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Yersiniosis in Captive Exotic Mammals

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SUMMARY

Within a 2½-month period, *Yersinia pseudotuberculosis* infection occurred in 3 blesbok (*Damaliscus dorcas*), 1 dik-dik (*Madoqua kirkii*), and 1 giant anteater (*Myrmecophaga tridactyla*) at the National Zoological Park, Washington, DC. Lesions consisted of fibrinonecrotic enteritis and peritonitis, mesenteric lymphadenitis, and embolic pyogranulomatous lesions in the liver, spleen, and lungs. Feed contaminated with the feces of wild rats and pigeons was thought to be the source of infection.

YERSINIA (formerly *Pasteurella*) *pseudotuberculosis* is a gram-negative, pleomorphic coccobacillus that is closely related to the plague bacillus *Yersinia pestis*.³ *Yersinia pseudotuberculosis* infection (yersiniosis, pseudotuberculosis) has been reported in rodents, carnivores, ruminants, subhuman primates, reptiles, birds, and man in many parts of the world.^{2,4,6-14} Clinical signs of the disease are variable. Many affected animals die without premonitory signs; in others, clinical signs include lethargy, anorexia, diarrhea, and emaciation. Frequently reported lesions include small granulomatous nodules disseminated throughout the liver and spleen, mesenteric lymphadenitis, and necrotic gastrointestinal lesions. The organism has been associated with abortion in cattle.⁷ Feed contaminated with the droppings of carrier animals (rodents and birds) is often the principal source of infection.¹¹ On the basis of somatic O antigenic structure, *Y. pseudotuberculosis* has been divided into 5 serotypes. Only serotypes IA, IB, and III have been isolated from mammals in North America.^{6,14}

This report is concerned with the clinical, pathologic, and epidemiologic features of an outbreak of *Y. pseudotuberculosis* serotype IA infection among several types of exotic mammals at the National Zoological Park (NZN), Washington, DC.

Clinical History

The outbreak of yersiniosis occurred from mid-January through early April of 1976 and resulted in the death of a dik-dik (*Madoqua kirkii*), 3 blesboks (*Damaliscus dorcas*), and a giant anteater (*Myrmecophaga*

tridactyla). The dik-dik and blesbok were located in the hardy hoof stock yards. The anteater was located in another part of the zoo.

The giant anteater, a 4-year-old male that had been at the NZP for 3½ years, was the 1st to die. It was found dead inside its heated house in mid-January, 1976. It had been in the same enclosure for the past 2 years and reportedly was eating and behaving normally the previous day.

The dik-dik, a 4-year-old female, had been in the zoo for 3 years and had had no previous illness. It had given birth to its 5th offspring a few months prior to death, and both dam and calf were doing well. Five days after the anteater died, the dik-dik was found dead in its outside enclosure.

Two months later, 3 blesbok (2 females and 1 male) developed an acute illness and died. The females, both 3 years old, had arrived from southwest Africa about 6 months previously. They had been quarantined for 30 days at Clifton, NJ. The male had arrived a month later from the Overton Zoo, Memphis, Tn. In late March, one of the females (MO 1382) was severely depressed and had a rectal temperature of 40.3 C (104.6 F). Blood samples were taken, and a mixture of penicillin and streptomycin was administered intramuscularly. The blesbok's condition improved slightly the next day, and its temperature returned to normal (38.3 C (101 F)). The antibiotics were continued, and the blesbok appeared to be responding to treatment, but it was found dead on the 3rd day of illness. One day before this blesbok died, one of its female penmates (MO 1381) was found dead. The latter blesbok had not manifested any signs of illness prior to death. The clinicopathologic findings on the 1st female blesbok were those of dehydration (elevated hematocrit) and leukopenia, with a left shift. A 3rd female blesbok, housed separately, was given prophylactic oxytetracycline in the feed for 3 days and remains well to date (14 mo later).

