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Comparative studies on the detection of *Theileria annulata* infection by clinical, parasitological and molecular techniques in buffaloes (*Bubalus bubalis*)

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ABSTRACT

During the period of 2019-2021, a total of 715 buffaloes were screened for *Theileria* infections. A set of clinical parameters were used to identify the theileriosis suspected animals which were later confirmed through microscopic tests (peripheral thin blood smear examination and lymph node biopsy smear examination) and molecular assay (cytochrome b based *T. annulata* specific polymerase chain reaction test). On the basis of clinical evaluation, 33.43% (239/715) buffaloes were suspected for the presence of theileriosis. Through laboratory examination, 63.60% (152/239), 7.48% (16/214) and 100% (239/239) were found positive for *Theileria* infection by blood smear examination, lymph node biopsy and PCR, respectively, among the clinically suspected theileriosis animals. The efficiency of PCR was recorded to be highest, followed by microscopic examination of peripheral blood smears, and the lowest value was obtained for lymph node biopsy smear examination. The results indicated that PCR is a highly sensitive diagnostic technique for confirmatory diagnosis of *T. annulata* parasitic infection in buffaloes, even at very low level of parasitemia. Analysis of the data based on thin blood smear examination revealed that the field incidence of theileriosis was 11.55% in the south-western region of Gujarat, India. The high incidences of *T. annulata* infection in buffaloes may be due to the sub-tropical climate of the region which is favorable for the growth and development of vector ticks and parasites.

Key words: buffaloes; incidence; molecular diagnosis; PCR; theileriosis

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Introduction

In tropical countries such as India, tick-borne hemoprotozoan diseases have serious implications for the health and productivity of domestic animals (EL HUSSEIN et al., 1991; DE CASTRO, 1997). Among the hemoprotozoan, *Theileria* spp. is very common in ruminants worldwide. The presence of *Theileria* organisms in a geographic location is assessed by clinical signs of the disease, laboratory diagnosis, the presence of susceptible host and tick vectors. The *Theileria* parasite is small round, ovoid, irregular or bacilliform shaped parasite, of the Phylum Apicomplexa, Sub-class Piroplasmia, Order Piroplasmida and Family Theileriidae. In India, *T. annulata* is mainly responsible for theileriosis in cattle and buffaloes. Clinically inapparent to rapidly fatal symptoms have been recorded in theileriosis (DARGHOUTH et al., 1996). They cause acute, sub-acute or chronic types of diseases in all breeds and all ages of cattle and buffaloes. In the acute form, animals show a marked rise in body temperature, depression, lacrimation, nasal discharge and enlargement of superficial lymph nodes. The mortality rate with *T. annulata* may reach 70% with a case fatality rate of 10-20% in new-born calves (MOORHOUSE et al., 2001). Loss of productivity and mortality from theileriosis causes major economic loss (CAMPBELL and SPOONER, 1999; RAZMI et al., 2003). The annual cost of *T. annulata* infection in India has been estimated to be 384.3 million US$ (MINJAUW and MCLEOD, 2003). Conventionally, diagnosis of theileriosis in the field is based on clinical signs and symptoms, and laboratory microscopy, where both methods have serious limitations in terms of specificity and sensitivity. However, many PCR assays have been developed based on 18S rRNA, Cytochrome b, Tams1, etc. gene fragments, to diagnose bovine theileriosis with high sensitivity and specificity, that can even diagnose carrier *T. annulata* infection (BISHOP et al., 1992; D'OLIVEIRA et al., 1995; TAHAR et al., 1997; COLLINS et al., 2002; BILGIC et al., 2010). In India, theileriosis is reported from various regions of the country, and its incidence varies from 16 to 45.4% (ANANDAN et al., 1989; MURALEEDHARAN et al., 1994; NAIR et al., 2011; KOHLI et al., 2014). The studies on theileriosis in buffaloes are very scarce, especially from the south-western region of Gujarat, India, which is known for its well-known buffalo breed, the Jaffrabadi buffalo. Moreover, confirmatory diagnosis of theileriosis is essential for effective therapy. Accordingly, the objective of the present investigation was to assess different diagnostic tools, such as parasitological and molecular tests, for confirmatory diagnosis of *T. annulata* infection in buffaloes in south-western, Gujarat, India.

