NOTE *Parasitology*

A Coprological Survey of Intestinal Helminthes in Stray Dogs Captured in Osaka Prefecture, Japan

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ABSTRACT. This study aimed to investigate intestinal helminth infection in stray dogs in Osaka Prefecture by surveying coprological samples from dogs captured from 2006–2011. Of 212 fecal samples collected, overall prevalence of infection was 39.2%. The most common species was *Toxocara canis* (25.0%), followed by *Trichuris vulpis* (8.0%), *Spirometra erinaceieuropaei* (3.3%), Taeniidae (2.4%), *Ancylostoma caninum* (1.9%) and *Toxascaris leonine* (0.5%). In the molecular analysis, all of the taeniid eggs were negative for *Echinococcus multilocularis* and were identified as other taeniid species (e.g., *Taenia pisiformis*). Our results suggest that stray dogs remain important infection reservoirs of zoonotic parasites in Osaka Prefecture. Therefore, control of stray dogs is crucial for reducing the risk of public health problems due to parasitic infections.

KEY WORDS: coprological survey, intestinal helminth, stray dog, Taeniidae, *Toxocara canis*.

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Dogs are recognized carriers of various zoonotic diseases. Among them, parasitic diseases caused by intestinal helminthes, such are *Echinococcus* spp. and *Toxocara canis*, are known to be potential public health threats. In Japan, recent surveys have reported a decreasing prevalence of intestinal helminthes in various owned and stray dog populations [1, 12, 17]. However, all of those studies were performed in the eastern part of Japan, and there have been no new prevalence data on intestinal helminthes in stray dogs in the western part since 1982 [15]. The aim of this study was to clarify the current prevalence of intestinal helminthes among stray dogs in Osaka, the most densely populated prefecture of western Japan. A coprological survey was performed through conventional microscopy, and additional molecular tests were performed when necessary.

From June 2006 to February 2010, fecal samples were collected from 212 stray dogs admitted to 4 public animal shelters in Osaka Prefecture (northern, northeastern, southeastern and southern). Location of dogs captured included both urban and rural residential areas as well as riverbanks and public parks. The data, sex and age-class were recorded at the time of collection. On the basis of tooth development (dogs retain their deciduous teeth until 4–5 months of age), the dogs were classified into two age classes as juvenile and adult. All samples were stored at 4°C until examined. A formalin-ethyl acetate sedimentation technique [19] with 0.5 g of fecal material was employed for separating and concentrating helminth eggs, and approximately half of the sediment volume was used for microscopy. Species identification of the helminth eggs was made on the basis of morphological characteristics. Because the eggs of taeniid tapeworms morphologically resemble each other [14], they were subjected to a further molecular discrimination. First, ovum DNA was extracted from a batch of 10 taeniid eggs using a protocol described by Bretagne *et al*. [3]. Either of the 2 mitochondrial DNA regions, the 12S ribosomal RNA (12S rRNA) gene or the cytochrome *c* oxidase subunit 1 (*cox1*) gene, were amplified using previously published primers [2, 16]. The success of amplification was verified by electrophoresis in a 1.5% agarose gel. Each polymerase chain reaction product was then directly sequenced in both directions with a BigDye terminator (Applied Biosystems, Foster City, CA, U.S.A.). A homology search was performed with Basic Local Alignment Search Tool software from the National Center for Biotechnology Information home page (http://www.ncbi.nlm.nih.gov). If the sample showed superimposed peaks on an electropherogram, up to 20 eggs were collected and reexamined individually by molecular analysis. A Pearson's χ^2 test with Yates' correction for continuity (2-tailed) was performed to examine differences between groups. When the overall P-value was significant ($P<0.05$), a post hoc multiple comparison was done using confidence intervals (CIs) to find the difference in proportions between any 2 groups. Statistical analysis and calculation of CIs were carried out using S-PLUS 6.1 (Insightful Corp, Seattle, WA, U.S.A.) and Microsoft Excel 2010 (Microsoft Corp, Redmond, WA, U.S.A.), respectively.

Of the 212 dogs, 83 were positive (39.2%) for at least 1 intestinal helminth. The helminth species identified were *T.*

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Table 1. Age-related prevalence of helminth eggs in feces of stray dogs captured in Osaka Prefecture, Japan

Species	Age class			
	<5 months (n=132)		≥5 months (n=80)	
	No. of positives (%)	95% CI of positives	No. of positives (%)	95% CI of positives
Ancylostoma caninum	1 (0.8)	0.02–4.2	3 (3.8)	0.8–10.6
Toxascaris leonina	1 (0.8)	0.02–4.2	0 (0.0)	0–3.7
Toxocara canis	46 (34.9)	27.3–43.3	7 (8.8)	4.3–17.0
Trichuris vulpis	3 (2.3)	0.5–6.5	14 (17.5)	10.7–27.3
Spirometra erinaceieuropaei	2 (1.5)	0.2–5.4	5 (6.3)	2.7–13.8
Taeniidae	1 (0.8)	0.02–4.2	4 (5.0)	1.4–12.3
Total	51[a)] (38.6)	30.8–47.2	32[b)] (40.0)	30.0–51.0

a) Three dogs were infected with 2 helminth species. b) One dog was infected with 2 helminth species.

