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Short Communication

Mortality in Captive Rhesus Monkeys (*Macaca mulatta*) in China Due to Infection with *Yersinia pseudotuberculosis* Serotype O:1a

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Abstract: The most common serotypes of *Yersinia pseudotuberculosis* infecting non-human primates are serotypes O:1b, O:3, O:4, and O:7. The O:1a serotype has never been reported in non-human primates. The present study describes an outbreak of serotype O:1a with high fatality (6/18) in captive rhesus monkeys in China. Bacteria were isolated from different organs of the carcasses using standard microbiological procedures. The strain was identified using conventional and molecular techniques such as morphological and biochemical identification, serotype determination, PCR-sequence analysis based on the 16S rRNA gene, detection of virulence genes, and antimicrobial susceptibility testing. The pathogenicity was determined after experimental infection in mice. Taken together, the obtained data indicate that *Y. pseudotuberculosis* O:1a is a pathogen of concern and represents a potential threat to monkey conservation efforts.

Keywords: yersinia pseudotuberculosis, Serotype O:1a, Rhesus monkey, antimicrobial, resistant

INTRODUCTION

Non-plague yersiniosis, mainly caused by *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*, is known as a zoonotic disease. *Y. pseudotuberculosis* has been detected in a wide range of hosts, including fish, reptiles, birds, and mammals (Mair 1973; Obwolo 1976; Helmer et al. 2015; Nakamura et al. 2015, 2016). Outbreaks of *Y. pseudotuberculosis* infections have been described worldwide in more than 14 species of non-human primates as causing acute fatal or chronic debilitating disease (Kageyama et al. 2002; Iwata et al. 2008; Nakamura et al. 2009). Notably, several vaccine preparations against *Y. pseudotuberculosis,* including Pseudovac® and Yersiniavax® (a mixture of killed strains of various serotypes), as well as an attenuated strain (IP32680) of *Y. pseudotuberculosis* are available for use in zoo animals, but the efficacies of these vaccines are not well established (Quintard et al. 2010). Therefore, close surveillance is necessary.

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Y. pseudotuberculosis is a Gram-negative bacillus with serotypes ranging from O:1 to O:15 (Fukushima et al. 2001). Serotypes O:1b, O:3, O:4, and O:7 have been reported as important infectious agents to non-human primates worldwide. Infections due to these serotypes manifest as gastrointestinal illness and/or lesions in the parenchymal organs (Bronson et al. 1972; Buhles et al. 1981; Kageyama et al. 2002; Iwata et al. 2008; Nakamura et al. 2009; Zao et al. 2013). The pathogenicity of *Y. pseudotuberculosis* is associated with several virulence factors, including the 70-kb virulence plasmid (pYV) and the high pathogenicity island (HPI; Cornelis et al. 1998; Lesic and Carniel 2005; Hammerl et al. 2012). The virulence plasmid pYV encodes a number of important virulence factors and virulence-associated proteins, and HPI encodes an iron absorption system represented by the siderophore yersiniabactin. Certain serotypes produce a superantigenic toxin designated *Y. pseudotuberculosis*-derived mitogen (YPM), which allows for efficient entry into host cells and plays important roles in eliciting severe systemic symptoms (Fukushima et al. 2001; Thoerner et al. 2003).

Although *Y. pseudotuberculosis* serotype O:1a has been reported in birds, including crows and parrots (Hacking and Sileo 1974; Galosi et al. 2015; Magistrali et al. 2015), and mammals such as deer, rabbits, sheep, and cattle, as well as humans (Deacon et al. 2003; Lai et al. 2014; Ljungberg et al. 1995), no reports were available on the incidence in non-human primates worldwide. Herein, we report an outbreak of *Y. pseudotuberculosis* serotype O:1a in captive rhesus monkeys in China. This report uncovers the occurrence of this serotype in rhesus monkeys and its impact on monkey conservation efforts.

