Comparative Medicine
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Vol 64, No 6
December 2014
Pages 496–500

Case Report

Extraintestinal Campylobacteriosis in Rhesus Macaques (*Macaca mulatta*)

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Two cases of clinical disease associated with extraintestinal *Campylobacter* infection were recently encountered in rhesus macaques (*Macaca mulatta*). The first case was that of a 3-y-old, male, rhesus macaque experimentally infected with SIV, who presented with abdominal pain and a midabdominal mass and was euthanized. Pathology findings included an abscess within the median liver lobe, fibrinopurulent peritonitis, and intestinal serositis with isolation of *Campylobacter fetus* from the blood, liver, and the hepatic abscess. The second case was that of a 1-mo-old, female, rhesus macaque who died with no apparent history of illness. Gross pathology findings included thin body condition and diarrheic staining of the perineum; histologically, acute multifocal hepatitis with intralesional bacteria was noted. *Campylobacter coli* was isolated from the liver and colon. Extraintestinal *Campylobacter* infection is uncommon in humans, usually occurring in immunocompromised subjects and most commonly manifesting as bacteremia. Extraintestinal *Campylobacter* infections in animals are rare but have been associated with bacteremia and cholecystitis. The macaques presented here were either immunocompromised due to SIV infection (case 1) or more vulnerable due to young age (case 2). These factors likely contributed to the extraintestinal spread of *Campylobacter*.

Campylobacter spp. are curved or spiral, gram-negative, microaerobic, typically motile bacteria with a single flagellum at one or both ends of the cell.[25] *Campylobacter* is one of the most common bacterial causes of gastroenteritis in humans worldwide.[3] The Centers for Disease Control and Prevention estimate it to affect more than 1.3 million people in the United States each year.[11] *Campylobacter* spp. colonize the intestinal tract of primates, other mammals, birds, reptiles, and shellfish, but infection is not always associated with clinical signs of disease.[2,23] Contaminated or undercooked poultry represents the largest potential source of human infection.[22] Campylobacteriosis is a zoonosis, and other significant sources of human infection include livestock, wildlife, pets, and contaminated water.[22] The species most commonly isolated from humans and nonhuman primates are *C. jejuni* and *C. coli*; other species like *C. fetus* are less commonly found.[2,4,9,23] Extraintestinal campylobacteriosis in humans usually occurs in immunocompromised or elderly persons with underlying medical problems and most commonly manifests as bacteremia.[23] There have been a few reports of extraintestinal *Campylobacter* infections in animals, including bacteremia and cholecystitis in 2 dogs.[17,30]

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History. Yerkes National Primate Research Center of Emory University is a fully AAALAC-accredited facility. All animals in the colony are managed under IACUC-approved protocols in accordance with the applicable USDA Animal Welfare Regulations and the *Guide for the Care and Use of Laboratory Animals*.[1,18]

Case 1 involved a 3-y-old, male, indoor-housed, rhesus macaque who was experimentally infected with $SIV_{mac}251$. Five months after inoculation, he presented for lethargy and presumptive abdominal pain. On physical exam, he was in fair body condition and had a palpable, fluctuant midabdominal mass. Radiographs revealed generalized loss of abdominal serosal detail, severe hepatomegaly, and distended, gas-filled intestines (Figure 1). Abdominal ultrasonography revealed a hypoechoic to anechoic mass within the liver, which appeared to be full of fluid and some hyperechoic debris. All erythrocyte values were normal, and the leukogram showed a leukopenia, characterized by neutropenia, regenerative left shift, lymphopenia, and slight monocytosis (Table 1). Serum chemistry revealed hypoproteinemia and hypoalbuminemia, increased liver enzyme values, slight azotemia, hyperphosphatemia, hypocalcemia and hyponatremia (Table 2). Due to the severity of signs and poor prognosis, euthanasia was elected.

Case 2 concerned a 1-mo-old, female, rhesus macaque who died without any apparent history of illness. The infant was housed with her dam in an indoor–outdoor enclosure among a large social group. A physical examination performed 1 wk prior to her death did not reveal any obvious clinical abnormalities.

