Prevalence of Toxocara Eggs in Sandpits in Tokushima City and its Outskirts

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ABSTRACT. To demonstrate the prevalence of Toxocara eggs in sandpits in Tokushima Prefecture, Japan, sand samples were examined. Of 46 sandpits surveyed, 29 sandpits (63.3%) had Toxocara eggs. Sandpits in public parks and playgrounds in residential areas were more contaminated (87.5%) with the eggs than those in kindergartens, schools, and children's centers (36.4%). To examine the seasonal change in the contamination, sand samples obtained from five sandpits were examined monthly from May 1990 to April 1991. Toxocara eggs were found in the majority of sandpits (3/5-5/5) in the spring, early summer, and autumn. In the summer and winter, the rates of sandpits contaminated were lower (2/5-3/5) than in other seasons. Egg counts became smaller in the summer and winter, especially in summer. The ratio of T. canis to T. cati eggs recovered was 2:3. Fecal examination of 144 puppies revealed that 98 (68.0%) were positive for T. canis eggs. These findings show that Toxocara eggs, a possible causative agent of visceral larva migrans, commonly present in sandpits of the urban area. To prevent the contamination of sandpits with these eggs, control measures are needed. —KEY WORDS: contamination, sandpit, Tokushima city, Toxocara egg.

Toxocara species are most common roundworms of Canidae and Felidae. Both T. canis and T. cati are considered to be the causative agents of human toxocariasis. T. canis is well-known by its unique transmission mode, transplacental migration, in the definite host. The infection rates are very high in puppies and become lower with the age of host. Age-resistance of Felidae is not reported against T. cati.

Human toxocariasis develops by ingesting embryonated eggs. Beaver et al. [2] proposed the term visceral larva migrans (VLM), and since then many cases of visceral larva migrans were reported in Europe and North America [7]. In Japan, the cases reported are increasing in number [13].

Children are particularly exposed to the risk of the infection with Toxocara species, because of their pica habit and their close contact with infected pets and contaminated playgrounds. For this reason many studies have been carried out to examine the prevalence of Toxocara eggs in the ground of children's playgrounds and public parks [3, 4, 6, 11], and the incidence of the eggs in sandpits was reported to range from 10 to 87%. In Japan, Uga et al. [16], Man-nami et al. [12], Yamada et al. [17], and Igarashi and Yatomi [8] also reported the contamination of sandpits in public parks with Toxocara eggs. The contamination rate varied from 19.2 to 68.8% and was the highest in urban parks [16]. The environmental contamination with Toxocara eggs was surveyed under the supervision of Tokushima Health Center, Tokushima Prefecture, by systematic and seasonal examinations of sand samples from sandpits. To estimate the infection rates with ascarids among puppies, one of the main sources of environmental contamination, puppies were also examined for the eggs in the feces.

MATERIALS AND METHODS

A total of 46 sand samples were collected from 24 sandpits in the public parks and playgrounds in the residential areas (hereafter referred to as "parks sandpits") and 22 sandpits in kindergartens, grade-schools, and children's centers (hereafter referred to as "children's facilities sandpits").

After removing the superficial layer, a total of 1,000 cm³ of sand was taken from the center and four corners of each sandpit.

In order to determine the seasonal change in the contamination, five sandpits, A, B, C, D, and E, were selected and examined monthly from May 1990 to April 1991 by the same method described above. Sandpit “A” is located at a children's playground in a shrine in a built-up residential area. The playground is surrounded by tall trees and a wisteria trellis hangs over the sandpit. Sandpits “B” and “C” are situated in old parks in a residential quarter, that are now used as playgrounds. Sandpit “D” is in the newest park, built in 1989, in a residential area. Sandpit “E” is in a park located in the central area of the city and is surrounded by office buildings, shops, and housing.

Sand samples were washed with running tap water through a series of four sieves of 1, 0.3, 0.095, and 0.045 mm meshes respectively. The final residue on the 0.045 mm mesh was transferred to several 50 ml glass centrifuge tubes. After adding 30 ml of saturated sodium nitrate solution (specific gravity 1.39 at 20°C), tubes were shaken by hand for three minutes. Ten ml of distilled water was then layered onto the sodium nitrate mixture. The tubes were centrifuged at 800 g for three min. The aqueous fractions from the interface layer were transferred to other tubes. After adding 30 ml of distilled water, tubes were centrifuged at 800 g for three min. After washed again by the same procedure as above, the sediments was microscop-
typically examined for ascarid eggs. When eggs were detected, egg-containing sediments were transferred to a test tube and fixed with 10% hot formalin. The fixed sediments were dehydrated through a series of 30%, 40%, and 70% ethanol and transferred to glycerine-alcohol. The specimens thus obtained were mounted on slides with glycerine jelly and covered with slips. Eggs were then counted under a microscope.

