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Research Article

EVALUATION OF MOLECULAR BIOLOGY FOR IDENTIFICATION THEILERIOSIS DISEASE IN CROSSBRED CATTLE

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Abstract- Theileriosis is hemoprotozoan disease spread in most of developing countries including India. In this presence study was done with cattle of north Gujarat India 44 and 40 blood sample were collected from crossbred cattle respectively clinical case and subclinical case out of 282 crossbred cattle. Only 13 sample was conform Theileriosis positive detected in microscopic examination in group of clinical case but not in sub clinical case but the detection done by polymerase chain reaction (PCR) were showed 30 and 21 sample positive respectively in clinical case and subclinical case. In other word 15.4% and 60.7% sample showed positive respectively microscopic and molecular technique. It means the PCR was fine technique for detection of Theileriosis. The different group of cattle was treated with different antibiotic combination, Buparvaquone was proved effective than other drug.

Keywords- Screening for sample, Microscopy observation, PCR, Drug efficacy

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Introduction

Population of crossbred cattle has been on rise in Gujarat state with simultaneous increase in milk production and incidence of hemoprotozoan infections. Theileriosis has become endemic in the state and prevalent as clinical, subclinical or carrier sates. Therefore early diagnosis is importance to reduce economic loss and healthy milking animals [1]. Diagnosis of Theileriosis is usually carried out by blood smear staining technique, which is not sufficiently sensitive to detect the piroplasms in the carrier animals. In this study, a total of 116 sample were collected from infected as well as apparently healthy cattle and buffaloes. Screening of blood smears by Giemsa staining detected 15 sample (12.93 %) positive for Theileria piroplasms out of 116 sample. However, the PCR based screening using the specific primers from the major merozoite-piroplasm surface antigen sequence of *T. annulata* (Tams1) gene detected 74 sample(63.79 %) positive for *T. annulata* which included 59 sample found negative by Giemsa staining. Our study suggests that the PCR based screening is more sensitive and accurate method for diagnosis of tropical theileriosis in cattle and buffaloes [2] *Theileria annulata* were seen in 16 of 150 (10.66 %) by examination the blood smears with light microscope, whereas 68 of 150 (45.33 %) cattle were positive by PCR method. All animals that were positive by blood smears were also positive by PCR [3] PCR showed that 42 sample were Theileria spp. positive, while routine microscopy showed erythrocytes harboring Theileria like structures in 11 blood sample. Examination of 50 microscopic fields showed 57 % sensitivity and 99 % specificity compared to 100 % sensitivity and specificity for PCR [4] Hemoprotozoan diseases transmitted by the ticks cause devastating losses to the livestock industry throughout the world. The hot and humid climate is highly favourable for the development and survival of ticks. In particular, ticks spread Theileria, which pose a serious challenge to the exotic crossbred cattle population. Tropical theileriosis is one of the most prevalent diseases of cattle caused by *T. annulata* and is transmitted through Ixodid tick of genus Hyalomma. Molecular method polymerase chain reaction [PCR] has been developed to diagnose the theileriosis from the persistently infected cattle and the prevalence of *T. annulata* being recorded using microscopic and PCR methods.

Material and Method

A total of 84 sample were collected of which 44 sample were from clinical cases while 40 from suspected theileriosis cases without overt clinical signs out of 282 crossbred cattle (Kankrej X HF). Sample from Ten healthy animals were collected for comparison as control.

The crossbred cattle under study were subjected for thorough clinical examination inclusive of history, respiratory rate, pulse rate, rectal temperature, mucous membrane, enlargement of lymph nodes etc. Animals with pyrexia (body temperature 104°F and above), anaemia, prescapular lymph node enlargement and off feed were considered as clinical cases whereas those with moderate temperature rise, pale mucus membrane and moderate or no rise of prescapular lymph node were considered as subclinical cases of theileriosis. These animal maintained their appetite and continued to eat.

Blood sample were collected from the jugular vein of the animals in a tube containing Tripottasium ethylene diamine tetra acetic acid (K_3EDTA). Samples were collected on day of examination and 4th day post treatment.

The blood samples were also obtained in plain tubes without containing anticoagulant. They were centrifuged at 3,000 rpm for 15 minutes to separate serum. The serum sample were stored at -20°C until further used for biochemical examination. Simultaneous blood smears were prepared.

Thin blood smears were prepared and examined for theileria piroplasm. The air dried Giemsa stained blood smears were examined under the high power and oil immersion lenses. The percentage of infected erythrocytes (parasitaemia) was expressed as + (scanty upto 2%), ++ (mild 2 to 10%), +++ (moderate 10 to 40%), ++++ (high 40 to 60%) and +++++ (very high >60%) after examining 300

erythrocytes from 5-10 different microscopic fields under oil immersion lens [5]. The detection of species specific theileria was carried out as per method suggested [6]. Genomic DNA of the parasite was extracted from whole blood by using DNA purification kit (Qiagen blood and tissue DNA extraction kit cat. No-) as per the manufacturer's instruction and stored at -20^{0}c.

