The Pharma Innovation Journal 2020; 9(9): 568-571

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(9): 568-571

www.thepharmajournal.com
Received: 27-06-2020
Accepted: 25-08-2020

K Sudhakar Goud
Ph.D. Scholar, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

K Vijayakumar
Professor and Head, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Corresponding Author:
K Sudhakar Goud
Ph.D. Scholar, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Molecular diagnosis and treatment of oriental theileriosis in calves

K Sudhakar Goud and K Vijayakumar

DOI: https://doi.org/10.22271/tpi.2020.v9.i9h.5208

Abstract
Bovine theileriosis caused by *Theileria orientalis* is a tick borne haemoprotozoan disease and causes high morbidity and mortality in young calves. This paper deals with molecular diagnosis and treatment of oriental theileriosis in calves. The disease occurrence was found to be higher in calves aged between 3 to 8 months. The infected calves had low to moderate level of parasitaemia, except one calf which had high level of parasitaemia. Majority of infected calves were having mild anaemia with packed cell volume ranging between 15-23%. Molecular diagnosis using species specific primers confirmed the presence of *T. orientalis* in infected calves. Treatment with buparvaquone along with haematinics was found to be effective in calves.

Keywords: *Theileria orientalis*, calves, diagnosis, PCR

Introduction
Oriental theileriosis is a tick-borne haemoprotozoan disease of bovids caused by parasites within the *Theileria orientalis* complex. This group of organisms variously designated as *T.sergenti/ buffeli/ orientalis* were considered as benign parasites, now responsible for serious production losses and mortality in cattle worldwide. These parasites exert their major pathological effect through erythrocyte destruction. The clinical signs of oriental theileriosis include lethargy, fever, anorexia, anaemia, jaundice, abortion and decreased milk production (Eamens *et al*., 2013a) [3]. Once the animal is infected with *T. orientalis* it becomes carrier and harbour the organism lifelong (Sugimoto and Fujisaki, 2002) [10]. Stress acts as an important predisposing factor for the appearance of clinical signs of disease. Hence pregnant and recently calved animals are at high risk for developing clinical disease (Watts *et al*., 2015) [13]. Young calves are highly susceptible for theileriosis with clinical disease and mortalities in herds which are endemic for oriental theileriosis (Swilks *et al*., 2017b) [12] *T. orientalis* is mainly transmitted by bush tick *Haemophysalis longicornis* (Heath, 2016) [5] and the role of other vectors in transmission is not very clear. Transplacental transmission of *T. orientalis* to calves in pregnant dams and subsequent abortion has been demonstrated by experimental transmission via infected ticks (Beak *et al*., 2003) [1]. But Lawrence *et al*. (2016) [7] opined that vertical transmission of *T. orientalis* is highly unlikely and the infection status of the dam at calving has no influence on the infection status of the calf at four months of age. The present paper describes the molecular diagnosis and treatment of oriental theileriosis in calves.

Materials and Methods
The present study was conducted in 52 crossbred calves aged below 8 months of age showing clinical signs of inappetence, pyrexia, anaemia and enlargement of superficial lymphnodes suggestive of theileriosis. Whole blood in ethylene diamine tetra acetic acid (EDTA) coated vials, peripheral blood smears and lymph node aspirate smears were collected from all the calves. General clinical examination including recording of rectal temperature, pulse, respiration, examination of visible mucous membranes, auscultation of heart and lungs were done. Tick infestation was noticed on some of the calves.
The blood smears and lymphnode aspirate smears were stained by Field staining method and examined under oil immersion objective of the microscope. Approximately 50 microscopic fields were checked per smear before declaring it as negative. The level of parasitaemia was determined by counting the number of parasitized erythrocytes per 2000 erythrocytes in stained smear and expressed as percentage (Shiono *et al*., 2003) [9].

The smear positive samples were further graded as low (0.1-0.9%), moderate (1-5%) and high (6-10%) level of parasitaemia based on proportion of erythrocytes showing theilerial piroplasms. The fresh whole blood samples were subjected for determination of packed cell volume (%) by automatic haematology analyser. The samples were also graded based on packed cell volume (PCV) as categories of severely anaemic cases (PCV<15), mildly anaemic cases (PCV 15-23) and cases without anaemia (PCV>23) (Eamens *et al*., 2013b) [4]. The dung samples of the calves were examined for the presence of parasitic ova.

