

Prevalence of Antibodies to Five Selected Zoonosis Agents in Monkeys

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(Received 16 January 1991/Accepted 20 March 1991)

ABSTRACT. The prevalence of antibodies against 5 zoonosis agents was determined in serum samples of 443 breeding monkeys. Of the monkeys, 296 were bred or kept for a long time at R institute, and the remaining 147 were newly imported from the Philippines and kept at S institute for quarantine. Antibodies to simian virus 40 were highly prevalent at 89.1% among monkeys of R institute, whereas no antibody could be detected in those of S institute. Antibodies to *Chlamydia psittaci* and *Yersinia pseudotuberculosis* were detected in 14.4 and 11.6% at R institute, and in 9.0 and 3.5% at S institute, respectively, evidencing a significant difference ($P<0.05$) between those of the two institutes for both agents. Antibodies to *Toxoplasma gondii* and *Leptospira interrogans* were found in 3.6 and 2.9% of the overall, respectively, showing no difference in positive rates in relation to breeding place. Even in cases positive to the latter 4 zoonosis agents, the antibody titers were low. The results obtained suggest that all zoonoses tested do not seem to be so serious diseases among monkeys at the present time except simian virus 40 infection, which is highly prevalent among breeding monkeys in Japan.—**KEY WORDS:** antibody prevalence, epidemiology, monkey, zoonosis.

J. Vet. Med. Sci. 53(4): 553–559, 1991

Many monkeys, bred or imported, have been used as experimental animals in medical and veterinary research and also as zoo animals in Japan. However, no detailed information about the infectious disease prevalent in these monkeys is available. It is necessary to know whether the apparently healthy monkeys run the potential risk of zoonosis in order to protect humans in close contact with them.

At this time, we had an opportunity to collect serum samples from monkeys when they were given their regular medical check-up at R institute, where mainly Japanese monkeys are bred and maintained as experimental animals. In addition, we obtained stock serum samples which were collected from newly imported crab-eating monkeys from the Philippines at S institute while they were quarantined.

The present study was undertaken to determine and compare the prevalence of antibodies to five selected zoonosis agents such as simian virus 40 (SV 40), *Chlamydia psittaci*, *Leptospira interrogans*, *Yersinia pseudotuberculosis* and *Toxoplasma gondii*, among monkeys of the two institutes.

MATERIALS AND METHODS

Monkey sera: Serum samples were collected from 296 monkeys reared and maintained at R institute at the time of their medical check-up, and also from 147 newly imported monkeys from the Philippines while quarantined at S institute. The species of the monkeys were Japanese monkey (*Macaca fuscata fuscata*), rhesus monkey (*M. mulata*), bonnet monkey (*M. radiata*), crab-eating monkey (*M. fascicularis*) and Yaku-Japanese monkey (*M. fuscata yakui*). The sex and age of the monkeys tested are shown in Table 1. In R institute, the majority of the Japanese monkeys were reared as 3 (T, W and A) isolated groups and rhesus monkeys as one isolated group in outdoor open enclosures. Other monkeys including those of S institute were kept in indoor pens or indoor cages. The sera were collected from 1985 to 1987 and kept at -20°C until use.

Serological survey: The serological survey was performed for evidence of antibodies to the following 5 pathogens.

Antibodies to SV 40 were detected by the immune adherence hemagglutination (IAHA) test as previously described [15]. Antibodies to *Chlamydia psittaci* were detected by the complement fixation (CF) test with a commercially available antigen (Denka-Seiken Co., Tokyo), and a titer of 1:10 or

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Table 1. Species, sex and age of the monkeys tested^{a)}