Pathology. At necropsy, case 1 had an abscess (diameter, approximately 5 cm) within the median liver lobe, and the omentum had multifocal adherence to the hepatic serosa. Severe multifocal fibrinopurulent peritonitis and intestinal serositis also were present. *C. fetus* was isolated from the blood, liver, and hepatic abscess. Due to the prolonged postmortem interval, the infant in case 2 was moderately to severely autolyzed. The animal was in a thin body condition and had diarrheic staining of the perineum.

Received: 14 Mar 2014. Revision requested: 21 Apr 2014. Accepted: 20 Jun 2014.
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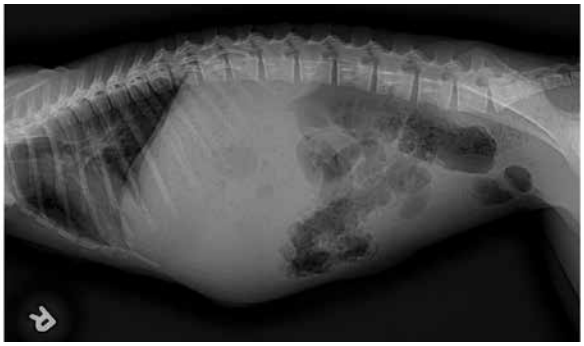

Figure 1. Case 1. Radiograph, right lateral, abdomen. Generalized loss of abdominal serosal detail, severe hepatomegaly, and distended, gas-filled intestines.

Table 1. Leukocyte abnormalities, case 1

Parameter	Result ($\times 10^3/\mu L$)	Reference[a]
WBC	6.8	15.66 ± 3.16
Neutrophils	2.37	6.18 ± 2.40
Bands	1.21	
Lymphocytes	1.95	8.94 ± 2.46
Monocytes	0.54	0.36 ± 0.20

[a]Reference values from reference 12 reported as mean ± 1 SD.

Table 2. Serum chemistry abnormalities, case 1

Parameter	Result	Reference range[a]
AST (U/L)	66	27–45
ALP (U/L)	3500	55–237
GGT (U/L)	196	51–85
BUN (mg/dL)	36	22–30
Phosphorous (mg/dL)	7.4	3.8–5.6
Calcium (mg/dL)	7.4	9.9–10.9
Sodium (mmol/L)	133	147–155
Total protein (g/dL)	5.1	7.3–8.3
Albumin (g/dL)	1.8	3.8–4.8

[a]Reference range for adult indoor-housed male rhesus macaques obtained from reference 5.

No additional gross lesions were noted. *C. coli* was isolated from the liver and colon.

Tissue samples collected at necropsy were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin. In case 1, the median hepatic lobe parenchyma had multifocal necrosis intermixed with degenerate and viable neutrophils and bacteria (Figure 2). Steiner silver staining of liver samples showed frequent spiral-shaped *Campylobacter* organisms within the lumen of the bile duct (Figure 3). The apical surface of the bile duct epithelial cells was often lined with minute (2 to 6 µm), basophilic, apicomplexan protist dots (*Cryptosporidium* spp.; Figure 3). The pancreatic duct had aggregates of neutrophils in its lumen and occasional cryptosporidia on the apices of the ductular epithelial cells. The lung alveolar lumina occasionally contained moderate aggregates of neutrophils intermixed with mucin. Cryptosporidia were noted occasionally at the apical surfaces of bronchiolar epithelial cells.

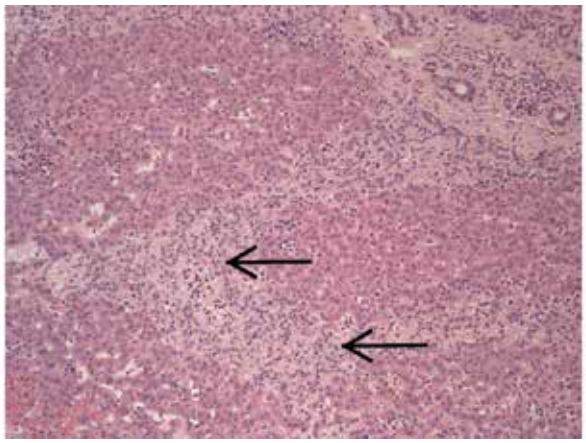

Figure 2. Case 1. Liver. The hepatic parenchyma has multifocal necrosis intermixed with degenerate and viable neutrophils (arrows) and bacteria. Hematoxylin and eosin stain; magnification, 100×.