canis (25.0%), *Trichuris vulpis* (8.0%), *Spirometra erinaceieuropaei* (3.3%), Taeniidae (2.4%), *Ancylostoma caninum* (1.9%) and *Toxascaris leonine* (0.5%). Age-related significant differences were observed in *T. canis* and *T. vulpis* (Table 1). For *T. canis*, the prevalence of juvenile (age<5 months old) and adult (age≥5 months old) dogs was 34.9% (46 of 132) and 8.8% (7 of 80), respectively. In contrast, the prevalence of *T. vulpis* in juvenile and adult dogs was 2.3% (3 of 132) and 17.5% (14 of 80), respectively. In the molecular analysis, all of the taeniid eggs detected from 5 dogs were negative for *E. multilocularis*. The eggs were identified as *Taenia hydatigena* (n=1), *T. pisiformis* (n=3), *T. serialis* (n=1) and *T. taeniaeformis* (n=2) (AB704400–AB704406). Two dogs with Taeniidae were found to be infected with 2 different species (*T. hydatigena* and *T. taeniaeformis*, and *T. pisiformis* and *T. serialis*).

The overall prevalence of intestinal helminthes found in our study (39.2%) revealed a high level of infection that is comparable with the findings from recent studies carried out in the eastern part of Japan [12, 17]; however, it appears to be lower compared with the >80% prevalence found in a study conducted approximately 3 decades ago in Hyogo, a neighboring prefecture of Osaka [15]. One-fourth of the stray dogs examined in the present study were infected with *T. canis*, a causative agent of toxocariasis in humans. The prevalence rate of *T. canis* in this study (25.0%) was relatively higher than that seen in studies performed in Hyogo Prefecture (18.4%) and Saitama Prefecture (12.5%) [15, 17], probably due to the difference in age classes of the animal subjects. It is well known that patent *T. canis* infection is much more common in juvenile dogs than in adult dogs [8]. Our results concord with this age-related difference and suggest the important role of juvenile dogs in environmental contamination [8]. In addition, we also emphasize that a certain number of patent infections were found in the adult dog group. Experimental studies have clearly demonstrated that a low infective dose of embryonated eggs can induce patent infection in adult dogs [6, 10]. Therefore, ascarid infection among adult dogs should be reconsidered and given much more attention.

Like *T. canis*, *E. multilocularis* is another important zoonotic parasite of Japanese dogs. Although *T. canis* occurs nationwide, the distribution of *E. multilocularis* is thought to be limited to Hokkaido Prefecture, the northernmost island prefecture of Japan. However, Morishima *et al*. [11] found *E. multilocularis* infection in dogs transported from Hokkaido to other prefectures. In addition, a dog harboring *E. multilocularis* was found in Saitama Prefecture [18]. These findings raised the concern that infected dogs from Hokkaido transported to remote areas might become a source of indigenous alveolar echinococcosis in humans. In our study, all of the taeniid eggs examined using the molecular method were identified as species belonging to the genus *Taenia*; no *E. multilocularis* infection was found. The result suggests that, at present, the possibility that *E. multilocularis* has become established in Osaka Prefecture is quite low. However, continuous surveying must be done for dogs originating from Hokkaido.

The detection rate of taeniid eggs in our study (2.4%) was higher than that seen in other studies done in Japan (0.2% in Hyogo Prefecture and 0.3% in Saitama Prefecture) [15]. Since all of the taeniid-positive cases involved dogs from rural areas, the difference is probably attributable to a higher opportunity for predation of their hosts in these areas. Interestingly, *T. taeniaeformis*, commonly found in cats, was detected in 2 dogs (0.9%). Two previous studies have detected *T. taeniaeformis* in dogs; one demonstrated the adult tapeworms by autopsy, and the other found the eggs by microscopy [4, 5]. Because no postmortem examinations were undertaken in the present study, we could not confirm that the occurrence of *T. taeniaeformis* eggs in canine samples was due to presence of an adult tapeworm. A possible explanation may be that dogs shed *T. taeniaeformis* eggs in their feces in coprophagic behavior, as previously suggested in the cases of other feline parasites seen in dogs [7, 9, 13].

In conclusion, we found that stray dogs in Osaka Prefecture were infected with several species of intestinal helminthes, including zoonotic species. Although *E. multilocularis* was not detected, the present results suggest that the stray dogs in the study area can still act as a reservoir for transmitting zoonotic parasitic diseases both to humans and to other dogs. Therefore, both control of stray dogs and appropriate public health education for residents are continually needed to reduce the risks of zoonotic infections.

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