

Case Study

***Yersinia enterocolitica* Infection in a Vervet Monkey (*Cercopithecus aethiops*) in Iran**

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ABSTRACT

A vervet monkey (*Cercopithecus aethiops*) housed at quarantine of Razi vaccine and serum research institute died following a few days of sickness. Multiple abscesses on the surface and inside the liver and enlargement of spleen with pinpoint white spot were the most prominent macroscopic features. We examined the organs of the dead monkey and *Yersinia enterocolitica* was isolated. In histopathologic examination of the liver, spleen and intestine sections, acute hepatitis, splenitis and enteritis were evident. This is the first report of fatal case of *Y. enterocolitica* infection in vervet monkey from Iran.

Keywords: *Vervet monkey, Yersinia enterocolitica*

INTRODUCTION

Yersinia is a genus of bacteria in the family of Enterobacteriaceae. This genus, *Yersinia*, contains several species: *Y. pestis*, the etiologic agent of human plague; *Y. pseudotuberculosis* and *Y. enterocolitica* are the major species of this genus and considered primarily enteric pathogens (Bottone 1997, Hubbert 1972, Mair 1973). *Y. enterocolitica* is widely distributed in nature in aquatic and animal reservoirs. Swine serve up as a major reservoir for human pathogenic strains (Bucher *et al* 2008, Bowman *et al* 2007, Bhaduri *et al* 2006). Infection with pathogenic strains of *Y. enterocolitica* occurs in all age groups, but clinical illness is more frequently reported in children and young adults, with asymptomatic infection being common in adults. Human yersiniosis is quite rare in United State, but

it is still the third most frequently reported zoonosis in Europe. In 2006, the highest incidences between 6.2 and 15.1 per 100,000 inhabitants were reported in Sweden, Germany, Lithuania, and Finland. Common symptoms of *Y. enterocolitica* infection, especially in children, are fever, abdominal pain, and diarrhea, which can be bloody. Sometimes long-term sequences such as reactive arthritis will occur, especially in HLA-B27-positive individuals (Bucher *et al* 2008). There are several reports of diarrhea due to *Y. enterocolitica* infection in children from Iran (Garveriani *et al* 2007, Soltan-dallal 2001). Although, *Y. enterocolitica* is primarily a gastrointestinal tract pathogen but under defined host condition has a strong tendency for extraintestinal spread. Moreover, it is thought to be a food-borne pathogen, having been recovered from a food source in several outbreaks (Bottone 1997). Milk and pork are commonly implicated in

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outbreaks of yersiniosis (Bucher *et al* 2008). Foods from which *Y. enterocolitica* have been isolated include milk and non-ripened/ non-fermented dairy products, various meats, poultry, sea foods, vegetables, prepared foods and salads (Bottone 1997, Schiemann 1978). Transmissible pathogenic and opportunistic zoonotic enteric bacteria, like as *Y. enterocolitica*, comprise a recognized occupational health threat to exposed humans from non-human primates (NHPs) (Vore *et al* 2001).

CASE HISTORY

Six month after coming of a colony of 30 vervet monkey to the quarantine of the Razi vaccine and serum research institute, one male monkey in good body condition, after a short period of illness, died. The most outstanding signs of the disease were as depression, anorexia, diarrhea and dehydration. All the monkeys were kept in cages, individually. The animal was suspected to have tuberculosis. Samples of different organs were collected and referred to the Departments of Bacteriology, Parasitology and Pathology of Razi Institute.

Bacteriologic examination. Samples of the liver transferred, aseptically, to mycobacteria growth indicator tube (MGIT), (Difco laboratories-Michigan-USA), and Lowenstein-Jensen medium. Concurrently, other samples of the liver was cultured in nutrient broth medium and kept in 37°C for 24 hour. Then it was transferred to the three solid media (blood agar, MacConkey agar and nutrient agar) and incubated at 37 °c. After 24 hours pure colonies was appeared on the blood agar as well as MacConkey agar media. Direct smear from the colonies revealed that they are gram negative coccobacilli. Then the colonies transferred to BBL (BBL CRYSTAL™, Enteric/Nonfermenter ID system, Becton Dickinson and company, Cockeysville, Maryland 21030 USA) and Remel (RAPID ONE SYSTEM, Remel Inc, 12076 SantaFe Dr. Lenexa, Ks 66215 USA) commercial kits for

determining the isolated bacteria. Moreover, urease and oxidase tests as well as motility test were performed on the isolated bacteria.

Parasitologic examination. Samples of the liver, intestine and lung were checked by direct observation, dissecting tissue, preparation of smear and flotation procedure.

Histopathologic examination. Samples of different organs were kept in 10% formalin until fixation. After completing of fixation the tissues routinely processed to paraffin blocks, sectioned at 5 µm, deparaffinized, stained with H&E and finally examined by a light microscope.

