Outbreak of *Yersinia Pseudotuberculosis* in a Zoo in Israel

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ABSTRACT

Here we report an outbreak of *Yersinia pseudotuberculosis* in the National Zoo of Ramat Gan in Israel. The outbreak took place between February and April 2012, and affected multiple species during different sessions of the zoo, namely addax (*Addax nasomaculatus*), agouti (*Dasyprocta* spp.), antbear (*Myrmecophaga tridactyla*), oryx (*Oryx leucoryx*), marmoset monkey (*Callithrix* spp.) and macaque (*Macaca* spp.). The most common gross pathology findings were observed in the small intestine, mesenteric lymph nodes, and liver. *Y. pseudotuberculosis* was the only pathogen consistently found. Thirteen isolates that were available for phenotyping were assigned to biovar 1, and were sensitive to 13 out of 14 antibiotics tested. Two isolates were further assigned to serotype O:1b and positive by PCR for genes *inv* and *yadA*, thus confirming their pathogenic genotype.

Keywords: *Yersinia pseudotuberculosis*; Zoo Animals; Outbreak; Israel Typing.

INTRODUCTION

Yersinia pseudotuberculosis is a member of the Enterobacteriaceae family of gram-negative bacteria that can cause enteritis and septicemia in a variety of mammals and birds. Stress factors such as cold, starvation, transport, and pre-existing gastrointestinal damage, are believed to trigger the disease (1,2). The bacteria can lead to enterocolitis, mesenteric lymphadenitis, and rarely, septicemia. Yersiniosis has been reported globally in livestock and wildlife (3). The lesions caused by *Y. pseudotuberculosis* and *Y. enterocolitica*, a similar species, cannot be distinguished through gross pathology alone (4).

CASE REPORT

In 2012, 15 animals from the National Zoo of Ramat Gan in the center of Israel died during the months of February and March. The National Zoo houses over 1,600 animals from 68 species of mammals, 130 species of birds and 25 species of reptiles. Mortality was first detected in addax and oryx over a period of 2 weeks in February. Mortality of antbear, agouti and monkeys was detected in the following month, several days apart from one another. The deceased animals underwent a full *post-mortem* examination at the Kimron Veterinary Institute in Israel, and samples were collected for bacterial and histopathological analysis.

MATERIAL AND METHODS

The bacteriological examinations were performed on internal organ samples using various culture conditions and media after surface burning (5). All samples were inoculated on blood agar (tryptone agar [Merck, Darmstadt, Germany] enriched with 5% washed sheep blood cells), non-enriched tryptone agar, and McConkey agar (Becton-Dickinson, Heidelberg, Germany), and incubated aerobically at 37°C for 24-48 hours. Intestinal contents, necrotic or purulent materials were also inoculated on blood agar and incubated anaerobically at 37°C for 48 hours. Samples from ruminants' lungs were also inoculated on blood agar and incubated at 37°C in microaerophilic (5% CO_2 enriched) atmosphere for 72 hours. Mesenterial lymph nodes or intestinal contents

were tested for the presence of *Salmonella* spp. by enrichment for 24 hours in tetrathionate broth (Neogen, Lansing, MI, USA) with 1% iodine, and then inoculated on McConkey and Brilliant Green agars and incubated at 37°C for 24 houts. Lung samples were further tested for the presence of *Mycoplasma* spp. by enrichment in *Mycoplasma* Friis FF selective broth (6) incubated in microaerophilic conditions at 37°C, and inoculated on *Mycoplasma* Friis FF selective agar 3 and 10 days thereafter, which were incubated under the same conditions. Following the first isolations of *Y. pseudotuberculosis*, further samples were also inoculated on Yersinia selective agar (Becton-Dickinson, Heidelberg, Germany).

Bacterial species identification was performed by standard bacteriological techniques (3). Anaerobic isolates were identified with ID23 API Anaerobes (bioMérieux, Marcy-l'Étoile, France). *Mycoplasma* spp. isolates were identified at the Division of Avian Diseases in the KVI as described before (5).

