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Incidence of Anaplasmosis in Buffaloes in and Around Navsari and Efficacy of Diagnostic Tests

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Abstract

Incidence of anaplasmosis in buffaloes in and around Navsari district was studied over a period of one year (2016-17). Clinical cases with history of fever, anaemia, icterus, anorexia and progressive debility presented at Teaching Veterinary Clinical Complex, Livestock Research Station and in field were suspected for presence of haemoprotozoan infections and specially examined for anaplasmosis by Giemsa stained thin blood smear (GSTBS), cELISA and PCR. The information related to epidemiological parameters was also collected for risk factor' analysis. The overall incidence of anaplasmosis in buffaloes in and around Navsari district was 37.50%. Comparatively higher incidence was observed in field cases (63.63%) followed by cases from TVCC (60%) and LRS (25%). Seasonwise incidence was 43.75, 39.13 and 22.22% in winter, summer and monsoon, respectively. Incidence in buffaloes aged above 3 years and below 3 years was 40.47 and 16.66%, respectively. The overall effect of place, season and age on incidence of anaplasmosis in buffaloes was non-significant. Considering PCR as gold standard, the sensitivity, specificity and accuracy of GSTBS were 44.44, 100 and 79.16 %, respectively, whereas, corresponding figures for cELISA was 100 %. Results indicated fairly presence of anaplasmosis in buffaloes in and around Navsari but difficult to diagnose with routine smear examination. Therefore, use of advanced diagnostic techniques (PCR/ELISA) is advocated for confirmatory diagnosis.

Introduction

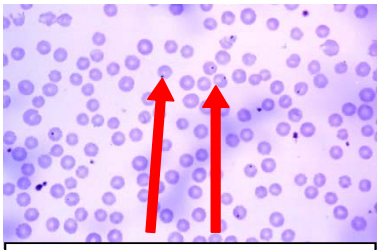
Bovine anaplasmosis is an infectious but non-contagious vector-borne disease of bovine and usually caused by *A. marginale.* The disease is generally transmitted by ticks (*Rhipicephalus* spp.; *Boophilus* spp. and *Hyalomma* spp.) and mechanically by biting flies (*Tabanus* spp.) or blood contaminated fomites. Anaplasmosis is responsible for a severe haemolytic disease characterized by fever, severe anaemia, icterus, loss of appetite, dullness or depression, rapid deterioration of physical condition, muscular tremors, pale mucous membrane and laboured breathing (Radostits *et al.,* 2007). Based on reports, the prevalence of bovine anaplasmosis in Indian sub-continents varies from 0.77 to 70% depending on the test performed. Whereas, the incidence of bovine anaplasmosis in suspected case varies from 1.33 to 48.75% (Muraleedharan *et al.,* 2005; Vahora *et al.,* 2012; Ashuma *et al.*, 2013; Kumar *et al.,* 2015a & b). In India, reports on prevalence of anaplasmosis in buffaloes are available but very little information on incidence of anaplasmosis especially in buffaloes is available. Therefore, a study on incidence of

anaplasmosis in buffaloes in and around Navsari and efficacy of diagnostic tests was initiated.

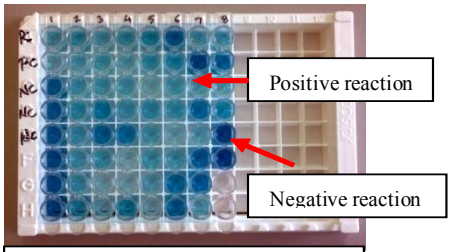
Materials and Methods

The present study was conducted at Department of Veterinary Medicine, Vanbandhu College of Veterinary Science and A.H., Navsari Agricultural University, Navsari. Total 48 clinical cases from Teaching Veterinary Clinical Complex(TVCC), Livestock Research Station (LRS) and field with symptoms such as fever, anaemia, icterus, anorexia and debility were suspected for anaplasmosis. These cases were screened for the presence of *Anaplasma* infection using GSTBS, PCR and cELISA tests. Approximately 5 ml of blood was drawn from jugular vein in vacuutainer with EDTA as well as serum clot activator. Whole blood was used for thin blood smear preparation as well as DNA extraction for PCR whereas serum was used for performing cELISA. Giemsa stained thin blood smears from all suspected cases examined under 100X oil immersion magnification of microscope and the organism identified as per standard method in use. Further, cELISA and PCR were also performed for confirmatory diagnosis (Plate-1). cELISA test kit for bovine anaplasmosis was procured from VMRD, USA and the test was performed as per the protocol outlined in the user manual. The diagnostic PCR was performed using custom synthesized primers to amplify 576 bp of msp 5 gene of *A. marginale* with DNA template isolated from suspected cases using QIAGEN®DNeasy® blood & tissue kit (Kumar *et al.,* 2015c). Data pertaining to incidence and associated risk factors were analyzed on IBM SPSS statistical software version 20.0 using chi-square test (at 5% level and confidence interval at 95% level) as per method described by Snedecor and Cochran (1990). Considering PCR as a gold standard test, sensitivity, specificity and accuracy of GSTBS and cELISA were calculated as per formula given by Samad *et al.* (1994).

