Canine gastrointestinal parasitic zoonoses in India

Rebecca J. Traub\textsuperscript{1}, Ian D. Robertson\textsuperscript{1}, Peter J. Irwin\textsuperscript{1}, Norbert Mencke\textsuperscript{2} and R.C.A. Andrew Thompson\textsuperscript{1}

\textsuperscript{1}World Health Organization Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch 6150, Australia
\textsuperscript{2}Bayer HealthCare AG, Animal Health Division, D-51368 Leverkusen, Germany

Although well recognized and studied in developed countries, canine parasitic zoonoses pose a lowly prioritized public health problem in developing countries such as India, where conditions are conducive for transmission. A study of the most recent parasite survey determining prevalence and epidemiology of canine parasitic zoonoses among tea-growing communities of northeast India demonstrated the endem-icity of the problem. This particular study serves as a model using conventional, as well as molecular parasitological, tools to provide novel insights into the role of dogs as mechanical transmitters of human parasites such as \textit{Ascaris} and \textit{Trichuris}, and discusses the risks dogs pose with regards to zoonotic transmission of hookworms and \textit{Giardia}.

The potential role of companion animals as reservoirs for zoonotic diseases has been recognized as a significant public health problem worldwide \cite{1}. The subject has received attention, priority and coverage in medical literature of developed countries primarily because of the availability of resources for research and the importance of the human–animal bond within the companion animal industry \cite{2}. The attitudes and economic status of pet owners in these developed countries also ensure that the available veterinary resources are well used \cite{3}. Canine parasitic zoonoses, however, are far from confined to developed countries. In developing countries such as India, uncontrolled populations of stray and semi-domesticated dogs exist in close proximity to increasing densities of human populations in urban environments \cite{4}, and humans often share a close relationship with semi-domesticated dogs in rural settings (Figure 1). In these socioeconomically disadvantaged communities, the poor levels of hygiene and overcrowding, along with a lack of veterinary attention and zoonotic awareness, exacerbates the risks of disease transmission \cite{5}. There is an estimated 19.2 million stray dogs in India alone and, despite control efforts, the dog population is believed to be rising \cite{6}. Current animal welfare legislation in India comprises a program of sterilization, vaccination and re-homing, together with humane euthanasia of terminally

\begin{figure}[h]
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\includegraphics[width=\textwidth]{image1.png}
\caption{Children in rural Assamese communities share a close relationship with semi-domesticated dogs. Photographs taken by Rebecca J. Traub.}
\end{figure}
ill or rabid animals (http://envfor.nic.in/awb/awb.html). Because of restricted resources, the program primarily targets major cities with high densities of stray animals rather than rural populations, where interactions between domestic and silvatic cycles of zoonoses thrive [7]. Rabies is by far the most serious, well-recognized and well-documented canine zoonosis in India. At least 30,000 human deaths are reported each year, mainly in children [6]. However, the surveillance and control of canine zoonoses in countries like India are lowly prioritized in contrast to other public health problems with greater numbers of morbidity and mortality, such as malaria, tuberculosis, childhood diseases and HIV/AIDS [8].

Disease recognition and priority

Resource allocation to diseases with high levels of morbidity and mortality is obviously understandable. The WHO Global Burden of Disease studies draw on a wide range of data sources to develop estimates of incidence, prevalence, severity, duration and mortality of diseases worldwide. However, in some regions, data are sparse or evidence is uncertain, and therefore estimates of disease burden might not be accurately calculated [8]. This emphasizes the importance of adequate and ongoing surveillance and research to gain a more accurate estimate of disease burden and, therefore, to define priorities at a local level. Most studies of canine parasitic zoonoses in India are of sporadic case reports, or based on seroprevalence studies of the more common canine zoonoses such as hydatid disease [9–11] and toxocariasis [12,13]. However, despite the strong evidence to show the endemcity of these serious zoonoses, knowledge of the prevalence and risk factors associated with zoonotic parasites of dogs in India is largely lacking. Until recently, the only general prevalence studies of gastrointestinal (GI) parasites in dogs [14–17] focused on strays in urban settings and were conducted >30 years ago. There is an urgent need to obtain more-recent parasite data. A more comprehensive and accurate approach to studying the transmission of canine parasitic zoonoses necessitates simultaneous parasite surveys conducted in humans and animals from the same environment to correlate data on an epidemiological and molecular level. Epidemiological observations such as statistically significant univariate and multivariate analyses, associated with the prevalence of parasites in both humans and dogs, allow identification of significant risk factors for infection. Molecular tools can then provide the level of discrimination that is often not achieved by microscopy alone and which is needed to differentiate between species or intraspecific variants of the relevant parasites. Molecular characterization can provide important information about the parasite’s zoonotic potential and transmission dynamics within a community. A survey aimed at unraveling epidemiological and zoonotic relationships of canine GI parasites in an endemic teagrowing community in Assam (Figures 1a,b) [18–21] provides an example of such a study, and highlights the advantages of combining conventional and molecular epidemiological tools. This study will be discussed in context to the current knowledge of canine parasitic zoonoses in India.