Early in April, a male blesbok (101514), penned adjacent to the females that died, appeared slightly bloated and depressed. A blood sample was cultured, and the blesbok was given an injection (1 g) of oxytetracycline. Three days later it was unable to rise; despite intensive antibiotic and fluid therapy, it died within 24 hours. Paracentesis prior to death revealed peritonitis.

Materials and Methods

All animals that died were necropsied. Representative specimens of all organs were fixed in buffered 10% formalin,

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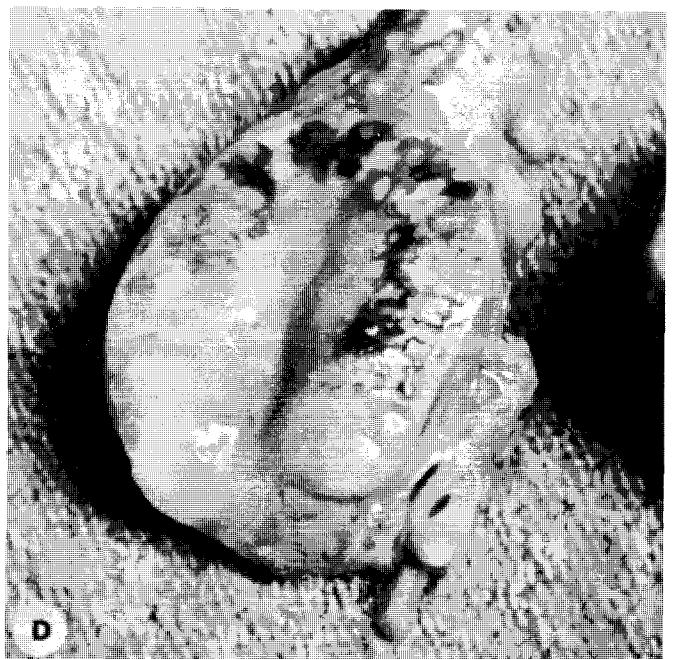
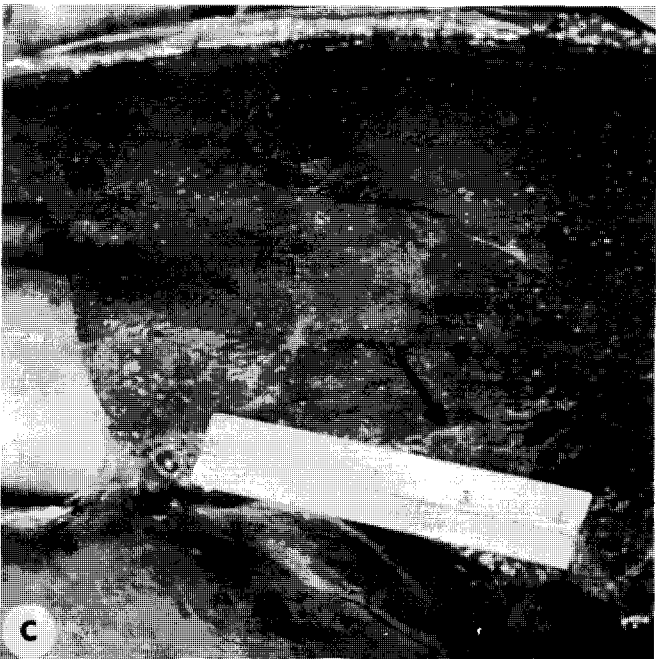
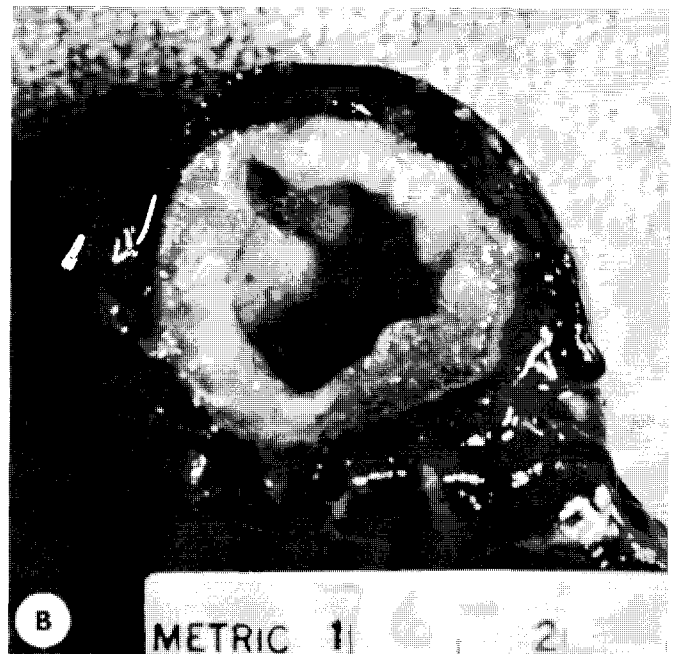
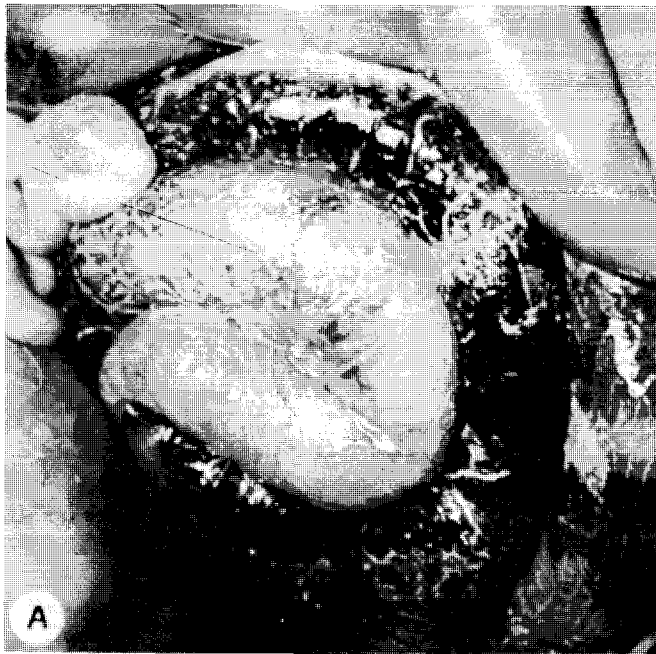