Materials and methods

A study was undertaken with respect to *Theileria annulata* detection in buffaloes at the Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Junagadh, from January 2019 to December 2021. The screening of *Theileria* parasites was carried out on the animals presented to the Veterinary Clinical Complex (VCC), Veterinary College, Junagadh and the animals present on farms, Gaushalas, in and around Junagadh district, Gujarat.

Sample collection. A total of 715 blood samples were collected from buffaloes during the study period. About a 3 ml blood sample was collected from the jugular vein of each animal using a sterile 16G needle, in a clean vial containing EDTA as the anticoagulant. To check for any hemoprortozoa parasites, samples were immediately transferred to the disease diagnostic laboratory, VCC, Junagadh, for microscopic examination and for molecular diagnosis, samples were processed for isolation of genomic DNA at the Molecular Protozoology laboratory, Veterinary Parasitology Department, Junagadh. Additionally, swollen superficial lymph nodes (mainly prescapular) were aspired to check for the presence of Koch's blue bodies in the lymphocytes. The animal associated data, such as age, sex and breed, and the address of the animal's owner were also recorded for every sample collected.

Clinical Examination. All the animals in the study were thoroughly examined clinically and the following signs and symptoms recorded: fever, inappetence, superficial lymph node enlargement, lacrimation, salivation, coughing, corneal opacity,

nasal discharge, pale mucous membranes, the presence of ticks, any drop in milk production, congested mucous membranes, emaciation, diarrhea, jaundice, conjunctivitis and lack of appetite. Animals having any symptoms such as fever, enlargement of superficial lymph nodes, lacrimation, nasal discharge, the presence of ticks, pale mucous membrane, and the lack of appetite were considered to be theileriosis suspects.

Microscopic examination. All 715 animals were tested for *Theileria* infection by thin blood smear examinations. Accordingly, a thin blood smear was prepared on a clean glass slide, air dried and fixed with methanol for 3-5 min, followed by flooding with 1:20 diluted ready-to-use Giemsa's solution (Himedia, India) for 30-40 min. After washing and air drying, the blood smear was carefully examined for *Theileria* parasites under an oil immersion lens (100× magnification). At least 100 microscopic fields were observed before declaring a sample negative. Similarly, enlarged superficial lymph nodes found in animals were aspirated and tested microscopically, where lymph node biopsy smears were stained and observed under a light microscope for the presence of Koch's blue bodies in lymphocytes.

DNA extraction and PCR. Whole blood genomic DNA was extracted from 300 blood samples of buffaloes (239 unhealthy animals, suspected of having theileriosis, and 61 clinically normal animals) using a commercially available QIAamp DNA mini kit (Qiagen, Germany). The DNA extraction was done as per the manufacturer's guidelines. Briefly, a 200 µl blood sample was mixed with 20 µl proteinase K, followed by 200 µl of lysis buffer, and incubated in a water bath at 56°C for 10 min. Subsequently, 200 µl ethanol (96–100%) was added, mixed gently and loaded into the spin column. The column was centrifuged and washed twice, as per the recommendations. Finally, DNA was eluted in 200 µl of elution buffer. The quality of DNA was assessed through agarose gel electrophoresis and stored at -20 °C until further use.

The molecular confirmation of *T. annulata* was carried out by specific amplification of a fragment of about 312 bp of cytochrome b gene in a conventional PCR (BILGIC et al., 2010). The primer pair, $Tctb_1$ (forward) 5'ACTTTGGCCGTAATGTTAAAC3' and $Tctb_2$ (reverse) 5'CTCTGGACCAACTGTTTGG3' was synthesized (Eurofins Genomics India Pvt. Ltd., Bangaluru) and diluted in nuclease water to make a 10 mM working solution. PCR was performed in a final reaction volume of 25 µl reaction mixture containing 4 µl DNA sample, 12.5 µl 2x DreamTaq Green PCR master mix (Thermo Scientific, Lithuania), 1 µl each of $Tctb_1$/$Tctb_2$ (primers) and 6.5 µl nucleus water. The reaction was carried out in a thermocycler (Applied BioSystem, USA) as follows: initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation (96°C for 15 s), annealing (60°C for 30 s) and extension (72°C for 30 s). At the end, final extension was held for 5 min at 72°C. No template control (NTC) was kept as a negative control. To identify the amplification of the targeted sequence, 20 µl PCR product was loaded in 1.5% agarose gel containing 0.5 µg/ml ethidium bromide with a DNA ladder, and electrophoresis was done in 1x Tris-Acetic acid-EDTA (TAE) buffer at 120 V for 20 min. The amplified products were visualized using a gel documentation system (Syngen, USA). 50 µl of amplified PCR product was directly submitted along with forward and reverse primes to a commercial service provider (Eurofins Genomics India Pvt. Ltd., Bengaluru, India) for bi-directional Sanger sequencing. Obtained sequences were aligned and checked using the BioEdit program and BLASTn (NCBI, USA). Finally, the sequence was submitted to GenBank (National Center for Biotechnoloy Information, USA).

