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Prevalence of Paramphistomosis in Domestic Ruminants in Chittoor District of Andhra Pradesh, India

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ABSTRACT

A total of 1169 dung samples from domestic ruminants were examined to record the prevalence of paramphistomosis infection. In cattle, the prevalence of infection by direct smear (DS) and sedimentation method (SD) was 17.43% and 31.19%, respectively. In sheep, the prevalence rates were recorded higher than cattle (26.09% and 33.18%). In goats, the prevalence of paramphistomosis was 20.66% and 30.52%. Out of 109 cattle carcasses, 47 were found with flukes in rumen, reticulum and bile duct during slaughterhouse examination (SH). In sheep and goat, the prevalence rates were 42.15% and 40.85%, respectively by SH. Age-wise the highest prevalence was recorded in cattle of 2-4 years (50.0%) by SH. In goats, the prevalence of infection was lower in the age group of <1 year, when compared to their counterparts in sheep. In contrast, the higher prevalence was noticed in >1-2 years old goats (63.27%) than sheep of the same age group (36.85%). Sex-wise, statistically no significant difference was observed between male and female animals. Morphologically identified, *Cotylophoron cotylophorum*, *Paramphistomum cervi*, *Gastrothylax crumenifer*, *Fischoederius elongatus* and *Gigantocotyle* spp. In conclusion, an overall prevalence of 24.29%, 32.51% and 42.0% of paramphistomosis infection was recorded in cattle, sheep and goats by DS, SD and SH examinations, respectively.

Key words: Domestic ruminants, Paramphistomosis, Prevalence.

INTRODUCTION

India has the largest livestock population and contributes substantial income to the nation, but the animals are infected by multifarious parasites resulting into economic losses. Parasitological prevalence studies are important to know about the status and transmission of diseases (Moudgil *et al.* 2016). Further parasitism produces ill effects and predisposes the animals to various potential pathogens (Moudgil *et al.* 2018; Kaur *et al.* 2019). Establishing a data base to predict any infection is of utmost importance and needs attention (Bal *et al.* 2014; Kaur *et al.* 2016). Among the parasitic diseases, paramphistomosis is one of the most important infections of domestic animals with considerable significance in Asia and Africa. The disease causes heavy economic loss to the livestock industry to the tune of several thousand crores of rupees annually (Shabih and Juyal, 2005).

Paramphistomosis is caused by a digenetic trematode belonging to the family paramphistomatidae and considered to be one of the most important diseases in tropical and subtropical regions (Godara *et al.,* 2014). Diagnosis of helminth infections in livestock is mainly relying on conventional microscopic methods in low and middle income group countries. Early diagnosis of trematode infections is so difficult as the egg output is not present in faeces until the fluke reaches the maturity (Hafeez *et al*. 2006; Kaur *et al.,* 2008). However, immunodiagnosis seems to more useful under such circumstamces (Kaur *et al.,* 2009; Kaur *et al.,* 2013). The adult paramphistomes are the major parasites of rumen and reticulum of ruminants, which cause localized loss of rumen papillae, responsible for ruminitis, irregular rumination, unthriftness, lower feed conversion and loss of body condition. Various reports on paramphistomosis from different states representing different geographical locations of the country are available (Samanta and Santra, 2009; Bandyopadhyay 2010; Kumari *et al*., 2010; Swarnkar *et al*., 2010; Yadav *et al*., 2010; Khajuria *et al*., 2011; Rahman *et al*., 2012; Maitra *et al*., 2014). A study on prevalence limited only to cattle amphistomosis has also been conducted from Andhra Pradesh by Kumari and Hafeez, (2005). No further reports exist on this infection in domestic ruminants from this area. Nearly about 40 species of amphistomes have been reported from India and predominant species are *Paramphistomum cervi, Gigantocotyle explanatum, Gastrothylax crumenifer* and *Fischoederius elongatus* (Agrawal, 2003). The amphistome egg morphology and immature flukes are not much useful for identification of paramphistomes because of very similar morphological characters. To overcome this, adult flukes are to be identified

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morphologically. Proper identification is an important aspect to define pathogenicity, treatment and control. Keeping in view of the paucity of information in the proposed area, the present study aimed with an objective to determine the 'prevalence of paramphistomosis in domestic ruminants of Chittoor district of Andhra Pradesh'.

