ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 5, pp.4235 -4239, May, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

MOLECULAR DETECTION AND ASSOCIATED PATHOGENESIS IN A FATAL CASE OF THEILERIA ORIENTALIS INFECTION IN INDIA: PROBABLE CIRCULATION OF A VIRULENT STRAIN AND STRESS ASSOCIATED FACTORS

Parikshit Kakati, Prabhat Chandra Sarmah, Kanta Bhattacharjee*, Dipak Bhuyan[1] and Bhaben Chandra Baishya[1]

Department of Veterinary Parasitology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India
[1]Teaching Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India

ARTICLE INFO

Article History:

Received 2nd, April, 2015
Received in revised form 10th, April, 2015
Accepted 4th, May, 2015
Published online 28th, May, 2015

Key words:

Theileria orientalis, Ikeda, Oriental theileriosis, heifer, PCR, India.

ABSTRACT

Oriental theileriosis, a tick-borne entity caused by the haemoprotozoan parasite Theileria orientalis, presumably a benign Theileria species appears to be an important disease problem in cattle nowadays. The present communication reports a fatal case caused by T. orientalis in a pregnant heifer from Northeast India. The animal, with history of high fever, inappetance, nervine symptoms followed by recumbency and nonresponsive to subsequent symptomatic treatment was brought to the outpatient unit of the author's institute's Clinical Complex for blood examination and further treatment on 3rd day of illness. Microscopic examination of blood smear followed by Polymerase Chain reaction (PCR) analysis confirmed the presence of Theileria orientalis infection and involvement of Ikeda variant. Administration of anti theilerial compound Buparvaquone at the terminal stage of illness was however unsuccessful and the animal succumbed to infection within 8 hours of parasite specific treatment. Ulcers in abomasal mucosa, distended gall bladder, lung oedema, tarry colored sticky intestinal contents indicating hemorrhage from abomasal ulcers were the important pathological findings observed at necropsy. On the basis of clinical symptoms, microscopic and molecular study along with postmortem findings, the cause of death of the pregnant heifer was attributed to be due to Theileria orientalis infection.

INTRODUCTION

*Theileria orientalis***,** a tick-borne haemoprotozoan parasite of cattle is widely distributed in tropical and subtropical region of the world. This species of *Theileria*, earlier considered to be non-pathogenic has been presently receiving great importance due to emergence of pathogenic strains and involvement in outbreaks of clinical disease characterized by fever, anaemia, jaundice and abortion and even mortality recorded from several countries (McFadden *et al*., 2011; Eamens *et al*., 2013; Aparna *et al*., 2011; Sivakumar et. al., 2012; Kamau *et al*., 2011). In India also, the parasite was considered non-pathogenic (Shastri *et al*., 1988) for which not much attention was paid till the recent report of Aparna *et al*. (2011) who recorded fatal disease due to *T. orientalis* in crossbred adult bovines infested with *Haemaphysalis bispinosa* in Southern India. A 3 years old indigenous heifer from a cattle herd belonging to the Department of Animal Reproduction, Obstetrics and Gynecology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam was showing unusual behavior like intense bellowing, excitement, uncontrolled running and hitting against objects as soon as it was released for grazing. The animal was put on supportive treatment based on clinical symptoms but in the absence of visible improvement, the recumbent animal was shifted to the clinics on 3rd day of illness for further investigation. In order to get a confirmatory diagnosis and initiate proper treatment to the heifer in view of the serious condition of the animal, immediate blood sampling was done and subjected to both microscopic and molecular analysis along with complete blood count (CBC). The present communication reports the clinical, microscopic, molecular and pathological findings recorded during post mortem after the animal ultimately succumbed owing to *T. orientalis* infection.

