The Journal of Veterinary Medical Science

NOTE

Pathology

Yersinia infection in two captive guereza colobus monkeys (*Colobus guereza*)

Hayate NISHIURA[1)], Akiko YAMAZAKI[2)], Koichi WAKAKURI[1)], Jun SASAKI[1)], Jun TERAJIMA[2)] and Kenji OCHIAI[1)]*

[1)]Laboratory of Veterinary Pathology, Co-department of Veterinary Medicine, Iwate University, Morioka, Iwate 020-8550, Japan
[2)]Veterinary Public Health, Co-department of Veterinary Medicine, Iwate University, Morioka, Iwate 020-8550, Japan

J. Vet. Med. Sci.
81(8): 1201–1204, 2019
doi: 10.1292/jvms.19-0039

Received: 18 January 2019
Accepted: 30 May 2019
Advanced Epub: 15 July 2019

ABSTRACT. Two guereza colobus monkeys (*Colobus guereza*) reared in a zoological garden in Japan suddenly died of multifocal fibrinonecrotic gastroenteritis and septicemia associated with infection by *Yersinia spp*. It was necessary to microbiologically differentiate *Yersinia frederiksenii* and *Y. enterocolitica*. We described the pathological findings and discuss the causal agent to emphasize the need to revert to using a combination of multiple examinations for diagnosis.

KEY WORDS: bacterial zoonoses, gastroenteritis, guereza, monkey, *Yersinia*

Yersinia spp. are gram-negative, coccobacilli that are facultative anaerobes in the family *Enterobacteriaceae*. *Yersinia enterocolitica* is one member of a generic triad of pathogenic *Yersinia* spp. with *Y. pestis* and *Y. pseudotuberculosis*. *Y. pestis* causes a disease plague in humans and animals. The term "yersiniosis" refers to infections caused by either *Y. enterocolitica* or *Y. pseudotuberculosis*, which both produce similar lesions that appear as enteritis and sometimes septicemia in humans and animals [11]. *Y. enterocolitica* hosted a large number of biochemically different strains until the 1980s. However, DNA-DNA hybridization studies showed that these strains were distinct species from each other and from *Y. enterocolitica* [4]. The new 8 species, including *Y. frederiksenii*, are collectively referred to as *Y. enterocolitica*-like bacteria. They are generally considered to be nonpathogenic bacteria because of the absence of classical *Yersinia* virulence markers [17]. However, several species have the potential to cause gastrointestinal infections in humans [5]. The virulence-related genes were also found in *Y. enterocolitica*-like species, which indicates the pathogenic potential of these strains [9].

The guereza (*Colobus guereza*) is an old-world monkey that is native to equatorial Africa. This species inhabits a diverse range of habitats, including deciduous forests, savanna woodland, and montane forests [13]. The guereza has a large, multi-chambered stomach that contains bacteria which enables it to digest leaves and other plant fibers [14]. Nonhuman primates are considered to be quite susceptible to infection with *Y. enterocolitica* and fatal cases of yersiniosis have been reported worldwide, e.g., in an owl monkey [2], squirrel monkeys [10, 12], agile gibbons [10, 12], patas monkeys [16], and marmosets [6]. However, detailed information of the disease in guerezas is limited. The present report describes two guerezas that died of *Yersinia* infection and discuss the etiological agent based on the pathology and the molecular biological features of the isolates.

Five guereza colobus monkeys were reared in a zoological garden in Japan. They were kept at outdoor enclosure during daylight and housed inside during the hours of darkness. They fed ordinary vegetables and fruits, including green leafy vegetables, Chinese cabbage, cabbage, lettuce, boiled sweet potato, boiled Irish potato, banana, orange, and apple, with a proper amount of leaves of Japanese oak. The stockyard could have a rodent invasion. Two of the five monkeys, which were sisters, suddenly died over two days in June. One (Case 1) of these cases was a 5-year-old female that was lethargic and lacked an appetite, and died later at the same evening. The second case (Case 2) was a 4-year-old female that showed symptoms of vomiting and bloody stool the following morning. Although antibiotic-free fluid therapy was administered, the symptoms progressively worsened and she died later at the same evening. Necropsies were conducted at the zoological garden.

