RESEARCH ARTICLE

Molecular Detection of Anaplasmosis in Crossbred Cattle

Vandna Bhanot[1]*, Naresh Jindal[2]

Abstract

Bovine anaplasmosis is an infectious, non-contagious, tick-borne disease of domestic as well as wild ruminants caused by obligate intraerythrocytic parasites of family Anaplasmataceae and genus *Anaplasma*. Fifty blood samples were collected from crossbred cattle in a dairy farm with a history of a drastic decrease in milk yield and fever in a few animals. Six (12%) samples showed characteristic *Anaplasma* organisms when stained with Giemsa's stain, whereas 19 (38%) samples were found positive for *A. marginale* by PCR assay. Low hemoglobin and total erythrocyte count were observed, whereas an increase in total leucocyte count was noted in *Anaplasma* affected animals compared to healthy animals at the same farm.

Keywords: Anaplasmosis, Cattle, Hematology, Molecular diagnosis, PCR.

Ind J Vet Sci and Biotech (2022): 10.21887/ijvsbt.18.2.23

Introduction

Anaplasmosis is the most common cause of economic losses in the livestock industry throughout the world. Bovine anaplasmosis is a parasitic disease of cattle caused by the obligate intracellular (erythrocytic) rickettsia *Anaplasma marginale*, primarily occurring in tropical and subtropical regions of the world. Although cattle, sheep, goats, buffalo, and some wild ruminants can be infected with the erythrocytic Anaplasma, cattle have been more susceptible than buffaloes (Rajput *et al*., 2005). Most commonly, Rhipicephalus (Boophilus) microplus (Asian blue tick) are biological vectors of A. marginale, but the pathogen is often transmitted mechanically to susceptible cattle by blood-contaminated mouthparts of biting flies or by needles and veterinary instruments that become contaminated with blood. In cattle aged > 2 years, *A. marginale* causes persistent fever, lethargy, icterus, weight loss, abortion, decreased milk yield, and death in more than 50% of untreated animals (Kocan *et al*., 2003 and M'Ghirbi *et al*.,2016)

The standard technique for diagnosing bovine anaplasmosis has traditionally been done by microscopic examination of Giemsa-stained blood smears revealing > 10^6 infected erythrocytes/ml (Gale *et al*., 1996). In the acute phase, inclusion bodies are easily detected in bovine erythrocytes (Maharana *et al*., 2014) by microscopic examination. In contrast, this technique is not easily applicable for detecting chronic and sub-clinical infections. Such infected animals serve as a reservoir for spreading *A. marginale* (Eriks *et al*.,1989).Polymerase Chain Reaction assays allow diagnosis of parasite at levels far below the detection limit of the frequently used parasitological techniques and have an advantage in separating clinical and subclinical forms of parasitic infection (Almeria *et al*., 2001). In the present study, a PCR assay was used along with microscopy to detect anaplasmosis in crossbred cattle.

[1] Disease Investigation Laboratory, Ambala, Department of Veterinary public health and epidemiology, Lala Lajpat Rai University of Veterinary and animal Sciences , Hissar, Haryana, India

[2]Department of Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar , Haryana, India

Corresponding Author: Vandna Bhanot, Asstt. Disease Investigation Officer, Disease Investigation Laboratory, Ambala, Department of Veterinary public health and epidemiology, Lala Lajpat Rai University of Veterinary and animal Sciences, Hissar, e-mail: vandna.van@gmail.com

How to cite this article: Bhanot, V. & Jindal, N. (2022). Molecular Detection of Anaplasmosis in Crossbred Cattle. Ind J Vet Sci and Biotech. 18(2), 109-112.

Source of support: Nil

Conflict of interest: None.

