



Molecular Diagnosis and Therapeutic Management of Concurrent Infection with Theileriosis and Babesiosis in Holstein Friesian Crossbred Cattle

K. Sudhakar Goud^{1*} and K. Vijayakumar²

¹Ph.D Scholar, Department of Veterinary Epidemiology & Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Kerala, INDIA

²Professor & Head, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Kerala, INDIA

*Corresponding Author: drsudhabet@gmail.com

How to cite this paper:

Sudhakar Goud, K., & Vijayakumar, K. (2020). Molecular Diagnosis and Therapeutic Management of Concurrent Infection with Theileriosis and Babesiosis in Holstein Friesian crossbred Cattle. *International Journal of Livestock Research*, 10(10), 158-163. doi:

<http://dx.doi.org/10.5455/ijlr.20200518093609>

Received : May 18, 2020
Accepted : Aug 13, 2020
Published : Oct 31, 2020

Copyright © Goud *et al.*, 2020

This work is licensed under the Creative Commons Attribution Inter-National License (CC BY 4.0). <http://creativecommons.org/licenses/by/4.0/>



Abstract

Molecular diagnosis and therapeutic aspects of concurrent infection of Babesiosis and Theileriosis in five Holstein Friesian crossbred cattle are described. Pyrexia, decreased milk production and pale mucus membrane were prominent clinical signs observed. In the peripheral blood smear intra erythrocytic piroplasms Babesia and Theileria were identified. Polymerase chain reaction was performed to confirm the blood smear findings. Haematological examination revealed low haemoglobin and RBC counts. All animals were successfully treated with Inj Diminazene aceturate @ 3.5mg/kg body wt. and Inj. Buparvaquone @ 2.5mg/kg body wt. along with supportive therapy. Ticks present on infected animals were identified as Haemophysalis longicornis.

Keywords: Babesiosis, Cattle, Diagnosis, Polymerase Chain Reaction, Theileriosis

Introduction

Theileriosis and Babesiosis are tickborne haemoprotozoan diseases that have major impact on livestock production particularly in cattle and small ruminants (Mehlhorn and Schein, 1984). Theileriosis caused by *Theileria parva* and *T. annulata* is characterised by lymphoproliferative changes and high mortality (Aktas *et al.*, 2006; McKeever, 2009). Oriental theileriosis caused by *T. orientalis* is previously considered as benign and believed to cause mild disease, recently emerged as agent capable of outbreaks of clinical theileriosis (Kamau *et al.*, 2011, Proctor *et al.*, 2016). The major manifestations of oriental theileriosis in cattle are anaemia, jaundice, lethargy and late term abortion in pregnant animals (Eamens *et al.*, 2013). Bovine Babesiosis is mainly caused by *Babesia bigemina* and *B. bovis* in tropical and sub-tropical countries (Uilenberg, 2006). Babesiosis in cattle is clinically characterised by fever, inappetance, anaemia, jaundice, haemoglobinuria, abortions and occasional mortality (Banerjee *et al.*, 2005). The anemia in babesiosis is due to intravascular hemolysis (Saleh, 2009) and in theileriosis it is due to erythrophagocytosis (Eamens *et al.*, 2013). Both *Theileria* and *Babesia* spp. are capable of causing anemia of various types viz. haemolytic, hypochromic as well as normochromic leading to reduction in erythrogram parameters.

Cattle that survive acute infections with *Theileria* and *Babesia* species may remain persistently infected and serve as an important reservoir of infection for the ticks that transmit the infection to other susceptible animals (Hussein *et al.*, 2007). Clinical disease occurs when these carrier cattle are transported to non-endemic areas (Bilgic *et al.*, 2013). Traditionally in field conditions blood smear examination is routinely used for identifying these hemoparasites (Altay *et al.*, 2008). But this technique is useful for detection of acute infections only. For diagnosis of these diseases in carrier animals and animals with low level of parasitemia polymerase chain reaction (PCR) is the highly sensitive and specific method (Aktas *et al.*, 2006, d'Oliveria *et al.*, 1995). This study describes molecular diagnosis and successful therapeutic management of concurrent infection with theileriosis and babesiosis in five Holstein Friesian crossbred cattle.

History and Clinical Observations

The present study includes five Holstein Friesian crossbred cattle aged between three to five years with history of inappetance, decreased milk production and high fever. Clinical examination revealed high rectal temperature (103.2 to 104°F), pale conjunctival mucous membrane, swollen prescapular lymphnodes, tachycardia and tick infestation.

