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Prevalence of haemoprotozoal diseases in cattle: A review of 6000 cases

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Abstract
The present study was undertaken to study the prevalence of haemoprotozoans in cattle. A total of 6000 blood samples were examined for the presence of different haemoprotozoan parasites by Leishman-Giemsa staining, out of which 1899 cattle were found positive for either one or more haemoprotozoans accounting to a prevalence rate of 31.65%. *Anaplasma marginale* was found to be most predominant haemoprotozoan 1298(68.35%) followed by *Theileria annulata* 516(27.17%), *Babesia bigemina* 77(4.05%), *Trypanosoma evansi* 28(1.47% %), Microfilaria 12(0.63%), *Ehrlichia bovis* 8(0.42%) and *Babesia bovis* 4(0.21%). Mixed infection was observed in 44(2.31%) cattle. The highest number of cases were recorded in Jersey cross 1249(65.77%) followed by Holstein Friesian cross 321(16.90%) and indigenous cattle 329(17.32%). Of the cases recorded, 56(2.95%) were males and 1843(97.05%) were females. The highest prevalence of haemoprotozoans was observed in female cattle were 9.22% in cattle of 1st parity, 25.17% in 2nd parity, 27.54% in 3rd parity, 17.06% in 4th parity, 10.16% in 5th parity, 2.16% in aged cattle more than 5th parity and 5.74% in cattle aged less than 1 year. In males, the highest incidence was observed in cattle belonging to the age group of 1.95% in young male cattle less than one year, 0.47% in 1 to 3 years age group, 0,21% in 3-5 years and 0.16% each in 5-8 years and more than 8 years old male cattle. Season wise, highest prevalence was observed in monsoon season 791(41.65%), followed by summer 576(30.33%) and post monsoon 532(28.01%) season.

Keywords: Cattle, bovine, haemoprotozoans, *Anaplasma marginale*, *Babesia bovis*, *Babesia bigemina*, *Theileria annulata*, *Ehrlichia bovis*, *Trypanosoma evansi*, Microfilaria, incidence, prevalence

Introduction
Haemoprotozoan infections are a great menace leading to illness and mortality in farm animals mainly cattle, sheep and goat. Most of the haemoprotozoan parasites are tick borne and they have significant importance to the world animal health (Uilenberg, 1995) [1]. India being located in the tropics, the climatic condition is very conducive for the proliferation and survival of the tick vectors which propagate the transmission of haemoprotozoan diseases.

The major haemoprotozoans infecting cattle are *Anaplasma marginale*, *Babesia bovis*, *Babesia bigemina*, *Theileria annulata* and *Trypanosoma evansi*. Haemoprotozoan diseases are diagnosed based on clinical signs exhibited by the animals, blood smear and lymph node aspiration smear examination, serological and molecular tests (Julie *et al.* 2021) [2]. Cattle affected with haemoprotozoans exhibit clinical signs like anorexia, pyrexia, listlessness, anaemia, enlargement of lymph nodes etc. Bock *et al.* (2004) [3] also reported high grade fever, anaemia, hemoglobinuria, ataxia, and sometimes death in haemoprotozoon infection. Subclinical haemoparasitic infections which appear apparently normal are also of concern as they markedly impair the health and production performance of cattle as well as act as carriers (Julie *et al.* 2021) [4].

The ailing animals are an economic burden to the farmers due to poor returns on investment in terms of reduction in the yield of milk, meat, hide and impaired draft power for ploughing and other farming related practices, money spent to commute the animal for medical assistance, treatment costs etc. Heavy financial losses occurs due to mortality of cattle with severe parasitemia and when ailing cattle with haemoprotozoal infections succumb their lives by acquiring other bacterial and viral infections due to immunosuppression which is a big blow to the livelihood of farmers.

