PREVALENCE AND ZOONOTIC POTENTIAL OF ANCYLOSTOMIASIS IN DOGS IN BUMTHANG DISTRICT, BHUTAN

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ABSTRACT: Canine hookworm infection is endemic in Southeast Asian countries. Canine hookworms include *Ancylostoma caninum*, *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Uncinaria stenocephala* which represent a potential public health concern. In human, canine hookworms can cause patent enteric infections and cutaneous larval migrans. However, there are limited data available on the prevalence of canine hookworms in Bhutan. This study was conducted to determine the prevalence of ancylostomiasis among dogs in Bumthang district. Faecal and whole blood samples were collected from 177 dogs (100 free-roaming and 77 pet dogs) in Bumthang. Faecal samples were processed and examined for hookworm eggs using standard parasitological methods. The overall prevalence of ancylostomiasis in Bumthang was 5.6% (95% CI; 2.25-9.05). The prevalence was highest in Chhoekhor Toed with 9.1% followed by 8.8% in Chamkhar town, 5.4% in Tang, 2.6% in Ura and none in Chumey. The prevalence was comparatively higher in pet dogs (6.5%, 95% CI; 0.73-9.27) than free-roaming dogs (5.0%, 95% CI; 0.99-11.99). However, there was no statistical significance between the prevalence in pet dogs and free-roaming dogs ($p > 0.05$). This study indicates that dogs in Bhutan are known reservoir hosts of human hookworm infection. The finding necessitates the inclusion of dogs in any interventions to combat human hookworm infections in the country. Further, the molecular characterization of the *Ancylostoma* species needs to be carried out in order to understand *Ancylostoma* species and species-specific potential public health risks in the country.

Keywords: ancylostomiasis; dogs; prevalence

1. INTRODUCTION

The canine hookworm infection is endemic in Southeast Asian countries with a prevalence ranging from 70% to 100% (Thompson et al 2011). Some of the dog parasites, such as *Toxocara canis* and *Ancylostoma spp.* are reported of significant public health concern, especially in developing countries and communities that are socioeconomically underprivileged (Brahmbhatt et al 2015). In these communities, poor levels of hygiene and overcrowding combined with the lack of veterinary attention and zoonotic awareness are attributed to aggravating the risk of disease transmission (Craig et al 2000). The global infection prevalence for any hookworm in human in 2016 was around 450.68 million with a disease burden of around 1.8 million DALYS in 2015 (Stracke et al 2020). In Southeast Asia and the Pacific, *Ancylostoma ceylanicum* is highly endemic and is estimated to infect around 100 million people, challenging the current hookworm paradigm (Stracke et al 2020).

Dog hookworms include *Ancylostoma caninum*, *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Uncinaria stenocephala* (Mahdy et al 2012). These hookworms are zoonotic and can be transmitted to humans from dogs (Stracke et al 2020). The adult hookworms live in the intestines and shed eggs in the environment; which then develop into infective third stage larvae (Murphy et al 2013). In dogs, these hookworm larvae normally mature in the intestinal tracts and cause anaemia and hemorrhagic diarrhoea depending on the parasitic burden and age of the dogs (Brahmbhatt et al 2015).

In humans, dog hookworms can cause cutaneous larval migrans and eosinophilic enteritis, either through percutaneous or oral transmission (Murphy et al 2013). *Ancylostoma braziliense* is the predominant species that causes cutaneous larval migrans although *Ancylostoma caninum*, *Ancylostoma ceylanicum* and *Uncinaria stenocephala* may be involved less frequently (Mahdy et al 2012). *Ancylostoma ceylanicum* is the only zoonotic hookworms known to produce patent intestinal infections in humans (Murphy et al 2013). *Ancylostoma caninum* can cause eosinophilic enteritis, but does not seem to become patent (Murphy et al 2013). Thus, the free roaming (stray) and owned (pet) dogs play an important role in transmitting the disease and act as reservoirs for human hookworm infections (Brahmbhatt et al 2015).

