Yersiniosis in a Cotton Top Tamarin (*Saguinus oedipus*)

Kayla J. Alexander

Mississippi State University College of Veterinary Medicine

Class of 2019

Clinicopathologic Conference

October 5, 2018

Advisor: Timothy Morgan, DVM, PhD, DACVP

Introduction

When composing a list of differential diagnoses for an animal that has been found acutely dead, it is important to consider the environment and time of year. Due to the inherent microbiology of certain organisms, seasonality can alter the degree of pathogenicity making an otherwise localized, self-limiting infection turn deadly. It is also important to recognize the environment in which the animal has been maintained. Many diseases can be controlled or avoided with proper environmental management strategies.

History and Pathological Findings

On January 10th, 2018, a 14-year-old male Cotton Top Tamarin ("Batch") from the Alabama Gulf Coast Zoo was received by the MSU-CVM Diagnostic Laboratory Service for necropsy. It was reported that Batch had loose stool from March to June 2017, which was treated with a sulfonamide antimicrobial. In October 2017, Batch and his colony were moved to temporary housing, so their housing exhibit could be rebuilt. Batch began losing weight at that time. He weighed 480 grams at his final recorded weight in December 2017 (down from an initial weight of 500 grams in October). Batch was seen "acting strangely" on January 7th, 2018 and was found dead the following morning.

Upon gross examination, the animal was emaciated with a body condition score of 1/5. There was no evidence of external trauma, parasites, or fecal staining. The first-most apparent findings were the lack of subcutaneous and intraabdominal fat and serous atrophy of the pericardial fat. Within the thoracic cavity, there was approximately five milliliters of serosanguinous fluid. The lungs were consolidated with a dark red to purple mottled appearance. There was also severe pulmonary emphysema and scattered pulmonary bullae with the right lung lobes being most affected.

The intestines were severely gas distended with marked venous congestion. The gastrointestinal lumen contained yellow to light pink, mucoid, sticky digesta and fecal material. The hepatic capsule had several fibrin tags on the surface. The hepatic parenchyma had a multifocal, widely disseminated, sometimes coalescing pattern of firm, white-to-tan discoloration. The left medial hepatic lobe was nearly totally necrotic. The tracheobronchial, iliac, and mesenteric lymph nodes were all grossly enlarged and hemorrhagic.

Differential Diagnoses

Tyzzer's disease is demonstrated by a triad of three classical lesions: necrotizing enteritis, hepatitis, and myocarditis and often causes acute death. Given the degree of hepatic necrosis and enteritis, our primary differential diagnosis was Tyzzer's disease caused by the bacteria *Clostridium piliforme*. Tyzzer's has also been reported to have an increased incidence of occurrence following administration of sulfonamide antibiotics in laboratory rabbits.[13] Our animal had a history of sulfonamide administration, however, there was no evidence of myocarditis, a pillar in the triad of Tyzzer's pathologic lesions.

Shigella should be considered a differential diagnosis in any primate with a history of diarrhea. Animals become predisposed to Shigellosis after stressful events. They rapidly dehydrate and may die if not quickly treated. Hemorrhagic, necropurulent colitis is a classic pathological finding of Shigellosis. Periodontitis is also a typical lesion found in monkeys infected with Shigella.[2] The animal in this case had no evidence of oral disease, so Shigellosis was moved further down our list of differentials.

In any case of septicemia in a non-human primate, Klebsiella should be suspected. Klebsiella is an opportunistic pathogen that normally inhabits the oropharyngeal cavity of

monkeys. Primates with fatal Klebsiellosis tend to develop pneumonia before they succumb to septicemia. Klebsiella can be easily identified on cytology or histopathology. These organisms produce a large, mucoid capsule that is a characteristic identifying feature of the species.[2] In this case, the organisms found on histopathology lacked capsules.

Salmonella should be considered a differential diagnosis in any species that has died following diarrheal disease. Salmonellosis manifests as pseudomembranous enterocolitis, which produces watery, bloody, and mucus-laden diarrhea. When the organism becomes septic, it causes the formation of pyogranulomatous lesions in affected organs.[2] The use of commercially available, routinely used media can quickly verify or dismiss Salmonellosis via culture, and in this case, Salmonella was not isolated.