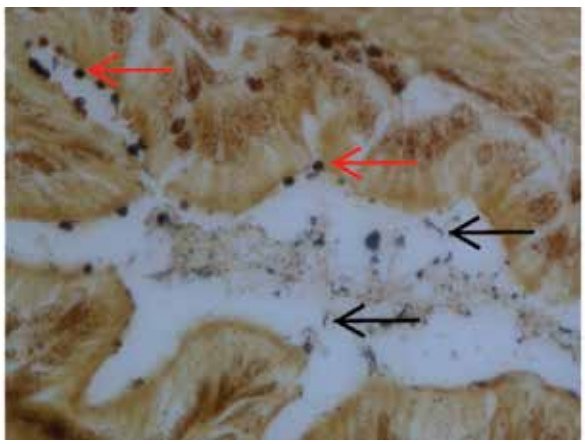

Figure 3. Case 1. Bile duct. Spiral-shaped *Campylobacter* organisms within the lumen of the duct (black arrows). *Cryptosporidia* spp. located at the apical surfaces of epithelial cells (red arrows). Steiner stain; magnification, 600×.

In case 2, the hepatic parenchyma had severe diffuse infiltrates of neutrophils, lymphocytes, and macrophages intermixed with spiral-shaped bacteria (Figure 4). The lamina propria of the small intestine from both cases had moderate diffuse infiltrates of lymphocytes, macrophages, and few plasma cells along with rare dilated lymphatics. Case 1 had moderate multifocal proliferation of goblet cells.

Electron microscopy was performed on sections of liver from both cases. Formalin-fixed tissue sections were trimmed into 1 × 1 mm sections, postfixed in 1% phosphate-buffered osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (model 1011, JEOL, Peabody, MA). Electron microscopy identified *Campylobacter* within the liver in both cases (Figure 5).

To determine whether there was an increase in microbial translocation through a breach in intestinal mucosa,[14] immunohistochemistry (IHC) was performed using an antibody against the LPS core antigen (catalog no. 2D7/1, anti*E. coli* LPS antibody, Abcam, Cambridge, MA) to observe the bacterial products

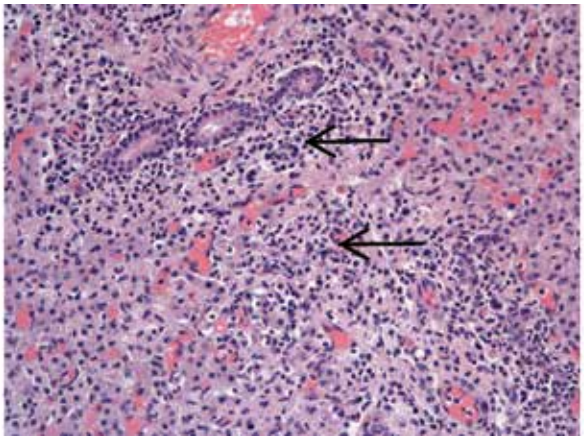

Figure 4. Case 2. Liver. The hepatic parenchyma has severe diffuse infiltrates of neutrophils, lymphocytes, and macrophages (arrows) intermixed with bacteria. Hematoxylin and eosin stain; magnification, 200×.

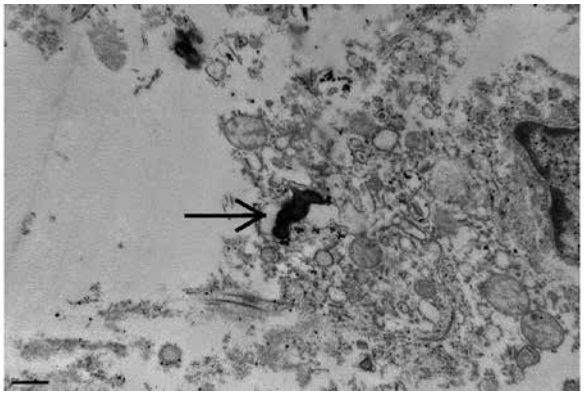

Figure 5. Case 2. Liver. A spiral, curved bacterium (arrow) is present in the hepatic parenchyma. Transmission electron microscopy; bar, 0.5 µm.

