HHS Public Access

Author manuscript

J Zoo Wildl Med. Author manuscript; available in PMC 2021 March 17.

Published in final edited form as:

J Zoo Wildl Med. 2020 March 17; 51(1): 170–176. doi:10.1638/2019-0062.

CLONAL SPREAD OF *YERSINIA ENTEROCOLITICA* 1B/O:8 IN MULTIPLE ZOO SPECIES

Christie L. Hicks, DVM, **Julia E. Napier, DVM**, **Douglas L. Armstrong, DVM**, **Lori M. Gladney**, **Cheryl L. Tarr, PhD**, **Molly M. Freeman, PhD**, **Peter C. Iwen, PhD D (ABMM)**

1431 South 54th Street, Omaha, Nebraska 68106, USA (Napier); 1515 Madison Street, Omaha, Nebraska 68107, USA (Armstrong); Centers for Disease Control and Prevention-Enteric Disease Laboratory Branch, 1600 Clifton Road NE, Mailstop C-03, Atlanta, Georgia 30329, USA (Gladney, Tarr, Freeman); and University Nebraska Medical Center, 985900 Nebraska Medical Center, Omaha, Nebraska 68198-5900, USA (Iwen).

Abstract

Yersinia enterocolitica (YE) bioserotype 1B/O:8 (YE 1B/O:8) was identified in routine culture of a variety of zoo species housed at Omaha's Henry Doorly Zoo and Aquarium (OHDZA) from April to July 2011. Animal cases representing 12 species had YE detected from 34 cases during routine fecal monitoring and/or during postmortem examination: Coquerel's sifakas (*Propithecus coquereli*, two cases), black & white (BW) ruffed lemurs (*Varecia variegata variegata*, six cases), red ruffed lemurs (*Varecia rubra*, seven cases), white handed gibbon (*Hylobates lar albimana*, one case), black lemurs (*Eulemur macaco*, three cases), mongoose lemurs (*Eulemur mongoz*, two cases), African hunting dogs (*Lycaon pictus*, five cases), agile gibbons (*Hylobates agilis*, three cases), siamangs (*Hylobates syndactylus*, two cases), colobus monkey (*Colobus angolensis palliates*, one case), argus pheasant (*Argusianus argus*, one case), and orangutan (*Pongo pygmaeus*, one case). Most species were not symptomatic; however, three symptomatic cases in Coquerel's sifakas (two) and a white handed gibbon (one) showed clinical signs of diarrhea and lethargy that resulted in death for the Coquerel's sifakas. One unexpected death also occurred in a BW ruffed lemur. To the authors' knowledge, this is the first report of YE 1B/O:8 in such a large variety of zoo species. The source of the YE could not be identified, prompting the initiation of a diseases surveillance program to prevent further cases for the species that are sensitive to YE. To date, no additional cases have been identified, thus suggesting a single introduction of the YE 1B/O:8 strain into the zoo environment.

Keywords

Black & white (BW) ruffed lemurs (*Varecia variegata variegata*); Coquerel's sifaka (*Propithecus coquereli*); PFGE; *Yersinia enterocolitica* 1B/O:8 (YE 1B/O:8)

Correspondence should be directed to Dr. Hicks (flipperdvm@hotmail.com).

INTRODUCTION

Yersinia enterocolitica (YE) is a gram-negative coccobacillus, comprised of six biotypes (1A, 1B, 2, 3, 4, and 5) and 60 serotypes.[8] YE is a known cause of gastrointestinal yersiniosis in humans and is also recognized as a cause of disease in more than 110 species of mammals, birds, and reptiles.[8] Serotypes O:3, O:8, and O:9 express virulence factors and are noted for causing yersiniosis in both animals and humans. In the United States, bioserotype 1B/O:8 is the most common cause of disease in animals, with the 4B/O:3 bioserotype emerging as an important pathogen[11] in recent years. Outbreaks of yersiniosis have been reported in a breeding unit of Cynomolgus monkeys (*Macaca fascicularis*) in the United Kingdom,[7] Japanese macaques (*Macaca fuscata*) at the Tokyo Tama Zoo,[10] and squirrel monkeys (*Saimiri sciureus*) and agile gibbons (*Hylobates agilis*) from Tobu Zoo Park in Japan.[6,8] In addition, YE 1B/O:8 has been isolated from wildlife in a gray fox (*Urocyon cinereoargenteus*) and a porcupine (*Erethizon dorsatum*) within the state of New York.[12]

This study describes a clonal spread of YE bioserotype 1B/O:8 at OHDZA in multiple zoo species with varying severity from shedding, mild clinical signs, acute disease, and latent disease to, in some cases, death.

