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Detection of haemoparasitic infections in cattle by parasitological techniques

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Abstract
A research was carried out to assess the frequency of haemoparasitic diseases of cattle in Bengaluru districts of Karnataka state from February to September 2022. A total of 222 cattle blood samples have been examined by microscopic methods *viz*., wet mount and Giemsa staining to diagnose haemoparasitic infections of cattle. Out of 222 blood samples examined, 20.20 and 26.57 percent samples have been observed to be positive for haemoparasites comprising *Anaplasma* (7.65% and 8.55%), *Theileria* (10.81% and 13.96%), *Babesia* (0 and 1.35%) and co-infection of *Anaplasma* and *Theileria spp*. (1.80% and 2.70%) by wet mount and Giemsa staining methods, respectively. Out of 19 samples that tested positive for *Anaplasma* spp. using the Giemsa staining technique, all 19 tested positive for *A. marginale*, and there was a coinfection of *A. marginale* and *A. platys* in two samples.

Keywords: Prevalence, haemoparasites, microscopy

1. Introduction
Haemoparasitic diseases, especially anaplasmosis, babesiosis and theileriosis are regarded as significant impediments in cattle health and productivity (Rajput *et al*., 2005) [28]. Tick and tick-borne diseases (TTBDs) are thought to be the leading source of economic losses in the cattle industry, impacting the growth of Indian dairy farming directly or tangentially (Ghosh *et al*., 2007) [15]. It is believed that roughly 80 percent of the globe's cattle are susceptible to TTBDs (De Castro 1997) [12]. Anaplasmosis, babesiosis, and theileriosis were among the top ten most prevalent cattle illnesses in India from 2014 to 2015, transmitted through ticks which are strongly favoured by the region's agroecological and geoclimatic conditions for growth and reproduction.
Bovine anaplasmosis is an illness that affects both farmed and feral ruminants and is caused by *Anaplasma* spp. of the order rickettsiales. *Anaplasma marginale* and *Anaplasma centrale* are the primary pathogens of the disease (Pierre *et al*., 2010) [26]. The organisms observed as dense, blue-purple bodies of size varying from 0.3 to 1.0 µm. However, *Anaplasma phagocytophilum* (*Ehrlichia phagocytophila*), *Anaplasma platys* (*Ehrlichia platys*) and *Anaplasma bovis* (*Ehrlichia bovis*), have recently been been added to the genus *Anaplasma* (Dumler *et al*., 2001) [13]. *Theileria spp*. (*Theileria parva*, *Theileria annulata* and *Theileria orientalis*) are round, ovoid, rod-like, or irregular-shaped organisms found in erythrocytes and lymphocytes and cause theileriosis (Soulsby, 1982) [31]. *Babesia* spp. (*Babesia bigemina*, *Babesia bovis*, *Babesia major* and *Babesia divergens*) organisms are amoeboid to pyriform shaped and found in RBC's and are responsible for babesiosis.
In order to establish strategic and tactical control measures of these parasites, the present research work was conducted on the detection of haemoparasitic infections in cattle from Bengaluru districts of Karnataka state.

2. Material and Methods
2.1 Collection of blood samples
In the period from February 2022 and September 2022, 222 blood samples from cattle were collected in EDTA vacutainer tubes from the urban and rural districts of Bengaluru. Out of 222 animals, 18.46 (41/222) percent were ailing with clinical signs such as pale mucous membrane, high temperature (104-106 ^{0}F), weakness, reduced appetite and tick infestation. The remaining 81.53 (181/222) percent of the animals were apparently healthy without any clinical signs but all were infested with ticks.

2.2 Microscopic Examination

Wet mount examination was carried out as per the standard protocol (Soulsby, 1982) [31] and Giemsa staining examination was performed within 3 to 4 hours of collection as described by Benjamin (1998) [6]. The samples were examined by using binocular compound microscope under oil immersion and haemoparasitic organisms were recognized on the basis of morphological characters (Soulsby, 1982; Bowmann DD, 2009) [31].

