The Indian Journal of Veterinary Sciences & Biotechnology ***(2017) Volume 13, Issue 2, 62-66***

ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT

http://dx.doi.org/10.21887/ijvsbt.v13i2.10054

Submitted : 20-07-2017 ***Accepted : 07-09-2017*** ***Published : 15-11-2017***

Incidence and Haematological Changes in Haemoprotozoan Infections in Bovines of South Gujarat

M.D. Patel, Niranjan Kumar, P.H. Rathod, K.K. Tyagi and L.M. Sorathiya

Department of Veterinary Medicine, Vanbandhu Veterinary College,

Navsari Agricultural University, Navsari-396450, Gujarat

Corresponding Author: mdpatel@nau.in

Abstract

A study was conducted to record the incidence, risk factors and haematological changes during haemoprotozoan infections of bovines over a period from January, 2014 to December, 2016. A total of 193 suspected cases (141 cattle and 52 buffaloes) presented at Teaching Veterinary Clinical Complex and Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat, were examined for the presence of haemoprotozoan parasites by Giemsa staining technique. Total 46 (23.83%) cases (23 each of cattle and buffaloes) were found positive for presence of haemoparasite infection. The effect of source of sample, season and species on incidence of haemoprasitic infections was significant whereas effect of year was non-significant. Higher incidence was observed in rainy (34.92) followed by winter (25.42 %) and summer (12.68%) season. Significantly higher incidence of Anaplasmosis (8.29%) was observed followed by Babesiosis (6.74%), Theileriosis (3.63%) and Trypanosomosis and Ehrlichiosis (2.59% each). All positive cases showed comparatively lower values of haemoglobin (Hb.), packed cell volume (PCV) and total erythrocyte count (TEC) whereas values of total leukocyte count (TLC) and differential leukocyte count (DLC) were within the normal range.

Key words : Haematological Changes, Haemoprotozoan, Incidence, Bovine.

Introduction

Haemoprotozoan diseases such as babesiosis, theileriosis and anaplasmosis are the three major tick borne haemoprotozoan diseases of bovines in tropical and sub-tropical regions of the world (Velusamy *et al*., 2014).The remaining one, trypanosomosis is mainly transmitted mechanically by the bites of haematophagous flies. They cause significant morbidity and mortality in cattle and buffaloes. Dairy animals, especially bovines, which are bearing production stress along with other diseases, are potential viable host to these infections (Sharma *et al*., 2013). Generally, the hot and humid climate is very conducive for the development and survival of potential vectors such as ticks and flies and is a constant source of infection to susceptible animals (Chowdhury *et al.,* 2006). Most of the haemoprotozoan parasites are of great economic importance in Asia and has always been a formidable barrier to the survival of exotic and cross bred cattle in India. Most of blood protozoan parasites cause anemia by inducing erythrophagocytosis. These infections are responsible for

causing pyrexia, anaemia, loss of body weight, decrease in milk yield and thus lead to severe economic losses to livestock owners . Many animals die and undergo a long period of convalescence entailing loss of meat and milk production. Haemoprotozoan infections have been reported in bovines of different parts of India (;Vahora *et al*., 2012; Velusamy *et al*., 2014; Bhatnagar *et al*., 2015, Maharana *et al*., 2016). However, true status of haemoprotozoan infections has not yet been explored in bovines of south Gujarat, India. Hence a study on incidence, risk factors and haematological changes in haemoprotozoan infections of bovines of south Gujarathas been undertaken .

Materials and Methods

A total of 193 bovine blood samples from suspected cases (includes 141 cattle and 52 buffaloes) presented at Teaching Veterinary Clinical Complex, Veterinary College and Livestock Research Station, NAU, Navsari for haemoprotozoan infections on the basis of clinical signs viz., high temperature, anaemia, anorexia, enlargement of lymph nodes, progressive debility, respiratory distress, grinding of teeth, decrease in milk yield were collected over a period of three years (January, 2014 to December, 2016). 5 ml of blood sample from each animal were collected aseptically from jugular vein in EDTA vacutainers and the blood samples were immediately brought to the laboratory for detection of haemoprotozoan parasites.Thin blood smears were prepared and fixed with methanol stained with Giemsa's stain and thereafter examined under microscope (100x) with oil immersion for the identification of blood parasites as described by Soulsby (1982). Blood samples were also examined by PCR with little modification (Radwan *et al*., 2013**)** for *A.marginale*. Various haematological parameters including haemoglobin (Hb gm/dl), packed cell volume (PCV %), total erythrocyticcells (TEC, x 10^6/cumm), total leukocytic cells (TLC, cells/cumm) and Differential leukocyte count (DLC, %) were analyzed in fully automatic haematology cell counter (Exigo Vet, Swiden). The epidemiological information was collected from each case and recorded in a proforma developed for present study. The obtained data were analyzed in IBM SPSS statistical software version 20.0 as per methods described by Snedecor and Cochran (1994).