Y. pseudotuberculosis was first identified by means of colony morphology, triple-sugar iron, urea, lysine and indole reactions, and biochemical profile using API 20E (bioMérieux, Marcy-l'Étoile, France), and biotyped using reactions for raffinose, melibiose, and citrate (7). Identification was later confirmed by MALDI-TOF (Bruker, Germany) following the manufacturer's direct protocol. Antibiotic sensitivity was assessed by a standard disk diffusion test following the CLSI standards and using the following antibiotics (Oxoid): amikacin, amoxicillin-clavulanic acid, ampicillin, cefotaxime, cephalotin, enrofloxacin, gentamicin, trimethoprim-sulfamethoxazole, cefuroxime, florfenicol, tilmicosin, ceftiofur, tetracycline, and penicillin G. In addition, two representative isolates were serotyped (cordially performed by Dr. Mikael Skurnik, University of Helsinki, Finland) and screened in the KVI for the presence of genes *inv*, encoding invasion, and *yadA*, encoding a collagen-binding protein, as chromosomal and plasmid markers of pathogenicity, respectively (8).

Virological exames were performed by PCR following the Dept. of Virology protocols in the KVI. Histopathological exams were performed as described previously (5).

RESULTS

Macroscopic lesions and bacteriological results are summarized in table 1. *Y. pseudotuberculosis* was the main pathogen consistently found in the examined animals.

The most common gross findings were observed in the small intestine, mesenteric lymph nodes, and liver (Figures 1 and 2) in all species. Severe fibrino-hemorrhagic enterocolitis was present, characterized by necrotic debris and serosal edema. The mesenteric lymph nodes were greatly enlarged, appearing necrotic and hemorrhagic on the cut surface with a caseous content in some cases. The liver showed diffuse dissemination of miliary foci of necrosis measuring approximately 0.5-1 mm in diameter. Seven cases showed peritoneal involvement, characterized by a yellowish exudate and fibrin strands in the peritoneal cavity. Other occasional lesions included caseous-necrotic laryngitis, pleuritis, and suppurative pneumonia.

Histologically, the intestinal lesions consisted of extensive areas of necrosis and hemorrhage affecting the mucosa, lamina propria, submucosa, and, in some cases, the muscularis and serosal surface (Figure 3). A pseudo membrane made up of necrotic material, blood, and numerous bacterial colonies was present on the mucosal surface. Bacteria were also present within blood vessels. The mesenteric lymph nodes showed masses of gram-negative coccobacilli forming microcolonies, surrounded by neutrophils, with multifocal to coalescing areas of necrosis (Figure 4). The hepatic lesions consisted of randomly distributed foci of hepatocytic necrosis of varying sizes, accompanied by neutrophils and few macrophages, with intralesional bacterial colonies (Figure 5).

A total of 13 *Y. pseudotuberculosis* isolates were biotyped and tested for antibiotic sensitivity. All isolates were assigned to biovar 1 (raffinose- and citrate-negative, melibiose-positive). All isolates were sensitive to all drugs tested but tilmicosin, against which the isolates were resistant. In addition, two isolates were serotyped and screened for the presence of chromosomal and plasmid markers of virulence. These isolates were assigned to serotype O:1b, and both isolates were positive for the presence of *inv* and *yadA*, thus confirming their pathogenic genotype.

DISCUSSION

Yersiniosis is a widespread disease affecting various animal species, including ruminants, primates, rodents, lagomorphs, birds, and, less commonly, carnivores (1). *Yersinia pseudotuberculosis*, the causative agent, has been reported globally in animals as well as in soil, water, and food. The primary

Table 1. Macroscopic lesions and bacteriological results during an outbreak of *Y. pseudotuberculosis* in the National Zoo in Ramat Gan, 2012.

