大韓獸醫學會誌(2010) 第50卷 第4號
Korean J Vet Res(2010) 50(4) : 273~277

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Investigation of helminths and protozoans infecting old world monkeys: captive vervet, cynomolgus, and rhesus monkeys

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(Accepted: November 30, 2010)

Abstract : The objective of this study was to investigate the infection rate of gastro-intestinal tract parasites on acquired laboratory nonhuman primates, Vervet monkey, Cynomolgus monkey, and Rhesus monkey acquired from Japan and China. These monkeys have been acclimating at an individual housing condition after our legal quarantine period. We examined 133 fecal samples to investigate parasitic infection using direct smear and formalin-ether-sedimentation technique. As a result, total parasitic infection rate was 33.8% (n = 45/133) for all monkeys. Two species of macaques, cynomolgus and rhesus, were infected with *Trichuris trichiura (4), Giardia lamblia (4)* and *Balantidium coli (41).* Vervet monkeys, which had been controlled by individual housing system for a long time, were clear for parasitic infection. The protozoan, *Balantidium coli* was one of the most frequently detected in these monkey colonies. Double infection was noted in only 4 monkeys and involved with *Trichuris trichiura* and *Balantidium coli*. Serious clinical symptoms were not observed in the most of the infected monkeys, but the monkeys infected by *Giardia lamblia* showed intermittent or chronic watery diarrhea. Consequently, the prophylactic anthelmintic treatment and periodic monitoring are essential to preserve the SPF colonies in the laboratory facility.

Keywords : *Balantidium coli*, *Giardia lamblia*, nonhuman primate, *Trichuris trichiura*

Introduction

Many studies of parasites have investigated wild or captive and New World or Old World nonhuman primates [3-5, 8, 12, 17]. Actually, both helminth and protozoal parasites are common, as indicated by several surveys of nonhuman primates imported for research purposes [6, 9, 11, 14, 20]. Parasitic infections may affect animal health and ultimately the studies for which these animals are used. Moreover, many of these parasites are zoonotic pathogens and therefore pose a risk to animal personnel [13]. Numerous protozoan and metazoan genera have been described as infecting the members of all major nonhuman primate groups [5, 9, 19]. Some of these are considered to be nonpathogenic, or at least their detrimental effects on the host have yet to be elucidated [19]. A large number, however, can result in physiologic disturbances, nutritional loss, or may produce lesions that result in serious debilitation, and can create opportunities for secondary infections that may be fatal [19]. Transmission of microorganism can occur due to humans and nonhuman primates sharing the same ecosystems, which may lead to sporadic outbreaks of zoonosis. Therefore, the control of the parasitic infection is very important in the nonhuman primate laboratory facilities, especially, used the animals in the preclinical research which could suppress the immunity. Previous studies have been reported about captive or wild nonhuman primates, however, investigation for the laboratory nonhuman primates have rarely been reported.

In this study, we investigated infection rate of gastrointestinal parasites in the 3 Old World monkeys for research acquired from Japan and China. We also

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identified the clinical symptoms that occur by gastrointestinal tract parasitic infection.

Materials and Methods

Animals

We investigated three colonies of nonhuman primates which imported from Japan and China. Vervet monkeys acquired from Japanese laboratory facility rearing for several years, and cynomolgus and rhesus monkeys were acquired from Chinese laboratory animal farm. Cynomolgus and rhesus monkeys were housed in combination facility which provided indoor and outdoor area, while vervet monkeys were only housed in individual cage. In this study, total numbers of 133 monkeys including 81 rhesus monkeys, 24 vervet monkeys, and 28 cynomolgus monkeys were investigated. The average age was 3 years 9 months in rhesus monkeys, 13 years 6 months in vervet monkeys, and 4 years 6 months in cynomolgus monkeys. Sex ratio of total population was 36 males and 97 females. The animals were housed in individual home cages and had daily access to food (PS DIET; Oriental Yeast, Japan and fresh fruits) and unlimited access to water. The animals were housed in a room maintained at 24 ± 4°C and a relative humidity of 50 ± 10%, with artificial lighting from 12 : 12 light-dark cycle (7:00 AM onset) and with 13-18 air change per h. All animals were reared in strict accordance with the National Institutes of Health "*Guide for the Care and Use of Laboratory Animals*".

Procedure

Formalin-ether-sedimentation technique and direct smear for determining the presence of parasite eggs, cysts and larval stages were used in this study. The date, age, sex and species of the monkey were labeled on the collecting tubes. At first 1 to 5 g of fresh feces was collected. If samples were more than 2 h old, samples were stored at 4°C until examined. It was mixed 1 g of feces in 15 mL of water and then strained the mixture, and poured into a 15 mL centrifuge tube. The samples were centrifuged at 1,000 rpm for 1-2 min and decanted the supernatant and added fresh water, then centrifuged again for 1-2 min. The supernatant was decanted and added 10 mL of 10% formalin, then let stand for 10 min. Added 3 mL of ether in the tube, applied a stopper and then shook the contents vigorously. It was centrifuged the mixture for 2 min and removed the debris on top of the tube with a cotton-tipped swab. Lastly, it decanted the rest of the fluid and collected the sediment with a pipette, placed it on a microscope slide, and examined it microscopically for parasite eggs. The direct smear was also performed by common method.

