

## THE ROLE OF DOGS IN TRANSMISSION OF GASTROINTESTINAL PARASITES IN A REMOTE TEA-GROWING COMMUNITY IN NORTHEASTERN INDIA

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**Abstract.** The prevalence and risk factors associated with canine gastrointestinal parasitic zoonoses and the role of dogs in the mechanical transmission of human *Ascaris* infection was examined in three tea estates in Assam, India. Nearly all (99%) dogs harbored one or more zoonotic species of gastrointestinal parasites, with hookworm infection being most common (94%). Parasitic stages presumed to be host-specific for humans such as *Ascaris* spp. (31%), *Trichuris trichiura* (25%), and *Isospora belli* (2%) were also recovered from dog feces. A polymerase chain reaction–linked restriction fragment length polymorphism technique was used to differentiate the species of *Ascaris* eggs in dog feces. The results of this study demonstrate the role of the dog as a significant disseminator and environmental contaminator of *Ascaris lumbricoides* in communities where promiscuous defecation by humans occurs.

### INTRODUCTION

The role of the dog as a definitive host for a number of zoonotic parasites has been widely studied and recognized as being a significant public health problem worldwide, especially in developing countries and communities that are socioeconomically disadvantaged.<sup>1</sup> In these communities, poor levels of hygiene and overcrowding, together with a lack of veterinary attention and zoonotic awareness, exacerbates the risk of disease transmission.<sup>2,3</sup> Recently, it has been demonstrated that dogs are capable of mechanically transmitting *Toxoplasma gondii* when experimentally fed sporulated oocysts from cat feces. Viable sporulated oocysts were present in the dog feces for up to two days post-inoculation.<sup>4</sup> The study supported the hypothesis that dogs may be involved in the mechanical transmission of *T. gondii* to humans. Similarly, Hymenolepid<sup>5</sup> and *Ascaris*<sup>6</sup> eggs have been observed in the feces of dogs in communities in Australia and India, respectively, but the public health implications of these findings were not investigated. It is possible that in developing communities, where promiscuous defecation by humans is common, the dog may act as a significant vector of human parasitic stages via coprophagy of human feces.

This present study forms part of an ongoing project aimed at investigating the prevalence and epidemiology of gastrointestinal parasites among humans and dogs in tea-growing communities of Assam. We examined the prevalence and risk factors associated with canine gastrointestinal parasitic zoonoses in dogs, and the role of dogs in the mechanical transmission of human *Ascaris* infections in three separate tea estates in Assam. We also compared the prevalence and fecal egg counts (intensities) of *Ascaris lumbricoides* in humans and dogs to explore the extent to which dogs may be influencing the dynamics of transmission of *Ascaris* within the human population via coprophagy. A polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique based on a four base pair nucleotide difference in the first internal transcribed spacer region of *A. lumbricoides* and *A. suum*<sup>7,8</sup> was used to determine the species of *Ascaris* eggs in microscopically positive dogs.

### MATERIALS AND METHODS

**Study area and design.** The tea-growing communities of Assam were chosen for this project for the following reasons.

1) Anecdotal evidence suggested that the community was heavily infected with gastrointestinal parasites such as *Ascaris* and hookworms, with malnutrition and anemia being commonly encountered, especially among women and children. Medical practitioners at Phulbari Central Hospital also reported at least one case of hydatidosis in a six-year-old child and one case of blindness due to *Toxocara* larval migrans, in the year prior to commencing the study. 2) These people share a close relationship with semi-domesticated dogs, often allowing them into their houses. The complete lack of veterinary attention places the community at increased risk of acquiring canine parasitic zoonoses. 3) No previous parasite surveys have been conducted in this community. 4) The tea estates are small and isolated with limited between-estate contact. 5) The consent, cooperation, and assistance of the managerial and medical staff of Williamson Magor & Co. were offered to help conduct fieldwork.

**Area.** The three tea estates under study, Phulbari, Addabarie, and Balipara, lie within the Brahmaputra Valley, on the north bank of the Brahmaputra River, approximately 10 km from the nearest town, Tezpur. The Valley is overshadowed by the Himalayas to the north and the Shillong Plateau to the south. It has fertile alluvial soil and experiences heavy monsoon rains (June to October) that vary between 180 and 250 cm per year. Average temperatures range from 23°C in January to 33°C in June. The region is predominated by rice paddies and tea plantations surrounded by scattered villages and rainforests. The three tea estates used in the current study are located within a 10-km radius of each other, all connected by road. Phulbari, Addabarie, and Balipara had a reported human population of 6,531, 4,839 and 2,004, respectively (Census 2000).