Statistical analysis. The $\chi 2$ test was used for comparing the PCR test with microscopy, and $P<0.05$ was considered as a significant difference between the tests. Cohen's kappa was used to monitor the agreement between PCR and microscopy (LANDIS and KOCH, 1977).

Results and discussion

*Clinical examination***.** The clinical vitals recorded in 239 animals were found to be infected with theileriosis either through microscopy or PCR. 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 important clinical signs/symptoms observed were: mild to moderate fever, inappetence, lymph node enlargement, lacrimation of the eyes, salivation, coughing, corneal opacity, nasal discharge, pale mucous membranes, the presence of ticks, a drop in milk production, congested mucous membrane, emaciation, diarrhea, jaundice, conjunctiva and lack of appetite. The majority of *Theileria annulata* infected buffaloes showed fever, inappetence, lymph node enlargement, lacrimation of the eyes, nasal discharge, pale mucous membranes, the presence of ticks, emaciation and lack of appetite. The clinical signs/symptoms were consistent with those reported earlier by OSMAN and AL-GAABARY, 2007; FAROOQ et al., 2019; CHARAYA et al., 2021. The pathogenicity in tropical theileriosis is mainly due to parasite development and multiplication in leukocytes, and to some extent the piroplasm stage in erythrocytes. GLASS et al. (2005) reported that the production of large quantities of IFN-γ, along with other pro-inflammatory cytokines (including IL-1α, IL-1β, IL-6 and TNF-α) in response to the schizont stage of *Theileria* might be the main cause of pathogenesis, which is manifested as fever, loss of appetites, salivation, lacrimation, coughing, etc. The mucus membranes become pale in color, indicating that the animal is suffering from an anemic condition, showing that the reduction in hemoglobin and also total erythrocyte count could be due to hemolysis. Corneal opacity could be responsible for WBC infiltration, whereas diarrhea may be due to the inflammatory reaction and ulceration of the abomasal and gastro intestinal tract. Respiratory signs occur due to the accumulation of edematous fluid inside the lungs and thoracic cavity (OSMAN and AL-GAABARY, 2007; KAUR et al., 2021). Moreover, OSMAN and AL-GAABARY (2007) reported nervous manifestations, such as hyperesthesia, head pressing, convulsions, tremors and paddling before death in a few *T. annulata* infected buffaloes, which was not observed in the present investigation. This might be because all the animals were treated immediately after diagnosis and no deaths were recorded. Theileriosis is a tick-borne disease and a few species of *Hyalomma* are considered as a biological vector of *T. annulata* (KUMAR et al., 2020). They are very common in India, including this region. Here, in the present investigation around 94% diseased animals were found with visible tick infestations. A similar association between tick infestations and bovine theileriosis was reported by PARVEEN et al. (2021). The ticks were identified as *Hyalomma* spp. on the basis of the standard key. However, the absence of visible tick infestations in a few diseased animals does not mean that they did not have tick infestations. They might have the small immature stage of ticks (larvae/or nymph) that are very difficult to observe on animals during general inspection, or the ticks might have been repleted and left the host before observation.