Submitted: 30/10/2021 **Accepted:** 05/01/2022 **Published:** 10/04/2022

Materials and Methods

The present study was conducted on a dairy farm of district Ambala, Haryana, in the year 2019. Blood samples (n = 50) in EDTA vial were collected from crossbred cattle having fever in a few animals and a drastic decrease in milk yield. Blood smears were prepared on clean glass slides. Smears were fixed with methanol, and let to dry in the air, then stained with Giemsa's stain and examined under a microscope (X1000) with immersion oil for the presence of haemoparasites infections. The haemoparasites were identified to species level as per morphological characters as described by Soulsby (1982). Various hematological parameters such as hemoglobin (Hb), total erythrocyte count (TEC), and total leucocyte count (TLC) were compared between healthy (n=8) and affected animals (n = 19). The blood samples were kept at 4°C and further analyzed by PCR. DNA was extracted from the blood samples using a DNA extraction

kit by Qiagen. *Anaplasma marginale* specific primers for the gene *msp4* (Forward primer 5'- CTGAAGGGGGAGTAATGGG-3' and Reverse primer 5'- GGTAATAGCTGCCAGAGATTCC-3') were used for diagnosis and amplification of 344 bp gene product (Torina *et al*., 2012). The amplified PCR products were separated by electrophoresis on 1% agarose gel and visualized under UV transilluminator for detection of 344 bp amplified product in positive cases. A sample with heavy parasitemia of *A. marginale* was used as a positive control for the standardization of PCR assay. A no-template reaction was also used as a negative control for all the PCR reactions.

Results and Discussion

Out of 50 blood samples, only six (12%) samples showed characteristic rickettsial organisms when stained with Giemsa's stain as well as PCR (Fig. 1). The PCR product of positive control yielded a specific band of 344 bp on gel electrophoresis while there was no band in the negative control. In addition, 13 more samples were positive for *A. marginale* by PCR. Overall, 19 (38%) samples were found positive for *A. marginale* by PCR. Gel electrophoresis of PCR products from these 19 samples showed a specific fragment of 344 bp (Fig. 2). The blood samples found negative by PCR were also found negative for *A. marginale* on blood smear examination. These samples belonged to animals whose production performance was poor and gradually decreased. Various studies revealed that anaplasmosis is prevalent in the cattle population in Haryana. Ganguly *et al*. (2017) observed *T. annulata* (33.32%), followed by *B. bigemina* (4.76%), *A.marginale* (1.67%), and mixed infection of these parasites (1.58%) in crossbred cattle in Haryana. Bhanot (2020) also reported haemoparasitic infection in crossbred cattle and found theileriosis (44.6%), followed by anaplasmosis (3.4%) and babesiosis (2.6%) to be the major disease problems.

Recent molecular technology has gained a lot of importance in the diagnosis of haemoparasitic infection. Conventional parasitological techniques like microscopic examination of Giemsa stained thin blood smear is always a standard gold test for diagnosing anaplasmosis in clinically suspected animals, while in subclinical and chronic infections Giemsa stained thin blood smear is not suitable (Eriks *et al*., 1989; Ashuma *et al*., 2013). The 2 X 2 contingency table for blood smear examination and PCR is shown in Table 1. The result revealed that the PCR assay is 100% sensitive (true positive (TP)/true positive (TP) + false negative (FN) × 100) and 70.4% specific (true negative (TN)/true negative (TN) + false positive (FP) × 100) when compared with microscopy based detection method. This further asserts its usefulness in rapid

Table 1: 2 x 2 contingency table for blood smear examination and PCR

	Blood Smear Examination		
	Positive	Negative	Total
PCR			
Positive	6[a]	13[b]	19
Negative	0[c]	31[d]	31
Total	6	44	50

[a] True positive, [b] False positive, [c] False negative ,[d] True negative
Sensitivity of PCR = a/ (a+c) x 100 = 100 %; Specificity of PCR = d/ (b+d) x 100 = 70.4 %

