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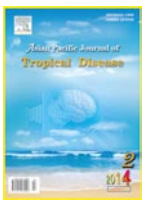
Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(14)60720-9

Seasonal prevalence of paramphistomosis in domestic ruminants in different agro-climatic zones of Uttarakhand, India

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ARTICLE INFO

Article history:
Received 6 Dec 2013
Received in revised form 25 Dec 2013
Accepted 20 Mar 2014
Available online 27 Aug 2014

Keywords:
Paramphistomes
Prevalence
Hills
Tarai
Ruminants

ABSTRACT

Objective: To establish the epidemiology of paramphistomosis in Hills and Tarai region of Uttarakhand state, India for the period from 2005 to 2007.
Methods: For this purpose, 11 278 faecal samples (4 391 cattle, 2 197 buffaloes, 1 760 sheep and 2 930 goats) were collected from Hills and Tarai region of Uttarakhand for 2 years. Faecal samples were examined for the presence of paramphistomes eggs, both qualitatively and quantitatively. The data were recorded on the basis of months and prevailing seasons of the state.
Results: The overall prevalence of paramphistomosis during this period in domestic ruminants was 9.69%. Also, bovines (cattle-12.4% and buffaloes-12.3%) were found to be more prone to infection as compared to small ruminants (sheep-7.4% and goats-4.9%). The peak infection was observed in the monsoon and post monsoon season (17.42%). This trend was similar for both Tarai (15%) and Hills (6.7%). The overall prevalence in Tarai was found to be 12.3% and in Hills it was 3.9%. In both Tarai and Hills region, maximum prevalence was seen in cattle with infectivity of 14% and 7.2% respectively and minimum prevalence in goat with infectivity of 8.7% and 2% respectively. Maximum mean eggs per gram was recorded in buffalo (22.05 in winter) followed by cattle (19.7 in summer), goat and sheep (18.7 and 16.33 in monsoon and post monsoon). The most common species of paramphistomes recorded were *Paramphistomum cervi*, *Gastrothylax crumenifer* and *Fischoederius elongatus*.
Conclusions: The analysis of the data obtained revealed that prevalence was more in the Tarai region as compared to the Hills region and this pattern remained same after quantitative analysis of the samples.

1. Introduction

Helminth parasitism, especially gastrointestinal parasitism, is one of the major health problems severely limiting the animal productivity. Epidemiological pattern of the parasitic diseases in different agro climatic zones of the country would provide a basis for evolving strategic and tactical control of these diseases. The economic losses in livestock due to diseases differ from season to season[1-3]. Some of the parasites cause morbidity and mortality in domesticated ruminants and lower their production substantially, while most animals suffer continuously with sub clinical state of parasitiasis[4,5].

Paramphistomosis is one of the most important diseases in domesticated animals causing heavy economic losses to livestock industry, widely prevalent in India and many other countries[2,6-10]. The mortality rate due to immature paramphistomosis is very high and may go up to 80%-90% in domestic ruminants. Approximately 40 species of amphistomes have been reported by Agrawal[11], but the predominant species are *Paramphistomum cervi* (*P. cervi*), *Gigantocotyle explanatum*, *Gastrothylax crumenifer* (*G. crumenifer*), and *Fischoederius elongatus*. Several of these amphistomes species have been reported in domesticated animals in India. Incidence of amphistomosis in cattle, buffaloes, sheep and goats has been reported

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Foundation Project: Supported by Indian Council of Agricultural Research (ICAR), NATP on Diagnosis of Parasitic Diseases of Domestic Animals (Grant No. IXX08847).

in different states of India[12-14]. Also, the humid areas of Tarai and Hills of Uttarakhand are known to have high incidence of helminth infections[14]. Paramphistomosis causes major production loss in ruminants, often without clinical manifestation. Adult flukes are less pathogenic as compared to immature flukes because of their plug feeding on the duodenal mucosa. Keeping in view, the endemicity, economic impact of paramphistomosis in ruminants and paucity of documented data in the region, the study aimed to determine the seasonal prevalence and intensity of infection in domesticated ruminants in Tarai and Hills region of Uttarakhand.