Table 1. Frequency distribution of clinical signs and symptoms observed in theileriosis in buffaloes

Clinical sign	No. of animals	Percentages
Fever	239	100%
Inappetence	197	82.42%
Lymphnode enlargement	214	89.42%
Lacrimation of eye	199	83.26%
Salivation	104	43.51%
Coughing	148	61.92%
Corneal opacity	48	20.08%
Nasal discharge	204	85.35%

Table 1. Frequency distribution of clinical signs and symptoms observed in theileriosis in buffaloes (continued)

Clinical sign	No. of animals	Percentages
Pale mucus membrane	187	78.24%
Visually ticks present	224	93.72%
Drop milk production	182	76.15%
Congested mucus membrane	27	11.29%
Emaciation	212	88.70%
Diarrhoea	132	55.23%
Jaundice	154	64.43%
Conjunctivitis	139	58.15%
Lake of appetites	229	95.81%

Microscopic examination. Among 715 cases investigated through Giemsa's stained thin blood smear, 152 buffaloes (21.26%) were found positive for *Theileria* infection. These animals were also found to be clinically ill. Positive blood smears showed intracellular forms of parasites that are morphologically comparable with the theilerial piroplasms. The different intraerythrocytic morphological stages observed in the present study were circular, oval, piriform, comma-shaped or parachute forms of different sizes. They were observed as individual, double, triple and tetra-forms. On lymph node biopsy, Koch's blue bodies were readily recorded in 7.48% (16/214) clinically ill animals, and 10.53% (16/152) buffaloes were found to be piroplasm positive in blood smear examination. The presence of Koch's blue bodies is a characteristic diagnostic feature of acute infection with *T. annulata* and *T. parva*. In the present investigation, *Theileria* schizonts were recorded in fewer buffaloes compared to clinically ill and piroplasm positive animals. A similar observation was recorded by ZAITOUN et al. (2019), where 13 out of 30 clinically ill, piroplasm positive and cattle with enlarged lymph nodes were found positive for *Theileria* schizonts. This might be because of the lower level of schizont-infected leukocytes in lymphoid tissue in well-adapted buffaloes and local breeds of cattle (ROBINSON, 1982; GLASS et al., 2012; LARCOMBE et al., 2019) that was not detected in microscopy. Moreover, the appearance of Koch's blue bodies in the lymphocytes of *Theileria* infected buffaloes depends on the stage of infection, the immunity of the individual animal, and the severity of infection. The Giemsa's staining of blood smear is still the most useful technique for identification of *Theileria* piroplasms. However, this technique has some limitations in that it cannot detect the sub-acute and carrier states where parasitemia is very low (FRIEDHOFF and BOSE, 1994; ULLAH et al., 2021).

PCR amplification. The PCR test is considered to be more precise than Immunosorbent assays (ELISA), Immunofluorescent Antibody Test (IFAT) and Indirect Haemagglutination Assay (IHA), as well as microscopic detection of *Theileria* parasites (GUBBLES et al., 2000). In the present investigation, blood samples from 239 theileriosis suspected and 61 randomly selected, clinically normal buffaloes were subjected to PCR to assess the clinical and sub-clinical status of the animals. The presence of 312-bp amplified DNA fragments was recorded in all the 239 clinically theileriosis suspected samples (100.00%) and 16.39% clinically normal buffaloes by employing *T. annulata* specific PCR. PCR reactions, amplifying a band of 312

bp, were considered positive for the *T. annulata* infection (Fig 1). Positive control samples always showed the requisite band size of 312 bp. PCR is a sensitive, specific and more accurate diagnostic tool for diagnosis of bovine theileriosis, and its superiority over microscopic methods is well established (KHAMINSOU et al., 2008; KAUR et al., 2021). PCR, as a diagnostic technique, not only helps to detect clinical cases of bovine theileriosis, but also helps in the detection of carrier buffaloes where microscopically undetectable parasitemia is often present in carriers as well as in chronic cases (NOAMAN, 2014; ULLAH et al., 2021). The same was observed in the present investigation where PCR assay was able to detect *T. annulata* infection in all clinically ill and a few clinically normal animals, which may have had a subacute or carrier stage infection. The two partial sequences (310 bp) of the cytochrome b gene of *T. annulata* amplified from PCR positive samples of buffaloes were confirmed by BLASTn analysis (NCBI, USA), and GenBank (NCBI, USA) accessions were generated (ON755205 and ON755206). The sequences were found to be highly conserved, and more than 99% sequence homology was recorded with other published sequences (GenBank, NCBI, USA) of the cytochrome b gene of *T. annulata*.

