Veterinary Microbiology 166 (2013) 681–685

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Fatal atypical O:3 *Yersinia pseudotuberculosis* infection in cynomolgus macaques

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ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form 27 June 2013
Accepted 10 July 2013

Keywords:
Yersinia pseudotuberculosis
Cynomolgus macque
O:3
wbyK

ABSTRACT

Fatal *Yersinia pseudotuberculosis* infection in cynomolgus macaques was diagnosed based upon pathology, microbiology and PCR for this study. Pathological findings included acute, erosive to ulcerative, necrohemorrhagic enterocolitis. Genotyping by PCR showed an O:3 pattern (*gmd-fcl*$^+$, *ddhC-prt*$^+$, *manB*$^+$, *ddhA-B*$^+$), but an additional gene, *wbyK*, was detected. This is the second report to identify *wbyK*+ O:3 genotype as the cause of fatal yersiniosis. The first case was reported in 2008, and involved farm deer in the U.S. As the frequency of *wbyK*+ O:3 genotype is found more often in different carriers, O:3 genotype is proposed to be divided into two subtypes: O:3a without *wbyK* and O:3b with *wbyK*. Virulence gene analysis showed the presence of *inv*, *ypmC*, *irp1*, *ybtP-ybtQ*, *yadA*, *yopB*, *yopH*, *lcrF*, and suggested that this O:3b isolate could be a highly pathogenic strain to cynomolgus macaques.

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1. Introduction

Yersinia pseudotuberculosis is a gram-negative coccobacillus *Enterobacteriaceae* found in soil, water, foods, birds, and mammals. In cynomolgus macaques (*Macaca fascicularis*), *Y. pseudotuberculosis* causes various symptoms, ranging from diarrhea, enterocolitis, to fatal disease (Rosenberg et al., 1980).

The pathogenicity of *Y. pseudotuberculosis* is determined by several virulence factors. O-antigen (O-polysaccharide), a side chain of lipopolysaccharide (LPS) on the bacteria's outer membrane, plays a role in the virulence by causing *Y. pseudotuberculosis* to invade Peyer's patches and epithelial cells more efficiently (Skurnik and Bengoechea, 2003). Traditionally, immune sera against O-antigen was used to diagnose different serotypes (Tsubokura and Aleksić, 1995). Based on the genetic composition of O-antigen gene clusters between *hemH* and *gsk* genes, 21 O-serotypes or O-genotypes have also been identified (Bogdanovich et al., 2003).

Besides O-antigen, virulence factors derived from the chromosome include invasin (Inv), *Y. pseudotuberculosis*-derived mitogen (3 variants, YPMa, YPMb, and YPMc) and high-pathogenicity island (HPI); plasmid-born virulence factors include *Yersinia* adhesin A (YadA,), *Yersinia* outer membrane proteins (Yops), low-Ca^{2+} response F protein (LcrF), etc. The loss of virulence plasmids will either decrease the pathogenicity or cause the bacteria to become nonpathogenic.

Although the bacteriological, biochemical, and histopathological properties of *Y. pseudotuberculosis* infections in cynomolgous monkeys have been well studied (Bronson et al., 1972; Rosenberg et al., 1980), little is known about their O-genotyping and virulence genetics. In 2011, a group of our cynomolgus macaques suffered from acute fatal yersinosis. Here we reported the isolation of *Y. pseudotuberculosis*, the pathological findings, the typing of the O-antigen gene cluster and its virulence gene distribution.

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http://dx.doi.org/10.1016/j.vetmic.2013.07.013

