Infection Status of Paramphistomes in Cattle at Northern Area of Bangladesh

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Abstract

Objectives: Paramphistomiasis is a common disease of ruminant causes heavy economic losses, distributed all over the world and caused by different genus and species of paramphistomes. This study was conducted to investigate the epidemiology of paramphistomiasis in cattle in northern areas of Bangladesh.

Materials and methods: A cross sectional study was conducted from July, 2018 to October, 2019 and a total 300 faecal, 150 visceral and 300 snail samples were examined. The faecal samples were examined by Modified Stoll’s Dilution Technique. The paramphistomes were preliminary identified under microscope using low powder objectives and snails were identified by their characteristics shell characters.

Results: The overall prevalence of paramphistomiasis was recorded as 79.66% (faecal sample) and 92.67% (visceral sample). Prevalence was higher in adult and female than young and male. Cross breed and pasture grazing cattle were more infected than indigenous and stall feeding cattle. Infection rate was high in poor health cattle and rainy season than good health cattle and summer season. It was observed that prevalence of paramphistomiasis in cattle was significantly affected by age, sex, breed, feeding habit of cattle but seasons of the year had no significant effect. It was found that 6.33% snail (Indoplanorbis sp.) of the research areas were infected with amphistomes cercariae and infection rate was high in rainy season. Denudation of the rumen papillae and severe haemorrhagic enteritis was also observed.

Conclusion: It may conclude that age, sex, breed, feeding habit of cattle had significant effect on the prevalence of paramphistomiasis in cattle.

Keywords: Paramphistomiasis, Cattle, Prevalence, Faeces, Viscera, Snail

I. INTRODUCTION

In Bangladesh there are many constrains in cattle production, among them malnutrition and parasitism are the major limiting factors [10]. Parasitic diseases are one of the major causes of hindering the livestock development around the globe including Bangladesh. It has been estimated that about 10% animals die annually due to parasitic diseases [29]. Gastrointestinal parasites are a major constraint to health and productivity in grazing livestock production systems [28]. The losses due to parasitism take in the form of mortality, poor general health condition, retard growth, lower output of work, decrease in the production of milk and meat [4]. ADB report (1984) clearly mentioned that the loss of productivity of animals in terms of mortality, milk, meat, generations loss and other productive traits due to parasitism (50%) in Bangladesh. The geo-climatic conditions together with the water logged and low-lying areas in Bangladesh are conducive to parasitic diseases in domestic ruminants.

In fact, the cattle of Bangladesh are affected by various types of parasitic infection [18]. Helminthiasis is one of the most important groups of parasitic diseases in several countries. Among these infections, paramphistomes are the most common and pathogenic [14]. Paramphistomiasis is caused by digenetic flukes belong to the family paramphistomatidae. Adult paramphistomes are the main parasites in the rumen and reticulum of sheep, goats, cattle and water buffaloes etc. Paramphistomiasis is distributed all over the world, but its highest prevalence has been reported in tropical and subtropical regions, particularly in Africa, Asia, Australia, Eastern Europe and Russia [30]. Light infection does not cause serious damage to the animals, but massive number of immature paramphistomes can migrate through the intestinal tract causing acute parasitic gastroenteritis with high morbidity and mortality rates, particularly in young animals [7]. Mature paramphistomes are also responsible for ruminitis, irregular rumination, unthriftiness, loss of body condition, decrease in milk production and reduction of fertility [31]. Diagnosis of paramphistomiasis is mainly based on faecal examination [7]. The present investigation was therefore aimed to investigate infection status of paramphistomes in cattle and study gross lesions caused by paramphistomes.

II. MATERIALS AND METHODS

Study area, period and animal

The investigation was carried out in different districts of northern areas of Bangladesh during the period from July, 2018 to October, 2019. The experimental period was divided
into these three seasons such as summer (March-June), rainy (July-October) and winter (November-February). Three hundred cattle were selected randomly. The age of the cattle were above 6 months and were determined by examining the teeth (Sharma, 1981) and birth record. During collection of samples the age, sex, breed, place of farming and season of the year were carefully recorded. The cattle were categorized into two groups: young (< 4 years) and adult (>4 years) animals.

