NOTE *Parasitology*

Prevalence of *Giardia intestinalis* Infection in Dogs of Breeding Kennels in Japan

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ABSTRACT. *Giardia intestinalis* antigen in fecal samples was examined in 361 dogs of 14 breeding kennels located at various areas in Japan, using a commercial enzyme-linked immunosorbent assay (ELISA) kit. *G. intestinalis* antigen was detected in 37.4% of the fecal specimens. All of the 14 breeding kennels were positive for *G. intestinalis* antigen with the range from 6.7 to 59.3%. The prevalence in puppies (54.5%) was significantly ($p < 0.01$) higher than that in adults (30.9%). There was no difference in prevalence between males and females, and between the puppies from the mother dogs positive and negative for *Giardia* antigen. In conclusion, *G. intestinalis* widely invaded the breeding kennels in Japan.

KEY WORDS: breeding kennel, canine, *Giardia intestinalis*.

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Giardia intestinalis is a pathogenic protozoan parasite found in the small intestine of dogs and other mammals including humans, and often causes acute or chronic diarrhea [5, 9–11]. *G. intestinalis* infection in dogs, especially in puppies, is recognized to be clinically important due to its high prevalence [3, 4, 6], serious symptoms in some occasions [5, 10], and possibility as zoonosis [9, 11]. There have been several epidemiological reports including our articles [3, 4] and others [10, 12], suggesting that pet shops and/or breeding kennels play a significant role as a source of pathogen in *G. intestinalis* infection of puppies. Nevertheless, only a few reports have been described on the presence of *G. intestinalis* infection in dogs of breeding kennels in Japan [10] and other countries [2]. The presence of *G. intestinalis* in dogs of breeding kennels has not been established in Japan. The purpose of the present study was to determine the prevalence of *G. intestinalis* infection in dogs of breeding kennels located at various areas in Japan and to partially elucidate the etiology of *G. intestinalis* infection in puppies.

The present study was conducted during the period from October, 2003 to July, 2004. Fecal specimens were obtained from 361 dogs kept in 14 breeding kennels of various areas in Japan. The animals included 110 male and 251 female dogs consisted of 27 breeds, aged between 1 month and 14 years old. The number and location of breeding kennels were 5 in Aomori (Aomori #1–#5), 2 in Akita (Akita #1, #2), 1 in Iwate, 1 in Niigata, 1 in Nagano, 2 in Tokyo (Tokyo #1, #2), 1 in Kanagawa and 1 in Tokushima Prefectures.

Fecal samples were collected immediately after passed and examined grossly for their appearance (normal, soft or diarrhea). Then they were stored at –20°C until analysis. *G. intestinalis* specific antigen in fecal samples was detected by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (RIDASCREEN® Giardia, R-Biopharm AG, Germany), which employed a monoclonal antibody against cell wall proteins of *G. intestinalis* cysts and trophozoites, according to the manufacture's instruction. The statistical significance of differences ($p<0.05$) was analyzed by Fisher's exact probability test.

G. intestinalis antigen was detected in all the 14 breeding kennels, though the positive rate in each kennel widely varied from 6.7 to 59.3% (Table 1). Differences in the prevalence among the breeding kennels did not relate to the breeds of dogs (data not shown). Positive rate in 361 dogs of the 14 kennels was 37.4% and showed no difference between males (37.3%) and females (37.5%), and among the appearance of their feces, namely, *Giardia* antigen was detected in 37.4% (111/297), 37.3% (19/51) or 38.5% (5/13) of normal, soft or diarrhea feces, respectively. However the positive rate of puppies from 1 to 9 months old (54.5%, 54/99) was significantly ($p<0.01$) larger than that of adults over 10 months old (30.9%, 81/262). Although *Giardia* antigen was detected in 36.8% (7/19) and 48.0% (24/50) of the pups from the mother dogs positive and negative for *Giardia* antigen, respectively, the difference between both positive rates was not significant.