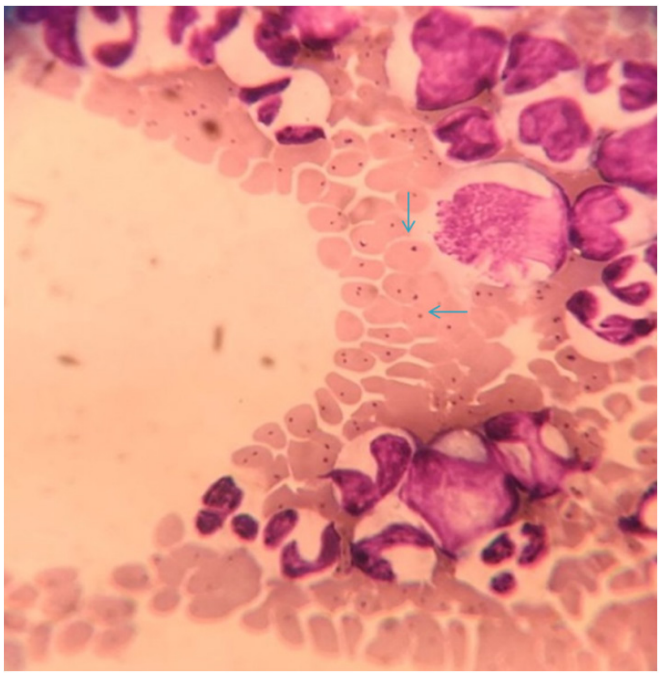

Fig. 1*: Anaplasma* organisms on the margin of RBC by microscopic examination (X1000)
blood smears

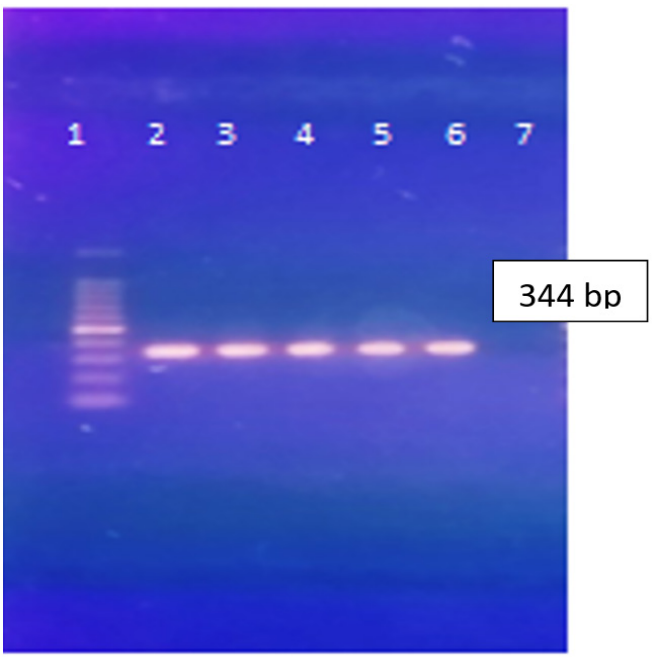

Fig. 2: Agarose gel electrophoresis of amplified 344 bp product by PCR using *msp4* specific primers for *A. marginale*Lane1: DNA marker (100bp DNA Ladder), Lane 2: Positive Control, Lane 3-6: Sample tested, Lane 7: Negative Control

diagnosis of *A. marginale* with high specificity and sensitivity especially in carrier animals/subclinical infections with very low parasitemia. In the case of a carrier state with low levels of parasitemia, *Babesia* and *Anaplasma* organisms may not be found on microscopic examination (Atif *et al*., 2012b). For confirmatory diagnosis, PCR is used for detection even in case of a low level of parasitemia (Ge *et al*., 1995; Torina *et al*., 2008). Bisen *et al*., 2021 also reported molecular detection by msp5 nested-PCR as highly sensitive and reliable for detecting carrier cattle for Anaplasma infection.