MATERIALS AND METHODS

Clinical signs observed during examination of the animal were recorded thoroughly. Blood sample was collected in a vaccutainer (Beckett Dickinson; BD) with EDTA for

Corresponding author:* **Kanta Bhattacharjee
Department of Veterinary Parasitology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India

haematological and parasitological examination. Complete Blood Count (CBC) was done in an automatic blood cell counter and thin blood smears were immediately prepared for Giemsa staining after fixing with methanol. Stained blood smears were examined under oil immersion objective of the microscope for the detection of haemoparasite(s). The other part of the blood sample was used for DNA extraction using DNeasy Blood and Tissue kit (Quiagen® Kit, Catalogue No. 69504) as per manufacturer's protocol and subjected to PCR analysis using published primers and cycling conditions (Table-1) against *Babesia bigemina* (Laha *et al.*, 2012), *Anaplasma marginale* (Figueroa *et al.*, 1993), *Theileria annulata* (d'Oliveira *et al.*, 1995) and *Theileria orientalis* (Kamau *et al.*, 2011). Conditions used for PCR amplifications were 12.5µl of DyNAzyme II PCR Master Mix (Fermentas), 10 mM of each primer scaled up to 25 µl reaction mix. PCR was carried out in a Techne-500 thermal cycler (Bibby Scientific) and the products were subjected to electrophoresis in an agarose gel prestained with Ethidium Bromide and subsequent visualization done in gel documentation system (DNR Mini Lumi, Applied Bioimaging). Dung sample was subjected to parasitological examination using sedimentation and floatation techniques for presence of any endoparasites. Bacillary haemoglobinuria, leptospira infection, bovine viral diarrhea (BVDV), poisoning, clostridial infection, history of dog bite, intussusception, torsion of the uterus and post parturient haemoglobinuria were ruled out for differential diagnosis prior to molecular confirmation of the disease due to *T. orientalis.* The animal was promptly treated with anti theilerial compound Buparvaquone (Carter, 2011) @ 2.5 mg per kilogram of body weight intramuscularly in the neck muscle. Necropsy was performed and gross pathological changes observed were recorded.

RESULTS

Clinical examination revealed lateral recumbency with high body temperature (107˚ F), intermittent bellowing with hyperesthesia, paddling of legs, highly congested mucous membrane of the eyes, nasal discharge, head and neck pulled out more dorsally, anaemic conjunctiva, sticky and tarry colored dung and reddish mucoid vaginal discharge. Microscopic examination of giemsa stained blood smear revealed small intraerythrocytic piroplasms of varying shapes viz. rod shaped (Fig 1), comma shaped bodies with trailing cytoplasm. (Fig 2), and crescent shaped (Fig 3) similar to that of *T. orientalis* and confirmed later on by PCR which amplified the Merozoite Piroplasm Surface Protein Gene (MPSP), showing a product size of 776 bp (Fig 4). Further PCR analysis using type specific primers for *T. orientalis* revealed the presence of Ikeda type showing a product size of 826 bp (Fig 5). The blood smear did not reveal any organisms similar to *B. bigemina*, *A. marginale* and *T. annulata* which were also supported by PCR. Although prompt treatment was initiated, the animal could not be saved and ultimately succumbed to the infection. Post mortem examination was carried out and significant findings included presence of an about 5 months old and dead male fetus (Fig 6), congestion and oedema in lung, distended gallbladder (Fig 7), ulcer in abomasal mucosa (Fig 8) and presence of tarry colored blood mixed sticky contents in the entire intestinal tract (Fig 9). Thus, microscopic examination of blood sample supported by molecular assay carried out simultaneously along with gross pathological lesions at necropsy confirmed the cause of death of the animal due to solitary infection with the haemoparasite *T. orientalis*.