Materials and Methods

Thin blood smears were prepared with the blood collected from ear vein before and after 48 hours of treatment. The blood smears were fixed with methanol for three minutes and stained by Field staining method (Himedia, India) and examined under oil immersion objective (100X) of the microscope. Whole blood samples were collected from jugular vein into EDTA coated vials for haematology and for molecular diagnosis.

DNA extraction and polymerase chain reaction

Genomic DNA was extracted from the whole blood using DNeasy Blood and Tissue kit (QIAGEN) according to the manufacturer's instructions. The final eluted DNA was stored at -20°C until use. The PCR assay was performed using primers BaF and BaR targeting 18S rRNA of *Babesia* spp. *Theileria* genus specific PCR was performed using primers 989 and 990 targeting the SSU rRNA of *Theileria* spp. (Table 1).

Table 1: Oligonucleotide sequences of primers used in the study

Primer	Sequence	Organisam	Reference
BaF	AATACCCAATCCTGACACAGGG	<i>Babesia</i> spp.	Maharana <i>et al.</i> (2018)
BaR	TTAAATACGAATGCCCCAAC		
989	AGTTTCTGACCTATCAG	<i>Theileria</i> spp.	d'Oliveira <i>et al.</i> (1995)
990	TTGCCTTAAACTTCCTTG		

The reaction mixture for each PCR was shown in Table 2. The reactions were performed in an automatic DNA thermal cycler (Bio-Rad). The reaction conditions comprised of initial denaturation for 5 min at 94°C, denaturation

for 1 min at 94°C, annealing for 1 min at 55°C for *Babesia* spp. and for 1 min at 52.6°C for *Theileria* spp. extension for 1 min at 72°C and final extension for 5 min at 72°C. The denaturation, annealing and extension were repeated for 30 cycles for *Babesia* spp. and for 40 cycles for *Theileria* spp. The PCR products were electrophoresed in 1.2% agarose gel in 1X TBE buffer. The agarose gel was transferred on to UV transilluminator (GeNei™) for visualization of expected bands and gel documentation done.

Table 2: Components of a 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
6	Total	25

Results

Blood Smear Examination

Rod shaped piroplasms with trailing cytoplasm suggestive of *Theileria* organism and pear-shaped pyriform bodies of *Babesia* spp. were observed inside the red blood corpuscles in the stained blood smears of all the five animals.

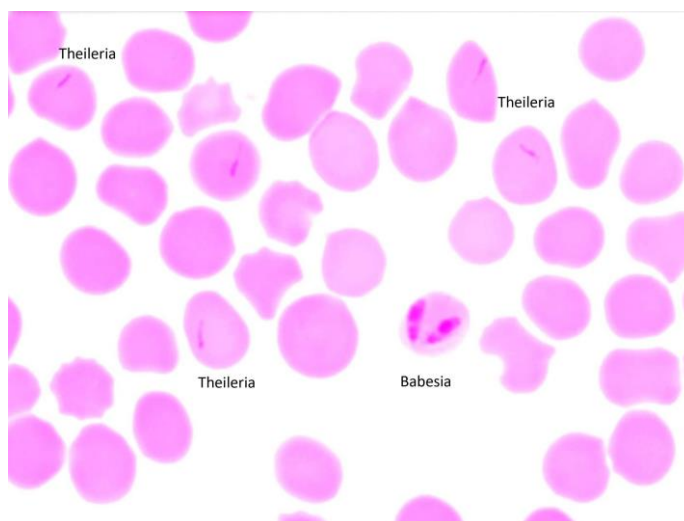


Figure 1: *Babesia* and *Theileria* piroplasms in blood smear

The ticks were identified as *Haemaphysalis longicornis*. Hematology revealed reduced haemoglobin (< 6 g/dl) and red blood cell count (< 4 X 10⁶/cumm) in all animals.

Polymerase Chain Reaction

In PCR all the five DNA samples were amplified by both the primer sets yielding amplicon size of 410bp suggestive of *Babesia* spp. using BaF and BaR and 1098bp suggestive of *Theileria* spp. by 989 and 990 primers (Fig. 2).

