Diagnosis and therapeutic management of bovine theileriosis

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Abstract

Bovine Theileriosis is a haemoprotozoan disease caused by Theileria spp in cattle lead to huge depreciation in terms of economy of farmer and mainly transmitted by Hyalomma and Rhipicephalus ticks. In the present study a total of ten cases suspected for theileriosis with the clinical signs such as elevated body temperature, enlarged superficial lymphodes, anaemia, tachycardia and presence of ticks over the body were studied. Further they were subjected to haematological examination, thin blood smear examination and further confirmed by PCR assay. The results revealed that, haematological examinations implicated severe anaemia whereas thin blood smear examination showed presence of pleomorphic theileral organisms in the RBC’s and amplification of expected PCR product of 1098 bp by targeting theleria genus specific primers for the SSU rRNA gene confirming theileriosis in cattle. The affected animals were treated with Inj Buparvaquone @ 3.5 mg/Kg given deep intramuscularly once as anti-haemoprotozoan drug along with other supportive therapy followed for five days. The complete recovery was achieved from bovine theileriosis based on haematological examinations and amelioration of clinical signs in all the animals.

Keywords: Bovine theileriosis, Theileria spp, PCR assay, haematological examinations, anaemia

Introduction

Theileriosis is one of the major tick-borne diseases in tropical and subtropical countries. The climatic variation poses a threat of many infectious, metabolic and parasitic diseases in India. In India, theileriosis is widely spread among the cattle and is endemic in nature constituting 30-60 per cent sero-positivity throughout different states of the country (Sachin Kumar, 2018) [1]. Approximately 250 million cattle are verge of susceptibility to disease which act as major constraint in developing countries tend to cause severe economic loss to farmers and indirectly affecting national GDP. An intra erythrocytic protozoan parasite theileria annulata of the genus Theileria, is the cause for thileriosis in domestic and wild animals mainly transmitted through the bite of Hyalomma and Rhipicephalus ticks. Whereas, higher incidence is commonly observed in the intensive dairy herds (Balha, 1989) [2] and crossbred cows of all age groups with the general epidemiology of the disease in tropical areas (Jithendran, 1987 and Gitaub et al., 1999) [3, 4]. Diagnosis of haemoprotozoan diseases often poses a challenge to clinician and microscopic demonstrations of the infective stages in the blood cells were traditional methods with lesser sensitivity and time consuming. In order to diagnose with good sensitivity and specificity PCR assay was used to diagnose theileriosis (Atley et al., 2005) [5]. Molecular diagnosis of haemoprotozoan diseases involves several PCR- based diagnostic procedures, which may help in the identification of the parasites up to the species or even strain level (Criado-Fornelio et al., 2003) [6]. The present study was aimed at diagnosis by PCR assay and therapeutic efficacy in theileriosis affected cattle.

Materials and Methods

A total of ten suspected cases of theileriosis were presented to the out-patient ward at department of Veterinary Medicine, Veterinary College Bidar with the history of rise in body temperature ranging from 103°F-107°F, anaemia due to haemolytic crisis, enlarged superficial lymph nodes accompanied by dullness, anorexia, salivation, larication, discharge from nostrils, tachycardia, decreased milk production and presence of ticks all over the body. Among them eight animals were adult (aged between 4-8 years) and 2 were calves (aged
between 5-8 months). All the cases were further subjected to haematological examination, thin blood smear examination, and PCR assay for confirmation.

**Hematological examination**
A total of 10 ml of blood samples were collected through jugular venipuncture from the all ten suspected animals in to the sterile vials containing disodium salts of EDTA as an anticoagulant under aseptic conditions for determination of haematological parameters using automatic haematological analyser (Automatic blood cell counter, Model PCE-210, ERMA Inc).

**Blood smear examination**
A thin blood smear examination was performed by Giemsa staining technique to visualize the pleomorphic organisms in the RBC’s

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence [Forward and reverse (5’-3’)]</th>
<th>Denature Temp. (°C)</th>
<th>Anneal Temp. (°C)</th>
<th>Extend Temp. (°C)</th>
<th>Product Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSU rRNA</td>
<td>F: “AGTTTCGTGACCTATCAG” R: “TGCCCTAAACTTTCTTG”</td>
<td>92</td>
<td>55</td>
<td>72</td>
<td>1098</td>
</tr>
</tbody>
</table>

Later the amplified products were separated by gel electrophoresis on a 1 per cent agarose gel. Gel preparation was done by adding 1gm of agarose powder in 100 ml of TAE buffer along with ethidium bromide solution (0.1%) and mixed thoroughly. Five micro litre of DNA marker (100bp) with dye was added in to the first well for comparison whereas, PCR products were mixed with five micro litre of bromo-phenol blue dye on parafilm by pipetting and loaded in to rest of the wells. The gel was allowed to run for 30 to 50 minutes at 100 volts and then gel was observed and photographed in Gel Doc System (BioRad).