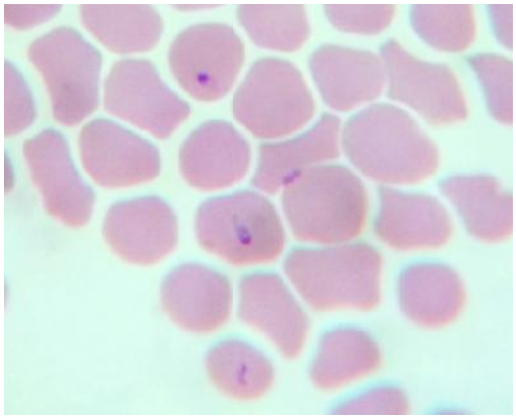

Fig 1

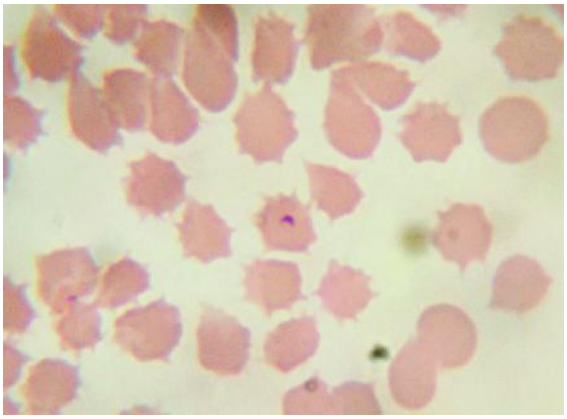

Fig 2

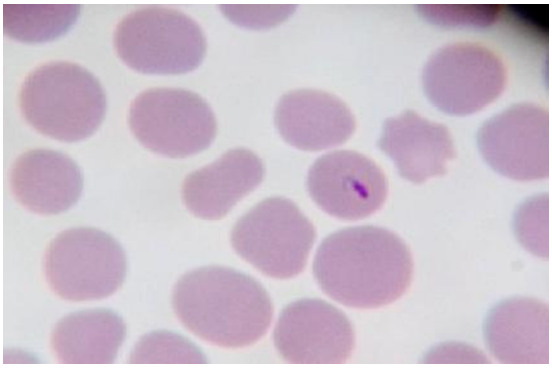

Fig 3

DISCUSSION

Bovine tropical **t**heileriosis caused by pathogenic *T. annulata* in cattle has been well documented from different parts of India (Bansal, 2005) but another species, *T. orientalis* although reported from India (Shastri *et al*., 1988) long back, not much attention was paid to this species. There was no local knowledge on the prevalence of haemoparasite other than *Babesia* and *Anaplasma* in Assam till the report of Kakati (2013) who recorded predominance of *Boophilus microplus* tick vector and incidence of *T. orientalis* besides *B. bigemina* and *A. marginale* in indigenous and crossbred cattle and record of mortality among crossbreds. There are very scanty reports on *T. orientalis* in India which might be due to the fact that this

