



Case Report

Spontaneous outbreak of *Yersinia enterocolitica* infection and co-infection with *Escherichia coli* in black-tufted marmosets (*Callithrix penicillata*)

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Abstract

Yersiniosis is a zoonotic bacterial disease that affects humans and animals, including primates. The aim of the study was to report one case of fatal *Yersinia enterocolitica* infection and two cases of co-infection with *Escherichia coli* in captive black tufted marmosets (*Callithrix penicillata*) in Apucarana, Paraná, south Brazil. The affected animals presented severe diarrhea and progressed to death. Gross examination showed multifocal to coalescing necrosis in the liver, mild diffuse hepatomegaly, mild diffuse necrotizing enteritis, moderate splenomegaly and focally extensive hyperemia of the leptomeninges. Microscopically, the liver lesions comprised multifocal areas of lytic necrosis with intralesional colonies of gram-negative coccobacilli, characterizing a severe, random, multifocal to coalescing necrotizing hepatitis. A moderate multifocal lymphocytic cholangiohepatitis, severe focally extensive mucosal necrosis in the small intestine, and mild multifocal lymphoplasmacytic leptomeningitis in the brain were observed. Two cases of co-infection by *Y. enterocolitica* and *E. coli*, and one case by *Y. enterocolitica* were confirmed through bacterial culture, biochemical characteristics and 16S rRNA. To the best of the author's knowledge, it is the first report of an infection of *Y. enterocolitica* and co-infection with *E. coli* in black-tufted marmosets resulting in diarrhea, septicemia and death.

Key words: Necrotizing hepatitis; non-human primates; yersiniosis.

Introduction

Non-human primates are susceptible to numerous bacterial diseases, and some of these, including *Yersinia* spp., have zoonotic potential. The genus *Yersinia* is a member of the family Yersiniaceae (1), and non-human primates are extremely susceptible to infection (3).

The black-tufted marmoset (*Callithrix penicillata*) is a small neotropical primate (300-450g) endemic in Brazil. It has a wide geographical distribution, occurring in

the Atlantic Forest and in savanna areas (15). The species is found in secondary forests and highly disturbed areas, where they seek food and protection from predators (9).

Fatal cases of infections caused by *Y. enterocolitica* in different species of non-human primates have been reported worldwide (8, 16, 17). However, there are no reports approaching the disease in *Callithrix penicillata*. This study aimed to describe an infection by *Y. enterocolitica* and co-infection with *E. coli* in three *Callithrix penicillata* kept in captivity in Paraná, south Brazil, confirmed by isolation and molecular characterization.



Figure 1. Necrotizing hepatitis caused by *Yersinia enterocolitica* in a black tufted marmoset (*Callithrix penicillata*). **A.** Animal #1 during an external examination at the autopsy. **B.** *In situ* evaluation of the abdominal cavity evidencing severe, multifocal to coalescing, necrotizing hepatitis.

Case report

Nine wild caught black-tufted marmosets (Fig. 1) (*Callithrix penicillata*), males and females, had severe intermittent greenish diarrhea for four to five days. During this period, they received antibiotic treatment, but all died. The animals were maintained in the same temporary enclosure, under the responsibility of the official conservation and environmental protection department of the municipality of Apucarana, located in the northern region of Paraná, Brazil, specialized for rescued animals and a later attempt to reintroduce them into nature.

A standard autopsy was performed on three (two males and one female) of the nine animals. Samples of liver, intestine, kidneys, spleen, lung, lymph nodes, heart, and brain were collected, fixed in 10% buffered formalin solution, routinely processed for histopathologic evaluation and visualized with hematoxylin and eosin (HE) stain.

Specific sections were stained with Gram stain by Brown and Brenn Method (5) to identify intralosomal bacteria.