In the present study, hematological parameters (Hb, TEC $x10^6$ and TLC$x10^3$) were compared between healthy and affected animals. The results of the study revealed Low Hb (5.6 ± 0.25) and TEC (4.02 ± 0.275) levels with increase in TLC (9.4 ± 0.412) count as compared to healthy animals (Hb-10.25 ± 0.72; TEC -7.02 ± 0.58; TLC-6.01 ± 0.35). The values for different cells of leucocyte (Mean ±SE) are presented in Table 2 reveals that neutrophil and monocyte count in the infected cattle were low whereas lymphocytes and eosinophil count were high as compared to healthy animals. The major sign of anaplasmosis infection is extravascular hemolytic anemia in cattle (Ajayi *et al*., 1978; Kuttler, 1984; Atif *et al*., 2012a). A high risk of anemia could be due to phagocytosis of infected erythrocytes by the bone marrow cells and spleen (Jain, 1993). Normally there is simultaneous hemolysis of intravascular and extravascular RBCs (Riond *et al*., 2007). Destruction of the non-parasitized RBCs also starts in addition to the breakdown of parasitized RBCs due to immune-mediated autolysis. The disease also adversely affects the milk production of cattle. This alterations in haematological parameters is in agreement with Ganguly *et al*., (2018) who observed significantly decreased levels of TEC, Hb and PCV in animals infected with *Anaplasma* than healthy control animals. Ashuma *et al*. (2013) reported that haemogram of the *Anaplasma* infected animals revealed anaemia as indicated by significant decrease ($P < 0.05$) in RBC, Hb and PCV in both the infected groups positive by GSTBS and PCR as compared to the healthy control group.

It can be deduced that Anaplasmosis is associated with alteration in haemogram which may results in production loss. Although microscopy is widely accepted and cost effective technique for the diagnosis of haemoprotozoans and haemorickettsial organisms, the technique is not highly sensitive. Further, subclinical infection of anaplasmosis can be better detected by molecular methods. Appropriate tick control strategies are also to be adopted for containing the infection at reduced level in the herd in endemic areas.

Table 2: Haematological parameters of Anaplasma positive and healthy animals

Parameter	*Positive (N=19) (Mean ±SE)*	*Healthy control (N=8) (Mean ±SE)*
Hb(g/dl)	5.6 ± 0.25	10.25 ± 0.72
TLC($x 10^3$)	9.4 ± 0.41	6.01±0.35
TEC ($x10^6$)	4.02± 0.27	7.02 ± 0.58
Neutrophil	38.15± 1.9	52.15 ± 3.6
Lymphocyte	58.84 ± 1.7	46 ± 3.8
Monocyte	0.63 ± 0.16	0.75 ± 0.16
Eosinophil	1.89± 0.24	0.5 ± 0.19

Acknowledgment

The authors express their sincere sense of gratitude to the Vice-Chancellor, LUVAS, Hisar, for providing research facilities and financial support.

References

Ajayi, S. A., Wilson, A. J. and Campbell, R. S.(1978). Experimental bovine anaplasmosis: Clinicopathological and nutritional studies. *Research in Veterinary Science,*1,76-81.

Almeria, S., Castella, J., Ferrer, D., Ortuno, A., Estrada-Pena, A. and Gutierrez, J. F. (2001). Ovine piroplasms in Minorca (Balearic Islands, Spain): a comparison of PCR-based and light microscopy detection. *Veterinary Parasitology,*99, 249–59.

Ashuma, A.S., Singla, L.D., Kaur, P., Bal, M.S., Batth, B.K. and Juyal, P.D.(2013). Prevalence and haemato-biochemical profile of *Anaplasmamarginale* infection in dairy animals of Punjab (India). *Asian Pacific Journal of Tropical Medicine,* 10, 139-144.

Atif, F.A., Khan, M.S., Khan, M.A., Ashraf, M. and Avais, M. (2012a). Chemotherapeutic efficacy of oxytetracycline, enrofloxacin and imidocarb for the elimination of persistent *Anaplasma marginale* infection in naturally infected Sahiwal cattle. *Pakistan Journal of Zoology.,* 44, 449-256.

Atif, F.A., Khan, M.S., Iqbal, J.I., Arshad, G.M., Ashraf, E. and Ullah, S. (2012b).Prevalence of *Anaplasma marginale, Babesia bigemina* and *Theileria annulata* infections among cattle in Sargodha District, Pakistan. *African Journal of Agricultural. Research,7*, 3302-3307.