Six out of 18 rhesus monkeys (3–5 years of age) died unexpectedly or after a short illness (presented as fever, mucoid stool and anorexia) from March through April 2014 at a rhesus monkey breeding farm in Beijing, China. The affected animals were quarantined and treated with an injection of ceftazidime, amoxicillin-clavulanate, and ampicillin for a 1-month period, with no success. The postmortem examination revealed hemorrhagic manifestations in the liver, kidneys, and gut. A total of 31 tissue samples were collected, under aseptic conditions, from different organs of the dead monkeys including lung, liver, intestine, and kidney. Samples were homogenized in sterile phosphate buffered saline and plated on trypticase soy agar supplemented with 5% sheep blood. Gram-negative bacilli were isolated from 19 (61%) of 31 samples and identified as *Y. pseudotuberculosis* by the Phoenix 100 identification system (Becton Dickinson, Sparks, MD, USA). The isolated bacteria were designated as *Y. pseudotuberculosis* BJ01 through BJ19, as BJ denotes the place of isolation, ''Beijing,'' followed by the number of the isolate. A molecular identity was determined by PCR-sequence analysis based of the 16S rRNA gene fragment. The generated sequences were aligned with each other and reference sequences in the database using Clustal X (ftp://ftp-igbmc.u-trasbg.fr/pub/ClustalX/) to determine the identity of the isolated *Yersinia* spp. The homology search showed 97–99% similarities to *Y. pseudotuberculosis* sequences. The obtained sequences were deposited in the GenBank database under accession numbers KT429579 and KU991663-991680.

The virulence factors of YPM, pYV, and HPI were detected by amplification of the *ypmA*, *ypmB*, and *ypmC* genes of YPM, the *yadA* and *virF* genes of pYV and the *IS100*, *psn*, *yptE*, *irp1*, *irp2*, *ybtP-ybtQ*, *ybtX-ybtS*, *int*, and *asnT-Int* genes of HPI (Supplementary Table 1). The results revealed the occurrence of *inv*, *yadE*, and *virF* genes in the 70 kb pYV and *IS100*, *psn*, *yptE*, *irp1*, *irp2*, *ybtP-ybtQ*, *ybtX-ybtS*, *int*, and *asnT-Int* virulence genes in HPI, which indicates a high degree of pathogenicity (Supplementary Fig. 1). Collectively, the genetic identity of the collected isolates revealed that they belonged to genetic group 2 ($YPMs^-$, HPI^+), reported as European gastroenteric pathogenic type *Y. pseudotuberculosis* (Fukushima et al. 2001).

The O-antigen genotyping of the isolates was performed by intergenic multiplex PCR (containing *gmd-fcl*, *ddhC-prt*, *manB*, *abe*, *wbyL*, *wbyH*, *ddhA-B*, *wbyK*, and *wzx* genes), as described by Bogdanovich et al. (2003). The generated data indicated the O:1a pattern for the obtained *Y. pseudotuberculosis* isolates; this pattern is characterized by the presence of the *ddhC-prt*+, *wbyH*+, *ddhA-B*+, and *wzx*+ genes (Supplementary Fig. 2). Furthermore, the isolates were also identified by conventional serotyping agglutination as serogroup O1, and the antibiotic susceptibility was determined using the BD Phoenix Automated Microbiology and antimicrobial susceptibility testing system (Becton Dickinson, Sparks, MD, USA) with panel NMIC/ID-5 (Table 1).

The virulence of the isolates in experimental animals was assessed by mouse inoculation testing. Six groups of 5-week-old female C57/l mice ($n = 9$) were inoculated intraperitoneally or intragastric with 0.2 ml of a representative isolate (BJ01) in phosphate buffered saline containing 10^6, 10^7, or 10^8 colony-forming unit per milliliter (CFU). The infected mice were sacrificed at 36 h post-inoculation. The necropsy showed that the infecting bacteria were highly pathogenic to

Table 1. Results of Antimicrobial Susceptibility Tests of *Y. pseudotuberculosis* Serotype O:1a Isolates

Antimicrobial agents	BD Phoenix	
	MIC (μg/ml)	Susceptibility
Amikacin	≤8	S
Gentamicin	≤2	S
Imipenem	≤1	S
Meropenem	≤1	S
Cefazolin	8	R
Ceftazidime	>16	R
Cefotaxime	>16	R
Cefepime	>16	R
Aztreonam	>16	R
Ampicillin	>16	R
Piperacillin	>64	R
Amoxicillin-Clavulanate	>16/8	R
Ampicillin-Sulbactam	>16/8	R
Piperacillin-Tazobactam	>64/4	R
Colistin	>2	ND
Trimethoprim-Sulfamethoxazole	≤0.5/9.5	S
Chloramphenicol	≤4	S
Ciprofloxacin	≤0.5	S
Levofloxacin	≤1	S
Moxifloxacin	≤1	ND
Tetracycline	≤2	S

