BHUTAN JOURNAL OF ANIMAL SCIENCE
Rai et al. (2017). Comparative immunological and coprological screening of Fasciolosis in cattle. Bhutan J. Anim. Sci. 1(1): 70-72.

Short Communication

Comparative immunological and coprological screening of Fasciolosis in cattle

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ARTICLE HISTORY

Received: 22/12/16
Peer reviewed: 24/12/16-10/01/17
Received in revised form: 15/01/17
Accepted: 20/01/17

KEYWORDS

Coprological screening
Cost-effectiveness
Fasciolosis
Immunological screening
Pourqueir ELISA
Prevalence rate

ABSTRACT

The main objectives of this study were to establish comparative advantage of immunological screening over coprological screening of Fasciolosis in cattle, find the true prevalence rate of Fasciolosis in cattle, and establish the cost-effectiveness of post-screening treatment over current unsystematic treatment practice. A total of 228 faecal and serum samples were collected from one Geog each of three Dzongkhags and two government cattle farms. The immunological testing was carried out using Pourqueir ELISA screening kit for Fasciola f2 antibodies. Sedimentation method followed by Stoll method was used for coprological testing. The study revealed an extremely significant difference ($p \leq 0.01$) in the results between the types of tests. The study detected Fasciola prevalence rate of 42.5% (95%CI: 36.09-49.25) and 18% (95%CI: 13.5-23.72) by immunological and coprological screenings, respectively indicating that the immunological method is more sensitive than coprological screening. Coprological screening two weeks post-screening treatment of positive animals with triclabendazole (TCBZ) in one study location showed 100% clearing of infestation. This indicates cost-effectiveness of "test and treat" approach to bovine Fasciolosis control. Thus, the early immune-diagnosis of bovine fasciolosis using the specific f2 antigen could be an ideal alternative to the current faecal egg count method.

INTRODUCTION

Fasciolosis or Liver Fluke, also known as distomatosis and liver rot, is an important helminthiasis caused by a trematode *Fasciola hepatica* (the common liver fluke) or *F. gigantica*, a parasite that settles in the biliary ducts of many different species of animals. This parasite is one of the zoonotic trematodes. In Europe, the Americas and Oceania only *F. hepatica* is a concern, but the distributions of both species overlap in many areas of Africa and Asia (Mas-Coma et al. 2005). The definitive host range is very broad and includes many herbivorous mammals, including humans. The life cycle includes freshwater snails as an intermediate host of the parasite. The economic impact of fasciolosis is becoming even more important because infestation often develops in a pernicious way; it progresses slowly and is not immediately recognized. Worldwide losses in animal productivity due to fasciolosis is conservatively estimated at over US$3.2 billion per annum (Spithill et al.1999). In addition, fasciolosis is now recognized as an emerging human disease: the World Health Organization (WHO) has estimated that 2.4 million people are infected with Fasciola, and a further 180 million are at risk of infection (WHO 1995).

The clinical signs of this disease in cattle are anaemia and enteritis resulting, eventually in cachexia. More often, it progresses slowly and presents as a chronic disease. This disease causes chronic cholangitis (or cholecystitis). In young cattle, it can be acute or subacute with hemorrhagic phenomena due to the massive migration of the larvae or be toxicoinfectious from pathogens such as *Clostridium* (necrosing hepatitis) or *Corynebacterium* (liver abscess) etc.

In Bhutan, although this infestation is prevalent in marshy rice growing areas of both sub-tropical and temperate zones, no scientific screening either at herd or individual level followed by treatment with efficacy follow-ups have been conducted. Such a study was urgently required to determine prevalence rate as a baseline for future reference and, efficacy of post-screening treatment. This is also pertinent at this stage in view of possible import and spread of this infestation through the ongoing large scale import of dairy cattle from fasciola endemic states of India. The animals are neither subjected to coprological examination nor treated for fasciolosis during quarantine period. Routinely, only need-based coprological examinations from clinical cases in the field are undertaken. Coprological examinations alone are generally not adequate because while coprological diagnosis of fasciolosis is possible from 8 to 12-week post infection (WPI), fasciola *species* specific-antibodies are recognized through the use of ELISA or Western blot since 2-4 WPI (Zimmerman et al. 1982; Duménigo et al. 2000). Thus, this herd disease can be rapidly diagnosed by serology facilitating early and appropriate choice of therapy. The specific objectives of this study were to establish comparative advantage, if any, of immunological screening against coprological screening for fasciolosis in cattle and understand the true prevalence rate of fasciolosis in cattle in Bhutan. The general objective was to find the cost-effectiveness of post-screening treatment over current unsystematic treatment practice.

MATERIALS AND METHOD

Study locations

The study area included one warm sub-tropical district of Samtse and two temperate rice growing districts of Wangdue and Punakha. In each district, one suitable location was identified in consultation with concerned Dzongkhag Livestock Sector (DLS) and Regional Livestock Development Centre (RLDC). In addition, National Jersey Breeding Centre (NJBC), Samtse and Wangkha Calf Rearing Centre (CRC), Chukha were also included.

Samples and Sample collection

Serum and faecal samples were collected from individual animals at herd level (individual house-hold or farm). In total, 228 faecal and serum samples were collected as shown in Table 1. Both the types of samples were collected, processed and preserved as per the sampling standard operating procedure (SOP) of National Centre for Animal Health (NCAH).