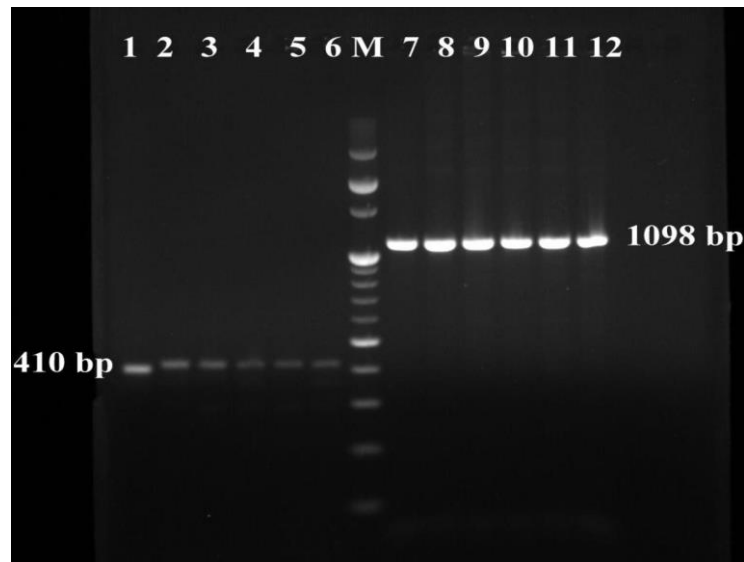


Figure 2: PCR gel doc image showing bands at 410 bp (*Babesia* spp.) and 1098 bp (*Theileria* spp.); Lane 1 – *Babesia* positive control, Lane 2 to 6 - Samples positive for *Babesia* spp, Lane M – 100bp Ladder, Lane 7 – *Theileria* positive control, Lane 8 to 12 – Samples positive for *Theileria* spp.

Treatment

All the animals were treated for *Babesiosis* with Inj Diminazene aceturate^a @ 3.5mg/kg body wt. deep IM along with liver extract, iron supplements on first day. On third day the animals were treated with Inj Buparvaquone^b @ 2.5mg/kg body wt for theileriosis. Butox^c liquid (1ml diluted in four litres of water) was applied as spray for control of ticks. By fifth day of treatment all the animals were found to be negative for *Babesia* and *Theileria* on blood smear examination. Ferritas^d bolus (two per day) was continued for one week and uneventful recovery is noticed in all the animals. Post treatment haematological analysis was done after three weeks and values were within normal range.

- Inj. Berenil® RTU Vet, Brand of MSD Animal Health, Pune
- Inj. Zubion®, Brand of Intas Animal Health, Ahmedabad
- Brand of Intervet, Pune
- Brand of Intas Animal Health, Ahmedabad

Discussion

Tick transmitted diseases *Theileriosis* and *Babesiosis* are responsible for huge economical losses to livestock farmers globally. In our study the ticks present on the animals were identified as *Haemophysalis longicornis*. Minami and Ishihara (1980) in their study concluded that *Haemophysalis longicornis* can act as a vector for transmission of both *Theileria* and *Babesia* spp. in cattle. Laboratory diagnosis of clinical infection by piroplasms of cattle is usually based on stained blood smear examination. Carrier animals with less number of erythrocytes infected are important contributors for transmission of infection through tick bites. But detection of infection in carrier animals by microscopy is not easy and generally it is not possible to discriminate between pathogenic from non-pathogenic species that occur simultaneously with in the same host.

Theileria piroplasms detected in our study resemble with that of non-transforming *Theileria* spp. which are rod shaped with trailing cytoplasm. The pathogenesis of these *Theileria* spp. was not clearly established, but piroplasms exert major effects through destruction of erythrocytes. Along with infected erythrocytes, non-infected erythrocytes were also cleared from circulation by erythrophagocytosis leading to severe anaemia in infected cattle. In addition, even though very few *Babesia* piroplasms (2-3 in fifty fields examined per slide) were detected in infected animals, *Babesia* spp. induce anemia by intravascular hemolysis. Combination of these two mechanisms of anaemia could have led to the development of clinical anaemia among the co-infected animals in this study. The significant reduction in haemoglobin values and RBC counts in co-infected animals were in agreement with Sivakumar *et al.* (2012) who also stated that development of anemia in *Theileria* infected animals will be potentiated by co-infection with *Babesia*.

Marked improvement in haemogram was detected after 3 weeks of treatment and by which time milk production also restored back to normal. Diminazene aceturate is the drug of choice for treatment of Babesiosis (Benerjee *et al.*, 2005) and buparvaquone is effective against theileriosis (Abdela and Bekele, 2016). In our study both the drugs were used to treat the co-infected animals and all the five animals were found to be negative for both *Theileria* and *Babesia* on blood smear examination by 5th day of treatment.