The morbidity and mortality losses of cattle in backyard farming in addition to affecting the socioeconomic status of the farmers, as, profits from cattle are the major source for their income and livelihood; the losses in dairy industry have a detrimental effect on the economy of

the nation as a whole. Ananda *et al.* (2009) [5] stated that haemoprotozoans have always been a formidable barrier to the survival of exotic and crossbred cattle in India. (Malayar and Farid, 2018) [6] Also reported that haemoprotozoan diseases hamper the health and productivity of cattle greatly.
Therefore, the present study was carried out to have an insight on the prevalence and intensity of haemoprotozoan infection in cattle by Leishman-Giemsa stained blood smear examination.

Materials and Methods
The study was conducted on 6000 cattle presented with clinical signs suggestive of haemoprotozoan infection to the Large Animal Medicine-Out Patient ward of Madras Veterinary College Teaching Hospital (MVCTH), Chennai, Tamilnadu, India during the period 2014-2020. The cattle belonged to different breeds, age group and of both sexes. Particulars of animal like breed, sex age, parity were recorded to study the effect of breed, sex and age on the prevalence of haemoprotozoans. As Tamilnadu receive rainfall from both southwest and northeast monsoon, to study the effect of season wise prevalence, the year was divided into four seasons as Summer (April-May), South west monsoon season (June to September), North east monsoon (October – December) and Post Monsoon season (January to March).

Examination of Leishman - Giemsa stained blood smears
Peripheral blood smears collected from the ear tips of clinically ailing animals were stained with Leishman - Giemsa stain for 30 minutes, washed, dried and examined under low, high and oil immersion objective of the binocular microscope. Microscopic identification of haemoprotozoans was done in accordance to Souslby (1982) [7]. The smears were graded as mild positive (+) when only few parasites were demonstrable in the entire smear, moderate positive (+ +) when few parasites were demonstrable in every microscopic field examined and highly positive (+ + +) when many parasites were demonstrable while spanning many fields. The smears were rated as negative (-) on failing to demonstrate a single parasite in the entire smear.
In selected cases, wet film examination was also carried out to identify the bigger parasites like Trypanosomes and Microfilaria followed by routine Leishman - Giemsa staining.

Analysis of Data
Results of the blood smear examination inclusive of all animal particulars and period of collection were plotted in Microsoft Excel and analysed to discern the total prevalence of haemoprotozoan infection, breed, sex, age and season wise prevalence of hemoprotozoan infection in cattle.

Results and Discussion
Total Prevalence
Microscopic examination of the Leishman-Giemsa stained blood smears revealed one or more of the haemoprotozoans in 1899 out of 6000 smears screened. This accounted to an overall prevalence of haemoprotozoans as 31.65%. The year wise details of smears screened and percentage of prevalence are presented in Table-1 and Fig.1. Along with less parasitic prevalence by percentage, the total number of smears screened itself reduced to a minimum of 500 in the year 2020, as less number of animals were presented to the ward due to the Covid-19 crisis.
The total prevalence of 31.65% observed in our study was less than the findings of Hosen *et al.* (2020) [8] who reported an overall prevalence of 52% in cattle population in Sylhet District of Bangladesh and Jaryal *et al.* (2018) [9] who recorded a prevalence of 78.57% haemoprotozoan infections in cows in Kangra district of Himachal Pradesh. On the contrary, Harish *et al.* (2006) [10] have reported a prevalence of only 8.93% in their study on the incidence of haemoprotozoan diseases of cattle in Karnataka.

Table 1: Year wise prevalence of Haemoprotozoal infections in bovine during 2014 to 2020

Year	No. of smears screened	No of positive smears	Prevalence (%)
2014	904	325	35.95
2015	835	256	30.66
2016	819	242	29.55
2017	979	365	37.28
2018	1015	339	33.40
2019	938	285	30.38
2020	510	87	17.06