2. Materials and methods

2.1. Animals' history

A group of 40 cynomolgus monkeys of Chinese origin (age: 2 to 4 years) were received into an indoor holding facility on May 25, 2011. Animals were fed a commercial High Protein Monkey Diet (PMI LabDiet) and were supplemented with fresh fruits, vegetables and other enrichment items. On June 12, 2011, one female (animal A) was found dead with no previous signs of illness other than being slightly underweight. A fecal sample from the cage mate (animal B) was submitted to Antech Diagnostics (Irvine, CA) and results showed negative for *Yersinia* sp., *Salmonella* sp., *Shigella* sp., *Cryptosporidium* sp., and all ova and parasites (including *Giardia* sp.). On June 16, 2011, *Campylobacter* sp., Coliforms, and non-pathogenic *Enterococcus* sp. (*E. faecalis*/*E. faecium*) were isolated from routine fecal samples, and were considered as normal stool flora. On July 9, 2011, animal B also died and appeared to be slightly emaciated and dehydrated at the time of death. A prophylactic treatment regimen consisting of azithromycin (Bio-Serv, Frenchtown, New Jersey), acidophilus (1 wafer containing 1 billion active *Lactobacillus acidophilus* and *Bifdobacterium bifidium*), and fenbendazole (Intervet, Millsboro, Delaware) was initiated for all of the remaining animals in the colony. At this time, our differential list included rotavirus, *Salmonella*, *Shigella*, *Campylobacter*, *Klebsiella* and *Yersinia* sp., therefore a PCR panel screening for these organisms was performed using the formalin-fixed Peyer's patches obtained from animal B. *Yersinia* sp. was PCR identified, and the colony was given 30 mg sulfamethoxazole and trimethoprim for 10 days (Bio-Serv, Frenchtown, New Jersey). On October 23, 2011, a third monkey (animal C) was found dead, with slight emaciation and dehydration. *Y. pseudotuberculosis* was identified via PCR. On November 7, 2011, two animals (animals D and E) were showing the following clinical signs: lethargy, lateral recumbency, hypothermia, mucoid stool with and without blood, bradycardia, cyanotic mucous membranes, dehydration and decreased body weight. Both animals were humanely euthanized. *Y. pseudotuberculosis* was identified via PCR and microbiological culture. Two additional animals with clinical signs consistent with gastric ulcer development (reluctance to eat/anorexia, weight loss, pale mucous membranes) were placed on a treatment regimen consisting of coconut water ad libitum (Vita Coco), fiber bites (Bio-Serv, Frenchtown, New Jersey), Pepto Bismol (Procter and Gamble, Cincinnati, Ohio), metoclopramide, and omeprazole (Dr. Reddy's Laboratory Limited, Bachepalli, India). Following the above treatment, all clinical signs relating to ulcer development resolved, and the animals remained healthy.

2.2. Histopathology

Tissues collected from animals B, C, D and E at necropsy were fixed in 10% neutral-buffered formalin and processed to hematoxylin and eosin-stained tissue sections for examination by a board-certified veterinary pathologist.

2.3. Yersinia genus and Yersinia pseudotuberculosis-specific PCR

Formalin-fixed paraffin-embedded (FFPE) tissues, stool, fresh or frozen tissues were processed for DNA extraction by using QuickExtract FFPE DNA extraction kit (Epicentre, Madison, WI), QiaAmp Stool DNA Extraction Kit (Qiagen, Valencia, CA), Generation Capture Column Kit (Qiagen, Valencia, CA) or PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA). *Yersinia* genus and *Yersinia pseudotuberculosis*-specific PCR were performed using GoTaq Green (Promega, Madison, WI) and primers (University of Texas Health Science Center [UTHSC], San Antonio, TX) described by Stenkova et al. (2008).

2.4. Yersinia isolation

Fresh tissue samples (Table 1) collected from animals D and E were homogenized and inoculated onto Blood, MacConkey and *Yersinia* selective (CIN) agar plates (Remel, Lenexa, Kansas). After incubation at 25 °C for 48 h, colonies with biochemical tests spot indole negative and oxidase negative (Remel, Lenexa, Kansas) were further identified by the API-20E (Biomerieux, Inc., Durham, NC). Catalase production was qualitatively detected by catalase reagent droppers containing approximately 3% hydrogen peroxide (Becton, Dickinson and Company, Sparks, MD).

2.5. Antimicrobial susceptibility testing

Antimicrobial disk susceptibility testing was performed by following CLSI M02-A10 guidance.

2.6. O-antigen genotyping and serotyping

Genotyping of O-antigen was performed using GoTaq Green master mix (Promega, Madison, WI) and 9 sets of primers (UTHSC, San Antonio, TX) in individual PCR tubes (Bogdanovich et al., 2003). DNA sequencing from *wbyK* PCR products were carried out on an ABI 3130xl Genetic Analyzer (Foster City, CA) (Nucleic Acids Core Facility, UTHSC, San Antonio, TX). The conventional serotyping agglutination by using anti-serum against O-antigen of *Y. pseudotuberculosis* was performed in Microbial Diseases Laboratory, California Department of Public Health (Richmond, CA).

2.7. Virulence gene PCR

DNA was prepared from *Y. pseudotuberculosis* isolates grown in Luria Bertani (LB) broth (Sigma, Saint Louis, MO) and extracted by Qiagen Generation Capture Column Kit (Qiagen, Valencia, CA). PCR was performed using GoTaq Green (Promega, Madison, WI) and primers (UTHSC, San Antonio TX) (Supplemental Table 1).

3. Results

3.1. Pathology

Bacterial-related gross findings were similar in necropsied animals B, C, D and E, which included enlarged

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