DNA Extraction

Genomic DNA was extracted from 100µl of whole blood using DNeasy Blood and Tissue kit (Qiagen) as per the manufacturer's instructions. The resulting 200µl of eluted DNA samples were stored at -20 ^{0}C for subsequent analyses.

Polymerase chain reaction

To confirm the presence of Theileria organisms genus specific PCR was performed to amplify the SSU rRNA gene of Theileria using the primers set 989/990 (Table 1) described by d'Oliveria *et al*. (1995) [2]. The polymerase chain reactions were carried out in 25µl reaction mixture, the components of each reaction were shown in Table (2). The reactions were carried in thermal cycler (Bio-Rad Laboratories, USA) with initial denaturation at 94 ºC for 5 min. followed by 40 cycles of each denaturation at 94 ºC for 1 min., annealing at 52.5 ºC for 1min. and extension at 72 ºC for 1 min. Final extension was allowed at 72 ºC for 5 min. after the last cycle.

Amplification of Major Piroplasm Surface Protein (MPSP) gene by PCR

Species specific PCR targeting the major piroplasm surface protein (MPSP) gene was performed to confirm the presence of *Theileria orientalis*. Primers used were mentioned in Table (1). The amplification reactions were performed with initial denaturation at 94 ºC for 4 min. followed by 44 cycles of each denaturation at 94 ºC for 1 min., annealing at 58 ºC for 1 min. and extension at 72 ºC for 1 min, with final extension at 72 ºC for 5 min.

Table 1: Oligonucleotide sequences of primers used in the study

Primer	Sequence	Genus/Species	Reference
989 F	5'-AGTTTCTGACCTATCAG-3'	*Theileria* spp.	d'Oliveira *et al*. (1995) [2]
990 R	5'-TTGCCTTAAACTTCCTTG-3'		
MPSP-F	5'-CACGCTATGTTGTCCAAGAG-3'	*T.orientalis*	Baek *et al*., (2003) [1]
MPSP-R	5'-TTGGAGACTCAATGCGCCCTA-3'		

Table 2: Components of single PCR reaction mixture

S.no.	Name of the reagent	Quantity (µl)
1	2 X PCR Master mix (Sapphire, Takara)	12.5
2	Forward Primer (10 pmol/µl)	1
3	Reverse Primer (10 pmol/µl)	1
4	Nuclease free water	5.5
5	Template DNA	5
Total		25

The PCR products were electrophoresed in 1.2% agarose gel stained with ethidium bromide. The agarose gel was transferred on to UV transilluminator (GeNei ^{TM}) for visualization of expected bands and gel documentation done.

Results

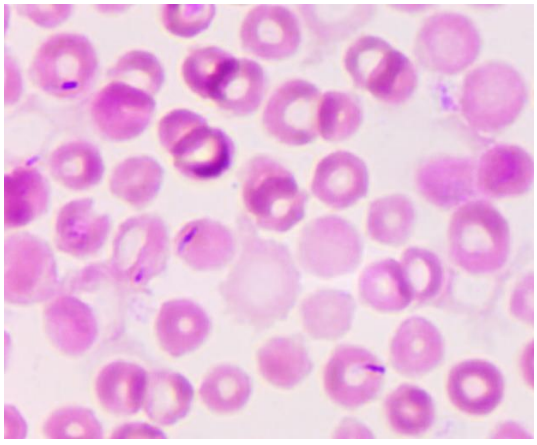

Fig 1: Blood smear showing Theileria piroplasms

Blood smear examination revealed the presence of Theileria piroplasms in 28 calves. The theilerial piroplasms appeared as thin or thick rods with light staining trailing cytoplasm in erythrocytes (Fig 1). All the lymphnode aspirate smears were found to be negative. All the dung samples of calves were found to be negative for parasitic ova.

Clinical manifestations

The twenty eight calves infected with oriental theileriosis were having history of inappetance, weakness, pale mucous membranes, dyspnoea and tachycardia. Pyrexia was noticed in ten calves (temperature above 103ºF), the remaining calves had normal body temperature. Swelling of pre-scapular lymphnodes was observed in seven calves, but lymphnode aspirate smear was negative for Koch blue bodies (KBB). Ticks were identified as *Haemaphysalis longicornis* based on morphological features.

Age wise distribution of clinical cases of oriental theileriosis in calves revealed, higher incidence (71.4%) in calves aged between 3 to 8 months, compared to the calves below 3 months of age (28.5%) (Fig 2).

