RESEARCH ARTICLE

Epidemiology of Haemoparasites Causing Infections in Cattle of Junagadh, Gujarat, India

Bhupendrakumar J. Thakre[1]*, Vivek Kumar Singh[2], Nilima N. Brahmbhatt[1], Krishna C. Gamit[3], Vijay L. Parmar[4], Avinash K. Bilwal[4], Sohil H. Kachara[1]

Abstract

The current investigation used microscopy to determine the incidence and epidemiology of key haemoprotozoans, including *Theileria, Babesia, Trypanosoma*, and rickettsia *Anaplasma* in cattle from the Junagadh region of Western Gujarat, India. Whole blood samples (948) from cattle were obtained between January 2023 and May 2024 at several places, including the Veterinary Clinical Complex, Gaushala, Pashumela, Panjarapole, and farmers' homes. According to microscopic examination of samples, 22.46% (213/948) of the population had haemoparasitic infections. In particular, the prevalence rates for mixed infections, *Babesia* spp., *Trypanosoma* spp., *Anaplasma* spp. and *Theileria* spp. were 2.00%, 2.42%, 5.69%, 1.26%, and 11.07%, respectively. Cattle above the age of 4 years had the highest frequency of haemoparasitic infections (35.37%), followed by cattle between the ages of 1-4 years (16.50%) and < 1 year (10.89%). Male animals (17.24%) and female animals (24.45%) showed a significantly different prevalence. Compared to cattle from organised farms (11.91%), the prevalence of infection was much greater in unorganised farms (28.96%) due to poor flooring, fractured walls, and poor sanitation. The infection rate of crossbred animals was greater (33.89%) than that of the Gir breed (13.53%), indicating that crossbred animals are more vulnerable than native breeds. The summer (31.01%) had the highest frequency of infection, followed by the monsoon (16.55%) and winter (16.63%) seasons. The results of this study highlight the importance of haemoparasites to cattle health in this area.

Key words: Cattle, Epidemiology, Junagadh, Prevalence, VCC, Western Gujarat.

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.6.11

Introduction

India is a country that relies heavily on agriculture, and the estimated losses caused by tick-borne diseases (TBDs) to cattle are 8.7 million USD mostly affecting small and marginal farmers. In tropical and subtropical regions, babesiosis, species *Babesia bovis* and *Babesia bigemina*, is regarded as a significant economic haemoprotozoan disease (Jayalakshmi *et al*., 2019; Pradeep *et al*., 2019). Major tick-borne illnesses affecting cattle in tropical and subtropical areas include haemoprotozoan diseases such theileriosis, babesiosis, and anaplasmosis (Velusamy *et al.,* 2014; Mahmoud *et al.,* 2024). Trypanosomosis is another serious illness that is primarily spread by the bites of haematophagous flies. Significant morbidity and death are brought on by these illnesses in cattle. Animals raised for dairy products, particularly bovines suffering from illnesses and production stress, are especially vulnerable to these infections (Sharma *et al*., 2013). These areas' hot, humid climates favour the growth and survival of vectors like flies and ticks, which puts vulnerable animals at constant risk of infection (Ezenwaka *et al.,* 2024). In addition to being commercially important in Asia, haemoprotozoan parasites constitute a major threat to the survival of exotic and crossbred cattle in India. The majority of these parasites induce erythrophagocytosis, which results in anaemia. Aside from reduced milk yield, weight loss, pyrexia, and anaemia, infections cause livestock owners to suffer significant financial losses. There are losses in meat and milk production as a result of the protracted convalescence or death of several affected animals. There have been reports of haemoprotozoan infections in a number of Indian states (Maharana *et al* 2016; Mahmoud *et al.,* 2024).

[1]Department of Veterinary Parasitology, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh-362 001, Gujarat, India

[2]Department of Physiology and Biochemistry, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh-362 001, Gujarat, India

[3]Department of Livestock Production Management, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh-362 001, Gujarat, India

[4]Department of Veterinary Medicine, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh-362 001, Gujarat, India

Corresponding Author: Bhupendrakumar J. Thakre, Department of Veterinary Parasitology, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Junagadh-362 001, Gujarat, India. e-mail: drbhupendrakumarthakre@gmail.com

How to cite this article: Thakre, B. J., Singh, V. K., Brahmbhatt, N. N., Gamit, K. C., Parmar, V. L., Bilwal, A. K., & Kachara, S. H. (2024). Epidemiology of Haemoparasites Causing Infections in Cattle of Junagadh, Gujarat, India. Ind J Vet Sci and Biotech. 20(6), 58-63.