Each tea estate comprises three distinct socioeconomic groups, the executives, staff, and labor. The executives make up less than 1% of the total population and are managerial and medical staff. This group was excluded from the study. The staff make up approximately 5% of the total population and consist of factory workers, health workers, and teachers. Most are literate and have completed secondary education. They are provided with separate staff living quarters within the estate. The houses are arranged in fenced compounds. They are constructed of solid brick and cement and contain separate cooking, living, dining, sleeping, and bathing areas with a veranda overlooking a backyard. Each house is pro-

vided with an enclosed Asian-style latrine connected to a septic tank. The labor make up the majority of the population and are predominantly tea-pickers. Most are illiterate having attended no formal schooling. They are also provided with specific, mostly brick housing, similar in design to the staff quarters, however with smaller dimensions (average compound area = 2,500 square feet), a separate enclosed area for bathing, together with an Asian-style latrine connected to a septic tank. However, in the majority of cases the latrine facilities are not used due to the acquired habit of defecating outdoors. Most labor quarters are provided with running water and electricity. The labor community often live in overcrowded conditions with poor standards of hygiene.

Both staff and labor communities keep domesticated animals such as cattle, goats, and poultry for milk, eggs, and meat. Company regulations disallow the rearing of pigs within the estate. Each tea estate is provided with a school and a local hospital with free education, medical, surgical, and dental services. A Health Officer is appointed to each estate to coordinate a biannual anthelmintic program targeted at children. Medical practitioners also hold regular meetings at the Mother's Club aimed at educating women tea-pickers on issues such as birth and disease control.

**Design.** To aid cooperation, sampling was initiated by a series of preliminary lectures, given by the primary investigator (RJT) aimed at the assigned health workers for each labor housing group among the estates. The purpose of these was to explain the research project and provide a poster presentation demonstrating practical and simple measures of parasite control.

Both households owning and those not owning dogs were included in the study and randomly sampled from each tea estate. In the evening, after returning from their duties, a questionnaire was used to determine risk factors influencing the prevalence of parasites in themselves and in their dogs. The potential for interviewer bias was limited by having one interviewer, the primary investigator, and a questionnaire with ordered and specific questions and procedures to follow. Specific data were collected first on household information including the number of residents, socioeconomic grouping, religion, water source, and pets, particularly dog ownership. A second questionnaire was aimed at individuals and information relating to age, gender, level of education, defecation habits, health status and the frequency of deworming, and personal contact with dogs. Individuals were also questioned about their awareness and knowledge of rabies and canine parasite zoonoses. Participants owning dogs were required to answer an additional questionnaire about their dogs. Data was collected on the dogs' demographic characteristics (age, gender, breed), diet (including whether raw meat or offal were fed), defecation and roaming patterns, frequency of deworming, vaccination status, and access to a veterinarian. Owners of dogs were handed collars and leashes and advised to feed their dogs rice and lentils in the evening and then keep their dog restrained within the compound for the duration of the night. Fecal pots were given to each human participant with their name and a picture drawing for those participants who were illiterate. Feces were collected from the rectum of the dogs by the primary investigator in the morning, together with the human fecal samples. Written or verbal consent was obtained from each human adult participant prior to participation and from parents of minors. The study was approved

by the Murdoch University Human and Animal Ethics Committees of Western Australia and the medical and managerial staff of Williamson Magor & Co. Feces from both humans and dogs were stored separately in 5% formal saline and 2.5% potassium dichromate. Samples were transported to Murdoch University (Perth, Western Australia) for further processing.

An adult *A. lumbricoides* worm was obtained from a participant at the tea estates who had reported coughing it up earlier. An adult *A. suum* worm was obtained from a local pig abattoir in Perth. The adult worms were fixed in 80% ethanol and used as a positive and negative controls for molecular studies.

**Sample sizes.** Single fecal samples were collected from a total of 101 dogs and 328 humans from the three tea estates over a three-month period (July-September 2000). Humans that owned dog, as well as those that did not own dogs, were sampled to assess if dog ownership or contact constituted a risk with regard to prevalence of *A. lumbricoides* in humans (Table 1).

