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STREPTOCOCCUS ZOOEPIDEMICUS INFECTIONS OF POSSIBLE HORSEMEAT SOURCE IN RED-BELLIED TAMARINS AND GOELDI'S MONKEYS

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Abstract: An outbreak of septicemia caused by *Streptococcus zooepidemicus* occurred in two red-bellied (white-lipped) tamarins (*Saguinus labiatus*) and two Goeldi's monkeys (*Callimico goeldii*) at the National Zoological Park in Washington, D.C. The tamarins were housed indoors in a mixed exhibit along with two species of small mammals, and the Goeldi's monkeys were in a single-species exhibit within the same facility. One of the Goeldi's monkeys infected with *S. zooepidemicus* became depressed and dehydrated but responded to antibiotics and recovered uneventfully. The three remaining animals all developed septicemia and died; lesions consisted of suppurative cervical lymphadenitis and splenitis in the tamarins, and enteritis with mesenteric lymphadenitis in the Goeldi's monkey. Infections were most likely associated with an uncooked horsemeat product from which *S. zooepidemicus* was isolated. When uncooked horsemeat is to be fed to exotic species, the products should be routinely cultured for *S. zooepidemicus,* and strict sanitation practices carried out to prevent inadvertent streptococcal infection in susceptible species.

Key words: Streptococcus zooepidemicus, tamarin, marmoset, *Saguinus* sp., *Callimico goeldii,* horsemeat, septicemia, zoonosis.

INTRODUCTION

Streptococcus zooepidemicus is a commensal bacterium of the skin and upper respiratory tract in horses and is a frequent wound contaminant and a secondary invader following viral respiratory infections.[6] In mares it is often associated with endometritis and abortion.[10] *Streptococcus zooepidemicus* has been reported as a pathogen in domestic farm animals,[11] birds,[12,13,15] dogs,[19] laboratory animals,[1,16] humans,[3,5,8] and recently was responsible for three fatal cases of meningoencephalitis in farmed red deer (*Cervus elaphus*).[7] In 1984, fatal *S. zooepidemicus* infections, attributed to feeding an uncooked horsemeat product, occurred in small carnivorous marsupials and insectivores at the National Zoological Park (NZP) in Washington, D.C.[18] This paper reports additional cases of *Streptococcus zooepidemicus* infections at NZP in red-bellied (white-lipped) tamarins (*Saguinus labiatus*) and Goeldi's monkeys (*Callimico goeldii*) occurring within a 3-wk period. Infections in these cases are strongly suspected to be associated with a horsemeat source from which *S. zooepidemicus* was isolated.

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CASE REPORT

The *Streptococcus* outbreak occurred in the NZP's Small Mammal House (SMH), which is constructed of multiple completely separated glass-fronted exhibits and which houses a variety of small carnivores, primates, insectivores, and rodents. The affected red-bellied tamarins, a 7½-yr-old female and infant daughter, were kept in a mixed exhibit as a family group with three three-banded armadillos (*Tolypeutes matacus*) and an acouchi (*Myoprocta pratti*); the mate and twin male sibling of the red-bellied tamarin group were apparently unaffected. The affected Goeldi's monkeys were kept in a separate exhibit down the aisle from the red-bellied tamarins. Diets for all the inhabitants of the SMH were prepared in the same kitchen and distributed to animals in stainless steel pans. The tamarins and Goeldi's were fed Marmoset Diet (Zupreem, Hill's Pet Products, Inc., P.O. Box

148, Topeka, Kansas 66601, USA) supplemented with fruits and vegetables daily, and live crickets and fetal mouse pups weekly; food pans were mounted to trees and were inaccessible to other species in the mixed exhibit. The armadillos received a frozen horsemeat product (Nebraska Brand Feline Food for Carnivores, Central Nebraska Packing, Inc., North Platte, Nebraska 69101, USA) supplemented with fruit and vegetables. The horsemeat product was routinely thawed at room temperature overnight and put in pans on the floor daily. These pans were accessible to the tamarins in the mixed exhibit.