Fig 1—Typical gross lesions from blesbok from which *Yersinia pseudotuberculosis* was isolated.

A—Intestinal tract. Notice fibrinous peritonitis, edema, hemorrhage, necrosis, and distension of serosal lymphatics with exudate.

B—Cross section of small intestine shown in Figure 1A, demonstrating swelling of all layers, with thickened necrotic mucosa and hemorrhage of the muscular wall.

C—Liver with multiple white spots and fibrinous deposits on the surface (arrow).

D—Edematous mesenteric lymph node with necrosis and hemorrhage, mainly in the cortex.

embedded in paraffin, sectioned at 6 μ , and stained with hematoxylin and eosin. Additional staining of selected tissues with acid-fast, gram, Gormori methenamine-silver, or Giemsa stains was done when indicated.

Specimens of lungs, liver, spleen, small intestine, heart blood, and peritoneal exudate were routinely cultured for bacteria (including acid-fast organisms) and fungi. Media used for initial isolation included selenite medium for enteric pathogens, brain-heart infusion, tryptose blood agar plates, MacConkey, and Mycosel^a and Sabouraud dextrose

for fungi. Biochemical tests were conducted, using a profile.^b Cultures of *Y pseudotuberculosis* were sent to the Veterinary Services Laboratory, Animal and Plant Health Inspection Service (APHIS), US Department of Agriculture (USDA), Ames, Ia, and to the Center for Disease Control (CDC), Vector-Borne Diseases Division, Fort Collins, Co, for confirmation and serotyping.

^a Mycosel Agar, Bioquest, Cockeysville, Md.

^b API 20 Enterobacteriaceae, Analytab Products, Inc, Plainview, NY.