Source of support: Nil

Conflict of interest: None.

Submitted: 20/07/2024 **Accepted** 29/08/2024 **Published** 10/11/2024

Local climate and management techniques impact the prevalence of haemoparasite infections in cattle. Precise mapping of these parasites over various agro-climatic zones is essential for efficient control. It is unclear, therefore, how common haemoparasitic diseases are in cows in Junagadh, Gujarat (Maharana *et al.,* 2016; Kaur *et al*., 2021). Conventional microscopy, which has drawbacks such low sensitivity, the inability to distinguish between species, and the misreading of cryptic cases and carrier states, is frequently used in the region to diagnose tick-borne illnesses (TBDs) (Subapriya *et al*.. 2021; Chamuah *et al*., 2023). The purpose of this study was to determine the risk variables linked to the disease and to give baseline data on the incidence of haemoprotozoan and rickettsial infections and the risk factors linked to these diseases in cattle in the Junagadh region of Western Gujarat, India,

Materials and Methods

Sample Collection and Processing

In the Saurashtra region of Gujarat, India, blood samples of 948 cows were gathered at random between January 2023 and May 2024 from various locations, including the Veterinary Clinical Complex, Panjrapole, Gaushala, Pashumela, and farmer houses. Interviews with owners and farmers provided information on animal attributes (age, sex, breed), as well as husbandry practices followed. The animals were divided into three age groups: calves (< 1 year), young (1-4 years), and adults (> 4 years). The animals included both native Gir cattle (*Bos indicus*) and non-descript breeds or crossbreds (Holstein Friesian, Jersey X local Gir breed). The winter (November-February), summer (March-June), and rainy (July-October) seasons were all considered when analysing seasonal prevalence. Based on clinical signs such as fever, anorexia, lymph node enlargement, pale mucous membranes, and presence of tick, the prevalence of haemoparasitic illnesses was evaluated. The animal owners also verified the history of anorexia and the decline in milk yield. Taking into account the type of flooring, drainage, and ventilation of the shed, the housing system (organised or unorganised) was also taken into consideration as a variable for disease prevalence.

Microscopic Detection of Haemoparasites

Jugular vein blood samples were drawn from animals displaying general health problems such as fever, anaemia, and chronic starvation, and placed in EDTA-coated vacutainer tubes, which were then refrigerated and brought to the laboratory for parasitological analysis. Thin blood smears were made using conventional method. The slides were then allowed to air dry, fixed in methanol, and stained with 10% Giemsa for 30 min at pH 7.2. After that, slides were viewed using an Olympus Microscope (Germany) at × 100 oil immersion magnification in order to detect blood parasites based on their morphology (Maharana *et al*., 2016).

Statistical Analysis

The resulting data were combined, organised for frequency, and then transformed into a percentage. The SPSS 13.0 programme was utilised to analyse the data statistically. The t-test was applied, and statistical differences were deemed significant when $p<0.05$.

Results And Discussion

Prevalence based on Microscopy

Overall prevalence of haemoparasites infection was found to be 22.46% (213/948) in the animals screened by microscopic examination. Out of 948 blood samples, 11.07% (105/948) of the population had *Theileria* spp., which were followed by 5.69% (54/948) *Anaplasma* spp., 2.42% (23/948) *Babesia* spp., 2.00% (19/948) mixed infection (19/948), and 1.26% (12/948) *T. evansi* (Fig. 1). In the present study, the prevalence of all haemoparasites were recorded mainly because of abundance of vector population, *i.e., Hyaloma anatolicum anatolicum, Rhipicephalus* (*Boophilus*) *microplus*, and *Tabanus* spp. (Maharana *et al*., 2016). The incidence of haemoprotozoan infections in present study was comparatively lower than previous reports of 29.31% (Maharana *et al*., 2016; Kumar *et al.,* 2016) and 58.55% (Kumar *et al.,* 2018), whereas, lower incidence (<20%) than present was also reported by Velusamy *et al*. (2014).