3. Results and Discussion

An overall of 222 cattle blood samples were analyzed by wet mount method indicated 47 (20.20%) samples to be positive for haemoparasites *viz*., *Anaplasma* (7.65%), *Theileria* (10.81%) and mixed infection of *Anaplasma* and *Theileria* (1.80%) (Fig.1, 2 and 3; Table.1). The *Anaplasma* organisms observed were round and showed rotatory movements at the margins of red blood cells and *Theileria* organisms showed slow and gentle movement in the RBC's. However, no sample was found to be positive for *Babesia* organisms by wet mount method. The present findings indicated that wet mount might be a rapid and easy method for detection of organisms during acute infection. Similar findings were described by Soulsby (1982) and Bhatia and Bhatnagar (2004) [31, 7] for detection of *Trypanosoma* spp. by wet mount method.

Giemsa stained blood smears revealed 26.57 (59/222) percent samples to be positive for haemoparasites (Table 1) which is on par with the results of Singh *et al*., 2012 from Punjab who recorded 22.90 percent positive. While, Alim *et al.* (2011) reported lower prevalence of 12.02 and 16.18 percent in indigenous and crossbreed cattle, respectively from Bangladesh. Whereas, Lalchandni, (2001) from Pakistan (39.21%), Ananda *et al*. (2009) and Krishna murthy *et al*. (2014) [2, 32] of Karnataka region has reported the higher prevalence of haemoparasites of 43.1 and 43.3 percent respectively in bovines. The differences in the prevalence of haemoparasites in different regions by microscopy could be probably due to lack of higher sensitivity, although is widely accepted and cost effective technique (Nair *et al*. 2013) [24]. However, the percent positivity depends largely on the variation in geo-climatic condition, breed, dissemination, and abundance of carrier animals and tick vectors (Ogden *et al*., 2002; Bhatnagar *et al*. 2015) [25, 8]. The data pertaining to the district wise prevalence by microscopy is given below (Table 1). During this study, Microscopic analysis of 222 cattle Giemsa stained thin blood smears indicated 8.55 percent positivity for *A. marginale* and mixed infection of both *A. platys* and *A. marginale* was found in 2 samples (Fig. 4a b& c; Table. 1). Similarly, Ge *et al*. (1997) [14] observed *A. marginale* in 13.2 percent of the samples from three counties of Oklahoma state. Kakati *et al.* (2015) [16] from Assam reported *A. marginale* in 14.03 percent of the samples and Baswaraj *et al*. (2021) [5] reported dense rounded intraerythrocytic bodies at the margin of the erythrocytes in 11.33 percent of the samples from Bidar (Karnataka). However, Birdane *et al*. (2006) [9] found a greater prevalence of 34.3 percent from Aegean region of Turkey. Whereas, comparatively lower prevalence was reported by Muraleedharan *et al*. (2005) [22] from Coorg, Mysore and Mandya region of Karnataka and Pradeep *et al*. (2019) [27] from Kerala, South India observed 1.33 and 3.0 percent of *A. marginale* organisms in cattle, respectively. The lower positivity rate in the current research could be attributed to management practices, medicines administered by field vets, and acaricides applied to suppress ticks in different regions (Sharma *et al*., 2013) [29].

Further, Out of 222 samples examined 13.96 (31) percent samples were positive for *Theileria* organisms (Fig. 4d & e; Table. 1). Various authors from different geographical regions has reported theileriosis *viz*., In the year 1989, Anandan *et al*. from Tamil Nadu recorded 21.1 percent, Muraleedharan *et al*. from Karnataka (1994) [21] reported 17.7 percent, Lalchandni, (2001) from Pakistan (58.82%), Nair *et al*. from Northern Kerala (2011) recorded 16%, Mahajan *et al*. (2013) [20] from Punjab (4.86%), Kohli *et al*. (2014) [17] from Dehradun (45.4%) and Velusamy *et al*. (2014) [32] from Tamil Nadu (13%).

In the present study, *Babesia* organisms were detected in 3 (1.35%) of the samples (Fig. 4f & g; Table. 1). The current research findings are on par with Singh *et al*. (2012) [30] from Punjab and Chowdary *et al*. (2006) from Bangladesh recorded 1.56 and 3.3 percent respectively. Whereas, higher prevalence was reported by Banerjee *et al.* 1983 (14.53%) from Bangladesh, Bhatnagar *et al*., 2015 [8] (15.65%) from Pakistan, Ananda *et al*. 2014 (45.31%) and Krishnamurthy *et al*. 2016 (12.5%) from Karnataka. These changes in prevalence could be attributed to differences in regions, seasons, and study methodologies. (Velusamy *et al*. 2014) [32].