Conclusion

Theileriosis and babesiosis are the most important disease of bovines that affect the productivity of livestock in humid tropics. The diagnosis of these diseases is made routinely by light microscopy under field conditions. The major disadvantage is that animals with low levels of parasitaemia could not be diagnosed as positive by this method. Lack of expertise in identifying the parasite morphology makes it difficult to diagnose the pleomorphic forms of *Theileria* piroplasms by this method. So, some of the important pathognomonic clinical signs seen in acute cases of babesiosis infected animals are not found in chronically infected cattle. The enlargement of lymph node is not characteristic in clinical cases of benign theileriosis which makes the tentative diagnosis of these cases difficult. Molecular methods even though time consuming and expensive, could be used for confirmative diagnosis. In the present study concurrent infection with theileriosis and babesiosis was confirmed by PCR and successfully treated with buparvaquone and diminazine aceturate.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Abdela, N. and Bekele, T. (2016). Bovine theileriosis and its control: a review. *Advances in Biological Research*, 10(4), 200-12.
2. Aktas, M., Altay, K. and Dumanli, N. (2006). A molecular survey of bovine *Theileria* parasites among apparently healthy cattle and with a note on the distribution of ticks in eastern Turkey. *Veterinary Parasitology*, 138(3-4), 179-185.
3. Altay, K., Aydin, M.F., Dumanli, N. and Aktas, M. (2008). Molecular detection of *Theileria* and *Babesia* infections in cattle. *Veterinary Parasitology*, 158(4), 295-301.
4. Banerjee, P. S., Dabas, S., Vatsya, S., Bhatt, P., & Yadav, C. L. (2005). Babesiosis in a crossbred cow with clumping of parasitised red blood cells. *Journal of Veterinary Parasitology*, 19(2), 153-154.
5. Bilgic, H.B., Karagengç, T., Simuunza, M., Shiels, B., Tait, A., Eren, H. and Weir, W. (2013). Development of a multiplex PCR assay for simultaneous detection of *Theileria annulata*, *Babesia bovis* and *Anaplasma marginale* in cattle. *Experimental Parasitology*, 133(2), 222-229.
6. d'Oliveira, C., Van der Weide, M., Habela, M.A., Jacquiet, P. and Jongejan, F. (1995). Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. *Journal of Clinical Microbiology*, 33(10), 2665-2669.
7. Eamens, G.J., Gonsalves, J.R., Jenkins, C., Collins, D. and Bailey, G. (2013). *Theileria orientalis* MPSP types in Australian cattle herds associated with outbreaks of clinical disease and their association with clinical pathology findings. *Veterinary Parasitology*, 191(3-4), 209-217.
8. Hussein, A.H., Mohammed, N.A.E.S. and Mohammed, H.K. (2007). Theileriosis and babesiosis in cattle: haemogram and some biochemical parameters. In Proceedings of XIII International Congress of International Society of Animal Hygiene. Tartu, Estonia. pp.143-150.
9. Kamau, J., de Vos, A.J., Playford, M., Salim, B., Kinyanjui, P. and Sugimoto, C. (2011). Emergence of new types of *Theileria orientalis* in Australian cattle and possible cause of theileriosis outbreaks. *Parasites & Vectors*, 4(1), 22-32.
10. Maharana, B.R., Patel, B.R., Patel, J. and Hirani, N.D. (2018). Parasitological and molecular based detection of cerebral babesiosis in Kankej bullock and its successful therapeutic management. *Asian Journal of Animal and Veterinary Advances* 13(2), 122-127.

11. McKeever, D.J. (2009). Bovine immunity—a driver for diversity in *Theileria* parasites? *Trends in Parasitology*, 25(6), 269-276.
12. Mehlhorn, H. and Schein, E. (1984). The piroplasms: life cycle and sexual stages. In *Advances in Parasitology*, (Volume 23, pp.37–103). Academic Press.
13. Minami, T. and Ishihara, T. (1980). *Babesia ovata* sp. n. isolated from cattle in Japan. *National Institute of Animal Health Quarterly*, 20(3), 101-113.
14. Proctor, A.K.K., Ball, M., Freeman, P., Jenkins, C. and Bogema, D.R. (2016). Prevalence of *Theileria orientalis* types in beef cattle herds on the North Coast of New South Wales. *Australian Veterinary Journal*, 94(4), 117-120.
15. Saleh, M.A. (2009). Erythrocytic oxidative damage in crossbred cattle naturally infected with *Babesia bigemina*. *Research in Veterinary Science*, 86(1), 43-48.
16. Sivakumar, T., Tagawa, M., Yoshinari, T., Ybanez, A.P., Igarashi, I., Ikehara, Y., Hata, H., Kondo, S., Matsumoto, K., Inokuma, H. and Yokoyama, N. (2012). PCR detection of *Babesia ovata* from cattle reared in Japan and clinical significance of coinfection with *Theileria orientalis*. *Journal of Clinical Microbiology*, 50(6), 2111-2113.
17. Uilenberg, G. (2006). *Babesia*—a historical overview. *Veterinary Parasitology*, 138(1-2), 3-10.