Cattle have been found to have an extremely low prevalence of infections with *A. marginale* and *Babesia* spp. The low frequency of the vectors, *Boophilus microplus* and *Ixodid* ticks, or the scarcity of exotic and hybrid animals in this area could be the cause of the low incidence (Maharana *et al*., 2016). On the other hand, a greater incidence of *Theileria* spp. infections in cattle was noted in Punjab, where the majority of dairy animals are either exotic or crossbred, with reports of 14.65% and 15.38%, respectively (Singh *et al*., 2012). In Punjab, *H. anatolicum* is the predominant tick species. According to Ezenaka *et al.* (2024), bovine anaplasmosis, which is caused by *A. marginale*, is a serious rickettsial disease that infects cattle both mechanically and biologically by biting flies and blood-contaminated fomites. It causes major economic losses in tropical and subtropical regions. However, according to Sumbria *et al*. (2024), the distribution and density of tick vectors and reservoir hosts determine the prevalence to a great extent. 2.42% of the animals in the current investigation tested positive for the inclusion bodies of *A. marginale*. Anaplasmosis in cows has been found to have varying incidence rates throughout country, with reports ranging from 0.45% to 9.09% (Ezenwaka *et al*., 2024).

It is commonly known that the occurrence of vector-borne illnesses rises with increase in the population of vectors, which is impacted by weather factors like humidity and temperature (Mahmoud *et al.,* 2024), as was seen in this investigation. According to the data, infections are very low to nonexistent in the winter and spring when both temperature and humidity are low, and quite high during the summer, rainy, and fall seasons when temperature and humidity are high.

Clinical Examination in Haemoparasite Infected Cattle (n=98)

The clinical vitals were recorded in 98 animals found to be typically infected with haemoarasites through microscopy. Considerable variation was observed in the clinical signs of the infected animals. The frequency distribution of clinical signs observed in the present study is presented in Table 1. The majority of haemoparasites infected cattle showed important clinical signs/symptoms ranging from 26.46 to 100%, *viz.,* tick infestation, mild to moderate fever, lymph node enlargement, lack of appetites, pale mucous membranes, nasal discharge, lacrimation from the eyes, a drop in milk production, inappetence, emaciation, salivation, pale conjunctiva, congested mucous membrane, diarrhea, corneal opacity, haemoglobinurea, and jaundice (Table 1). Haemoglobinuria was noted in cases (23.46%) with babesiosis, and lymph node enlargement was observed in 94 cases (95.91%) with theileriosis. Tick infestation was noted in all 98 animals (100%) positive for haemoparasites, and 76 (77.55%) animals with decreased milk production. Of the 98 haemoparasites positive animals 95 exhibited fever and severe anaemia symptoms. Fever and inappetence are caused by endogenous pyrogens that are released into the bloodstream as a result of cellular lysis (Thakre *et al.,* 2023; Sumbria *et al.,* 2024). Erythrophagocytosis, which is the lysis of red blood cells resulting from parasite proliferation, and subsequent clearance by the reticuloendothelial system is the cause of anaemia (Modi *et al.,* 2015). The ongoing blood loss from tick feeding exacerbates the altered essential blood parameters, particularly reduced haemoglobin levels (Debbarma *et al.,* 2017; Thakre *et al.*, 2023). A significant economic setback is the effect that rickettsial and haemoprotozoan illnesses have on milk output. Our findings are consistent with Aulakh *et al*. (2005) and Sharma *et al*. (2013) in that they showed variations in blood parameters after this cascade.