In late October 1988, an adult female Goeldi's monkey was hospitalized for marked depression and dehydration. Her tongue was diffusely swollen and pale and a rectal swab for *Salmonella/Shigella* screen was taken. She was treated with 20 ml s.c. fluids (0.9% saline), injectable multiple B vitamins, 24 mg trimethoprim sulfadiazine (Di-Trim, Syntex Animal Health, Inc., West Des Moines, Iowa 50265, USA), and placed in an incubator with 50% O_2. Later that day, fluids (0.9% saline) were administered via an indwelling catheter along with 5 mg prednisolone sodium succinate (Solu-Delta-Cortef, The Upjohn Co., Kalamazoo, Michigan 49001, USA). Fluids and antibiotic therapy were continued through the next day and her condition improved. A beta-hemolytic *Streptococcus* that was sensitive to trimethoprim sulfadiazine was isolated from the rectal swab. Treatment with trimethoprim sulfadiazine at 12 mg b.i.d. was continued for the next 10 days and the animal was returned to her exhibit fully recovered. Two days after clinical signs appeared in this animal, an adult male Goeldi's monkey from the same exhibit was found dead on the floor.

Two wk later, a female infant red-bellied tamarin was found dead hanging from a vine in the mixed exhibit. The following day, her mother was hospitalized because of a distended abdomen and swelling around the neck. For the past 2 wk she had appeared weak and had periods of a voracious appetite. It was assumed that she was pregnant. The tamarin was anesthetized with 7 mg ketamine HCl (Ketaset, Veterinary Products, Bristol Laboratories, Syracuse, New York 13201, USA), and 3 ml of pale-yellow clear fluid were removed by abdominocentesis. The swelling in the neck appeared to be edema of the submandibular area, and there was perineal soiling indicative of previous diarrhea. Her temperature was 40.4°C, which decreased to 35.3°C over the next few hours. An accurate hemogram could not be performed because the blood sample obtained was hemolyzed, but a blood smear indicated an increased white blood cell count, many immature segmented neutrophils, and some atypical lymphocytes. There were also many nucleated red blood cells, and basophilic stippling and marked polychromasia were present. The tamarin was given 24 mg of trimethoprim sulfadiazine s.c. and was noted alert and eating 1 hr later; 2 hr later she was found dead.

Pathology

Gross findings: The bodies of the two red-bellied tamarins and Goeldi's monkey appeared fairly well nourished. The adult female tamarin had enlarged edematous and hyperemic cervical lymph nodes (submandibular, 3 × 2.5 cm; retropharyngeal, 2 × 2.5 cm) and numerous scattered white pinpoint foci throughout the liver. The spleen was congested and had a prominent follicular pattern on cross section. The infant tamarin had numerous bite wounds in the skin of the ventral thorax and abdomen and moderate to severe autolysis had taken place. The Goeldi's monkey had a catarrhal exudate throughout the gastrointestinal tract and reddened mesenteric lymph nodes with prominent follicles.

Representative sections from all organs were taken, fixed in 10% buffered formalin, and processed in paraffin. Sections 4-μm thick were made and stained with hematoxylin and eosin and Brown and Brenn stain for bacteria.

Figure 1. Spleen from adult red-bellied tamarin with *Streptococcus zooepidemicus* infection showing chains of gram-positive cocci intracellularly and in the splenic sinuses. Brown & Brenn, ×320.