Data analysis

The presence of any parasite in any of the samples examined was noted and the number of samples found to contain a particular species of parasite was then expressed as a percentage of the total number of samples examined. In addition, the distribution of species of parasites in the both sex of our laboratory monkeys was noted.

Results

The fecal examination was conducted on all monkeys of the 3 species. As a result, *Trichuris trichiura, Giardia lamblia* and *Balantidium coli* were detected in 2 species of macaques (cynomolgus and rhesus monkeys). *Balantidium coli* was the most frequent parasite followed by *Trichuris trichiura* and *Giardia lamblia*. The population of total parasitic infection was 45 of 133 monkeys (33.8%). Although rhesus monkey colony was infected by more species of parasites, higher prevalence of parasitic infection was shown in cynomolgus monkeys (50%, n = 14/28) than in rhesus monkeys (38.3%, n = 31/81). The prevalence of parasitic infection of male was relatively high in 50% (n = 18/36). Double infection of parasites was observed in 4 macaques (1 male and 3 females) and identified with *Trichuris trichiura* and *Balantidium coli* (Table 1).

In microscopes, the *Balantidium coli* of both cysts and trophozoites with sausage shaped macronucleus were identified and the size documented approximate 40-60 μm. Trophozoites of balantidium revealed active movement by ciliate in direct smear field, and the shape of oval or round type as circumstance revealed occasionally. The egg size of *Trichuris trichiura* (unembryonated stage) was approximate 50-60 μm and found in only 4 monkeys co-infected with *Balantidium coli*. In the fecal samples infected with *Giardia lamblia*, a huge number of spirochaetes were observed and showed a very fast twisting motion. *Giardia lamblia* also revealed very fast and active swimming

Table 1. Summary for the gastrointestinal parasites found in the fecal samples of Vervet, Cynomolgus, and Rhesus monkeys

Items		Vervet monkey	Cynomolgus monkey	Rhesus monkey	Total
Subjects (male/female)		24 (4/20)	28 (5/23)	81 (27/54)	133 (36/97)
Age range		5y 8m - 18y 4m	3y 8m - 6y 3m	3y 5m - 4y 10m	–
Detected parasites		None	*Trichuris trichiura* (2) *Balantidium coli* (14)	*Trichuris trichiura* (2) *Balantidium coli* (27) *Giardia lamblia* (4)	*Trichuris trichiura* (4) *Balantidium coli* (41) *Giardia lamblia* (4)
Infection rate		0.0% (0/24)	50.0% (14/28)	38.3% (31/81)	33.8% (45/133)
Sex	Male	0.0% (0/4)	100.0% (5/5)	48.1% (13/27)	50.0% (18/36)
	Female	0.0% (0/20)	39.1% (9/23)	33.3% (18/54)	27.8% (27/97)
Double infection rate		None	14.3% (2/14)	6.5% (2/31)	8.9% (4/45)

y: Years, m: Months.