Table 1 Numbers of samples collected at different locations.

Location	Nos of serum	Nos of faeces
Samtse	62	62
CRC, Wangkha	51	51
Wangdue	65	65
Punakha	50	50
Total	228	228

Laboratory procedures

Faecal samples were examined by sedimentation method followed by Stoll Dilution method (modified) (MAF, UK 1986); a quantitative technique to quantify the number of eggs per gram (epg) of faeces. Staining with 1% Methylene Blue was mandatorily done for all positive samples as a differentiation technique to rule out the paramphistomes species. The serum samples were tested using Pourqueir ELISA screening kit for Fasciola f2 antibodies following the manufacturer's instruction. The exact kit used was POURQUIER® ELISA BOVINE FASCIOLOSIS SERUM AND MILK VERIFICATION VERSION P05120/04. This test is reliable since the "f2" antigen purified from *Fasciola* extracts is used. The "f2" antigen is very immunogenic and highly specific for *Fasciola hepatica*. All the positive animals by coprological examination in one location were treated with drug of choice (triclabendazole @12mg/kg body weight) by the research team. This lot of animals were re-examined by coprological method two weeks post treatment.

Data analysis

The data were managed using Microsoft excel and conducted descriptive statistics using online statistical tools.

RESULTS

The results obtained by both the methods were classified as negative or positive. The ELISA result may be classified as strong, medium, low or no (negative) infestation. This classification was not used due to lack of corresponding categorization for coprological results.

Coprological and serological results

The result obtained by coprological examination is presented in Table 2. The number of animals detected positive for fascioliasis was highest in Wangdue followed by Punakha. In Samtse, none of the animals were tested positive.

The result obtained by serological examination is shown in Table 3. Similar to coprological result, the serological result also showed the highest number of animals positive for fascioliasis in Wangdue, followed by Punakha whilst Samtse had the lowest number of animals tested positive for fascioliasis.

Table 2 Test result of coprological examination.

Location	Nos tested	Nos positive	+ve (%)
Samtse	62	0	0
CRC, Wangkha	51	2	3.9
Wangdue	65	32	49.2
Punakha	50	7	14
Total	228	41	18

Table 3 Test result of Serological (ELISA) examination.

Location	Nos tested	Nos positive	+ve (%)
Samtse	62	10	16
CRC, Wangkha	51	14	27.5
Wangdue	65	47	72.3
Punakha	50	26	52
Total	228	97	42.5

DISCUSSION

This study revealed fasciola prevalence rate of 42.5% (95%CI: 36.09-49.25) ranging from 16% to 72.3% at different sampling sites indicating higher prevalence in cattle in the country. Therefore, prevention and control program against fasciolosis should be implemented to reduce the production losses for the farmers and the government. Our study indicates that the serological diagnosis is more sensitive with a 24.5% higher detection rate than the coprological examination (Tables 2 and 3). The prevalence rate by coprological method is much lower at 18 % (95%CI: 36.09-49.25) with a range of 0% to 49.2%. Specific antibody detection by ELISA had a 'good' sensitivity and specificity (Rapsch et al. 2006). However, the sensitivity of traditional faecal examination can be significantly improved by repeated sampling and use of higher amount (10gm) of faeces instead of 3gms used in our study. The result obtained in this study is in agreement with the findings of Brockwell et al. (2013) where serum ELISA was found to be more sensitive and detected all positive animals by 28-day post infection (dpi) in the comparative kinetics of serological and coproantigen ELISA and faecal egg count (FEC) in cattle. In the same study, all treated animals returning to negative status by coprological examination within one week is in full concurrence with our findings of 100% efficacy by two-weeks post treatment. In another study conducted by Brockwell et al. (2014), both faecal egg count and coproantigen reduction tests (FECRT and CRT) revealed widespread resistance to triclabendazole (TCBZ) in South-eastern Australia. Although our study showed TCBZ to be highly effective against fasciolosis, it should be used judiciously to avoid development of resistance. The effectiveness of Pourqueir ELISA screening kit in detection of Fasciola in live animals is comparable to findings of Damwesh et al. (2013) that used the same kit in detection of Fasciola in Nigeria. The higher sensitivity but a lower specificity of antibody detection method over FEC method for detecting fasciola infestations in cattle and goat were proven by Levieux et al. (1994) and Anderson et al. (1999).

CONCLUSIONS

This study demonstrates higher prevalence of fasciola in cattle in Bhutan and needed to implement a suitable control programme. Among others, 'test and treat' approach is recommended to be effective and economical as demonstrated in this study. However, further research with more study sites needs to be undertaken to obtain accurate prevalence rate. The analysis of the ELISA's performance demonstrated higher sensitivity as compared with faecal egg count. Both of these have comparative advantages of immunological diagnosis over coprological examination. Therefore, we can exploit these advantages in the control of fasciolosis by undertaking treatment even before clinical manifestation of the disease. In future, early immunodiagnosis of bovine fasciolosis using the specific f2 antigen could be an ideal alternative to the current faecal egg count method. Finally, some of the gaps in this study may be improved by designing proper research plan including nationwide sampling, repeat faecal sampling at different time interval to understand the seasonal prevalence of infestation.

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