Campylobacterosis is a common diarrheal disease in primates. Diseased animals present with extremely watery, bloody diarrhea that quickly leads to dehydration. It is also known to cause abortions in pregnant animals. Asymptomatic carriers serve as a continuous source of infection for the colony. Campylobacter can easily be identified with the use of silver stains. The organism has a characteristic spiral shape when viewed microscopically.[2] In this case, these organisms were not appreciated on histopathology.

Yersiniosis should be considered a top differential in cases of acute death in primates. Animals rapidly become septicemic following the initial development of necrotizing enteritis. The bacteria invade mesenteric lymph nodes and disseminate systemically via the lymphatics. Necrotic hepatitis, splenitis, and myelitis are the most pronounced lesions of Yersiniosis. The presence of large, gram negative bacterial colonies contained within necrotic foci of affected organs strongly supports a diagnosis of Yersiniosis and can be considered pathognomonic for the disease.[2]

Laboratory Findings

When examined histologically, the pulmonary vasculature was dilated, congested, and frequently contained discrete bacterial colonies and one intra-arteriolar septic thrombus. Fibrin and neutrophils filled alveolar spaces in a sporadically focused pattern. Multiple bullae were present, especially towards the periphery of the lung lobes. Multifocal hepatic necrosis was confirmed. Within the necrotic foci, large, discrete bacterial colonies were contained. There were also numerous granulomas distributed throughout the hepatic parenchyma in addition to the necrosis. There was frequent bile duct hyperplasia with intraluminal neutrophillic infiltrates. The hepatic sinuses contained a moderate to marked number of neutrophils and some macrophages.[9]

Other histological findings included dilated and congested renal vasculature. There were bacterial colonies located within several renal capillaries and glomeruli. The intestines were autolytic and contained lymphocytes and plasma cells within the lamina propria. The testicles were atrophic, and spermatogenesis was decreased. No other abnormalities were described histologically.[9]

Culture and sensitivity of several organs were performed. Both gram negative and gram positive enteric bacteria were grown repeatedly from several sources. The most grossly affected organ, the liver, was not excluded from this description, however, *E. coli, Klebisella pneumoniae*, and *Yersinia enterocolitica* were specifically isolated. The lungs also cultured a mixed bacterial population including the previously mentioned species. The organ with the least number of cultured organisms was the mesenteric lymph node, and *Yersinia enterocolitca* was the only bacteria identified.[9]

Pathophysiology

Yersinia enterocolitca is a gram negative enteric coccobacillus. The organism is peritrichously flagellated but is only motile at cooler than typical incubation temperatures of 22-30^{o} C (72-80^{o} F). Yersinia grows best in culture at temperatures of 25-28^{o} C (77-82^{o} F) either under aerobic or anaerobic conditions. Yersinia is widespread throughout the environment and is commonly found in the gastrointestinal tracts of several natural animal reservoirs. Rodents and wild birds are frequently recognized as carriers.[6]

Y. entercolitica is classified into biogroups and serotypes based on phenotypic characteristics and O polysaccharide side chains, respectively. Of the six biogroups, five are considered pathogenic, and only a few serotypes have been positively correlated with clinical disease. In the United States, biogroup 1B, serotype O:8 is the most clinically important strain of *Y. enterocolitca* and possesses the potential to become septic.[6, 10, 11]

Cefsulodin-Irgasan-Novobiocin (CIN) agar, also known as Yersinia selective agar, is a selective and differential media used to grow and identify *Y. enterocolitica*. Colonies appear as light pink to rose-colored with dark red centers, commonly referred to as "bull's eye" colonies. The dark red centers are a result of Mannitol fermentation. "Cold enrichment" at temperatures as low as 4^{o} C may be needed to selectively populate *Y. entercolitica* from samples of multiple bacterial species.[6,3] Once positively cultured, the use of PCR, immunohistochemical stains, or pulsed field gel electrophoresis can be used to confirm the biogroup and serotype.[6, 10]

Yersinia enterocolitca typically causes a self-limiting localized enteritis in the distal ileum, unless the host is immunocompromised. The bacteria cross the intestinal epithelium preferentially through M cells and invade Peyer's Patches. Once in the Peyer's Patches, the

organisms are internalized by local macrophages and transported to the mesenteric lymph nodes or disseminated systemically via the lymphatics. Several virulence factors prevent the complete phagocytosis of *Y. entercolitica*, allowing the organism to persist and replicate within macrophages. The bacteria also replicate extra-cellularly in organs, such as the liver, and form micro-abscesses that preclude necrosis. Within the necrotic foci, large gram negative bacterial colonies exist and are nearly pathognomonic for the disease.[6, 11]