MATERIALS AND METHODS

Species sampled

Species sampled are listed in Table 1. Table 1 includes individuals that were tested for YE, the dates of fecal collection, the number of individuals positive, the location where the individuals were housed, the animal identification numbers by International Species Information System (ISIS), their clinical definition of either shedder or clinically affected, and their outcome.

Specimen collection

Approximately 2 g feces was collected from each individual as a routine process for those entering quarantine and leaving the institution, routine surveillance, and/or when a clinical condition warranted collection. A sterile swab (BBL™CultureSwab™, Collection and Transport System) (Becton, Dickinson and Company, Franklin Lakes, NJ 07417, USA) was used to sample the collected feces, which were subsequently transported in ambient air to an offsite clinical laboratory within the same day for culture.

Postmortem examinations

Postmortem examinations were performed on the animals within 12 hr after death. Representative tissues were collected in accordance with the Prosimian Taxon Advisory Group guidelines (www.AAZV.org). All samples were placed in 10% buffered formalin and submitted to Northwest ZooPath for histopathologic analysis (Monroe, WA 98272, USA). Tissue swabs (BBLCultureSwab, Collection and Transport System, Becton, Dickinson and Company) were also collected aseptically during the postmortem examination and submitted for bacterial culture.

Bacterial culture and identification

Fecal swabs were inoculated onto Cefsulodin-Irgasan-Novobiocin (CIN) agar (Remel™ CIN Agar Base, Thermo Fisher Scientific, Waltham, MA 02451, USA) and MacConkey (MAC) agar (Thermo Fisher Scientific). The CIN and MAC agar plates were incubated in ambient air. The CIN agar was incubated for up to 5 days, whereas the MAC agar was incubated for up to 48 hr. Suspect YE colonies that were pink to red on CIN agar or clear, nonlactose fermenters on MAC agar were chosen for further characterization. Suspect YE colonies that were also oxidase negative and urease positive were subsequently inoculated to a MicroScan Dried Conventional Gram Negative Panel (Siemens, Sacramento, CA 95828, USA) according to the manufacturer's instructions for species identification.

Biotyping and serotyping

A subset of eight YE isolates collected at different time points and from different animals were chosen for further characterization. These isolates were sent to the Enteric Diseases Laboratory Branch at the Centers for Disease Control (CDC) and Prevention (Atlanta, GA 30329, USA) for additional testing and are listed in Figure 1.

Isolates were grown overnight at 25°C on trypticase soy agar (TSA) II with 5% sheep's blood agar (Thermo Fisher Scientific). YE isolates were biotyped according to the approach of Wauters.[13,15] Isolates were serogrouped by slide agglutination.[14]

Pulsed-field gel electrophoresis method

YE isolates were prepared for pulsed-field gel electrophoresis (PFGE; Fig. 1) according to the standardized PulseNet protocol for *Escherichia coli/Salmonella/Shigella*.[9] Enzyme digestion (Roche Diagnostics Corp, Indianapolis, IN 46250, USA) was carried out on plug slices using either 25 U *Apa*I at 25°C (2 hr) or 40 U *Not*I at 37°C (2 hr). Gels were run for 19.5 hr with initial switch time of 1.29 sec and final switch time of 18.66 sec. Gels were stained and imaged as described previously and analyzed using BioNumerics v6.6.11.

RESULTS

YE was detected in 34 cultures from 12 different species (Table 1). YE was identified in six separate areas within the zoo and with all cases occurring over a period of 111 days, ranging in seasonality, from early spring to summer. Eight YE isolates were further analyzed and identified as YE bioserotype 1B/O:8 with indistinguishable PFGE patterns when tested with *Not*I; however, small differences were observed with the *Apa*I enzyme (Fig. 1).