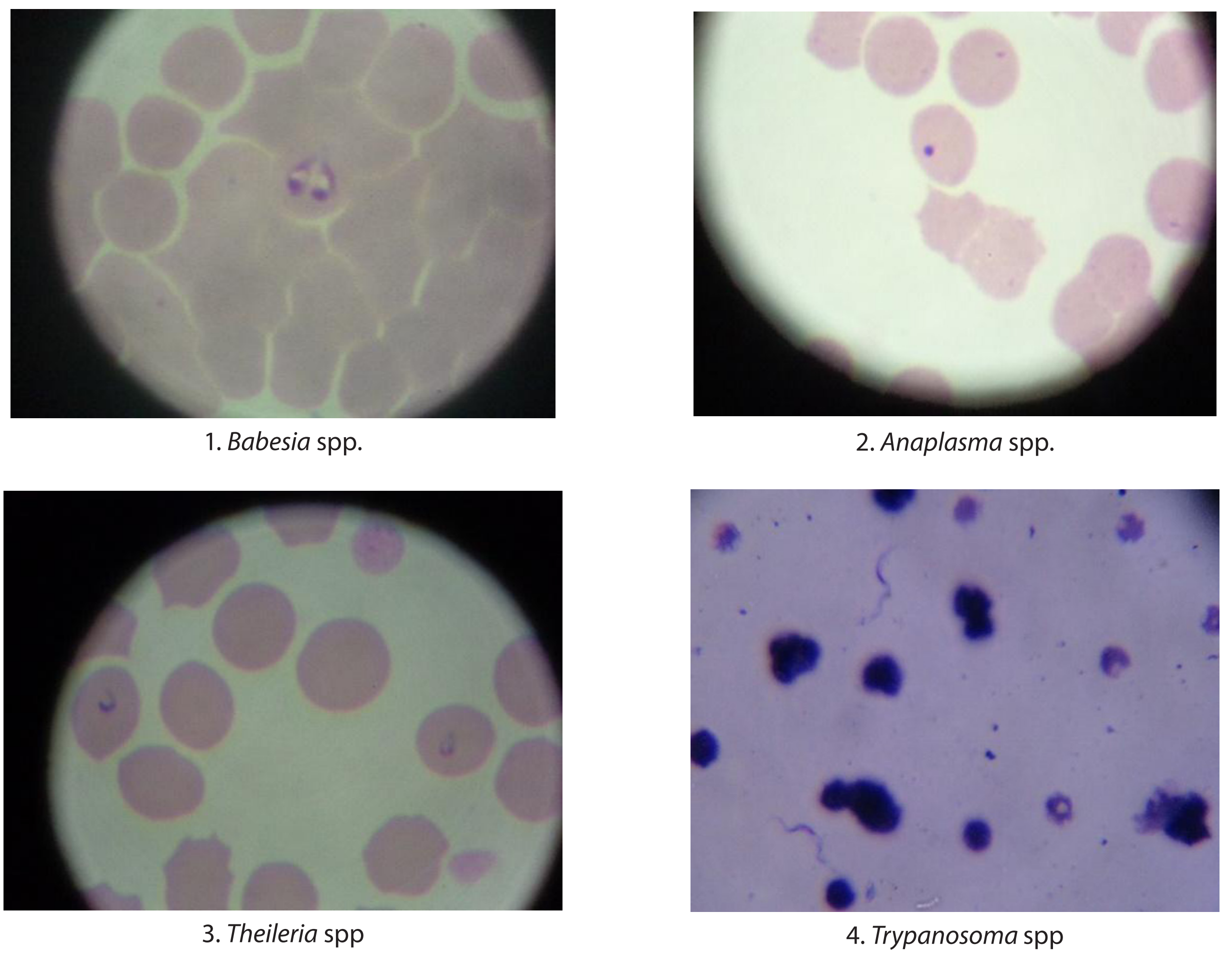

Fig. 1: Blood smears positive for: 1. *Babesia* piroplasms (100x). 2. *Anaplasma* spp. (100x). 3. *Theileria* piroplasms (100x), and 4. *Trypanosoma* spp. (100x).