PCR based detection of *Theileria annulata* was done using the cyt b gene specific PCR reaction by primer pair, forward primer 5' CCAGGACCACCCTCAAGTTC3' and revere primer 5'GCATCTAGTTCCTTGGCGGA3' with 25ul PCR reaction by using (Qiagen top taq PCR master mix Cat. No-) and cycle condition of thermal cycler was denaturation , annealing and extension temperature was respectively 95°C, 55°C and 72°C for 30sec upto 30 cycle. Amplification product size in 1.5% Agarose gel electrophoresis was visualized at 430bp compared with standard DNA marker.

Treatment of affected cases was carried out using Oxytetracycline, Oxytetracycline + Diminazineaceturate and Buparvaquone in different groups. Effectiveness of therapy was worked out using statistical tools where the $P<0.05$ has been considered as statistical significant.

Result

Theileriosis is a major tick borne haemoprotozoan disease of crossbred cattle in several developing countries of the world including India [7]. In the present study, a total of 282 crossbred cattle were screened in the Deesa taluka of Banaskantha district, of which 44 animals showed clinical signs whereas 40 animals with anaemia and ticks on body were suspected to be subclinical cases of theileriosis. From the clinically diagnosed 44 theileriosis cases, only 13 were positive in blood smear examination while 30 were positive in PCR examination. No positive cases were detected during blood smear examination in suspected theileriosis cases while 21 were positive in PCR. Thus out of the 84 cases, 51 cases were positive for theileria in PCR examination, making an overall prevalence of theileriosis to be 29.55 per cent on the blood smear examination basis while 60.25 percent by PCR examination basis.

Discussion

The overall prevalence of Theileriosis has been reported in the range of 15 and 30 percentage in India [8].

The analysis of retrospective data revealed prevalence of theileriosis highest (21.57%) during the month of June while lowest (2.42%) in January. The prevalence was highest in rainy season (44.70%) while the lowest in winter season (17.00%) which is in agreement with earlier workers [9]. [10] stated that the incidence of theileriasis was not seasonal but occurred irregularly throughout the year depending upon prevailing macroclimate. The retrospective data of the area is in agreement with [11-13] reported maximum incidence in summer followed by monsoon and minimum in winter. They stated that the disease was related to hot and humid climate due to higher infectivity of vector ticks.

Theileriosis is traditionally diagnosed on clinical findings or stained blood smear examination for positive identification of the case. However, they are not sensitive enough for diagnosis of subclinical or carrier animals. Molecular tests are sensitive and useful in such cases. Molecular diagnosis involves several PCR based diagnostic procedures, which help in identification of parasite/organism up to the species or strain level [14].

Present study also revealed that PCR has a higher sensitivity (60.71 per cent) over microscopic examination of blood smear (29.55 per cent) which is in agreement with [6, 15, 16, 17] reported PCR to be most profound method for detection of theileria spp.

Evaluation of therapeutic response to different treatments in theileria affected subgroups were inform of clinical, haematological and biochemical improvements.

No significant improvement was noticed in the clinical, hematological and biochemical parameters four days post treatment in oxytetracyclin treated animals. Similar, results with oxytetracycline was reported by [18].

The combined therapy of Oxytetracycline + Diminazineaceturate showed some improvement in reducing body temperature, pulse and respiratory rate. The haematological values also marginally improved after treatment, suggesting cessation of intravascular haemolysis but was statistically non-significant. [19] reported that oxytetracycline treatment given for five days with diminazine aceturate on first and third day improved the clinical cases in calves. Similar results have been obtained with combined oxytetracycline+diminazineaceturate treatment [8, 20].

Buparvaquone is a second generation hydroxynaphthoquinone compound and drug of choice for the therapy of all forms of theileriosis. The values of Hb, RBC, WBC and lymphocytes improved significantly post treatment while neutrophils and eosinophil did not in Buparvaquone treated cases. Buparvaqon 100 percent effective in clinical theileria cases while [20, 21]. Obtained similar results during experimental infection.

Several authors described Buparvaquone was highest effective specific treatment for theileriosis [22].

Conclusion

PCR is useful in diagnosis of Theileriosis during subclinical or carrier stage compared to conventional blood smear examination. The drug Buparvaquone is effective then compared to oxytetracycline alone or in combination with diminazine acculturate in the treatment of Theileriosis

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Author Contributions

All authors are associated with this works like sample collection, guide, technical support, literature collection and manuscript writing respectively.

Abbreviations: polymerase chain reaction (PCR)

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: None declared

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