship between yersiniosis and iron-overload states, such as hemochromatosis, has been suggested [2]. Similar correlations have been reported in the domestic chicken under experimental conditions [3]. Yersiniosis occurs less frequently in avian than in mammalian species, but toucans are highly susceptible to hemochromatosis [14], and this may suggest greater susceptibility to *Y. pseudotuberculosis*. Additionally, correlations between bacterial serotype and pathogenicity are still unclear in avian species. Further investigations are needed to establish these correlations.

In this report, we describe four cases of fatal yersiniosis caused by *Y. pseudotuberculosis* in three captive toucans (Ramphastidae) and a Japanese squirrel (*Sciurus lis*). To our knowledge, this is the first report of fatal yersiniosis caused by *Y. pseudotuberculosis* in a Japanese squirrel and/or its closely related species in the world as well as in toucans (Ramphastidae) in Japan. Since sudden death in avian and rodent species may be caused by several important zoonotic diseases, differential diagnosis using immunohistochemical and bacterial examinations is useful to establish an accurate diagnosis. This report could provide important information regarding sudden death in captive avian and rodent species.

ACKNOWLEDGMENTS. The authors would like to thank the zoo veterinarian and the zoo staff at the zoological gardens for providing the animals for pathological and bacterial examinations.

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