Original Research Article   https://doi.org/10.20546/ijcmas.2018.706.103

Hospital-Prevalence of *Theileria annulata* Infection in Cattle-Calves Determined by Blood Smear and Lymph Node Aspirate Smear Examination in Bikaner, Rajasthan, India

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A B S T R A C T

One hundred cattle-calves irrespective of their age, sex and breed brought to Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, Bikaner were screened for *Theileria annulata* infection. Blood and lymph node aspirate smears were prepared from the ear vein and enlarged superficial lymph nodes of suspected cattle-calves and stained with Giemsa's stain for detection of piroplams and schizonts under oil immersion, respectively. Blood smear examination revealed presence of piroplasms in seven cases. There was anisocytosis and poikilocytosis observed in erythrocytes structure and infected erythrocytes were appeared as echinocytes. Lymph node aspirate smears examination revealed presence of schizonts in lymphocytes only in three cases and presence of releasing merozoites from the infected cell only in one out of three cases. Thus, the hospital prevalence of *Theileria annulata* infection in cattle-calves in Bikaner was 7% and 3% by Giemsa stained blood smear and lymph node aspirate smear examination, respectively.

Introduction

Bovine tropical theileriosis is a protozoan disease caused by blood protozoa *Theileria annulata* and it is transmitted by tick *Hyalomma anatolicum anatolicum.* It causes significant economic losses in large parts of Asia (Hasanpour *et al.,* 2013). It is mainly seen in cattle, sheep and goat as well as in wild and captive ungulates (Radostits *et al.,* 2007). This intracellular infection inflicts economic burden on cattle breeders in terms of mortality and morbidity as well as expenses spent on prophylactic measures against disease and treatment (Durrani *et al.,* 2008).

Theileria spp. infection can cause acute, subacute or chronic disease pathology (Gill *et al.,* 1977). In *T. annulata* infection, the most common clinical symptoms are weakness, weight loss, anorexia, high body temperature, petechia on the conjunctival mucosa, swollen lymph nodes, anaemia and cough. On later stages of theileriosis, infected animals cannot stand up, their body temperatures are under normal values (< 38.5°C), and icterus,

dehydration and blood in faeces are the occasional clinical symptoms (Bakheit *et al.,* 2004). Calves (≤ 4 months of age) infected with *Theileria annulata* suffered from emaciation, anaemia, unilateral or bilateral exophthalmia, petechiae in conjunctiva, oral and nasal mucosa, and occasionally in the pinnae. Widespread subcutaneous nodules with 0.5 to 3.0 cm diameter are also detected, as well as enlarged superficial lymph nodes, particularly the submandibular, the retropharyngeal and sometimes the prescapular (Branco *et al.,* 2010).

Tanwar *et al.,* (1984) reported 48.85 per cent prevalence of theileriosis in Rathi calves during 1979-1980 from Bikaner region by Giemsa stained blood smear examination. Martin-Sanchez *et al.,* (1999) analyzed 214 samples out of which, 78.04 per cent, 69.86 per cent, and 62.26 per cent were found to be positive by nested PCR, indirect immuno-fluorescent antibody test, and optical microscopy of Giemsa-stained smears, respectively. Omer *et al.,* (2002) examined 403 adult and young Holstein Friesian cattle clinically and parasitologically out of which, 62 (15.4 per cent) were found positive for *T. annulata* microscopically. An average of 1–5 piroplasmic forms in the RBC were observed in all cases with a range of 10–45 per cent parasitemia. Sayin *et al.,* (2003) conducted blood smear and serological examination of the 198 cattle in March, before the start of the first disease season. The prevalence of piroplasmosis was 11.1 per cent (22 out of 198) and the seroprevalence of *T. annulata* was 10.6 per cent (21 out of 198). Dumanli *et al.,* (2005) reported 19.7 per cent (293/1483) prevalence of *Theileria annulata* by microscopic examination. Aktas *et al.,* (2006) examined 252 blood samples out of which, 41(16.26 per cent) were positive for piroplasms upon microscopic examination. Ananda *et al.,* (2009) screened a total of 132 clinically suspected blood samples from cross-