At necropsy, Case 1 showed bloody pleural effusion and ascites accumulated up to 50 and 120 m*l* of fluid, respectively. Hemorrhages and petechiae were observed in the thoracic wall, pulmonary pleura, diaphragm, and epicardium. There was ecchymoses, up to 5 × 2 cm in diameter, on the serosal surface of the glandular stomach with adhesion to the surrounding tissues. The mucosa of glandular stomach and proximal small intestine was diffusely hyperemic and had multiple raised nodules with ulceration, up to 1 cm in diameter, with fibrinous exudate and peripheral hemorrhages. The gastrointestinal content was bloody. Mucosal petechiae were also noted in the ileum, cecum, and colon. Case 2 had moderate epicardial petechiae, moderate gastric

*Correspondence to: Ochiai, K.: kochiai@iwate-u.ac.jp

serosal hemorrhage, and multiple raised mucosal lesions of the stomach, up to 4 × 2 cm in diameter, with fibrinonecrotic exudate in center and frequent peripheral halo-like hemorrhages (Fig. 1a). Liver, spleen, and gastric lymph nodes in the both cases were mildly to moderately enlarged by hyperemia and congestion.

Tissue samples of the major organs, including the liver, spleen, kidney, heart, lung, stomach, and intestine, were fixed in 10% neutral buffered formalin and sent to our laboratory. Tissue samples were routinely processed for histopathology and sections were stained with hematoxylin and eosin (HE) and Gram staining (Brown-Hopps method). Histologically, glandular stomachs in the both Case 1 and Case 2 revealed multiple ulcers. These ulcerative foci contained numerous bacterial colonies intermixed with fibrinonecrotic debris associated with severe infiltration of neutrophils (Fig. 1b). The intralesional bacteria were gram-negative coccobacilli (Fig. 1c). Neutrophilic infiltration occasionally extended deep in the muscularis mucosa and submucosal tissue. Fibrin thrombi with bacterial clumps were frequently observed in dilated veins. The both cases revealed multifocal hepatocytic necrosis with mild to moderate infiltration of neutrophils and macrophages and intralesonal bacterial colonies in the liver (Fig. 1d). Similar necrotic foci were also noted in the spleen of both animals and in the gastric lymph nodes of Case 2. The kidney, heart, lung, and pancreas showed no significant lesions.

For identification of bacteria, samples were collected from the liver, spleen, stomach, gastric lymph nodes, intestines, and feces using sterile transport swabs (Health Sciences Research Institute, Yokohama, Japan) and analysed by the institute using an automated microbiology system (VITEK®2 Compact, bioMerieux, Marcy l'Etoile, France). *Yersinia frederiksenii* were detected in all of the examined samples from Case 1 and in the spleen and gastric lymph nodes from Case 2. *Escherichia coli* was also detected in the liver from Case 1 and the gastric lymph nodes and intestinal contents from Case 2. *Clostridium perfringens* was detected in the gastric ulcer and gastrointestinal contents from Case 1.

Next, the affected gastric lymph nodes from Case 2 were homogenized in brain heart infusion (BHI) broth (Becton, Dickinson and Co., Franklin Lakes, NJ, U.S.A.) and incubated at 37°C for 12 hr in our laboratory. The suspension was spread across the surface of Cefsulodin-Irgasan-Novobiocin (CIN) agar plates (Becton, Dickinson and Co.) and incubated at 27°C for 24 to 48 hr. Slightly protruding red colonies, which were 4–5 mm in size, dark pink in the center with semitransparent in edge, were formed on the CIN agar plates. All of the isolates from three colonies were identified as *Y. enterocolitica* by the Vitek® system performed by