MATERIALS AND METHODS

Dung samples and amphistome specimens were collected from cattle, sheep and goats aged, >6 months to ≥4 years from local slaughterhouses of Chittoor district of Andhra Pradesh during the period from February to September, 2016. Prior to slaughter, dung samples (n=1169) were collected directly from the rectum of animals using a disposable glove and transferred into a zip lock cover. These samples were labelled with details of age, sex, and date of collection. The collected samples were transferred to the laboratory for parasitological examination (Table 1). Adult amphistomes (n=500) from the rumen and reticulum from slaughtered cattle, sheep and goats were collected (samples from postmortem conducted animals were very few and were clubbed with the slaughtered animal's figure). In addition, bile ducts were also collected from amphistome infected cattle. The collected amphistomes were processed in the laboratory and 10 of the randomly selected and processed amphistome specimens from each animal were identified. Thus, a total of 100 amphistome specimens from 10 slaughtered cattle was processed and identified. Similarly, 250 and 150 amphistome specimens were identified from 25 sheep and 15 goats, respectively (Table 1).

Table 1: Details of sample collection.

Kind of animal	Type of sample		
	Dung	Adult amphistomes	Total
Cattle	109	100	209
Sheep	847	250	1097
Goat	213	150	363
Total	**1169**	**500**	**1669**

The prevalence of paramphistomosis infection in the slaughtered domestic ruminants was estimated by detecting

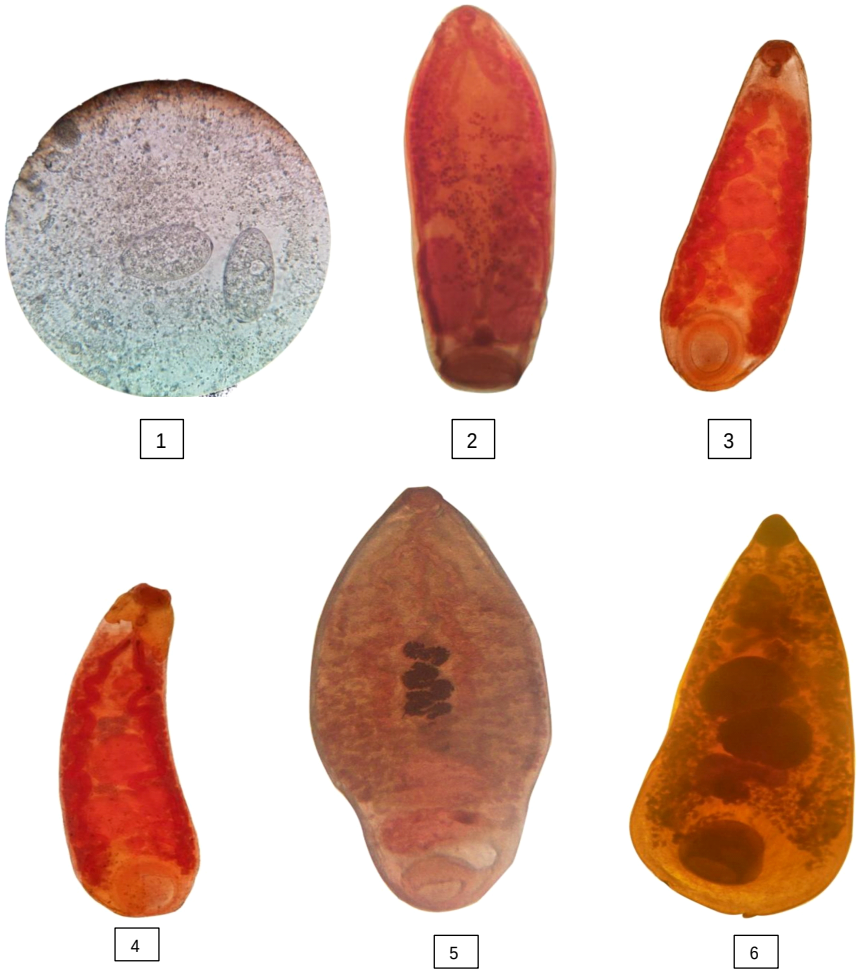

Fig 1: Amphistome eggs (400x); **Fig 2:** *Gastrothylax crumenifer* (50x); **Fig 3:** *Cotylophoron cotylophorum* (50x); **Fig 4:** *Paramphistomum cervi*; **Fig 5**: *Fischoederius elongatus* (50x); **Fig 6:** *Gigantocotyle* spp.

the amphistome eggs (Fig 1) in the dung samples by DS and SD methods (Soulsby, 1982; Gupta and Singla 2012). The prevalence of paramphistomosis was also recorded by finding the flukes in the tissues of rumen, reticulum and bile duct of the slaughtered domestic ruminants. The collected adult flukes were processed by following standard protocol and were identified based on morphological characters (Soulsby, 1982). The prevalence rates recorded by DS, SD and SH examinations were compared and statistically analyzed. Statistical analysis of data was done following Chi-square test and Marasculio procedure using Microsoft Excel software version 2013.

