Coproscopic and Haematological Approaches to Determine the Prevalence of Helminthiasis and Protozoan Diseases of Red Chittagong Cattle (RCC) Breed in Bangladesh

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ABSTRACT
While at present many crossbred cattle are available throughout Bangladesh, there are few original varieties of cattle localized in some areas of the country. They also have better performance compared to other available indigenous breeds. Red Chittagong Cattle (RCC) is thought to be the only recognized breed of Bangladeshi origin. An attempt has been made in this study to identify the prevalence of parasitic diseases in Red Chittagong cattle kept in different areas of Chittagong. The study showed that crossbred cattle and the RCC animals were infected at similar level by parasitic infestations. However, in the absence of proper management, RC cattle were generally resistant to some parasitic infections which were common in indigenous/crossbred cattle. It was also notable that in some areas the parasite prevalence was very low, indicating possible geographic factors responsible for parasite survival. A number of blood protozoan infections were also less prevalent in RC cattle than crossbred animals as found in most areas. This study is the first of its type to understand the prevalence of parasitic diseases in RCC breed of cattle which is thought to be the only indigenous cattle breed available in Bangladesh.

INTRODUCTION
Parasitic diseases are responsible for significant losses through morbidity and mortality in cattle in Bangladesh. Parasitism is the major cause hindering the development of livestock population in the country (Shahiduzzaman et al., 1999). Several studies have indicated the incidence of different parasitic diseases and their seasonal prevalence in cattle in Bangladesh (Rahman, 1969; Rahman and Razzak, 1973). These parasitic infections are more severe in younger animals than adults. Fasciolosis is reported to be one of the important diseases of cattle and small ruminants in the country (Qadir, 1981). Similarly, gastrointestinal nematodes are also serious problems for ruminants, especially young animals. Previous reports suggest that 50% calves up to 1 year of age died due to gastrointestinal parasites that cause digestive disturbances and malnutrition leading to calf mortality (Debnath et al., 1995). Different helminth infections are responsible for about 54.22% calf mortality in Bangladesh. Strongyles are another harmful group of bovine parasites due to their feeding habit or development process in the digestive system (Shahiduzzaman et al., 1999). The prevalence of blood protozoa in animals and birds of Bangladesh also has been reported by a number of authors (Banerjee et al., 1983; Samad and Shahidullah, 1984). They are the common anemia producing agents in indigenous cattle causing great economic losses to the farmers of Bangladesh. It was recorded that Trypanosoma theileri was prevalent in 5 out of 857 cattle in different parts of Bangladesh (Rahman et al., 1982). Another study revealed that bovine babesiosis, caused by Babesia bigemina and transmitted by the tick Boophilus microplus, is widespread in Bangladesh (Samad et al., 1988). There were several other studies where bovine babesiosis was reported in different parts of the country (Ahmed, 1976; Shahidullah, 1983).

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Bangladesh is one of the densely populated developing countries of the world, where more than 80% rural people rear indigenous cattle. While present many crossbred cattle are available throughout the country, there are a few original varieties of cattle localized in some areas of Bangladesh. They also have better performance compared to other available indigenous breeds. The Red Chittagong (RC) cattle is one of such varieties of cattle which are found usually in Chittagong district and Chittagong hill tract region and are rare in other parts of Bangladesh. Most of the RC cattle are found in the Thanas (administrative units) of Raozan, Anowara, Patia, Chandanaish and Bashkhali. There are few literatures available on the performance of RC cattle which is not internationally considered as a pure breed but as a variety (Mason and Buramendram, 1982). However, it has been reported that the RC cattle require lower input support than other indigenous cattle with high quality milk and beef production (Bhuiyan, 2007). The production performances of indigenous cattle like RC is not as high as those of the crossbred animals reared in the country but it is easy to be adopted by the existing farming system under harsh environmental conditions. Alongside good management issues, the disease incidence and susceptibility is a significant factor to gain maximum benefit from the RC cattle reared in Bangladesh. The present study was, therefore, designed to explore the status of parasitic diseases prevalent in RC cattle through classical coproscopic and blood smear analysis.

