PRIMATOLOGY

Etiologic Structure of Bacterial Intestinal Infections in Monkeys of Adler Breeding Center

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 6, pp. 681-684, June, 2011
Original article submitted March 3, 2011

We studied etiologic structure of bacterial intestinal infections in monkeys of Adler nursery. A total of 533 monkeys with diarrhea syndrome and monkeys dead from intestinal infections, as well as clinically healthy monkeys and animals dead from other pathologies were examined by bacteriological and molecular-genetic methods. Pathogenic enterobacteria *Shigella* and *Salmonella* and microaerophile *Campylobacter* were found in 5 and 19%, respectively. A high percentage (49%) of intestinal diseases of unknown etiology was revealed in monkeys. The fact that the number of detected opportunistic enterobacteria did not differ in healthy and diseased monkeys suggests that they are not involved into the etiology of intestinal disease.

Key Words: *monkey; acute intestinal infection; Shigella; Yersinia; Campylobacter*

Long-term bacteriological studies of intestinal diseases in different monkey species carried out at the Laboratory of Infectious Pathology of Sukhumi and Adler Breeding Center revealed an etiologic relationship between these diseases and pathogenic enterobacteria and, first of all, with various serological types of *Shigella flexneri* [14]. Thus, *Shigella flexneri* and much less frequently, *Salmonella,* enteropathogenic *Escherichia coli* and *Yersinia* were isolated from ~75-80% sick animals with severe diarrhea syndrome and monkeys dead from acute intestinal infections (AII) [3,4,8]. Similar results were obtained in the primatological institutions and foreign zoos, mainly in the USA, Japan, the Netherlands and others [7,11,12]. Since the mid-1980s, the ethological connection between pathogenic enterobacteria and AII tends to weaken against the background of high prevalence of these diseases and high mortality associated with them [9,10].

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Thus, the etiologic structure of intestinal infections is now changing towards an increase in the incidence of diseases with unknown etiology (from 50 to 70% in different years) [5,6,10]. However, the relevance and effectiveness of treatment, prevention, and antiepidemic measures in primatological institutions strictly depend on identification of AII etiology.

Here we studied etiologic structure of bacterial intestinal infections in monkeys of Adler breeding center over the last 3 years.

MATERIALS AND METHODS

The objects of the study were 533 different monkeys of both sexes and different species aged from 7 days to 30 years. Of these, 235 monkeys had severe diarrhea syndrome, 163 died from AII, 87 were clinically healthy, and 48 died from other pathologies (trauma, pneumonia, fatty liver and kidney degeneration, *etc*.).

Three hundred and seventy-nine monkeys were 379 macaques of different species (*M. mulatta, M. fascicularis, M. nemestrina, M. assamensis*), 122

0007-4888/11/1516-0734

monkeys were baboons (*Papio hamadryas* and *Papio anubis*), 21 were green monkeys (*C. aethiops*), and 11 monkeys belonged to rare species (Table 1).

The animals were kept in group cages of 5-7 animals and in open cages of 30-50 animals with natural earth or covered with gravel. Sick animals were placed in an isolator and were housed in individual cages equipped with feeders and automatic waterers.

The material for the study was fecal samples taken with a sterile tampon from healthy and diseased monkeys. Fecal samples from sick animals were taken at admission and then repeatedly during treatment and convalescence. In dead animals, feces from three parts of the intestine (ileum, caecum, and rectum) were samples, as well as mesenteric lymph nodes, liver, kidney, and spleen were examined bacteriologically.

Feces were inoculated in Endo mediums, Ploskirev medium, bismuth-sulfite agar, medium for isolation of *Yersinia*, and 5% blood and meat-peptone agar and cultured at 37°C for 18-20 hours; dishes with *Yersinia* medium were incubated at 28°C for 48 hours. For identification of the isolated cultures, conventional methods were used: determination of microbial saccharolytic and proteolytic properties as well as a set of plates, consisting of 20 tests for enterobacteria identification: biochemical plate, differentiating enterobacteria (PBDE, LLC Scientific-Production Association). In accordance with the instruction, record of the results was performed visually using a table of biochemical properties of enterobacteria as well as catalog codes. Serological identification was carried out of with special and standard diagnostic sera (Pasteur Institute of Epidemiology and Microbiology).