Table. 1: Frequency distribution of clinical signs and symptoms observed in descending order in haemoparasites infected cattle (n=98)

Clinical signs	No. of animals	Percentage
Ticks infestation	98	100.00
Fever	95	96.93
Lymphnode enlargement	94	95.91
Lack of appetites	91	92.85
Pale mucus membrane	79	80.61
Nasal discharge	78	79.59
Drop milk Production	76	77.55
Inappetence	75	76.53
Emaciation	69	70.40
Lacrimation of eye	62	63.26
Coughing	58	59.18
Salivation	55	56.12
Pale conjunctiva	52	53.06
Congested mucus membrane	43	43.87
Diarrhoea	43	43.87
Corneal opacity	35	35.71
Haemoglobinurea	23	23.46
Jaundice	23	23.46

Assessment of Risk and Impact of Environmental Variables

The impacts of age, season, breed, sex, and kind of farm were modelled in order to assess risk variables for illness occurrence in this study. The findings are presented in Table 2. Season and host age had a substantial impact on the occurrence of the disease; animals > 4 years (35.37%) had the highest prevalence of haemoparasitic infection, followed by those 1-4 years (16.50%) and < 1 year (10.89%) old. Summer had the largest occurrence of infections (31.01%), followed by the monsoon (16.55%) and winter (16.63%). The prevalence in male animals (17.24%) was considerably lower than in female animals (24.45%). Compared to organised farms (11.91%), unorganised farms (without suitable flooring, fractured walls, and inadequate sanitation) had considerably higher infection prevalence (28.96%). The infection rate in ND/crossbred animals was greater (33.89%) than in the Gir breed (13.53%), indicating that crossbreds are more susceptible than native breed.

Varying incidences of babesiosis have been reported in India, ranging from 0.76% to 18.50% (Muraleedharan *et al*., 2005; Singh *et al*., 2012; Sharma *et al*., 2013). According to Eriks *et al*. (1993), *Anaplasma* is linked to a long-term or lifetime carrier status, which increases the possibility of finding positive animals, particularly in endemic instability areas (Sumbria *et al*., 2024). Tick infestation, fever, and lymph node enlargement were all present in animals that tested positive for theileriosis; *H. anatolicum* and *T. annulata* were particularly common (Singh *et al.,* 2012). The Junagadh region has a noticeably high prevalence of theileriosis despite the vector tick *H. anatolicum's* restricted presence (Maharana *et al*., 2016). Due to medication resistance and previous treatment in the field, which makes identification

Table. 2: Risk factor associated with prevalence of haemoparasites among cattle in Saurashtra region of Gujarat, according to different variables

Risk factors	Variable	Samples	*Theileria*	*Babesia*	*Anaplasma*	*Tryps*	Mix infect	Total
		No. of animals positive by Microscopy (n=948)						
Farm Management	Organized	361	21 (5.81)	4 (1.10)	12 (3.32)	2 (0.55)	4 (1.10)	43 (11.91)
	unorganized	587	84 (14.31)	19 (3.23)	42 (7.15)	10 (1.70)	15 (2.55)	170 (28.96)
Age	< 1 year	257	12 (4.66)	3 (1.16)	8 (3.11)	2 (0.77)	3 (1.16)	28 (10.89)
	1-4 years	315	21 (6.66)	6 (1.90)	16 (5.07)	4 (1.26)	5 (1.58)	52 (16.50)
	>4 year	376	72 (19.14)	14 (3.72)	30 (7.97)	6 (1.59)	11 (2.92)	133 (35.37)
Season	Winter	243	17 (6.99)	2 (0.82)	13 (5.34)	2 (0.82)	4 (1.64)	38 (15.63)
	Summer	403	62 (15.38)	16 (3.97)	29 (7.19)	7 (1.73)	11 (2.72)	125 (31.01)
	Monsoon	302	26 (8.60)	5 (1.65)	12 (3.97)	3 (0.99)	4 (1.32)	50 (16.55)
Breed	Gir	532	35 (7.14)	8 (1.50)	20 (3.75)	3 (0.56)	6 (1.12)	72 (13.53)
	ND/CB cattle	416	70 (16.82)	15 (3.60)	34 (8.17)	9 (2.16)	13 (3.12)	141 (33.89)
Sex	Male	261	19 (7.27)	4 (1.53)	11 (4.21)	3 (1.14)	8 (3.06)	45 (17.24)
	Female	687	86 (12.51)	19 (2.76)	43 (6.25)	9 (1.31)	11 (1.60)	168 (24.45)
	Overall	**948**	**105 (11.07)**	**23 (2.42)**	**54 (5.69)**	**12 (1.26)**	**19 (2.00)**	**213 (22.46)**

Figures in parentheses indicate percent values.

challenging under the microscope, this may be the reason for fewer instances identified by microscopy in our study than molecular approaches reported by others.