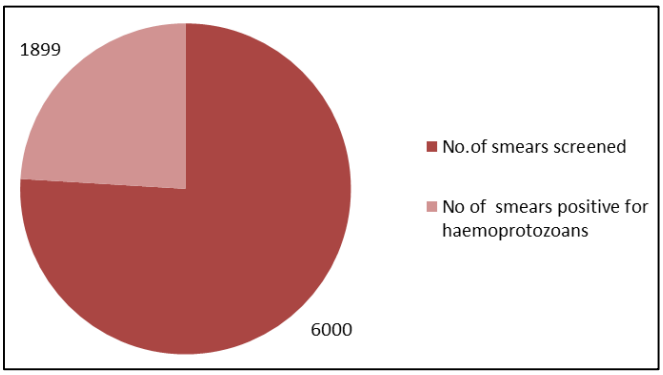

Fig 1: Total prevalence of haemoprotozoal infections in bovine during 2014-20

Generawise prevalence
The haemoprotozoans identified were *Anaplasma marginale* 68.35% (1298/1899), followed by *Theileria annulata* 27.17 % (516/1899), *Babesia bigemina* 4.05% (77/1899), *Babesia bovis* 0.21%(4/1899), *Ehrlichia bovis* 0.42% (8/1899), *Trypanasoma evansi* 1.47% (28/1899) *and Microfilaria* 0.63%(12/1899) Fig.2. This is in agreement with the findings of Khan *et al.* (2004) [11] who observed highest prevalence rate *of Anaplasma marginale* compared to other blood parasites in cattle and buffaloes in Pakistan. Atif *et al.*(2012) [12] also reported *Anaplasma marginale* (9.71%) as the most prevalent haemoparasite of cattle followed by *Theileria annulata* (6.86%) and *Babesia bigemina* (6.57%) among cattle in Sargodha District, Pakistan.
However, Prameela *et al.* (2020) [13] and Krishna Murthy *et al.* (2016) [14] observed highest prevalence of *T. Annulata* in their study on the prevalence of Haemoprotozoan infections in bovines in Andhra Pradesh and Karnataka State respectively. Krishna Murthy *et al.* (2016) [14a] further reported that the lowest prevalence was observed with *A. marginale* infection which is contrary to our findings. This could be attributed to the difference in the climate and population of vectors in such places compared to our place of research.
Among the 1899 animals found positive for haemoprotozoan infections, 44(2.31%) animals had mixed infection. Our findings is in close agreement with Singh *et al.* (2012) [15] who have also observed 2.13 % (15/703) mixed infection in cattle by screening of blood smears of cattle in Punjab. Concomitant

infection of *Anaplasma marginale* and *Theileria annulata* was of highest frequency 37(2.32%) followed by 4 cases of *Theileria annulata* and Babesia bigemina and one cases each of *Anaplasma marginale* and *Babesia bigemina*, *Anaplasma marginale* and *Babesia bovis* and *Anaplasma marginale* and *Ehrlichia bovis* Fig.3. This is in agreement with the findings of Shashi Kala *et al.* (2018) [16] who also reported a majority of concomitant infection of theileriosis and anaplasmosis (21.29%) in their study on the prevalence of haemoprotozoan disease in cattle.

