The risk of helminth infections at Endangered Primate Rescue Center, Cuc Phuong National Park Vietnam

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Abstract. To clarify the transmission route of parasitic infections in primates at Endangered Primate Rescue Center, Cuc Phuong National Park, we investigated the prevalence of intestinal helminths in leaf and dog fecal samples collected in the surrounding areas of the center. By morphological analysis, only Ancylostoma spp. and Isospora spp. were detected in leaf samples, with the infection rate of 7.07% and 1.01% respectively. In addition, survey on 156 dog feces samples showed that the overall prevalence of gastrointestinal parasites was quite high (73.55%). Ancylostoma spp has the highest prevalence (68.39%), followed by Toxocara spp. (25.81%), Trichuris spp. (10.97%), Isospora spp. (7.1%) and Taenia spp (3.87%).

1. Introduction
The Endangered Primate Rescue Center (EPRC) is located in northern Vietnam and housing more than 150 individuals of 15 primate taxa, of which there are 7 species of leaf-eating monkeys (langurs). The center provides sanctuary and rehabilitation to primates of the Genus Nomascus, Nycticebus, Trachypithecus and Pygathrix with their IUCN Red List status ranging from ‘Endangered’ to ‘Critically Endangered’. The medical problems associated these primates have included: parasites, enteritis (gastrointestinal inflammation), pneumonia, and occasional trauma. They are known to susceptible to many gastrointestinal parasites that affect their survival and reproductive activity by causing gastro-enteritis, watery diarrhea, haemorrhage, dysentery and death [1]. Considering the growing contact between people and non-human primates, understanding parasite dynamics and transmission route is important to wild species conservation, and implementation of effective prevention and control strategies against parasites.

Previous parasitological surveys of langurs housed at the center showed high prevalence of intestinal helminths, including Strongyloides spp., Trichuris spp., Ancylostoma spp. and Capillaria spp. [2]. Primates are vulnerable to parasitic infestations since they are in close contact with animal keepers. In addition, egg-contaminated food is another probable route of infection for primates kept in zoos. Leaves are harvested daily by keepers in the surrounding areas of the center and prepared as food for primates. However, leaves from such sources, if consumed raw and improperly washed, can increase the risk of foodborne transmission [3], since infective stages of these parasites are able to survive in the environment.
In order to clarify infection status and improve the effectiveness of disease prevention and treatment, the aim of this study was to investigate the prevalence of parasites in leaf and dog fecal samples in the surrounding areas of the EPRC.

2. Materials and methods

2.1. Sample collection
The study was conducted at the Endangered Primate Rescue Center (EPRC), Ninh Binh province from July to November 2019. The leaf samples used in the experiment were collected from the surrounding area of the center where leaves frequently collected as food for primates, including those located at the foothills and along the forest edge, and on hills higher than 10 meters.

During the same period, following verbal consent from owners, dog fecal samples were collected freshly off the ground, put into a sterile zipper bag, and then sent to the laboratory. Households were randomly chosen among seven villages surrounding the center (Nga, Sam, Bai Ca, Ao Luong, Met, Dong Tam, Ky Phu). The majority of investigated dog breeds was Indochina Dingo. A total number of leaf and fecal samples for this study are 99 and 114 samples, respectively.

2.2. Parasite examination
Fecal samples were examined for intestinal helminths using flotation technique with saturated sugar solution (SG: 1.28) [4] using a light microscope, under 400x magnification for helminth eggs and 100x magnification for tapeworm eggs. The same procedure was used to examine leaf samples, after leaves were cut into small pieces by a scissor.

2.3. Data analysis
All data was recorded and statistical analysis was performed using Excel 2019.

3. Result

3.1. Parasites in the foodstuffs
All leaf samples were collected within a 20 km radius of the center, scattered from the ground to high hills (>10m). Eight out of ninety-nine leaf samples were infested with *Isopora* spp. and *Ancylostoma* spp., at 1.01% and 7.07% respectively (table 1).

| Location/altitude | Number of samples | Isopora spp. positive | Ancylostoma spp. positive |
|-------------------|-------------------|-----------------------|--------------------------|
| 2-20 km at 0-10 m | 55                | 0                     | 4                        |
| 2-20 km at > 10 m | 44                | 1                     | 3                        |
| Total             | 99                | 1                     | 7                        |