Collection of samples and preservation

Three types of samples were collected, these are-

- Faecal samples- 300 sample from 300 cattle
- Visceral samples- 150 viscera (GIT) from 150 slaughtered cattle
- Snail samples- 300 snails from different regions of research area

About 20-25 grams of faecal samples were collected randomly directly from the rectum and also from the recently voided faeces of 100 cattle in every season and kept in separate polythene bag, tied carefully and numbered properly. The samples were preserved in 10% formalin and examined as early as possible.

After slaughtering, the entire digestive tract of 150 cattle of both sexes were collected and examined carefully for any gross pathological changes caused by the parasites and after removing the rumen content, rumen, reticulum & duodenum carried to the laboratory.

A total of 300 aquatic snails (Indoplanorbis spp.) were collected from different pools, canals, ponds, beels, irrigation channels, rice fields and the edge of the river. The snails were collected ones in each pre-monsoon and post-monsoon period from a particular place. The snails were either hand-picked or collected with the help of scooped-net [13]. After collection the snails were kept in polythene bags containing water, brought to the laboratory.

Examination of samples

The faecal samples were examined by Modified Stoll’s Dilution Technique as described by Soulsby [23]. The paramphistomum eggs were indentified on the basis of their characteristics morphological features as described by Soulsby [23].

After removing the content, the rumen, reticulum and duodenum thoroughly washed and cleaned off ingesta and put in a separate jar containing luke warm normal saline and left for an hour or two to release the attachment of parasites from the wall of rumen reticulum and duodenum. Parasite found in the internal surface (usually in rumen) was scraped off with the finger and washing of both contents and scrapings were examined to record the parasite. The mucosal surface of the rumen reticulum and duodenum was rubbed carefully between the fingers to remove any remaining worms adhering to the wall of rumen reticulum and duodenum [16].

The snails washed in running tap water for 15 minutes. Snails containing different water vegetation and other debris’s on the shells were cleansed with soft brush and washed in running tap water. The snails were identified by their characteristics shell characters as described by Hubendick [8] and Malek and Cheng [12].

Collection and Identification and preservation of the parasites

The parasitic counts of gastro-intestinal contents and washings of the viscera were made by the methods followed by Taylor [24] and Islam [9] with little modifications.

The worms (paramphistomes) were preliminary identified under microscope using low powder objectives. The paramphistomes were then preserved in 10% formalin in separate vials.

The parasites were finally identified following the standard key [26] after preparation of permanent slides as described by Cable [3].

Study of gross lesions

To detect gross lesions and attachment of parasites, the mucosal surface of the rumen, reticulum and duodenum was examined carefully before washing and detected gross lesions were recorded.

Statistical analysis

The influence of age, sex and season on the prevalence of paramphistomiasis was analyzed by chi-square \( \left( X^2 \right) \) test as described by Mostafa [32]. Odds ration were calculated according to the formula given by Schleselman (21).

III. RESULTS AND DISCUSSIONS

Overall prevalence of paramphistomiasis in cattle

In the present study a total 300 faecal and 150 visceral samples were examined of which 239 (79.66%) faecal sample and 139 (92.67%) visceral samples were found to be infected with paramphistomiasis fluke (Fig. 1). The present findings are near about similar to the earlier findings of Uddin [25] who reported 72.19% prevalence of paramphistomiasis through visceral examination. The result is higher than the earlier report of Garrels [5] who recorded 64.4% prevalence of paramphistomiasis in cattle. This variation in the prevalence of paramphistomiasis in cattle may be due to agro ecological conditions, animal husbandry practices, lack of deworming practice, wrong method of deworming, prevalence of infected intermediate snail hosts, selection of samples, techniques of samples collection etc.