Date of Death	Affected Organs	Bacteria findings [Virological findings]
Addax (*Addax nasomaculatus*)		
Feb 1st	Lungs, liver	Mixed culture
Feb 9th	Small intestine, liver, peritoneum	*Y. pseudotuberculosis* (liver, spleen)
		Clostridium septicum (intestine)
Feb 12th	Small intestine, liver, peritoneum	*Y. pseudotuberculosis* (spleen, liver, intestine)
		[RSV]
Feb 12th	Small intestine, lungs, peritoneum	*Y. pseud.* (spleen, liver)
		C. septicum (intestine)
		[Akabane virus]
Feb 14th	Small intestine, mesenteric lymph nodes, liver, spleen	*Y. pseudotuberculosis* (lymph nodes, spleen, intestine)
Feb 20th	Larynx, lungs, liver, spleen, mesenteric lymph nodes, intestine	No bacteria found
		[BTV]
Mar 7th	Larynx, kidney, systemic lymph nodes, mammary gland, liver, lungs	*Y. pseudotuberculosis* (spleen, lungs, lymph nodes)
Apr 3rd	Small intestine, lungs	*Y. pseudotuberculosis* (liver)
		Mycoplasma arginini (lungs)
Mar 4th	Small intestine, liver, peritoneum	*Y. pseudotuberculosis* (liver, spleen)
Mar 5th	Small intestine, mesenteric lymph nodes, peritoneum	*Y. pseudotuberculosis* (liver, spleen, lung)
		M. arginini (lung)
Agouti (*Dasyprocta* spp.)		
Mar 4th	Lungs, liver, kidney, brain stem	*Y. pseudotuberculosis* (lung, liver, kidney)
Antbear (*Myrmecophaga tridactyla*)		
Mar 11th	Large intestine, mesenteric lymph nodes, peritoneum, tracheobronchial lymph node	*Y. pseudotuberculosis* (spleen, liver, lung, lymph nodes)
		Mycoplasma spp. (lung)
Oryx (*Oryx leucoryx*)		
Feb 16th	Lungs, liver, small intestine, peritoneum, pleura	*Y. pseudotuberculosis* (lung, liver, spleen, intestine)
		Arcanobacterium pyogenes (lung)
		C. septicum (spleen, peritoneum, liver, kidney, intestine)
Mar 6th	Mesenteric lymph nodes, small intestine, liver	*Y. pseudotuberculosis* (liver, spleen, intestine)
Mar 11th	Liver, intestine	*Y. pseudotuberculosis* (liver)
		Salmonella grp. C2 (intestine)
		[BTV]
Mar 11th	Small intestine, peritoneum, mesenteric lymph nodes	Not suitable for bacterial culture
		[BTV]
Mar 18th	Small intestine, liver, spleen	*Y. pseudotuberculosis* (liver)
Marmoset monkey (*Callithrix* spp.)		
Mar 22th	Lungs, liver	*Y. pseudotuberculosis* (spleen)
		C. bifermentans (liver)
Mar 28th	Autolytic, no lesions detected	Mixed culture
Mar 27th	Liver, meninges, brain	*Y. pseudotuberculosis* (liver)
Apr 1st	No lesions observed	No bacteria found
Macaque (*Macaca* spp.)		
Mar 29th	Liver	*Y. pseudotuberculosis* (liver)

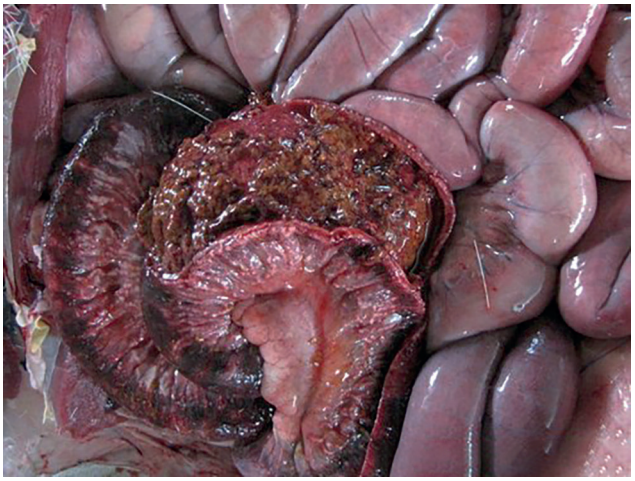

Figure 1: Pseudomembranous enteritis and serosal edema.

