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Epidemiological investigation of helminth infection of cattle from different districts of Mizoram, India

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Abstract
A study on incidence of helminth parasites of cattle was carried out from eight different districts of Mizoram, India from June 2018 to May 2019. A total of 1153 faecal samples from different age groups of cattle were examined microscopically using salt flotation and sedimentation method. Some positive samples were copro-cultured for proper identification of *Strongyle* larvae. The overall prevalence of gastro intestinal (GI) helminths was 33.39% (385/1153). The identified species revealed nematodes, cestodes and trematodes with a total of 11 species. The percentage prevalence of various helminthes in descending order include *Haemonchus* sp (20.25%), *Toxocaravitulovum* (15.84%), *Moniezia* sp (13.76%), *Strongyloidespapillosus* (10.38%), *Trichuris* sp (8.57%), *Oesophagastomum* sp (6.75%), *Trichostrongylus*sp (6.75%), *Nematodirus* sp (5.97%), *Fasciolagigantica* (5.19%), *Paramphistomum*sp (3.89%) and *Eurytrema* sp (2.59%). A total of 225 cattle slaughtered at local abattoirs were also inspected for the presence of any helminth and helminth induced lesions. At post-mortem examination, mixed infection with trematodes of *Fasciolagigantica*, *Paramphistomum* sp and nematodes of *Haemonchus* sp, *Oesophagostomum* sp and *Nematodirus* sp were observed. A significant seasonal difference was observed with higher prevalence during monsoon compared to other seasons. Similarly prevalence varied according to age and sex of the animals. Male and young calves were more commonly infected with GI parasites than female and older cattle. The epidemiological data obtained in the present study warranted proper examination of cattle for proper controlling of parasites.

Keywords: GI Parasites, cattle, Mizoram, India

1. Introduction
Gastro intestinal (GI) parasitism is one of the major constraints in economy for dairy cattle in several tropical and sub-tropical countries including India, Economic losses due to GI parasites include morbidity, mortality, loss of body weight, condemnation of affected organ and in severe cases even death of the affected animals [1, 2]. It has been observed that the prevalence of GI parasites is related to agro climatic conditions, management practices as well as host factors like age, breed, species, health status, pregnancy and previous exposure to parasites [3, 4]. Studying epidemiology of helminthes is very important because of wide geographical distribution in different regions of the globe [5]. Epidemiology of parasitic diseases in which the borderline between the safe and dangerous worm load is indefinite and where manifestation of disease is generally subclinical and insidious in onset, it might be more desirable to think on the epidemiology in forms of population dynamics [6]. Epidemiological studies are used to know the causes of disease so that most appropriate control measures can be applied.

Many parasitic infections exhibit rhythmic pattern due to intrinsic and extrinsic factors. Interactions between hosts and parasites are embedded within environmental rhythms. But variation of parasitism due to seasonal changes is unknown. It is a rare chance that parasites can detect and respond directly to environmental stimuli like temperature, light etc. Parasites by living inside a host can overwinter in the internal organs like liver, intestine, muscle etc of endothermic hosts. Parasites fail to show periodicity because of circannual clock to time emergence but relapse in animals as a result of constant photoperiod regimes [7, 8].

The epidemiological study of GI parasites in cattle has been reported in several countries [9, 10] but veryfew reports are available from Mizoram, India. The aim of this report is to provide information on the prevalence of GI parasites of cattle from this part of India.

2. Materials and Methods

2.1 Study area

The study was conducted in eight districts of Mizoram India. The state of Mizoram is located on the North Eastern part of India and is surrounded by Myanmar and Bangladesh on the South and on the north by other North Eastern (NE) states of India like Manipur, Assam and Tripura (Fig. 1). The state receives an average rainfall of 350 mm.