2. Materials and methods

2.1. Study area and site

The study was conducted in seven districts (three from Tarai and four from Hills region) of Uttarakhand, India which extends from 28°43′N to 31°27′N longitude and 77°34′E to 81°02′E latitude. The state is surrounded by Nepal in the east, China in the north, Himachal Pradesh in the west and Uttar Pradesh in the south. The state covers two major agro-climatic zones, *viz*. Tarai (up to 1 000 m elevation) and Hills (above 1 000 m elevation). The climate in these zones differ significantly with respect to the data regarding mean monthly minimum and maximum temperatures, monthly rainfall and morning and evening relative humidity of Tarai region (year 2005-2007) as per the database from Crop Research Centre, Pantnagar, Uttarakhand. The Hills zone comprises of four districts, *viz*. Nainital, Almora, Bageswar and Pithoragarh and the Tarai region encompasses three districts of Udham Singh Nagar, Dehradun and Haridwar. To facilitate the study of seasonal variation, the year was apportioned into three seasons, *i.e*. monsoon and post-monsoon (July to October), winter (November to February) and summer (March to June).

2.2. Collection and processing of samples

The study was conducted from July 2005 to June 2007. During the study period, a total of 11 278 fresh faecal samples (4 391 cattle, 2 197 buffaloes, 1 760 sheep and 2 930 goats) were collected either directly from the rectum or from top of freshly defecated uncontaminated faeces. Cold chain was maintained while bringing the samples in the laboratory.

2.3. Qualitative and quantitative analysis of faecal samples

Faecal examination of each animal was separately conducted regularly for a period of 2 years. Primary screening was done by preparing direct smears from the faecal samples to search eggs or larvae of helminth parasite. The samples were further examined by both floatation and sedimentation techniques for qualitative analysis.

Stoll's egg counting technique[15] with some modifications was used for egg counting or quantitative analysis. The eggs per gram (EPG) of a fecal sample was calculated by the following formula:

$$\text{EPG} = \frac{\text{Total number of eggs counted in 0.15 mL}}{\text{Volume of sample taken (0.15 mL)}} \times \text{Dilution factor}$$

2.4. Collection of samples from slaughter houses

Fresh amphistomes were also collected from local slaughterhouses; stained amphistome whole mounts were prepared. These slides were examined and the genuses were identified with the help of key furnished by Dutt[16].

3. Results

Out of the total 11 278 faecal samples, 1 093 (9.69%) of the total samples were found to be positive for paramphistome eggs. The infectivity was the highest during monsoon and post monsoon (12.77%) followed by summer (9.74%) and winter (6.54%). The overall seasonal prevalence of paramphistomes irrespective of species or zone with their sample size and mean EPG has been shown in Table 1.

Table 1

Overall season wise prevalence of paramphistomes in domesticated ruminants of Uttarakhand.

Season	No. of samples	Percent positive (%)	Mean EPG
Summer	3 519	343 (9.7%)	16.02
Monsoon and post monsoon	3 890	497 (12.7%)	17.42
Winter	3 869	253 (6.5%)	16.06
Total	11 278	1 093 (9.6%)	16.49

With respect to the overall host species wise prevalence of paramphistomes, cattle (12.4%) and buffalo (12.3%) were found to be the most infected among the four ruminants, followed by sheep (7.4%) and the least infected was goat (4.9%). Details of overall host species wise prevalence of paramphistomes in Uttarakhand along with the species wise mean EPG is presented in Table 2.

Table 2

Overall host species wise prevalence of paramphistomes in Uttarakhand.

Species	No. of samples	Percent positive (%)	Mean EPG
Cattle	4 391	545 (12.4%)	18.0
Buffalo	2 197	271 (12.3%)	18.1
Sheep	1 760	131 (7.4%)	15.1
Goat	2 930	146 (4.9%)	14.5
Total	11 278	1 093 (9.6%)	16.4

Subsequently, the seasonal prevalence of paramphistomes with respect to different host species was deduced which revealed that all the four ruminant species showed the highest infectivity during the monsoon and post monsoon with cattle (14.8%) showing the maximum percent positivity followed by buffalo (14.09%), sheep (10.5%) and goat (9.2%). In summer, buffalo showed the highest infectivity followed by cattle, sheep and goat. Winter showed the least infectivity ranging from the highest infectivity in cattle (8.5%) to the least in goat (3.08%). Details have been depicted in Table 3.

Table 3
Overall host species wise seasonal prevalence of paramphistomes of Uttarakhand.

Species	Summer	Monsoon and post monsoon	Winter	Total
Cattle	189/1 389 (13.6%)	235/1 586 (14.8%)	121/1 416 (8.5%)	545/4 391 (12.4%)
Buffalo	98/696 (14.0%)	120/852 (14.0%)	53/649 (8.1%)	271/2 197 (12.3%)
Sheep	17/288 (5.9%)	64/608 (10.5%)	50/864 (5.7%)	131/1 760 (7.4%)
Goat	39/1 146 (3.4%)	78/844 (9.2%)	29/940 (3.0%)	146/2 930 (4.9%)
Total	343/3 519 (9.7%)	497/3 890 (12.7%)	253/3 869 (6.5%)	1 093/11 278 (9.6%)

Further analysis of the data revealed that the highest mean EPG was found in winter for buffalo (22.05) followed summer for cattle (19.70) and during monsoon and post monsoon for goat (18.70) and sheep (16.33). Overall mean EPG was the highest for buffalo, followed by cattle, sheep and goat respectively (Table 4).