RESULTS AND DISCUSSION

In the present study, three screening methods were adopted to record the prevalence of paramphistomosis infection in domestic ruminants. An overall prevalence of 24.29%, 32.51% and 42.0% of infection was observed by DS, SD and SH examinations, respectively. Statistically, a significant difference was noticed (Table 2).

The prevalence of infection in cattle by DS and SD methods were 17.43% and 31.19%, respectively (Table 2). In contrast, earlier studies reported a high prevalence of paramphistomosis infection in cattle (Paul *et al.,* 2011; Saha *et al.*, 2013). Numerous studies reported a lower prevalence rate of infection in cattle from India. From Andhra Pradesh, 5.94% of infection was reported (Kumari and Hafeez, 2005) and similar reports were recorded from Punjab (Singh *et al.,* 2012), Maharashtra (Gadre *et al*., 2008), Rajasthan (Choubisa and Jaroli, 2013), Telangana state (Murthy and Rao, 2014) and Uttarakhand (Maitra *et al*., 2014). In a study, none of the cattle were found positive for paramphistomosis in the Nilgiri district of Tamil Nadu, probably this may be due to insufficient number of samples (n=20) (Allwin *et al.,* 2016).

In the current study, 26.09%, 33.18% and 42.15% of prevalence of paramphistomosis in sheep was noticed by DS, SD and SH examinations, respectively (Table 2). Differing with the present findings, lower prevalence of ovine paramphistomosis was reported from various parts of India (Shabih and Juyal, 2005; Choubisa and Jaroli, 2013; Balakrishnan *et al*., 2014; Tramboo *et al*., 2015). This may be due to the free grazing habit of sheep leads to heavy infection by ingesting the metacercaria than cattle and goats (Swarnakar and Kumawat, 2013). In the present study, the infection rate in goats was between 20.66 - 40.85% by all three detection methods (Table 2). In comparison, a higher prevalence of infection was noted by Uddin *et al.,* (2006). In north India, a low prevalence of infection in goats was observed by earlier workers (Shabih and Juyal, 2005; Choubisa and Jaroli, 2013; Godara *et al.,* 2014; Maitra *et al*., 2014). The fluctuations in prevalence of infection among the domestic animals could arise as a result of drinking from drying water bodies where the levels of metacercariae tend to increase (Rolfe *et al*., 1991).

Slaughterhouse survey is a complex way of gathering information on epidemiology of parasite infections in livestock. Based on abattoir study, the prevalence of paramphistomosis in cattle, sheep and goats showed not much variation (40.85 to 43.12%) (Table 2). For this, one of the reasons may be the common grazing areas and most of the infections in sheep and goats are due to transmission through bovines (Dunn, 1969). The present study reported a prevalence of infection in cattle at 43.12%. Previous reports

Table 2: Prevalence of paramphistomosis in domestic ruminants of Chittoor district of Andhra Pradesh.

Kind of Animal	Number positive/Number screened (%)		
	Direct smear method	Faecal Sedimentation method	PM/Slaughterhouse examination
Cattle	19/109 (17.43)^{Aa} (8.07 - 26.79)	34/109 (31.19)^{Aab} (19.76 - 42.62)	47/109 (43.12)^{Ab} (30.90 - 55.34)
Sheep	221/847 (26.09)^{Aa} (22.21 - 29.98)	281/847 (33.18)^{Ab} (22.21 - 29.98)	357/847 (42.15)^{Ac} (33.78 - 46.52)
Goat	44/213 (20.66)^{Aa} (13.51 - 27.80)	65/213 (30.52)^{Aab} (22.39 - 38.64)	87/213 (40.85)^{Ab} (32.17 - 49.52)
Total	**284/1169 (24.29)^{a} (21.06 - 27.53)**	**380/1169 (32.51)^{b} (28.98 - 36.04)**	**491/1169 (42.0)^{c} (38.28 - 45.72)**

Values are actual proportions with percentages and 95% confidence intervals in parenthesis; Proportions with different alphabet superscripts are significantly different (P<0.05); Capital alphabets are for vertical comparison (between species) and lower case alphabets are for horizontal comparison (between methods); Marasculio procedure using Microsoft Excel software version 2013.