Zoonotic ancylostomiasis

Cutaneous contact with the infective stages of canine hookworms can lead to the development of cutaneous larva migrans (CLM) in humans. Infections with Ancylostoma caninum can result in eosinophilic enteritis (EE) [22,23]. Patent adults of Ancylostoma ceylanicum have been shown to produce patent infections in humans [24,25], and such infections in dogs in Southeast Asia are being increasingly recognized [26]. CLM is commonly reported in travelers returning from the tropics [27] and in areas endemic for canine hookworm infection [28]. Reports on the occurrence of CLM in India are sporadic and restricted to case reports presented by dermatology clinics and hospitals. Individuals usually have a history of prolonged exposure to wet surfaces in public places such as beaches or bus stops where stray dogs are known to roam freely [29–32]. Although CLM appears to be uncommon, it is probably endemic in socioeconomically disadvantaged communities that are unable to afford proper footwear and often sleep in environments heavily contaminated by faeces of stray dogs [33]. The relatively benign character of infection in humans and the low socioeconomic strata of people it most commonly affects are probably reasons why CLM is considered as of minor importance in India. There have been no reports of A. caninum-induced EE in India but, because of the widespread nature of A. caninum and the obscure clinical presentation, it is plausible that the condition is more widespread than reported and is not actively investigated or misdiagnosed.

Parasite surveys of stray dogs conducted in Madhya Pradesh [15] and tea-growing communities in Assam [21] revealed that A. caninum is the most common infection with a prevalence of 89% and 72%, respectively. Unidentified hookworm species were also found to be common in Miraj (prevalence 50.5%) [34], Bangalore (prevalence 73%) [16], Uttar Pradesh (prevalence 71%) [14], and Calcutta (prevalence 91–99%) [17]. Development and application of a species-specific PCR restriction fragment length polymorphism (PCR–RFLP) assay for screening Ancylostoma spp. directly from eggs in faeces detected Ancylostoma braziliense in India for the first time [21]. Up to 60% of dogs in tea-growing communities of Assam harbored A. braziliense, with 37% of dogs having mixed infections with A. braziliense and A. caninum [21]. This was believed to account for the high incidence of CLM reported in the human population among the tea estates, exacerbated by the fact that the majority of individuals (67%) admitted to walking barefoot while outdoors [35]. There are no reports of A. ceylanicum infections in dogs in India despite the isolation of the hookworm in 16 out of 173 humans from the Hoogly region on the outskirts of Calcutta [36]. Civet and jungle cats were the most common hosts for this parasite in and around this region [36].