**Statistical analysis.** The prevalence was calculated for each parasite. Associations between parasitism and host and management factors were initially made using chi-square tests for independence. Continuous data (age) was analyzed using one-way analysis of variance. Logistic multiple regression was then used to quantify the association between parasite prevalence and each variable after adjusting for other variables. Only variables significant at  $P \leq 0.25$  in the univariate analyses were considered eligible for inclusion in the logistic multiple regression.<sup>9,10</sup> Backward elimination was used to determine which factors could be dropped from the multivariable model. The likelihood ratio chi-square statistic was calculated to determine the significance at each step of the model building. The level of significance for a factor to remain in the final model was set at 10%.

Statistical comparisons were performed using Statistix for Windows (Analytical Software, Tallahassee, FL) and Excel® 97 (Microsoft, Redmond, WA).

**Parasitologic techniques.** Fecal samples were examined for parasites using a standard sedimentation in water technique followed by centrifugal flotation in saturated zinc sulfate, sodium nitrate, and microscopy. A malachite green background stain was used to screen for *Cryptosporidium* oocysts.<sup>11</sup> Particular attention was paid to *Trichuris* eggs visualized in the feces of dogs and measurements were taken to distinguish between the eggs of *Trichuris vulpis* and *T. trichiura*.

Eggs of *Ascaris* were concentrated and purified from one gram of feces using a saturated salt and glucose method (Meinema JM, unpublished data). The concentration of *Ascaris* eggs was determined and expressed in eggs per gram (epg) of feces.

Dog fecal samples that were positive for *Ascaris* eggs were

TABLE 1  
Number of humans and dogs sampled at each tea estate

Tea estate	Dogs	Dog owners	Non-dog owners
Phulbari	41	79	69
Addabarie	40	72	51
Balipara	20	32	25
Total	101	183	145

washed repeatedly with 1× phosphate-buffered saline (PBS) and allowed to incubate at room temperature (18–22°C) in aerated petri dishes. Eggs were suspended in 1× PBS solution to which was added 6 mg/ml of penicillin, 20 µg/ml of gentamicin, and 2.5 µg/ml of amphotericin B. The development of eggs was evaluated microscopically once a week.

**Molecular methods.** Fecal samples of dogs microscopically positive for *Ascaris* eggs were subjected to molecular analysis using human-derived *Ascaris* eggs and the adult *A. lumbricoides* worm as positive controls. The pig-derived adult *A. suum* worm was used as a negative control.

**Extraction of DNA from eggs.** The purified *Ascaris* eggs were diluted in 1× PBS and 50 µL of this suspension was transferred into a 1.5-mL tube and centrifuged at 14,000 rpm for one minute. The supernatant was removed and 180 µL of ATL tissue lysis buffer (Qiagen, Hilden Germany) and 5 µL of proteinase K (10 µL/ml) (Qiagen) was added to the plug of eggs and incubated overnight at 56°C. This suspension was then subjected to 15 cycles of freeze-thawing at liquid nitrogen temperatures, followed by freeze-fracturing at liquid nitrogen temperatures.<sup>12</sup> One hundred seventy microliters of 170 µL AL buffer (Qiagen) and 10 µL of glassmilk matrix was added to the suspension, mixed, and incubated at 72°C for 10 minutes. Samples were then centrifuged at 14,000 rpm for one minute and the supernatant was discarded. Pellets were washed with 800 µL of AW buffer containing ethanol (Qiagen), vacuum-dried for five minutes to remove any remaining ethanol, and resuspended in 30 µL of elution buffer (Qiagen). The suspension was then incubated at 72°C for 15 minutes, centrifuged at 14,000 rpm for three minutes, and the supernatant was removed into a fresh tube and stored at -20°C until required.

**Extraction of DNA from *Ascaris* adult worms.** Twenty-five milligrams of tissue from each worm was suspended in 180 µL of ATL tissue lysis buffer (Qiagen) and 5 µL of proteinase K (10 µL/ml) and incubated overnight at 56°C. The suspension was centrifuged at 10,000 rpm for one minute and the supernatant transferred to a fresh 1.5-mL tube and boiled for 10 minutes. The suspension was then subjected to the glassmilk method previously described for the extraction of DNA from *Ascaris* eggs.