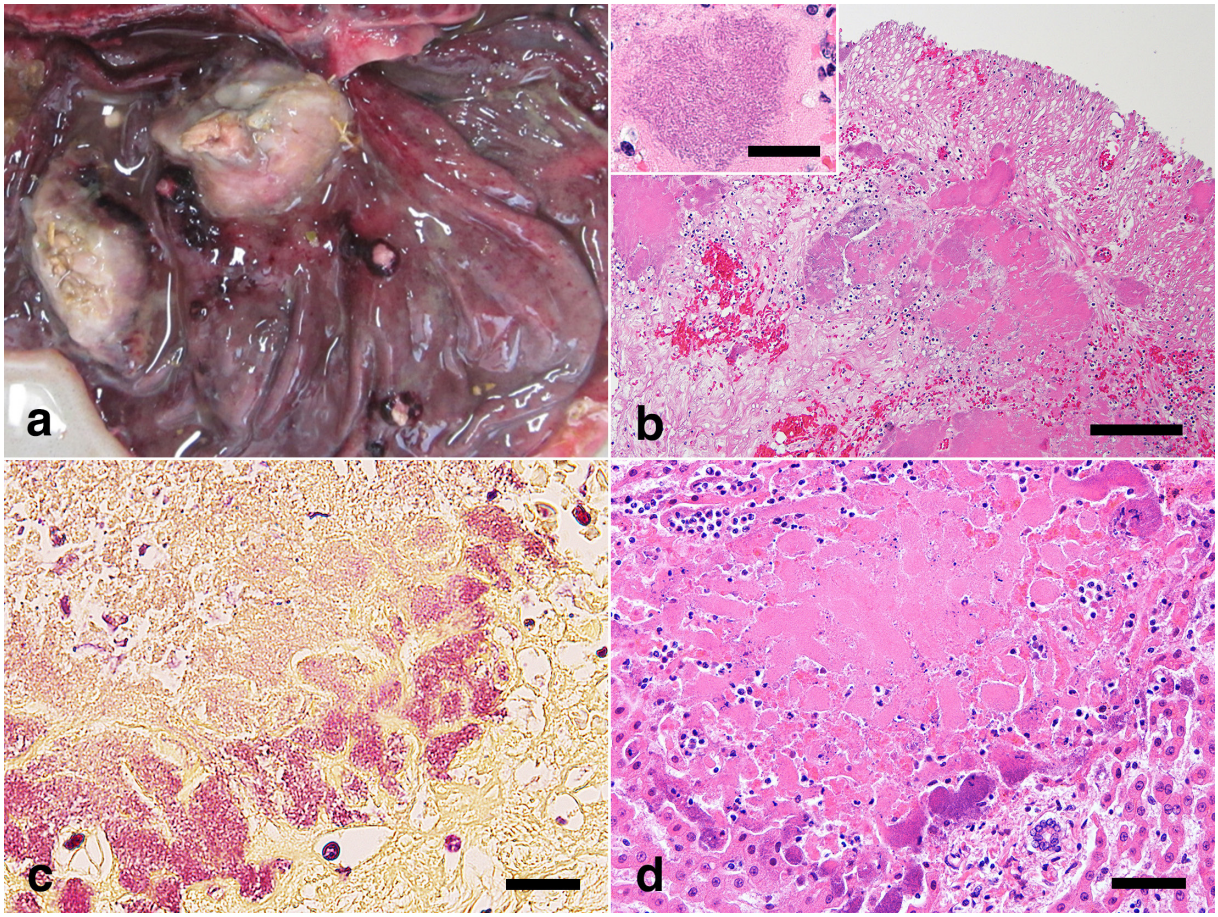

Fig. 1. *Yersinia* infection in guereza colobus. (a) Stomach, Case 2. Multiple raised mucosal lesions with fibrinonecrotic exudate in center. Smaller foci (arrows) have peripheral halo-like hemorrhages. (b) Stomach, Case 1. Mucosal hemorrhages and necrosis intermixed with bacterial clumps. HE. Bar=100 μm. Inset: high magnification image of a clump of bacteria. HE. Bar=10 μm. (c) Stomach, Case 2. Intralesional coccobacilli are gram-negative. Gram stain (Brown-Hopps method). Bar=10 μm. (d) Liver, Case 1. Hepatocytic necrosis with bacterial clumps. HE. Bar=40 μm.

Health Sciences Research Institute. Additionally, genomic DNA from the isolate was extracted using a DNeasy tissue kit (Qiagen, Hilden, Germany) for 16S ribosomal RNA gene sequence analysis and polymerase chain reaction (PCR) amplification was performed using the following primer set: 10F (5′-GTTTGATCCTGGCTCA-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′). These primers were designed to amplify ~800 bases on the 5′ side of bacterial the 16S small subunit ribosomal RNA gene. The sequence of isolates showed 100% homology with *Y. enterocolitica* (GenBank accession no., CP009846.1), whereas it had 96.0 to 97.5% homology with *Y. frederiksenii* (GenBank accession no., Af366379, AJ639876 and AJ639875).