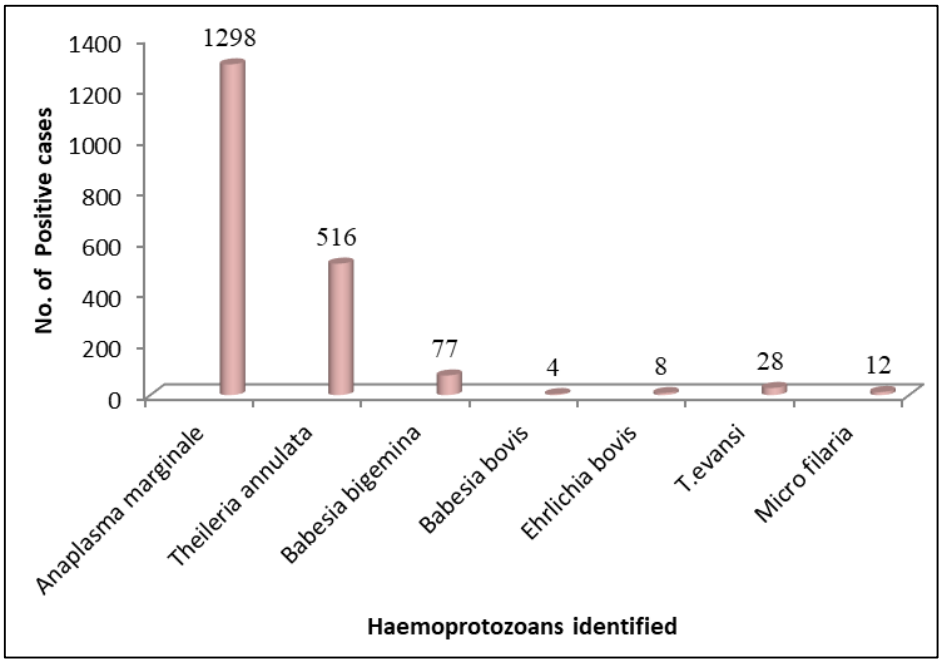

Fig 2: Generawise prevalence of haemoprotozoal infections in bovine Total prevalence during 2014-20

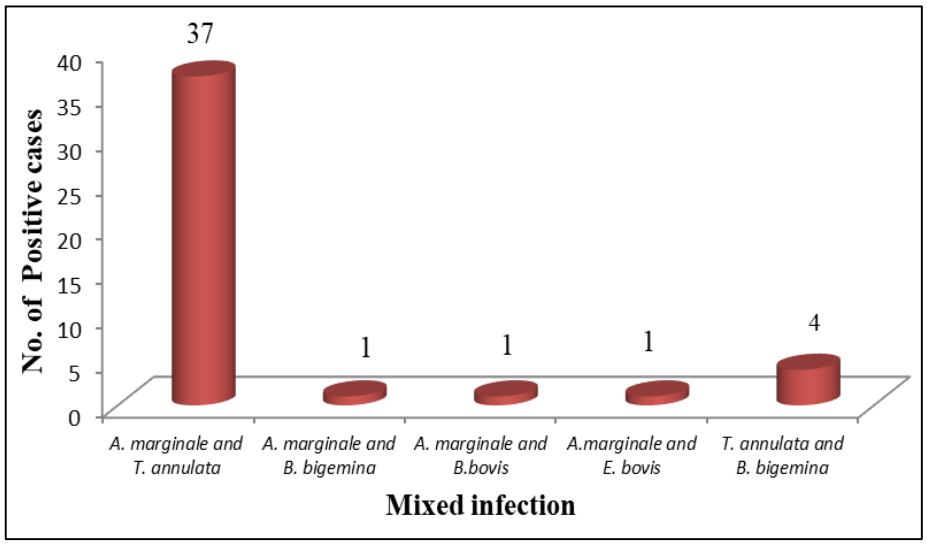

Fig 3: Total prevalence of mixed haemoprotozoal infections in bovine during 2014-20

Breed wise prevalence

The prevalence of haemoparasitic infection was 82.68% in cross bred cattle and 17.32% in indigenous cattle (Table-2 and Fig.4). Within the cross bred cattle, the prevalence was more in Jersey cross (65.77%) compared to the Holstein Friesian cross bred cattle (16.90%). Our findings of higher prevalence in cross bred cattle is in agreement with Velusamy *et al.* (2014) [17] who reported that cross-bred animals were mostly affected than indigenous animals in their study on influence of season, age and breed on prevalence of haemoprotozoan diseases in cattle of Tamil Nadu, India. Siddiki *et al.* (2010) [18] claimed constant exposure of infections and development of immunity against such infections as reasons for the lower prevalence in indigenous cattle.