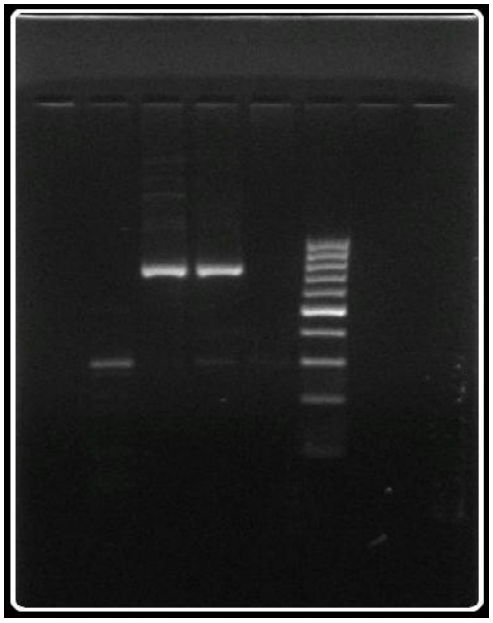

Fig 4

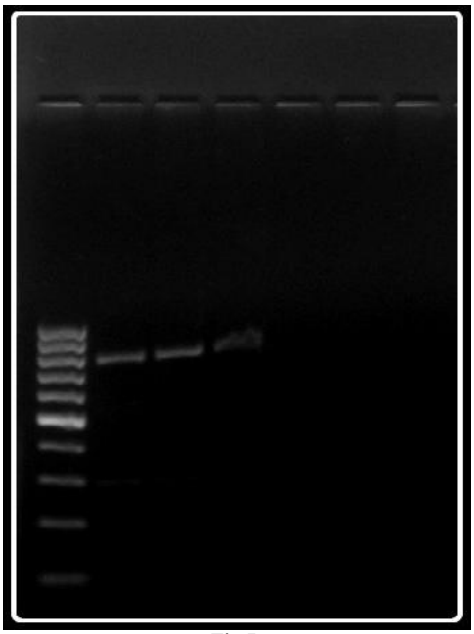

Fig 5

Fig 6

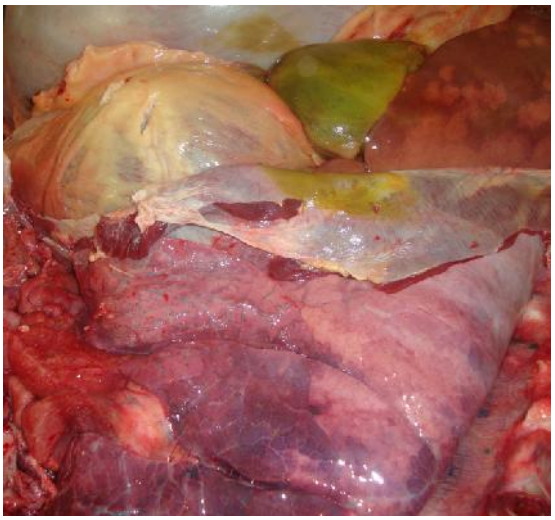

Fig 7

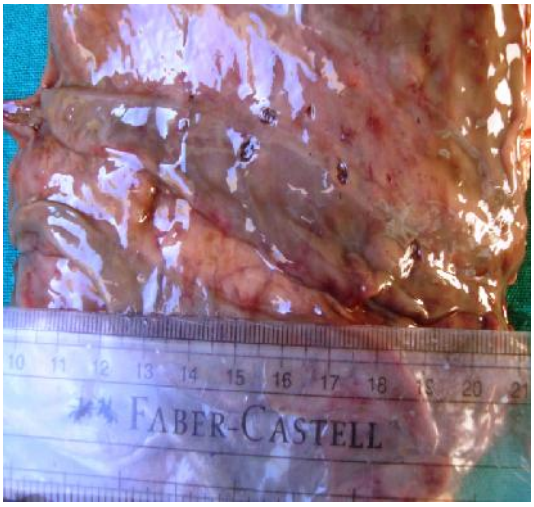

Fig 8

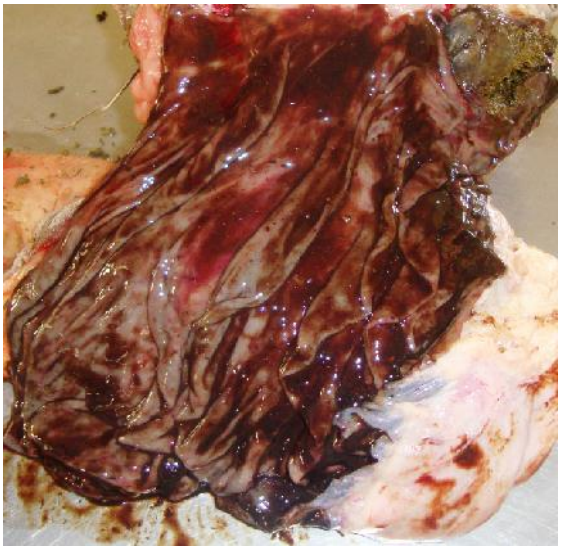

Fig 9

parasite responsible for oriental theileriosis was earlier considered as a mild one and had usually no ill effects in cattle. However, on the basis of clinical history, laboratory analysis, mortality and post mortem findings recorded in the present case due to *T. orientalis* confirmed its virulence similar to recent reports made by Aparna *et al*. (2011) from India and many other countries like Australia (Burney and Lugton, 2009;

Table 1 standard primer used for identification of *B. bigemina, T. annulata, A. marginale* and *T. orientalis* along with their amplification targets, product size and cycling conditions.