There are a few virulence factors that contribute to the successful invasion, persistence, and pathogenicity of *Y. enterocolitca*. Some are present simultaneously only in the pathogenic biogroup 1B, serotype O:8 strain. The protein Inv is responsible for the initial invasion of Yersinia into intestinal epithelial cells, preferentially M cells. Once the bacteria have crossed the basolateral membrane into Peyer's Patches, YadA becomes the major protein responsible for the attachment to epithelial cells, phagocytes, and the extracellular matrix. YadA also protects the bacteria from destruction by neutrophils and is required for successful replication. A third adhesion protein, Ail, functions in much the same way as YadA but has only been detected in pathogenic Yersinia strains, whereas, the other two proteins have been associated with all strains of Yersinia. An additional set of genes, Ysa T3SS, functions in highly similar fashion as the aforementioned proteins but is uniquely identified within the genome of highly pathogenic *Y. entercolitica*.[6]

As with any gram negative enteric bacteria, the presence of the O polysaccharide side chain of the LPS antigen structure enhances pathogenic and antigenic properties. It has been shown that the adhesion and invasion proteins, Inv, YadA, and Ail, are down-regulated and display impaired function in the absence of O antigen in laboratory created mutant strains of *Yersinia entercolitica*.[6]

Collectively known as Ysc T3SS, Yersinia Outside Proteins (Yops) are a group of effector proteins responsible for the persistence of the bacteria within host organs. Yops proteins have a range of responsibilities including the formation of the injectisome complex, down-regulation of inflammation, and inhibition of phagocytosis. YopE is responsible for the disruption of neutrophilic and macrophagic cytoskeleton assembly preventing complete phagocytosis of *Y. enterocolitica*. YopP and YopM function to decrease the host inflammatory response by blocking the production of the pro-inflammatory cytokines TNF-alpha, IL-8, IL-10, and IL-18. YopH further prevents inflammation by blocking the recruitment of neutrophils and macrophages to the site of bacterial colonization.[5, 6]

High-Pathogenicity Island (HPI), a set of genes found only within the biotype 1B strain of *Y. enterocolitica,* contributes to virulence and discriminates low-pathogenicity from high-pathogenicity strains of Yersinia. HPI is an iron-capture island and also encodes for the production of yersiniabactin. Yersiniabactin allows the bacteria to intake and metabolize iron more efficiently, allowing for growth and replication within the host cell. It also dampens the production of reactive oxygen species by host innate immune cells further protecting the bacteria from destruction.[5, 6]

Management and Treatment Options

In primates, problematic *Yersinia entercolitica* infections cause septicemia, and animals are often found acutely dead. Therefore, control measures to prevent further mortalities are more important than individual treatment in suspected outbreaks. Rodents and wild birds are known carriers of the bacteria and are some of the most common sources of infection. Animals become infected via fecal-oral transmission.[1, 12] Vermin control in and around facilities where food and water is stored is a primary control measure. Personal hygiene is also an important aspect to the

control of transmission. Washing hands, wearing personal protective equipment (PPE), and changing PPE between animals prevents the potential spread of contaminants.

In one report, the authors discussed a series of *Yersinia enterocolitica* outbreaks and mortalities over a three-year period in a non-human primate facility housing Marmosets and Tamarins. The outbreaks generally occurred during colder months of the year. The group began vaccination of the surviving colony members annually during autumn with Pseudovac® and instated stricter hygienic guidelines for caretakers. After beginning the vaccination program, the group no longer experienced high mortality levels attributable to *Y. entercolitica*.[1] Further research is needed to conclude the efficacy of the Pseudovac® vaccine against pathogenic *Y. entercolitica* as it is labeled to prevent disease from Pseudomonas infection.