Approximately 0.5 g of brain and liver were sampled from the marmosets and sent to microbiological analysis. Samples were plated in Muller Hinton agar enriched with defibrinated sheep blood (5%) and in MacConkey Agar, followed by incubation for 24h at 37°C in aerobic condition. The isolation of colonies, suggestive of *Escherichia coli*, were identified according to morphological characteristics and biochemical profile (11, 24) and submitted to DNA extraction. The supernatants were used for polymerase chain reaction (PCR) using primers from 16S rRNA (25) and the products of gene amplification were sequenced, and phylogenetic tree built according to maximum likelihood method (12).

The main gross lesions are summarized in Table 1. All marmosets had hepatic, enteric, and splenic alterations. Hepatomegaly and severe multifocal to coalescing necrotizing hepatitis were observed in all animals (Fig.

Table 1. Principal gross and histopathologic findings of infection by *Yersinia enterocolitica* and co-infection with *Escherichia coli*.

Animals	Main gross lesions	Histopathologic lesions	Isolation organs	Bacterium	Type of infection
# 1	Severe multifocal necrotizing hepatitis	Severe multifocal to coalescing necrotizing hepatitis	Liver	<i>Y. enterocolitica</i> .	Single
	Mild diffuse necrotizing enteritis	Marked multifocal to coalescing lymphocytic cholangiohepatitis			
	Moderate diffuse splenomegaly and congestion	Marked multifocal to coalescing necrotizing enteritis			
	Mild hepatomegaly	Marked diffuse membranous glomerulonephritis			
	Prominence of jejunal Peyer's patches	Marked diffuse lymphoid hyperplasia in lymph node			
		Moderate multifocal pulmonary edema			
# 2	Severe multifocal necrotizing hepatitis	Severe multifocal to coalescing necrotizing hepatitis	Liver	<i>Y. enterocolitica</i> and <i>E. coli</i>	Dual
	Mild diffuse necrotizing enteritis	Marked multifocal to coalescing lymphocytic cholangiohepatitis			
	Mild splenomegaly	Marked multifocal to coalescing necrotizing enteritis			
	Moderate pulmonary edema	Marked diffuse membranous glomerulonephritis			
	Marked hepatomegaly				
	Prominence of jejunal Peyer's patches				
# 3	Severe multifocal necrotizing hepatitis	Severe multifocal to coalescing necrotizing hepatitis	Liver and brain	<i>Y. enterocolitica</i> and <i>E. coli</i>	Dual
	Moderate focally extensive hyperemia in the right cerebral hemisphere	Marked multifocal to coalescing lymphocytic cholangiohepatitis			
	Mild diffuse necrotizing enteritis	Marked multifocal to coalescing necrotizing enteritis			
	Marked hepatomegaly	Mild multifocal lymphoplasmacytic leptomeningitis			
	Mild splenomegaly	Marked diffuse membranous glomerulonephritis			
	Moderate pulmonary edema				
	Mild brain congestion				
	Prominence of jejunal Peyer's patches				

2). Mild diffuse necrotizing enteritis and moderate diffuse hyperemia of the intestinal mucosa was observed only in one animal, while the prominence of jejunal Peyer's patches was present in all animals. Furthermore, all marmosets showed mild splenomegaly, and only one of them revealed mild focally extensive hyperemia of the leptomeninges and mild diffuse brain congestion.

Histopathologic alterations were predominantly hepatic, enteric, encephalic and renal (Table 1). The liver in all animals revealed multifocal to coalescing areas of lytic necrosis, characterized by numerous gram-negative coccobacillus colonies in the center of the lesions, and admixed with marked eosinophilic cellular, karyorrhectic debris and fibrin, characterizing a severe, random, multifocal to coalescing necrotizing hepatitis (Fig. 3). Also, all of them presented moderate multifocal lymphocytic cholangiohepatitis. In the small intestine, severe mucosal

necrosis was observed affecting focally extensive areas mixed with cell debris and intraluminal gram-negative coccobacillus colonies in all animals (Fig. 4). Peyer's patches showed no microscopic changes. The brain of one animal presented mild multifocal lymphoplasmacytic leptomeningitis. Marked and diffuse membranous glomerulonephritis was observed in all animals.