Parasite	Primer sequence pair	Amplification Target	Product Size	Cycling conditions	Reference
Babesia bigemina	Bbi:F1:5'-TGG CGG CGT TTA TTA GTT CG-3' Bbi:R1:5'-CCA CGC TTG AAG CAC AGG A-3'	A portion of the *Babesia bigemina* mitochondrial DNA	1124 bp	94˚C,2min 94˚C,30sec 55˚C,30sec 72˚C,1min 72˚C,5min	Laha *et al.*, 2012
Theileria annulata	TaF1:5'-GTA ACC TTT AAA AAC GT-3' TaR1:5'-GTT ACG AAC ATG GGT TT-3'	30 kDa major *T.annulata* merozoite surface antigen gene	721 bp	95˚C,3min 94˚C,1min 55˚C,1min 72˚C,1min 73˚C,10min	d'Oliveira *et al.*, 1995
Anaplasma marginale	Am:F1:5'-CAC ATT TCT TGG AGC TGG-3' Am:R1:5'-TCT CTG GCA CTT TGA ACC-3'	A fragment of genomic parasite DNA	160 bp	95˚C,1 min 95˚C, 1 min 55˚C, 1min 73˚C,1.5min 73˚C,15min	Figueroa *et al.*, 1993
Theileria orientalis (Entire Theileria orientalis group)	Tor:F1:5'-CTT TGC CTA GGA TAC TTC CT-3' Tor:R1:5'-ACG GCA AGT GGT GAG AAC T-3'	Gene encoding a polymorphic merozoite piroplasm surface protein (MPSP)	776 bp	95˚C, 2min 95˚C, 15sec 57˚C, 30sec 72˚C, 1min 72˚C, 10min	Kamau *et al.*, 2011

Kamau *et al.*, 2011; Eamans *et al.*, 2013), Missouri, USA (Stockham *et al.*, 2000), Michigan, USA (Bayugar *et al.*, 2002), New Zealand (Mc Fadden *et al.*, 2011), Mongolia (Altangerel *et al.*, 2011) and Japan (Fuujisaki, 1992; Yokoyama *et al.*, 2010). Pathological lesions recorded in the present case were found consistent with the report of Aparna *et al.*, (2011) who recorded in addition hemorrhagic duodenitis in fatal cases and Carbon and Forshaw (Livestock Biosecurity Factsheet, Government of Australia, 2013). Record of tarry colored intestinal contents in the present case was also seen several cases of *T. orientalis* infection reported from Assam and some of which responded to Buparvaquone treatment provided at the initial stage (Kakati, 2013). Infection associated anaemia and concomitant pregnancy in the heifer might have acted as stress factors as stated by Aparna *et al.*, (2011) and Kakati (2013) which acted as predisposing and precipitating factors for severe clinical manifestation of the disease and resultant mortality consistent with reported outbreaks of *T. orientalis* infection in pregnant and recently introduced cattle (Kamau *et al.*, 2011). Buparvaquone has been proved to be highly effective against *T. orientalis* (Carter, 2011). Administration of the drug in the present case at the terminal stage however could not save the the animal. Similar to record of mortality were observed in few treated case by Kakati (2013).This indicated towards poor response to treatment in severely affected and animals in advanced stage of infection. Perusal of literature reveals presence of variant types of *T. orientalis viz.*, Type Buffeli, Chitose, Ikeda and five unnamed types globally based on MPSP sequence of the parasite of which Ikeda strain has been proved to be highly pathogenic and associated with several outbreaks in cattle (Mc Fadden *et al.*, 2011; Kamau *et al.*, 2011). PCR analysis in the present study revealed amplification of 826 bp fragment of DNA of Type Ikeda consistent with the earlier reports of Kamau *et al.*, 2011 which suggests the involvement of the Ikeda strain and also strains different from Buffeli and Chitose type. These need further confirmation and subsequent phylogenetic analysis to establish the pathogenic property of different isolates of the parasite circulating amongst the cattle population in India.