Breeding place	Species (common name)	Number of monkeys	Sex		Age (year)		
			male	female	0-2	3-4	≥5
R institute	Japanese monkey (m.)	231	90	141	65	39	127
	Rhesus m.	54	19	35	11	12	31
	Bonnet m.	5	4	1	0	1	4
	Crab-eating m.	3	1	2	0	1	2
	Yaku-Japanese m.	3	1	2	0	0	3
	Total	296	115	181	76	53	167
S institute	Crab-eating m.	147 ^{b)}	74	73	0	71	68
Grand total		443 ^{b)}	189	254	76	124	235

a) These data were kindly provided by staffs of the two institutes.

b) Include 8 monkeys whose ages are unknown.

more was referred to as positive. Antibodies to *Leptospira interrogans* serovars *icterohaemorrhagiae*, *autumnalis*, *hebdomadis*, *australis* and *canicola*, were examined by the latex agglutination test with commercially available antigens (Denka-Seiken Co., Tokyo). For screening test at the serum dilution of 1:10, a modified microplate method, whose specificity was confirmed beforehand was applied [18]. As to the sera positive to any of the five serovars in the screening test, further tests to determine corresponding serovars were performed by the manufacturer's indicated test procedure using the tube agglutination test. Titers of 1:10 or higher were taken as positive. Antibodies to *Yersinia pseudotuberculosis* were detected by the CF test using microtiter technique. Polyvalent antigen used was prepared by mixing an equal volume of supernatant fluids of sonicated bacterial suspensions of each serotype (1a, 1b, 2a, 2b, 3, 4a, 4b, 5a, 5b and 6) of *Y. pseudotuberculosis*. Titers of 1:10 or more were taken as positive. Antibodies to *Toxoplasma gondii* were detected by the latex agglutination test with a commercially available antigen (Eiken Chemical Co., Tokyo). Titers higher than 1:10 were referred to as positive.

Statistical analysis: Differences in the serological test results based upon origin, sex and age of the monkeys were analyzed with the chi-square test.

RESULTS

Prevalence of antibodies to SV 40: Of a total of 440 monkeys tested, 262 (59.5%) were seropositive to SV 40, and the geometric mean (GM) antibody titer was 1:35 (Table 2). All 262 seropositive monkeys were detected from the 294 monkeys maintained at

R institute (positive rate; 89.1%), whereas no seropositive monkey was detected at S institute where only 146 imported crab-eating monkeys were kept. Of the 262 positive monkeys, 26 (9.9%) were found to have high antibody titers of 1:640 or more.

Next, we analyzed the antibody prevalence to SV 40 in relation to sex and age in all 229 Japanese monkeys kept at R institute. As shown in Table 3, the positive rate and GM titer of baby monkeys under 1 year of age were significantly lower ($P < 0.01$) than those of any older age group. And in terms of GM antibody titers, that of 1-2 years old monkeys was somewhat higher than that of other older age groups. No sex difference in antibody prevalence was observed.

Prevalence of antibodies to *Chlamydia psittaci*: As shown in Table 4, 55 (12.6%) of the 436 monkeys were seropositive, but in general, antibody titers were low. The positive rate of Japanese monkeys (17.2%) was significantly ($P < 0.05$) higher than that of any other species.

We then analyzed antibody prevalence of Japanese monkeys in relation to 3 separate breeding groups, T, W and A. As indicated in Table 5, there were significant differences ($P < 0.05$) among positive rates of the 3 groups. But there was no difference in antibody prevalence by sex and age (data not shown).

Prevalence of antibodies to *Leptospira interrogans*: Table 6 shows the prevalence of antibodies to the 5 serovars of *L. interrogans*. Thirteen (2.9%) of the 443 monkeys tested were positive for at least one of the 5 serovars. Ten of them reacted to more than one serovar. There was no difference between positive rates of the two institutes. Of the 13 positive sera, reactors to *icterohaemorrhagiae* (8 sera) were