The most common species affected included the red ruffed lemurs (*Varecia rubra*, seven cases), and the black & white (BW) ruffed lemurs (*Varecia variegata variegata*, six cases), that were all located within the Madagascar exhibit. Other species in the Madagascar exhibit that tested positive for YE included Coquerel's sifakas (*Propithecus coquereli*, two cases), black lemurs (*Eulemur macaco*, three cases), and mongoose lemurs (*Eulemur mongoz*, two cases). Additional cases occurred in African hunting dogs (*Lycaon pictus*, five cases) at the Small Mammals exhibit, siamangs (*Hylobates syndactylus*, two cases), and an orangutan (*Pongo pygmaeus*, one case) at the New Orangutan exhibit, agile gibbons (*Hylobates agilis*,

three cases) at the Old Orangutan exhibit, a white handed gibbon (*Hylobates lar albimana*, one case) in the Jungle building, and an argus pheasant (*Argusianus argus*, one case) and a colobus monkey (*Colobus angolensis palliates*, one case) at the veterinary hospital.

YE was first detected in two Coquerel's sifakas (ISIS #19548 and #19549) and two mongoose lemurs (ISIS #19553 and #19555) during routine fecal culture surveillance at the onset of the outbreak (day 0). These animals appeared to be clinically normal at the time of fecal cultures. Several more clinically normal species presented with YE-positive fecal cultures on routine exam: 1 colobus monkey (ISIS #20667; day 22), five African hunting dogs (ISIS #15912, #16786, #20439, #20440, and #20583; day 23), one argus pheasant (ISIS #20744; day 44), six BW ruffed lemurs (ISIS #18368, #18369, #18370, #18373, #18374, and #18375; day 58), and seven red ruffed lemurs (ISIS #18376, #18377, #18378, #18379, #18380, #18381, and #18382; day 58). Follow-up cultures on a cohort of animals indicated that the animals continued to be YE positive: one Coquerel's sifaka (ISIS #19548; day 81), five BW ruffed lemurs (ISIS #18368, #18369, #18370, #18374, and #18375; day 82), one BW ruffed lemur (ISIS #18369; days 82, 90, 111), six red ruffed lemurs (ISIS #18376, #18377, #18378, #18380, #18381, and #18382; day 82), and one red ruffed lemur (ISIS #18379; days 82, 90).

Feces was also collected from a white handed gibbon (ISIS #18547) located within the Jungle building on day 66 due to clinical signs of lethargy and diarrhea. YE was detected on this day and in a follow-up culture on day 71.

YE was also detected in several other species that were cultured only for disease surveillance: two siamangs (ISIS #17910 and #18540; day 76), one orangutan (ISIS #13973; day 85), three agile gibbons (ISIS #15353, #17561, and #18466; day 86), and three black lemurs (ISIS #19541, #19542, and #19543; day 90).

The severity of yersiniosis at OHDZA became evident on day 70 when a BW ruffed lemur (ISIS #18373), from the Madagascar exhibit died unexpectedly with no symptoms and was discovered to have YE in fecal culture. During postmortem examination, gross abnormalities were seen within the liver, small and large intestines, and within the region of the gastric mesentery. Histopathologic diagnosis revealed acute and chronic abscesses of the large intestines, lymph nodes, and liver. The changes seen in these tissues were characteristic of yersiniosis and correlate with the culture results that were performed at the time.

One of the institutions Coquerel's sifakas (ISIS #19548) presented on day 80 with dehydration, lethargy, not taking medications well, and a decreased appetite. Oral erythromycin (80 mg/mL solution, Arbor Pharmaceuticals, Atlanta, GA 30328, USA; 88 mg po, bid) had been started on day 75 due to a positive fecal culture of *Campylobacter jejuni* only; this individual was negative for YE at this time. Supportive care and antibiotics were given, including lactated Ringer's solution (1-L solution, Nova-Tech Inc, Grand Island, NE 68801, USA; 120 ml sc, bid), erythromycin (500-mg solution, Hospira, Inc, Lake Forest, IL 60045, USA; 120 mg sc, bid), ceftazidime (1-g solution, Hospira, Inc; 210 mg im), and meloxicam (5-mg/ml solution, Bimedia, Inc, LeSueur, MN 56058, USA; loading dose of 0.8 mg sc, sid, followed by a maintenance dose of 0.4 mg sc, sid). Unfortunately, this sifaka's