MATERIALS AND METHODS

Selection of animals
The study was conducted in the Department of Pathology and Parasitology, Chittagong Veterinary & Animal Sciences University (CVASU) with collaboration from the RCC breed upgradation and improvement team of Bangladesh Livestock Research Institute (BLRI). The RCC and crossbred animals from which samples were collected during this study were selected from a number of dairy farms located in different areas (Chittagong sadar, Anowara, Patia, Chandanaish, Raozan and Satkania) under Chittagong district of Bangladesh. For each animal, respective farm record was observed to determine their entity as a crossbred animal. To identify the RCC from different thanas of Chittagong, active support from the BLRI research team was received, who had earlier selected original RC breed of cattle and kept their pedigree record. The information from the BLRI research team was used to locate the RCC owners in the study area and samples were collected from these animals with prior permission of the owners.

Collection of biological samples
Two different types of samples (blood and faeces) were collected during this study. For crossbred animals, samples were collected from different dairy farms under Chittagong Metropolitan area. Samples of RC cattle were derived from animals of 5 thanas of Chittagong (Satkania, Anowara, Patia, Chandanaish, Raozan). Strict aseptic procedures were followed to keep the animal safe and healthy while collecting biological samples. All necessary ethical considerations were followed. Approximately 5-6 ml of blood was collected from jugular vein using 10 ml disposable plastic syringe from each animal and then preserved in Vacutainer® tube containing anticoagulant. Faecal samples (approximately 10 gm) were collected directly from the rectum of the animal using polyethylene gloves and stored in small plastic container. The container was then filled with 10% formalin after labeling with specific identification number. The gross examination of the faeces was also done. Each sample was carried out to the Parasitology laboratory of CVASU for further examination.

Examination of faecal samples (coproscopy)
In addition to gross examination of faecal samples (color, odor, consistency etc) two different types of qualitative analyses, namely direct & floatation technique were followed to detect the parasitic eggs, ova, cyst, tapeworm segments and larvae in the faecal materials. The direct smear method for faecal examination was performed, as described by Rahman et al. (1996) and Hossain and Ali (1998). At least three smears were prepared for each sample and eggs were identified on the basis of their morphological features (Thienpont et al., 1980). In addition to direct smear approach, all the faecal samples were further analysed using concentration (floatation) technique, using saturated salt solution to achieve maximum floatation of the eggs or ova (Souslby, 1986). This concentration method usually recovers most nematode eggs and protozoan cysts, still many trematode and cestode eggs, as well as Giardia cysts may not be recovered.

Examination of blood smear
Blood samples collected were examined in the laboratory through thin smear technique. Blood samples were collected on microscopic slides (as direct smear) in duplicate from each animal and then taken to the laboratory for subsequent staining.

After preparation, thin smear was fixed with ethanol for 10 minutes and stained with Giemsa stain. The stained blood smears were examined under binocular microscope (Olympus) at different magnifications (X40 and X1000) using immersion oil, when necessary.

Data analysis
The data derived from the microscopic examination of faecal and blood samples were recorded systematically in a separate log book. For statistical analysis, the data derived during this study were analyzed using Chi-square test.

RESULTS AND DISCUSSION

Faecal examination
Comparative analysis of positive cases of faecal samples revealed that Satkania (50%) was the most vulnerable place for trematode parasite infection amongst RCC animals (Fig. 1). Lowest infection was recorded in Chittagong sadar area (25%), where most of the animals were crossbred and were reared under controlled and intensive management. Almost similar percentages of infection levels were observed in animals of Anowara, Patia and Raozan, while those of Chandanaish were comparatively more susceptible to trematode parasitic...
infections. However, statistically no significant differences were found between samples collected from six study areas.

While fasciolosis is commonly found in cattle in different parts of Chittagong (Unpublished observation), no faecal samples were found positive for Fasciola sp. infection. This might be due to factors like lower susceptibility of RCC to fasciolosis and needs further investigation. In addition, the epidemiological study towards distribution of snail populations in different parts of Chittagong is essential to understand the risk factors of trematode infection in the livestock. Unfortunately, no relevant information was available during this study.