**Polymerase chain reaction.** A 734-base pair region of the internal transcribed region-1 (ITS-1), 5.8S, and ITS-2 genes of *Ascaris* was amplified using a modification of the previously published primer ZX5R<sup>8</sup> as a forward primer (5'-GATGTAATAGCAGTCGGCG-3') in the ITS 1 region and a newly designed reverse primer RTITSR (5'-GCCATTCACACAATATACGCTG-3') in the ITS 2 region. The PCR amplification was performed in 25-µL volumes with the final mixture containing 10–50 ng of DNA, 12.5 pmol of each primer, 800 µM of each dNTP, 1.5 mM MgCl<sub>2</sub>, 67 mM Tris-HCl (pH 8.8), 16.6 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.45% Triton X-100, 0.2 mg/ml gelatin, and one unit of Tth plus polymerase (Biotech International, Ltd., Perth, Australia). Reactions were performed on a PE 2400 (Perkin Elmer, Foster City, CA) thermal cycler. Samples were heated to 94°C for two minutes, 60°C for one minute, and 72°C for two minutes, followed by 50 cycles of 94°C for 30 seconds, 60°C for 20 seconds, and 72°C for 45 seconds and one cycle of 72°C for 7 minutes.

**PCR-RFLP.** All 31 dog-derived *Ascaris* egg samples, five human-derived *Ascaris* egg samples, and the human-derived

and pig-derived adult worms were subjected to digestion with the restriction endonuclease *Hae* III. This method was based on a previously published PCR-RFLP method.<sup>7</sup> Purified PCR ZX5R-RTITSR products (2 µL) were digested directly with 12 units (0.5 µL) of *Hae* III (Biotech International, Ltd.) at 37°C for 16 hours in a volume of 20 µL. Restriction fragments were separated by electrophoresis on 2.5% agarose gels, stained with ethidium bromide, and photographed. The size of the fragments were estimated using the 323-1S 100-base pair DNA ladder (New England BioLabs, Beverly, MA).

**Sequencing.** Five dog-derived *Ascaris* egg samples and the human-derived *A. lumbricoides* worm and eggs were subjected to sequencing following digestion with *Hae* III. The PCR products were purified using Qiagen spin columns (Qiagen) and sequenced using an ABI Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) according to manufacturer's instructions, except that the annealing temperature was increased to 60°C. The PCR products were sequenced in a forward direction. Sequences were analyzed using SeqEd version 1.03 (Applied Biosystems) and aligned with each other as well as the previously published sequences for *Ascaris*<sup>8</sup> using Clustal V.<sup>13</sup>

## RESULTS

**Dogs.** The prevalence of gastrointestinal parasites in dogs from the three tea estates sampled is summarized in Table 2. Nearly all (99%) dogs were infested with one or more zoonotic species of gastrointestinal parasites. Parasitic stages of 13 zoonotic gastrointestinal parasites were observed. More than 90% of the dogs were infected with hookworm. Infection with three species of parasites was more common (28.7%) than infection with two (27.7%), four (16.8%), one (10.9%), five (9.9%), or six species (5%).

Apart from encountering gastrointestinal parasites that use dogs as their definitive host, parasite stages presumed to be host-specific for humans, such as *Ascaris* spp., *Trichuris* spp., *Hymenolepis diminuta*, and *Isospora belli*, were also detected in dog feces. The *Trichuris* eggs in the dog feces morphologically resembled those of *T. trichiura*, with an average length

TABLE 2  
Prevalence of parasitic stages found in dog feces

Parasite	Phulbari (n = 41)	Addabarie (n = 40)	Balipara (n = 20)	Total prevalence (n = 101)
Hookworm spp.	95%	90%	95%	94%
<i>Spirocerca lupi</i>	24%	53%	45%	40%
<i>Ascaris</i> spp.	37%	18%	35%	31%
<i>Trichuris</i> spp.	20%	20%	60%	25%
<i>Spirometra</i> spp.	15%	38%	45%	28%
<i>Opisthorchis</i> spp.	12%	18%	25%	17%
<i>Taenia</i> spp.	22%	8%	10%	14%
<i>Toxocara canis</i>	20%	5%	5%	11%
Coccidia spp.	10%	5%	15%	9%
<i>Isospora belli</i>	5%	0%	0%	2%
<i>Hymenolepis diminuta</i>	12%	5%	5%	8%
<i>Dipylidium caninum</i>	7.5%	5%	5%	6%
<i>Sarcocystis</i> spp.	2.5%	2.5%	5%	3%
<i>Giardia duodenalis</i>	2.5%	0%	10%	3%
<i>Gnathostoma spinigerum</i>	0%	0%	10%	2%
<i>Strongyloides</i> spp.	0%	2.5%	5%	2%
<i>Cryptosporidium parvum</i>	2.5%	0%	0%	1%
<i>Paragonimus</i> spp.	0%	0%	5%	1%
<i>Entamoeba</i> spp.	0%	0%	5%	1%

of 52.7  $\mu\text{m}$  (range = 47.5–62.5  $\mu\text{m}$ ) and an average width of 25  $\mu\text{m}$  (range = 22.5–27.5  $\mu\text{m}$ ).<sup>14</sup>