Table 2. Prevalence of antibodies^{a)} to SV 40 in monkeys

Breeding place	Species	Number of monkeys		Positive rate	Number of monkeys with antibody titers											G.M. titer ^{c)}
		tested	positive ^{b)}		<10	10	20	40	80	160	320	640	1280	≥2560		
R institute	Japanese monkey	229	205	89.5	23	1	8	23	52	61	44	11	5	1	100	
	Rhesus m.	54	49	90.7	3	2	7	8	11	9	7	7	0	0	84	
	Bonnet m.	5	4	80.0	0	1	2	0	0	0	0	2	0	0	70	
	Yaku-Japanese m.	3	3	100	0	0	0	0	0	2	1	0	0	0	200	
	Crab-eating m.	3	1	33.3	2	0	0	1	0	0	0	0	0	0	10	
	Total	294	262	89.1	28	4	17	32	63	72	52	20	5	1	92	
S institute	Crab-eating m.	146	0	0	146	0	0	0	0	0	0	0	0	0	<10	
Grand total		440	262	59.5	174	4	17	32	63	72	52	20	5	1	35	

a) Detected by IAHA test.

b) Titers of 1: 20 or more are taken as positive.

c) Geometric mean titer.

Table 3. Prevalence of IAHA antibodies to SV 40 in Japanese monkeys in R institute by sex and age

Age	Male				Female				Total			
	Number of monkeys		Positive	G.M.	Number of monkeys		Positive	G.M.	Number of monkeys		Positive	G.M.
	tested	positive	rate	titer	tested	positive	rate	titer	tested	positive	rate	titer
<1	10	2	20.0	10	9	2	22.2	<10	19	4	21.1	<10
1-2	19	16	84.2	114	26	25	95.2	249	45	41	91.1	179
3-4	15	14	93.3	100	24	21	87.5	141	39	35	89.7	124
≥5	45	45	100	96	81	79	97.5	117	126	124	98.4	109
Total	89	77	86.5	78	140	127	90.7	116	229	204	89.1	100

Table 4. Prevalence of antibodies^{a)} to *Chlamydia psittaci* in monkeys

Breeding place	Species	Number of monkeys		Positive rate	Number of monkeys with antibody titers			
		tested	positive ^{b)}		<10	10	20	40
R institute	Japanese monkey	227	39	17.2	188	36	3	0
	Rhesus m.	54	3	5.6	51	2	1	0
	Bonnet m.	5	0	0	5	0	0	0
	Yaku-Japanese m.	3	0	0	3	0	0	0
	Crab-eating m.	3	0	0	3	0	0	0
	Total	292	42	14.4	250	38	4	0
S institute	Crab-eating m.	144	13	9.0	131	10	3	0
Grand total		436	55	12.6	381	48	7	0

a) Detected by CF test using commercially available antigen.

b) Titers of 1: 10 or more are taken as positive.

the most common, followed by those of *hebdomadis* (6), *autumnalis* (5) and *canicola* (4), but none to *australis*. There was no difference in antibody prevalence to *L. interrogans* by sex and age.

Prevalence of antibodies to Yersinia pseudotuberculosis: Totally, 39 (8.9%) of the 438 monkeys

tested were positive, and the positive rate of the monkeys of R institute (11.6%) was significantly ($P<0.05$) higher than that of S institute (3.5%) (Table 7). Even in positive cases, their antibody titers were usually low, ranging from 1:10 to 1:20. No sex or age difference in antibody prevalence was

Table 5. Comparison of antibody prevalence to *Chlamydia psittaci* among 3 separate breeding groups of Japanese monkeys

Breeding group	Number of monkeys		Positive rate
	tested	positive	
T	109	23	21.1
W	40	0	0
A	36	11	30.6
Total	185	34	18.4

