Journal of Animal Research: v.12 n.1, p. 69-74. February 2022

DOI: 10.30954/2277-940X.01.2022.10

Prevalence Rate of Haemoprotozoan Infection and Assessment of Associated Risk Factors in Dairy Animals from Bikaner Region of Rajasthan, India

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Received: 11 Jan., 2022 **Revised:** 29 Jan., 2022 **Accepted:** 31 Jan., 2022

ABSTRACT

Haemoprotozoan diseases have deleterious impact on health and production of animals causing death in acute cases, production losses in chronically affected animals which decrease economic share of livestock sector. Thus with aim to record prevalence rate of haemoprotozoan infection in diary animals from Bikaner region of Rajasthan (India) present study was conducted from January to September 2021 on 200 (117 cattle and 83 Buffalo) dairy animals. Conventional microscopy method of Giemsa's stained blood smears was used for screening blood samples for haemoprotozoans. Out of total screened 24(20.5%) cattle and 13(15.7%) buffalo were found positive for haemoprotozoans with highly prevalent *Theileria* spp. 12.5% (25) followed by *Anaplasma* spp. 3.0% (6), *Babesia* spp. and mixed infection 1.5% (3) each. Age wise highest prevalence showed by below 6 months aged (22.3%) followed by > 2 years (20.9%) and 6 months to 2 years (19.7%) in cattle and below 6 months (23.1%) followed by 6 months to 2 years (16.7%) and > 2 years (10.7%) in buffalo. Breed wise crossbred cattle showed highest infection rate of 23.5% with female more infected in both species 24.1% (20/83) in cattle and 18.7% (11/59) buffalo and highest prevalence rate in monsoon season in both cattle(28.4%) and buffalo(24%).

HIGHLIGHTS

- Crossbred cattle and female animals are at higher risk of getting disease with more infection in monsoon season.
- Earlier diagnosis of carrier animals is important to prevent losses

Keywords: Bikaner, haemoprotozoan, prevalence, dairy animals

Dairy animals are backbone of animal husbandry sector. Rajasthan state has agricultural economy and rank 2nd in milk production with production of 23.67 million tonnes milk (NDDB, 2018-2019). It has total cattle and buffalo population of 13.9 and 13.7 million respectively (DADHF, 2019). Parasitic diseases that occur as subclinical infection affect health and vigour will ultimately cause production losses. Haemoprotozoans are parasitic protozoans belonging to phylum Apicomplexa, that multiply in blood cells and affect haemato-biochemical characteristics of host. All vector borne diseases cause annual economic loss of US$18.7 billion globally, whereas in India it is US$ 498.7 million/annum (Ghosh and Nagar, 2014). An estimated economic loss due to tropical theileriosis alone is US$ 1560.5 million/annum in India (Narladkar, 2018) while babesiosis and anaplasmosis cause loss of about US$ 57 million (Anwar, 2018). Genetically improved cattle are at higher risk of getting infection and chances of survivability in these animals will be less (Radostits *et al*., 1994). This may result in increased cost of control measures (Makala *et al*., 2003). All over world ticks are major transmitter of these infectious protozoans of both humans and animals viz., *Babesia* spp., *Theileria*

How to cite this article: Bharti, V., Pilania, P.K., Choudhary, P. and Joshi, S.P. (2022). Prevalence Rate of Haemoprotozoan Infection and Assessment of Associated Risk Factors in Dairy Animals from Bikaner Region of Rajasthan, India. *J. Anim. Res.*, **12**(01): 69-74.