2.2 Screening of animals for sample collection

The study was carried out from June 2018 to May 2019. One thousand one hundred and fifty three (1153) faecal samples were collected on four different seasons namely: monsoon (June, July, August) Post monsoon (Sept, Oct, Nov), Winter (Dec, Jan, Feb) and pre monsoon (Mar, April, May) irrespective age, breed or sex from both organized and in organized farms. Approximately five gram of faecal samples were collected directly from the animals' rectum individually into clean and sterile polyethylene disposable bags using a handbag. Animals of three different age groups (<1 year, 1 – 5 years and > 5 years) were selected randomly. In some cases, the samples were kept in 10% formalin solution.For recording the parasitic load in slaughtered animals' random inspections was carried out in slaughtered houses as well as in different meat markets.

2.3 Faecal sample examination

All the faecal samples were processed in the laboratory of Veterinary Parasitology Department, CVSc & AH, CAU, Selesih, Mizoram, India. Faecal samples were examined microscopically using flotation and sedimentation techniques as per method described earlier [11]. Faecal eggs were counted in fresh faecal sample in a McMaster slide using saturated salt solution as floatation fluid (sp. gr. -1.2). The number of eggs per gram of faeces was obtained by multiplying the total number of eggs counted in two chambers of the McMaster slide by the dilution factor of 50. The results are expressed as egg per gram of faeces (EPG).

2.4 Culture of faecal Samples

About 100gm of positive faecal samples were triturated using pestle and mortar and then mixed with animal charcoaland water is added until a pasty consistence was made. The mixture was then put into a culture jar and its mouth was inverted into a large petridish containing water. After 14 days the water in the petridish was pipetted out in centrifuge tubes, centrifuge and the larvae concentrated were killed by applying heat so that the larvae could die in a stretched condition. The larvae were then examined under microscope after placing the dead larvae on a slide by adding one or two drops of lugol's iodine. The larvae were identified with the help of the key provided by HMSO (1979) [12].

2.5 Post Mortem Examination

During the study period different slaughter areas were visited from time to time and carefully examined different viscera, muscle, skin mesenteric vein etc. To search for GI parasites, all ingesta were transformed to a bucket or a plastic container with slight scrubbing or light scrapping of the mucosal surface. Then water was added, allowed to stand for 5 minutes and decanted the supernatant. The process was repeated until clear sediment was obtained. After that a small amount of sediment was transferred into a petridish and examined under stereo zoom microscope. All parasites were cleaned with normal saline and identified accordingly [13]. In some caseshelminths were identified after putting them in lactophenol for 3-4 hours.

2.6 Prevalence and Statistical analysis

The prevalence was recorded according to age, sex, and season. The prevalence (P) was estimated according to standard method [14] and by the formula as given below:

$$P = \frac{\text{No. of infected cattle during specified period}}{\text{Total no. of cattle surveyed}} \times 100$$

The epidemiological data were subjected to Pearson's Chi-square (χ^2) test to assess if there was a statistically significant association in the various groups [15].

The upper and lower limits of the confidence intervals for the infection values of each pathogen species were calculated by the following formula [16]:

$$\hat{p} \pm z \cdot \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

Where, P-hat is dividing the numbers of events by the number of trials; z-score is the table value and n is the number of samples.

2.7 Ethical permission

All animal experiments were carried out strictly as per the guidelines issued by ARRIVE and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and was approved by Institutional Animal Ethics Committee with reference No.CVSc/CAU/IAEC/no. 6641, dtd, Selesih, the 25th March, 2018).

3. Results

Faecal sample examination revealed an overall prevalence of 33.39% (385/1153). The overall prevalence of GI parasites of cattle is shown in Table 1. Eggs of eleven species of helminthes were recorded in the present study. These included *Haemonchus* sp (20.25%), *Toxocaravitulovum* (15.84%), *Moniezia* sp (13.76%), *Strongyloidespapillosus* (10.38%), *Trichuris* sp (8.57%), *Oesophagastomum* sp (6.75%), *Trichostrongylus* sp (6.75%), *Nematodirus* sp (5.97%), *Fasciolagigantica* (5.19%), *Paramphistomum* sp (3.89%) and *Eurytrema* sp (2.59%). These are identified according to their egg and larval characters (Figures 2-15). Among the GI nematodes *Haemonchus* sp, *Trichostrongylus* sp and *Oesophagastomum* spwere more common in cattle while *Toxocaravitulorum* and *Strongyloidespapillosus* were only observed in calves.Co-infection with more than one GIparasite was recorded from some animals.