DISCUSSION

In necropsy of the dead monkey, most prominent macroscopic lesions were as dehydration, multiple abscesses on the surface and inside the parenchyma of the liver (Figure 1).

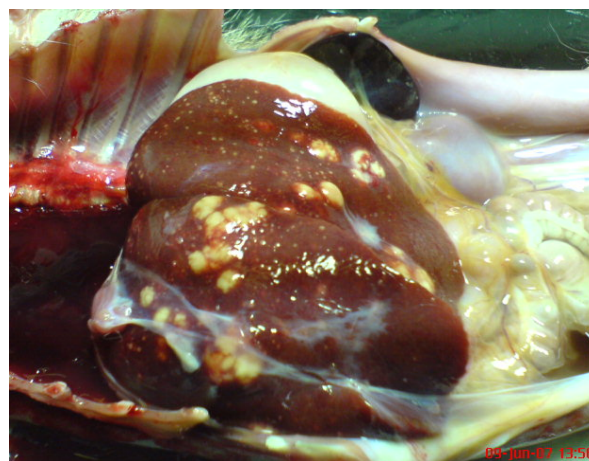


Figure 1. Multiple abscesses due to *Y. enterocolitica* on the surface of the monkey's liver.

The spleen was enlarged, splenomegaly, and several pinpoint white spot was evident on its surface (Figure 2). In direct smears from the liver no acid fast bacilli were seen in Ziehl-Neelsen staining method. Moreover, no mycobacterium was isolated after 14 days from mycobacteria growth indicator tube (MGIT) as well as after 2 month of culture on Lowenstein-Jensen medium. But in routine

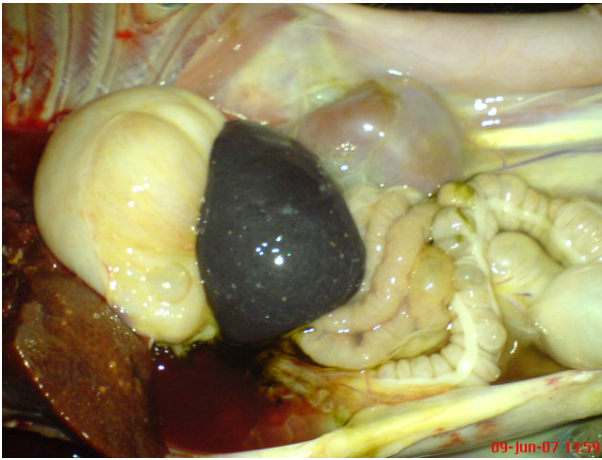


Figure 2. Enlargement of the monkey's spleen. There are several pinpoint white spot on the surface of the spleen.

bacteriologic examination of the liver, by using commercial kits, the isolated bacteria determined as *Yersinia enterocolitica*. Additional confirmatory biochemical test showed that the isolated bacterium is urease positive and oxidase negative. Moreover, it has the ability of motility in 22 °c. No parasite or parasitic cyst was seen in examination of the intestinal contents, liver and lung. In histopathologic examination of the liver and spleen sections, acute hepatitis and splenitis with diffuse and/or multifocal necrosis and abscessation, only in liver, was noticed (Figures 3, 4).

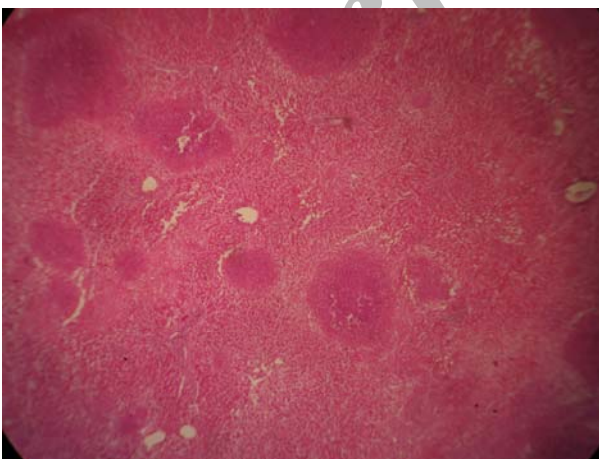


Figure 3. Acute hepatitis. Large foci of liver necrosis are seen (arrows head) (H&E × 40).

In the intestine acute necrotic enteritis, especially in the ileum, was evident. No prominent histopathologic lesions were seen in the lung and

kidney. Based on the results of the necropsy, microbiologic and histopathologic examinations the causative agent was diagnosed *Y. enterocolitica*. This study is the first report of fatal Yersiniosis in vervet monkey from Iran. Data on the prevalence of pathogenic *Y. enterocolitica* in animals and foodstuffs are very limited and old (Bucher *et al* 2008).