bred cattle by Giemsa's stain out of which, 57 (43.18 per cent) animals were found positive for haemoprotozoan parasites. Out of 57 positive cases, 41 (31.06 per cent) were found positive for *Theileria annulata* alone. Durrani *et al.,* (2010) collected blood samples from three districts of Punjab province (Pakistan) to examine presence of haemoprotozoans in cattle and reported 6.8 per cent prevalence of *Theileria* parasite by microscopy. Shahnawaz *et al.,* (2011) reported 3 per cent prevalence of *Theileria annulata* in large ruminants in Southern Punjab (Pakistan). Khattak *et al.,* (2012) collected 95 examined blood samples from two districts of Southern Punjab. Only five (5.2 per cent) of 95 blood samples were found parasite positive during microscopic examination of Giemsa stained blood smears. Prevalence of *T. annulata* was significantly (P = 0.053) higher in Kohat district as compared to Peshawar. Saeid *et al.,* (2013) examined 150 smears microscopically out of which, 16 (10.66 per cent) were positive for piroplasmic forms of *Theileria annulata*. Ariyaratne *et al.,* (2014) reported 7.31 per cent (3/41) prevalence of *Theileria* infection by light microscopic examination of thin blood smears. Kohli *et al.,* (2014) reported 27.2 per cent prevalence of theileriosis by blood smear examination. Singh *et al.,* (2014) carried out study on evaluation of clinical markers for diagnosis of bovine theileriosis and reported that blood smear examination revealed presence of only schizonts in mononuclear cells of 14.29 per cent (3/21) and presence of only piroplasms in the RBCs of 42.86 per cent (9/21) samples. Modi *et al.,* (2015) screened 117 cows for *Theileria annulata* infection out of which, 20 (17.09 per cent) were found positive for infection on the basis of cytoplasmic inclusions in Giemsa stained peripheral blood smear examination. Tuli *et al.,* (2015) collected a total of 1278 blood samples from twenty districts falling in five major agro-climatic zones of Punjab. Out of which 118 samples (9.23 per cent) were found

positive for *Theileria* spp. by Giemsa stained blood smear (GSTBS) examination

Materials and Methods

One hundred cattle-calves irrespective of their age, sex and breed brought to Teaching Veterinary Clinical Complex of College of Veterinary and Animal Science, Bikaner for treatment were screened for bovine tropical theileriosis. Blood smears were prepared from ear vein of suspected cattle-calves. Lymph node aspiration fluid was collected from the superficial lymph nodes which were infected and enlarged, adopting all aseptic precautions for detection of Koch's blue bodies (K.B.B.) in the lymph node aspirate smear. The 22 gauze needle (sterilized) was used for this purpose. After grasping the affected lymph node between thumb and index finger, the needle was allowed to penetrate inside the lymph node and then moved forward and backward in the lymph node tissue. A small quantity of the fluid was then aspirated with syringe. Immediately after aspiration of lymph fluid, smears were prepared on clean, greaseless glass slides and air dried. Smears were stained with Giemsa's stain as per procedure described by Soulsby (1982) and examined under oil immersion.

Results and Discussion

Examination of Giemsa's stained blood smears under oil immersion lens revealed intra-erythrocytic piroplasms of *Theileria annulata* only in seven out of one hundred cases examined. Mostly, the piroplasms were appeared as ring or oval shaped with a little percentage of dots. There was anisocytosis and poikilocytosis observed in erythrocytes structure and infected erythrocytes were appeared as echinocytes (Fig. 1, 2 and 3). Similar findings have been reported by Khan *et al.,* (2011) and Al-Emarah *et al.,* (2012). Abnormalities in erythrocytes structure

including anisocytosis, poikilocytosis, basophilic stippling and presence of reticulocytes were also observed by Durrani and Kamal (2008).

Examination of Giemsa's stained lymph node aspirate smears under oil immersion lens revealed presence of schizonts (Koch's blue bodies) in and outside of lymphocytes only in three out of one hundred cases examined and presence of releasing merozoites from the infected cell only in one case (Fig. 4, 5 and 6).

Thus, the hospital prevalence of *Theileria annulata* infection in cattle-calves was 7 % and 3% by blood smear and lymph node aspirate smear examination, respectively in Bikaner, Rajasthan. Prevalence of *Theileria annulata* infection by microscopic examination has been reported by many researchers namely Martin-Sanchez *et al.,* (1999) as 62.26 per cent; Omer *et al.,* (2002) as 15.4 per cent; Sayin *et al.,* (2003) as 11.1 per cent; Dumanli *et al.,* (2005) as 19.7 per cent; Aktas *et al.,* (2006) as 16.26 per cent; Ananda *et al.,* (2009) as 31.06 per cent; Durrani *et al.,* (2010) as 6.8 per cent; Shahnawaz *et al.,* (2011) as 3 per cent; Khattak *et al.,* (2012) as 5.2 per cent; Saeid *et al.,* (2013) as 10.66 per cent; Ariyaratne *et al.,* (2014) as 7.31 per cent; Kohli *et al.,* (2014) as 27.2 per cent; Singh *et al.,* (2014) as 14.29 per cent schizont form and 42.86 per cent piroplasmic form; Modi *et al.,* (2015) as 17.09 per cent schizont form and Tuli *et al.,* (2015) as 9.23 per cent.