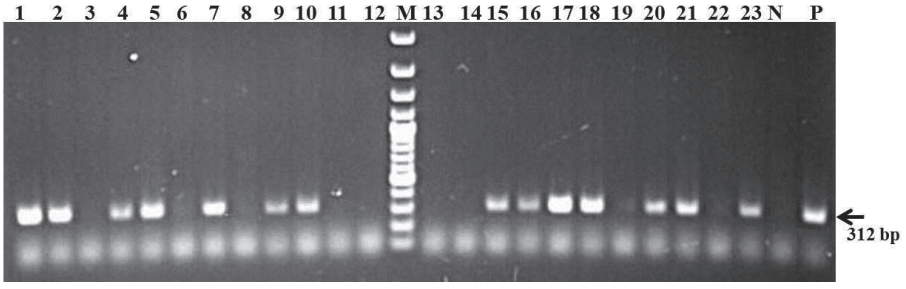

Fig. 1. Amplification of Cytochrome b gene fragment (312 bp) for PCR based identification of *Theileria annulata* infection in buffaloes [Lane 1- 23: representative field samples; N- none template control; P- positive control; M- 100 bp plus DNA ladder (Thermo Scientific, Lithuania)]

Diagnostic efficacy of different tests. On the basis of clinical evaluation, 33.43% (239/715) buffaloes were suspected for the presence of theileriosis. Three laboratory tests were compared for confirmatory diagnosis of theileriosis in buffaloes. On laboratory examination 63.60% (152/239), 7.48% (16/214) and 100% (239/239) were found positive for *Theileria* infection by blood smear examination, lymph node biopsy and PCR, respectively, among the clinically suspected theileriosis animals. The efficiency of PCR was recorded to be highest, followed by microscopic examination of peripheral blood smears, and the least was lymph node biopsy smear examination. The results indicated that PCR is a highly sensitive and specific diagnostic technique for detection of *T. annulata* parasitic infection in buffaloes, even at very low levels of parasitemia (ARIYARATNE et al., 2014). Further, the Cochen's kappa analysis revealed the slight agreement of lymph node biopsy examination with thin blood smear examination [k = 0.064 ±0.017 SE (0.030 to 0.097 at 95% confidence interval)] and PCR [k = 0]. However, a fair agreement was recorded between PCR and thin blood smear examination [k = 0.348±0.041 SE (0.267 to 0.429 at 95% CI) for detection of *Theileria* infection in buffaloes, which indicates the inherent deficiency of microscopic tests in the detection of *Theileria* parasites in buffaloes.

Incidence of Theileria annulate. A total of 715 buffalo blood samples were collected along with their clinical history, and they were subjected to microscopic examination. *Theileria* infection was found significantly more often (P<0.05) in buffaloes presented to the clinic (27.40%; 120/438) compared to the buffaloes in the field/ farms (11.55%; 32/277). The overall incidence was recorded as 21.26% (Table 2). However, through molecular assay *T. annulata* was recorded in all clinically ill, as well as in 16.39% (10/61) randomly selected clinically normal animals. The animals found to be *Theileria* positive infection were either clinically ill with various degrees of fever, inappetence, lymph node enlargement, lacrimation of eyes, and nasal discharge, or were without clinical signs and symptoms. Similarly, the incidences of *Theileria* infection in buffaloes were recorded at various levels by BANSAL et al., 1977; KHATTAK et al., 2007; SINGH et al., 2012a, as 0.07%; 2% and 2.32%, respectively. Earlier, from the same geographical region, the incidences of hemoparasites such as *Babesia bigemina, Theileria*, and *Anaplasma marginale* were recorded as 54.0; 3.4 and 1.1% in cattle and 38.8; 1.2 and 1.2% in buffalo, respectively, by blood smear examination (KUMAR et al., 2016). At the same time, another report from the same Gujarat state indicated the incidence of 12.93% and 63.79% of *Theileria* infection in buffaloes, obtained by blood examination and PCR, respectively (KUNDAVE et al., 2015; KAUR et al., 2021). The results of the present study indicated that PCR is more efficient in detecting theileriosis than the conventional staining technique, and this is in agreement with previous studies (MAHMMOD et al., 2010; ROY et al., 2000; HOOGHOOGHI-RAD et al., 2011; NOUROLLAHI-FARD et al., 2015; KAUR et al., 2021). The high incidence of *T. annulata* infections in buffaloes maybe due to the favorable atmosphere for ticks to grow in this tropical region, so there is a high prevalence of vectors i.e., *Hyalomma anatolicum* (PATEL, 2018). A higher prevalence of *T. annulata* infections in bovines was recorded earlier, as 14.65% (SINGH et al., 2012b) and 15.38% (HAQUE et al., 2012) in Punjab where *H. anatolicum* was reported as the major tick species.