Because of the recent importation of some of the affected blesbok from southwest Africa (Namibia), specimens of lungs, liver, kidney, spleen, and small intestine were aseptically removed, placed in sterile plastic bags, and shipped in dry ice to Plum Island Disease Center, Agricultural Research Service (ARS), USDA, Greenport, Long Island, NY, where they were examined for rinderpest and foot-and-mouth disease viruses. Sera collected from affected animals, experimental animals, and unaffected animals in the area were sent to CDC to determine antibody titers against *Y pseudotuberculosis*. Sera were titrated at CDC against a homologous strain (blesbok 101514) and against CDC's serotyping *Y pseudotuberculosis* IA strain, using a passive hemagglutination test.⁵

Because of the implication of wild rodents and birds in the transmission of *Y pseudotuberculosis*, 6 rats (*Rattus norvegicus*) and 2 pigeons (*Columba livia*) from the area where the outbreak occurred were trapped alive. The pigeons appeared lethargic at the time of capture. Four of the rats and both pigeons were anesthetized with sodium pentobarbital, and blood samples were obtained via cardiac puncture. The animals were then killed and necropsied, and tissue specimens were collected for bacteriologic and fungal culture, as was done for the afflicted captive mammals.

The remaining 2 rats were placed in separate cages, given food and water, and treated with 12.5 mg of prednisolone^c intramuscularly every 3 days for 2 weeks. This was done to provide immunosuppression in an attempt to "unmask" any subclinical infection by *Y pseudotuberculosis*. Both animals died of bacterial septicemia after 2 weeks and were necropsied.

Results

Cultural Findings—*Yersinia pseudotuberculosis* was isolated from all animals examined except the anteatr and the blesbok (MO 1382). Organs and other material positive for this organism included lungs, liver, spleen, blood, peritoneal fluid, and intestinal tract. All isolants that were serotyped by CDC proved to be serotype IA. Viruses were not isolated from any of the tissues.

Serologic Findings—Sera from 2 of the blesbok that died and from the surviving female were positive for antibodies to *Y pseudotuberculosis*. The surviving blesbok was seronegative 3 months later. Neither the exposed animals in the area nor the pigeons and rats trapped in the affected area developed antibody titers.

Pathologic Findings—The peritoneal cavities of the anteatr and blesbok contained several liters of thin, yellowish tan fibrinopurulent exudate. The peritoneal surfaces were dull and granular because of a layer of fibrinopurulent exudate.

The gastrointestinal tracts of all animals except the rats and pigeons contained lesions that were restricted to portions of the ileum and jejunum. These lesions varied from a 3 cm-annular band of necrotic mucosa in the dik-dik to multiple segments of completely necrotic small intestine in blesbok (Fig 1A and B). The intestinal mucosal lesions in the blesbok usually had thick, fibrinonecrotic pseudomembranes, and the serosal lymphatics were often dilated and filled with white, purulent exudate (Fig 1A and B). The large intestines were unaffected and contained normal feces.



Fig 2—Photomicrograph of small intestine specimen from a blesbok with yersiniosis, showing fibrinonecrotic pseudomembrane and extension of the inflammatory reaction through the entire thickness of the intestine. H&E stain; $\times 6$.

The livers of all animals except the rats contained numerous pinpoint-sized to 2-mm whitish spots scattered throughout the parenchyma (Fig 1C). One rat had a few spots. The lesions were raised and firm in the dik-dik and the rat but nonelevated in the other animals affected. Slightly larger but less numerous spots were seen within the red pulp of the spleens. In 1 blesbok, the spleen was enlarged and contained capsular hemorrhages. The lungs of the anteatr and 2 of the blesbok contained a few randomly distributed small white spots; the spots were larger and more firm in the dik-dik. The mesenteric lymph nodes of all animals were generally enlarged. Those of the blesbok were edematous, hemorrhagic, and often necrotic (Fig 1D), whereas those of the dik-dik and rats were abscessed.