The abnormality in erythrocytes shape is mainly due to toxic action of parasite in the erythrocytes, erythrocyte oxidation, and immune-mediated process as reported by Stockham *et al.,* (2000) and Singh *et al.,* (2001). Conventional diagnosis of tropical theileriosis depends on examination of Giemsa stained thin blood and lymph node aspirate smears.

Fig.1 Dominant ring shaped intra-erythrocytic piroplasms of *Theileria annulata* in Giemsa stained blood smear (100X)
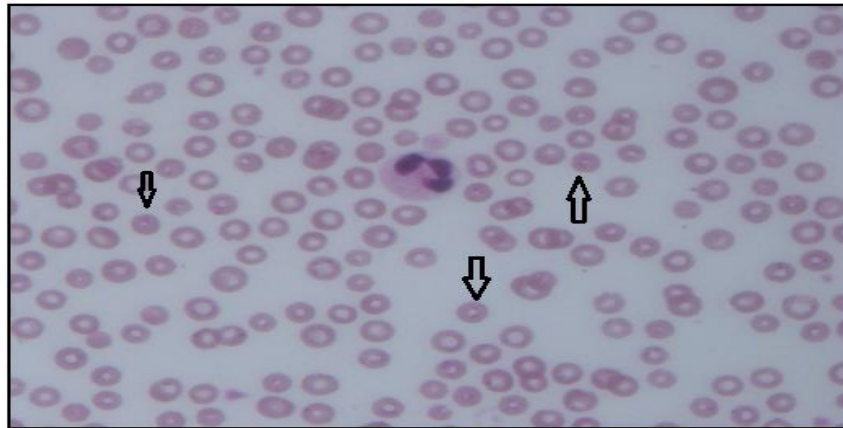

Fig.2 Dot shaped intra-erythrocytic piroplasms of *Theileria annulata* (Black arrows), anisocytosis and poikilocytosis in erythrocytes structure (White arrows) in Giemsa stained blood smear (100X)
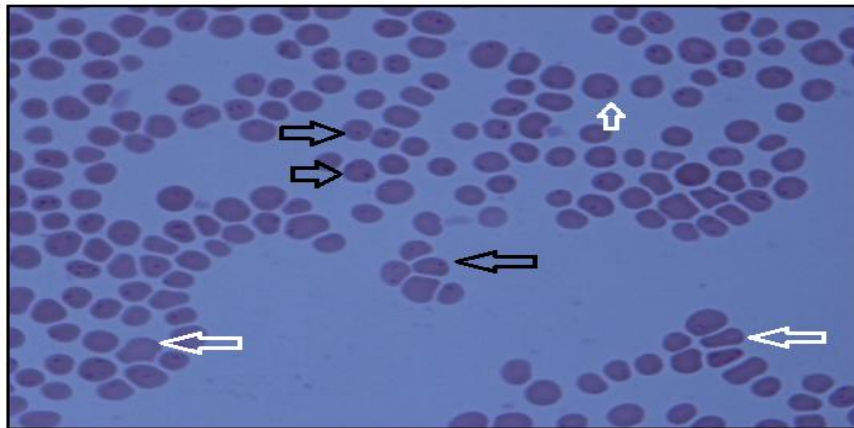

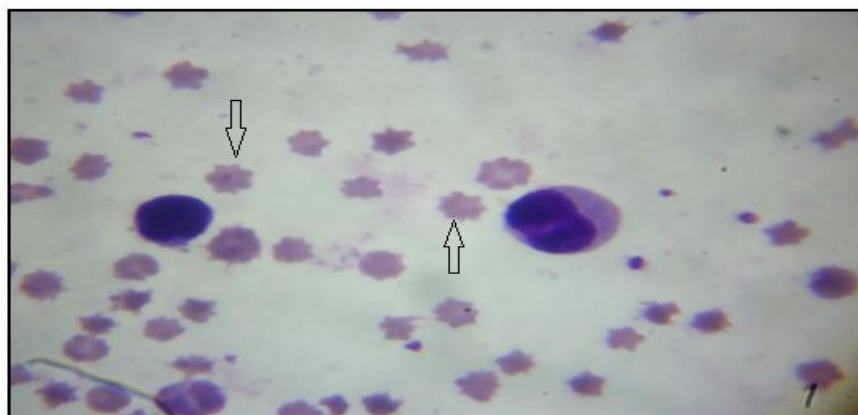**Fig.3** Echinocytes in Giemsa stained blood smear (Under 100X)