The mean  $\pm$  SD age of dogs sampled was 2.56  $\pm$  2.31 years (range = three months to 12 years). Most (96%) dogs sampled were allowed to roam freely within the tea estate and 57% of the dog owners allowed their dogs access into the house. Only 2% of the households owning a dog had access to a veterinarian and had dewormed their dogs within the last six months. The majority (79%) of dogs were reported to defecate in areas other than the immediate surroundings of their owners' property. Of the remaining dog owners who did report observing their dogs defecate within or surrounding their properties, 17% never disposed of their dog's feces, 2% did so rarely, 2% once every week and 1% daily.

Multifactorial risk factor analysis showed a higher than expected prevalence of *Dipylidium caninum*, and *Coccidia* spp. in younger dogs ( $P < 0.10$ ). The average age of dogs positive for *D. caninum* and *Coccidia* was 1.0 and 1.1 years compared with 2.67 and 2.56 years for unaffected dogs. Dogs fed offal on a regular basis were 12.5 times more likely to harbor Taeniid eggs (prevalence = 13%) than those not fed offal by their owners (prevalence = 1%) ( $\chi^2 = 0.02$ , degrees of freedom [df] = 1,  $P < 0.02$ ). Worming and socioeconomic status of the owner did not influence the prevalence of parasitism ( $P > 0.10$ ). Dogs belonging to households where at least one person defecated outside were 3 and 2.6 times more likely to harbor eggs of *Ascaris* ( $\chi^2 = 3.66$ , df = 1,  $P < 0.02$ ) and *Trichuris trichiura* ( $\chi^2 = 2.82$ , df = 1,  $P < 0.05$ ), respectively. No sex predilection was found for other parasites.

The mean  $\pm$  SD intensity of *Ascaris* eggs in dog feces was 1,830  $\pm$  4,711.86 epg (range = 12.5–28,750 epg).

**Humans.** Hookworm spp. were the most common parasite in humans (42%), followed by *A. lumbricoides* (36%) and *T.*

*trichiura* (32%). Figure 1 shows the mean egg intensities for *Ascaris* among the different age groups in humans. The highest *Ascaris* egg intensities were found in children 3–5 and 6–10 years old. The mean  $\pm$  SD *Ascaris* egg count in infested humans was 2,016  $\pm$  4,887.35 epg (range = 12.5–35,150 epg).

Forty-five percent of individuals surveyed admitted to having direct contact with dogs on a regular basis. Although 90% of individuals were aware of rabies and its mode of transmission, only 6% were aware of canine parasite zoonoses. Multifactorial risk factor analysis showed no significant relationship between prevalence of *Ascaris lumbricoides* in individuals and dog ownership or contact.

**Results of *Ascaris* egg incubation.** *Ascaris* eggs isolated from fecal samples of dogs were shown to be viable. Between 50% and 80% of eggs embryonated and produced motile third-stage larvae after 2–6 weeks of incubation.

**Molecular characterization.** The amplified ITS from the *Ascaris* eggs found in dogs following digestion with *Hae* III is shown in Figure 2. For comparison, digestion patterns for human-derived *Ascaris* eggs and human-derived and pig-derived adult *Ascaris* worms are also shown. Two types of ITS have been previously reported;<sup>7,8,15</sup> the human-derived *A. lumbricoides* has a single *Hae* III site and gives rise to two bands, while the pig-derived *A. suum* has shown to possess an additional *Hae* III restriction site and gives rise to three bands. In some cases, predominantly pig, but occasionally in human-derived parasites, ITS regions with allelic polymorphism<sup>7,8,15</sup> at the first *Hae* III restriction site exist. All 31 dog-derived *Ascaris* egg samples showed a digestion pattern consistent with those described for human-derived *Ascaris*. A single *Hae* III restriction site gave rise to two bands measuring 258 and 467 base pairs. In comparison, the pig-derived parasite from Perth produced a five-band pattern measuring