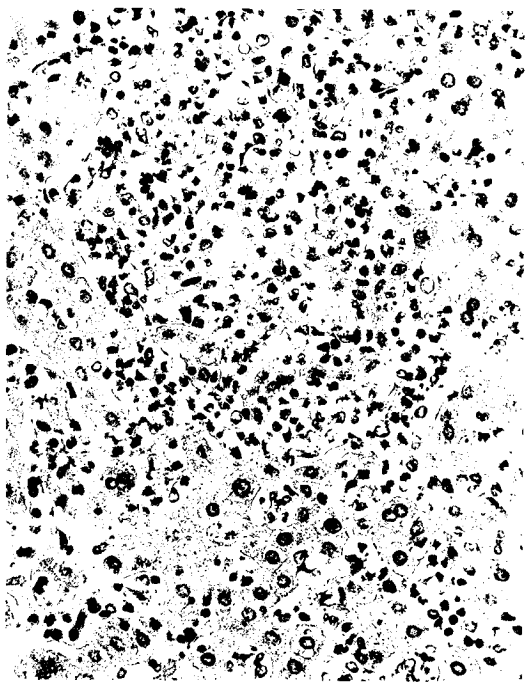

Figure 2. Liver from adult red-bellied tamarin with *Streptococcus zooepidemicus* showing sinusoidal neutrophilic infiltration and necrosis of adjacent hepatocytes. H&E, ×82.

Microscopic findings: There was extensive suppurative lymphadenitis of the cervical lymph nodes in the two red-bellied tamarins with clusters and chains of gram-positive cocci evident in the lymphatic sinuses. The adult tamarin had splenitis, characterized by an infiltration of the red pulp with numerous often degenerate neutrophils accompanied by fibrin and edema and lymphoid depletion. There was a wide zone of splenic subcapsular necrosis that contained many clusters and chains of gram-positive cocci (Fig. 1). The infant tamarin had similar inflammatory changes with gram-positive cocci in the spleen and numerous coccal emboli in the cerebral vasculature.

The adult tamarin also had a suppurative hepatitis characterized by sinusoidal infiltration with abundant neutrophils and necrosis of surrounding hepatocytes (Fig. 2). This was accompanied by thrombotic portal phlebitis and occasional intrasinusoidal thrombosis. Membranous glomerulonephritis, considered to be incidental, was also present.

Lesions in the Goeldi's monkey involved primarily the upper small intestine with necrosis and heavy colonization of the villous tips by gram-positive cocci (Figs. 3, 4). There was extensive edema and neutrophilic infiltration of the submucosa with exudation into the lumen. Gram-positive coccal emboli were present in the muscularis of the stomach accompanied by acute submucosal inflammation. In addition, there was suppurative mesenteric lymphadenitis with necrotic foci containing gram-positive cocci.

Microbiologic findings: Specimens of liver, heart blood, spleen, and ascitic fluid from the adult female tamarin were cultured on 5% sheep blood agar. Colony growth from each of these cultures indicated beta-hemolytic streptococci. Beta-hemolytic *Strep-*

Figure 3. Small intestine from Goeldi's monkey with *Streptococcus zooepidemicus* showing necrosis of villous tips and heavy bacterial colonization. H&E, ×82.

Figure 4. Small intestine from Goeldi's monkey with *Streptococcus zooepidemicus* showing abundant gram-positive cocci (arrows) in villous tip and within the lamina propria. Brown & Brenn, ×320.

tococcus was also isolated from the heart blood and small intestine of the Goeldi's monkey that died and from the rectal swab from the Goeldi's that survived. All beta-hemolytic streptococci obtained were typed as Lancefield Group C and identified as *S. zooepidemicus* at the National Veterinary Services Laboratory (NVSL), Ames, Iowa. Mixed bacterial cultures were obtained from heart blood and large intestine of the infant red-bellied tamarin (postmortem), but beta-hemolytic *Streptococcus* was not isolated.

Selected cultures were taken from the recently fed horsemeat product and from pans in the involved enclosures in the SMH. *Streptococcus zooepidemicus* was isolated from a number of batches of the refrigerated horsemeat product approximately 24 hr after it had been thawed. A beta-hemolytic *Streptococcus* compatible with *S. zooepidemicus* (based on colony morphology) was recovered from a water bowl in the Goeldi's monkey exhibit and from a swab of the armadillo's food tray.