Table 6. Prevalence of antibodies^{a)} to *Leptospira interrogans* in monkeys

Breeding place	Species	Number of monkeys		Positive rate	Number of positive monkeys with the highest titer to serovars ^{c)}				
		tested	positive ^{b)}		ict.	aut.	heb.	aus.	can.
R institute	Japanese monkey	231	7	3.0	6	3	2	0	3
	Rhesus m.	54	1	1.9	1	0	0	0	0
	Bonnet m.	5	2	40.0	1	1	1	0	0
	Yaku-Japanese m.	3	0	0	0	0	0	0	0
	Crab-eating m.	3	0	0	0	0	0	0	0
	Total	296	10	3.4	8	4	3	0	0
S institute	Crab-eating m.	147	3	2.0	0	1	3	0	1
Grand total		443	13	2.9	8	5	6	0	4

a) Detected by latex agglutination test using commercially available antigens.

b) Titers of 1:10 or more are taken as positive and 10 out of 13 positive sera reacted with 2 or more serovars.

c) ict.: icterohaemorrhagiae, aut.: autumnalis, heb.: hebdomadis, aus.: australis, can.: canicola.

Table 7. Prevalence of antibodies^{a)} to *Yersinia pseudotuberculosis* in monkeys

Breeding place	Species	Number of monkeys		Positive rate	Number of monkeys with antibody titers			
		tested	positive ^{b)}		<10	10	20	40
R institute	Japanese monkey	229	24	10.6	203	20	4	0
	Rhesus m.	54	9	16.7	45	9	0	0
	Bonnet m.	5	0	0	5	0	0	0
	Yaku-Japanese m.	3	0	0	3	0	0	0
	Crab-eating m.	3	1	33.3	2	1	0	0
	Total	294	34	11.6	258	30	4	0
S institute	Crab-eating m.	144	5	3.5	139	5	0	0
Grand total		438	39	8.9	397	35	4	0

a) Detected by CF test using polyvalent antigen.

b) Titers of 1:10 or more are taken as positive.

observed (data not shown).

Prevalence of antibodies to Toxoplasma gondii: Antibody prevalence to *T. gondii* is shown in Table 8. Sixteen (3.6%) of the 443 tested were seropositive. Even in positive cases, antibody titers were lower than 1:160. There was no difference in

positive rates in relation to breeding place, species, sex and age of the monkeys.

DISCUSSION

In this study, we selected 5 microbial pathogens

Table 8. Prevalence of antibodies^{a)} to *Toxoplasma gondii* in monkeys

Breeding place	Species	Number of monkeys		Positive rate	Number of monkeys with antibody titers					
		tested	positive ^{b)}		<10	10	20	40	80	160
R institute	Japanese monkey	231	8	3.5	223	3	3	0	2	0
	Rhesus m.	54	2	3.7	52	1	1	0	0	0
	Bonnet m.	5	0	0	5	0	0	0	0	0
	Yaku-Japanese m.	3	2	66.7	1	2	0	0	0	0
	Crab-eating m.	3	0	0	3	0	0	0	0	0
	Total	296	12	4.1	284	6	4	0	2	0
S institute	Crab-eating m.	147	4	2.7	143	2	2	0	0	0
Grand total		443	16	3.6	427	8	6	0	2	0

a) Detected by latex agglutination test using commercially available antigen.

b) Titers of 1:10 or more are taken as positive.

for antibody prevalence, in which it was previously demonstrated by the present authors that the antibody to SV 40 was highly prevalent among breeding monkeys in Japan but not among monkeys imported from Southeast Asia [15]. Therefore, it was of interest to know whether or not the similar results can be obtained because the serum samples used in the present study were collected after the previous study. Four other pathogens tested were known as popular zoonosis agents in Japan, and we have been investigating serologically the prevalence of these agents among wild and experimental animals [2, 18]. But little is known about it among monkeys in Japan.

From the standpoint of the recent popularity of monkeys as zoo and experimental animals, and the use of their tissues for in vitro culture, it seemed worthwhile to clarify antibody prevalence to these zoonosis agents in monkeys.