Table 2: Breed wise prevalence of haemoprotozoal infections in bovine Year wise prevalence during 2014 to 2020

Year	Cross Bred cattle		Indigenous cattle
	Jersey cross	HF cross	
2014	153	82	90
2015	163	37	56
2016	177	30	35
2017	258	59	48
2018	224	59	56
2019	207	43	35
2020	67	11	9

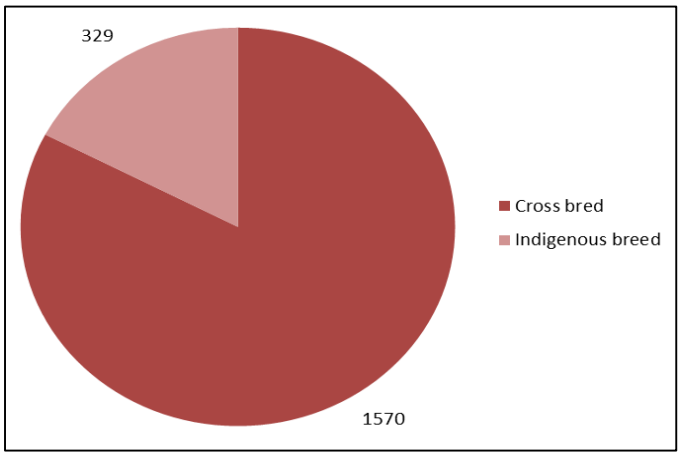

Fig 4: Breed wise prevalence of haemoprotozoal infections in bovine Total prevalence during 2014 to 2020

Sexwise prevalence

Sexwise analysis of positive cases of haemoprotozoans revealed a prevalence of 2.95% in males and 97.05% in females (Table.3 and Fig.5). A higher infection rate in females as compared to male cattle recorded in the present study differed with Singh *et al.*, (2012) [15a] who reported significantly higher incidence of haemoprotozoans in male cattle. The higher prevalence of haemoparasitic infection in our study is attributed to the fact that the sample population itself was dominated by females, as cattle are reared in the state mostly for milk production than for meat. Kamani *et al.* (2010) [19] stated that female animals are prone to tick borne diseases due to the use of contaminated needles for injecting drugs for milk let down. However, Atif *et al.* (2012) [12a] observed no statistical difference in gender wise prevalence of haemoprotozoan infection.

Table 3: Sexwise prevalence of haemoprotozoal infections in bovine Year wise prevalence during 2014 to 2020

Year	Male	Female
2014	11	314
2015	7	249
2016	14	228
2017	9	356
2018	11	328
2019	4	281
2020	0	87

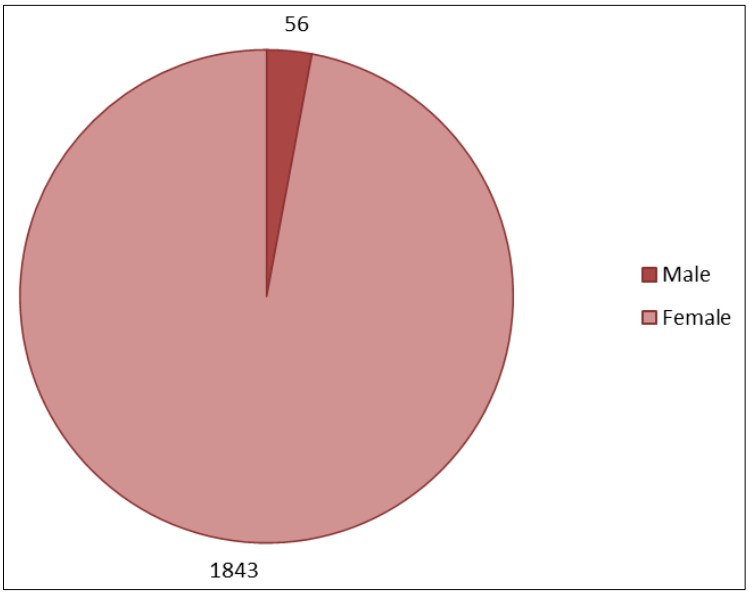

Fig 5: Sexwise prevalence of haemoprotozoal infections in bovine Total prevalence during 2014 to 2020