Bhutan has an estimated population of 72621 free-roaming dogs out of which, 37.1% (26951) are free-roaming owned dogs and 62.9% (45670) are free-roaming un-owned dogs as per the nationwide dog population survey carried out in December 2021 (unpublished data). The dog population and KAP surveys conducted in Bumthang during July 2020 observed a total of 2278 dogs in the district; out of which, 60.4% (1376) were free-roaming un-owned dogs and 39.6% (902) were owned dogs (RLDC 2021).

Dogs are culturally and socially accepted by the Bhutanese community (DoL 2020). Although most Bhutanese don't own specific dogs, they do feed them and consider them a friendly presence (DoL 2020). In rural places in Bhutan, owned dogs are used for guarding crops from wild animals and herding the livestock, while in urban areas dogs are mainly kept as pets and for guarding premises (DoL 2020). There are also free-roaming un-owned or stray dogs which are mainly seen in the urban areas posing numerous dog-related issues and public health threats (DoL 2020). Limited studies have been done on the prevalence of hookworm infections in dogs in Bhutan and most of the surveys of intestinal helminthes conducted in the past have been limited to clinical settings.

The survey conducted on prevalence of ancylostomiasis in stray dogs in Trong sub-district under Zhemgang district showed an overall prevalence of 35.9% (n=128) during the year 2018-19 (RLDC 2019). There is also little information on human hookworm infections from hospitals although statistical analysis identifies contacts with dogs and cats as significant predictor of human hookworm infections. The present study was carried out with the objective to determine the prevalence of ancylostomiasis in canine population in Bumthang district that may be perceived as possible reservoir hosts of human hookworm infections.

2. MATERIALS AND METHODS

2.1 Study area

A cross-sectional study was carried out in four sub-districts (Gewogs) and one Municipal Authority (Thromde) under Bumthang district, which is located in east-central part of Bhutan. The dog population survey was conducted in July 2020 using Epicollect5 mobile apps in order to calculate the sample size. The sampling was done during 15th March 2021 to 30th April 2021 at the time of Dog Population Management

(DPM) program in Bumthang district. The entire free-roaming and pet dogs presented during the Catch, Neuter, Vaccinate and Release (CNVR) program was used as the sampling frame for this study.

2.2 Sampling and microscopy

The sample size was calculated using Win Episcope 2.0 software based on the total dog population in the district and probability proportional to size (PPS) sampling frame was worked out for each sub-district and municipal. Depending on the PPS sampling size, sampling was done from every tenth free-roaming (stray) dog and every fifth owned dog (pet) presented to the clinic during the entire DPM program.

Fresh faecal samples were collected in 20 ml screw capped scintillation vials. Whole blood samples were collected in 7 ml EDTA vacutainer. The samples were transported to Regional Livestock Development Centre (RLDC) in Zhemgang for further laboratory analysis. The faecal samples were processed and examined for presence of hookworm-like eggs and other parasites through direct wet mount, sedimentation and floatation techniques as per SOP for parasitology of National Centre for Animal Health (NCAH), Serbithang. However, the sedimentation technique was considered while analyzing the prevalence of ancylostomiasis. The whole blood samples were subjected to complete blood count as per SOP for haematology of NCAH, Serbithang. Simultaneously, the faecal samples were preserved in 10% formalin and 100 µl of EDTA blood were dispensed and archived in the FTA® cards (Whatman) and stored at room temperature for further molecular characterization.

2.3 Statistical analysis

All data entry, management and analysis were done using Microsoft Excel program 2007 (Microsoft® Office Excel 2007, Professional Edition). Descriptive statistics was used to analyze the data and the results of laboratory findings.