As to SV 40 antibody, similar results with those previously reported were obtained [15]. Thus, a high antibody prevalence (89.1%) to SV 40 was obtained in monkeys bred and maintained in Japan, whereas no antibody was detected in monkeys newly imported from the Philippines. We also demonstrated in this study that both the seropositive rate (89.1%) and GM antibody titer (1:100) of Japanese monkeys kept at R institute were higher than those (80.4% and 1:50) of the previous report [15]. The data seem to indicate that the SV 40 is gradually spreading among Japanese monkeys. SV 40 has not only been suspected as a causative agent for human malignancy but also for progressive multifocal leucoencephalopathy [11], and Shah [23] reported a relatively high prevalence of SV 40 neutralizing antibodies in humans in contact with rhesus monkeys. Hence,

further study is needed to clarify SV 40 epidemiology among monkeys including wild Japanese monkeys.

Chlamydia psittaci is known as an agent of zoonosis infected from mainly birds. Recently, Hirai and colleagues demonstrated serologically and etiologically that the agent was widely distributed among many kinds of birds and mammals in Japan [8, 9, 13]. They reported a CF antibody positive rate of 30.2% in 1,048 bovine sera [9] and 37.1% in 568 feral pigeon sera [13] collected from the same areas where R institute is located. We also demonstrated chlamydial antibody in 10.7% of 335 wild Japanese serows captured in mountain areas and in 10.8% of 223 wild rats in urban areas, both not so far from R institute [2, 18]. In the present study, a total 12.6% of the monkeys were found to have chlamydial antibody, which was comparable with the case for wild serows and rats. The aforementioned and our own data together suggest that the chlamydial reservoirs were widely distributed and continuously maintained in these areas. However, it is interesting that if the data are limited to Japanese monkeys kept in 3 separate open enclosures in R institute, the positive rates of 3 groups are quite different at 0, 21.1 and 28.9%, respectively. Though confirmation is necessary, chlamydial infection in monkeys is more likely reflects direct or indirect contact with reservoir birds around enclosures. To our knowledge, no information about the incidence of the disease among monkeys is available. Because monkeys seem to be susceptible to chlamydial infection like humans, further study is needed to clarify its epidemiology.

Leptospirosis is widespread among many animal species in Japan [27]. Although all serovars are

potential pathogens for all species of domestic animals [25], in this experiment, we examined the antibodies against only 5 major serovars; *icterohaemorrhagiae*, *autumnalis*, *hebdomadis*, *australis* and *canicola*, which are known to be the main causative serovars of human leptospirosis in Japan [24]. Thirteen (2.9%) of 443 sera reacted with any one of the 5 antigens tested but even in positive cases, antibody titers were low at 1:20 or less. Though an attempt was made to determine the corresponding serovars, many of these sera had similar titers to more than one serovar. Of them, 8 reacted to serovar *icterohaemorrhagiae*, 6 to *hebdomadis*, 5 to *autumnalis* and 4 to *canicola*, in descending order, but none to *autumnalis*. The difficulty of determining the infecting leptospiral serovar by serologic means has been indicated by other workers [3, 6], but it should be clarified by further cross-agglutinin-absorption-agglutination tests between cross-reacted sera [25]. Though no report is available about the prevalence of leptospirosis in monkeys in Japan, there have been several reports of natural or experimental infection of monkeys with leptospira in the other countries [3, 19, 20]. These data seem to indicate that monkeys were either naturally resistant or infrequently exposed to leptospira in their natural habitat, but might acquire such infections during captivity, particularly when exposed to the microorganisms prevalent among rodents. As to serological surveys, Minette [19] showed agglutinins in 107 (7.9%) of 1,346 sera from Old World and one (1.4%) of 74 from New World monkeys. Baulu *et al.* [3] however, noted a relatively high antibody prevalence at 28.5% (184/646) among wild-caught vervet monkeys. The low antibody prevalence obtained in the present study would indicate that the breeding places are free from leptospira infected rodents or contaminated inanimate vehicles such as soil and water.