MIC = minimal inhibitory concentration; ND = no interpretative data based on Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing; S = susceptible; R = resistant.

mice, as revealed by obvious hemorrhaging in the gut, swelling of the mesenteric lymph nodes and enlargement of the kidneys and liver with multifocal yellowish-white nodules. The histopathological examination showed that the hepatic nodules were necrotic foci accompanied by polymorphic and mononuclear cell infiltration (Fig. 1).

In conclusion, the *Y. pseudotuberculosis* serotype O:1a isolated from dead monkeys in China was previously reported in other hosts in Europe, Australia, S. America, N. America, and Russia (Fukushima et al. 2001). In China, *Y. pseudotuberculosis* primarily belongs to serotype O:1b, O:1c, O:2a, O:2b, O:3, O:4b, O:5b, O:6, but serotype O:1a has been reported in rabbits, rats, swine, and squirrel monkeys (Zheng et al. 1995; Lai et al. 2014). The post-mortem findings of this outbreak, including hemorrhagic manifestations in the liver, kidneys, and gut accompanied by the clues pYV and HPI sequences, indicate that the isolated *Y. pseudotuberculosis* strain was highly pathogenic to rhesus monkeys. Notably, antibiotic sensitivity results indicated that quinolones (moxifloxacin, ciprofloxacin, and levofloxacin) were effective inhibitors of the growth of *Y. pseudotuberculosis* in vitro (Table 1). However, the effectiveness of quinolones in the treatment of rhesus monkeys is questionable, which may or may not be due to the difference in pharmacodynamics of these drugs in the monkeys under study and/or other host-related factors.

The mode and/or source of transmission of *Y. pseudotuberculosis* in captive monkeys throughout this outbreak remain unclear. Previous studies have shown that some animals such as birds and rodents could be asymptomatic carriers of *Y. pseudotuberculosis* (Buhles et al. 1981). Additionally, *Y. pseudotuberculosis* can also survive for months to years in the soil, water, and vegetation (Pedersen and Nadler 2013). Therefore, these animals, food sources, and the abiotic environment were potential sources of the infection. During this outbreak, healthy rats near the cages were trapped but were negative for *Y. pseudotuberculosis*. Taken together, the zoonotic and anthroponotic nature of *Y. pseudotuberculosis* show that extensive attention should be paid to the conservation of wild animals as well as to potential public health issues.

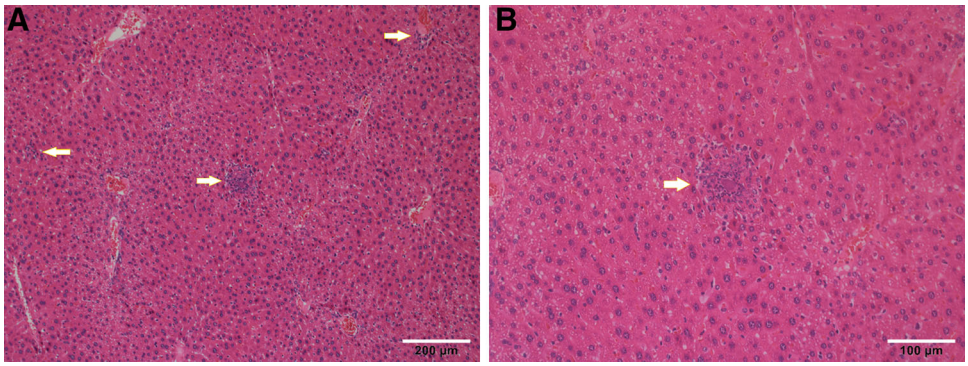

Figure 1. Light micrograph of a liver section of a mouse infected with *Y. pseudotuberculosis*. *Arrows* point to multifocal necrosis accompanied by infiltrating neutrophils and macrophages. Hematoxylin and eosin (HE) ×100 (**A**) and ×200 (**B**)

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