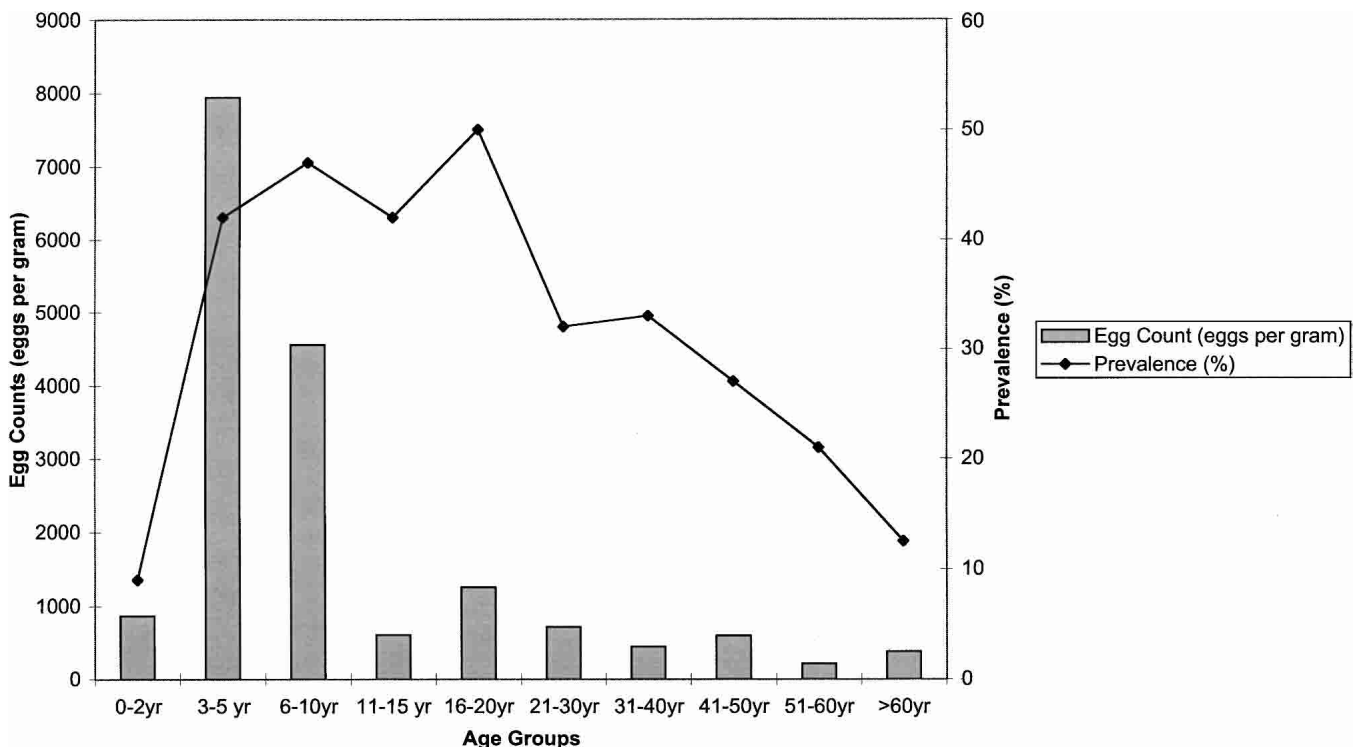


FIGURE 1. Prevalence and intensity (in eggs per gram of feces) of *Ascaris* infection among different age groups in humans.