Age related prevalence of paramphistomiasis in cattle

From this study, it was observed that the age of cattle had significant effect on the prevalence of paramphistomiasis in cattle. The highest rate of infection was found in adult cattle aged > 4 years (82.67% in faecal sample and 98.67% in visceral sample). The lower rate of infection was found in...
young aged <4 years (76.96% in faecal sample and 86.67% in visceral sample). Adult cattle were 7.94 times (in visceral sample) more likely to be infected than young animals (Table 1). This result is in agreement with the earlier findings of Alim et al. [2], Okafor et al. [17]. Alim et al. [2] reported the infection rate is increased with the increases of age which was 7.8% in calf and 65.6 in older animals. Okafor et al. [17] noted prevalence of paramphistomiasis was significantly higher in animal more than two years old (57.0%) than calf (13.19%). The reason for this variation in the prevalence of infection in different age groups in cattle in difficult to explain but it might be due to an immunological phenomenon [1], grazing habit and managemental variation.

**Sex related prevalence of paramphistomiasis in cattle**

Sex of the host had a significant effect on the prevalence of paramphistomiasis. From both type of samples this study revealed a relatively higher prevalence of paraphistomiasis in female (89.33% in faecal sample and 96.00% in visceral sample) than male (74.67% in faecal sample and 89.33% in visceral sample). Females were 2.87 times (in visceral sample) and 1.87 times (faecal sample) more likely to be infected than males (Table 2). The study is supported by the findings of Alim et al. (2004) who reported the female cattle (52.8%) were more commonly affected than male (47.5%). This result is in agreement with the earlier report of Saifuzzaman (2011) in cattle, who reported the female (37.5%) were more affected with paramphistomiasis than male (24.9%). But this finding is dissimilar with the findings of Khan et al. (2011), who reported the prevalence of paramphistomiasis in cattle in cattle is higher in males (29.6%) than female (23.61%). The higher percentage of infection in the females may be due to the alteration in the physiological condition of the animals during pregnancy and lactation (Production activity) and also the lack of feed supplement for production, which may lead to the lowering of body resistance of the females.

**Breed related prevalence of paramphistomiasis in cattle**

In this study it was observed that breed of cattle had significant effect on the prevalence of paramphistomiasis. The higher rate of prevalence (84.67% in faecal sample and 97.33% in visceral sample) was recorded in cross breed cattle than in indigenous cattle (74.67% in faecal sample and 88.00% in visceral sample). Cross breed cattle were 1.87 times (faeces) and 4.98 times (visceral sample) more likely to be infected than Indigenous cattle (Table 3). It is suggested that cross breed cattle were mostly prone to any infection including parasite infection [1].

**Seasonal prevalence of paramphistomiasis in cattle**

In the present study it was observed that incidence of faecal sample the prevalence of paramphistomiasis was higher in rainy season (85.0%) than winter (81.0%) and summer (73.0%) season, visceral sample also revealed the similar result, i.e. prevalence of paramphistomiasis was higher in rainy season (96.00%) than winter (94.00%) and summer (88.00%). There exist no statistically significant (P>0.05) associations of the season with the prevalence of paramphistomiasis. Cattle were (1.33 in feaces & 1.53 in viscera) times and (2.1 in feaces & 3.27 in viscera) times more likely to be infected in rainy season than winter and summer season (Table 4). The results are supported by the earlier reports of Uddin, who reported the highest prevalence of amphistomes in rainy season (83.63%) followed by winter (69.23%) and lower in summer (64.00%) season. The present finding is also in agreement with the earlier records of Khan et al. (2011), who reported that the incidence of paramphistomiasis was highest during the rainy season (74.4%) followed by winter (57.2%) and summer (24.4%). This variation might be due to different climatic factors such as temperature, rainfall, humidity etc, which influenced the availability of intermediate host, other agro-climatic condition, ecology of the vector & host and geographical location of the experimental area.