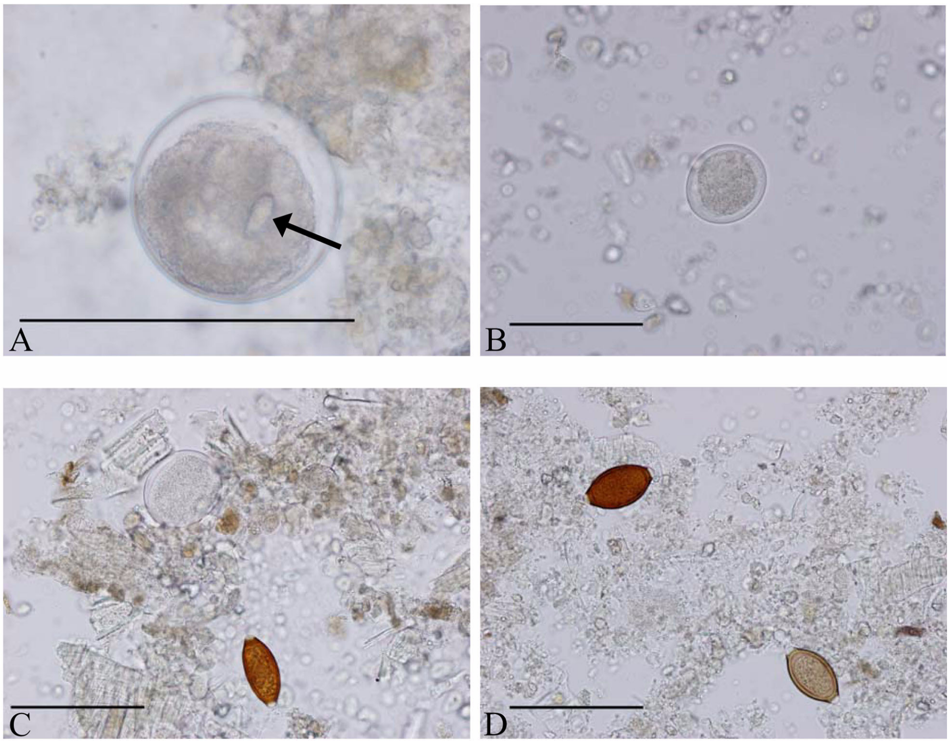

Fig. 1. Eggs, fecal (flotation from Rhesus) monkeys (A, B) and Cynomolgus monkeys (C, D). (A) *Balantidium coli* with sausage shaped (macronucleus (arrow)) was identified. Bar = 100 µm, ×400. (B) The size of cyst (*Balantidium coli)* was 62.40 × 55.63 µm. Bar = 100 µm, ×200. (C) *Trichuris trichiura* of approximate 50-60 µm and *Balantidium coli*. Bar = 100 µm, ×200. (D) The size of egg was 56.39 × 30.82 µm. Bar = 100 µm, ×200.

trophozoite by flagella (Fig. 1).

Clinical disorders by parasitic infection were rarely observed in other monkeys, except for some rhesus monkeys infected by *Giardia*. The monkeys infected with *Giardia* revealed persistent severe watery diarrhea which emitted a foul odor and brown color. In direct smear, a large quantity of spirochetes which have long, helically coiled shape was accompanied by *Giardia*. One of them gradually showed symptom of severe abdominal distension and weight loss. Thus, the monkey was prescribed a suitable medical treatment as metronidazole (50 mg/kg, bid, PO) until completely cured.

Discussion

There has been increasing concern about the gastro-intestinal tract parasites in nonhuman primates probably because of pathogenic conditions like cryptosporidiosis and tuberculosis, which are transmissible to humans [10, 15]. In addition, such gastro-intestinal tract parasites have considerable implications on conservation and management of nonhuman primates [1]. In this study, we found the existence of 1 helminth and 2 protozoans, parasites which are known to be pathogenic to primates. According to some reports, epidemics of colonic balantidiasis in tropical countries have been reported, and it may be a primary pathogen causing typhlitis in great apes [7]. Eggs of *Trichuris* spp. have been experimentally transmitted from nonhuman primates to humans [8], and provide evidence for the zoonotic potential of nonhuman primates. *Giardia* spp. has also been reported to cause diarrhea in monkeys [19].

It is important to document increased parasite burdens, but as these organisms are rarely primary pathogens and their incidence varies considerably among animals [16]. For instance, *Balantidium coli* is frequently observed in the lumen of the macaque cecum and colon, but despite differences in incidence and parasitic load among cynomolgus macaques from Mauritis, Vietnam, and the Philippines, *Balantidium* is not associated with mucosal alteration [2]. In another survey from a domestic rhesus monkeys colony, three parasites detected in this study were not significantly associated with diarrhea [18]. In other words, *Balantidium* and *Trichuris* may exist as normal flora in nonhuman primates unless there is a breach in the mucosal barrier. But in this study, only *Giardia* caused persistent diarrhea symptom in four rhesus monkeys, and one of them showed severe clinical problem as abdominal distension and weight loss. The monkey may have a mucosal barrier problem by inflammation or immune compromise.

Unlike the 2 macaque colonies, the vervet monkey colony there was no parasitic infection noted. Actually, this colony had been raised in a laboratory facility where managed with an individual housing system in Japan. While rhesus and cynomolgus colonies were raised in outdoor field cage with shelter, group housing system in China. In previous report, vervet monkeys were infected with various parasites, however that was document of monkeys bred in a semi-breeding colony [9]. It means that outdoor housing condition can more elevate contact and ingestion possibility of parasite ova against other husbandry system. In other words, individual housing system rather than group housing system is likely to minimize the opportunity of parasitic infection. However, it is considerable that transmission of parasites between neighboring monkeys can also occur by direct contact of long tail or hand through the mesh of cage even though it is individual housing system.

Conclusion

The conservation of high quality of laboratory nonhuman primate, colony managements including housing, sanitation and veterinary interventions such as regular screening and deworming programs are essential. And also the result of this study could provide useful reference for parasitic infection status of laboratory nonhuman primates which play an indispensable role in biomedical research.

Acknowledgments

This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Korea (Project No.: A040004).