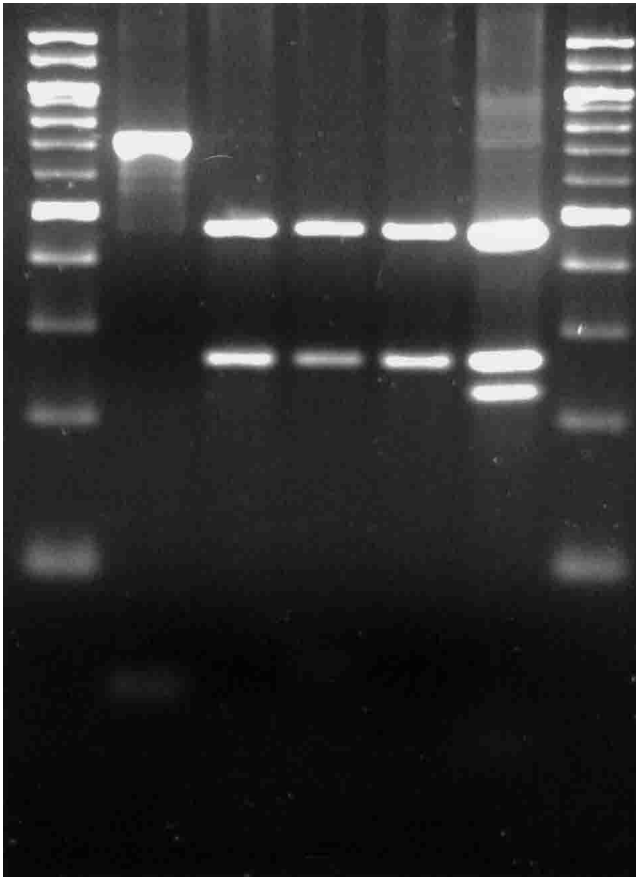


FIGURE 2. Determination of the source of *Ascaris* eggs in dog feces using restriction fragment length polymorphism analysis of the ZX5R-RTITSR polymerase chain reaction (PCR) products following digestion with the restriction endonuclease *Hae* III. From left to right, the gel shows representatives of undigested PCR product in lane 2, followed by digested products of dog-derived *Ascaris* eggs (lane 3), human-derived *Ascaris* eggs (lane 4), human-derived *Ascaris* adult worm (lane 5), and pig-derived *Ascaris* adult worm (lane 6). The size standards (lanes 1 and 7) are 100-base pair ladders (New England Biolabs, Beverly, MA).

35, 226, 258, 465, and 467 base pairs, showing it to possess allelic polymorphism at the first *Hae* III restriction site.

**Sequence analysis.** Sequences of the ITS-1, 5.8S, and ITS-2 regions of the five dog-derived *Ascaris* eggs showed 100% homology with the human-derived *Ascaris* eggs and the adult worm from Assam. Both dog-derived and human-derived sequences originating from Assam had a total length of 736 base pairs in comparison with the expected 734 base pairs, based on previously published sequences for *A. lumbricoides* ITS-1, 5.8S, and ITS-2 (GenBank accession numbers: AJ00895; AJ001507; AJ000895).<sup>8</sup> This was due a two base pair GC insertion in the ITS-1 region.

## DISCUSSION

The frequent finding of eggs of *A. lumbricoides* in dog feces and at a high intensity suggests that dogs are acting as significant mechanical transmitters of *Ascaris* infection for humans. *Ascaris lumbricoides* is estimated to infect 25% of the world's population<sup>16</sup> with 73% of the global infections occurring in

Asia<sup>17</sup> alone. Ascariasis has been widely recognized as a major contributor to childhood malnutrition and in heavy infections may also result in life-threatening complications such as intestinal obstruction<sup>18</sup> hepatobiliary and pancreatic disease.<sup>19,20</sup> The *Ascaris* eggs found in dog feces were still viable, even after passage through the intestine of the dog. Multifactorial analyses indicates that *Ascaris*-positive dogs are ingesting the feces from their owner's household, since a higher than expected prevalence was found in dogs that belonged to households where at least one human member defecated outdoors. Most dogs were allowed to roam freely within the estate and were observed to defecate in areas other than the surrounding property of their owners. It is therefore certain that dogs are also acting as disseminators and environmental contaminators of *Ascaris* eggs by increasing the net exposure of infective stages in contact with the human population. Eggs of *Ascaris* have been shown to adhere to items such as utensils, furniture, money, fruit, vegetables, door handles, and fingers in highly endemic areas<sup>21</sup> due to their sticky and resistant nature. Eggs become infective to humans within a 6–12-week period under favorable conditions.<sup>22,23</sup> Similarly, it is likely that the sticky-coated *Ascaris* eggs may adhere to the dog's coat during both coprophagy and defecation. Due to their highly resistant nature, survival of *Ascaris* eggs on the dog's coat for prolonged periods is possible. During this period the eggs may undergo further development and maturation to infectivity. The mode of transmission of *Echinococcus* to humans is primarily via direct contact with the dog. Matoff and Kolev demonstrated that the hairs of the anal region and paws possessed the most eggs, followed by the hairs of the thighs and belly, and lastly, the hairs of the back and muzzle.<sup>24</sup> In a similar fashion, it is possible that the dog may be acting as an incubator and direct vector of *A. lumbricoides*. However, there appeared to be no increased risk of infection with *Ascaris* in humans that admitted to having direct contact with dogs on a regular basis. It would therefore appear that the most important role of the dog in this endemic focus is in the contribution to environmental contamination.