Table 2. Parasitological incidence (thin blood smear examination) of theileriosis in buffaloes in south-western Gujarat, India during July 2019 to June 2021

Seasons	No. of Animals screened	Age			Breed		Sex	
		0-6 months	6 m- 2 years	> 2 years	Jaffrabadi	Non-Discript	Male	Female
Monsoon	175	2/14 (14.29%)	8/48 (16.67%)	22/113 (19.47%)	18/126 (14.29%)	6/49 (12.24%)	2/11 (18.18%)	44/164 (26.83%)
Winter	229	2/19 (10.53%)	16/67 (23.88%)	30/143 (20.98%)	27/139 (19.42%)	21/100 (21.00%)	1/15 (6.67 %)	38/214 (30.65%)
Summer	311	7/32 (21.88%)	20/88 (22.73%)	45/191 (23.56%)	61/225 (27.11%)	20/86 (23.26%)	1/24 (4.17%)	66/287 (23.00%)
Total *Theileria* positive	152/715 (21.25%)	11/65 (16.92%)	46/203 (22.66%)	95/447 (21.25%)	105/480 (21.87%)	47/235 (20.00%)	4/50 (8.00%)	148/665 (22.25%)

More cases of bovine theileriosis were observed during the summer (March-June) than in winter (November- February) and than other seasons of the year (Table 2). However, the differences were not statistically significant (P>0.05). Similar findings were recorded in the neighboring country Pakistan, and Kashmir in India (MUHAMMAD et al., 1999; FAROOQ et al., 2019; ULLAH et al., 2021). However, VAHORA et al. (2012) reported a higher prevalence of theileriosis during the monsoon season in the Punjab states of India. The high prevalence of theileriosis during the summer may be due to the hot and humid climate which favors *Hyalomma* tick growth and development. The lower temperature and humidity of winter months are less favorable for the growth and multiplication of tick vectors, which might contribute to the lower frequency of vector borne diseases (ZAHID et al., 2005; MA et al., 2020). It appears that the moderate winter in the Junagadh region makes the environment conducive for the survival of the ticks responsible for the transmission of the disease. This is corroborated by the fact that the disease was recorded to be more frequent predominantly during the summer months.

In relation to age, an insignificantly (P>0.05) higher incidence of theileriois was recorded in the 6 months to 2 years age group of animals (22.66%), followed by those older than 2 years of age (21.25%), and was lowest in the 0-6 months age group (16.92%) (Table 2). A high prevalence of *Theileria* infection was recorded in buffaloes >6 months of age, which is in agreement with the study by ANANDA et al. (2009), who recorded the highest prevalence in adult buffaloes because the physiological factors such as estrus, pregnancy and lactation lead to temporary suppression of immunity which in turn leads to an increased rate of disease occurrence in adult animals, as suggested by DURRANI, 2003. Usually, young calves (0-6 months) suffered less from theileriosis compared to those aged >6 months, which may be due to maternal immunity transfer through colostrum to the calves in endemic areas (MORZARIA et al., 1988). The resistance of young animals to theileriosis as compared to adults was also recorded by UTECH and WHARTON, 1982. Similarly, age-related resistance in young buffaloes to most tick-borne protozoan and rickettsial diseases has been reported by BAILEY (1955) and DUMANLI et al. (2005).

A high prevalence of bovine theileriosis was observed in older buffaloes in the >2 years age group, where the disease is well established and they might be carriers of the infection. These findings are in agreement with the observations of ANANDA et al., 2009; RATHER et al., 2015; CHARAYA et al., 2021. Similar observations were also made by DARGHOUTH et al. (2004) who reported that the exposure of calves to infection in the first season was shown to be significantly lower than for older buffaloes.