In general, microscopic findings in all of the affected animals consisted of severe necrosis of the mucosa of the small intestine, with edema of the lamina propria and submucosa and massive infiltration of neutrophils, lymphocytes, and macrophages (Fig 2). Some bowel segments were hemorrhagic, and in some areas the necrotizing process penetrated the smooth muscle layers and extended into the serosa, resulting in peritonitis. Lymphatics, especially those beneath the serosa, were distended and filled with neutrophils, mononuclear cells, and necrotic debris. Many veins contained thrombi. Numerous colonies of gram-negative pleomorphic coccobacilli were seen throughout the lesions.

The spots noticed grossly in the livers and spleens were foci of necrosis containing neutrophils and large monocytes. Giant cells were not seen. Many of the lesions, except those of 1 of the blesbok (MO 1382), contained numerous colonies of pleomorphic, gram-negative bacilli (Fig 3). Occasionally, the foci of necrosis extended through the serosal surface of the organs.

^c Meticorten, Schering Corporation, Kenilworth, NJ.

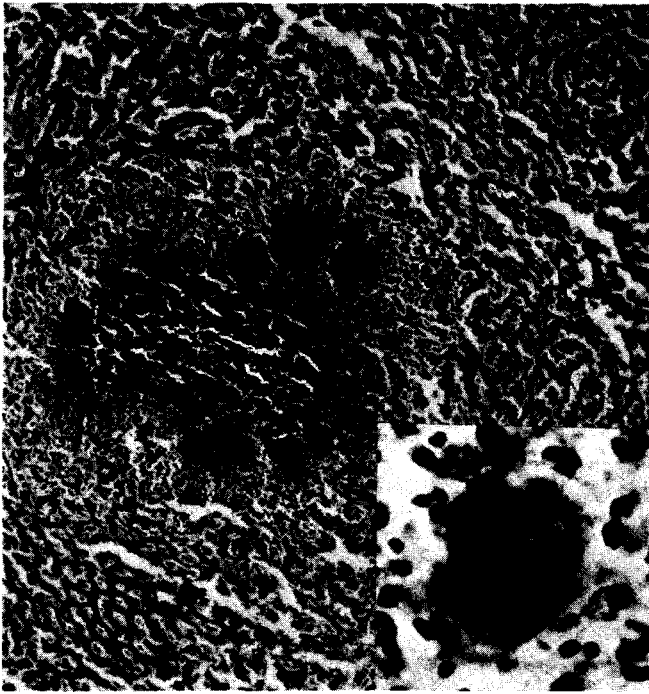


Fig 3—Photomicrographs of hepatic lesion from same blesbok as in Figure 2. H&E stain; $\times 60$. Inset—Notice bacterial colonies and numerous neutrophils. $\times 900$.

Within the blesbok spleens were numerous venous thrombi. The lesions in the dik-dik and rat were more circumscribed, contained fewer neutrophils and necrotic areas, and were surrounded by connective tissue capsules (Fig 4, 5, and 6). The lesions in these animals contained far fewer organisms than those of the blesbok and anteater.

Microscopically, the pulmonary lesions varied from small infiltrations of mixed inflammatory cells into the

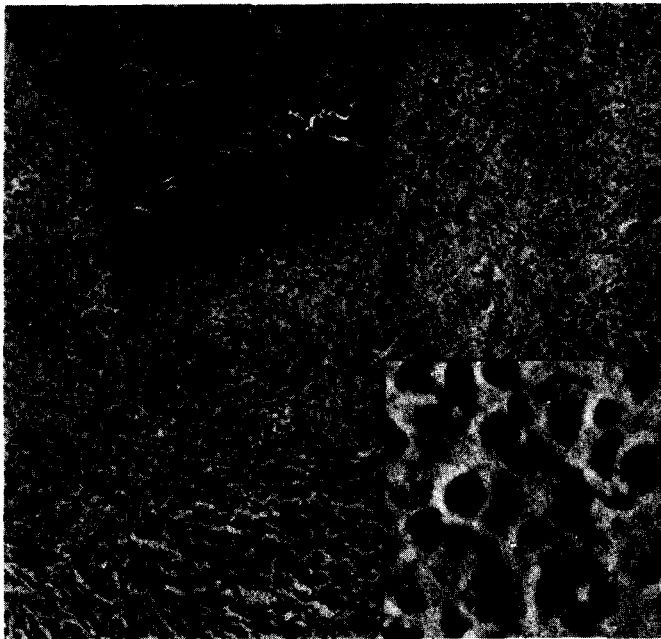


Fig 4—Photomicrograph of liver lesion from a dik-dik with yersiniosis. H&E stain; $\times 75$. Inset—Inflammatory cell infiltrate is mostly mononuclear. $\times 750$.