condition worsened, and it was found dead 2 days after clinical presentation of the symptoms. YE was isolated from postmortem materials that matched other isolates from the start of the outbreak (Table 1; Fig. 1). The small and large intestines were noted to be ulcerated and hyperemic grossly during postmortem examination. On histopathology, a diagnosis of a multifocal, severe, necrotizing, ulcerative, and hemorrhagic enteritis with intralesional bacilli was made. One of the differentials for the bacteria present was yersiniosis. Cultures taken at the time of gross postmortem examination revealed a few colonies of YE.

The second and remaining Coquerel's sifaka (ISIS #19549), also located in the Madagascar exhibit, was likewise started on oral erythromycin 85.2 mg po, bid on day 75 due to the positive fecal culture of *C. jejuni* only; this individual was also negative for YE at this time. The keeper staff noted abnormal behavior and lethargy in the afternoon on day 83. Clinically this animal was dehydrated and had "raspiness" on inspiration. Supportive care and antibiotics were started immediately after a thorough diagnostic workup was performed. Lactated Ringer's solution (240 ml sc, sid), meloxicam (0.2–0.4 mg im, sid), ranitidine (25-mg/ml solution, Teligent Pharma, Inc, Singapore, Singapore; 1.95 mg im), metoclopramide (5-mg/ml solution, Cardinal Health, Dublin, OH 43017, USA; 0.78 mg im, sid), and ceftazidime (195 mg im, sid) were administered and continued over 3 days. The inspiratory noise appeared to improve initially; however, it took a turn for the worse and a brown/red crusty material could be seen surrounding the nasal opening. This material quickly turned into copious amounts of a bloody mucopurulent bilateral nasal discharge. This animal died on day 87. The postmortem examination revealed reddened lungs with grossly normal intestines. Histopathologic findings suggested liver necrosis and disseminated intravascular coagulation. YE was not cultured from the lung or intestinal tract tissues after postmortem examination of this individual.

The remaining BW ruffed lemurs (five animals) and red ruffed lemurs (seven animals) in the outdoor Madagascar exhibit were treated with differing oral antibiotics to include the following: trimethoprim/sulfadiazine (400 mg, Neogen Corp, Lexington, KY 40511, USA; 29.4 mg/kg po, sid), trimethoprim/sulfamethoxazole (48 mg/ml, Pharmaceutical Associates, Inc, Greenville, SC 29605, USA; 30 mg/kg po, sid), and enrofloxacin (100 mg/ml, Taylors Pharmacy, Winter Park, FL 32789, USA; 9.8 mg/kg po, sid) prophylactically, to prevent further disease within the enclosure. Although additional animal cultures were collected on day 90 (one BW lemur ISIS #18369 and one red ruffed lemur ISIS #18379) and on day 111 (one BW lemur ISIS #18369), the animals remained clinically normal, and follow-up fecal cultures in these treated animals remained negative for YE.

A subset of YE isolates ($n = 8$) recovered from YE-positive animals were confirmed as YE 1B/O:8 at the CDC (Atlanta, GA 30329, USA), and these isolates were also characterized by PFGE (Fig. 1). The isolates from the Coquerel's sifaka, agile gibbon, and orangutan were indistinguishable from each other by *Apa*I and differed from isolates from the other five species only by a single band shift around 240 kb. All isolates were indistinguishable from each other by *Not*I. PFGE-matched isolates from the start of the outbreak were also recovered from other asymptomatic animals in the Madagascar exhibit on day 58: BW ruffed lemurs (six cases) and red ruffed lemurs (seven cases) and on day 90 in a single BW

ruffed lemur and red ruffed lemur. To the authors' knowledge, no human cases of yersiniosis were associated with this zoo outbreak.

DISCUSSION

This report describes clonal spread of YE 1B/O:8 in numerous species at one zoo and provides details on disease progression, diagnostic testing, treatment, and follow-up. This report differs from other reports of YE in zoo animals in that most cases were detected in shedders during routine fecal cultures and a few from postmortem examination.[3,6,8]

Nearly indistinguishable PFGE patterns of YE IB/O:8 were observed across the eight animals tested. Although this observation suggests a common exposure by all infected animals to a single strain, it is not conclusive due to the lack of discriminatory power of PFGE for YE.[4] Alternative molecular methods are needed to more accurately and definitively determine an epidemiologically meaningful relationship among strains. Although a source of YE was not identified, prevention and surveillance efforts remain vigilant to prevent future outbreaks.