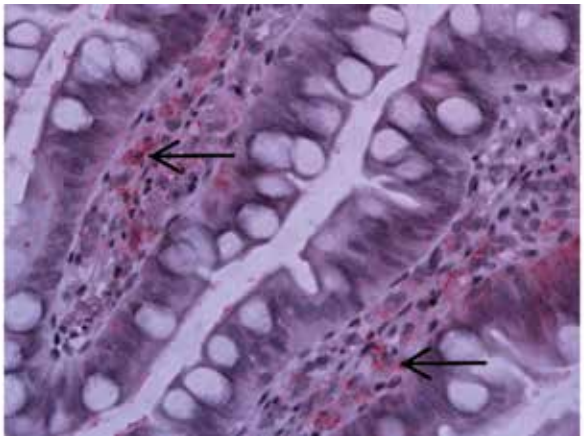

Figure 6. Case 1. Duodenum. Cells positive for LPS core antigen (arrows) occur frequently in the lamina propria. Immunohistochemistry for LPS; magnification, 400×.

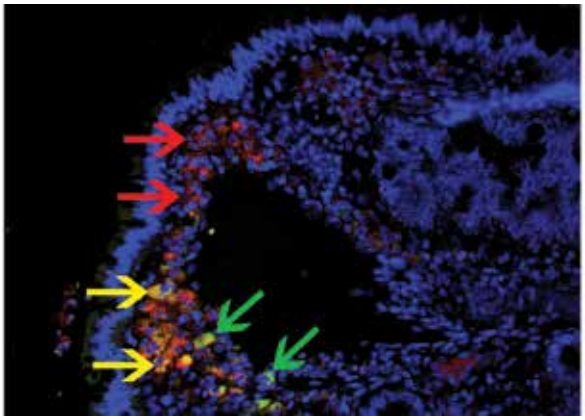

Figure 7. Case 1. Duodenum. Red arrows indicate $CD68^+$ cells (macrophages, red). Green arrows indicate cells showing LPS staining only (green). Yellow arrows indicate dual-labeled macrophages containing LPS (yellow). Nuclei were stained with DAPI (blue). Dual immunofluorescence for CD68 and LPS; magnification, 200×.

within the lamina propria. Frequently, LPS-positive cells, especially macrophages (stained with antiCD68 antibody; Pierce, Thermo Fisher Scientific, Waltham, MA), were present in the lamina propria of the duodenum in both cases (Figures 6 and 7). Sections of small intestine from an SIV-infected macaque with severe campylobacteriosis served as both a positive control (when incubated with LPS- and CD68-specific antibodies) and a negative control (when incubated with irrelevant, isotype-matched control immunoglobulins).

Bacteriology. Samples were cultured under microaerophilic conditions on either *Campylobacter*-specific blood agar or CVA agar at 42 °C or 37 °C, respectively. Gram staining of suspected *Campylobacter* colonies allowed the visualization of small, curved, gram-negative bacilli. A positive oxidase test confirmed that the bacilli were *Campylobacter* spp. Antimicrobial susceptibility testing with nalidixic acid and cefalotin followed by hippurate hydrolysis was used to differentiate among the *Campylobacter* species most commonly encountered at YNPRC. Colonies resistant to nalidixic acid but susceptible to cefalotin were identified as *C. fetus.* Colonies susceptible to nalidixic acid, resistant to cefalotin, and negative for hippurate hydrolysis were identified as *C. coli.*