Results and Discussion

Of 193 blood samples collected from suspected cases of 141 cattle and 52 buffaloes, 46 were found positive for presence of different haemoprotozoan infections indicating 23.83% overall incidence of haemoprotozoan infection in bovines of south Gujarat. Individually, incidence in cattle and buffaloes was 16.31 and 44.23%, respectively. The detail on incidence of haemoprotozoan infection in bovine and their risk factors are given in table-1. The incidence of haemoprotozoan infections in present study was comparatively lower than previous reports (Maharana *et al.,* 2016), whereas, lower incidence (<20%) than present was also reported by Chaudhri *et al.* (2013) and Velusamy *et al*. (2014) .

During the present study, the effect of source of sample, season and species was significant whereas effect of year was non-significant. Higher incidence was observed in rainy (34.92) followed by winter (25.42 %) and summer (12.68%) season. The finding of higher occurrence of haemoprotozoan infection in monsoon is in agreement with previous reports (Chaudhari *et al.,* 2013; Maharana *et al.,* 2016). Generally, the prevalence depends largely on the distribution and density of the reservoir hosts, season and vectors. Further, most of the animals suffering during monsoon and summer months might be due to high abundance of vectors in these seasons of the year (Radostits *et al*., 2007; Velusamy *et al.,* 2014). In present study, higher incidence was observed in buffaloes (44.23%) than cattle (16.31%). Generally, higher incidence in cattle than buffaloes were reported previously (Chowdhary *et al*., 2006; Atif *et al*., 2012; Vahora *et al*., 2012) which might be due to more susceptibility of cattle especially crossbred cattle to tick borne diseases as a result of more production stress whereas buffaloes generally carry subclinical infection (Ashuma *et al*., 2013).

Further, significantly higher incidence of Anaplasmosis (8.29%) was observed followed by

Table-1:Incidence and risk factor analysis of haemoprotozoan infections in bovines of south Gujarat

Sr. No.	Parameters		Samples	Negative	Positive	Percent positive	P value
1	Year	2014	84	63	21	25.00	0.546
		2015	54	40	14	25.93	
		2016	55	44	11	20.00	
			193	**147**	**46**	**23.83**	
2	Source	TVCC	175	144	31	17.71	0.000*
		LRS	18	03	15	83.33	
			193	**147**	**46**	**23.83**	
3	Season	Summer	71	64	09	12.68	0.002*
		Rainy	63	41	22	34.92	
		Winter	59	42	15	25.42	
			193	**147**	**46**	**23.83**	
4	Species	Cattle	141	118	23	16.31	0.000*
		Buffalo	52	29	23	44.23	
			193	**147**	**46**	**23.83**	
5	Type of infection	Anaplasma spp.	193	147	16	8.29	0.000*
		Babesia spp.			13	6.74	
		Trypanosoma spp.			05	2.59	
		Theileria spp.			07	3.63	
		Ehrlichia spp.			05	2.59	
			193	**147**	**46**	**23.83**	

* significance at $p<0.05$.

Babesiosis (6.74%), Theileriosis (3.63%) and Trypanosomosis and Ehrlichiosis (2.59% each). But comparatively higher occurrence of Babesiosis followed by theileriosis, anaplasmosis and trypanosomosis was previously reported in bovine (Chaudhari *et al.,* 2013; Maharana *et al.,* 2016). The difference observed in the occurrence may be due to the different geographical locations of the study areas, time periods and various methods of sample analysis (Velusamy *et al.,* 2014). Identified haemoprotozoan parasites on smear examination have been presented in Fig. 1.