The selection of antibiotics and the supportive care provided to the animals was based initially on the history of what has been successful within this collection previously but also the ease of which medications could be reliably given and accepted by these animals. The literature suggested that varying antibiotic resistance in YE due to broad spectrum enzyme A production is possibly, making certain classes of antibiotics partially to completely resistant to B-lactams such as the cephalosporins and/or oxacillin. This suggested that the antibiotic of choice should be within the fluoroquinolone family. Other antibiotics that have proven helpful to treat yersiniosis are tetracyclines, trimethoprim-sulfonamides, and chloramphenicol.[5] A variety of these antibiotics were administered in this outbreak with varied success. The Coquerel's sifakas appeared to be the most sensitive to YE regardless of the medication that was administered. This correlates with the suggestions in the literature that certain animal species are more susceptible to YE than others.[16] The Coquerel's sifakas also presented with the most severe clinical symptoms (approximately 2–7 days before death), and despite supportive care, including antibiotics and fluid therapy, neither animal survived. Both animals were found to have positive cultures for *C. jejuni* during the outbreak; however, they were negative for YE at the time.

Exposure to YE is thought to be primarily through a "cold" source (ingestion of water and/or food) rather than a "warm source" (fecal/oral transmission from a mammal). Reptiles, fish, and invertebrates appear to not be carriers of YE, and although birds are not known to carry the YE O:8 strain, they may carry other strains of YE.[12] Humans could also be considered as a source of YE; however, strain 1B/O:8 is not as common or as prevalent within the United States today as it was several decades ago.[11] Zoonotic transfer to humans should be considered when YE is identified in zoo animals. The seasons of the year also may play a role, with early spring, late autumn, and winter being when the highest frequencies of this bacteria are present.[16] The zoo investigation began in mid-April 2011, which correlates with a higher frequency of occurrence in early spring. It is possible that the environment did play a role in the outbreak source; for example, the animals may have ingested a contaminated

food source and later defecated in their environments,[8] thus creating an opportunity for rodents[1] to spread the bacteria to other enclosures. However, without a definitive culture from any of these potential sources for confirmation, it is difficult to confirm this hypothesis. Others have also encountered similar challenges as this institution in determining a source for outbreaks, and in general, most cases of yersiniosis appear to occur intermittently and without an identifiable source.[10] This may be due to low numbers of the pathogenic bacteria present within a large amount of background bacterial flora, making isolation from either a food source or the environment unlikely.[2] Therefore, when the source of the infection is not known, preventing entrance of this bacteria within a zoologic institution is increasingly more difficult. However, prevention is key in the control of yersinia and can be achieved with a good rodent control program, as well as practicing good hygiene within exhibits and kitchens.

CONCLUSION

This report describes a clonal spread of YE 1B/O:8 in multiple zoo species housed in different locations of one zoologic institution. Certain susceptible species showed dramatic symptoms in response to carriage or infection with this strain of YE compared with other species. YE 1B/O:8 presented with the greatest severity in the nonhuman primates[5] of the Madagascar exhibit and correlates well to what has been previously stated in the literature. This disease[16] represented all clinical stages from acute as in the BW ruffed lemur that was found dead unexpectedly on day 70 to latent as with the two Coquerel's sifakas that tested positive on fecal culture with YE at the start of the outbreak but did not show clinical disease until days 80 and 83, respectively. Twelve different species tested positive for YE in fecal culture. To the authors' knowledge, this is the first report of YE 1B/O:8 in an institution that involves such a large variety of species. Disease surveillance continues by performing routine cultures in a variety of species and with the development of protocols in place when YE is detected from an individual and/or a group. Fecal cultures are performed monthly for any animal that comes into contact with the public and quarterly for those that do not have direct contact. To date, no additional positive cultures have been detected in this animal population.

Acknowledgments:

The authors thank the Madagascar exhibit keepers and the laboratory and veterinary technicians for support in the collection of samples and in the treatment of the affected animals during the outbreak investigation.