**Feeding habit related prevalence of paramphistomiasis in cattle**

From this study, it was observed that the prevalence rate of paramphistomiasis was higher in grazing cattle (86.0% in faecal sample and 96.67% in visceral sample) than stall feeding cattle (76.5% in faecal sample and 90.00% in visceral sample). This study revealed that feeding habit had significant (P<0.01) effect on prevalence rate of paramphistomiasis and also observed that the grazing cattle were 1.89 times (faeces) and 2.86 (viscera) times more likely to be infected than stall feeding cattle (Table 5) and it is in the conformity with previous reports of Alim, et al. [2] who reported the higher rate of amphistomes infection was in grazing animals (68.00%) than stall feeding animals (22.2%).

**Health status-related prevalence of paramphistomiasis in cattle**

Health status-related prevalence of paramphistomiasis in cattle is shown in Table. The present study revealed that health status had significant (P<0.05) effect on the prevalence of paramphistomiasis in cattle. It was found that the prevalence of paramphistomiasis was higher in poor health cattle (88.0% in faecal sample and 97.92% in visceral sample) than good health cattle (75.5% in faecal sample and 90.00% in visceral sample). It was also observed that poor health cattle were 2.4 times (faeces) and 5.11 (viscera) times more likely to be infected than good health cattle (Table 6). There exist no statistically significant (P>0.05) associations of the health status with the prevalence of paramphistomiasis. Cattle were (1.33 in feaces & 1.53 in viscera) times and (2.1 in feaces & 3.27 in viscera) times more likely to be infected in rainy season than winter and summer season (Table 4). The results are supported by the earlier reports of Uddin, who reported the highest prevalence of amphistomes in rainy season (83.63%) followed by winter (69.23%) and lower in summer (64.00%) season. The present finding is also in agreement with the earlier records of Khan et al. (2011), who reported that the incidence of paramphistomiasis was highest during the rainy season (74.4%) followed by winter (57.2%) and summer (24.4%). This variation might be due to different climatic factors such as temperature, rainfall, humidity etc, which influenced the availability of intermediate host, other agro-climatic condition, ecology of the vector & host and geographical location of the experimental area.

**Intensity of paramphistomes infection in cattle regarding egg release**

The range of EPG (Egg per gram) in paramphistomiasis varies among the cattle population. From the present study it observed that the highest, 44.54% cattle had the EPG range between 401-800 while 7.47% cattle had the EPG range between 1201-1600 and only 2.12% cattle had EPG over 1600 (Fig. 2). This finding also contrasts with the findings of Jithendan and Bhat [11].
Seasonal incidence of amphistomes cercariae in the snails

In this study 300 (100 in each season) aquatic snails (Indoplanorbis spp.) were examined for the presence of amphistomes cercariae. It was also observed that 6.33% snail (Indoplanorbis sp.) of the research areas are infected with amphistomes cercariae and infection rate was higher in rainy (9.0%) season than winter (6.0%) and summer (4.0%) (Table 7). There exists no statistically significant association of the season with the prevalence of Paramphistomiasis. The proportions of infected animals in different seasons differ insignificantly. The seasonal high prevalence of amphistomes cercariae in the snails found in this study confirms the reports of Mondal et al. [15] who reported there was significant variation in the distribution of vector snails in the different season of the year. The present finding of snail (Indoplanorbis spp.) infection rate with amphistome cercariae was higher than the reports of Rahman et al. [19].

Gross pathological change associated with paramphistomiasis

In the rumen and reticulum adult and young paraphistomes found attached to walls in colonies. A heavy burden of worms was seen in the oesophageal and/or reticular end of rumen. The gross pathological changes associated with the mature stage of paramphistomiasis flukes in naturally infected cattle were mostly observed in the rumen. At the attachment sites of the worms the rumen papillae were severely denuded (Fig 3). These denudations even destroyed about 30% to 40% of the rumen papillae.