In 15 positive cases of Anaplasmosis in buffaloes only 5 cases were found positive on Giemsa's stained thin blood smear examination (GSTBS) whereas all were found positive for presence of *A.marginale* infection by PCR (Fig. 2) which proved better efficacy of PCR for detecting chronic and persistent infection which generally goes undetected on routine smear examination as it has ability to diagnose haemoprotozoan infection in blood with minimum infective unit of one organism

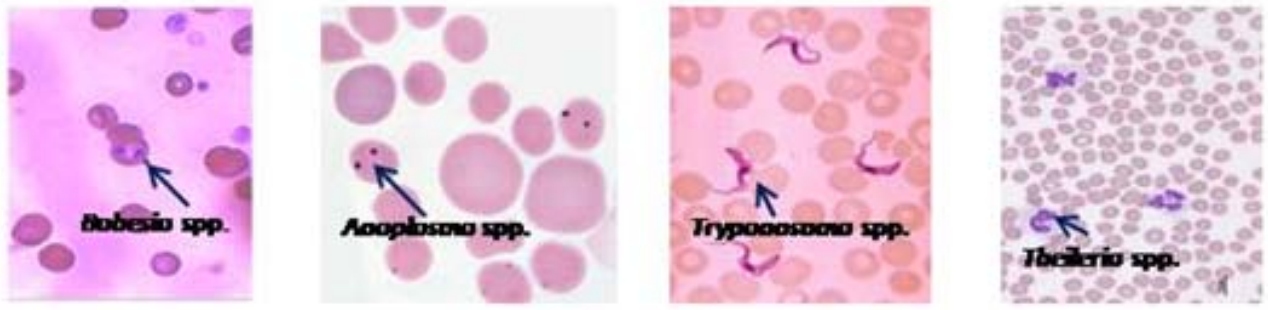
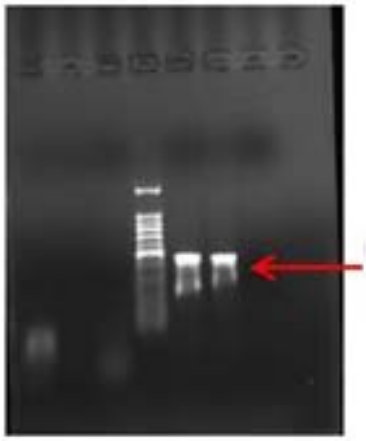


Fig. 1 Identification of major haemoprotozoan infections based on thin blood smear examination stained by Giemsa's stain

(Jaseem and Agaar, 2015). In this regard, Carelli *et al*. (2007) had also stated that Giemsa's stained thin blood smear examination only give better result in acute clinical form of disease but have low to poor efficacy to detect haemoprotozoan infections in chronic or persistent infections with low parasitemia and many times it is difficult to indentify positive cases even by experienced hand. Therefore, they advocated use of molecular based techniques for confirmatory diagnosis of subclinical or persistent haemoprotozoan infections which further helpful for effective treatment.



Amplification of 576 bp of msp5 gene of A. marginale

Fig. 2 Confirmation of *A. marginale* infection by PCR

All positive cases showed comparatively lower values of Hb, PCV and TEC whereas values of TLC and DLC (Neutrophil, Lymphocyte, Eosinophi and Monocyte) were within the normal range (Table 2). Lower Hb, PCV and TEC were in an agreement with previous reports (Chaudhri *et al*., 2013; Maharana *et al*., 2016). Mild leukocytosis may be observed due to stress associated with infection. During present study, it was also observed that hematological data of suspected cases found negative on smear examination were more or less similar to infected cases indicated that the suspected animals might be in carrier stage of disease which was not detected under microscopic examination (Maharana *et al*., 2016).

Table-2: Haematological changes in various haemoprotozoan infections in bovines of south Gujarat

Sr. No.	Type of infection	Hb (g/dl)	TLC (/cumm)	PCV (%)	TEC ($x10^6$/ cumm)	DLC (%)			
						N	L	E	M
1	Anaplasma spp.	6.25	8268.42	17.58	3.16	29.89	67.11	1.63	1.37
2	Babesia spp.	7.97	12069.23	23.89	4.11	45.92	52.46	0.69	0.92
3	Trypanosoma spp.	7.66	7940.00	20.84	4.39	49.40	46.60	1.60	2.40
4	Theileria spp.	8.39	10450.00	23.67	4.85	51.86	41.43	5.00	1.71
5	Ehrlichiaspp	6.98	14600.00	21.60	3.22	44.60	51.20	2.20	2.00
	Infected- Overall	**7.23**	**10201.00**	**20.87**	**3.79**	**40.76**	**55.84**	**1.92**	**1.47**
6	Negative but suspected	**7.13**	**11988.19**	**21.24**	**4.00**	**44.98**	**50.81**	**2.41**	**1.37**
	Overall	7.16	11534.45	21.14	3.95	43.91	52.09	2.28	1.39
	F value	0.338	0.147	0.227	0.089	0.021*	0.005*	0.001*	0.179
Reference value from Merck's Vet.Manual		8-15	5000-14000	24-46	5-10	15-35	45-75	0-20	0-8

* Significance at $p<0.05$.