Age wise prevalence

Age wise prevalence of female cattle

Age wise analysis of female cattle revealed the highest prevalence of haemoprotozoans in cattle of third parity (Table.4 and Fig.6). The prevalence were 9.22% in cattle of 1st parity, 25.17% in 2nd parity, 27.54% in 3rd parity, 17.06% in 4th parity, 10.16% in 5th parity and 2.16% in aged cattle more than 5th parity. A low prevalence of 5.74% was observed in cattle aged less than one year. Cynthia *et al.* (2011) [20] stated that newborn calves were protected by colostral immunity and endemic instability of the study areas could be responsible for frequent infections in adult cattle. Hosen *et al.* (2020) [8a] also stated age resistance in combination with maternal antibodies could be the reason for the reduced number of clinical outbreaks in young animals.

The reason for the lowest prevalence in adult cattle more than the 5th parity (2.16%) could be due to less cattle population due to death of cattle from haemoprotozoans as well as other infectious diseases. Moreover, owners seek skimpy medical attention for aged cattle with reduced milk yield or even abandon the aged cattle due to the cost incurred in feeding them.

Age wise prevalence of male cattle

The highest prevalence among males was observed in the age group of less than a year old (Table.5 and Fig.7). The prevalence recorded was 1.95% in young male cattle less than one year, 0.47% in 1 to 3 years age group, 0.21% in 3-5 years and 0,16% each in 5-8 years and more than 8 years old male cattle. The reason for the decrease in the number of cases presented and the number of positive cases with increasing age in males might be due to the culling of male animals.

Table 4: Age wise prevalence of haemoprotozoal infections in bovine Year wise prevalence during 2014 to 2020

Year	Female							Male				
	< 1 Yr	1C	2C	3C	4C	5C	>5C	<1 Yr	1-3 Yrs	3-5 Yrs	5-8 Yrs	> 8 Yrs
2014	15	32	73	117	40	41	3	3	-	-	-	1
2015	16	22	58	68	61	20	4	5	1	1	-	-
2016	20	23	61	51	37	29	4	10	2	2	2	1
2017	24	39	100	90	49	36	20	5	1	-	-	1
2018	20	34	88	87	61	36	2	9	2	-	-	-
2019	9	18	65	96	56	26	5	5	3	1	1	-
2020	5	7	33	14	20	5	3	-	-	-	-	-

