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Diagnosis and molecular confirmation of oriental theileriosis in a cow: A case report

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Abstract

Oriental theileriosis is responsible for benign or non-transforming theileriosis and exerts its major pathogenic effect through the destruction of erythrocytes. The disease is caused by an Apicomplexa haemoprotozoan called *Theileria orientalis*. Oriental theileriosis in general causes a benign disease but some strains of this organism are capable of causing a fatal disease. This paper deals with a case of oriental theileriosis in a two year old cross-bred heifer at its last trimester of pregnancy. The animal was brought to the University Veterinary hospital, Mannuthy, with the history of high temperature (104°F), moderate enlargement of lymph nodes, inappetance for last one week, salivation, respiratory distress and lethargy. On examination the animal was found to be anaemic with pale mucous membranes and was infested with ticks on the inner aspect of the ear margin and also on the dorsal aspect of the poll region. Microscopic examination of Giemsa stained peripheral blood smears revealed *Theileria* piroplasm within the erythrocytes. The peripheral blood smears stained with Acridine orange stain also revealed the presence of *Theileria* piroplasm. Polymerase chain reaction (PCR) using species specific primers confirmed the presence of the organism. The animal was treated with combination of intramuscular injections of long acting oxytetracycline @ 20 mg/kg BW and Buparvaquone @ 2.5 mg/kg BW twice with the interval of 48 hours along with other supplemental therapy. On the third day, the animal resumed feed intake, and other clinical signs were also resolved. The animal made an uneventful recovery after five days of therapy. Peripheral blood smears were examined after two weeks to determine the presence of the organism and was found to be negative.

Keywords: Oriental theileriosis, *Theileria orientalis*, Buparvaquone, oxytetracycline

1. Introduction

Oriental theileriosis is caused by an Apicomplexa haemoprotozoan *Theileria orientalis* which was previously recognized as a mild and benign disease but recently have been associated with disease outbreaks in Asia and Australia (Constable *et al.*, 2017) [3]. Some virulent strains have caused fatal disease (Aparna *et al.* 2011) [1]. Oriental theileriosis have been reported from many countries like Ethiopia, New Zealand, Iran, Central Africa, Greece, Italy, Japan and Turkey. It has also been reported in cattle from Kerala (Nair *et al.*, 2011) [10], Assam (Kakati *et al.*, 2015), Andhra Pradesh and Telangana (George *et al.*, 2015) [5].

The epidemiology of oriental theileriosis corresponds to the geographical distribution of the tick vector (Watts *et al.*, 2016) [14]. Ticks of genus *Haemaphysalis* spp. and *Boophilus* spp. have been responsible for the transmission of this fatal disease in Kerala (Aparna *et al.*, 2011) [1]. The

disease is characterized by non-specific signs such as reluctance to walk, abortion and weakness (Watts *et al.*, 2016) [14]; other signs such as pallor, pyrexia and elevated heart and respiratory rates were also described (Izzo *et al.*, 2010) [6]. Once affected, animals remain infected and infectious for life (Watts *et al.*, 2016) [14] and consequently, relapses occur during times of stress such as pregnancy, lactation or rapid changes in environmental condition (Sugimoto and Fujisaki, 2002) [12].

Case History

A two year old cross-bred heifer weighing about 300 kg was presented to the University Veterinary hospital, Mannuthy, with the history of high temperature, weakness, lethargy, reluctance to get up, panting, respiratory distress and inappetance for a week. Water intake was normal and no change in colour of urine was noticed. There was no report of change in feed and management of the animal and was regularly dewormed. On clinical examination, conjunctival mucosa appeared moderately pale, faeces was loose in consistency and tarry

And stringy salivation with an anxious expression could be colored, ticks could be observed on the inner aspect of the ear margin as well as poll region noticed in the animal.

Materials and Methods

Ticks were collected and identified as *Rhipicephalus microplus* and peripheral blood smears were stained with Giemsa stain as well as Acridine orange stain and examined under the microscope. Complete blood count was performed

with whole blood. Serum was also collected and subjected to biochemical analysis for estimation of AST, albumin, total protein and creatinine. The DNA was extracted from the whole blood and subjected to polymerase chain reaction (PCR) using primers specific for *Theileria orientalis* (Table 1). The reaction mixture was prepared as per Table 2 and the PCR programme was carried out as per Table 3. The PCR products were visualized using Gel Documentation system.