Table 4
Overall mean EPG of paramphistomes in domesticated ruminants of Uttarakhand.

Species	Summer	Monsoon and post monsoon	Winter	Total
Cattle	19.70	18.60	15.95	18.08
Buffalo	16.46	16.03	22.05	18.18
Sheep	15.27	16.33	13.85	15.15
Goat	12.68	18.70	12.40	14.59
Total	16.02	17.42	16.06	16.49

As per the records of agro-climatic zone-wise seasonal prevalence of paramphistomes in domesticated ruminants of Uttarakhand (Table 5), it was found that the infectivity of Tarai region was higher than that of Hills region. In both the regions, the prevalence was maximum during monsoon and post monsoon followed by summer and winter respectively.

Table 5
Zone-wise seasonal prevalence of paramphistomes in domesticated ruminants of Uttarakhand.

Zones	Summer	Monsoon and post monsoon	Winter	Total
Tarai	287/2 133 (13.4%)	426/2 831 (15.0%)	240/2 778 (8.6%)	953/7 742 (12.3%)
Hills	56/1 386 (4.0%)	71/1 059 (6.7%)	13/1 091 (1.1%)	140/3 536 (3.9%)
Total	343/3 519 (9.7%)	497/3 890 (12.7%)	253/3 869 (6.5%)	1 093/11 278 (9.6%)

Subsequently, the agro-climatic zone-wise prevalence ruminant hosts revealed the highest prevalence of paramphistomes in the Tarai region for the domesticated ruminant following a similar trend of infectivity with the Hills region with the maximum percentage of infectivity in cattle, followed buffalo, sheep and goat (Table 6).

Table 6
Agro-climatic zone-wise host species prevalence of paramphistomes in domesticated ruminants of Uttarakhand.

Zones	Cattle	Buffalo	Sheep	Goat	Total
Tarai	471/3 363 (14%)	253/1 926 (13.1%)	116/1 161 (9.9%)	113/1 292 (8.7%)	953/7 742 (12.3%)
Hills	74/1 028 (7.2%)	18/271 (6.6%)	15/599 (2.5%)	33/1 638 (2%)	140/3 536 (3.9%)
Total	545/4 391 (12.4%)	271/2 197 (12.3%)	131/1 760 (7.4%)	146/2 930 (4.9%)	1 093/11 278 (9.6%)

From the monthly records obtained irrespective of species, a definite pattern in the percentage of incidence could be ascertained. As a whole, the percentage of infection had increasing trend from the month of March, reaching peak during June, and minor peak noticed in August/September and then gradually started declining from October and came to a minimum in the month of January. Host species wise monthly prevalence showed that cattle showed the highest infectivity in the month of June (19.41%) and lowest was during November (6.93%). Buffaloes showed peak of infection in June (30.00%) and the lowest was observed in January (6.19%). Sheep showed maximum infection in June (26.67%) and the lowest was observed in April (0.67%). Goats had the highest infectivity in July (15.20%) and the lowest was seen in May (1.84%). Graphical presentation are depicted in Figures 1 and 2.

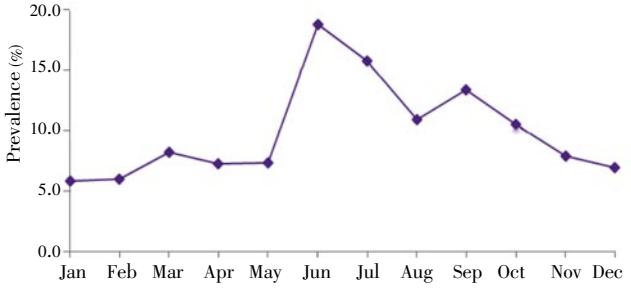

Figure 1. Overall monthly prevalence of paramphistomes in domesticated ruminants of Uttarakhand (2005-2007).

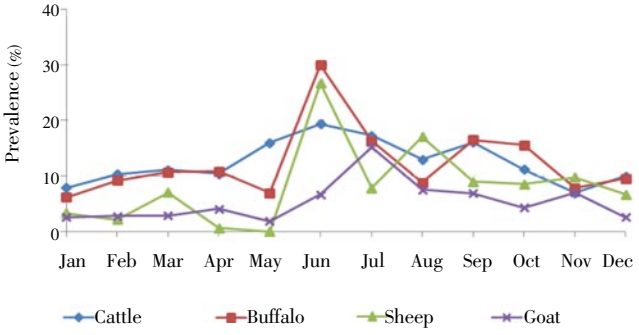

Figure 2. Monthly prevalence of paramphistomes in domesticated ruminants of Uttarakhand (2005-2007).