From these results, both cases were diagnosed as acute multifocal fibrinonecrotic gastroenteritis due to *Yersinia* spp. and they died from septicemia. These pathologic findings in the both cases were consistent with those of yersiniosis. There was no histologic change associated with coliform infection, including *E. coli* adhering on the apical surface of the mucosal epithelial cells and neutrophilic colitis with crypt epithelial cell hyperplasia and attenuation of surface epithelium, in the present cases. *Yersinia enterocolitica* usually causes intestinal infections in primates, which results in symptoms such as vomiting, diarrhea, and depression [8]. In mammals, enteric *Yersinia* migrate to the ileum, cecum, and ascending colon of the intestine and the associated lymph tissues after oral ingestion [3]. *Y. enterocolitica* can disseminate to the visceral organs via portal circulation or the lymphatic system, or both; causing suppurative inflammation and acute death [3]. Characteristic postmortem findings, previously reported in nonhuman primates, include severe acute segmental suppurative erosive enterocolitis and necrotic and suppurative foci within the liver, spleen, and mesenteric lymph nodes [8]. The jejunum and ileum are generally the most frequently affected segments of the intestine but stomach is also affected [3]. Although severe inflammation was observed in the stomach than in the lower intestines in the present cases, it was difficult to determine whether the distribution of lesions is peculiar to the disease of this species.

In the present cases, *Y. frederiksenii* was initially suspected to be the pathogen by an automated system. The Vitek® system utilizes a colorimetric method, identifies bacterial species according to their different biochemical profiles. The system has high level of accuracy for identifying *Y. enterocolitica*, *Y. enterocolitica*-like species, and other clinical gram-negative bacilli [15]. In fact, the isolates on *Yersinia*-selective agar was identified as *Y. enterocolitica* by both the device and the sequence analysis, which indicated the reliability of the system. Therefore, we could not strictly have denied that *Y. frederiksenii* was the causative agent. *Y. enterocolitica*-like species are regarded as avirulent bacteria but the pathogenic potential is suggested because some strains of them contain virulence-related genes [9] and *Y. enterocolitica*-like species are likely to be simultaneously detected with *Y. enterocolitica* in pediatric patients [7]. With regard to the inconsistency between the initial microbiology and the following sequence analysis, three possibilities may be considered. (1) Both the *Y. frederiksenii* and *Y. enterocolitica* existed within the gastrointestinal lesions and indeed a considerable number of *Y. frederiksenii* was collected for examination. But *Y. enterocolitica* might have disappeared or have been missed because the inspection laboratory had not been requested to use the selective agars for *Yersinia* spp. (2) The growth ability of the microorganisms may have been affected by the selective agars when we have performed the bacterial isolation. CIN agar is a highly selective medium designed to isolate *Y. enterocolitica. Y. enterocolitica* is able to rapidly proliferate on the agar whereas the growth of *Y. frederiksenii* may be inhibited in the culture condition. (3) The last is simple sampling error during the necropsy. However, the third speculation is highly unlikely, since the initial automated analysis needs tissue cultures superior to swab cultures [1] and *Y. frederiksenii* has been detected in several organs in precisely the same way.

Our results indicated that there is a risk of failure to accurately identify causal pathogens and to overlook important bacterial species, especially when *Yersinia* infection is suspected. To our knowledge, this is the first report case of *Yersinia* infection in two guereza colobus monkeys.

ACKNOWLEDGMENT. The authors thank Sendai Yagiyama Zoological Park for donating the samples.