CONCLUSION

A case of fatal *Theileria orientalis* infection in a pregnant heifer has been reported in the present communication along with the gross and associated pathological lesions. The findings of the case study indicate towards stress associated precipitation of a fulminating form of the otherwise mild pathogen in cattle or circulation of a virulent strain in the population. The result of the current study warrants a detailed and systematic study of the parasite involved in clinical outbreaks. Identification of possible cytokines released in response to possible stress factors and isolation of a virulent strain of the parasite should be envisaged aimed towards control and elimination of the disease in the country.

Acknowledgement

The authors would like to acknowledge the Director of Teaching Veterinary Clinical Complex, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam for giving the opportunity to diagnose the case and providing us the necessary help and resources.

Figure captions

Fig 1:Giemsa stained blood smear showing intraerythrocytic rod shaped form of *T. orientalis* (X1000).
Fig 2: Giemsa stained blood smear showing intraerythrocytic comma shaped form of *T.orientalis* with trailing cytoplasm (X1000).

Fig 3: Giemsa stained blood smear showing intraerythrocytic crescent shaped form of *T.orientalis* (X1000).
Fig 4: Picture showing 776 bp fragment of *T. orientalis* DNA in 1.5 % agarose gel.
Fig 5: Picture showing 826 bp fragment of *T. orientalis*(type Ikeda) DNA in 1.5 % agarose gel.
Fig 6: Dead fetus recovered from the heifer at post mortem.
Fig 7:Viscera of the dead heifer showing congested, edematous lungs along with distended gall bladder.
Fig 8: Abomasal mucosa showing numerous haemorrhagic ulcers.
Fig 9: Tarry colored sticky contents recovered from the intestine**.**

References

Altangerel, K., Battsetseg, B., Battur, B., Sivakumar, T., Batmagnai, E., Javkhlan, G., Tuvshintulga, B., Igarashi, I., Matsumoto, K., Inokuma, H. and Yokoyama, N. 2011. The first epidemiological survey of *Theileria orientalis* infection in Mongolian cattle. Vet. Parasitol., 182: 343–348.

Aparna, M., Ravindran, R., Vimalkumar, M.B., Lakshmanan, B., Rameshkumar, P., Kumar, K.G., Promod, K., Ajithkumar, S., Ravishankar, C., Devada, K., Subramanian, H., George, A.J. and Ghosh, S. 2011. Molecular characterization of *Theileria orientalis* causing fatal infection in crossbred adult bovines of South India. Parasitol. Int., 60: 524–529.

Bansal, G.C. 2005. Bovine Theileriosis in India: An overview. Proc. Nat. Acad. Sci. India. 75: 134-43.

Bayugar, R.C., Pillars, R., Schlater, J. and Holmana, P.J. 2002. *Theileria buffeli* infection of a Michigan cow confirmed by small subunit ribosomal RNA gene analysis. Vet. Parasitol., 105: 105-110.

Burney, A. and Lugton, I. 2009. Prevalence and effect of *Theileria* infection in NSW South Coast cattle herds: A targeted surveillance study financially supported by NSW DII. Report prepared by Alissa Burney (Vet Intern, Sydney Univ.) and Ian Lugton (SDV SELHPA), South East Livestock Health and Pest Authority, New South Wales, Australia. (8/07/09).

Carbon, M. and Forshaw, D. 2013. Bovine anaemia due to *Theileria orientalis* group (BATOG)-information for veterinarians. In: Livestock Biosecurity Factsheet, Dept. of Agr. And Food, Govt. of Western Australia (May,2013). URL: agric.wa.gov.au

Carter, P. 2011. Assessment of the efficacy of Buparvaquone for the treatment of 'benign' bovine theileriosis. In: *Animal Health*, Meat and Livestock Australia Limited, Queensland Government.