Toxocariasis

Humans become infected with Toxocara when they accidentally ingest embryonated eggs through contamination of infected soil, food, fomites or by direct contact with dogs [37,38]. While most people infected by Toxocara do not develop overt clinical disease, three clinical syndromes
have been associated with *Toxocara* infection in humans: (i) visceral larva migrans (VLM); (ii) ocular larva migrans (OLM); and (iii) covert toxocariasis [39]. There is minimal literature available on the seroprevalence or the incidence of toxocariasis in humans in India. The only random survey conducted in a rural area in Haryana found that 6.4% out of the 94 individuals tested were seropositive to *Toxocara canis* [13]. History of pica (abnormal craving for non-food substances, e.g. dirt, paint or clay) or contact with dogs could not be obtained to ascertain risk factors for seropositive individuals. In two separate studies conducted in Chandigarh and New Delhi, 7 out of 30 and 14 out of 68 suspected ocular cases of toxocariasis were seropositive for *T. canis* [12,13]. Parasite surveys conducted >30 years ago found *T. canis* to be the most common parasite of stray dogs in Miraj (prevalence 55.8%) and Calcutta (prevalence 82%) [17,34]. Unfortunately, the ages of these dogs were unknown and it is possible that a younger population of dogs was surveyed. By contrast, the prevalence of *T. canis* in adult stray dogs in Madhya Pradesh was 2.7% [15]. The most recent study showed that the prevalence of *T. canis* in tea-growing communities in Assam was 11% [18]. In addition, male dogs were found to be 10.5 times more likely to harbor *Toxocara* (prevalence 18.3%) compared with that in female dogs (2.1%), in agreement with earlier studies [40,41]. In another study, 46% of public parks and 32% of school grounds in Andhra Pradesh were contaminated with *Toxocara ova* [42].

**Echinococcosis**

Dogs and other suitable canids (dingos, wolves and foxes) are the major definitive host for *Echinococcus granulosus*, the cause of unilocular hydatid disease (cystic echinococcosis) in livestock and humans. The disease is regarded as one of the most important and globally widespread helminth zoonoses. There are numerous reports of human cystic hydatid disease throughout India. Over 400 cases of cystic echinococcosis were sporadically reported in the medical literature between 1953 and 1972 [9,43-45] and, recently, a further 71 cases were reported over a seven-year period in Pondicherry [46]. An isolated case of alveolar echinococcosis caused by *E. multilocularis* was also reported from a man from the hill regions of Kashmir [47], but no animal reservoir has yet been identified. In India, ideal conditions exist for the establishment, propagation and dissemination of cystic echinococcosis in both humans and livestock. Several factors including biological, cultural, educational, socio-economic, agricultural, environmental and faunated related contribute to the transmission of infection [48]. A lack of education and knowledge about the life cycle of the parasite, as well as the lack of legislation for meat inspection and offal disposal at local abattoirs [49], significantly contributes to domestic cycles of transmission. Moreover, home slaughter, especially for religious events, is common throughout the country, and stray and semi-domesticated dogs are given ample opportunity to be exposed to infection [50]. A wide range of captive and wild animals such as deer, wild buffalo, elephants and wild boar are known to act as intermediate hosts, and wolves, foxes and jackals as definitive hosts for the parasite [48]. The existence of a silvatic cycle of perpetuation is therefore likely, but needs investigation. Examination of stray dogs throughout the country has shown the prevalence of *E. granulosus* to range between 3.5% in villages in Amritsar to 33% in the town of Kurnool. In Kurnool, the prevalence of infection was higher in stray dogs near slaughter houses [51] compared with those in stray dogs away from the area. In Kashmir, dogs that were associated with nomadic and semi-nomadic pastoral communities in hilly areas had a higher prevalence of *E. granulosus* (35%) than that in stray dogs near the vicinity of slaughter houses in urban areas (11–17%) [52]. In contrast to previous studies in other parts of India, none of the dogs belonging to tea-growing communities in Assam were found to harbor *Echinococcus granulosus* using a copro-antigen ELISA test (R. Traub, PhD Thesis, Murdoch University, 2003) despite access to offal from locally slaughtered goats, cattle and pigs [18].

The prevalence of cystic echinococcosis in each intermediate host varies considerably throughout India. Varying percentages of hydatid cysts have been recovered from sheep, goats, cattle and buffalo throughout the country, and camels in the west [49,52,53]. However, there are conflicting reports on the proportion of fertile cysts recovered from each host, even in similar geographical areas. Buffaloes in India can be infected with sheep (G1), cattle (G5) and buffalo (G3) strains of *E. granulosus* [54]. However, molecular characterization of cysts from other intermediate hosts, including humans, has yet to be carried out. The buffalo is generally considered the most significant host for sustaining the life cycle [55–57], which could be partly explained by the older age (12–16 years) at which they are slaughtered and the differences in strain biology of the parasite in the region. Despite the parasite’s significant economic and public health impact on the subcontinent, production losses in livestock and human morbidity and mortality as a result of cystic echinococcosis has yet to be assessed on a national scale [48].