When the faecal samples were examined for cestode parasitic infections, no positive case was found in samples collected from RC cattle (data not shown). However, there was a single positive case in crossbred cattle which indicates the risk of cestodal infection in animals even when they are reared in controlled management. The absence of positive cases in RCC does not necessarily indicate that they are fully resistant to cestodal infection but anyone can assume that the incidence of cestode infection is fewer compared to trematode or nematode infection. Infestation of paramphistomiasis varies from 0.70 to 88.89% from place to place (Georgiev et al., 1980; Gupta et al., 1985; Michalski et al., 1990). Further study on the prevalence of arthropod intermediate host in the study area will be useful to explore the different cestodal infections available in cattle in the area.

Faecal samples examined for nematodiasis in RC cattle showed that highest nematode infection was prevalent in Patia (25%), while no single infection was recorded at Anowara (Fig. 2), which is located in coastal area. However, statistically there were no significant differences in nematode infection among different study areas. As most of the pasture of Anowara region is often covered with tidal water, there is a possibility that the infective larval stage of different nematode worms is regularly washed out with the water, contributing minimum infective larvae in the pasture. Thus, there might be lower risk associated with ingesting nematode larvae, resulting in fewer nematodal infections. However, this fact warrants an exclusive survey to determine the geographic distribution and seasonal incidence of nematode larvae in different areas before making any such hypothesis.

Faecal sample examination is also useful to identify the extent of enteric protozoal infection in animals. Present study revealed that RCC from Anowara were more susceptible to intestinal protozoan infections compared to other study areas (Fig. 3). Out of 20 samples from Anowara, 13(65%) were found positive, whereas no single positive case was recorded in Chandanaish. Again, statistically there were no significant differences among study areas.

The enteric protozoa are usually transmitted through drinking water and food ingestion. Thus, it seems that animals of Anowara are at high risk of enteric protozoan infections compared to other regions. The local practice of feeding unsafe water is possibly the most important cause for its spread. However, there are no differences in practice between different study areas regarding access of safe water by the cattle. Further analysis can identify the most important enteric protozoan infections in the respective area. The difference in prevalence among various studies might be due to variations in geo-climatic conditions of these areas of the study areas (Kakar and Kakarsulemankhel, 2008).

During this study, two different types of faecal sample examination techniques were performed, namely direct smear and Floatation technique. A comparison of the success rate of two techniques was made to assess
their feasibility for future diagnostic or research endeavors (Table 1). Of the 198 samples examined, 89(45%) and 40(20%) were found positive in direct smear technique and Floatation technique, respectively, the difference was statistically significantly (P<0.01). It was found that direct smear technique was twice as effective as Floatation technique. It might be due to some parasitic eggs that did not float but readily seen in heavy infection. Considering that the eggs which are light usually float in floatation technique, one can easily follow direct smear technique for any survey or seasonal incidence study. This is also cost-effective and easy to perform, even under field conditions.

The gastrointestinal parasitic infections in RCC and crossbred cattle were also further analysed to detect any sex-specific variations (Table 2). It was observed that both male and female RCC and crossbred animals were equally susceptible to parasitic infections as there were no statistically significant differences in their susceptibility.

The overall prevalence of different gastrointestinal parasitic ova/larvae and tapeworm segments in the faeces of RCC and crossbred animals were analysed to identify their prevalence (data not shown). The comparative analysis showed variable results where several parasites were highly prevalent in crossbred animals and few were more prevalent in RCC animals. A total of 119 samples of RCC were tested, in which Fasciola (2%), Paramphistomum (38%), and coccidial oocysts (17%) were recorded. However, there was no statistical difference in the occurrence of parasitism between the RCC and crossbred animals. Highest infection was recorded with Paramphistomum, while coccidian parasites like Balantidium and Eimeria were highly prevalent in crossbred cattle. Compared to the RC cattle, most of the infections were lower in crossbred cattle except some nematode infections like Oesophagostomum, Cooperia, Chabertia, Ostertagia, Haemonchus, Mecistocirrus etc. Raza et al. (2009) recorded the prevalence of Paramphistomum cervi as 17.64 and 20.00% in cattle and buffaloes, respectively.

