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The prevalence and distribution of gastrointestinal parasites of stray and refuge dogs in four locations in India

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ABSTRACT

A gastrointestinal parasite survey of 411 stray and refuge dogs sampled from four geographical and climactically distinct locations in India revealed these animals to represent a significant source of environmental contamination for parasites that pose a zoonotic risk to the public. Hookworms were the most commonly identified parasite in dogs in Sikkim (71.3%), Mumbai (48.8%) and Delhi (39.1%). In Ladakh, which experiences harsh extremes in climate, a competitive advantage was observed for parasites such as *Sarcocystis* spp. (44.2%), *Taenia hydatigena* (30.3%) and *Echinococcus granulosus* (2.3%) that utilise intermediate hosts for the completion of their life cycle. PCR identified *Ancylostoma ceylanicum* and *Ancylostoma caninum* to occur sympatrically, either as single or mixed infections in Sikkim (Northeast) and Mumbai (West). In Delhi, *A. caninum* was the only species identified in dogs, probably owing to its ability to evade unfavourable climatic conditions by undergoing arrested development in host tissue. The expansion of the known distribution of *A. ceylanicum* to the west, as far as Mumbai, justifies the renewed interest in this emerging zoonosis and advocates for its surveillance in future human parasite surveys. Of interest was the absence of *Trichuris vulpis* in dogs, in support of previous canine surveys in India. This study advocates the continuation of birth control programmes in stray dogs that will undoubtedly have spill-over effects on reducing the levels of environmental contamination with parasite stages. In particular, owners of pet animals exposed to these environments must be extra vigilant in ensuring their animals are regularly dewormed and maintaining strict standards of household and personal hygiene.

1. Introduction

Canine gastrointestinal parasites can be divided into three broad categories; those of veterinary importance, for example *Spirocerca lupi*, those of public health importance, for example *Echinococcus granulosus* and those that produce morbidity in both canines and humans, namely hookworms and *Toxocara canis*. All three categories of gastrointestinal parasites are known to be endemic in India (Traub et al., 2005), especially among stray and semi-domesticated dogs. These parasites may be transmitted to humans either directly, through the ingestion of infective stages via close contact with a dog; or indirectly, through skin penetration or ingestion of infective stages in the

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environment, including those that may be food- or water-borne.

Although investigated, there appears to be a lack of widely accessible up-to-date information available on the prevalence and distribution of canine gastrointestinal parasites in India. The population of stray or community dogs in India is estimated as high as 20 million, despite efforts to curb numbers through sterilisation campaigns (Menezes, 2008). These uncared for animals not only pose an important source of parasites for the 5 million-odd 'owned' or 'pet' dogs, but also for the general public.

There is substantial evidence to show that canine intestinal parasites are a public health concern in India particularly in relation to hydatid disease, toxocarosis and zoonotic ancylostomosis (reviewed by Traub et al., 2005).

This study aimed to determine the prevalence and distribution of gastrointestinal parasites of veterinary and public health importance in stray dogs from four distinct geographical and climatic locations in India, the north-east (Sikkim), far north (Ladakh, Jammu and Kashmir), north (Delhi) and west (Mumbai).

2. Materials and methods

2.1. Study sites and sampling

The study was stratified to include four climatic zones, wet tropical (Mumbai), semi-arid (Delhi), arid mountainous (Leh, Ladakh) and humid temperate (Gangtok, Sikkim) based on information produced by The World Meteorological Organisation.

Field work for this project was conducted between June and September 2008 with in-kind support provided by veterinary charity-based organisations conducting animal birth control programmes in Ladakh, Sikkim, Delhi and Mumbai (Vets Beyond Borders, Jeevasharam, Krishanasharam and In Defence of Animals, India). The refuge centres provide shelter, de-sexing and veterinary care where appropriate, for dogs that are either rescued from the streets or abandoned by their owners. An estimate of each animal's age was made (based on dentition and body size) and classified as puppy (less than 6 months old), juvenile (between 6 months and 1 year old), adult (between 1 and 7 year old) and geriatric (more than 7 year old). Each animal's sex, body condition score and source (stray or refuge) was noted. A single stool sample was collected per-rectum from 411 dogs from Ladakh ($n = 86$), Sikkim ($n = 94$), Delhi ($n = 110$) and Mumbai ($n = 121$) and preserved separately in 10% formalin and in 90% ethanol for future microscopic screening and molecular analysis, respectively. This project was approved by the University of Queensland Animal Ethics Committee.

2.2. Parasitological techniques

Formalin preserved faecal samples were initially subjected to a sedimentation in water technique followed by faecal flotation using zinc sulphate ($ZnSO_4$) (S.G. 1.20). Faecal samples which were positive on microscopy for the presence of taeniid and hookworm eggs were further subjected to molecular analysis.

2.3. Extraction of the genomic DNA

Approximately 25 mg of faeces was washed once with 1 × TE buffer (40 mM Tris HCl, 10 mM EDTA), then boiled (100 °C) for 10 min to reduce the presence of inhibitors.

For taeniid egg-positive samples, DNA was extracted using QIAmp DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer's protocol except that samples were subjected to an initial overnight incubation step (200 μl ASL buffer and 30 μl proteinase K) followed by 5 cycles of freeze-thawing and 3 cycles of freeze-fracturing prior to DNA extraction.

For hookworm egg-positive samples, Zirconia beads (Daintree Scientific, Australia) were added to faecal samples and the samples homogenised for 5 min at high speed using a bench-top Beadbeater (Biospec Products, Bartlesville OK). Samples were then centrifuged at 11,700 × g for 1 min before 100 μl of supernatant was transferred to a clean 1.5 mL tube.

2.4. PCR identification of taeniid eggs with multiplex PCR

A multiplex PCR using primers 'Cest1-5' was utilised for the detection and identification of taeniid eggs (Trachsel et al., 2007). The PCR amplicons were electrophoresed on a 2% agarose gel run in 1× TE buffer, stained using ethidium bromide and visualised using a GelDoc system (Bio-rad).

2.5. PCR-RFLP for hookworm egg species identification

Due to faecal sample exhaustion, 75–80% of hookworm positive samples could be subjected to species identification using PCR-RFLP (Palmer et al., 2007; Traub et al., 2004b). PCRs were carried out using an inhibitor-resistant DNA polymerase on each crude faecal lysate. Lysates were diluted 1/5 in 1× TE buffer and the 25 μl reactions carried out using 0.2 U of Phusion Hotstart II High Fidelity DNA polymerase (Thermo Scientific, catalogue # F-5495), 12.5 pmol of each primer, 0.2 μl of 20 mg/mL bovine serum albumin and 2 μl diluted DNA. Cycling conditions included an initial denaturation at 99 °C for 30 s, then 50 cycles of 98 °C for 10 s, 60 °C for 15 s and 72 °C for 30 s. The RFLP products were run on 1–2% agarose gels in 1× SB (Sodium Borate) buffer, stained using SYBR Safe (Invitrogen/Life Technologies) and visualised using a GelDoc system (Bio-rad).

2.6. Presence of amplifiable DNA

Samples that were microscopy positive for hookworm but which failed to generate a result by PCR were tested for the presence of PCR inhibitors by using published primers (18SEUDIR and 18SEUINV) that amplify a 140 bp fragment of the 18SrRNA gene of eukaryotes (Fajardo et al., 2008). PCR products were visualised on a 1% agarose gel in 1× SB (Sodium Borate) buffer, stained using SYBR Safe