DISCUSSION

In this outbreak of *S. zooepidemicus* infection affecting red-bellied tamarins and Goeldi's monkeys, septicemia was attributed as the cause of death. The pathologic picture suggested that the streptococcal organisms gained entrance via the oral cavity or upper digestive tract, spread to regional lymph nodes, and subsequently progressed to septicemia. Although *S. zooepidemicus* was not recovered from the infant red-bellied tamarin, the strong histologic evidence of splenitis, cervical lymphadenitis, and gram-positive bacteremia indicated that streptococcal sepsis coupled with trauma (parental aggression) most likely lead to its death.

Streptococcus zooepidemicus is one of four

recognized species in Lancefield's Group C, and, along with *S. equi, S. dysgalactia,* and *S. equisimilus,* carries a unifying cell-wall determinant, N-acetyl galactosamine. There are at least 15 strains of *S. zooepidemicus,* most of which have shown pathogenicity in both humans and other animals.[2]

In the previous streptococcal outbreak in small carnivores at NZP,[18] *S. zooepidemicus* infections were associated with uncooked horsemeat that constituted part of the normal diets. In the current outbreak, it appears that exposure to the contaminated horsemeat in the small primate species affected was inadvertent or indirect.

In the case of the red-bellied tamarins, there was an opportunity for direct access to uncooked horsemeat product from the armadillos' pan that contained meat from a batch proven to contain *S. zooepidemicus.* A direct connection was not ascertained for the streptococcal infections in the Goeldi's monkeys, but transmission probably occurred by contamination of water bowls, feeding utensils, or some other fomite from the SMH kitchen where the horsemeat product is prepared for distribution.

Infection caused by *S. zooepidemicus* also occurred in a number of Egyptian spiny mice (*Acomys cahirinus*) housed in the SMH approximately 2 wk after the primate infections. Pathologic findings consisted of cervical lymphadenitis and enteritis, and *S. zooepidemicus* was recovered from cervical lymph nodes, stomach contents, cecal contents, and from heart blood. The similarity of the lesions to those of the primates and the microbiologic findings suggests a common mode of oral infection. These animals had no direct access to horsemeat and transmission is hypothesized to have occurred indirectly as in the Goeldi's monkeys.

The occurrence of *S. zooepidemicus* infections within a short period of time in this and the previously reported outbreak[18] suggests that contamination of the food product (horsemeat) may also be sporadic. Factors determining the virulence of *S. zooepidemicus* for small primates and other small exotic mammals are unknown at this time, but high concentrations of organisms ingested may be important in the establishment of infection.

Streptococcus zooepidemicus has recently been recognized as a serious pathogen in humans. Infection and death have occurred in people who have drunk unpasteurized milk in England[4,5,8] and consumed homemade cheese in New Mexico[9] that was contaminated with *S. zooepidemicus. Streptococcus zooepidemicus* infections in humans have also been related to caring for sick horses infected with the same organism.[3,14,17] In a number of recent cases, *S. zooepidemicus* was associated with poststreptococcal glomerulonephritis similar to that following infection with *S. pyogenes.*[2-4]

Because horses frequently harbor *S. zooepidemicus,* it is not surprising that uncooked horsemeat products may contain the organism. If zoos are feeding uncooked horsemeat products, periodic culturing for *S. zooepidemicus* as a potential pathogen is warranted. Cleaning and disinfecting exhibits and food preparation areas is important to reduce the possibility of fomite spread of the bacteria; cleaning and sanitizing all feed pans, utensils, and food preparation surfaces with dilute bleach rinses should be done daily. Also, food pans containing uncooked meat products should be removed or changed at regular short intervals to prevent overgrowth of potential pathogens.

In view of their zoonotic potential, all disease-causing streptococci isolated from zoo animals should be serotyped. At the time of this outbreak of *S. zooepidemicus* at NZP, a keeper from the SMH and two members of his family had throat cultures positive for beta-hemolytic streptococci, which were considered as a possible source of the infection in the small primates affected. The organisms were subsequently determined to be Group A and not Group C streptococci by the family's pediatrician (W. C. Rees, pers. comm.), which helped to rule out a human-borne strain and led to the uncooked horsemeat as the most likely

source of the infections in the tamarins and Goeldi's monkeys.

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