References

1. **Collet JY, Galdikas BM, Sugarjito J, Jojosudharmo S.** A Coprological study of parasitism in orangutans (Pongo pygmaeus) in Indonesia. J Med Primatol 1986, **15**, 121-129.
2. **Drevon-Gaillot E, Perron-Lepage MF, Clément C, Burnett R.** A review of background findings in cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins. Exp Toxicol Pathol 2006, **58**, 77-88.
3. **Gracenea M, Gómez MS, Torres J, Carné E, Fernández-Morán J.** Transmission dynamics of Cryptosporidium in primates and herbivores at the Barcelona zoo: a long-term study. Vet Parasitol 2002, **104**, 19-26.
4. **Hope K, Goldsmith ML, Graczyk T.** Parasitic health of olive baboons in Bwindi Impenetrable National Park, Uganda. Vet Parasitol 2004, **122**, 165-170.

5. **Karere GM, Munene E.** Some gastro-intestinal tract parasites in wild De Brazza's monkeys (Cercopithecus neglectus) in Kenya. Vet Parasitol 2002, **110**, 153-157.
6. **Karr SL Jr, Henrickson RV, Else JG.** A survey for intestinal helminths in recently wild-caught Macaca mulatta and results of treatment with mebendazole and thiabendazole. J Med Primatol 1980, **9**, 200-204.
7. **Lee RV, Prowten AW, Anthone S, Satchidanand SK, Fisher JE, Anthone R.** Typhlitis due to Balantidium coli in captive lowland gorillas. Rev Infect Dis 1990, **12**, 1052-1059.
8. **Monteiro RV, Dietz JM, Beck BB, Baker AJ, Martins A, Jansen AM.** Prevalence and intensity of intestinal helminths found in free-ranging golden lion tamarins (Leontopithecus rosalia, Primates, Callitrichidae) from Brazilian Atlantic forest. Vet Parasitol 2007, **145**, 77-85.
9. **Munene E, Otsyula M, Mbaabu DA, Mutahi WT, Muriuki SM, Muchemi GM.** Helminth and protozoan gastrointestinal tract parasites in captive and wild-trapped African non-human primates. Vet Parasitol 1998, **78**, 195-201.
10. **Muriuki SM, Farah IO, Kagwiria RM, Chai DC, Njamunge G, Suleman M, Olobo JO.** The presence of cryptosporidium oocysts in stools of clinically diarrhoeic and normal nonhuman primates in Kenya. Vet Parasitol 1997, **72**, 141-147.
11. **Muriuki SMK, Murugu RK, Munene E, Karere GM, Chai DC.** Some gastro-intestinal parasites of zoonotic (public health) importance commonly observed in old world non-human primates in Kenya. Acta Trop 1998, **71**, 73-82.
12. **Phillippi KM, Clarke MR.** Survey of parasites of rhesus monkeys housed in small social groups. Am J Primatol1992, **27**, 293-302.
13. **Purcell JE, Philipp MT.** Parasitic diseases of nonhuman primates. In: Sonia WC (ed.). The Laboratory Primate. pp. 579-593, Elsevier Academic, Amsterdam, 2005.
14. **Sano M, Kino H, de Guzman TS, Ishii AI, Kino J, Tanaka T, Tsuruta M.** Studies on the examination of imported laboratory monkey, Macaca fascicularis for E. histolytica and other intestinal parasites. Int J Zoonoses 1980, **7**, 34-39.
15. **Sapolsky RM, Else JG.** Bovine tuberculosis in a wild baboon population: epidemiological aspects. J Med Primatol 1987, **16**, 229-235.
16. **Sasseville VG, Diters RW.** Impact of infections and normal flora in nonhuman primates on drug development. ILAR J 2008, **49**, 179-190.
17. **Sato H, Une Y, Takada M.** High incidence of the gullet worm, Gongylonema pulchrum, in a squirrel monkey colony in a zoological garden in Japan. Vet Parasitol 2005, **127**, 131-137.
18. **Sestak K, Merritt CK, Borda J, Saylor E, Schwamberger SR, Cogswell F, Didier ES, Didier PJ, Plauche G, Bohm RP, Aye PP, Alexa P, Ward RL, Lackner AA.** Infectious agent and immune response characteristics of chronic enterocolitis in captive rhesus macaques. Infect Immun 2003, **71**, 4079-4086.
19. **Toft JD 2nd, Eberhard ML.** Parasitic diseaases. In: Bennett BT, Abee CR, Henrickson R (eds.). Nonhuman Primates in Biomedical Research Diseases. pp. 111-114, Academic Press, San Diego, 1998.
20. **Wong MM, Conrad HD.** Prevalence of metazoan parasite infections in five species of Asian macaques. Lab Anim Sci 1978, **28**, 412-416.