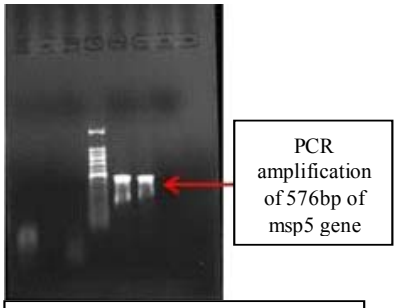
Plate-1 : Various tests used for diagnosis of A.marginale infection in buffaloes



***A. marginale* organism on blood smear stained by Giemsa stain**



Result of cELISA indicating presence of anaplasma antibody reaction



PCR amplification of msp5 DNA sequence of *A. marginale*

Results and Discussion

Over a period of one year, total 48 suspected cases of anaplasmosis in buffaloes were studied and subjected to different diagnostic tests i.e. GSTBS, cELISA and PCR. Based on results of confirmatory test (PCR), the overall incidence of anaplasmosis in buffaloes was 37.50 % (18/48). The finding of present study was in accordance to previous reports of Abou-Elnaga *et al.* (2009) and Sharma *et al.* (2015). During present study, comparatively higher incidence (63.63%) was observed in field cases followed by TVCC (60.00%) and LRS (25.00%). The season wise incidence was 43.75, 39.13 and 22.22% in winter, summer and monsoon, respectively. The anaplasmosis infection in winter in the absence of vector ticks suggests mechanical transmission of disease (Atif *et al.,* 2012). Jassem and Agaar (2015) stated that significant variation in prevalence of anaplasmosis based on area and season might be due to difference in agro-climatic condition and intensity of vector population. During the study, comparative higher incidence in buffaloes aged above 3 years (40.47%) was observed than buffaloes aged below 3 years (16.66%). The higher incidence in adult may be attributed to production and reproduction stress that lead to weak immune system (Sajid *et al.,* 2014). The overall differences

Table-1: Incidence and risk factor's analysis of anaplasmosis in buffaloes

Sr. No.	Risk factors	Particular	No. of suspected cases	No. of positive cases	Incidence (%)	P value
1	**Places**	**LRS**	32	08	25.00	**00.09**
		TVCC	05	03	60.00	
		Field	11	07	63.63	
		Total	**48**	**18**	**37.50**	
2	**Season**	**Winter**	16	07	43.75	**00.55**
		Summer	23	09	39.13	
		Monsoon	09	02	22.22	
		Total	**48**	**18**	**37.50**	
3	**Age**	**>3 Years**	42	17	40.47	**00.26**
		<3 Years	06	01	16.66	
		Total	**48**	**18**	**37.50**	

Figure-1: Frequency distribution of clinical symptoms in anaplasmosis infected buffaloes

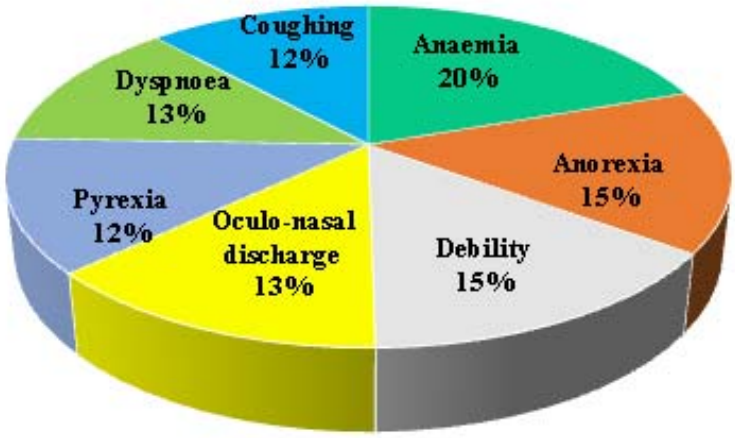


Table-2: Sensitivity, specificity and accuracy of Giemsa stained thin blood smear and cELISA

Sr. No.	Test	Result	PCR		Total	Sensitivity (%)	Specificity (%)	Accuracy (%)
			Positive	Negative				
1	**GSTBS**	Positive	08	0	08	**44.44**	**100**	**79.16**
		Negative	10	30	40			
		Sub-total	18	30	48			
2	**cELISA**	Positive	18	0	18	**100**	**100**	**100**
		Negative	0	30	30			
		Sub-total	18	30	48			

in incidence of anaplasmosis due to different risk factors (place, season and age) were non-significant (Table-1).

In present study, the most frequent symptom observed was anaemia followed by anorexia, debility, oculo-nasal discharge, pyrexia, dyspnoea and coughing (Figure-1). These findings were in accordance with observations recorded by Vahora *et al. (*2012); Ashuma *et al. (*2013) and Kumar *et al.* (2015a & b). Eventhough, clinical findings should be correlated with laboratory diagnosis (El-Ashker *et al.,* 2015).

The sensitivity, specificity and accuracy of GSTBS were 44.44, 100 and 79.16 (%), respectively. Whereas,the corresponding figures for cELISA was cent percent (Table-2). The finding on comparative superiority of cELISA over GSTBS for diagnosis of anaplasmosis was in agreement to the previous reports (Birdane *et al.,* 2006 and Sharma *et al*., 2015). Earlier, Noaman *et al.* (2009) stated that the traditional Giemsa stain method is not much applicable for determination of chronic or persistent anaplasmosis in cattle. Therefore, cELISA has been reported as most reliable test for screening of persistent infection based on its sensitivity, rapidity and repeatability of results and relatively low cost and ease of standardization (Al-Gharban and Dhahir, 2015). Meanwhile, Reinbold *et al*. (2010) reported PCR as more accurate and precise diagnostic tool for detection of *A. marginale* infection as compared to cELISA. They also stated that PCR has ability to diagnose *A. marginale* infection in a blood with minimum infective unit of one *A. marginale* organism. As a result, PCR has been suggested as most accurate and sensitive method for diagnosis of anaplasmosis in many reports (Jassem and Agaar, 2015; Sharma *et al*., 2015; Patel *et al*., 2017).

The results of present study indicated fairly presence of anaplasmosis in buffaloes in and around Navsari, Gujarat but difficult to diagnose with routine smear examination technique. Therefore, use of advanced diagnostic technique like PCR/ELISA is advocated for confirmatory diagnosis.

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Conflict of Interest:

All authors declare no conflict of interest.

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