Y. pseudotuberculosis is well known to affect a wide variety of avian and mammalian species, including man. In Japan, since 1970, human infection due to this organism has been increasing, and surveys of this organism from animals and environmental sources have been made by many investigators [2, 10, 17, 26]. However, little is known about the prevalence of the disease among monkeys in Japan. Hirai *et al.* [14] reported the loss of 41 patas monkeys, showing watery or hemorrhagic diarrhea, over an 8-year period (1964–1972) in the Japan

Monkey Center located near R institute, and they isolated *Y. pseudotuberculosis* from dead animals and dropping feces collected from the ground within the enclosure where the patas monkeys were kept. In contrast, several outbreaks of monkey pseudotuberculosis associated with severe clinical signs were reported in other countries [4, 22]. As to antibody prevalence, Fribourg-Blanc *et al.* [7] found that 45% of 150 cynomolgous monkeys had agglutinins to this organism. Currie *et al.* [5] also reported that 21.5% of 79 primates had hemagglutinating antibodies against polyvalent antigen of serotypes 1 through 6 of *Y. pseudotuberculosis*. In the present study, antibody was detected by the CF test using polyvalent antigen prepared by mixing each serotype (1 through 6) of *Y. pseudotuberculosis* antigen. In the preliminary experiments, the CF test used in this study was considered to be useful screening test for the detection of pseudotuberculosis antibody (data not shown). Totally, 8.9% (39/438) of the monkeys had been shown to have the antibody, in which 11.6% of the monkeys kept at R institute was significantly ($P < 0.05$) higher than 3.5% of those of S institute. But to which serotypes of the antigen the positive sera reacted remained unsolved. Even in positive sera, antibody titers were usually low at 1:20 or less. Judging from our own results and those of others, the disease due to *Y. pseudotuberculosis* does not seem so prevalent among monkeys in these two institutes at the present time.

Finally, toxoplasmosis is a widespread disease in man, other mammals, and birds throughout the world. Monkeys, especially New World monkeys, are also susceptible to infection of this organism [1, 12]. Naturally occurring toxoplasmosis in monkeys has been reported sporadically. In Japan, two spontaneous cases of toxoplasmosis which occurred in ring-tailed monkeys in the Japan Monkey Center were firstly reported by Itakura and Nigi [16]. However, limited information is available with respect to prevalence of toxoplasma antibodies among monkeys. Rao Bhau *et al.* [21] reported that out of 211 rhesus monkeys which were captured from the Himalayan region in India, 111 (52.6%) had a toxoplasma antibody detected by the IHA test. The present study showed a relatively low toxoplasma antibody prevalence (3.6%) among monkeys without any difference between those of the two institutes. Though 2 out of 3 Yaku-Japanese monkeys tested were found to have toxoplasma antibody, the titer was very low.

As presented here, we checked the antibodies to the 5 pathogens, and demonstrated relatively low antibody prevalences to them except to SV 40. The data suggested that the zoonoses caused by at least these 4 pathogens were not serious diseases among monkeys at the present time. However, even in such situation, the monkeys bred and maintained in Japan have more serological evidence of infection with SV 40, *C. psittaci* and *Y. pseudotuberculosis* than the newly imported monkeys. This may be due to the fact that the natural habitat of the crab-eating monkeys in the Philippines may be free from these diseases, because the majority of the imported monkeys were sent to Japan soon after capture.

On the other hand, there are other popular zoonoses such as tuberculosis, salmonellosis and shigellosis among monkeys, thus it needs more experiments to clarify the situation of zoonosis prevalent in monkeys. The authors are now pursuing further studies in this regard. In any case, monkeys are handled or maintained as experimental or zoo animals in Japan, they should always be screened for these and other diseases to guarantee safety.

ACKNOWLEDGEMENTS. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 01480104) from the Ministry of Education, Science and Culture of Japan, and by the Cooperative Research Service of the Primate Research Institute, Kyoto University.

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