In the present study, a commercial ELISA kit was applied to evaluate the prevalence of *Giardia* infection in dogs of breeding kennels, since the previous study suggested that the ELISA kit was more sensitive to detect *Giardia* infection than the conventional microscopic technique [4]. *Giardia* antigen was detected in all of breeding kennels examined in the present study. The result certified that *G. intestinalis* widely invaded breeding kennels in Japan. The high infection rate of *Giardia* was also recorded in household dogs originating from pet shops and/or breeding kennels, compared to those from private dog owners [3, 4]. These findings obviously suggest that breeding kennels would play an important role in *Giardia* transmission. The prevalence of *Giardia* infection showed no relation to fecal conditions. Mochizuki *et al.* [6] also reported that the posi-

Table 1. ELISA-positive rates (%) for *Giardia intestinalis* in dogs of 14 breeding kennels from different areas in Japan

Age\ Kennels	Aomori #1	Aomori #2	Aomori #3	Aomori #4	Aomori #5	Akita #1	Akita #2	Iwate	Niigata	Nagano	Tokyo #1	Tokyo #2	Kana-gawa	Toku-shima	Total
Puppies (1–9 months old)	0 (0/1)*	0 (0/4)	66.7 (8/12)	50.0 (3/6)	33.3 (5/15)	33.3 (1/3)	25.0 (1/4)	100 (1/1)	100 (9/9)	50.0 (3/6)	100 (12/12)	57.1 (4/7)	0 (0/7)	58.3 (7/12)	54.5 [a] (54/99)
Adults (10 months or older)	9.1 (1/11)	28.6 (2/7)	31.3 (10/32)	40.0 (2/5)	25.0 (3/12)	5.9 (1/17)	18.2 (2/11)	15.4 (2/13)	25.8 (8/31)	47.4 (9/19)	28.6 (10/35)	60.0 (12/20)	12.5 (1/8)	43.9 (18/41)	30.9[b] (81/262)
Overall (Puppies + Adults)	8.3 (1/12)	18.2 (2/11)	40.9 (18/44)	45.5 (5/11)	29.6 (8/27)	10.0 (2/20)	20.0 (3/15)	21.4 (3/14)	42.5 (17/40)	48.0 (12/25)	46.8 (22/47)	59.3 (16/27)	6.7 (1/15)	47.2 (25/53)	37.4 (135/361)

*: (Positive dogs/examined dogs) a) vs b): Significant statistical difference ($p<0.01$).

tive rates of *Giardia* were almost equal in fecal samples obtained from dogs with or without diarrhea. The clinical signs of giardiasis including diarrhea may be developed continuously or intermittently [5], and the stress may accelerate the appearance of symptoms [10]. On the other hand, it is undoubted that many of the asymptomatic carrier dogs in breeding kennels are also important for the transmission of *Giardia*, as the source of infection. Living together of pups with their mothers had no relation in *Giardia* infection. This may indicate less possibility of vertical transmission in *Giardia* infection than that of horizontal transmission via the environment contaminated with *Giardia* cysts.

Positive rate for *Giardia* antigen was relatively low in 3 breeding kennels (Aomori #1, Akita #1 and Kanagawa) which were small-scale facilities with fewer than 20 dogs. In addition, one (Kanagawa) of the 3 facilities has been opened recently. These facts may suggest that those kennels have been kept clean hygienically. In contrast, the higher prevalence rate was recorded in 7 kennels (Aomori #3, Aomori #5, Niigata, Nagano, Tokyo #1, Tokyo #2 and Tokushima) keeping over 25 dogs and 1 small-scale facility (Aomori #4). The cause for higher prevalence is not clear, because there are no data to assess the hygienic conditions and the area occupied by one dog in each breeding kennel. However, it is sure that the hygienic consideration of owners reflects the prevalence of *Giardia* infection in their breeding kennels.

G. intestinalis is classified into several genotypes on the basis of molecular markers, and at least 4 genotypes (assemblages A, B, C and D) have been isolated from dogs [7, 8]. Assemblages C and D appear to be specific for dogs, whereas assemblages A and B seem to have zoonotic potential [7–9, 11]. Abe *et al.* [1] reported that 4 *Giardia* isolates from dogs in Japan were assemblage D. However this finding will not always negate the possibility of zoonotic transmission of *G. intestinalis* from dog, since assemblage A has been recently detected in dogs in Japan (our unpublished data). The owners of breeding kennels should know well the hygienic management of dogs to prevent *Giardia* transmission from dogs to humans. Periodic fecal examination and treatment with giardicidal drugs will contribute to prevent the dogs of breeding kennels from *G. intestinalis* infection, whether the dogs show the symptoms or not. Further the genotypic studies will be necessary to confirm the zoonotic potential of *G. intestinalis* isolated from dogs of breeding kennels in Japan.

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