Prevalence for ancylostomiasis was estimated as the number of samples detected positive to hookworm-like eggs from the total samples analyzed. Data were analyzed using PHStat2 software and SPSS programmed for windows version 26. Chi-square and *t*-test were used to investigate the association between variables. The statistical significance was defined as $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Sample details

A total of 177 faecal and 177 whole blood samples were collected from dogs consisting of 100 each from free-roaming (stray) and 77 each from owned (pet) dogs. The details of samples in the study areas are illustrated in Table 1.

3.2 Prevalence of Ancylostomiasis

The overall prevalence of endo-parasites including *Ancylostoma spp.* in dogs in Bumthang district was 20.9% with the detection rate of 8.5% *Toxocara spp.*

Table 1: Study areas and demography of dogs

Study Area	Free-roaming dogs					Pet dogs				
	Age			**Gender**		**Age**			**Gender**	
	Adult	Juvenile	Pup	Male	Female	Adult	Juvenile	Pup	Male	Female
Chamkhar town	34	3	1	12	26	12	6	1	9	10
Chhoekhor Toed	7	0	0	4	3	4	0	0	2	2
Tang	16	7	0	5	18	33	0	0	28	5
Ura	18	2	0	8	12	19	0	0	15	4
Chumey	8	2	2	6	6	1	0	1	2	0
Total	**83**	**14**	**3**	**35**	**65**	**69**	**6**	**2**	**56**	**21**

followed by 5.6% *Ancylostoma spp*, 4.5% *Taenia spp.* 3.4% *Diphyllobothrium spp.* and 0.6% *Dipylidium spp.* (Figure 1).

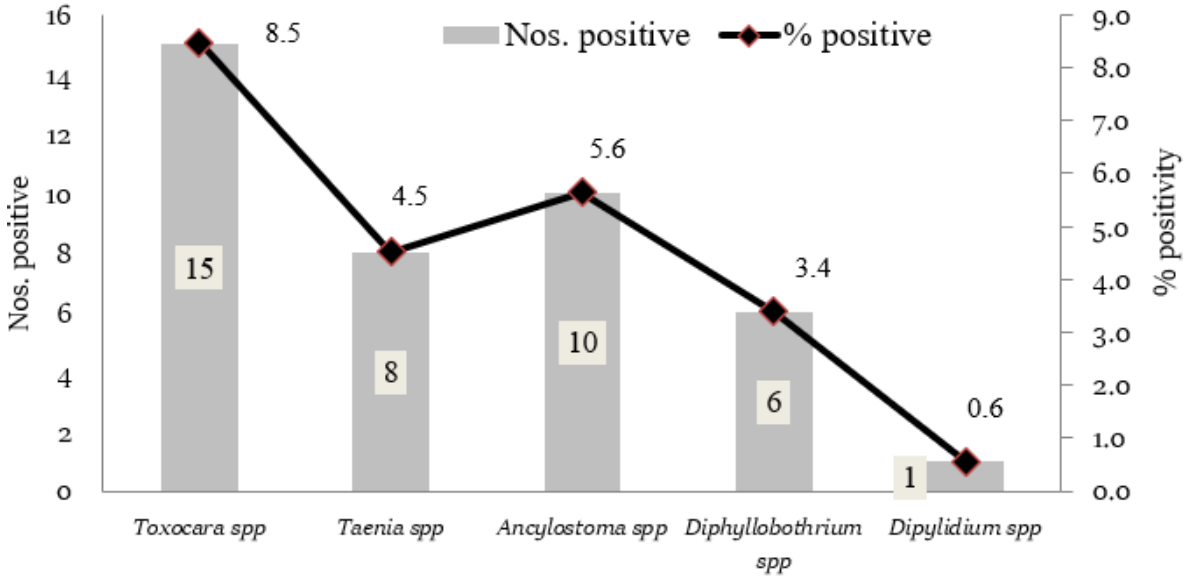

Figure 1: Prevalence of *Ancylostoma spp* and other endo-parasites in Bumthang district

The overall prevalence of ancylostomiasis in Bumthang district was 5.6% (95% CI; 2.25-9.05). The prevalence was higher in pet dogs (6.5%, 95% CI; 0.73-9.27) compared to free-roaming dogs (5.0%, 95% CI; 0.99-11.99). However, there was no statistical significance between the prevalence in pet dogs and free-roaming dogs ($p > 0.05$).