During the whole study period a total of 225 numbers of carcasses were inspected at slaughtered places as well as stomach and intestinal contents in laboratory out of which 79 numbers (35.11 %) of animals were positive for helminthiasis. Mixed infection with trematodes of *Fasciolagigantica*, *Paramphistomum* sp and nematodes of *Haemonchus* sp, *Oesophagostomum* sp and *Nematodirus* sp were observed.

The highest incidence was recorded from Aizawl district (Fig. 16). From table 2 it is clear that during monsoon period helminth infection was highest (37.37%) followed by pre-monsoon (33.33%), post monsoon (32.50%) and winter

(25.65%). The results varied with age and sex which is shown in the same table. Prevalence of *Toxocaravitulorum* and *Strongyloidespapillosus* was higher in young animals (<1 year old) than in older animals (>5 year old). In contrast, prevalence of *Moniezia* sp and *Paramphistomum* sp was lower in young animals compared to older age group. Further, data showed that the prevalence rate in male was slightly higher (33.63%) as compared to that of females (33.29%). In this study, mixed infection was also recorded in some animals. The egg per gram of faeces (EPG) was considered to be the severity of GI parasite infection in cattle. The EPG recorded in the present study ranged from 250 to 650.

4. Discussion

Biological rhythms are thought to have evolved to enable organisms to organize theiractivities according to circadian cycles. The parasitic infections vary according to fitness consequences and epizootiology of individual parasite and the correlation between host parasite relationship, host physiology and local environmental factors [17]. GI parasitic infection most commonly occurred during rainy season in many temperate and tropical climates because favourable environmental conditions ensure survival of parasitic stages and when hosts' vast amount of energy is devoted for reproduction. When the rhythms of parasites are not in synchrony with the rhythms of their environment, the parasite's capacity to reproduce and transmission is reduced considerably [18]. Unlocking the mysterious of how parasite rhythms are organized would greatly facilitate the role of rhythm in interactions between host and parasites and the fitness consequences of these interactions.

The present study revealed that the cattle in the study area suffer from a wide variety of GI helminthes, albeit in varying degree of intensity. The study clearly indicated parasitic burden is dominated by *Haemonchus* sp which might be due to favourable environmental conditions and frequent exposure to pasture contamination. The rare of helminthic infection vary from country to country and even within the same country. This could be due to factors like grazing habits, livestock management, frequency of deworming to animals etc. [19]

The prevalence rate of *Strongyloidespapillosus* in the present investigation at 10.38% was comparable to the results reported by previous workers at 7% [20]. *Toxocaravitulorum* is only recorded in calveswhich might be due to prenatal infections from their mother. The low level of fascioliasis and paramphistomiosis can be explained by epidemiological factors. The occurrence of these trematodal infections is related to the risk of ingestion of metacercariae. Mizoram, being a hilly state of India the chances of ingestion of infective stage from snail through food and drink is very rare.The slightly higher rate of infection in male compared to female is unexplainable but might be due to the less care of male animals by the farmers. This finding of course is in agreement with other previous workers [21-23].

Calves had higher number of individuals with *Toxocaravitulorum* and *Strongyloidespapillosus* compared to other age groups but this difference was not seen with other helminthes like *Trichuris*, *Fasciola*, *Nematodirus*, *Paramphistomes* and *Strongyles*. In fact, adult cattle tend to feed from the open grazing grounds, while the calves were most tied around homestead. At slaughter the prevalence of helminthes infections was higher because most animals examined were brought from adjoining state of Assam and even from Myanmar. As a matter of fact, most of the research data gathered on the occurrence of *Fasciolagigantica* infection is based on abattoir study and faecal examination of fluke eggs is biased (Sanyal 2001). The same may be true for other parasites also.

5. Conclusion

The result clearly indicates that the cattle of different age group harbor helminthic load at varying intensity. However, the study was conducted for one year only and restricted to one state of NE region of India. So, to get a true epidemiology the study should cover entire NE region of India for an extended period of time with the ultimate goal of preparing a check list and control strategy.

Declaration of interest

The authors declare no conflict of interest.

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