Source of Support: None; **Conflict of Interest:** None

spp., *Anaplasma* spp. and many more that hamper livestock production (Norval *et al*., 1984). For growth and propogation of these vectors hot and humid climate is favourable (Krishnamurthy *et al*., 2016) commonly found in tropical and subtropical regions thus tick borne infections are more common in these regions (Basu & Charles, 2017) so countries like India, China Iran and Turkey are at high risk of these diseases (Razmi *et al*., 2003). Clinical signs associated with haemoprotozoan infections include fever, enlargement of lymph nodes, nasal and ocular discharge (El - Deeb and Younis, 2009) haemoglobinuria (Yeruham *et al*., 2003) progressive haemolytic anaemia, fever, jaundice, decreased milk production, abortions, hyper excitability and sudden death in some cases (Richey and Palmer., 1990; Sharma *et al*., 2013). Conventional diagnostic methods for most of the infections rely upon microscopical demonstration of infective stages of different parasites in blood or tissue fluids (Terkawi *et al*., 2011). A record of prevalence rate is important to take appropriate control measures for these diseases as they continue to persist as subclinical infection in animal body and decrease their production and reproduction efficiency. So above study was designed to provide data regarding prevalence of these diseases in studied animals.

MATERIAL AND METHOD

Present study was conducted for period of 9 months from January to September 2021. Samples were collected in all seasons (winter, summer and rainy) to determine the prevalence of blood protozoans in dairy animals of Bikaner region of Rajasthan.

Study area

Study was conducted in Bikaner city located in northwest region of Rajasthan state which is located at 27.0238° North latitude, 74.2179° East longitude and 309 m above the sea level on the western side of the country.

Collection of samples

A total of 200 samples (including 117 cattle and 83 buffalo) were collected seasonally for a period of 9 months from January to September 2021 from dairy animals in Bikaner region of Rajasthan. Sample collection was done on basis of clinical signs including discoloration of mucus membrane, fever, nasal discharge, haemoglobinuria, swollen lymph nodes, laboured breathing, decreased milk production, progressive emaciation and debility. From juglar vein of suspected animals 2 ml of blood collected aseptically in EDTA vacutainers and transported in a cool transport box Post graduate laboratory of Department of Veterinary Parasitology, CVAS, Bikaner, for further examination.

Examination of blood smears

Blood smears are stained using Giemsa staining technique as described by Soulsby (1982). Blood smears were prepared, air dried and then fixed in methanol for 3-4 min so as to allow methanol to evaporate completely. Slides were then placed on the staining rack and working Giemsa's stain solution (1ml Giemsa stock solution + 9ml distilled water having pH 6.8–7.2) was added. Stain was allowed to act for 30-40 min. Stained smears were then rinsed with distilled water and air dried at room temperature. Completely dried smears were then subjected to microscopic examination under 100x oil immersion objective using compound microscope. Identification of haemoprotozoans was done by characters as described by Soulsby (1982).

STATISTICAL ANALYSIS

Statistical analysis was performed by using SPSS 20.0 software by applying Chi Square (χ^2) test and level of significant difference was at 95% confidence interval at p value ≤ 0.05.

Ethical standards

"The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant institutional guides on the care and use of dairy animals."

RESULTS AND DISCUSSION

Overall prevalence

Out of 200 samples screened 37 samples were found positive for haemoprotozoans infection indicating overall prevalence rate of 18.5%. These results of are in harmony

with those of Prameela *et al*. (2020) and Muniraja *et al*. (2021). Higher prevalence rates were Hosen *et al*. (2020) and Subapriya *et al.* (2021) while lower values for prevalence rate are reported by Bhatnagar *et al*. (2015) and Khillare *et al*. (2019).

Animal species wise prevalence

Infection rate of 20.5% and 15.7% in cattle and buffalo, respectively. On animals wise study it was concluded that cattle have higher infection rate as compared to buffalo. It can also deduced from past studies of Chaudhri *et al*. (2013), Muniraja *et al*. (2021). Variations in results of prevalence rate can be due to various reasons including animal species included for study, prevalence of tick vectors in studied area, breeds of animal selected for study, climatic conditions of area during study period.