According to the study, a number of epidemiological characteristics make the summer and the months after a wet season more probable for theileriosis outbreaks to arise. Environmental elements including the summer temperature, humidity, and microclimate of grazing areas promote the growth and development of ticks and flies. Previous results demonstrating prevalence rates of 29.31% (Maharana *et al*., 2016) and 58.55% (Kumar *et al.,* 2018) suggest that similar trends are probably present in other regions of the nation. Tick-borne diseases are more common in unorganised farms as compared to organised ones, with the former having greater prevalence rates due to the vector population. The Kuchha sheds/buildings found on these farms are either cemented or made entirely of bricks, and they include gaps, fractures, poor ventilation, and inadequate drainage, all of which are conducive to the survival and reproduction of vector ticks. Moreover, the proprietors of these disorganised farms frequently have lower educational backgrounds, which make them ignorant of the proper usage of acaricides and long-term strategies for managing tick populations. The age and breed of the animals are two factors that affect the likelihood of sickness. According to Soulsby (2005), inverse age resistance protects younger animals from the spread of clinical illness. According to Kumar *et al.* (2018), animals older than 4 years old had the highest prevalence (35.37%). Furthermore, mature cattle are subject to a number of stimuli that can worsen the pathophysiology of disease, including heat stress, production demands, immunisations, and reproductive status. ND/crossbred animals had a greater rate of infection (33.89%) than Gir breed (13.53%) in our study, which is in line with previous reports (Kumar *et al.,* 2016; Kumar *et al*., 2018). According to Glass *et al*. (2012), some studies link this vulnerability to variations in immunological responses, namely the generation of pro-inflammatory cytokines, which are more prevalent in exotic animals or native breeds carrying genetic loci for increased tolerance.

Conclusions

The study provided a holistic picture about the prevalence of haemoparasites infections in cattle and its correlation with the environmental variables and epidemiological determinants in the Junagadh region of Gujarat. The knowledge of pathogens in a particular region is very much essential for the formulation of effective control strategies to prevent these types of infections. The current work emphasized that *Theileria* spp, *Babesia* spp, *Anaplasma* spp and *Trypanosoma* spp. were the most prevalent haemoprotozoa in cattle of Junagadh region. Therefore, early diagnosis and strategic control and management programme should be adopted to reduce occurrence of haemoparasites and associated economic losses in cattle.

Acknowledgments

The authors are highly thankful to Director of Research and Dean P.G. studies, KU, Gandhinagar, and Principal, College of Veterinary Science & AH, KU, Junagadh for providing the necessary facilities.

References

Aulakh, G.S., Singla, L.D., Kaur, P., & Alka, A. (2005). Bovine babesiosis due to *babesia bigemina:* Haemato-biochemical and therapeutic studies. *The Indian Journal of Animal Sciences*, *75*(6), 617-622.

Chamuah, J. K., Awomi, L. T., Mollier, R. T., Singh, M., Aier, I., & Perumal, P. (2023). Haemoparasites of Domestic Bovines and their Diagnostic Pattern: A Review. *Biotica Research Today*, *5*(8), 600-605.

Debbarma, A., Pandit, S., Jas, R., Baidya, S., Mandal, S. C., Ralte, L. & Shit, N. (2017). Haematological impact of naturally occurring tick borne haemoparasitic infections in cattle of West Bengal, India. *Exploratory Animal & Medical Research, 7*, 175-178.

Eriks, I. S., Stiller, D., & Palmer, G. H. (1993). Impact of persistent *Anaplasma marginale* rickettsemia on tick infection and transmission. *Journal of Clinical Microbiology*, *31*(8), 2091-2096.

Ezenwaka, C.O., Udi, A., & Nzeako, S.O. (2024). Occurrence of haemoparasites in cattle from three selected abattoirs in Port Harcourt Metropolis, Rivers State, Nigeria. *Scientia Africana*, *23*(1), 189-196.

Glass, E.J., Crutchley, S., & Jensen, K. (2012). Living with the enemy or uninvited guests: functional genomics approaches to investigating host resistance or tolerance traits to a protozoan parasite, *Theileria annulata*, in cattle. *Veterinary Immunology and Immunopathology*, *148*(1-2), 178-189.