Selected colonies obtained from the liver of three marmosets were characterized as *Yersinia* spp. Co-infection of *Yersinia* spp. and *E. coli* were identified in two animals. Brain samples of one marmoset were characterized with co-infection with *E. coli* and *Yersinia* spp. (Table 1). The isolates were stored as BER 262 and BER 263 strains. The phylogenetic tree exhibits BER 262 strain grouping in the same cluster as other *Y. enterocolitica* strains (Figure 5), and in Figure 6, the phylogenetic tree displays BER 263 strain in the same cluster as other *Escherichia coli* strains.

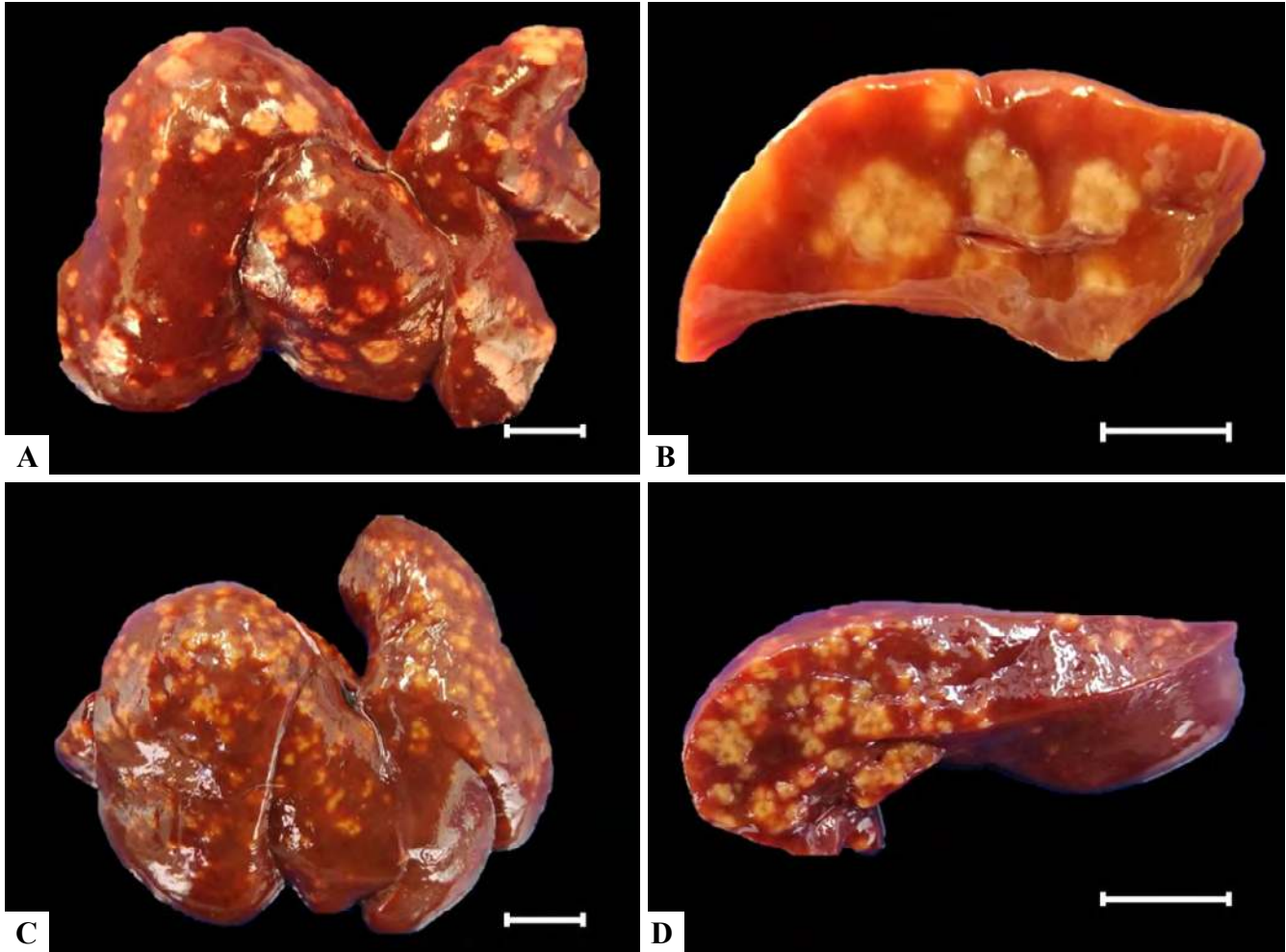


Figure 2. Necrotizing hepatitis in black-tufted marmoset (*Callithrix penicillata*) spontaneously infected by *Yersinia enterocolitica* and with a co-infection by *Escherichia coli*. **A & B.