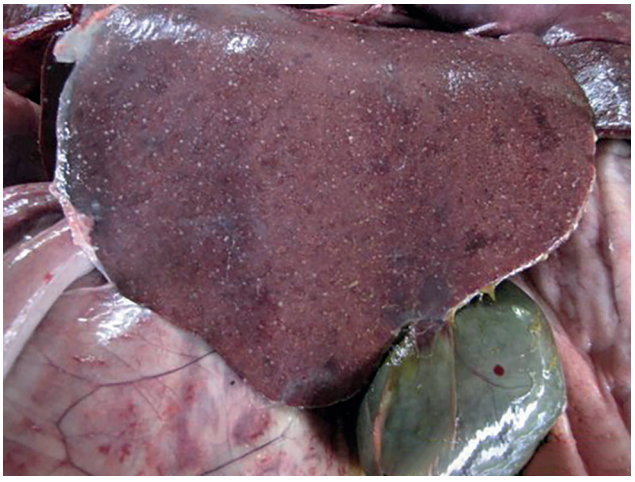

Figure 2: Hepatic necrosis, multifocal.

modes of transmission are through contaminated water and food sources and ingestion of infected meat. As previously reviewed, *Y. pseudotuberculosis* is also a human pathogen, with public health implications (9). Apart from two unrelated cases of sepsis in captive monkeys (D. Elad, unpublished data) and an outbreak of *Y. pseudotuberculosis* in bovine mastitis in dairy cattle (9), this species is rarely isolated from animals in Israel. The isolates in this outbreak were confirmed to be of pathogenic lineages by means of biotyping, serotyping and genotyping.

At the National Zoo, ruminants are kept in a large open area (the "Safari") and other animals, such as monkeys, antbears, and rodents, are kept in separate cages. The initial source of infection, which probably occurred in the ruminant yard, is unknown. However, during the outbreak, the

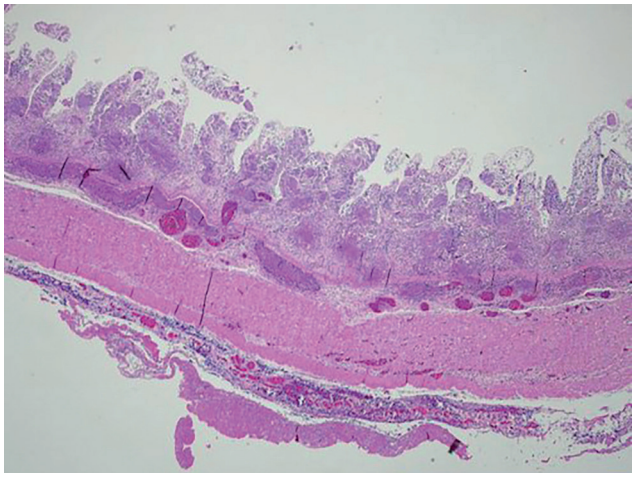

Figure 3: Jejunum: Denuded villi with necrosis and multifocal fibrinous peritonitis.

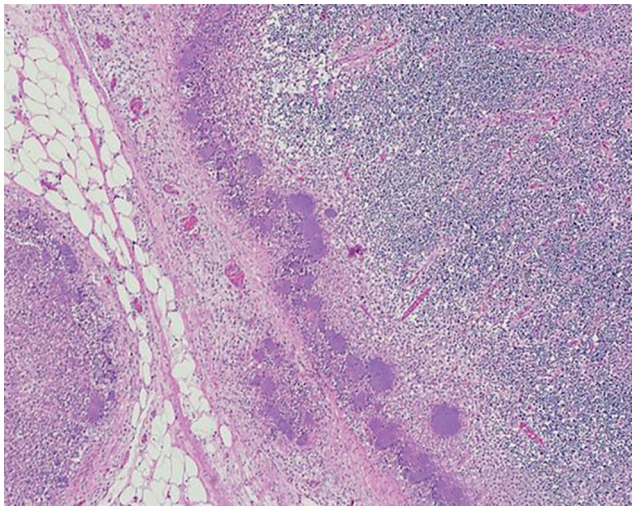

Figure 4: Lymph node: lymphadenitis necrotizing with subcapsular bacterial colonies.