D' Oliveira, C., Weide, M., Habela, M., Jacquit, P. and Jongejan, F. 1995. Detection of *Theileria annulata* in blood samples of carrier cattle by PCR. *J. Clin. Microbiol.,*10: 2665-2669.

Eamens, GJ., Bailey, G., Jenkins, C. and Gonsalves, J.R. 2013. Significance of Theileria orientalis types in individual affected beef herds in New South Wales based on clinical, smear and PCR findings. Vet Parasitol., S0304-(13)00011-3.

Figueroa, J.V., Chives, L.P., Johnson, G.S. and Buening, G.M. 1993. Multiplex Polymerase Chain Reaction based assay for the detection of *Babesia bigemina*, *Babesia bovis* and *Anaplasma marginale* DNA in bovine blood. Vet. Parasitol., 50: 69-81.

Fuujisaki, K. 1992. A Review of the Taxonomy of *Theileria sergenti/buffeli/orientalis* Group Parasites in Cattle. *J. Protozool. Res.,* 2: 87-96.

Kakati, P. 2013. Prevalence of ticks and tick borne haemoparasitic infection in cattle of Assam. Master Degree Thesis. Submitted to Assam Agricultural University, Jorhat, Assam, India. (Unpublished Data).

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: 22.

Laha, R., Das, M., Goswami, A. and Singh, P. 2012. A clinical case of babesiosis in a cross bred cow of Meghalaya. *Indian J. Anim. Res.,* 46 (3): 302-305

McFadden, A.M., Rawdon, T.G., Meyer, J., Makin, J., Clough, R.R., Tham, K., Mullner, P. and Geysen, D. 2011. An outbreak of haemolytic anaemia associated with infection of *Theileria orientalis* in naive cattle. N. Z. Vet. J., 59: 79–85.

Shastri, U.V., Pathak, S.V., Jadhav, K.V. and Deshpande, P.D. 1988. Occurrence of *Theileria orientalis* in bovines from Maharastra state and its *transmission by Haemaphysalis bispinosa ticks. Indian*. J. Parasitol., 12**:** 173-177.

Sivakumar, T., Kothalawala, H., Abeyratne, A.E., Vimalakumar, S.C., Meewewa, A.S., Igarashia, I. and Yokoyama, N. 2012. A PCR-based survey of selected *Babesia* and *Theileria* parasites in cattle in Sri Lanka. Vet. Parasitol., 190: 263– 267.

Song, Kun-Ho. and Sang, Byung-Chan. 2003. Prevalence of *Theileria sergenti* infection in Korean native cattle by polymerase chain reaction. *Korean J Parasitol*., 41(3): 141-145.

Stockham, S.L., Kjemtrup, A.M., Conrad, P.A., Schmidt, D.A., Robimson, T.W., Tyler, J.W., Johnson, G.C., Carson, C.A. and Cuddihee, P. 2000. Theileriosis in a Missouri beef herd caused by *Theileria buffeli*: A case report, herd investigation, ultra structure, phylogenetic analysis and experimental transmission. Vet. Pathol., 37: 11-21.

Yokoyama, N., Ueno, A., Izuno, D., Kuboki, N., Khukhuu, A., Igarashi, T., Miyahara, T., Shiraishi, R., Kudo, M., Oshiro, S., Zakimi, C., Sugimoto, K. and Inokuma, H. 2010. Genotypic Diversity of *Theileria orientalis* Detected from Cattle Grazing in Kumamoto and Okinawa Prefectures of Japan. *J. Vet. Med. Sci.,* 73(3): 305-312.

How to cite this article:
Kanta Bhattacharjee *et al*., Molecular Detection and Associated Pathogenesis In A Fatal Case Of Theileria Orientalis Infection In India: Probable Circulation Of A Virulent Strain And Stress Associated Factors. *International Journal of Recent Scientific Research Vol. 6, Issue, 5, pp.4235 -4239, May, 2015*

****