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

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Abstract: The objective of this study was to investigate the infection rate of gastrointestinal 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 anthelminitic 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
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