Jayalakshmi, K., Sasikala, M., Veeraselvam, M., Venkatesan, M., Yogeshpriya, S., Ramkumar, P.K. & Vijayasarathi, M.K. (2019). Prevalence of haemoprotozoan diseases in cattle of Cauvery delta region of Tamil Nadu. *Journal of Parasitic Diseases*, *43*, 308-312.

Kaur, R., Yadav, A., Rafiqi, S.I., Godara, R., Sudan, V., Chakraborty, D., & Katoch, R. (2021). Epidemiology, haematology and molecular characterization of haemoprotozoon and rickettsial organisms causing infections in cattle of Jammu region, North India. *BMC Veterinary Research*, *17*(1), 219.

Kumar, B., Maharana, B.R., Prasad, A., Joseph, J.P., Patel, B., & Patel, J.S. (2016). Seasonal incidence of parasitic diseases in bovines of south western Gujarat (Junagadh), India. *Journal of Parasitic Diseases*, *40*, 1342-1346.

Kumar, B., Mondal, D.B., & Jithin, M.V. (2018). Prevalence of babesiosis in cattle in Patna region, India. *International Journal of Current Microbiology & Applied Science*, *7*, 5167-74.

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

Mahmoud, H.Y., Rady, A.A., & Tanaka, T. (2024). Molecular detection and characterization of *Theileria annulata, Babesia bovis,* and *Babesia bigemina* infecting cattle and buffalo in southern Egypt. *Parasite Epidemiology and Control*, *25*, e00340.

Modi, D.V., Bhadesiya, C.M., & Mandali, G.C. (2015). Hematobiochemical changes in crossbred cattle infected with *Theileria annulata* in Banaskantha district of Gujarat. *International Journal of Scientific Research*, *5*(1), 1-4.

Muraleedharan, K., Ziauddin, K.S., Hussain, P.M., Pattabyatappa, B., Mallikarjun, G.B., & Seshadri, S.J. (2005). Incidence of *Anaplasma* sp., *Babesia* sp. and *Trypanosoma* sp. in cattle of Karnataka. *Journal of Veterinary Parasitology*, *19*(2), 135-137.

Pradeep, R.K., Nimisha, M., Sruthi, M.K., Vidya, P., Amrutha, B.M., Kurbet, P.S. & Ravindran, R. (2019). Molecular characterization of South Indian field isolates of bovine *Babesia* spp. and *Anaplasma* spp. *Parasitology Research*, *118*(2), 617-630.

Sharma, A., Singla, L.D., Tuli, A., Kaur, P., Batth, B.K., Javed, M., & Juyal, P.D. (2013). Molecular prevalence of *Babesia bigemina* and *Trypanosoma evansi* in dairy animals from Punjab, India, by duplex PCR: A step forward to the detection and management of concurrent latent infections. *BioMed Research International*, *1,* 893862.

Singh, N.K., Singh, H., Jyoti, Haque, M., & Rath, S.S. (2012). Prevalence of parasitic infections in cattle of Ludhiana district, Punjab. *Journal of Parasitic Diseases*, *36*, 256-259.

Soulsby, E.J.L. (2005). *Helminths, Arthopods and Protozoa of Domestic Animals*. 7th edn., Baillere Tindall, London, UK.

Subapriya, S., Senthil, N.R., Gowri, B., Chandrasekaran, D., Gopalakrishnan, A., Arunaman, C. S. & Vairamuthu, S. (2021). Prevalence of haemoprotozoal diseases in cattle: A review of 6000 cases. *Prevalence*, *904*, 325.

Sumbria, D., Sudan, V., & Kour, R. (2024). Prevalence of haemoparasites infection in diseased bovine and canine of Bhatinda district of Punjab, India. *Journal of Parasitic Diseases*, *48*(2), 301-307.

Thakre, B.J., Kumar, B., Vagh, A.A., Brahmbhatt, N.N. & Gamit K.C. (2023). Comparative studies on the detection of *Theileria annulata* infection by clinical, parasitological and molecular techniques in buffaloes (*Bubalus bubalis*). *Veterinarski Arhiv, 93*(6), 641-652.

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