Discussion

Several *Campylobacter* spp. can cause diarrhea due to gastroenteritis, but primarily *C. jejuni* followed by *C. coli* is associated with diarrhea in humans, nonhuman primates, and other domestic, wild, and laboratory animals.[2,17,23] Although *Campylobacter* is not always associated with disease and is sometimes considered a commensal organism, its presence outside the intestinal tract can cause serious systemic illness.[23] Compared with other species, *C. fetus* may be more invasive and more likely to spread beyond the intestinal tract in humans.[26,31] Extraintestinal *Campylobacter* infection in humans most commonly presents as bacteremia and occasionally as cholecystitis, hepatitis, pancreatitis, nephritis, myocarditis, cellulitis, meningitis, abortion, and abscesses.[10,13,20,21,23,25,31] Human patients with extraintestinal campylobacteriosis usually are immunocompromised, and one group identified the first month of life and immunosuppression as factors that potentially predispose humans to extraintestinal *Campylobacter* infection.[9,26,28] In our 2 macaques, SIV infection (case 1) and bacterial infection during infancy (case 2) were the most

likely causes of immunocompromise leading to the extraintestinal spread of C*ampylobacter* spp.

Campylobacter has been reported to have a propensity for colonizing the liver, biliary tract, and gallbladder in humans, mice, and rhesus macaques because it appears well suited to grow in bile.[7,8,13,15] Both macaques likely had intestinal colonization by *Campylobacter* spp. combined with an inadequate immune response, which allowed extraintestinal spread either by transcellular or paracellular[6] translocation across a compromised mucosal barrier, with subsequent hematogenous spread or as an ascending infection through the bile duct.

Presumptive diagnosis of *Campylobacter* can be made by microscopic identification of motile and curved or spiral-shaped rods in stool samples.[17] Other culture-independent methods such as serologic testing are used occasionally, but definitive diagnosis of *Campylobacter* infection usually is made by isolation of the organism from blood, feces, or other tissue samples.[2,9,17] Successful culture generally requires the use of selective media and incubation at 42 °C in a microaerophilic atmosphere.[2] Because some *Campylobacter* spp. are more fastidious than are *C. jejuni* and *C. coli*, several culture and biochemical methods may be required for diagnosis.[23] Antibiotic therapy may not be necessary in all cases of *Campylobacter*-induced diarrhea, but lack of appropriate antibiotic treatment has been associated with 88% mortality in humans diagnosed with *Campylobacter* bacteremia.[2,26] Macrolide antibiotics, such as erythromycin and the newer drugs clarithromycin and azithromycin, are the frontline *Campylobacter* treatment in nonhuman primates and humans, although low levels of resistance have been reported.[2,13,16,26] Fluoroquinolones are a frequently chosen empirical diarrhea treatment in humans and are generally effective against *Campylobacter,* but one study in humans found an increased risk of death with empirical use of fluoroquinolones in patients with *C. fetus*.[26] Regimens including an aminoglycoside, such as gentamicin, or a carbapenem, such as imipenem, have been used successfully to treat severe systemic *Campylobacter* infection in humans.[16,26] Several surveillance programs have reported high levels of *Campylobacter* resistance to tetracycline and ciprofloxacin.[16]

Cryptosporidium, identified in case 1, is a common opportunistic pathogen seen in immunodeficient rhesus macaques.[27,29] This organism can cause chronic diarrhea, anorexia, and weight loss[24] and is frequently isolated from the small intestine, gall bladder, and biliary duct.[19,32] We believe that, although cryptosporidiosis may have contributed to the loss of condition in this animal, the most significant pathology (hepatic abscess) which led to his euthanasia was associated with campylobacteriosis.

Finally, due to the frequent presence of *Campylobacter* spp. in the gastrointestinal tract and the number of nonhuman primates that are immunocompromised secondary to experimental or natural causes, extraintestinal spread of *Campylobacter* is a real and recurring risk factor for morbidity and mortality in such cases. Therefore, systemic campylobacteriosis needs to be included on a list of rule-outs for ill and potentially immunocompromised nonhuman primates, especially when hepatobiliary involvement is suspected.

Acknowledgments

This work was supported by a NIH-NCRR base grant (P51RR000165) to Yerkes National Primate Research Center. We thank the YNPRC animal care staff, necropsy staff, and veterinary technicians for their assistance with this work.

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