Fig.4 Koch's blue bodies (intracellular schizonts) in infected mononuclear cells in Giemsa stained lymph node aspirate smear (Under 100X)
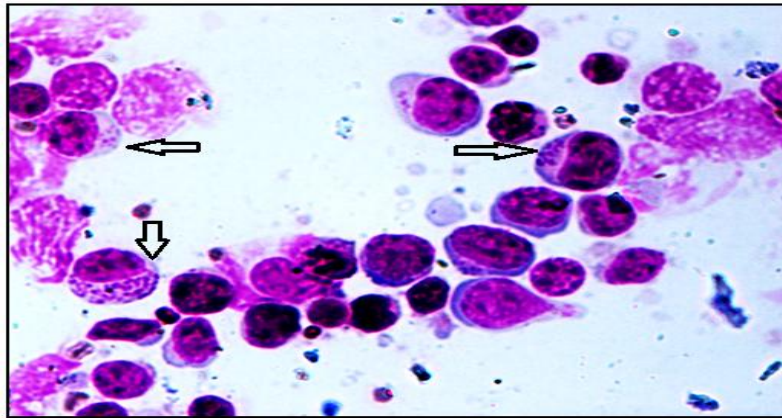

Fig.5 Extracellular schizonts (Koch's blue bodies) outside the lymphocytes (red arrow) and intracellular schizonts in infected lymphocytes (black arrow) in Giemsa stained lymph node aspirate smear (Under 100X)
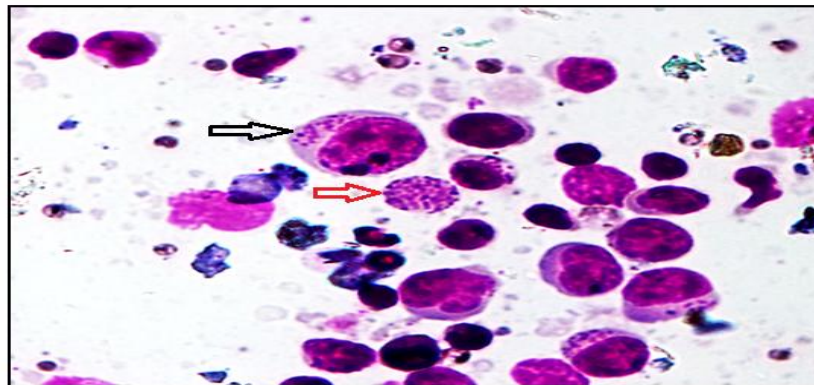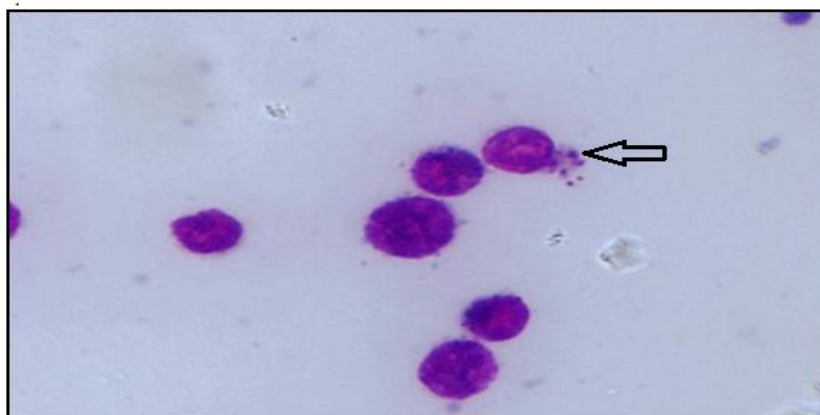

Fig.6 Releasing merozoites from the infected cell in Giemsa stained lymph node aspirate smear examination (Under 100X)

This method is limited to the acute stage of the disease where the parasitemia is high enough to be detected microscopically. During chronic and carrier stages the level of parasitemia usually below the microscopical detectable level.

Acknowledgements

Conflict of Interest

The author declares that he has no conflict of interest.

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