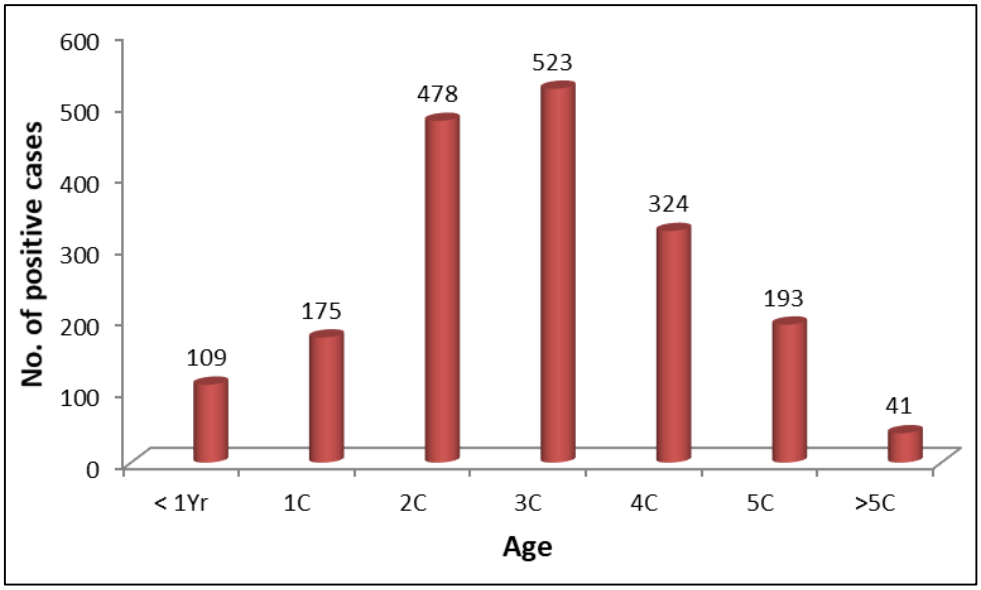

Fig 6: Age wise prevalence of haemoprotozoal infections in female cattle Total prevalence in during 2014 to 2020

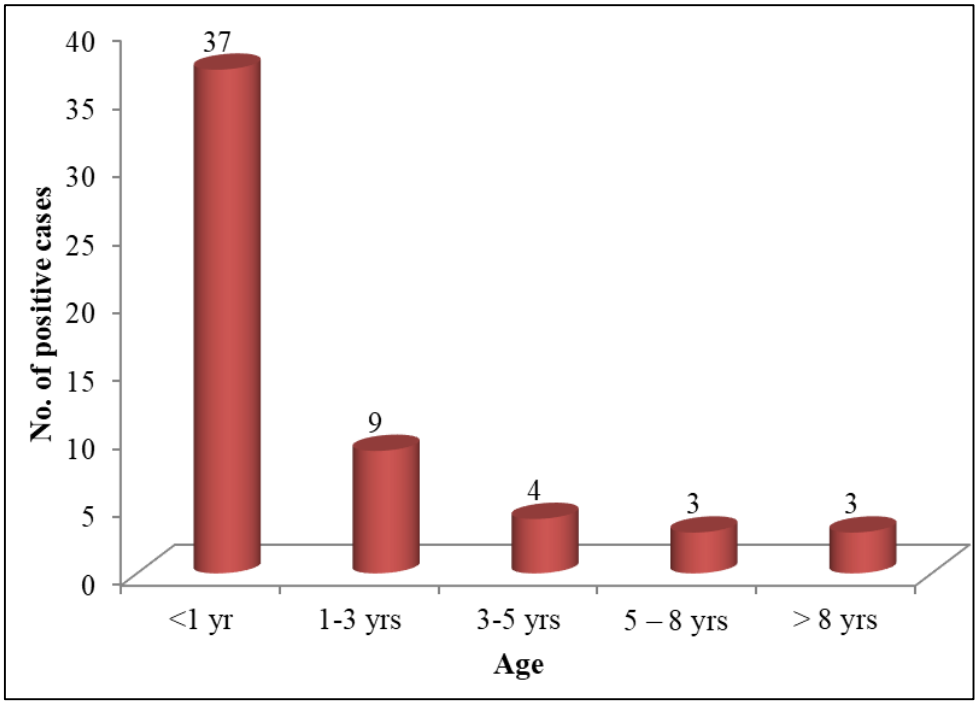

Fig 7: Age wise prevalence of haemoprotozoal infections in male cattle Total prevalence during 2014 to 2020

Season wise prevalence

Season wise analysis revealed a prevalence of 15.06% in southwest monsoon season (June to September), 26.59% in north east monsoon season (October – December), 28.01% in post monsoon season (January to March) and 30.33%. In summer (April to May) (Table.5 and Fig.8). Higher incidence of haemoprotozoal diseases in the monsoon period were reported earlier by Radostits *et al.* (1994), Roy *et al.* (2004) and Ananda *et al.* (2009) and Vahora *et al.* (2012) [21]. Ananda *et al.* (2009) [5] claimed that more animals suffered during monsoon months due to more number of ticks in the monsoon period. Krishna Murthy *et al.* (2016) [14a] recorded highest prevalence of 66.6% during monsoon months (June to September) followed by summer 58.9% and a lower prevalence of 27.1% during winter season in their study on prevalence of haemoprotozoan infections in bovines of Shimoga region of Karnataka State. Zahid *et al.* (2005) [22] claimed that the lower frequency of haemoprotozoal diseases in winter season was due to the lower temperature and humidity of winter season which is less favourable for the growth and multiplication of the disease transmitting tick vectors.