The results indicated presence of haemoprotozoan infections in bovines of south Gujarat. Further, use of advanced diagnostic tool is advocated for confirmatory diagnosis as subclinical or chronic infections asgoes unnoticed in routine smear examination technique.

Acknowledgements

The authors are highly thankful to Professor and Head(s), Department of TVCC and Parasitology, Vanbandhu College of Veterinary Science & A.H. and Research Scientist, Livestock Research Station, Navsari Agricultural University, Navsari for providing necessary help and facilities.

Conflict of Interest: All authors declare no conflict of interest.

References:

Ashuma, Sharma, A., 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*.**6(2)**: 139-144.

Atif, F. A., Khan, S., Iqbal, H.J. and Roheen, T. (2012). Prevalence of tick-borne diseases in Punjab, Pakistan and haematological profile of *Anaplasmamarginale*infection in indigenous and crossbred cattle.*Pakistan J. Sci*. **64**: 11–15.

Bhatnagar, C.S., Bhardawaj, B, Sharma, D.K. and Meena, S.K. (2015).Incidence of haemoprotozoan diseases in cattle in southern Rajasthan, *India. Int. J. Curr. Microbiol. App. Sci*. **4:** 509-514.

Carelli, G., Decaro, N., Lorusso, A, Elia, G., Lorusso, E., Mari, V. and Buonavoglia, C. (2007). Detection and quantification of *Anaplasmamarginale* DNA in blood samples of cattle by real-time PCR. *Veterinary Microbiology*.**124(1)**: 107-114.

Chowdhury, S., Hossain, M.A., Barua, S.R. and Islam S. (2006) Occurrence of common blood parasites of cattle in SirajgonjSadar area of Bangladesh.*J. Vet. Med.* **4(2)**:143-145.3].

Chaudhri, S.S.,Bisla,R.S.,Bhanot,V. and Singh,H. (2013). Prevalence of haemoprotozoan infections in pyretic dairy animals of eastern Haryana.*Indian J. Anim. Res.* **47(4)**: 344-347.

Jaseem, G.A. and Agaar, O.A. (2015).Moleculat and biochemical study of Anaplasmamarginale in Wassit Province of Iraq. *African Journal of Bacteriology Research*.**7(4)**: 36-41.

Maharana B.R. , Binod Kumar, A. Prasad, T.K. Patbandha, N.R. Sudhakar, J.P. Joseph and B.R. Patel. (2016). Prevalence and assessment of risk factors for haemoprotozoan infections in cattle and buffaloes of South-West Gujarat, India.*Indian J. Anim. Res*. **50(5)**: 733-739

Radostits, O.M, Gay, C.C., Hinchcliff, K.W. andConstble, P.D. (2007). Veterinary Medicine, A text book of the diseases of cattle, horses, sheep,pigs and goats. 8th edn. ELBS, Baillier, London.Pp.1455-1459.

Radwan M.E.I., Ali A. F., Hamied, O. A. (2013). Epidemiological studies,molecular diagnosis of *Anaplasmamarginale* in cattle and biochemical changes associated with it in Kaliobia Governorate, *Amr. J.Infe. Dis. Microbiol.*, **1(3):** 46-49

Sharma, A., Singla, L.D., Tuli, A., Batth, B.K., Kaur, P., Javed, M. and Juyal, P.D. (2013). Molecular prevalence of*Babesiabigemina*and *Trypanosoma evansi*in dairy animals from Punjab, India by duplex PCR: A step forwardto detection and management of concurrent latent infections. *Biomed Res Inter.* Article ID 893862, 8 pages.

Snedecor, G. W. and. Cochran W. G (1994).Statistical Methods.6th Edition.IOWAState University Press. Ames, IOWA.P.1956.

Soulsby, E.J.L. (1982). Helminthes, arthropod and protozoa of domestic animals.7th edition, The English Book Society andBailiere Tindall Ltd.,London.pp.752-53.

Vahora, S.P., Patel, J.V., Patel, B.B., Patel, S.B. and Umale, R.H. (2012).Seasonal incidence of haemoprotozoan diseases in crossbred cattle and buffalo in Kaira and Anand districts of Gujarat, *India. Vet. World*.**5:** 223–225.

Velusamy, R., Rani, N., Ponnudurai, G., Harikrishnan, T.J., Anna, T., Arunachalam, K., Senthilvel, K. and Anbarasi, P. (2014). Influence of season, age and breed on prevalence of haemoprotozoan diseases in cattle of Tamil Nadu, India, *VeterinaryWorld*.**7(8)**: 574-578.

❑