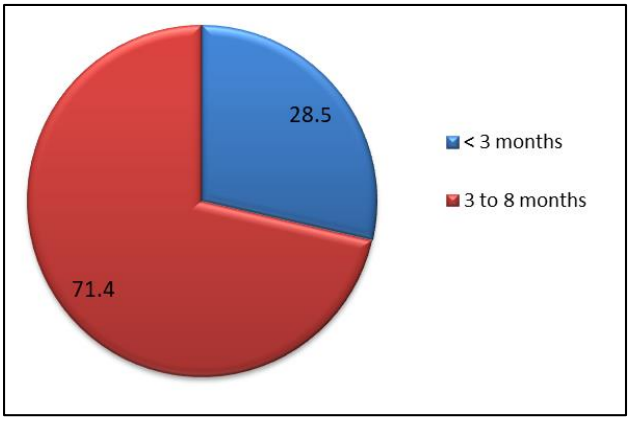

Fig 2: Age-wise distribution of oriental theileriosis cases in calves

Clinical Pathology

The level of parasitaemia varied from 0.5 to 10% in infected calves and majority of the infected calves had moderate level of parasitaemia with 1-5% of the infected RBC (Fig. 3). Majority of the infected calves were mildly anaemic and PCV ranged between 15-23% (Fig. 4)

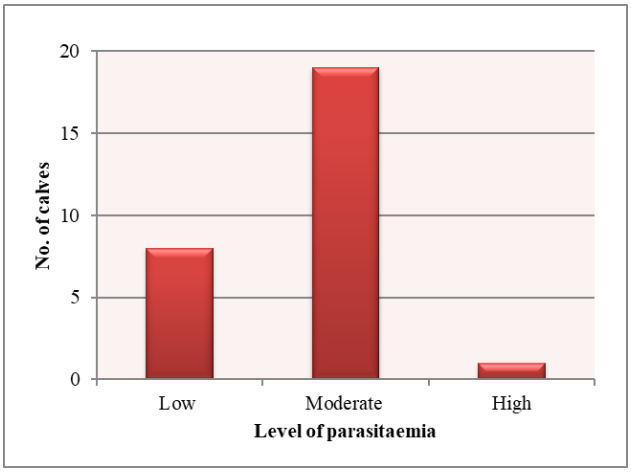

Fig 3: Level of parasitaemia in oriental theileriosis infected calves

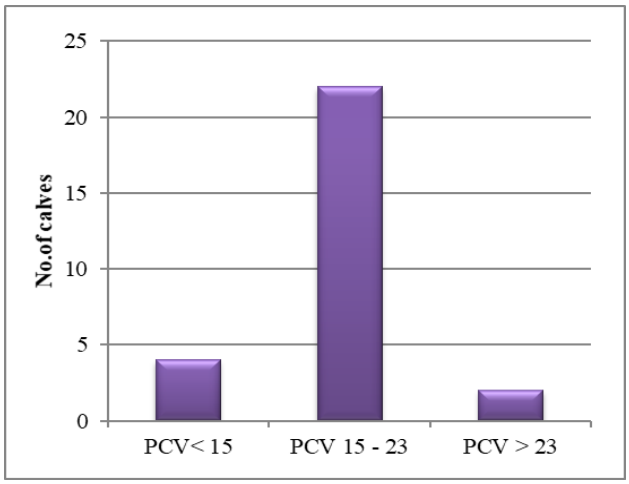

Fig 4: PCV% in oriental theileriosis infected calves

Molecular diagnosis

Amplification of 18SrRNA gene of Theileria was evident in PCR from all the 28 smear positive calves with typical bands of 1098bp region (Fig 5). The primers targeting the MPSP gene amplified the 875bp fragment specific for *T. orientalis* (Fig 6) in all 28 DNA samples and confirmed that all calves were infected with oriental theileriosis.

