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 29.66% 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 (38.85%). 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 database 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 circumstances (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; Swamkar et al., 2010; Yadav et al., 2010; Khajuria et al., 2011; Rahman et al., 2012; Maity 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...
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).

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

| Kind of animal | Type of sample | Total |
|----------------|----------------|-------|
| Cattle         | Dung           | 109   |
|                | Adult amphistomes | 100   |
|                | Total          | 209   |
| Sheep          | Dung           | 847   |
|                | Adult amphistomes | 250   |
|                | Total          | 1097  |
| Goat           | Dung           | 213   |
|                | Adult amphistomes | 150   |
|                | Total          | 363   |
| Total          | Dung           | 1169  |
|                | Adult amphistomes | 500   |
|                | Total          | 1669  |
the amphistome eggs (Fig 1) in the dung samples by DS and SD methods (Sousby, 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 (Sousby, 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 Marascuillo 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 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.

| Animal   | Direct smear method | Faecal Sedimentation method | PM/Slaughterhouse examination |
|----------|---------------------|-----------------------------|-------------------------------|
| Cattle   | 19/109 (17.43)ab (8.07 - 26.79) | 34/109 (31.19)ab (19.76 - 42.62) | 47/109 (43.12)ab (30.90 - 55.34) |
| Sheep    | 22/847 (26.09)ab (22.21 - 29.98) | 281/847 (33.18)ab (22.21 - 29.98) | 357/847 (42.15)ab (33.78 - 46.52) |
| Goat     | 44/213 (20.66)abc (13.51 - 27.80) | 65/213 (30.52)abc (22.39 - 38.64) | 87/213 (40.85)abc (32.17 - 49.52) |
| Total    | 284/1169 (24.29)b (21.06 - 27.53) | 380/1169 (32.51)b (28.98 - 36.04) | 491/1169 (42.0)c (38.26 - 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); Marascuillo procedure using Microsoft Excel software version 2013.

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

| Age     | Direct smear method | Faecal sedimentation method | PM/Slaughterhouse examination |
|---------|---------------------|-----------------------------|-------------------------------|
| <2 years | 5/42 (11.90)ab (0.00 - 24.78) | 9/42 (21.43)ab (5.12 - 37.74) | 19/42 (45.24)ab (25.46 - 65.02) |
| 2-4 years| 11/42 (26.19)ab (8.72 - 43.67) | 19/42 (45.24)ab (25.46 - 65.02) | 21/42 (50.0)c (30.13 - 69.87) |
| >4 years | 3/25 (12.0)a (0.00 - 28.74) | 6/25 (24.0)ab (2.00 - 46.00) | 7/25 (28.0)c (4.87 - 51.13) |
| Total   | 19/109 (17.43)b (8.07 - 26.79) | 34/109 (31.19)ab (19.76 - 42.62) | 47/109 (43.12)c (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); Marascuillo 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 (Shahib 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 5: Sex-wise prevalence of paramphistomosis in domestic ruminants.

| Kind of Animal | Male | | | Female | | |
|----------------|------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Direct smear method | Sedimentation method | PM/Slaughterhouse examination | Direct smear method | Sedimentation method | PM/Slaughterhouse examination |
| Cattle | 14/75 (18.67) | 23/75 (30.67) | 34/75 (45.33) | 5/34 (14.71) | 11/34 (32.35) | 13/34 (38.24) |
| (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) | 172/561 (30.66) | 218/561 (38.86) | 92/286 (32.17) | 109/286 (38.11) | 139/286 (48.60) |
| (18.42 - 27.57) | (33.56 - 44.16) | (29.64 - 39.51) | (25.05 - 39.28) | (30.71 - 45.51) | (31.84 - 41.86) | (40.99 - 56.21) |
| Goat | 29/131 (22.14) | 36/131 (27.48) | 55/131 (41.98) | 15/82 (18.29) | 29/82 (35.37) | 32/82 (39.02) |
| (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) | 231/767 (30.12) | 307/767 (40.03) | 112/402 (27.86) | 149/402 (37.06) | 184/402 (45.77) |
| (18.55 - 29.98) | (13.51 - 27.80) | (20.91 - 33.74) | (22.10 - 33.62) | (30.86 - 43.27) | (39.37 - 52.17) |

Values are actual proportions with percentages and 95% confidence intervals in parentheses; 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 Marascuilo procedure (Comparison between methods) using Microsoft Excel software version 2013.
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Table 6: Details of paramphistomes identified in slaughtered domestic ruminants.

| Paramphistome species                  | 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)  |
| Gastrolyax crumenifer                  | 19 (19.0)  | 29 (11.60)| 32 (21.33) |
| Fiscoederius 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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