**Treatment**
For the treatment of affected animals, Inj Buparvaquone was given once, and other supportive therapies were carried out for 5 days. Based on the clinical outcome and comparative haematological examinations on 0th day (before) and 5th day (after) of treatment were monitored and analysed to assess the recovery from theileriosis.

**Results and Discussion**
In the present study, all the cases were showing associated clinical signs of theileriosis such as elevated body temperature, enlarged superficial lymphodes, anaemia, tachycardia and presence of ticks over the body, which are thought to be classical signs exhibited by theileria infected cattle (Abdela and Bekele, 2016) [8]. Further they are confirmed as theileria positive by thin blood smear examination, and PCR assay. On haematological study there was significant (P<0.05) decrease in the haemogram (Total erythrocyte count, haemoglobin and haematocrit value) was observed in theileriosis affected cattle indicating of severe anaemia and concurrent results were observed by Hasanpour et al. (2008) [9]. The decrease in hemogram can be attributed to erythropagocytosis due to an immune-mediated mechanism directed against RBC’s might be responsible for the erythrocyte destruction (Uilenberg, 1981) [10]. In addition, pro-inflammatory cytokines, particularly TNFα, IL-1 and IL-6 have been incriminating anaemia associated with tropical theileriosis (Glass et al., 2003) [11]. Oxidized erythrocytes may be destroyed easily by erythropagocytosis therefore oxygen radicals may also be involved in the pathogenesis of anaemia (Yagi et al., 2002) [12]. Although there were fluctuations in the Mean ± SE (Table-1) values of erythrocytic indices, the values were within normal limits indicating normocytic normochromic regenerative anaemia in the affected cattle and results were in accordance with Lawrence et al. (2018) [13]. The total leucocyte count Mean ± SE (Table-1) values decreased significantly (P<0.05) in affected cattle and can be attributable to destruction of lymphocytes in lymphoid organs and infiltration of these cells into various organs (Sandhu et al., 1998) [14]. The schizonts infect lymphoid cells induce rapid and uncontrolled proliferation of both non-specific and specific T lymphoid cells resulting in enlarged lymphnodes (Gul et al., 2015) [15].

**PCR assay**
A molecular diagnostic tool, that helps in the identification of the parasites at it genomic level. In the present study, highly purified DNA was extracted from the whole blood by HiPur A” (Himedia laboratory diagnostics) Blood Genomic DNA Miniprep Purification Kit. The extracted DNA was stored at -20°C until further processing of PCR amplification. PCR was carried out by using following sets of primers as reported by Oliveira et al., (1995) [7]. The reaction was carried out using the *Theileria* genus specific primers for the SSU rRNA gene as, forward primer 989: 5’ AGTTTCGTGACCTATCAG 3’ - *Theileria* specific and reverse primer 990: 5’ TTGCCCTAAACTTTCTTG 3’ - *Theileria* specific. Then purified DNA was run through PCR amplification process for 3 cycles by following process
amelioration of clinical signs.

Conclusion
The theileriosis suspected animals were subjected to thin blood smear examination and confirmed diagnosis by PCR assay. Theilerosis affected animals were treated for five days completely recovered on 5th day along with supportive therapy.

Table 1: Comparison of haematological values before and after treatment in theileriosis affected cattle

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Theileriosis affected cattle (n=10) (Before treatment)</th>
<th>Theileriosis affected cattle (n=10) (After treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Range</td>
</tr>
<tr>
<td>TEC (×10⁶/µL)</td>
<td>3.41 ± 0.18a</td>
<td>2.20 - 4.10</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>5.90 ± 0.39a</td>
<td>3.60 - 7.20</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>23.40 ± 1.37a</td>
<td>17.00 - 32.00</td>
</tr>
<tr>
<td>Platelets (×10⁹/ µL)</td>
<td>248.90 ± 12.51a</td>
<td>191.00 - 302.00</td>
</tr>
<tr>
<td>TLC (×10⁶/µL)</td>
<td>7.42 ± 0.27a</td>
<td>6.50 - 9.20</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>69.57 ± 3.86e</td>
<td>54.84 - 86.36</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.58 ± 1.20a</td>
<td>9.73 - 23.10</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>25.93 ± 2.34a</td>
<td>16.36 - 40.5</td>
</tr>
</tbody>
</table>

*Means bearing different superscripts, differ significantly


References