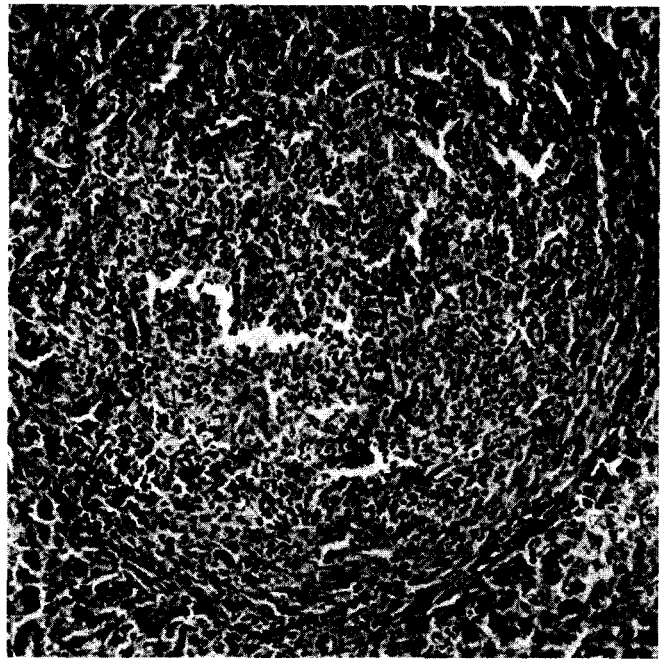


Fig 5—Photomicrograph of liver lesion from a wild rat from which *Y pseudotuberculosis* was cultured. Notice the absence of necrosis and the peripheral zone of fibroblastic cells. H&E stain; $\times 75$.

alveoli to more extensive lesions similar to those in the liver and spleen. The lesions in the dik-dik had large necrotic centers surrounded by an extensive pyogranulomatous inflammatory reaction.

The changes in the lymph nodes of the blesbok and anteater varied from mild infiltrations of the sinusoids by neutrophils and macrophages to extensive necrosis and hemorrhage, with mixed inflammatory cell infiltration and numerous bacterial colonies. The lymph nodes of the dik-dik and rats contained well-developed abscesses with necrotic centers. Organisms were difficult to find in these animals.

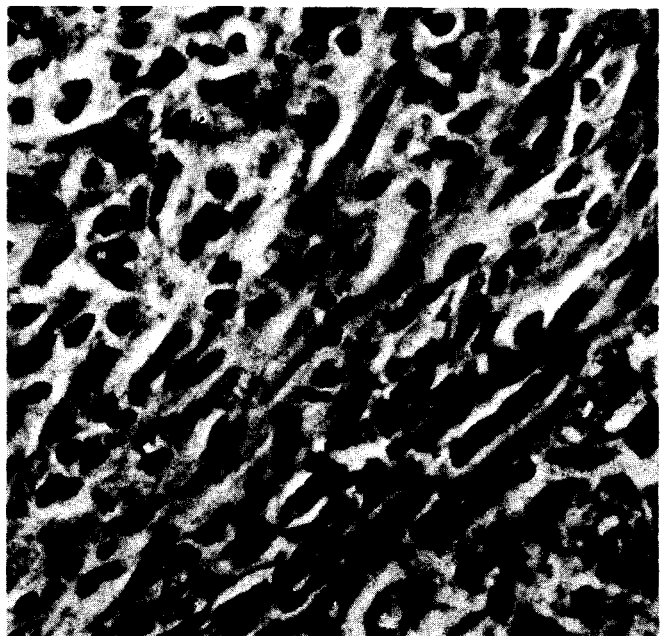


Fig 6—Higher magnification of Figure 5, showing macrophages, fibroblastic cells, and only occasional neutrophils. H&E stain; $\times 750$.