Table 3: Age-wise prevalence of paramphistomosis in cattle.

Age	Number positive/number screened (%)		
	Direct smear method	Faecal sedimentation method	PM/Slaughterhouse examination
<2 years	5/42 (11.90)^{Aa} (0.00 - 24.78)	9/42 (21.43)^{Aa} (5.12 - 37.74)	19/42 (45.24)^{Ab} (25.46 - 65.02)
2-4 years	11/42 (26.19)^{Aa} (8.72 - 43.67)	19/42 (45.24)^{Aa} (25.46 - 65.02)	21/42 (50.0)^{Ab} (30.13 - 69.87)
>4 years	3/25 (12.0)^{A} (0.00 - 28.74)	6/25 (24.0)^{A} (2.00 - 46.00)	7/25 (28.0)^{A} (4.87 - 51.13)
Total	**19/109 (17.43)^{a} (8.07 - 26.79)**	**34/109 (31.19)^{ab} (19.76 - 42.62)**	**47/109 (43.12)^{b} (30.90 - 55.34)**

Values are actual proportions with percentages and 95% confidence intervals in parenthesis; Proportions with different alphabet superscripts are significantly different (P<0.05); Capital alphabets are for vertical comparison (between age groups) and lower case alphabets are for horizontal comparison (between methods); Marasculio procedure using Microsoft Excel software version 2013.

published a higher prevalence ranging from 50.0 to 90.6% of infections in cattle (Bunza *et al.,* 2008; Azam *et al*., 2011). From India, Swarnakar *et al*. (2014) reported, the highest prevalence of infection in cattle slaughtered in Rajasthan. The higher infection in cattle may be due to large stomach, thus provides a wide surface area for fluke attachment (Barger 1993), improper deworming and regular recovery of flukes in slaughtered animals. Further, a higher prevalence in buffaloes may be due to the wallowing habit in contaminated water (Cheema *et al.,* 1997). Few studies from India reported a low prevalence of infection in cattle (Kumari and Hafeez, 2005; Chaudhary *et al*., 2014). Sheep carcasses were examined for finding the adult flukes by others in India and their findings were lower when compared to the present observations (Shahnawaz *et al*., 2011; Chaudhary *et al*., 2014; Godara *et al.,* 2014). The lower prevalence might be due to the sheep which were switched over to stall feeding (Shabih and Juyal, 2005; Singh *et al.,* 2012). Further, in the present study area, usually the large ruminants were confined to restricted grazing areas or mostly stall-fed, whereas small ruminants were of migratory behavior which made them susceptible to infection. Further, in this study visits to slaughterhouses were performed on weekdays, but more animals were brought on Sunday to cope up the demand. This may be one of the factors for higher infections as they form the bulk of animals brought to abattoir for slaughter, thus higher probability of being found positive for infection. Another reason is hot and dry season prevailed during the sampled period, which concentrated the snail population in water bodies leading to aggregation of snail hosts enhancing the chances of heavy infection (Shahnawaz *et al.,* 2011; Singla *et al.,* 2017). Caprine paramphistomosis detected in the current study was found to be higher than other studies (Chaudhary *et al*., 2014; Godara *et al.,* 2014). This may be explained by variation in genetic constitution, agro-ecological conditions, breed of animals and exposure to the source of infection (Uddin *et al*, 2006).

In cattle, age-wise prevalence of paramphistomosis showed a higher prevalence of infection in the age group of 2-4 years (Table 3). The present findings were in agreement with earlier observations (Kumari and Hafeez, 2005; Paul *et al.,* 2011; Swarnakar *et al*., 2014). In contrast, Khan *et al.* (2008) reported a higher prevalence of infection in young cattle. The low infections of biliary amphistomosis in adult cattle may be due to the mechanical barriers by fibrosis in bile ducts which may reduce the chances of establishment of the fluke (Alim *et al*., 2005). Prevalence in aged animals could be explained by the accumulation and longevity of infection would allow them to remain for a longer period, immunological phenomenon, grazing habits, method of study and management practice.