With respect to the monthly records of mean EPG obtained irrespective of host species, a definite pattern in the percentage of incidence could not be ascertained. As a whole, the mean EPG showed maximum in June (19.60) followed by January (19.50), August (19.10), July (18.10), while the least was observed in the months of February

and May with mean EPG of 13.6 each. In cattle, the highest mean EPG was found in June (24.53) followed by September (23.53). In buffaloes, the highest mean EPG was found in January (36.92) and the lowest mean EPG was in December (11.67). In sheep, the highest mean EPG was found in August (20.80) and the lowest mean EPG was in February and April (10.00). In case of goat, the highest mean EPG was found in July (25.00) and the lowest mean EPG was in January (10.00). Graphical presentation are depicted in Figures 3 and 4.

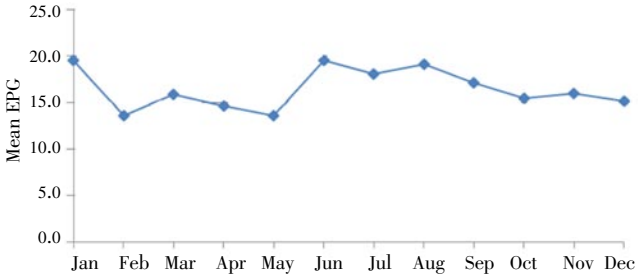

Figure 3. Overall monthly mean EPG of paramphistomes of domesticated ruminants in Uttarakhand (2005-2007).

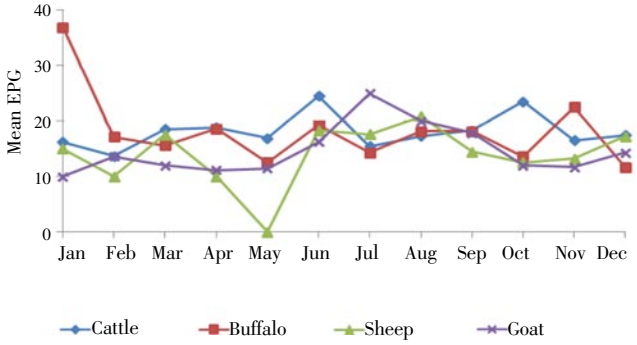

Figure 4. Monthly mean EPG of paramphistomes in domesticated ruminants of Uttarakhand (2005-2007).

The most common species identified from the fresh ruminal samples collected from local abattoirs were *P. cervi*, *Fischoederius elongates* and *G. crumenifer*. In addition to these, *Gigantocotyle explanatum* was also observed in the bile duct of buffaloes.

4. Discussion

Paramphistomosis is one of the most common parasites of ruminants, distributed globally with the highest incidence from tropical and sub tropical regions particularly the Asian subcontinent. It has an endemic status in India with persistent infection throughout the year in various geo-agroclimatic conditions with bouts of epidemic by amphistomes, causing a major production loss. Despite the scattered reports of the infection and the economic losses encountered, efforts are yet not successful in the control of the disease. Thus a holistic approach to understand the host, parasite and environment interaction in a large population is needed to generate an effective and conclusive database. Thus an endevour to elucidate the seasonal prevalence in domestic ruminants located in different agroclimatic zones of Uttarkhand has been undertaken.

The overall prevalence observed in our study revealed nearly four times less prevalence as compared to the observation of Kripali *et al.*[17], 2009 in the Tarai region of Uttarakhand. The differences noted were the large sample size (>75 times) in our study and moreover their findings were confined to small ruminants and limited to Pantnagar area only. Balachandran *et al.*[18] and Chandra *et al.*[19] have also reported acute outbreak amphistomosisi in organized sheep farm. Several reports of paramphostomis from various parts of the country is available such as Bihar, Delhi, Haryana, Jammu, Meghalaya, Punjab, Rajasthan, Sikkim, West Bengal with entirely different climatic and geographical condition[20-32]. Some of their findings showed variation in the prevalence or similar infectivity and susceptibility. As per the study of Khan[2], the incidence of paramphistomes was significantly high in buffaloes than cattle, and the trend of this and our findings were almost similar except that we did not observe much difference between cattle and buffalo and the infectivity was almost double.