*T. canis* and *T. cattii* could be differentiated from each other by a scanning electron microscope (SEM, Hitachi S-100), and the ratio of both species of eggs in a sandpit was determined with sand samples from "E" sandpit which contained 10^3 or more eggs per 1,000 cm³ of sand.

From April 1989 to May 1991, 144 "unnecessary puppies" of 79 litters, less than 5 months old, were examined for ascarid eggs. These puppies consisted of three kinds of animals: stray ones picked up on the street, abandoned puppies left in the street corner or the park, and unwanted puppies brought to the animal shelter by their owners. All the puppies were medium-sized mongrels.

Puppies were divided into three age groups according to Kirk [10], group I: those less than 3–4 weeks of age, before the development of deciduous teeth (Dc); group II: those from 3 to 8 weeks of age, before the development of the last deciduous teeth (Dp4); and group III: those from 6 weeks to 5 months, before the development of the first permanent teeth.

For fecal examination, a small amount of rectal feces was taken with a plastic stick, smeared onto a slide, and examined microscopically for eggs.

Differences in percentages were analyzed by significance by Chi-square test.

RESULTS

*Toxocara* eggs were detected from 29 (63.3%) of 46 sandpits (Table 1, Figs. 1, 2). Sand samples from park sandpits were more highly contaminated (87.5%) with ascarid eggs than those (36.4%) from children’s facilities (P<0.01). The contamination rate in the urban areas (93.3%) was higher than that (48.4%) in the rural areas (P<0.01).

The numbers of eggs recovered from each sandpit varied from 1 to 4,806/1,000 cm³ of sand; that is from 1 to 9 in 14 sandpits, 10 to 99 in 13 sandpits, and over 1,000 in 2 sandpits. Embryonated eggs were recovered from 22 of 29 contaminated sandpits (Figs. 3, 4).

Table 2 shows the seasonal changes in contamination and climatic conditions. *Toxocara* eggs were found in the majority of sandpits (3/5–5/5) in the spring, early summer, and autumn, whereas the rate of positive sandpits was lowered (2/5–3/5) in the summer and winter. The numbers of eggs detected were also decreased in both seasons, especially in the summer.

Although *Toxocara* eggs were recovered throughout the investigation period from "A" (except in July) and "E" sandpits, the numbers of eggs recovered ranged from 1 to 37 in "A", while those in "E" were more than 10^8 from November to February and decreased to 10^3 in the summer. Soon after the exchange of sands for new one in November 1990, eggs were recovered in "A" in the next investigation.

Scanning electron microscopy revealed clear morphological differences between *T. canis* (Fig. 5) and *T. cattii* eggs (Fig. 6). 38.7% of the 111 eggs recovered from "E" were identified as *T. canis*, and the remaining 61.3% were *T. cattii*.

68.0% of the 144 puppies examined were positive for *T. canis* eggs (Table 3). The infection rates were 89.0% and 80.5% in the group II and III, respectively, whereas all the puppies in the group I were negative for the eggs. There were no significant differences in the incidence among stray, abandoned, and unwanted puppies.

**DISCUSSION**

The contamination rates of sandpits were clearly higher in public parks than in children’s facilities. This difference may be attributed to the fact that humans, dogs, and cats can freely enter parks and playgrounds, while the facilities are usually enclosed by fences and their entry is limited to some extent. This might be supported by the fact that sandpit "A" was contaminated with ascarid eggs even one month after the exchange of sands for new one.

Because of their environmental differences, five sand-
PREVALENCE OF *TOXOCARA* EGGS IN SANDPITS

Fig. 1. Photomicrograph of a *Toxocara* egg recovered from a sandpit.
Fig. 2. Photomicrograph of the same egg as shown in Fig. 1, focused on the upper surface.
Fig. 3. Photomicrograph of an embryonated *Toxocara* species egg recovered from a sandpit.
Fig. 4. Scanning electron micrograph of an embryonated *Toxocara cati* egg recovered from a sandpit.
Fig. 5. Scanning electron micrograph of a *Toxocara canis* egg recovered from an infected puppy.
Fig. 6. Scanning electron micrograph of a *Toxocara cati* egg recovered from an infected cat.
Table 2. Seasonal changes in recovery of *Toxocara* species eggs

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Air Ave. (°C) 18.8 23.5 27.5 28.7 24.9 19.0 15.1 9.2 6.4 6.0 10.2 15.4

temp. Max. (°C) 22.7 27.5 31.6 29.5 28.5 22.8 19.3 13.3 10.0 10.1 14.2 20.1

No. days over 30°C 0 5 24 30 11 0 0 0 0 0 0 0

Total precipitation(mm) 148.0 189.0 51.0 81.0 520.0 278.5 335.5 21.0 15.0 32.0 127.5 219.5

Total hours of sunlight 193.1 187.3 242.6 262.2 143.9 145.6 165.4 188.5 147.6 145.1 128.6 195.9

+ +4 indicate the contamination grade indicated by numbers of eggs recovered. + : 1 9, +2: 10 99, +3: 100 999, +4: 1000

※ Sands were exchanged for the new one.