3.2. Parasites in the dog fecal samples
The prevalence of intestinal helminths in 156 dog fecal samples was quite high, accounting for 73.55% (n=114). *Ancylostoma* spp. has the highest infection rate (68.39%), followed by *Toxocara* spp. (25.81%), *Trichuris* spp. (10.97%) and *Isopora* spp. (7.1%). The eggs of *Taenia* spp. are also found in six samples (3.87%) (table 2). These species were all common intestinal parasites, which had been recorded previously in Vietnam.
Table 2. Prevalence of intestinal parasites among dog fecal samples collected in the surrounding of the center.

| Parasite species | No. of positive samples | Infection rate (%) |
|------------------|-------------------------|--------------------|
| *Taenia* spp.    | 6                       | 3.87               |
| *Ancylostoma* spp.| 106                     | 68.39              |
| *Toxocara* spp.  | 40                      | 25.81              |
| *Trichuris* spp. | 17                      | 10.97              |
| *Isospora* spp.  | 11                      | 7.1                |

4. Discussion
The aim of this study was to investigate parasitic egg-contamination in raw leaves fed to the primates at Endangered Primate Rescue Center and in dog fecal samples collected in the surrounding areas, to evaluate them as a possible transmission route of infections. Eighteen out of 55 leaf samples from the foothills and forest edge were collected around the landfill and places where dogs and cats frequently showed up (Nga, Bai Ca, etc.), making them easy targets to get contaminated. Even for those collected on hills higher than 10 meters, with less presence of dogs, three samples were still found to be positive with hookworm. During collecting leaves as food for primates, staff did not bring the leaves with them all the time and had to put on the ground, and this may be the reason why they could easily get contaminated with pathogens. Previously, a high prevalence of intestinal parasites in primates of the center was also reported (62.63%), in which *Strongyloides* spp. accounted for the highest percentage (48.48%), followed by *Toxocara* spp. (31.31%), *Ancylostoma* spp. (8.08%) and *Capillaria* spp (5.05%) [2]. This was mostly the case that primates frequently ingested contaminated food, thus they were vulnerable to these parasitic infections. However it is very difficult to determine the source of infection as there is little correlation between the number of investigated samples and the daily primate food intake.

On the other hand, 155 fecal samples of dogs were examined, in which the overall infection rate was quite high (73.55%). The figure for *Ancylostoma* spp. was the highest (68.39%), followed by *Toxocara* spp. and *Trichuris* spp. Similarly, previous studies on the prevalence of intestinal parasites in dogs in Hanoi and Ho Chi Minh city showed five common species, namely *Spirocerca lupi, Toxocara canis, Ancylostoma* spp., *Trichuris vulpis, Taenia* spp. [5,6]. In our study *Isopora* and *Taenia* eggs were also identified, at the rate of 7.1% and 3.87% respectively.

In the current study, we did not find *Strongyloides* spp. in both stool and leaf samples. This is probably due to the low sensitivity of microscopic examination or embryonated eggs mostly hatch into rhabditiform larvae in the intestinal wall and larvae were passed into feces [7], which makes microscopic detection of this pathogen in stool difficult. Until recently, the first molecular evidence of this nematode species in Northern Vietnam has been recorded [8].

The presence of *Taenia* spp. is of concern from a public health perspective due to its zoonotic potential. Differentiation of taeniids eggs is not possible because of their morphological similarity [9]. It is worth mentioning that, hydatidosis due to *Echinococcus ortleppi* appears to be one common cause of death of the langur population in EPRC, which was first reported in 2009 [10]. The disease is not only posing a health risk to primates, but also affects humans as intermediate hosts, especially for those who live in endemic areas. Thus, it is necessary to identify the causative agent - *Taenia* species using molecular method to evaluate the transmission risk as well as to select the most appropriate treatment.

The present study confirms the risk of parasite contaminated leaves fed to the primates at EPRC and the presence of helminths in the direct surrounding environment. Primates can easily acquire helmintic parasites by oral uptake of eggs in or by handling infected definitive hosts, or egg-contaminated plants/soil. During our investigation, we observed that each household normally owned one to three dogs. In addition, dogs were not routinely dewormed or kept in cages, posing the high risk of spreading pathogens in the environment.
These findings also strongly support the need for ensuring the food safety for captive animals, and additional biosecurity measures should be started in the center. These could include altering the way leaves are handled during daily harvest; or perhaps washing leaves prior to feeding out, requiring a system of hanging and spraying leaves or immersing leaves in water. In addition, increased frequency of deworming (including both animals and humans) to prevent the initial exposure to the parasite from becoming a chronic infection is necessary.

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