LITERATURE CITED

1. Bresnahan JF, Whitworth UG, Hayes Y, Summers E, Pollock J. *Yersinia enterocolitica* infection in breeding colonies of ruffed lemurs. J Am Vet Med Assoc. 1984; 185(11):1354–1356. [PubMed: 6511581]
2. Fredriksson-Ahomaa M, Korkeala H. Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: a methodological problem. Clin Microbiol Rev. 2003;16(2):220–229. [PubMed: 12692095]
3. Frediksson-Ahomaa M, Naglic T, Turk N, Seol B, Grabarevic Z, Bata I, Perkovic D, Stolle A. Yersiniosis in zoo marmosets (*Callitrix jacchuss*) caused by *Yersinia enterocolitica* 4/O:3. Vet Microbiol. 2007;121:363–367. [PubMed: 17223285]

4. Goering RV. Pulsed field gel electrophoresis: a review of application and interpretation in the molecular epidemiology of infectious disease. Infect Gen and Evol. 2010;10(7):866–875.
5. Hirsh DC. Enterobacteriaceae: Yersinia In: Hirsh DC, MacLachlan NJ, Walker RL (). Veterinary microbiology. 2nd ed. Ames (IA): Blackwell; 2004 p. 75–80.
6. Iwata T, Une Y, Okatani AT, Kaneko S-I, Namai S, Yoshida S-I, Horisaka T, Horikita T, Nakadai A, Hayashidani H. *Yersinia enterocolitica* serovar O:8 infection in breeding monkeys in Japan Microbio Immun. 2005;49(1):1–7.
7. MacArthur JA, Wood M. Yersiniosis in a breeding unit of *Macaca fascicularis* (Cynomolgus monkeys). Lab Anim. 1983;17:151–155. [PubMed: 6345931]
8. Nakamura S, Hayashidani H, Iwata T, Namai S, Une Y. Pathological changes in captive monkeys with spontaneous yersiniosis due to infection by *Yersinia enterocolitica* serovar O8. J Comp Path. 2010;143:150–156. [PubMed: 20207365]
9. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Path Dis. 2006; 3(1):59–67.
10. Sasaki Y, Hayashidani H, Kaneko K-I, Ogawa M, Tanabe K, Kono N, Shichiri S, Hashizaki F, Hiramatsu H. Occurrence of *Yersinia enterocolitica* in the Tokyo Tama Zoo. J Wildl Dis. 1989;25(2):287–290. [PubMed: 2716113]
11. Schubert S, Bockemuhl J, Brendler U, Heesemann J. First isolation of virulent *Yersinia enterocolitica* O8, Biotype 1B in Germany. Eur J Clin Microbiol Infect Dis. 2003;22:66–68. [PubMed: 12582749]
12. Shayegani M, Stone WB, DeForge I, Root T, Parsons LM, Maupin P. *Y ersinia enterocolitica* and related species isolated from wildlife in New York State. Appl Environ Microbiol. 1986;52(3):420–424. [PubMed: 3767355]
13. Wauters G Antigens of *Yersinia enterocolitica* In: Bottone EJ (). Yersinia enterocolitica. Boca Raton (FL): CRC Press; 1981 p. 41–53.
14. Wauters G, Charlier S, Shulze G, Shulze J. Somatic and flagellar antigens of *Yersinia enterocolitica* and related species. Contr Microbio Immun. 1991;12: 239–243.
15. Wauters G, Janssens K, Janssens M. Revised biogrouping scheme of *Yersinia enterocolitica*. Contr Microbio Immun. 1987;9:14–21.
16. Zwart P Yersiniosis in nondomestic birds and mammals In: Fowler ME (). Zoo and wild animal medicine, volume 3, Current Therapy. Philadelphia (PA): W. B. Saunders; 1993 p. 52–55.