** Animal #1 infected by *Y. enterocolitica*; the liver exhibits multifocal to coalescing white, irregular, necrosis with variable sizes (0,1 – 1,0 cm). **C & D.** Animal #2 co-infected; the liver exhibits multifocal to coalescing white, irregular, necrosis, however, they are smaller compared to single yersiniosis infection. Bar = 1 cm.

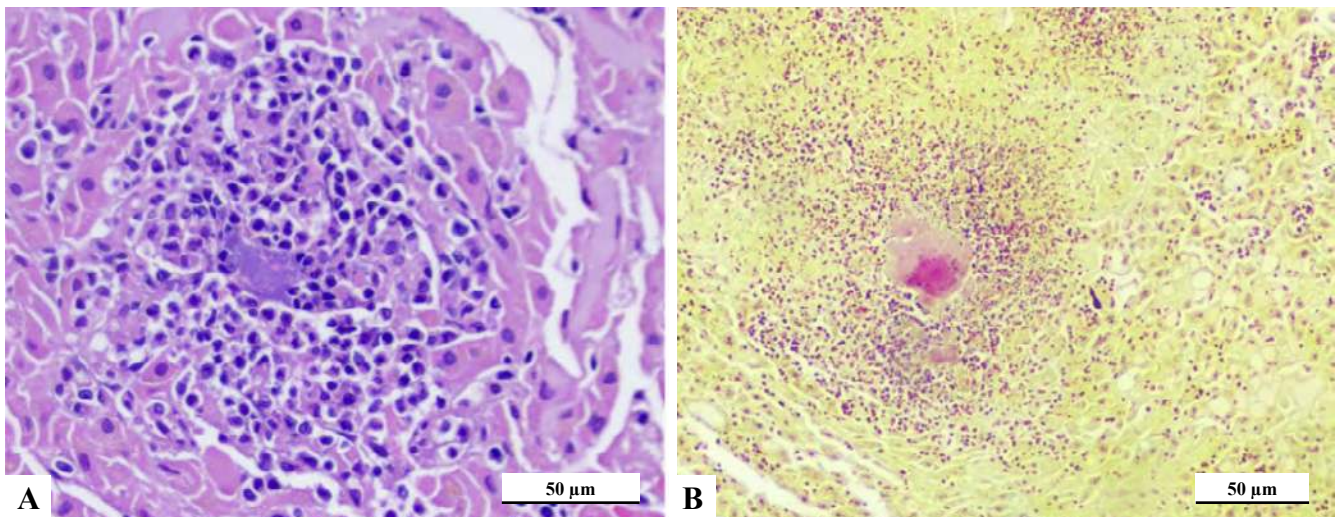


Figure 3. Necrotizing hepatitis in a black tufted marmoset (*Callithrix penicillata*) spontaneously infected by *Yersinia enterocolitica*. **A.** Necrotic areas characterized by numerous coccobacilli colonies in the center of the lesions, surrounded by lymphoplasmacytic infiltrate, [HE; scale bar= 50 µm]. **B.** Necrotic areas with intralésional colonies of gram-negative coccobacilli; [Brown-Brenn Gram; scale bar 50 µm].