During active outbreaks, antimicrobial treatment can be successful, however, antibiotic resistance is a growing concern. A recent survey among participating clinical and diagnostic primate institutions looked at the resistance patterns of common primate enteric pathogens. Results were consistent among the institutions and showed that *Y. entercolitica* was resistant to ampicillin, Clavamox, and erythromycin. *Y. entercolitica* was most susceptible to fluoroquinolones and third generation cephalosporins, both of which are considered of highest clinical importance in human medicine as defined by the World Health Organization.[7] It is important to appropriately and effectively treat *Yersinia enterocolitca* and avoid further resistance development. The most current treatment recommendation includes the use of fluroquinolone and third generation cephalosporins, both of which are commonly used clinically in non-human primate medicine.[7, 4]

Zoonotic Concerns

While non-human primates should be considered a potential zoonotic source of *Yersinia entercolitica,* the Centers for Disease Control and Prevention name improperly cooked and/or raw pork products as the main source of transmission to humans. Clinical signs in humans include bloody diarrhea with fever and abdominal pain. The infection is usually self-limiting in immunocompetent people, and infection is most common in young children. Patients with elevated blood iron levels are pre-disposed to septicemia caused by highly pathogenic *Y. enterocolitica*. Reactive arthritis and erythema nodosum are additional clinical signs and can present up to one month after the initial enteric infection.[4]

The 2017 preliminary report by the Foodborne Diseases Active Surveillance Network (FoodNet) shows an increase of 96% in the overall incidence of foodborne related illnesses, including those due to *Yersinia enterocolitica,* since the previous report in 2014-2016. Yersinia-related disease showed an increase of 166%, which roughly translates to 1 in 100,000 foodborne related illness. The recent employment of culture-independent diagnostic testing (CIDT), such as PCR, may be responsible for the report of increased incidence.[8] Although it is not the most common source of zoonotic enteric disease, *Yersinia enterocolitica* should remain a foodborne pathogen of concern.

References

1. Bakker, J., et.al. "A Report on Yersinia-related Mortality in a Colony of New World Monkeys." *Laboratory Primate Newsletter* July 2007: 46(3):11-16.

2. Baskin, Gary. "Pathology of Nonhuman Primates." 11 September 2008. *Primate Info Net Library and Information Service National Primate Research Center, University of Wisconsin - Madison.* http://pin.primate.wisc.edu/research/vet/pola6-99.html. 8 August 2018.

3. Becton Dickinson GmbH. "BD Yersinia Selective Agar (CIN Agar), BD Aeromonas Yersinia Agar." Heidelberg, Germany: BD, March 2013. PA-254056.06.

4. Centers for Disease Control and Prevention. *Yersinia enterocolitica (Yersiniosis).* 9 March 2018. https://www.cdc.gov/yersinia/healthcare.html. August 2018.

5. Drummond, Niall, et.al. "Yersinia Enterocolitica: A Brief Review of the Issues Relating to the Zoonotic Pathogen, Public Health Challenges, and the Pork Production Chain." *Foodborne Pathogens and Disease* (2012): 9(3):179-89. DOI: 10.1089/fpd.2011.0938.

6. Fabrega, Anna, and Vila, Jordi. "Yersinia enterocolitca: Pathogenesis, virulence, and antimicrobial resistance." *Enfermedades Infecciosas y Microbiologia Clinica* (2012): 30(1):24-32.

7. Kim, Jefferey, et. al. "Antimicrobial Use for and Resistance of Zoonotic Bacteria Recovered from Nonhuman Primates." *Comparative Medicine* (2017): 67(1):79-86.

8. Marder, EP, et. al. "Morbidity and Mortality Weekly Report (MMWR)." 23 March 2018. *Preliminary Incidence and Trends of Infections with Pathogens Transmitted Commonly*

Through Food — Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2006–2017. http://dx.doi.org/10.15585/mmwr.mm6711a3. 26 August 2018.

9. Morgan, Tim. "Finalized Necropsy Report: C18-00613." 21 January 2018.

10. Nakamaura, S., et. al. "Pathological Changes in Captive Monkeys with Spontaneous Yersiniosis due to Infection by Yersinia enterocolitica serovar 08." *Comparative Pathology* (2010): 143:150-56.

11. Soto, Esteban, et. al. "An Outbreak of Yersinia enterocolitica in a Captive Colony of African Green Monkeys (Chlorocebus aethiops sabaeus) in the Caribbean." *Comparative Medicine* (2013): 63(5):439-44.

12. Taketoshi, Iwata, et. al. "Yersinia enterocolitica Serovar O:8 Infection in Breeding Monkeys in Japan." *Microbiology and Immunology* (2005): 49(1):1-7.

13. Weisbroth, Steven, Ronald Flatt, and Alan Kraus. "Bacterial Diseases." *The Biology of the Laboratory Rabbit*. New York: Academic Press, 1974. 214-18.