For detection of microaerophilic bacteria *C. jejuni*, molecular genetic PCR method was used, which is now recognized as the most informative and simple [1,2,13]. For PCR, primers of Biokom to the conservative sites of the outer *C. jejuni* membrane were used included in the set of test systems GenePak. DNA was isolated according to the instructions. Amplification was performed in Tertsik thermocycler. PCR products were separated by horizontal electrophoresis in agarose gel containing ethidium bromide and visualized in UV light (λ=312 nm). Results were recorded visually using Gel Imager video system (Litekh). Positive and negative controls that were part of test system were run.

RESULTS

Bacteriological examination of feces repeated three times before and after treatment in monkeys with diarrhea syndrome (frequent loose stools with or without mucus and blood) revealed no pathogenic enterobacteria. In 87 clinically healthy monkeys, *Shigella flexneri* 4a was found in one case (1.1%) in Javanese macaque (Table 2).

In the group of monkeys dead from AII of different morphological forms, pathogenic enterobacteria were identified in 20 (12.2%) of 163 monkeys; of them, 16 (9.8%) were identified as *Shigella*, 2 (1.2%) as *Salmonella,* and 2 (1.2%) as *Y. pseudotuberculosis*. All 16 strains of *Shigella* and *Salmonella* were serologically typed. Of these, 6 (3.6%) strains belonged to *Shigella flexneri* 4a; 3 (1.8%) to *Shigella flexneri* 2a, 6 (3.6%) to *Shigella newcastl,* and 1 (0.6%) to *Shigella Shmitz-Shtuzeri.* In cases of *Shigella* detection, enterocolitis and gastroenterocolitis dominated morphologically. One strain of *Salmonella* was assigned to rare groups and the second to *Salmonella typhimurium*. Two strains identified as *Y. pseudotuberculosis* were isolated from two monkeys (Javanese macaque and baboon

TABLE 1. Characteristics of Examined Animals

Species	Living		Dead		Total
	sick	clinically healthy	from AII	from other diseases	
Rhesus monkeys (*M. mulatta*)	135	36	77	20	268
Javanese macaques (*M. fascicularis*)	61	5	24	10	100
Lapunder macaques (*M. nemestrina*)	4	1	3	0	8
Assamese macaques (*M. assamensis*)	2	0	1	0	3
Hamadryas baboons (*Papio hamadryas*)	7	36	50	15	108
Anubis baboons (*Papio anubis*)	5	0	8	1	14
Green monkeys (*C. aethiops*)	19	0	0	2	21
Other species	2	9	0	0	11
Total	235	87	163	48	533

TABLE 2. Results of Bacteriological Examination of Monkey Feces

Bacteria genus		Live				Dead			
		sick (*n*=235)		clinically healthy (*n*=87)		from AII (*n*=163)		from other diseases (*n*=48)	
		abs.	%	abs.	%	abs.	%	abs.	%
Pathogenic	*Shigella*	0	0	1	1.1	16	9.8	0	0
	Salmonella	0	0	0	0	2	1.2	0	0
	Yersinia	0	0	0	0	2	1.2	0	0
Opportunistic	*Proteus*	13	5.5	24	27.5	29	17.7	12	25
	Citrobacter	8	3.4	7	8	5	3	3	6.25
	Klebsiella	14	5.9	2	2.2	5	3	2	4.1
	Morganella	4	1.7	0	0	4	2.4	2	4.1
	Providencia	1	0.4	1	1.1	12	7.3	1	2
	Enterobacter	3	1.2	1	1.1	3	1.8	0	0
	Edwardsiella	0	0	0	0	1	0.6	0	0
	Pseudomonas	1	0.4	0	0	3	1.8	0	0
Microaerophile	*Campylobacter*	50	21.3	3	3.4	26	16	5	10.4

hamadryad) dead from generalized yersiniosis with typical lesions in the intestinal tract and parenchymatous organs. Cultures of *Y. pseudotuberculosis* were isolated from the liver, spleen, kidney, hyperplastic mesenteric lymph nodes, lungs, and in one case, stomach.

In sick and dead monkeys, the following opportunistic bacteria were most frequently detected: *Providencia spp*. in 0.4 and 7.3%, respectively, *Klebsiella spp*. in 5.9 and 3%, and *Proteus spp*. in 5.5% and 17.7%. The percentage of other conditionally pathogenic enterobacteria did not differ in sick and healthy animals. *Pseudomonas aeruginosa* was detected in four monkeys.

PCR testing revealed *C. jejuni* in all groups with different frequency. An example of *C. jejuni* detection in infected monkeys is shown in Figure 1. Thus, *C. jejuni* was verified in 84 of 533 monkeys (15.7%). Microaerophiles were most frequently found in monkeys with AII (sick and dead, 21.3 and 16%, respectively). In healthy animals, the percentage of carriers was 3.4% (by 6.2 and 5.2 times less frequently than in sick and dead animals, respectively). Interesting results were obtained during examination of animals dead from other pathologies. *C. jejuni* were detected in 5 (10.4%) of 48 monkeys; this can be considered as asymptomatic carriage against the background of pathologies not involving the gastrointestinal tract.