Table 5: Season wise prevalence of haemoprotozoal infections in bovine Year wise prevalence during 2014 to 2020

Year	Southwest Monsoon season	Northeast monsoon season	Post monsoon season	Summer
2014	34	102	118	71
2015	58	52	45	101
2016	18	37	58	129
2017	47	88	116	114
2018	55	85	114	85
2019	50	110	71	54
2020	24	31	10	22
Total	286	505	532	576

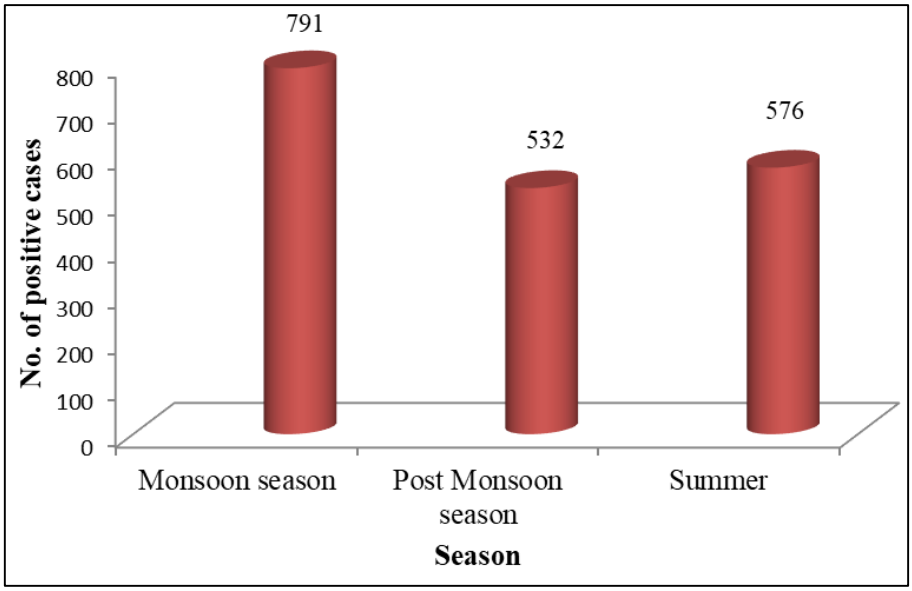

Fig 8: Season wise prevalence of haemoprotozoal infections in male cattle Total prevalence during 2014 to 2020

Conclusion

The present study revealed that out of 6000 samples screened, 1899 cases were found positive for haemoprotozoans recording an overall prevalence of 31.65%. *Anaplasma marginale*, *Theileria annulata*, *Babesia bigemina*, *Babesia bovis*, *Ehrlichia bovis*, *Trypanasoma evansi* and Microfilaria were identified with a prevalence of 68.35%, 27.17 %, and 4.05%, 0.21%, 0.42%, 1.47% *and* 0.63% respectively. Sexwise analysis revealed a higher infection rate in females (97.05%) as compared to male cattle (2.95%). Age wise analysis of female cattle revealed highest prevalence of haemoprotozoans in cattle of third parity (27.54%) and lowest prevalence of 2.16% in cattle more than 5th parity. The highest prevalence among males was observed in the age group of less than a year old (1.95%). Highest percentage of incidence was seen in monsoon (41.65%) followed by summer (30.33%) and post monsoon season (28.01%).

An insight of above findings shall serve as a guideline to adopt effective tick control measures, periodic screening of even apparently healthy animals which serve as a reservoir of haemoprotozoan infection to other susceptible animals in vicinity and to offer early and rationale treatment based on microscopic identification of the haemoprotozoans to mitigate morbidity and mortality in cattle population.

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Conflicts of Interest

There is no conflict of interest.

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