The overall prevalence of ancylostomiasis among dogs in Bumthang district was 5.6%. The prevalence was much lower compared to Trong sub-district in Zhemgang with a prevalence of 35.9% (RLDC 2019). This is most likely due to cold climatic conditions of Bumthang unlike Trong in Zhemgang, which experiences a hot and humid climate that is favourable for the survival of the parasites. Moreover, the District Livestock Sector (DLS) in Bumthang implements Community Animal Birth Control (CABC) program for free-roaming dogs on annual basis. These dogs are de-wormed against gastro-intestinal tract (GIT) endo-parasites using broad-spectrum anthelmentics, which may have attributed to low detection of GIT endo-parasite loads in this study.

The prevalence of *Ancylostoma spp* in dogs of Anand district, India was 22.95% (Brahmbhatt et al 2015); 23.6% in Sao Paulo State, Brazil (Oliveira-Sequeira et al 2002); 24.5% in Maracaibo, Venezuela (Ramirez-Barrios et al 2004); 19.06% in Himachal Pradesh, India (Agnihotri et al 2008); 17.9% in Ibadan, Nigeria (Sowemimo et al 2008); 19.32% in Punjab, India (Singh et al 2011); 17.84% in Jabalpur, India (Qadir et al 2011). The differences of prevalence level with that of our current study could be associated with environment and other anthropogenic factors of the community settings.

Worldwide, there is significant variation in the prevalence of hookworms reported in dogs. The study conducted in Malaysia reported an overall prevalence of 48.0% (Mahdy et al 2012) with the highest prevalence in rural dogs (71.4%) as compared to urban dogs (48.0%). In Thailand, the prevalence of hookworm infection was 58.9% among dogs in temple communities (Traub et al 2008). In China, 66.3% of dogs in 175 farms in Heilongjiang province had hookworm infections (Wang et al 2006). In rural areas of India, the prevalence of hookworm ranges from 93.0% to 98.0% (Traub et al 2002). In all cases, hookworm was found to be the most

common GI parasites detected in dogs; which is similar to the finding in our study. The prevalence of ancylostomiasis in dogs was highest in Chhoekhor Toed with 9.1% (n=11) followed by 8.8% in Chamkhar Throm (n=57), 5.4% in Tang (n=56), 2.6% in Ura (n=39) and 0.0% in Chumey (n=14). The area-wise prevalence of ancylostomiasis in free-roaming and pet dogs is shown in Table 2.

Although the area-wise prevalence is statistically insignificant, the higher rate of infection in Chhoekhor Toed might be associated with dry and dusty environment and poor hygienic conditions of the sub-district as compared to other areas. Climate and soil structure are crucial determinants of hookworm prevalence, as the parasite thrives in areas where moisture and temperature are ideal for larval development outside the host (Loukas et al 2016). Moreover, hookworm infection tends to be more prevalent in rural areas, where the favorable ecologies converge with poverty and weak sanitary infrastructures (Loukas et al 2016).