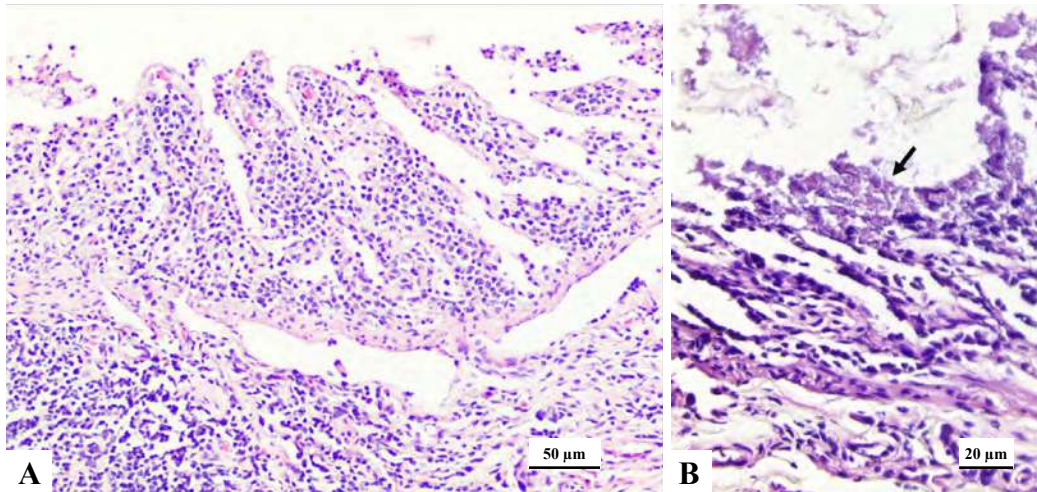


Figure 4. Severe mucosal necrosis in small intestine of marmoset (*Callithrix penicillata*). **A.** Focally extensive areas which exhibit loss of mucosal villous architecture; [HE; scale bar= 50 µm]. **B.** Admixed with cell debris and intraluminal coccobacillus colonies; [HE; scale bar= 20 µm].

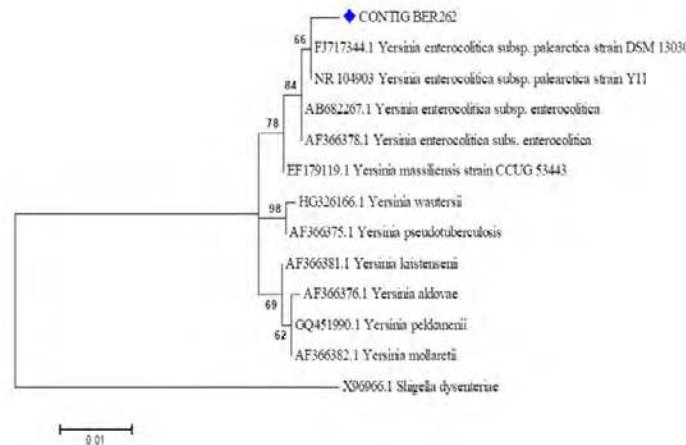


Figure 5. Molecular phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model [1] and the evolutionary analyses were conducted in MEGA7 [2].

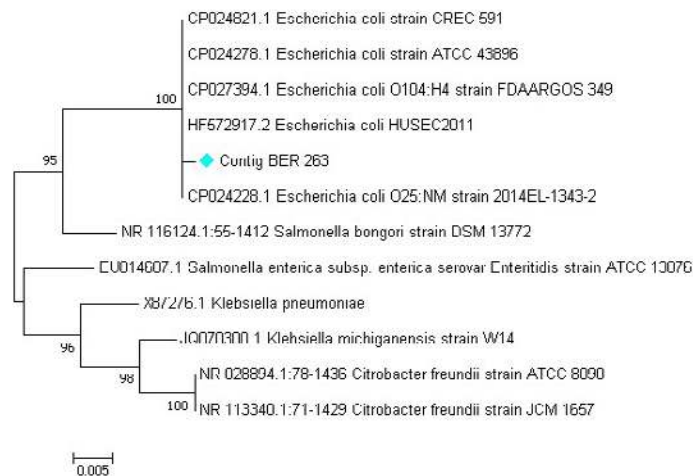


Figure 6. Molecular phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura 3-parameter model [1] and the evolutionary analyses were conducted in MEGA7[2].