In sheep and goats, age-wise prevalence of paramphistomosis was recorded in the present study. Except, sheep of <1 year age group, all other small ruminants of more than one year age group showed a higher prevalence of paramphistomosis infection (Table 4). As the age advances, the animals were more infected, except the sheep under one year age group which showed a significant higher prevalence rate by SH examination. The present findings were corroborated with Bunza *et al.* (2008). Low prevalence of infection in animals of 1-2 years may be due to the fact that, only healthy young animals were brought to slaughter, and those affected by acute paramphistomosis were dead even before they were brought to slaughter and long prepatent periods of fluke infection. In contrast, the higher prevalence of infection in young sheep was noted by Shahnawaz *et al*. (2011) and Godara *et al.* (2014). The prevalence of infection in goats of more than one year group was reported to be high in the present study by SH examination (63.27%) and this observation was similar to other findings (Uddin *et al.,* 2006; Bunza *et al.,* 2008).

The prevalence of infection in this study was reported to apparently higher in female sheep than males (Table 5) and is consistent with Godara *et al.*, (2014). The possible reason for higher infection in females may be due to loss of immunity during pregnancy, birth and lactation (Barger, 1993). In the present study, male goats showed higher prevalence of infection than females by SH examinations and it was similar to the reports of Uddin *et al.* (2006). In a study by Paul *et al.* (2011) reported female cattle were more infected by 1.79 times than males. Swarnakar *et al*., (2014), reported male buffaloes were more susceptible to the infection than female animals and this may be due to lack of care because of their future economic importance.

In cattle, five fluke species were identified morphologically *viz*., *C. cotylophorum, P. cervi, G. crumenifer, F. elongatus,* and *Gigantocotyle* species (Fig 2-6). But in small ruminants, only first three were noticed apart from mixed infections. The exact cause of mixed infection is difficult to explain, but might be competition among the parasite for accommodation and food (Alim *et al*. 2005). The variation among species may be explained that, cattle have an indiscriminate type of grazing behavior and goats do not usually graze in marshy areas. In addition, goats are browsers by nature and they tend to graze in very rare cases and there is low probability of picking of the infection. The present study supports the findings of Kumari and Hafeez (2005) and *G. crumenifer* was the predominant species in both the studies. Our findings were corroborated with Swarnakar *et al*. (2014) who reported *P. cervi* and *Gigantocotyle* spp. in buffaloes from Rajasthan. It is significant to note that the infection in cattle with *G. crumenifer* was more prevalent compared to rest of the amphistome species. The variations can be attributed to the densities of snails in the water bodies (Swarnakar and Kumawat, 2013). From Maharashtra, Gadre *et al.,* (2008) found only *Paramphistomum* spp. in cattle. Other studies observed various amphistome species in cattle with different combinations of mixed infection with one or more species, in which some were identical to the present study (Rolfe *et al.,* 1991; Dube *et al*., 2004). Many studies on the prevalence

Table 4: Age-wise prevalence of paramphistomosis in sheep and goats.

Age	Number positive/number screened (%)					
	Direct smear method		Faecal sedimentation method		PM/Slaughterhouse examination	
	Sheep	Goat	Sheep	Goat	Sheep	Goat
<1 year	56/231 (24.24)^{Aa}	16/115 (13.91)^{Aa}	68/231 (29.44)^{Aa}	22/115 (19.13)^{Aa}	130/231 (56.28)^{Ab**}	25/115 (21.74)^{Aa}
	(16.98 - 31.51)	(5.60 - 22.23)	(21.71 - 37.16)	(9.68 - 28.58)	(47.87 - 64.68)	(11.83 - 31.65)
>1-2 years	165/616 (26.79)^{Aa}	28/98 (28.57)^{Aa**}	213/616 (34.58)^{Ab}	43/98 (43.88)^{Ba}	227/616 (36.85)^{Bb}	62/98 (63.27)^{Bb**}
	(22.19 - 31.38)	(16.82 - 40.33)	(29.64 - 39.51)	(30.97 - 56.79)	(31.84 - 41.86)	(50.72 - 75.81)
Total	**221/847 (26.09)^{a}**	**44/213 (20.66)^{a}**	**281/847 (33.18)^{b}**	**65/213 (30.52)^{ab}**	**357/847 (42.15)^{c}**	**87/213 (40.85)^{b}**
	(22.21 - 29.98)	**(13.51 - 27.80)**	**(29.01 - 37.34)**	**(22.39 - 38.64)**	**(37.78 - 46.52)**	**(32.17 - 49.52)**

Values are actual proportions with percentages and 95% confidence intervals in parenthesis; Proportions with different alphabet superscripts are significantly different (P<0.05); Capital alphabets are for vertical comparison (between age groups), lower case alphabets are for horizontal comparison (between methods in each species), * or ** indicates significant difference between species in each method; Chi-square test and Marasculio procedure (Comparison between methods) using Microsoft Excel software version 2013.