In Chamkhar Throm, the high prevalence of ancylostomiasis can be attributed to an abundant number of free-roaming dogs in the town which scavenge rubbish and defecate all over the places. Both the pet and stray dogs are equally infected in this area because there is a close association between the pets and stray dogs as pets are rarely leashed and confined in Bhutan (Dorji et al 2020). The lower rates of prevalence in Tang and Ura sub-districts are probably because the dogs are mostly pets or utility dogs that are regularly de-wormed against GIT endo-parasites. Interestingly, no cases of ancylostomiasis were detected in Chumey sub-district although 21.4% of the samples were positive to taeniasis and ascariasis. The prevalence of ancylostomiasis was comparatively higher in pet dogs (6.5%) than in free-roaming dogs (5.0%) in this study. However, the overall GIT endo-parasite infections were found to be higher in free-roaming dogs (23.0%) compared to pet dogs (22.1%), which is consistent with other similar studies conducted in other regions. Pet dogs in Bhutan are rarely confined and they are in close interactions with free-roaming dogs most of the time, which makes them equally susceptible to GIT endo-parasite infections. In Spain, the prevalence of *Ancylostoma spp* in stray dogs was 43.1% and 29.7% in farm dogs (Regidor-Cerrillo et al 2020). Similarly, the prevalence was 28.7% in dogs in shelters, 48.0% in urban stray dogs and 71.4% in rural stray dogs in a study conducted by Mahdy et al (2012). There are also some variations in the findings wherein Azian et al (2008) reported higher hookworm infection rates in urban dogs compared to rural dogs.

The age-wise prevalence of *Ancylostoma spp.* was 5.9% in adult dogs followed by 4.8% in juvenile dogs and none in puppies. The gender-wise prevalence of *Ancylostoma spp.* was 3.3% in male dogs and 8.1% in female dogs. The breed-wise prevalence of *Ancylostoma spp* was 6.3% in local breed and 4.1% in improved breeds. The category-wise overall prevalence of ancylostomiasis in dogs of Bumthang district is shown in Table 3. The age-wise, gender-wise and breed-wise comparison of the prevalence indicated statistically non-significant ($p > 0.05$).

Table 2: Area-wise prevalence of ancylostomiasis in free-roaming and pet dogs in Bumthang

Study area	Free-roaming dogs		Pet dogs		*p*-value (*t*-test)
	Examined n	Infected (n) (%)	Examined n	Infected n (%)	
Chamkhar town	38	2 (5.3)	19	3 (15.8)	>0.05
Chhoekhor Toed	7	1 (14.3)	4	0 (0.0)	>0.05
Tang	23	2 (8.7)	33	1 (3.0)	>0.05
Ura	20	0 (0.0)	19	1 (5.3)	>0.05
Chumey	12	0 (0.0)	2	0 (0.0)	

The age-wise prevalence showed higher in adult dogs compared to juvenile dogs. This was in contrast to the findings of Sowemimo et al (2008) who recorded the prevalence of *Ancylostoma spp* to be highest in dogs of age group 0-6 months. Similarly, Das et al (2009) recorded that the hookworm infections were common in the age group of 2 months to 6 years (26.48%) in pet dogs and Lefkaditis et al (2006) reported the prevalence of *Ancylostoma spp* in 12 young dogs as compared to 6 in adult dogs. Brahmbhatt et al (2015) also reported that the occurrence of *Ancylostoma spp* was more frequent in dogs from young age (< 1 year) followed by middle age (1-7 years) and lowest in old age (> 7 years). The present study did not detect *Ancylostoma* infections in puppies although one pup was positive to *Toxocara* infection. The contrasting results in this study in comparison to the findings of other studies may have been attributed by small sample size of puppies and juvenile dogs.

The gender-wise prevalence was higher in female dogs compared to male dogs in this study. Similar studies conducted in other region showed that the prevalence was 29.4% in male dogs and 14.6% in female dogs in Anand district, India (Brahmbhatt et al 2015). Mitra *et al* (1990) and Oliveira-Sequeira et al (2002) also recorded higher infection rates in adult males than in adult females. This may be due to individual hormonal status of male and female dogs, which requires further investigation.

The breed-wise prevalence was higher in local (stray) dogs as compared to improved/exotic dogs which included breeds like Apsoo, Damtse, Alsatian and Mastiff breeds. This was in agreement with Brahmbhatt et al (2015), Ramirez-Barrios et al (2004), Das et al (2009) and Mahdy et al (2012) who reported the *Ancylostoma spp* infection was very common in mongrel dogs/urban stray dogs. This may be because the improved breed of dogs are kept under good hygienic conditions and are accessible to well-balanced nutrition as compared to stray dogs.