Discussion

Diarrhea is a noteworthy problem found in nonhuman primates colonies and is considered a problem in zoos due to animal welfare and public health concerns (7). The most frequently bacterial pathogens associated with gastroenteritis in nonhuman primates are *Shigella flexneri* and *Campylobacter jejuni*. However, other enteric pathogens sporadically isolated in gastroenteritis include *Yersinia* spp., enterotoxigenic *E. coli*, *Pseudomonas aeruginosa*, and *Aerobacter aerogenes* (21).

Bacteria genus *Yersinia* spp. have been the cause of several spontaneous outbreaks of acute fatal yersiniosis worldwide and demonstrated high morbidity and mortality in captive colonies of non-human primates (3, 10, 16). We document here a *Y. enterocolitica* infection and co-infection with *E. coli* affecting black-tufted marmosets (*Callithrix penicillata*). To the best of the author's knowledge, it is the first report in Brazil involving this marmoset species. This infection has also been reported in several other monkey species, including common marmosets (*Callithrix jacchus*) (3, 8).

In domesticated animals and non-human primates, the clinical disease of yersiniosis is variable and tends to present diarrhea, weight loss, lethargy, anorexia, and dehydration (16, 20). Overall, the pathomorphological findings usually reported in non-human primates are enterocolitis, mesenteric lymphadenitis, septicemia, and eventually hepatomegaly, splenomegaly, and hepatitis. Necrotic and suppurative foci in the liver, spleen, and mesenteric lymph nodes may be present (4, 8, 10, 16, 22), as seen in the marmosets in the present cases. All cases found in the literature in non-human primates exhibited hepatic lesions similar to those seen in the outbreak in Apucarana.

In this study, we have isolated and confirmed a co-infection by *Y. enterocolitica* and *E. coli* through 16S rRNA sequencing. A similar study reported a co-infection and death by *E. coli* and *Salmonella paratyphi* in non-symptomatic mona monkeys (*Cercopithecus mona*), tanzania monkey (*Chlorocebus tantalus*), pata monkeys (*Erythrocebus patas*), olive baboon (*Papio anubis*) and common chimpanzee (*P. troglodytes*) living in a Nigerian zoo (18).

The entry of the pathogen into outbreaks of yersiniosis has been reported in different ways. Most cases occurred in outdoor colonies and cages, and the presence of other animals such as rats, mice and pigeons were considered the main source of infection. These animals can play a role as a reservoir (6). Despite that, the introduction of asymptomatic infected non-human primates (14, 19) and contaminated food (23) are considered the likely source of infection in indoor cases. In the present study, the origin of the bacteria or the stressful events that may have triggered this outbreak are unknown. However, the entry of new animals in the enclosure area was frequent, since animal rescues from the city and region were frequent, and the possibility of introducing an asymptomatic infected animal that contaminated the environment and fomites can be highly considered.

E. coli infections are reported as the major cause of hemorrhagic diarrhea in marmosets kept in captivity. The infection is maintained through cohabitation with asymptomatic carriers releasing bacteria into the environment, persisting for several years even after the treatment. Infection occurs through the fecal-oral route and the introduction into marmoset colonies takes place through contaminated fomites and contact between humans and non-human primates (13). Non-human primates' feeding behavior is the cause of high rates of *E. coli* infections due to their constant habit of putting their dirty hands and possibly contaminated by feces in their mouths (2), reinforcing the hypothesis of this infection route in the present report.

In conclusion, as far as the authors are aware, this is the first report of yersiniosis and a co-infection with *E. coli* reported in Brazil in black tufted marmosets, resulting in deaths after diarrhea and septicemia. The main pathological finding was necrotizing hepatitis in all animals.

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