Discussion

This outbreak of yersiniosis illustrates many of the previously reported aspects of the disease and emphasizes its potential importance among captive animals that are exposed to free-living vermin and birds. The source of infection in most reported outbreaks of yersiniosis, most of which have occurred in laboratory animal colonies and zoos, has either been shown or suspected to be wild birds or rodents. Either or both of these types of animal appear to be involved in this outbreak, in that positive cultures were recovered from both pigeons and rats that were trapped in the affected areas and that had access to the feed and surroundings of the affected animals. Because of the chronic, subclinical nature of the disease in the rats, however, they were probably responsible for initiating the outbreak. *Yersinia pseudotuberculosis* has been a cause of fatal illness in feral birds in Pennsylvania and Maryland,⁴ which are in close geographic proximity to the NZP.

Yersinia pseudotuberculosis is not difficult to culture but is easily overlooked because of its preferred growth at 25 C. The organism is nonmotile at 37 C and can be overgrown by other organisms at that temperature. On blood agar the colonies are round, initially translucent, then gray, and have a raised opaque center and flatter periphery. *Yersinia* is a nonlactose fermenter, is oxidase-negative, and ferments glucose, mannitol, and arabinose. Most important, this organism decomposes urea and grows readily on MacConkey agar, on which it can be maintained for several days at 25 C.¹ It was possible to culture this organism from tissues that had been frozen for several weeks. We were unable to culture the organism from the anteater or from 1 of the blesbok (MO 1382). The culture from the anteater was apparently overgrown. We assume the blesbok was culture-negative as a result of the intensive antibiotic therapy, inasmuch as bacteria could not be demonstrated in the lesions histologically either.

The severity of the disease in cases reported here and elsewhere varies among different species and among individuals of the same species. For example, the lesions were chronic and mild in the rats, subacute and moderate in the dik-dik, and acute and severe in the blesbok, anteater, and pigeons. The blesbok died peracutely or acutely, while other exotic hoofed stock in the same area remained healthy. The rats in this instance were clinically normal even though mild lesions were found at necropsy. Factors that contribute to this variation in individual animals and in species susceptibility are unknown, but similar findings have been reported in outbreaks involving other species.^{2,9} Likewise, the frequent occurrence of the disease in winter has been emphasized.^{8,10,11} The spectrum of lesions in the affected animals in this outbreak is similar to that reported by others.^{2-4,10,11} It is unclear whether immunosuppression precipitated clinical disease in the rats. Both *Staphylococcus aureus* and *Y pseudotuberculosis*

were isolated from the rats treated with prednisolone.

Methods that aid in the prevention and containment of this disease have been reported. Foremost is the control of wild rodents and birds, especially in preventing their access to feed. In our case, we implemented increased surveillance and rat extermination by in-house vermin-control personnel and by a commercial firm. Improvements in feed storage were also made; for example, feed was not left out overnight. Quarantine measures were instituted as soon as an infectious disease was suspected. These measures included isolation of affected and exposed animals and prophylactic treatment of the nearby hoofed stock with tetracycline⁴ (to which the original isolant was susceptible) added to the feed at the rate of 4.4 mg/kg/day. Personnel caring for these animals disinfected their footwear and used other sanitary precautions before contacting other animals.

It should be noted that *Y pseudotuberculosis* infection is a zoonotic disease. Although human infection is infrequently reported in the United States,¹² personnel having contact with affected animals should be warned to use strict sanitary precautions for their own safety.

Institution of these measures has apparently stopped the outbreak at the NZP. New cases have not occurred to date (14 mo after the initial outbreak).

⁴ Neo-Terramycin, Pfizer, Inc, New York, NY.

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