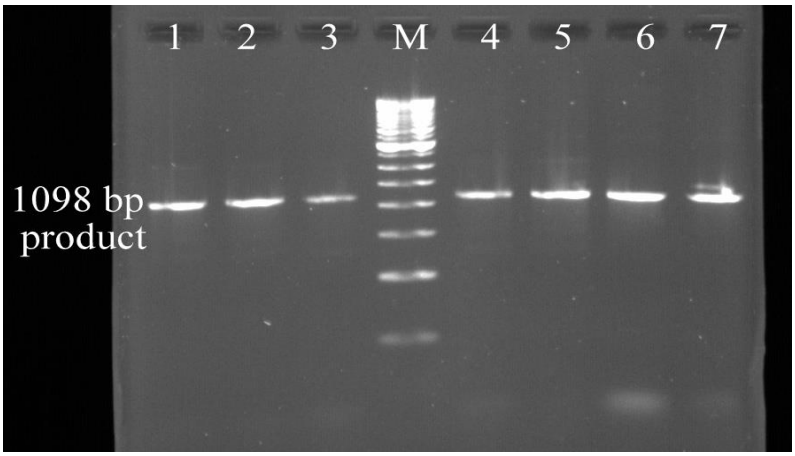

Fig 5: PCR gel doc image showing 1098 bp product specific for *Theileria* spp Lane 1. Positive control. Lane M – 250bp ladder Lane 2 to 7. Clinical samples positive for Theileria genus

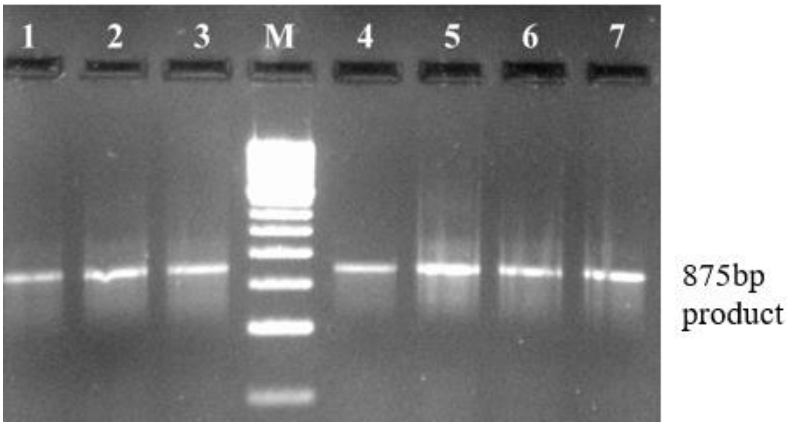

Fig 6: PCR gel doc image showing 875 bp product specific for *T. orientalis* Lane 1: Positive control Lane M – 250bp ladder Lane 2 to 7: clinical samples positive for *T. orientalis*

Therapeutic management

The infected calves were treated with Inj. Buparvaquone (Zubion®, Intas pharma, Ahmedabad) @ 2.5 mg per kg body weight along with supportive therapy with fluids, parentral supplements and hematinics. Two calves died during the course of the treatment despite intensive management. Blood smears examined after 48 hours of treatment were negative from 20 calves. The remaining six calves which were smear

positive after 48 hours of treatment were treated with second dose of Inj. Buparvaquone and were found to be negative on blood smear examination after 48 hours of second treatment.

Discussion

Twenty eight calves were found positive for Theileria piroplasms in blood smear examination and the infection was confirmed as oriental theileriosis caused by *T. orientalis* by PCR. Swilks *et al*. (2017a) [11] reported that transplacental transmission of *T. orientalis* occurs rarely in chronically infected animals and infection was not detectable in calves born to infected dams until one month of age and calves became positive for infection in PCR at three months of age. In contrast to that, infection was diagnosed in 45days old calf by blood smear examination and confirmed by polymerase chain reaction in our study. Eamens *et al*. (2013a) [3] detected *T. orientalis* infection in calves at 1 to 2 weeks of age by PCR in Australian herds. Swilks *et al*. (2017b) [12] detected *T. orientalis* infection by blood smear examination in calves between 4 and 20 days of age.

Mekata *et al*. (2018) [8] also found that calves born to *T. orientalis* PCR-positive dams were negative in PCR upto 30 days of age but 9.6% (3/31) of calves found to be positive for infection at 3 and 5 months of age. Maternal antibodies might control the level of parasitemia in calves born to dams having low level of parasitemia and thus the calves become undetectable for infection upto one month of age but the calves became PCR positive by three months of age (Mekata *et al*., 2018) [8]. In our study high proportion of the calves aged between 3 to 8 months were found positive for oriental theileriosis. Lawrence *et al*. (2016) [7] found a negative correlation between dam infection intensity and calf infection intensity at 4 months of age. High parasitaemia in dams close to parturition stimulates the production of better colostrum which protects the calf through passive immunity.