Table 3. Incidence of *Toxocara canis* in puppies

<table>
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<tr>
<th>Group of dogs</th>
<th>Group I &lt; 3 to 4 wks. old</th>
<th>Group II 3 to 4 wks. ≤ and &gt; 6 to 8 wks. old</th>
<th>Group III 6 to 8 wks. &lt; and &lt; 4 to 5 mo. old</th>
<th>Total</th>
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<tr>
<td>Stray dogs</td>
<td>10</td>
<td>8(80.0)%</td>
<td>4(75.0)%</td>
<td>14(78.5)%</td>
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<td>Abandoned dogs</td>
<td>17</td>
<td>16(94.6)%</td>
<td>12(87.5)%</td>
<td>35(86.6)%</td>
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<tr>
<td>Unwanted dogs</td>
<td>46</td>
<td>40(87.0)%</td>
<td>25(92.0)%</td>
<td>95(66.3)%</td>
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<tr>
<td>Total</td>
<td>30</td>
<td>73(89.0)%</td>
<td>41(80.5)%</td>
<td>144(98.0)%</td>
</tr>
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※ Not available.

Pits were not equal in seasonal changes of contamination. In the spring and early summer, the rate of contaminated sandpits was high and the numbers of eggs recovered were very large, while in the summer they both became lowered. High contamination rates in spring and autumn may be related to the increase of infected puppies and kittens, because in those seasons bitches and tabby-cats are usually delivered of offspring which are susceptible to parasite infection. The decrease in contamination rates of sandpits and numbers of eggs recovered in summer may also be due to the increased degeneration of deposited eggs under high temperature. Katakurak et al. [9] postulated the positive correlation between egg-degeneration and global solar radiation. This hypothesis was also supported by the present result that eggs were detected through the year round in "A" located in a shady place. The highest prevalence in "E" may be due to the fact that all the sites examined were paved in almost all the ground, so that owners will take their dogs for exercise and dogs and cats prefer to defecate on the soil and sand. As a result, *Toxocara* eggs might be concentrated in sandpits.

To exactly determine the prevalence of *Toxocara* eggs in the ground, large amounts of soil samples should be examined by the effective methods. Although Dada and Lindquest [5] and Quins et al. [14] recommended flotation methods with high recovery rates of 68.0 69.8%, using a small amount of sample, they reported the prevalence of *Toxocara* eggs to be 39 and 11% sandpits in Kansas and soils from public parks in Glasgow, respectively. However, Duwell [6], using large amounts of sand samples (200 300 g/m² sandpit), reported a much higher prevalence of 87% in sandpits in Frankfurt/M, even by a less effective technique with an efficiency of 55 60%. Although the recovery efficiency by this method has not
been tested in this study, a false negative result in frequency could be reduced by using large amounts of samples.

Uga et al. [16] reported that *T. canis* eggs were clearly distinguished from *T. cati* eggs by scanning electron microscopy. The ratio of *T. canis* to *T. cati* eggs in contaminated sand was 1:3 in Hyogo prefecture [16], and 2:3 in this study.

All the puppies less than 3 weeks old were negative for *T. canis* eggs. Even if they were infected with ascarids through transplacental or transmammary transmission, the parasites were not considered to be so mature to excrete eggs. In puppies above that age, infection rates increased up to 89.0%. These rates are much higher than those reported by Abe [1]. The difference between both rates may be ascribed to the fact that he surveyed pet dogs brought to veterinary hospitals by their owners, which were kept under good care and management. The infected puppies are the source of environmental contamination, but direct contact with these puppies causes no human infection because of more incubation time required for eggs to become infective.

There is no conclusive evidence that the contamination rates with ascarid eggs in sandpits were higher in Tokushima than in other areas, because different methods were used in different surveillance. Even so, it is apparent that sandpits in Tokushima city and its outskirts are highly contaminated with *Toxocara* eggs.

In order to prevent the contamination with *Toxocara* eggs, it needs not only to let pet owners know to deworm dogs and cats and to treat the feces sanitarily, but also to keep sandpits clean of ascarid eggs by some available means.

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**REFERENCES**