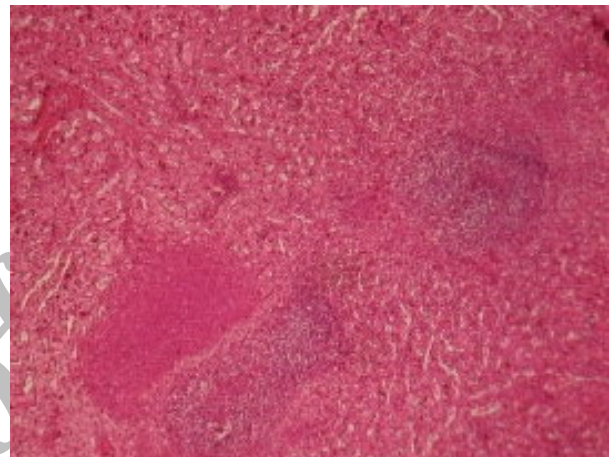


Figure 4. Acute splenitis. A Large focal necrosis of spleen tissue is evident (arrow head) (H&E × 100).

Several fatal outbreaks of yersiniosis due to *Y. pseudotuberculosis* (Buhles *et al* 1981, Kagayama *et al* 2002) and *Y. enterocolitica* (Baggs *et al* 1976, Iwata *et al* 2005, Skavian *et al* 1985) have been reported in different species of monkeys including patatas, squirrel and owl monkeys. Krylova *et al* (2000) described the pathomorphological picture and agents of spontaneous Yersinia infection in monkeys. They reported that between the 8 monkey species, red monkeys were the most sensitive to *Yersinia* infection. The infections with generalizes changes were usually due to *Y. pseudotuberculosis*. However, local changes involving the intestine was due to *Y. enterocolitica* infections (Krylova *et al* 2000). Acute enterocolitis due to *Yersinia* spp. Infection, frequently associated with septicemia and high mortality, have been frequently in sheep, goats and farmed deer in New Zealand and Australia (Philbey *et al* 1991, Slee *et al* 1992). Many different species of mammals and over 50 species of birds, including both captive and free-living birds, have

been reported with *Yersinia* spp infection (Mair 1973). Several outbreaks of yersiniosis have been reported in North American deer ranches, wildlife parks, and in free-ranging wildlife (Blake *et al* 1991, Sanford 1995, Welsh *et al* 1992). In a prevalence study conducted by Vore *et al* (2001), they showed that the prevalence of some significant human pathogens such as *Salmonella*/ *Shigella*, campylobacter and enteric *Yersinia* group in rhesus monkey is quite low. Soltan-Dallal (2001) in a one-year study conducted in cooperation with a local health center in Islamshahr (south of Tehran, Iran) reported that in 0.7% of children less than 5 years who presented with diarrhea the causative agent was *Yersinia*. In our study the most prominent histopathologic lesion was seen in liver, spleen and intestine. In general, *Yersinia* infection occur following orogastric inoculation, in which the bacteria enter the lamina propria of the distal ileum, cecum and colon with subsequent necrosis of Peyer's patches and eventual mucosal ulceration; extension to mesenteric lymph nodes and septicemia may also occur. If septicemia develops, suppurative lesions may develop in various organs (e.g. liver, spleen, lungs, meninges). Yersiniosis is frequently associated with a triad of gross lesions; hepatosplenomegaly and distended bowel. Infection is precipitated by changes in environmental conditions, such as a decrease in environmental temperature, poor nutrition or overcrowding. In a study which was performed in Ardebil province, north of Iran, *Y. enterocolitica* was the main causes of diarrhea in children under five years old in the cold weather (Garveriani *et al* 2007). Epizootics in domestic species are frequently preceded by outbreaks in rodents or birds (Bottone 1997, Hubbert 1972, Mair 1973). Fukai *et al* (1979) in an experimental study by infection of three species of animals, mice; rabbits; and monkey, with *y. enterocolitica* reported that severe intestinal lesions were common to the 3 animals. Apart from the intestine, the liver showed most remarkable

changes. Pai *et al* (1980) developed an animal model for *Y. enterocolitica* enteritis in which diarrhea could be produced consistently by pathogenic strains after orogastric challenge. They used rabbits because of their known susceptibility to diarrhea after intraduodenal infection. Crypt abscess, especially in ilea, localized at the depth of the intestinal glands were observed consistently. Wild rodent, particularly mice have been suggested as the source of infected animals (Iwata *et al* 2005, Kagayama *et al* 2002, Skavian *et al* 1985). Similarity of human and simian yersiniosis allows using monkeys as the experimental model for further studies (Krylova *et al* 2000).

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