The overall mean eggs per gram (EPG) of faeces of *Ancylostoma spp* detected in the faecal samples was 130±48.30 with a mean of 100±0.00 in free roaming dogs and 160±54.77 in pet dogs indicating higher severity of ancylostomiasis in pet dogs compared to free roaming dogs.

The mean haemoglobin (Hb) concentration was 12.26±2.47 g/dl with a median of 12.30 g/dl. Similarly, the mean packed cell volume (PCV) was 36.89±7.44% with a median of 37.00%. A total of 60 (33.9%) dogs were found to be anaemic in this study upon complete blood count examination. However, only three dogs positive to *Ancylostoma spp* were anaemic. The high degree of anaemia was attributed to other conditions such as poor health status

Table 3: Category-wise overall prevalence of ancylostomiasis in dogs of Bumthang district

Variable	Examined (n)	Infected (n)	Non-infected (n)	Prevalence (%)	Chi square test (χ^2)
Age					
Adult (> 6 months)	152	9	143	5.9	
Juvenile (3-6 months)	20	1	19	4.8	Chi square = 0.315, *df* = 2, *p* = 0.854 (non-significant)
Pup (< 3 months)	5	0	5	0.0	
Gender					
Male	91	3	88	3.3	Chi square = 1.736, *df* = 1,
Female	86	7	79	8.1	*p* = 0.188 (non-significant)
Breed					
Local	128	8	120	6.3	Chi square = 0.282, *df* = 1,
Improved/Exotic	49	2	47	4.1	*p* = 0.596 (non-significant)

(weakness) due to mange and transvenereal tumor (TVT) wherein the overall prevalence was found to be 7.6% and 2.2% respectively in the district. The co-infection of *Ancylostoma spp* with other endo-parasites was rare in this study with only one free-roaming dog showing co-infection.

Although the prevalence of ancylostomiasis among dogs in Bumthang district was comparatively low, the close association of free-roaming dogs and pet dogs with human population could play a significant role in contributing to the occurrence of zoonotic ancylostomiasis such as creeping eruption, eosinophilic enteritis or less frequently symptoms of localized myositis, erythema multiforme and ophthalmological manifestations in humans. It is difficult to compare the species-specific hookworm infections among dogs in Bhutan since there are limited studies conducted till date. The molecular characterization and phylogenetic analysis of *Ancylostoma spp* of the dogs would be beneficial to understand the existing dog hookworms and the potential species-specific public health risks in the country.

4. CONCLUSION & RECOMMENDATION

The study shows that hook worm infection is prevalent both in free-roaming and owned dog population. This finding highlights the risk of hookworm infection in human that can be transmitted through hookworm larvae contamination of public areas. Therefore, there is a need to create awareness among the general public on the parasite, its transmission mode, prevention measures and public health implications. In addition, to provide appropriate understanding on the species-specific public health risks, there is a need for further research.

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Funding source

The study was conducted during the time of Dog Population Management (DPM) program in Bumthang district and no separate costs were incurred for the study.

Availability of data and materials

All data generated or analyzed during this study are included in the result section. The full datasets will be made available from the corresponding author on request.

Ethical approval

As the study involved collecting samples as per standard sample collecting procedures without harming or giving stress to any animal, a separate ethical approval was not sought from the Livestock Research & Extension Division. However, this study was included in the concept note for implementation of Dog Population Management (DPM) program in Bumthang district and prior administrative approval was granted by the Department of Livestock vide letter no. 2(2) RLDC/ADM/ZG/2020-2021/125 dated 29th January 2021. Written consents were acquired from the participating pet dog owners at the time of sampling.

Competing interests

The authors declare that they have no competing interests.

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