**Giardiasis**

An estimated 1.5 million children in India die from diarrheal-related illnesses each year. *Giardia* is often one of the many pathogens that infect children together with rotavirus, bacterial pathogens such as *Shigella, Salmonella, Campylobacter*, enteroinvasive *Escherichia coli*, *Vibrio cholerae* and *protozoa* such as *Entamoeba histolytica* [58]. It is therefore often difficult to accurately attribute diarrheal symptoms to *Giardia* alone because many infections in children are often of mixed etiology [59]. *Giardia* was implicated as the cause of acute diarrheal in 11% and of persistent diarrhea in 15% of children presented to hospitals in Delhi [60,61]. In addition, a staggering 67% of children with recurrent abdominal pain were harboring the parasite in Kashmir [62]. Peak incidences of giardiasis usually occur during the monsoons or in dry seasons in drought-affected areas, when people are forced to obtain water from contaminated stagnant water supplies shared with animal sources. Until recently, there was no information regarding the prevalence of *Giardia* in dogs in India. Moreover, the zoonotic potential of canine *Giardia* remained largely an
unresolved issue. In tea-growing communities of Assam, 8% of humans were positive for Giardia measured by flotation and microscopy, and 20% of dogs were shown to harbor the protozoan by PCR [20]. Molecular and epidemiological tools applied with the aim of determining the zoonotic potential of canine Giardia infections within the communities in Assam simultaneously characterized isolates of Giardia recovered from both dogs and humans [20]. Phylogenetic analysis of the triosephosphate isomerase gene placed all isolates of Giardia recovered from dogs within the same genetic groups as those harbored by humans, Assemblages A and B. Further evidence for zoonotic transmission was supported by showing a highly significant relationship between the prevalence of Giardia in humans and the presence of a Giardia-positive dog in the same household. However, genetically similar isolates of Giardia could only be characterized in three out of the 11 instances where Giardia-positive dogs and humans shared the same household. An unusual dominance of Assemblage AII genotypes was found in dogs, which indicated that this genotype is most significant when dealing with zoonotic potential. The predominance of one genotype of Giardia in dogs also suggests that dogs harbored true infections with Giardia as opposed to mechanical passage of human Giardia cysts via coprophagy [20] and were therefore acting as true reservoirs for human infection. The application and careful interpretation of appropriate molecular tools, in conjunction with epidemiological data, in a locally defined endemic focus, allowed the fundamental question concerning the zoonotic transmission of canine Giardia to be proven for the first time in a natural setting [20].

The mechanical role of dogs as transmitters of human parasites

The role of the dog as a mechanical reservoir for human parasites was explored for the first time in tea-growing communities in Assam, where parasite stages presumed to be host-specific for humans such as Ascaris spp., Trichuris trichiura, Hymenolepis diminuta and Isospora belli were encountered in dog faeces [18]. The Ascaris eggs isolated from 30% of dog faeces were viable, producing motile larvae following two to six weeks of incubation at room temperature. A PCR–RFLP technique developed to identify the species of Ascaris eggs directly from faeces detected significantly high intensities of Ascaris lumbricoides in tested dogs [18], demonstrating the role of the dog as a significant mechanical transmitter and environmental disseminator of human Ascaris by increasing the net exposure of infective stages in contact with the human population. This important yet largely ignored aspect of mechanical transmission of zoonotic diseases by dogs is undoubtedly present in other socioeconomically disadvantaged communities in Asia, South America, Africa and Australia. Up to 25% of dogs in the tea-growing communities in Assam also harbored eggs that were morphologically identical to those of Trichuris trichiura rather than those of Trichuris vulpis [18,63]. The lack of T. vulpis in dogs in these tea-growing communities is unusual, but not unexpected. In >200 stray dogs in Bangalore [16], Uttar Pradesh [14] and Madhya Pradesh [15], T. vulpis was not recovered. Trichuris vulpis was recorded in dogs in Miraj by copro-examination [34], but 25% of these dogs were also shown to carry Ascaris eggs and it is possible that the species of Trichuris were incorrectly identified.