Bhanot, V. (2020). Retrospective study on occurrence of tick borne haemoparasitic diseases in dairy animals of eastern Haryana. *Indian Journal of Animal Science*, 90 (9), 1225-1228.

Bisen, S., Aftab, A., Jeeva, K., Silamparasan, M., Yadav, S., Chandra, D., Sankar, M., Garg, R., Raina, O.K., 2021. Molecular and serological detection of Anaplasma infection in carrier cattle in north India. *Vet. Parasitol. Reg. Stud. Reports*, 24, 100550

Eriks, I.S., Palmer, G.H., McGuire, T.C., Allered, D.R. and Barbet, A.F. (1989). Detection and quantitation of Anaplasma in carrier cattle by using a nucleic acid probe. *Journal of Clinical Microbiology,* 27, 179-184.

Ganguly A, Bisla R, Singh H, Bhanot V, Kumar A, Kumari S, Maharana B R and Ganguly I. (2017). Prevalence and Haematobiochemical changes of tick borne haemoparasitic diseases in crossbred cattle of Haryana, India. *Indian Journal of Animal Sciences, 87*(5),552–557.

Ganguly A, Maharana, B. R, Ganguly, I. Kumar A, Potliya, S., Arora, D. and Bisla, R. (2018). Molecular diagnosis and haemato-biochemical changes in Anaplasma marginale infected dairy cattle. *Indian Journal of Animal Sciences, 88*(9), 989–983.

Gale, K.R., Dimmock, C.M., Gartside, M. and Leatch, G. (1996). *Anaplasma marginale*: Detection of carrier cattle by PCR. *International Journal of Parasitology,* 26(10),1103–1109.

Ge, N.L., Kocan, K.M., Murphy, G.L. and Blouin, E.F. (1995). Detection of *Anaplasma marginale* DNA in bovine erythrocytes by slot-blot and in situ hybridization with a PCR-mediated digoxigenin-labeled DNA probe. *Journal of Veterinary Diagnostic Investigation,* 7,465-472.

Jain, N.C.(1993). *Essentials of Veterinary Hematology*.1st edn. Philadelphia.

Kocan, K.M., Fuente D.L.J., Guqlielmone, A.A. and Melendez, R.D. (2003). Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. *Clinical Microbiology Reviews,* 16, 698-712.

Kuttler, K.L. (1984). *Anaplasma* infections in wild and domestic ruminants: A review. *Journal of Wildlife Diseases,* 11, 12-20.

Maharana, B. R., Vala, K. B., Panigrahi, P. N. and Behra, S. K. (2014). Therapeutic management of anaplasmosis in a Jaffrabadibuffalo. *Indian Journal of Veterinary Medicine,* 34(2), 158–59.

M'Ghirbi, Y., Bèji, M., Oporto, B., Khrouf, F., Hurtado, A. and Bouattour,A.(2016).Anaplasma marginale and A. phagocytophilum in cattle in Tunisia. *Parasites Vectors*, 9, 1–8.

Rajput, Z.I., Hu S.H., Arijo, A.G., Habib, M. and Khalid, M.J. (2005) Comparative study of Anaplasma parasites in tick carrying buffaloes and cattle. *Journal of Zhejiang. University Science*,6, 1057 -1062.

Riond, B., Meli, M. L., Braun, U., Deplazes, P., Joerger, K., Thoma, R., Lutz, H. and Hofmann-Lehmann, R. (2007). Concurrent infections with vector-borne pathogens associated with fatal anaemia in cattle: haematology and blood chemistry. *Comparative ClinicalPathology,*17, 171-177.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. Bailliere Tindall, English LanguageBook Society, London.

Torina, A., Alongi, A., Naranjo, V., Estrada-Peña, A., Vicente, J. and Scimeca, S.(2008). Prevalence and genotypes of *Anaplasma* species and habitat suitability for ticks in a Mediterranean ecosystem. *Applied and. Environmental Microbiology,*74, 7578-7584.