In relation to breed, a significantly higher incidence of theileriosis was recorded in Jaffrabadi buffaloes (38.49%) compared to non-descriptive (24.71%) breeds (Table 2). It was observed that Jaffrabadi buffaloes were more susceptible to theileriosis as compared to non-descript buffaloes, which is in agreement with RATHER et al. (2015). However, the differences were not statistically significant (P>0.05). Similarly, in relation to other breeds observations have been reported for theileriosis in Murrah, ND and Marathwadi (3.70%; 1.70% and 0.84) buffaloes (BHOSALE et al., 2020). The effect of breed on the prevalence of theileriosis in buffaloes was found to be high due to exposure to some forms of physiological stress, such as parturition, lactation and starvation (RADOSTITS et al., 2007) which are greater in pure breeds compared to non-descript (ND). The incidence of theileriosis in females was significantly (P<0.05) higher (22.25%) than male buffaloes. The higher incidence of bovine theileriosis in females than males may be attributed to higher hormonal stress and immune-suppression in female animals during pregnancy and the lactating period (KOCAN et al., 2010; TULI et al., 2015).

Conclusions

The overall incidence of theileriosis in buffaloes was recorded as 21.26%, which was higher in animals presented to the clinic compared to farm/field animals. All ages, sexes and breeds of

buffaloes were found to be affected with theileriosis throughout the year in the south-western region of Gujarat, India. Although many clinical signs and symptoms were recorded in *T. annulata* infected buffaloes, they are not uniform and pathognomonic in nature. Laboratory diagnostics, such as microscopy of Giemsa stained peripheral blood smears and lymph node biopsy smears are quick and cheap but have low sensitivity. However a molecular test such as PCR was found to be an accurate and efficient diagnostic technique for confirmatory diagnosis of *T. annulata* infection in buffaloes, which can diagnose even asymptomatic theileriosis or the carrier stage.

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THAKRE, B. J., B. KUMAR, A. A. VAGH, N. N. BRAHMBHATT, K. C. GAMIT: Usporedba otkrivanja invazije parazitom *Theileria annulata* u bivola (*Bubalus bubalis*) kliničkim, parazitološkim i molekularnim metodama. Vet. arhiv 93, 641-652, 2023.

SAŽETAK

U razdoblju od 2019. do 2021. ukupno je 715 bivola testirano na invaziju parazitom *Theileria annulata*. Za otkrivanje životinja sumnjivih na tajleriozu upotrijebljen je skup kliničkih parametara koji su poslije potvrđeni mikroskopskim testovima (analizom razmaza periferne krvi i biopsijom limfnih čvorova) i molekularnim metodama (PCR specifičan za *T. annulata* na bazi citokroma b). Kliničkom procjenom posumnjalo se na tajleriozu u 33,43% bivola (239/715). Laboratorijskim je pretragama u životinja sumnjivih na tajleriozu utvrđeno sljedeće: 63,60% bivola (152/239) bilo je pozitivno na temelju analize krvnog razmaza, u 7,48% bivola (16/214) tajlerioza je otkrivena biopsijom limfnih čvorova, dok je PCR pokazao da je pozitivno 100% bivola (239/239). U otkrivanju tajlerioze najučinkovitiji je dakle bio PCR, zatim mikroskopski pregled razmaza periferne krvi, dok je najmanje pozitivnih životinja otkriveno biopsijom limfnih čvorova. Rezultati upućuju na visoku osjetljivost PCR-a za potvrdu invadiranosti parazitom *T. annulata* u bivola čak i u slučaju niske razine parazitemije. Analiza podataka temeljena na analizi razmaza periferne krvi pokazala je incidenciju tajlerioze na terenu u jugozapadnoj pokrajini Gujarat u Indiji od 11,55%. Visoka incidencija invazije parazitom *T. annulata* u bivola može biti posljedica suptropske klime tog područja koja pogoduje rastu i razvoju krpelja i parazita kao vektora.

Ključne riječi: bivoli; incidencija; molekularna dijagnoza; PCR; tajlerioza