The application of molecular tools

Despite the challenges and drawbacks of working in developing and isolated rural communities with limited laboratory facilities, the studies conducted in tea-growing communities in Assam [19] provide an example of how the most refined molecular tools can be applied to unravel epidemiological and zoonotic relationships. First, minimal quantities (~0.5–1.0 mg) of faeces are required for molecular screening and characterization of parasites, which makes their transportation by air convenient [20]. The chemical properties of potassium dichromate in solution or 20% v/v dimethyl sulfoxide (DMSO) make them a safe transport vehicle for biological material. Moreover, the DNA for all parasite species in whole faeces preserved in these samples can remain stable even at high temperatures (30–40 °C) for prolonged periods before analysis. Therefore, the hurdle of applying molecular-based tools when conducting parasite surveys does not lie in the misconception of having a specialized laboratory on-site, or time delays in sample processing, but in the considerable costs of equipment, reagent and personnel associated with processing the samples. However, development of highly sensitive, specific, rapid and automated PCR-based techniques capable of multiplex-system identification in future would permit molecular-based screening to be conducted in a more cost-effective and efficient manner [64].

The ability to selectively and sensitively detect and genetically characterize parasitic stages directly from faeces has served as a major advantage (Table 1) for studying the epidemiology of parasites within a population. Molecular tools developed and applied, in combination with classical parasitological and epidemiological methods, to detect, diagnose and genetically characterize parasites such as A. lumbricoides [18], canine hookworms [21] Giardia duodenalis [20,65], E. granulosus [66], E. multilocularis [67] and Opisthorchis [68] alleviates the need for tedious morphological identification of adult parasites following anthelmintic purging or at necropsy, and also the need for laborious laboratory amplification [66] of parasites such as Giardia using in vitro or in vivo cultivation techniques. Molecular tools also determine parasite prevalence with far greater accuracy than conventional microscopic methods, not only to species [21], but also at the genotype level [20,69,70].

The studies in Assam also demonstrate how conducting GI parasite surveys in dogs alone can provide a misleading picture with regards to the realistic threat dogs pose to a community, without correlating data with their human counterparts and vice versa. For example, Opisthorchis-like eggs were detected in 17% of dogs in Assam [18], but the prevalence of these flukes in the human population was negligible because of proper fish preparation practices by the people in these communities (R. Traub, PhD thesis, Murdoch University, 2003). The endemity of these foodborne trematodes in dogs despite their rare incidence among the human population is a reflection of how
reservoir hosts could on their own suffice in maintaining the life cycles of these zoonotic flukes.

**Control of canine parasitic zoonoses**

The role of education in preventing infection with parasitic zoonoses has been well documented and veterinarians have been identified as a potential provider for this education [2]. In tea-growing communities in Assam, nearly all (99%) dogs were infested with at least one or more zoonotic species of GI parasite [18]. Questionnaire results reflected a general lack of knowledge towards canine parasitic zoonoses. Although nearly 50% of the individuals surveyed had direct contact with dogs, only 6% had awareness and knowledge of canine parasitic
zoonoses. Almost all (98%) dogs lacked veterinary attention, were not vaccinated against rabies and were not dewormed [18]. In rural communities, implementation of a chemotherapy program to control GI parasites in dogs would not be highly realistic or logistically feasible because of the costs of implementation and frequency at which dosing would be required (every six to eight weeks). Instead, it would be advised that a combination of dog population control and public education be implemented. Local government-appointed veterinary officers could assist medical practitioners in educating the community about responsible pet ownership through already existing health education programs and provide government-funded outlets where owners could bring their dogs to be vaccinated and sterilized. Most non-chemotherapeutic measures taken to control geohelminth infections, such as improvements in education, sanitation and hygiene, would also help reduce the incidence of canine parasitic zoonoses in rural India.

Acknowledgements

We thank Bayer Health Care, Animal Health Division, Leverkusen, Germany, for providing financial support for studies conducted at the tea-growing communities of Assam, and to Williamson Magor and Co. for permission for research to be conducted at the tea estates owned by them.

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