C. jejuni infection was more prevalent in rhesus and Javanese macaques were (23.7%) and less frequent in baboons (8.3%) and green monkeys (5.2%). *C. jejuni* was isolated from 22.4% dead baboons and 12.3% macaques. Animals became ill in the autumn and winter months, in contrast to people who often get sick in the summer. All the age groups of monkeys were subjected to the disease.

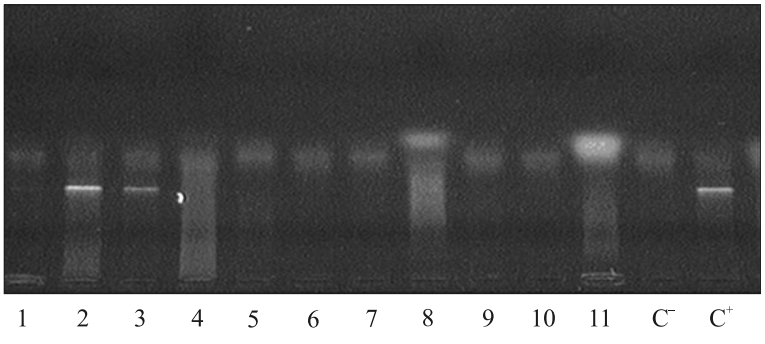

Fig. 1. Electrophoregram of PCR detection of *C. jejuni*. C^-: negative control; C^+: positive control; *2* and *3*: positive samples; *1*, *4-11*: negative samples.

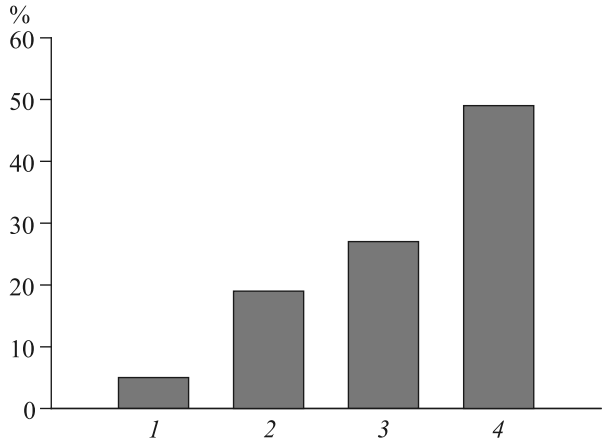

Fig. 2. Etiologic structure of AII in monkeys. *1*) pathogenic enterobacteria, *2*) *C. jejuni*, *3*) opportunistic bacteria, *4*) unknown etiology.

Etiologic structure of enteric bacterial infections is shown in Fig. 2.

Complex examination of sick, dead from intestinal infections, and healthy animals demonstrated circulation of different pathogenic enterobacteria and microaerofiles in various monkey species living in the breeding center. *C. jejuni* is now the leading cause of AII diseases and deaths from gastrointestinal diseases of different morphological forms and *Shigella* ranks second. In rare cases, the disease and death of monkeys can be attributed to *Salmonella* and *Yersinia*. It should be noted that in most cases, campylobacteriosis develops as monoinfection and only in 3 cases *C. jejuni* was isolated simultaneously with pathogenic enterobacteria. Mixed infections were associated with *Shigella* in two cases and with *Y. pseudotuberculosis* in one. In such cases, *Shigella* and *Yersinia* were considered as an etiologic factor. Moreover, intestinal infections were not epidemic. The analyzed cases were sporadic, because only single cases of sickness and deaths at different times and in different objects (cages and open cages) were observed. This was also confirmed by identification of various serological types of *Shigella* and *Salmonella*. Only in a small group of Javanese macaques died from intestinal pathology, *Shigella* was isolated in 3 cases. In sick and dead monkeys, opportunistic enterobacteria were detected in 18.7 and 38% cases, respectively, which practically did not differ from their percentage in the intestinal contents of healthy monkeys. It does not give grounds to assume their participation in the etiology of AII in monkeys, because opportunistic enterobacteria were detected at low dilutions (10^2-10^4). High percent of AII of unknown etiology in monkeys requires the search for new bacterial agents, including cocci and clostridia. Particular attention should be paid to the study of enteroviruses that cause intestinal diarrheal diseases.

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