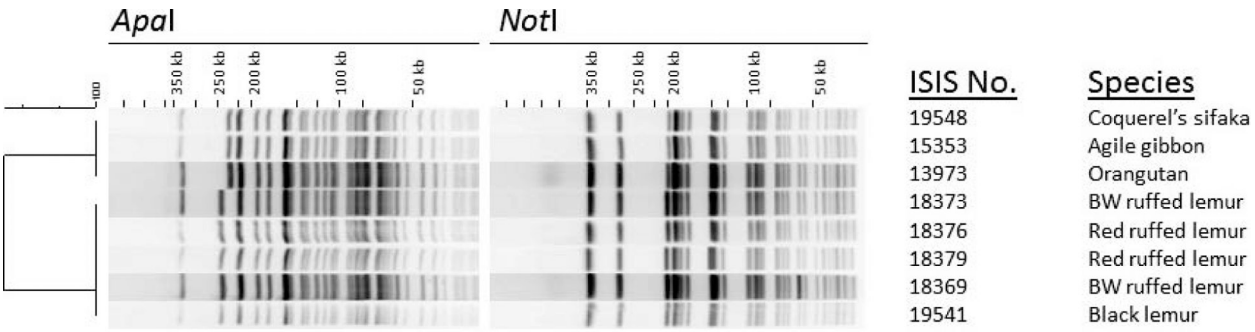

Figure 1.

Yersinia enterocolitica 1B/O:8 DNA fingerprints generated by pulsed-field gel electrophoresis (PFGE). Band size in kilobases (kb) indicated by the scale. PFGE patterns for isolates detected from three different animal species (ISIS #19548, #15353, and #13973) were indistinguishable by *Apa*I. They differed from isolates detected from lemur species (five isolates) by an ~10-kb band shift around 240 kb. PFGE patterns for all isolates are indistinguishable by *Not*I.

Table 1.

Timeline of species showing positive fecal results for YE and their location within the zoo.[a]

ISIS no.	Species	Location[b]	Day YE detected[c]	PFGE pattern	Clinical definition	Symptoms	Outcome
19548	Coquerel's sifaka	Madagascar exhibit	0, 81	Yes	Shedder to clinically affected	Diarrhea, dehydration, lethargy, decreased appetite	Died
19549	Coquerel's sifaka	Madagascar exhibit	0, 87	NA	Shedder to clinically affected	Lethargy, dehydration, inspiratory stridor	Died[d]
19553, 19555	Mongoose lemur	Madagascar exhibit	0	NA	Shedder	NA	Resolved
20667	Colobus monkey	Hospital holding	22	NA	Shedder	NA	Resolved
15912, 16786, 20439, 20440, 20583	African hunting dog	Small mammals exhibit	23	NA	Shedder	NA	Resolved
20744	Argus pheasant	Hospital holding	44	NA	Shedder	NA	Resolved
18368, 18370, 18374, 18375	BW ruffed lemur	Madagascar exhibit	58,82	NA	Shedder	NA	Resolved
18373	BW ruffed lemur	Madagascar exhibit	58, 70	Yes	Shedder	NA	Died
18369	BW ruffed lemur	Madagascar exhibit	58, 82, 90, 111	Yes	Shedder	NA	Resolved
18377, 18378, 18380, 18381, 18382	Red ruffed lemur	Madagascar exhibit	58, 82	NA	Shedder	NA	Resolved
18376	Red ruffed lemur	Madagascar exhibit	58, 82	Yes	Shedder	NA	Resolved
18379	Red ruffed lemur	Madagascar exhibit	58, 82, 90	Yes	Shedder	NA	Resolved
18547	White handed gibbon	Jungle exhibit	66, 71	NA	Shedder to clinically affected	Diarrhea, lethargy	Resolved
17910, 18540	Siamang	New orangutan exhibit	76	NA	Shedder	NA	Resolved
13973	Orangutan	New orangutan exhibit	85	Yes	Shedder	NA	Resolved
15353, 17561, 18466	Agile gibbon	Old orangutan exhibit	86	Yes	Shedder	NA	Resolved
19542, 19543	Black lemur	Madagascar exhibit	90	NA	Shedder	NA	Resolved
19541	Black lemur	Madagascar exhibit	90	Yes	Shedder	NA	Resolved

[a] BW, black & white; ISIS, International Species Information System; NA, not applicable; PFGE, pulsed-field gel electrophoresis; YE, *Yersinia enterocolitica*.

[b] Enclosure that housed the infected species.

[c] Number of days after initial cultured case of YE (day 0).

[d] Culture negative for YE.