It was observed that paramphistomosis in domestic ruminants has got a definite seasonal pattern, the infectivity was in peak during monsoon and post monsoon, followed by summer and the least was recorded in winter. Besides, all the four species revealed the highest infectivity during the monsoon and post monsoon followed by summer and winter respectively. Similar seasonal trend were also observed by many researchers in different parts of the country[2,21,26,27]. However, the previous study of Yadav *et al.* in Delhi area reported that the animals were most affected during summer then rains season and the least during winter[21]. Likewise, the study of Shahnawaz *et al.* also has differing observation with higher infection during winter followed by spring, summer and autumn[13].

It may be noted that in our study, the overall infection level was persistently above 5% throughout the study period, indicating an endemic carrier of the infection which anytime may flare up as an epidemic. The reason for more prevalence during monsoon has been well documented primarily due to the conducive environment of the intermediate host snail, dispersal of the infective stages wherein the metacercariae can infect host mostly in the early monsoon. Besides, the pre-patent period of amphistomes varies from 7-10 weeks depending upon the

parasite species, and further metacercariae remain viable for 3 months and continue their infection in the winter. There is no evidence of auto expulsion of amphistomes from the host body and this is the reason behind the year round prevalence of amhistomes[3,4].

A definite pattern of infection could be ascertained from the records of monthly data with species wise variation (cattle, buffalo, sheep) showing peak during June, and the lowest in November, January and April for the respective host species, whereas goats showed peak infection in July and the lowest in May. Our findings corroborated with the reports of Khan[2], who observed high incidence during June, July and declining trend towards the winter. Further, Yadav *et al*. reported monthly prevalence of amphistomosis in Delhi area with peak infection in cattle and sheep in the month of May, buffaloes in July and goats in February[21]. Variable percent of prevalence was noticed by other researchers[27,28,31-33].

Overall the trend of infectivity was similar in many studies, however slight variations were noted in the species or season/monthly prevalence which may be due to the differences in the agroclimatic conditions, availability or abundance of the intermediate host, animal husbandry/managemental practice, length of survey period, sample size and method of sampling. Other contributing factors may be the different animal behaviour or grazing pattern like buffalo dwells more in water bodies, goats are browser, sheep being grazer and various other determining factors either intrinsic host related like susceptibility, stress *etc.* or extrinsic environmental and climatic factors like temperature, humidity, rainfall *etc*.

It was observed that Tarai region had more infected population of domesticated ruminant as compared to the Hills region. Both the regions showed peak infection during monsoon and post monsoon followed by summer and winter respectively. This may be due to lower prevalence of snails in hilly areas as compared to the Tarai region. Also, the reason may be less availability of grazing grounds and less exposure to the paramphistomes in the hills. The harsh climatic conditions of the hilly areas in monsoon and winter might restrict the extensive grazing of ruminants and thereby reducing chances of infection.

Overall mean EPG was the highest for buffalo, followed by cattle, sheep and goat respectively. Further mean EPG was maximum during winter for buffalo (22.05), during summer for cattle (19.70) and during monsoon and post monsoon for goat (18.70) and sheep (16.33). Mean EPG records did not show a definite pattern in different host species, however it may be noted that the overall mean EPG was >10 throughout the study period, again indicating the existence of carrier animals. Khajuria *et al.* recorded that the highest EPG was during monsoon and the lowest during winter, with two peaks of EPG, one during April and the other during August[23].

Tehrani *et al.* reported that sheep carcass were infected with a single or multiple species of amphistomes, more specifically three species *viz. P. cervi*, *Cotylophoron cotylophorum* and *G. crumenifer* were identified[6]. Kumari *et al.* reported that the most common species of helminth recovered in the intestinal scrapping of sheep and goat was *Cotylophoron cotylophorum*[20]. The species of paramphistomes identified in the present study is in accordance with the above reports and many other researchers[2,20,25,26,31-33].

The present study clearly indicates that paramphistome infection in ruminants is a problem that can play a role in hindering the livestock development in the region specifically and the country at large. Thus, development of sustainable cost effective control strategies with interventions based on the basic epidemiological principle of host, agent and environment interaction is essential.

The epidemiological trend of parasitic infection in different agroclimatic zones of the region will provide a basis for evolving tangible control measures for paramphistomosis management in ruminants. Various interventions through chemotherapy, pasture management and good husbandry practices may ameliorate the present scenario.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This research was part of MVSc research of the first author. The study was supported by Indian Council of Agricultural Research (ICAR), National Association of Tax Professionals (NATP) on Diagnosis of Parasitic Diseases of Domestic Animals (Grant No. IXX08847). The authors are also thankful to the Dean of the college for providing necessary facilities and support to accomplish this work.

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