PRIMATOLOGY

Detection of *Campylobacter jejuni* in Healthy Monkeys and Monkeys with Enteric Infections by PCR

V. A. Kalashnikova, E. K. Dzhikidze, Z. K. Stasilevich, and M. G. Chikobava

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 9, pp. 346-348, September, 2002
Original article submitted February 26, 2002

Campylobacter were detected by PCR in feces of monkeys of different species (clinically healthy, with diarrhea, and dead from acute enteric infections). High prevalence of these bacteria in monkeys was revealed. The incidence of *C. jejuni* DNA in monkeys with acute enteric infections was higher than in healthy animals (69.6 and 51.3%, respectively). The highest percentage (92.3) of positive results was observed in *Macaca mulatta* with enteric diseases and in macaque dead of these diseases. The presence of *C. jejuni* in monkeys with diarrhea and the absence of pathogenic enterobacteria (*Shigella*, *Salmonella*, *Yersinia*) in feces probably attest to etiological relationship of acute enteric infections with *Campylobacter*.

Key Words: *monkeys; enteric infections; polymerase chain reaction; Campylobacter*

Enteric infections rank first among spontaneous diseases in monkeys. Acute enteric infections (AEI) associated with pronounced diarrhea are responsible for the majority of diseases and deaths of animals under conditions other than natural [16]. Earlier, 50-70% simian diseases associated with diarrhea were qualified as bacteriologically confirmed shigellosis or salmonellosis; however, starting from the middle of the 1980s the overwhelming majority (50-75%) of diseases remains etiologically unknown [6]. The same is true for human AEI. An important role in the etiology and pathogenesis of these infections is now assigned to some pathogenic and opportunistic microorganisms, which were poorly studied up to recent time. *Campylobacter* attract the major interest in this respect because of their geographical ubiquity and intense circulation in human and animal populations, including monkeys [3,7,8].

Laboratory of Infectious Diseases, Institute of Medical Primatology, Russian Academy of Medical Sciences, Sochi-Adler. ***Address for correspondence:*** iprim@sochi.net. Dzhikidze E. K.

The diagnosis of campylobacteriosis based on classical bacteriological methods is very difficult, because these microorganisms require specific media and strict microaerophilic conditions [3]. One of the most sensitive methods used in laboratory practice for detection of *Campylobacter* is molecular genetic diagnosis by PCR, based on amplification of a specific fragment of bacterial DNA by thermostable DNA polymerase and primer pair specific for the tested agent DNA [2,4].

We tried to detect *Campylobacter jejuni* in feces of monkeys of different species (healthy, sick, with diarrhea, and dead from AEI) by PCR.

MATERIALS AND METHODS

Clinically healthy male and female monkeys and monkeys with diarrhea of unknown origin of different age living in Adler Breeding Center were examined: 25 *M. mulatta*, 20 *M. fascicularis*, 4 *M. nemestrina*, 1 *C. aethiops*, 10 *P. anubis*, and 2 *P. hamadryas*.

Feces collected with sterile tampons from the rectum of live monkeys and the contents of 3 intestinal

0007-4888/02/1343-0299$27.00

TABLE 1. Incidence of *C. jejuni* DNA in Feces of Different Monkey Species

Monkey species	Healthy			Sick		
	total	PCR+		total	PCR+	
		abs.	%		abs.	%
M. mulatta	12	5	41.6	13(2)	12	92.3
M. fascicularis	13	8	61.5	7(2)	2	28.6
M. nemestrina	3	1	—	1	1	—
C. aethiops	1	0	0	—	—	—
P. anubis	10	6	60	—	—	—
P. hamadryas	—	—	—	2	1	—
Total	39	20	51.3	23	16	69.6

Note. The number of dead monkeys is shown in parentheses.

compartments (jejunum, caecum, and rectum) from monkeys dead from AEI were examined.

All monkeys were tested for enteropathogenic bacteria by bacteriological methods. The material was inoculated in differential diagnostic media (Endo's, Ploskirev's), 5% blood agar, and selenite broth with subsequent identification of isolated microorganisms by the routine methods.

PCR was used for detection and identification of *C. jejuni*. KAM-BAK test systems manufactured by Central Institute of Epidemiology, Ministry of Health of Russian Federation (Moscow), were used. PCR analysis was carried out in accordance with methodological recommendations.

The material was suspended in 5 ml normal saline, centrifuged, and a 0.1-ml aliquot was used for DNA isolation. DNA extraction and amplification was carried out using the reagents from the KAM-BAK kit. The results were interpreted in accordance with the manufacturer's recommendations; negative and positive controls were processed in parallel.

RESULTS

Bacteriological analysis of rectal smears and intestinal contents detected no pathogenic enterobacteria (*Shigella*, *Salmonella*, *Yersinia*) in none monkeys. On the other hand, *C. jejuni* DNA was detected in 36 specimens (58%). The incidence of *C. jejuni* DNA in feces of clinically healthy monkeys was high (Table 1).

The incidence of *C. jejuni* in sick monkeys and monkeys dead from AEI was higher than in healthy animals (Table 1). We should like to emphasize that the highest percentage of positive results 2-fold surpassing the corresponding parameter in healthy monkeys of this species was observed in sick and dead *M. mulatta*. These monkeys suffer from diarrheas of unknown origin most often (Table 1).

The study of intestinal contents from monkeys dead from AEI showed *C. jejuni* DNA in all three tested compartments of the intestine.

Hence, *C. jejuni* is highly prevalent in monkeys of different species, and the incidence of its DNA sequences is higher in sick animals than in healthy ones. Macaques (particularly *M. mulatta*) are the most sensitive to AEI. Detection of *C. jejuni* in sick animals with diarrhea in the absence of pathogenic enterobacteria (*Shigella*, *Salmonella*, *Yersinia*) in the feces indicates with high probability the etiological relationship of AEI with *C. jejuni*.

Our results indicate that the Russian KAM-BAK test system effectively detects specific sequences of *C. jejuni* DNA in primates and can be widely used for the diagnosis of *Campylobacter* infection.

These data widen our knowledge about etiological structure of AEI in monkeys, which is important for rational therapy and for the development of methods of eradication of *C. jejuni* as a possible etiological factor causing enteric diseases (enteritis, enterocolitis) often leading to death in monkeys.

REFERENCES

1. B. A. Lapin, E. K. Dzhikidze, and E. P. Fridman, *Manual of Medical Primatology* [in Russian], Moscow (1987).
2. *KAM-BAK Test System: Methodological Recommendations* [in Russian], Moscow (2000).
3. N. V. Safonova, L. B. Khazenson, and Z. N. Matveeva, *Etiology, Clinical Picture, and Diagnosis of Campylobacteriosis. Methodological Recommendations* [in Russian], Leningrad (1988).
4. S. Harington and J. MacGui, *Molecular Clinical Diagnosis. Methods* [in Russian], Moscow (1999), pp. 396-402.
5. N. A. Chaika, L. B. Khazenson, and J.-P. Buttsler, *Campylobacteriosis* [in Russian], Moscow (1988).
6. E. K. Dzhikidze, Z. K. Stasilevich, and R. I. Krylova, *Baltik. J. Lab. Anim. Sci.*, No. 11, 123-134 (2001).
7. W. R. Morton, M. Bronsdon, and G. Mickelsen, *Lab. Anim. Sci.*, **33**, No. 2, 187 (1983).
8. R. G. Russel, L. Krugner, C. C. Tsai, and R. Ekstoon, *Ibid.*, **38**, No. 6, 711 (1988).