Table 5: Sex-wise prevalence of paramphistomosis in domestic ruminants.

Kind of Animal	Number positive/number screened (%)					
	Male			Female		
	Direct smear Method	Sedimentation Method	PM/Slaughterhouse examination	Direct smear Method	Sedimentation Method	PM/Slaughterhouse examination
Cattle	14/75 (18.67)^{Aa}	23/75 (30.67)^{Aab}	34/75 (45.33)^{Ab}	5/34 (14.71)^{Aa}	11/34 (32.35)^{Aa}	13/34 (38.24)^{Aa}
	(7.08 – 30.26)	(16.95 – 44.38)	(30.53 – 60.14)	(0.00 – 30.35)	(11.69 – 53.02)	(16.77 – 59.70)
Sheep	129/561 (22.99)^{Aa}	172/561 (30.66)^{Ab}	218/561 (38.86)^{Ac}	92/286 (32.17)^{Ba}	109/286 (38.11)^{Aa}	139/286 (48.60)^{Ab}
	(18.42 – 27.57)	(25.65 – 35.67)	(33.56 – 44.16)	(25.05 – 39.28)	(30.71 – 45.51)	(40.99 – 56.21)
Goat	29/131 (22.14)^{Aa}	36/131 (27.48)^{Aa}	55/131 (41.98)^{Ab}	15/82 (18.29)^{Aa}	29/82 (35.37)^{Ab}	32/82 (39.02)^{Ab}
	(12.79 – 31.48)	(17.43 – 37.53)	(30.88 – 53.09)	(7.30 – 29.29)	(21.77 – 48.97)	(25.15 – 52.90)
Total	**172/767 (22.42)^{a}**	**231/767 (30.12)^{b}**	**307/767 (40.03)^{c}**	**112/402 (27.86)^{a}**	**149/402 (37.06)^{b}**	**184/402 (45.77)^{c}**
	(18.55 – 26.30)	**(25.85 – 34.38)**	**(25.85 – 34.38)**	**(22.10 – 33.62)**	**(30.86 – 43.27)**	**(39.37 – 52.17)**

Values are actual proportions with percentages and 95% confidence intervals in parenthesis; Proportions with different alphabet superscripts are significantly different (P<0.05); Capital alphabets are for vertical comparison (between age groups), lower case alphabets are for horizontal comparison (between methods in each species), * or ** indicates significant difference between gender in each method; Chi-square test and Marasculio procedure (Comparison between methods) using Microsoft Excel software version 2013.

Table 6: Details of paramphistomes identified in slaughtered domestic ruminants.

Paramphistome species	Animal		
	Cattle (%)	Sheep (%)	Goat (%)
Cotylophoron cotylophorum	15 (15.0)	24 (9.60)	16 (10.67)
Paramphistomum cervi	12 (12.0)	13 (5.20)	09 (6.0)
Gastrothylax crumenifer	19 (19.0)	29 (11.60)	32 (21.33)
Fischoederius elongatus	01 (1.0)	-	-
Gigantocotyle spp.	03 (3.0)	-	-
Mixed infection with two or more species	50 (50.0)	184 (73.6)	93 (62.0)
Total specimens	100	250	150

of infection are based on morphology of amphistome egg, which is not possible to identify the parasite species (Saha *et al.,* 2013). In future studies, this may be overcome by molecular techniques, even dung sample with amphistome ova is sufficient for species identification.

In the present study, *G. crumenifer* is the most predominant species in sheep and goats, followed by *C. cotylophorum* and *P. cervi* (Table 6) and is in agreement with earlier studies by Kabir *et al*. (2010). In India, Shahnawaz *et al*., (2011) identified *C. cotylophorum*, *G. crumenifer* and *Carmyerius spatiosus* in sheep of Kashmir valley. In goats, the present observations were in agreement with other reports of Uddin *et al.* (2006) and Kabir *et al*. (2010).

CONCLUSION

The present data concludes that, a very high prevalence of paramphistomosis in domestic animals of Chittoor district of Andhra Pradesh was observed. Infection is cycling among the domestic ruminants and not much variation was observed between cattle, sheep and goats. An alternative strategy involves periodical treatment of chronic cases of paramphistomosis is recommended to reduce the contamination of grazing lands and also to prevent the opportunity to pick up the infection by snail intermediate hosts.

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