Haemoprotozoan species wise prevalence

Blood protozoan species wise analysis showed that prevalence of *Theileria* spp. was highest (13.7%) in cattle followed by *Anaplasma* spp. (4.3%), mixed infection (1.7%) and *Babesia* spp. (0.9%) (Table 1). In Buffalo *Theileria* spp. (10.8%) was highly prevalent followed by *Babesia* spp. (2.4%) while *Anaplasma* spp. and mixed infection have equal prevalence rate of 1.2% each (Table 2) (Fig. 1). Haemoprotozoan wise highest prevalence of *Theileria* spp. could be due to higher number of vectors of this infection in area of study and less resistance of animals against disease. These results corresponds those of Prameela *et al*. (2020), Shah *et al*. (2020), Khatoon *et al*. (2021). Variation from above results was obtained by Muniraja *et al*. (2021) and Subapriya *et al.* (2021).

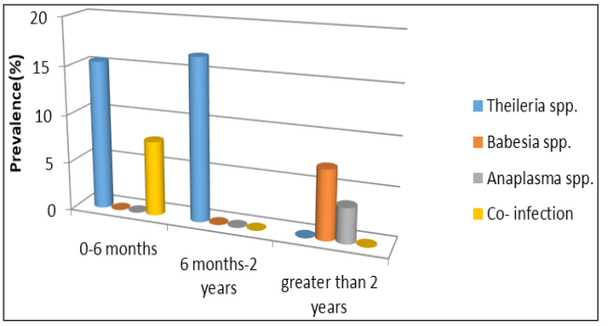

Fig. 1: Haemoprotozoan species wise prevalence

Age wise prevalence

Age wise a non significant ($p>0.05$) difference was observed in prevalence of haemoprotozoan diseases in cattle. Highest prevalence was observed in young cattle calves of age 0-6 months (22.3%) followed by above 2 years (20.9%) and 6 months to 2 years (19.7%) (Table 1). In buffalo significant ($p<0.05$) difference was observed in prevalence rate with highest prevalence in 0-6 months (23.1%) followed by 6 months to 2 years (16.7%) and

Table 1: Effect of various risk factors on blood protozoan infection in cattle in Bikaner region of Rajasthan

Parameters		Total samples	Samples negative	Samples positive	Percent positive	P value
Haemoprotozoan species	*Theileria* spp.	117	93	16	13.7%	0.585
	Babesia spp.			1	0.9%	
	Anaplasma spp.			5	4.3%	
	Mixed infection			2	1.7%	
Age	0-6 months	18	14	4	22.2%	0.218
	6 months -2 years	56	45	11	19.6%	
	>2 years	43	34	9	20.9%	
Sex	Male	34	30	4	11.8%	0.424
	Female	83	63	20	24.1%	
Breed	Crossbred	68	52	16	23.5%	0.853
	Indigenous	37	30	7	18.9%	
	Non-descript	12	11	1	8.3%	
Season	Winter	27	22	5	18.5%	0.303
	Summer	37	33	4	10.8%	
	Monsoon	53	38	15	28.3%	

above 2 years (10.7%) (Table 2). In cattle *Theleria* spp. have highest infection rate in 0-6 months (16.7%) and 6 months to 2 years (17.9%) however in adult animals above 2 years age *Anaplasma* spp. have highest prevalence 9.3%. *Babesia* spp. is found only in adult animals with prevalence rate of 2.3% in cattle and 7.1% in buffalo (Fig. 2). Mixed infection found in 0-6 months (5.6% in cattle and 7.7% in Buffalo) and above 2 years age group in cattle (2.3%). Age wise analysis revealed animals of age 0-6 months in both cattle and buffalo have more infection in comparison to other 2 age groups. *Theileria* spp. have highest infection rate in this age group less immunological resistance of young animals to theileriosis. These results are in accordance to those reported by Khawale *et al*. (2019) and Paul *et al.* (2016). Contrarily higher prevalence in adult animals is reported by Shah *et al*. (2020) from, Muniraja *et al*. (2021) and Khatoon *et al*. (2021).