Immature paramphistomes were found to be causes severe haemorrhagic enteritis mostly in the pyloric region of duodenum (Fig 3). In the duodenum, the upper part of duodenal mucosa was thickened, corrugated, congested and covered with blood stained mucus with the presence of numerous immature paramphistomes. The immature flukes burrowed into the mucosa of the duodenum. This finding is in conformity with the previous report of Mondal et al. [15]; Uddin [25]; Hossain and Baki [6], who reported pathological changes of amphistomiasis observed mainly in rumen, reticulum and duodenum which mainly includes denudation and destruction of rumen papillae and hemorrhagic lesions, slight thickening and corrugation in duodenum.

IV. CONCLUSION

Paramphistomiasis is one of the most common problems in cattle at the study area. The present study revealed that the overall prevalence of paramphistomiasis was recorded as 79.66% (faecal sample) and 92.67% (visceral sample). Prevalence was higher in adult, female, cross breed, pasture grazing and poor health cattle. Infection rate was high in rainy season than others.

REFERENCES


Fig. 1: Overall prevalence of paramphistomiasis in cattle

Fig. 2: Intensity of paramphistomes infection by counting egg (EPG)

Fig. 2: (A) Egg of paramphistomes (4X), (B) Egg of paramphistomes (10X), (C) Cercariae of paramphistomes (4X), (D) Cercariae of paramphistomes (10X), (E) Adult & preserved paramphistomes
Fig. 3: Pathological Changes in GIT - (A) Normal rumen papillae with paramphistomes, (B) Destruction of rumen papillae, (C) Sloughing off mucosa of reticular fold, (D) Hemorrhage in duodenum.

Table 1. Age-related prevalence of paramphistomiasis in cattle

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Age group</th>
<th>Samples examined</th>
<th>Positive cases</th>
<th>Negative cases</th>
<th>Odds ratio</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>Young (&lt;4 years)</td>
<td>150</td>
<td>115</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adult (&gt;4 years)</td>
<td>150</td>
<td>124</td>
<td>26</td>
<td>Adult vs Young = 1.45</td>
<td>1.667</td>
<td>0.197 (NS)</td>
</tr>
<tr>
<td>Viscera</td>
<td>Young (&lt;4 years)</td>
<td>75</td>
<td>65</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adult (&gt;4 years)</td>
<td>75</td>
<td>74</td>
<td>1</td>
<td>Adult vs Young = 11.38</td>
<td>7.946</td>
<td>0.005**</td>
</tr>
</tbody>
</table>

** means significant at 1% level of significance, NS means statistically not significant

Table 2. Sex-related prevalence of paramphistomiasis in cattle

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Sex</th>
<th>Samples examined</th>
<th>Positive cases</th>
<th>Negative cases</th>
<th>Odds ratio</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>Male</td>
<td>150</td>
<td>112</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>150</td>
<td>127</td>
<td>23</td>
<td>Female vs Male = 1.87</td>
<td>4.630</td>
<td>0.031*</td>
</tr>
<tr>
<td>Viscera</td>
<td>Male</td>
<td>75</td>
<td>67</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>75</td>
<td>72</td>
<td>3</td>
<td>Female vs Male = 2.87</td>
<td>2.453</td>
<td>0.117 (NS)</td>
</tr>
</tbody>
</table>

* means significant at 5% level of significance, NS means statistically not significant

Table 3. Breed-related prevalence of paramphistomiasis in cattle

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Breed</th>
<th>Samples examined</th>
<th>Positive cases</th>
<th>Negative case</th>
<th>Odds ratio</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>Cross</td>
<td>150</td>
<td>127</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indigenous</td>
<td>150</td>
<td>112</td>
<td>38</td>
<td>Cross vs Indigenous = 1.87</td>
<td>4.630</td>
<td>0.031*</td>
</tr>
<tr>
<td>Viscera</td>
<td>Cross</td>
<td>75</td>
<td>73</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indigenous</td>
<td>75</td>
<td>66</td>
<td>9</td>
<td>Cross vs Indigenous = 4.98</td>
<td>4.807</td>
<td>0.028*</td>
</tr>
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</table>