**Examination of blood smears**

When the blood samples collected from RCC and crossbred animals were examined for hemoprotozoan infection, it was found that there were no statistically significant differences in protozoan infection between the two breeds (data not shown). It is notable that in the absence of proper management and attention, RCC are equally susceptible as crossbred cattle. In addition, during this study an attempt was made to determine any sex specific prevalence of blood protozoan infections in crossbred and RC cattle (Table 3). It was recorded that no statistically significant differences were existed between male and female animals in respect to blood protozoan infection.

During this study, it was revealed that crossbred cattle were more susceptible to protozoan infection than RCC except Anaplasmosis and Babesiosis (data not shown). Incidences recorded during this study were *Anaplasma spp* (3%), *Babesia spp* (1%), *Theileria spp* (4%), *Eperythrozoon spp* (1%), while a few cases were uncharacterized (4%). The lower prevalence rate of blood protozoan infection in the RCC animals is possibly due to constant exposure to infection and development of immunity against different protozoan parasites. As the crossbred animals have less chance of pre-exposure, there is little chance of developing immunity thus contributing higher disease incidence.

The present study revealed that the frequency of parasite in any RC cattle is variable. Most gastrointestinal helminthes or protozoan infections were due to single parasite which indicates that broad-spectrum anthelmintic therapy is not necessary for effective treatment and control program. Thus, RCC owners can save a lot of money by using targeted therapy through routine faecal smear examination in the local veterinary hospital. The

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<th>Table 1: Comparative efficiency of two faeces examination procedures used during this study</th>
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<th>Table 2: Sex-specific prevalence of gastrointestinal parasites in RCC and crossbred cattle found through coproscopy</th>
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<th>Table 3: Sex-specific prevalence of blood parasites in crossbred and RC animals</th>
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distribution of vectors of different protozoal diseases like tick, mite and lice need to be further investigated to be correlated with present findings. The information will also be useful to take any preventive measures against hemoprotozoal infection in RCC of any designated area. Unfortunately, no previous record of the vector distribution in Chittagong was available during this study. The present study shows that the crossbred cattle and the RCC animals are infected at similar level by parasitic infestations. However, in the absence of proper management, RCC cattle are generally resistant to some parasitic infections which are common in indigenous/crossbred cattle. It is also notable that in some areas the parasite prevalence is very low, indicating geographic factors responsible for parasite survival. Further study for year-long seasonal incidence of parasitic ova or larvae in the pasture could be useful to identify the actual extent of parasitic infection in RC cattle. Proper management with regular anthelmintic therapy is necessary to gain maximum output from RC cattle. Different epidemiological factors including geological and ecological aspects of the RCC rearing areas (e.g. hilly areas, low-lying pasture, Riverside pasture, regular flooding of pasture with tidal water) need to be considered as contributing factors for incidence and prevalence study of parasitic diseases in RC cattle. Further study could also be directed to identify whether there are any age specificity existing in RC cattle and if any particular therapy has been conducted overwhelmingly leading to development of drug resistance in RC Cattle.

The present study was the first of its type to identify the range of helminthes and protozoan parasitic infections in RC cattle. For a comprehensive study, the relevant information of any existing infection along with seasonal trend and geographic distribution is highly essential. Unfortunately, these were not available during this study. There lies the need for a concerted work plan for further long-term study where researchers from different research backgrounds can contribute together for effective analysis of disease incidence and preventive strategy in RCC. The knowledge derived from these studies will increase our understanding whether RCC is really a unique breed with any degree of resistance against infectious diseases. The information will be quite important in planning future national breeding strategies directed toward conservation of this unique variety of cattle that is only available in Chittagong area of Bangladesh.

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