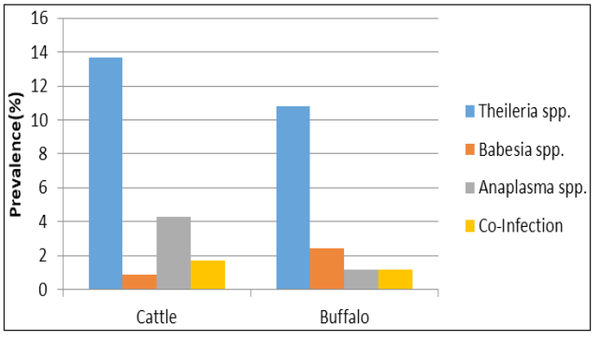

Fig. 2: Age wise prevalence (Buffalo)

Sex wise prevalence

Gender wise female have showed prevalence rate of 24.1% (20/83) in cattle (Table 1) and 18.7% (11/59) in buffalo (Table 2). In male animals *Theleria* spp. is only blood protozoan found which was 11.8% and 8.3% prevalent in cattle and buffalo, respectively. Sex wise analysis of data revealed that female were more infected than male animal. Gender is also a predisposing factor for diseases as female animals have to undergo more production and reproduction stress thus lowering their immunological resistance to diseases and these results are supported by studies of Shah *et al*. (2020), Khatoon *et al*. (2021) and Subapriya *et al.* (2021). On opposite higher infection rate in males is reported by Khawale *et al*. (2019 and Hosen *et al*. (2020).

Breed wise prevalence

Crossbred cattle showed highest prevalence rate 23.5% among all breed categories followed by indigenous breed (18.9%) while non-descript were least infected with infection rate of 8.3% (Table 1). Muniraja *et al*. (2021) and Subapriya *et al.* (2021) reported similar results. Contradictorily higher prevalence in indigenous and non - descript breeds was reported in studies of Hassan *et al*. (2019) and Malyar and Farid (2019).

Season wise prevalence

Study revealed that in cattle highest infection rate was in

Table 2: Effect of various risk factors on blood protozoan infection in Buffalo in Bikaner region of Rajasthan

Parameters		Total samples	Samples negative	Samples positive	Percent positive	P value
Haemoprotozoan species	*Theileria* spp.	83	70	9	10.8%	0.585
	Babesia spp.			2	2.4%	
	Anaplasma spp.			1	1.2%	
	Mixed infection			1	1.2%	
Age	0-6 months	13	10	3	23.1%	*0.042
	6 months -2 years	42	35	7	16.6%	
	>2 years	28	25	3	10.7%	
Sex	Male	24	22	2	8.33%	0.729
	Female	59	48	11	18.6%	
Season	Winter	16	15	1	6.2%	0.678
	Summer	38	33	5	13.2%	
	Monsoon	29	22	7	24.1%	

*- $p < 0.05$ (Significant).

monsoon season 28.4%, followed by winter 18.5% and summer 10.8% (Table 1). In buffalo order of prevalence was monsoon season 24% followed by summer 13.1% and lowest infection rate in winter 6.2% (Table 2). Highest prevalence in monsoon could be due to more conducive environment for proliferation of tick vectors in this season that help in transmission of haemoprotozoans season and it is supported by studies of Khatoon *et al*. (2021) and Subapriya *et al.* (2021) whereas higher infection rate in other seasons reported by Muniraja *et al*. (2021) and Sharma *et al.* (2021).

CONCLUSION

Haemoprotozons are major threat to animals especially animals especially animals reared for production purpose because they not only pose threat to health of animal but also cause production and reproduction losses. As the results of study showed that crossbred cattle and female animals are at higher risk of getting disease with more infection in monsoon season. So earlier diagnosis of carrier animals is important to prevent losses. Above results can be used to disease forecast on basis of seasonality, breed and to take proper measures to control vector population.

ACKNOWLEDGEMENTS

The authors thankfully acknowledge the financial support and facilities provided by RAJUVAS, Bikaner to carry out the research work.

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