* means significant at 5% level of significance
Table 4. Seasonal prevalence of paramphistomiasis in cattle

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Season of year</th>
<th>Samples examined</th>
<th>Positive cases</th>
<th>Negative cases</th>
<th>Odds ratio</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>Summer</td>
<td>100</td>
<td>73</td>
<td>27</td>
<td>Rainy vs Summer=2.1</td>
<td>4.609</td>
<td>0.100 (NS)</td>
</tr>
<tr>
<td></td>
<td>Rainy</td>
<td>100</td>
<td>85</td>
<td>15</td>
<td>Rainy vs Winter=1.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>100</td>
<td>81</td>
<td>19</td>
<td>Winter vs Summer=1.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viscera</td>
<td>Summer</td>
<td>50</td>
<td>44</td>
<td>6</td>
<td>Rainy vs Summer=3.27</td>
<td>2.55</td>
<td>0.279 (NS)</td>
</tr>
<tr>
<td></td>
<td>Rainy</td>
<td>50</td>
<td>48</td>
<td>2</td>
<td>Rainy vs Winter=1.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>50</td>
<td>47</td>
<td>3</td>
<td>Winter vs Summer=2.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS means statistically not significant

Table 5. Feeding habit-related prevalence of paramphistomiasis in cattle

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Feeding habit</th>
<th>Samples examined</th>
<th>Positive cases</th>
<th>Negative cases</th>
<th>Odds ratio</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>Grazing</td>
<td>100</td>
<td>86</td>
<td>14</td>
<td>Grazing vs Stall = 1.89</td>
<td>3.840</td>
<td>0.046*</td>
</tr>
<tr>
<td></td>
<td>Stall feeding</td>
<td>200</td>
<td>153</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viscera</td>
<td>Grazing</td>
<td>60</td>
<td>58</td>
<td>2</td>
<td>Grazing vs Stall = 2.86</td>
<td>2.354</td>
<td>0.125 (NS)</td>
</tr>
<tr>
<td></td>
<td>Stall feeding</td>
<td>90</td>
<td>81</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* means significant at 5% level of significance, NS means statistically not significant

Table 6. Health status-related prevalence of paramphistomiasis in cattle

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Health status</th>
<th>Samples examined</th>
<th>Positive cases</th>
<th>Negative cases</th>
<th>Odds ratio</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>Good health</td>
<td>200</td>
<td>151</td>
<td>49</td>
<td>Poor vs Good = 2.38</td>
<td>6.430</td>
<td>0.011*</td>
</tr>
<tr>
<td></td>
<td>Poor health</td>
<td>100</td>
<td>88</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viscera</td>
<td>Good health</td>
<td>102</td>
<td>92</td>
<td>10</td>
<td>Poor vs Good = 5.11</td>
<td>2.86</td>
<td>0.091 (NS)</td>
</tr>
<tr>
<td></td>
<td>Poor health</td>
<td>48</td>
<td>47</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* means significant at 5% level of significance, NS means statistically not significant

Table 7. Seasonal prevalence of amphistome cercariae in the snail (*Indoplanorbis* sp.)

<table>
<thead>
<tr>
<th>Season of year</th>
<th>samples examined</th>
<th>Positive cases</th>
<th>Negative cases</th>
<th>Odds ratio</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>100</td>
<td>4</td>
<td>96</td>
<td>Rainy vs Summer=2.67</td>
<td>2.861</td>
<td>0.239 (NS)</td>
</tr>
<tr>
<td>Rainy</td>
<td>100</td>
<td>9</td>
<td>81</td>
<td>Rainy vs Winter=1.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>100</td>
<td>6</td>
<td>94</td>
<td>Winter vs Summer=1.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS means statistically not significant