Table 1: Species specific primers of *T. orientalis*

Organism	Primer name	Gene sequence 18S rRNA	PRODUCT SIZE	REFERENCE
<i>Theileria orientalis</i>	Forward	5'-CACGCTATGTTGTCCAAGAG-3'	875 bp	Baek <i>et al.</i> (2003)
	Reverse	5'-TGTGAGACT CAATGCGCCCTA-3'		

Table 2: Reaction mixture for PCR

Sl. No	Reagents	Volume
1.	Nuclease Free Water (NFW)	11.25 µl
2.	MgCl2 (25 mM)	3 µl
3.	dNTP mix (2.5 mM each)	0.75 µl
4.	Forward Primer (25 pmol/µl)	1 µl
5.	Reverse Primer (25 pmol/µl)	1 µl
6.	Taq Buffer (10 X)	2.5 µl
7.	Taq DNA Polymerase (3U/µl)	0.5 µl
8.	template	5 µl
total volume		25 µl

Table 3: Programme for *Theileria orientalis* species specific PCR

Amplification of 875 bp fragment of 18S rRNA gene of <i>Theileria orientalis</i>	PCR Programme	Temperature	Time	Cycles
	Initial denaturation	94°C	4 min	45cycles
	Denaturation	94°C	1 min	
	Annealing	55.5°C	1 min	
	Extension	72°C	1 min	
	Final Extension	72°C	5 min	

Results

Giemsa stained peripheral blood smears revealed inclusions within erythrocytes suggestive of *Theileria* piroplasms (Fig. 1.), mostly thin or thick rod shaped with light staining trailing cytoplasm. Acridine orange stain revealed *Theileria* piroplasms within the erythrocytes with apple green fluorescence (Fig. 2). Species specific primers amplified 875 bp product suggestive of *Theileria orientalis* (Fig. 3). The DNA sequence were aligned and edited by Emboss Merger Website and global sequence comparisons were performed by using basic local alignment search tool (BLAST) hosted by the National Center for Biotechnology Information (NCBI). The Sequence revealed 100 per cent identity to major piroplasm surface protein like (MPSP) of *Theileria orientalis* isolate JX648207.



Plate 1: *Theileria orientalis* organism on Giemsa stain



Plate 2: *Theileria orientalis* organism on Acridine orange

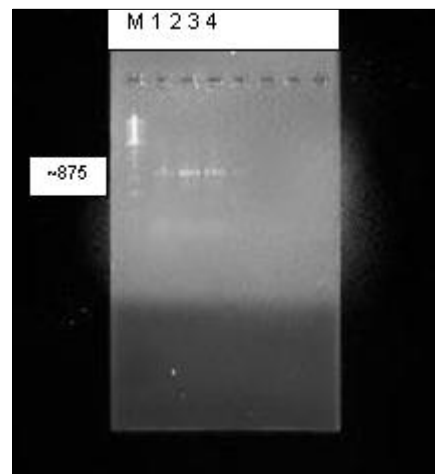


Plate 3: 875 bp amplification of *Theileria orientalis*

Treatment

The treatment was initiated with fluid therapy that included 2 litres each of Ringer’s Lactate and Dextrose Normal Saline and Inj. Beplex forte (10 ml). Combination therapy of intramuscular long-acting oxytetracycline injection @ 20mg/kg b.wt and Buparvaquone injection @ 2.5 mg/kg b.wt was given, which was repeated after 48 hours. This protocol was accompanied with daily one bolus of oral Feritas bolus (Intas) and Injection Belamyl (10 ml intramuscular). Remarkable recovery on the third day and resolution of clinical signs could be noticed and after 14 days post treatment, the animal became normal and examination of peripheral blood smears revealed absence of piroplasms in the erythrocytes.

Discussion

Oriental theileriosis is recognized as a cause of fatal anaemia

in cattle worldwide. Even though the parasite has been considered as non-pathogenic, during the recent past it has emerged as a cause of fatal disease characterized by fever, anaemia, jaundice and abortions (McFadden *et al.*, 2011) ^[9]. The present case showed clinical signs including fever, anaemia and inappetance. Microscopical examination of peripheral blood smears demonstrated the typical rod shaped piroplasms with trailing cytoplasm suggestive of *T. orientalis*. Light microscopy can be used for the diagnosis of theileriosis, but most of the time it is difficult to differentiate it from pleomorphic forms of other blood parasites. Acridine orange staining is found to be more sensitive and specific for detection of *T. orientalis* which clearly demonstrates the piroplasms with the nucleus of the organism being fluorescent green while the cytoplasm being light orange in color (Nair *et al.*, 2011) ^[10]. Application of molecular techniques helped in confirmatory diagnosis and differentiation of the species of organism (Kakati *et al.*, 2015) ^[7]. Treatment using a combination of Buparvaquone and oxytetracycline was effective in elimination of *Theileria* piroplasms and remission of clinical signs as reported by Vinodkumar *et al.*, (2016) ^[13] and Prasanth and Ajithkumar, (2016) ^[12].

Conclusion

Bovine oriental theileriosis is a very important disease as it causes huge economic losses to the dairy farmers in the form of sudden death or reduction in milk yield due to fever, anaemia and emaciation. Hence, tick control measures and the detection of carrier animals is of utmost importance in order to reduce the incidence of the disease.

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