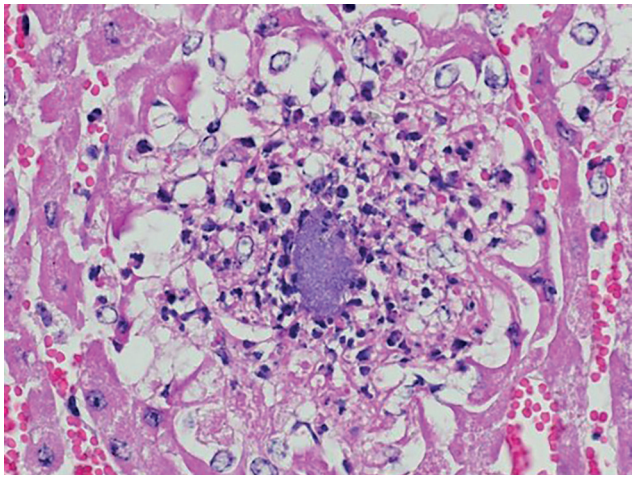

Figure 5: Liver-hepatocyte necrosis with intralesional bacterial colonies, degenerative neutrophils and peripheral macrophages.

bacterium was isolated from animals other than ruminants, suggesting cross-contamination from ruminant carcasses stored in the same fridge used for the food of other animals, given that *Y. pseudotuberculosis* is highly adapted to cold (10).

Outbreaks of yersiniosis can be triggered by stressors, such as cold and wet weather, changes in food availability, overcrowding, and capture (2). The incidence of the disease may be seasonal, with an increase in the colder temperatures of late autumn, winter, and early spring (11,12). In 2012, the winter in Israel was particularly cold and rainy. The outbreak period from early February towards the end of March correlates with a period of relatively heavy rains and cold temperatures measured in that area. No other changes in animal feeding or population were reported, and no outbreaks were reported in other zoos in Israel, despite lower temperatures. No further cases have been diagnosed in subsequent years. A nearby dump site that attracted migrating birds, which are potential carriers of the bacterium (13), was proposed as a possible source of infection. Although a significant number of rats were found dead in the National Zoo during the outbreak, no *Yersinia* bacteria were isolated from the examined rodent samples. Later attempts to treat rats trapped in the National Zoo with corticosteroids to induce immunosuppression failed to produce any bacterial infections.

In this outbreak, the lesions caused by yersiniosis were more widespread and involved various organs, including the small intestine, liver, spleen, lungs, meninges, brain, kidney, and mammary glands in one addax. The lesions found in early and later cases were not distinguishable in terms of distribution. Although *Y. pseudotuberculosis* was isolated from the intestine and other organs, intestinal lesions were not always present, suggesting a second possible route of dissemination besides the intestinal lumen (14). The concurrent infections with *Salmonella* spp. and *Porphyromonas* spp. in some animals may have resulted from a decline in host immune system defenses. In just a few instances in this study, positive PCR results were reported for Blue Tongue, Akabane and Respiratory Syncytial viruses. The importance of these findings to the overall outbreak remains uncertain. Acute necrotizing enteritis, similar to that seen in fulminant yersiniosis, can resemble lesions caused by *Yersinia pestis* and *Francisella tularensis*, both of which have not been reported in Israel. Other differential diagnoses to consider are *Salmonella* and *Clostridium piliforme* (Tyzzer's disease) in ruminants and lagomorphs, respectively (1,15).

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