In our study with the exception of three calves, all the remaining calves were above three months of age by the time they exhibited clinical signs and infection became detectable by blood smear examination and confirmed by PCR. Lawrence *et al*. (2016) [7] also reported that vertical transmission of *T. orientalis* can take three months to become detectable by PCR in calves. In contrary to this, we were able to detect infection by both blood smear examination and PCR as early as 45 days after birth in one calf and in two calves aged 2 months old.

Kawazu *et al*. (1991) [6] identified significantly higher level of parasitaemia in calves less than 3 months age compared to calves aged 6 months and above. But in our study all the infected calves had low to moderate level of parasitaemia (0.5 to 4.2%) except in one calf which had higher level of parasitaemia (10.5%).

Conclusion

Oriental theileriosis occurs in calves of any age and in disease endemic areas, anaemic calves should be first screened for *T. orientalis* infection. Buparvaquone along with supportive therapy was found to be effective in terms of clinical recovery for treatment of oriental theileriosis. Appropriate tick control measures and identification of carrier animals will be helpful in reducing the incidence of the disease in calves.

Ethical approval

The study was not conducted on experimental animals. We have taken clinical cases reported to clinics, so no need to take ethical approval. However, animals were examined and samples were collected after taking consent from the owner as per standard examination and sample collection procedures.

Acknowledgements

The authors greatly acknowledge Kerala Veterinary and Animal Sciences University (KVASU) for facilities provided for the research work.

References

1. Baek BK, Soo KB, Kim JH, Hur J, Lee BO, Jung JM *et al*. Verification by polymerase chain reaction of vertical transmission of *Theileria sergenti* in cows. Can. J Vet. Res. 2003; 67(4):278-282.
2. d'Oliveira C, Van Der Weide M, Habela MA, Jacquiet P, Jongejan F. Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. J Clin. Microbiol. 1995; 33(10):2665-2669.
3. Eamens GJ, Bailey G, Jenkins C, Gonsalves JR. Significance of *Theileria orientalis* types in individual affected beef herds in New South Wales based on clinical, smear and PCR findings. Vet. Parasitol. 2013a; 196(1, 2):96-105.
4. Eamens GJ, Gonsalves JR, Jenkins C, Collins D, Bailey G. *Theileria orientalis* MPSP types in Australian cattle herds associated with outbreaks of clinical disease and their association with clinical pathology findings. Vet. Parasitol. 2013b; 191(3, 4):209-217.
5. Heath ACG. Biology, ecology and distribution of the tick, *Haemaphysalis longicornis* Neumann (Acari: Ixodidae) in New Zealand. N.Z. Vet. J. 2016; 64(1):10-20.
6. Kawazu SI, Chandrawathani P, Kamio T, Minami T, Rajamanickam C, Fujisaki K. Serological survey of bovine theileriosis in Malaysia. Japan Agric. Res. Quart. 1991; 244:315-318.
7. Lawrence KE, Gedye K, McFadden AMJ, Pulford DJ, Pomroy WE. An observational study of the vertical transmission of *Theileria orientalis* (Ikeda) in a New Zealand pastoral dairy herd. Vet. Parasitol. 2016; 218:59-65.
8. Mekata H, Minamino T, Mikurino Y, Yamamoto M, Yoshida A, Nonaka N *et al*. Evaluation of the natural vertical transmission of *Theileria orientalis*. Vet. Parasitol. 2018; 263:1-4.
9. Shiono H, Yagi Y, Chikayama Y, Miyazaki S, Nakamura I. The influence of oxidative bursts of phagocytes on red blood cell oxidation in anaemic cattle infected with *Theileria sergenti*. Free Radical Research 2003; 37(11):1181-1189.
10. Sugimoto C, Fujisaki K. Non-transforming Theileria parasites of ruminants. In Theileria Springer 2002, 99-103
11. Swilks E, Fell SA, Hammer JF, Sales N, Krebs GL, Jenkins C. Transplacental transmission of *Theileria orientalis* occurs at a low rate in field-affected cattle: infection in utero does not appear to be a major cause of abortion. Parasites & Vectors. 2017a; 10(1):227-236.
12. Swilks E, Jenkins C, Poynting A, Collins D, Krebs GL. Prevalence and effect of *Theileria orientalis* infection in homebred calves in the Gloucester region of New South Wales, Australia. Aus. Vet. J. 2017b; 95(6):211-216.
13. Watts JG, Playford MC, Hickey KL. *Theileria orientalis*: a review. N.Z. Vet. J. 2016; 64(1):3-9.