In the current study mixed infection of *Theileria* and *Anaplasma* was observed in 6 samples examined by Giemsa staining method. The presence of mixed infections of theileriosis and anaplasmosis in the research could be attributed to the participation of the same tick species in the spread of both haemoparasitic diseases (Velusamy *et al*. 2014) [32]. Hence, In order to establish efficient measures of control against any haemoparasitic diseases in any geographical areas, prevalent studies must be conducted on a regular basis.

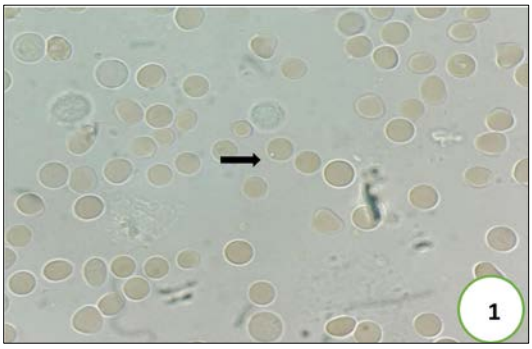


Fig 1: *Anaplasma* organism at margins of RBC's in wet mount method

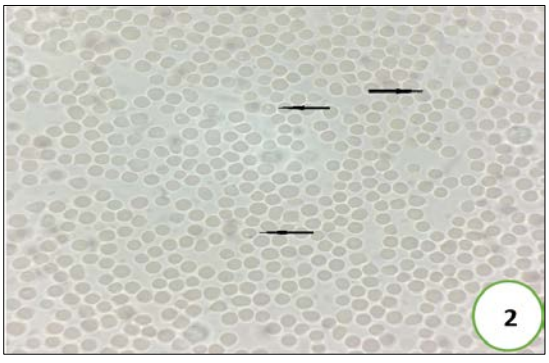


Fig 2: *Theileria* organism in RBC's in wet mount method

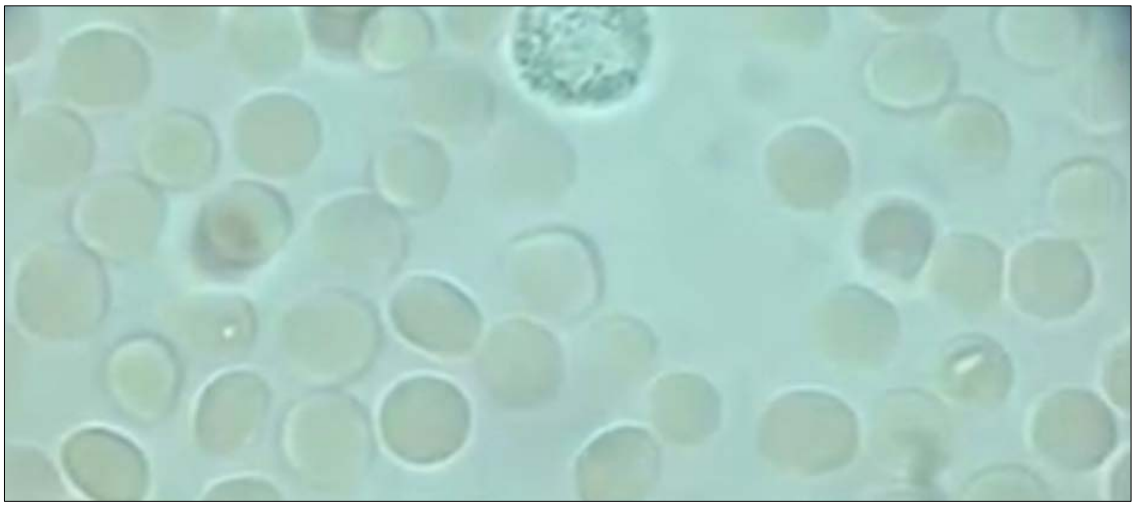


Fig 3: *Anaplasma* and *Theileria* organism in RBC's in wet mount method

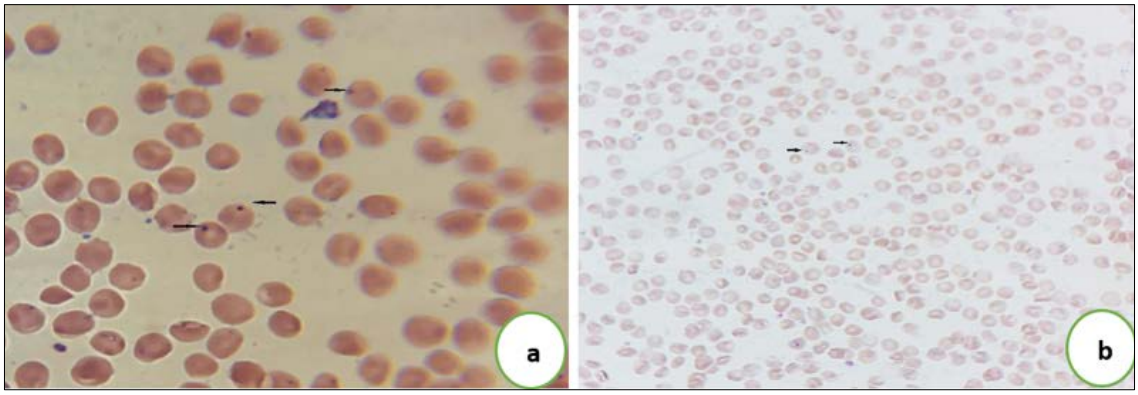


Fig 4 a & b): *Anaplasma* organisms at margins of RBC's in Giemsa stained blood smears

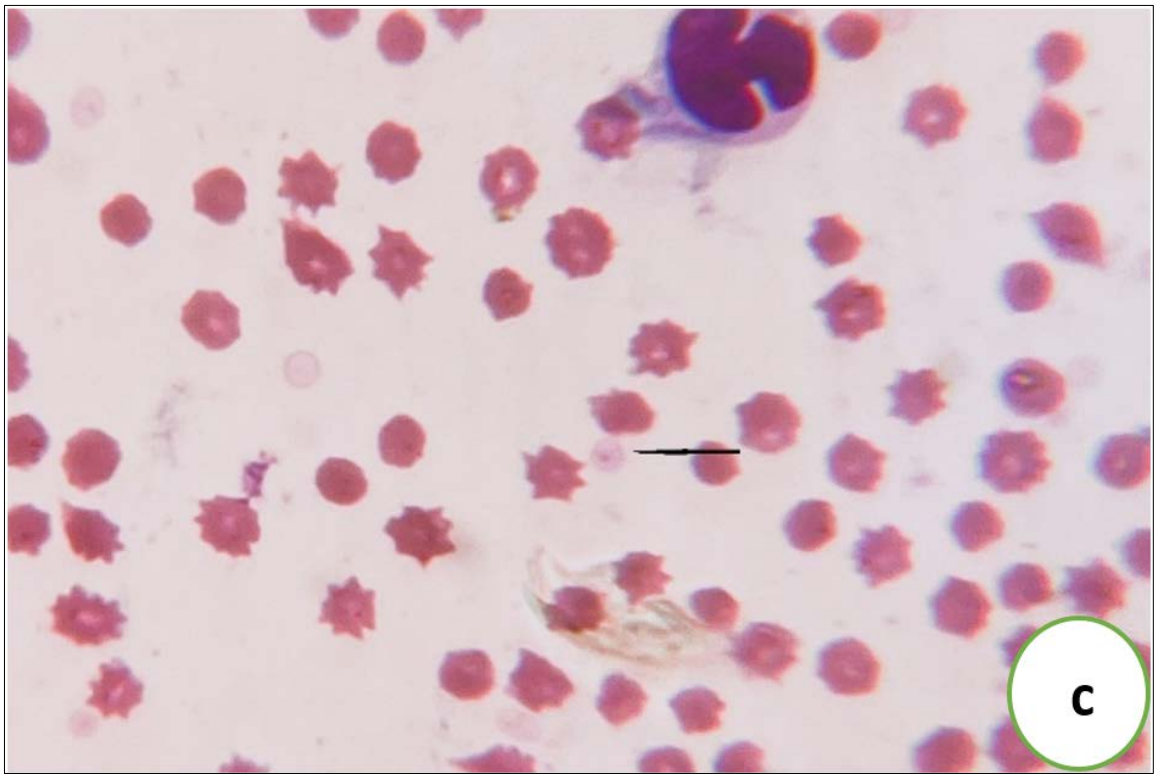


Fig 4 c): *A. platys* organism present in the platelets of Giemsa stained blood smears