Dogs were also shown to harbor eggs morphologically identical to *T. trichiura*. The dimensions for *Trichuris* eggs in dog feces are within the range described for eggs examined from female uteri of adult *T. trichiura* and those from human patients in a study conducted by Yoshikawa and others.<sup>14</sup> In contrast, the study found the parameters for *T. vulpis* to be larger (82 × 39 μm). The fact that there was a higher than expected prevalence of *Trichuris* eggs in the feces of dogs that belonged to households where at least one member defecated outside supports the possibility that these eggs were *T. trichiura*. Therefore, the role of the dog as a mechanical vector of *Trichuris* infection seems likely, although the absence of *T. vulpis* in these dogs remains to be explained. Autopsies on *Trichuris*-positive dogs to detect and differentiate adult worm morphology, or the advent of a molecular-based tool for identification of *Trichuris* eggs in fecal samples would prove useful in further investigating this finding.

It would appear that the *Hymenolepis* eggs recovered from the feces of dogs was of rodent origin, since none of the humans sampled were found positive for this parasite.

In this study, dogs belonging to both the staff and labor of the tea-growing communities of Assam were also shown to pose a significant public health threat to the community with regard to parasitic zoonoses. Similar observations have been

reported in surveys undertaken among stray or refuge dog populations worldwide.<sup>2-4,25</sup> In all cases, hookworms were found to be the most common parasite. The extremely high prevalence of hookworm infection in dogs in the tea estates could play a significant role in contributing to the incidence of creeping eruptions among the human population.

An unexpected finding was the high prevalence of *Spirometra* spp. and *Opisthorchis* spp. in dogs. This was in contrast to the study carried out on stray dogs in Bangkok where the related parasites *Dipyllobothrium mansonii* and *Opisthorchis viverrini* were not common (both had low prevalences of 1.9%).<sup>2</sup> The high prevalence of *Spirometra* spp. is a reflection of the fact that most dogs were allowed to roam and had access to paratenic hosts as food sources. It is probable that dogs acquire infection with *Opisthorchis* spp. by ingesting raw fish offal fed to them by their owners. Dogs are therefore acting as important indirect reservoirs of these parasites for humans. Conversely, the prevalence of the enteric parasite *Giardia duodenalis* is markedly lower than in previous surveys done in well-cared for dogs,<sup>26,27</sup> as well as semi-domesticated dogs.<sup>5,28</sup> It would appear that *Giardia* infection in dogs does not contribute a major zoonotic risk to humans in the community studied.

Age-related immunity was only found to affect infection with *Dipylidium* and *Coccidia* spp. in dogs with a higher than expected prevalence and in dogs less than one year of age. There is insufficient data available for risk factors associated with parasite prevalence in stray and uncared for dogs for a detailed comparison to be made. In the only similar study done in dogs belonging to Australian Aboriginal communities in the Kimberley region (Wilks KM, unpublished data) it was found that puppies less than six months old had a higher prevalence of hookworms *Toxocara canis* and *G. duodenalis*. It is unusual that other parasites in our study did not have a similar age effect. A possible explanation for a lack of age-related immunity in our study could be the low percentage (6%) of dogs sampled that were less than six months of age and the overall high prevalence of parasitism.

The species of taeniid eggs could not be distinguished using microscopy alone. It is likely that a gross underestimation of the prevalence of this family has been observed in this study due to the erratic nature of proglottid and egg shedding by these parasites. Nevertheless, a higher than expected prevalence of taeniid eggs was found in dogs that were fed offal on a regular basis by their owners. This finding is consistent with other studies carried out in Bangladesh<sup>29</sup> and Nepal,<sup>30</sup> where the highest prevalence of *Echinococcus* was observed in areas in and around slaughterhouses.

Questionnaire results showed that even though nearly half of the individuals surveyed had direct contact with dogs, they lacked awareness and knowledge of canine parasitic zoonoses. This, in addition to the complete lack of veterinary attention exacerbates the risk of transmission of canine parasitic zoonoses to the human community.

In summary, we have demonstrated that in communities where promiscuous defecation patterns exist, dogs play a major role in broadening the range of dissemination and environmental contamination of infective stages of *Ascaris*. Educating communities on the importance of using latrines would contribute in lowering the level of *Ascaris* infection among the population.

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