REFERENCES

1. Aggarwal, V. K., Higuera, C., Deirmengian, G., Parvizi, J. and Austin, M. S. 2013. Swab cultures are not as effective as tissue cultures for diagnosis of periprosthetic joint infection. *Clin. Orthop. Relat. Res.* **471**: 3196–3203. [Medline] [CrossRef]
2. Baggs, R. B., Hunt, R. D., Garcia, F. G., Hajema, E. M., Blake, B. J. and Fraser, C. E. 1976. Pseudotuberculosis (*Yersinia enterocolitica*) in the owl monkey (*Aotus trivirgatus*). *Lab. Anim. Sci.* **26**: 1079–1083. [Medline]
3. Bergman, M. A., Chafel, R. and Mecsas, J. 2010. Yersinia. pp. 309–323. *In*: Pathogenesis of Bacterial Infections in Animals, 4th ed. (Gyles, C. L., Prescott, J. F., Songer, J. G. and Thoen, C. O. eds.), Blackwell, Ames.
4. Brenner, D. J., Steigerwalt, A. G., Falcao, D. P., Weaver, R. E. and Fanning, G. R. 1976. Characterization of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by deoxyribonucleic acid hybridization and by biochemical reactions. *Int. J. Syst. Bacteriol.* **26**: 180–194. [CrossRef]
5. Cafferkey, M. T., Sloane, A., McCrae, S. and O'Morain, C. A. 1993. *Yersinia frederiksenii* infection and colonization in hospital staff. *J. Hosp. Infect.* **24**: 109–115. [Medline] [CrossRef]
6. Fredriksson-Ahomaa, M., Naglic, T., Turk, N., Šeol, B., Grabarević, Z., Bata, I., Perkovic, D. and Stolle, A. 2007. Yersiniosis in zoo marmosets (*Callitrix jacchuss*) caused by *Yersinia enterocolitica* 4/O:3. *Vet. Microbiol.* **121**: 363–367. [Medline] [CrossRef]
7. Greenwood, M. H. and Hooper, W. L. 1990. Excretion of *Yersinia* spp. associated with consumption of pasteurized milk. *Epidemiol. Infect.* **104**: 345–350. [Medline] [CrossRef]
8. Hirsh, D. C. 2004. Yersinia. pp. 75–81. *In*: Veterinary Microbiology, 2nd ed. (Hirsh, D. C., MacLachlan, N. J. and Walker, R. L., eds.), Blackwell, Oxford.
9. Imori, P. F., Passaglia, J., Souza, R. A., Rocha, L. B. and Falcão, J. P. 2017. Virulence-related genes, adhesion and invasion of some *Yersinia enterocolitica*-like strains suggests its pathogenic potential. *Microb. Pathog.* **104**: 72–77. [Medline] [CrossRef]
10. Iwata, T., Une, Y., Okatani, A. T., Kaneko, S., Namai, S., Yoshida, S., Horisaka, T., Horikita, T., Nakadai, A. and Hayashidani, H. 2005. *Yersinia enterocolitica* serovar O:8 infection in breeding monkeys in Japan. *Microbiol. Immunol.* **49**: 1–7. [Medline] [CrossRef]

11. Mair, N. S. 1973. Yersiniosis in wildlife and its public health implications. *J. Wildl. Dis.* **9**: 64–71. [Medline] [CrossRef]
12. Nakamura, S., Hayashidani, H., Iwata, T., Namai, S. and Une, Y. 2010. Pathological changes in captive monkeys with spontaneous yersiniosis due to infection by *Yersinia enterocolitica* serovar O8. *J. Comp. Pathol.* **143**: 150–156. [Medline] [CrossRef]
13. Oates, J. F. 1994. The natural history of African colobines. pp. 75–128. *In*: Colobine Monkeys: Their Ecology, Behaviour and Evolution (Davies, A. G. and Oates, J. F. eds.), Cambridge University Press, Cambridge.
14. Oates, J. F., Davies, A. G. and Delson, E. 1994. The diversity of living colobines. pp. 45–73. *In*: Colobine Monkeys: Their Ecology, Behaviour and Evolution (Davies, A. G. and Oates, J. F. eds.), Cambridge University Press, Cambridge.
15. Renaud, F. N. R., Bergeron, E., Tigaud, S., Fuhrmann, C., Gravagna, B. and Freney, J. 2005. Evaluation of the new Vitek 2 GN card for the identification of gram-negative bacilli frequently encountered in clinical laboratories. *Eur. J. Clin. Microbiol. Infect. Dis.* **24**: 671–676. [Medline] [CrossRef]
16. Skavlen, P. A., Stills, H. F. Jr., Steffan, E. K. and Middleton, C. C. 1985. Naturally occurring *Yersinia enterocolitica* septicemia in patas monkeys (*Erythrocebus patas*). *Lab. Anim. Sci.* **35**: 488–490. [Medline]
17. Sulakvelidze, A. 2000. *Yersiniae* other than *Y. enterocolitica, Y. pseudotuberculosis*, and *Y. pestis*: the ignored species. *Microbes Infect.* **2**: 497–513. [Medline] [CrossRef]