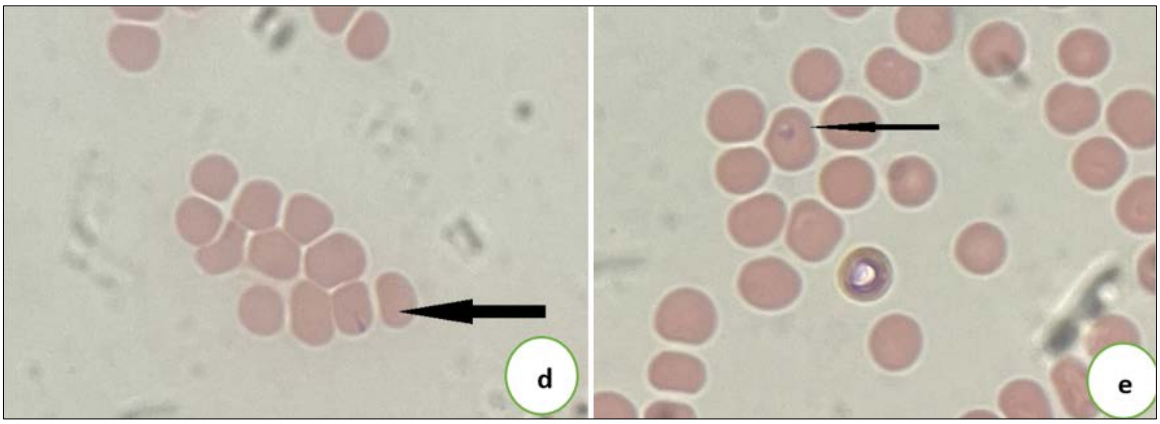
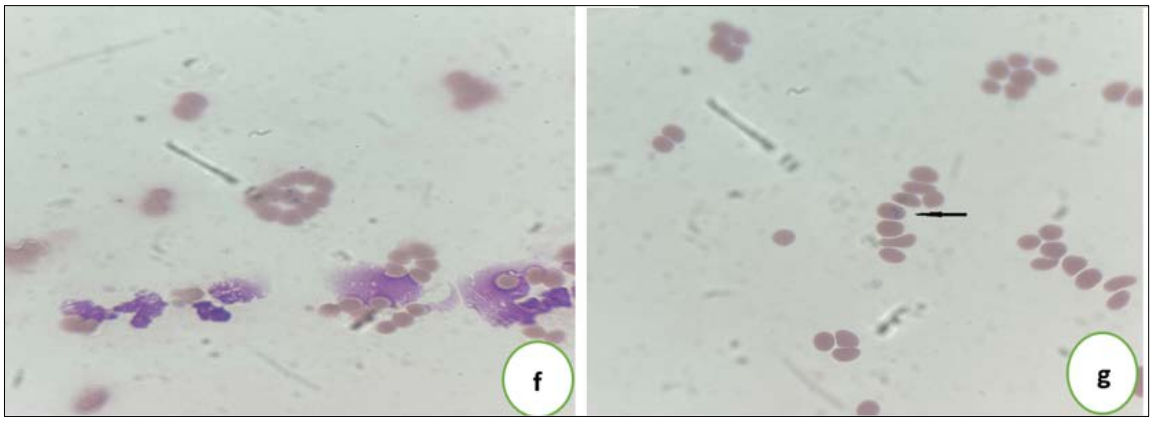


Fig 4 d & e): Giemsa stained blood smear showing *Theileria* rod form and ring form



4 f & g): Giemsa stained blood smear showing *Babesia* organisms

Table 1: District wise prevalence of haemoparasites by wet mount and Giemsa staining method

Sl. No	Districts	Number of samples examined	Number Positive		*Anaplasma*		*Babesia*		*Theileria*		*Theileria* & *Anaplasma*	
			Wet mount	Giemsa	Wet mount	Giemsa	Wet mount	Giemsa	Wet mount	Giemsa	Wet mount	Giemsa
1.	Bengaluru Urban	122	24 (19.67%)	31 (25.40%)	8	9	0	1	14	18	2	3
2.	Bengaluru Rural	100	21 (21.0%)	28 (28.0%)	9	10	0	2	10	13	2	3
Total		222	45(20.2%)	59 (26.57%)	17 (7.65%)	19 (8.55%)	0	3 (1.35%)	24 (10.81%)	31 (13.96%)	4 (1.80%)	6 (2.70%)

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5. Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

6. Author Contributions

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

7. Consent to participate

Informed consent was obtained from all individual participants included in the study. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author.

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