Biological Forum – An International Journal **16(1): 132-135(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Hospital Based Prevalence of *Theileria annulata* in Cattle-calves in Bikaner District of Rajasthan

Pavan Goyal

Department of Epidemiology and Preventive Veterinary Medicine, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner (Rajasthan), India.

(Corresponding author: Pavan Goyal)*

(Received: 20 November 2023; Revised: 28 November 2023; Accepted: 24 December 2023; Published: 15 January 2024)
(Published by Research Trend)

ABSTRACT: Cattle-calves were screened for *Theileria annulata* infection at the Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, Bikaner, regardless of their age, sex, or breed. Giemsa's stain was used to create blood smears and lymph node aspirate smears from the ear veins and enlarged superficial lymph nodes of suspected cow calves, respectively, in order to detect piroplasms and schizonts under oil immersion. Seven instances' blood smear analysis showed the presence of piroplasms. The structure of the erythrocytes showed anisocytosis and poikilocytosis, and the infected erythrocytes appeared as echinocytes. Examination of lymph node aspirate smears showed that only three instances had schizonts in the lymphocytes, and only one of the three cases had merozoites escaping from the infected cell. Accordingly, the lymph node aspirate smear examination and Giemsa-stained blood smear analyses revealed that the hospital-based prevalence of *Theileria annulata* infection in cattle-calves in Bikaner was 3.2% and 7.5%, respectively.

Keywords: Piroplasms, cattle-calves, *Theileria annulata*, schizonts, lymph node.

INTRODUCTION

Theileria annulata, a blood protozoan, is the source of bovine tropical theileriosis, a protozoan spread by the tick *Hyalomma anatolicum anatolicum*. In many regions of Asia, it results in considerable economic losses (Hasanpour *et al.*, 2013; Ullah *et al.*, 2021). It primarily affects cattle, sheep, and goats in addition to ungulates in the wild and captivity (Radostits *et al.*, 2007). Cattle breeders suffer financial consequences from this intracellular infection in the form of increased mortality and morbidity as well as costs associated with treatment and preventative measures (Durrani *et al.*, 2008) and also causes reduction in production (Zeb *et al.*, 2020).

According to Gill *et al.* (1977), *Theileria* spp. infections can result in acute, subacute, or chronic disease pathology. The most typical clinical signs of *T. annulata* infection include anemia, coughing, petechiae on the conjunctival mucosa, anorexia, weakness, and swollen lymph nodes(Ma *et al.*, 2020). Later stages of theileriosis are characterized by the inability of infected animals to stand, low body temperatures (<38.5°C), and sporadic clinical signs such as icterus, dehydration, and blood in the feces (Bakheit *et al.*, 2004). *Theileria annulata* infection in calves (≤4 months of age) resulted in emaciation, anemia, unilateral or bilateral exophthalmia, and petechiae in the nasal, oral, and conjunctival mucosa, as well as rarely in the pinnae. Broad subcutaneous nodules ranging in diameter from 0.5 cm to 3.0 cm are also observed, along with enlarged superficial lymph nodes, specifically the retropharyngeal, submandibular, and occasionally the prescapular (Branco *et al.*, 2010).

By using a Giemsa-stained blood smear test, Tanwar *et al.* (1984) observed a 48.85% prevalence of theileriosis in Rathi calves from the Bikaner region during the years 1979–1980. Martin-Sanchez *et al.* (1999) examined 214 samples; of these, 78.04 percent, 69.86 percent, and 62.26 percent were determined to be positive using optical microscopy of Giemsa-stained smears, nested PCR, and indirect immunofluorescent antibody test, respectively. In a 2002 clinical and parasitological examination of 403 adult and juvenile Holstein Friesian cattle, *Omer et al.* discovered that 62 (15.4%) of the animals tested positive for *T. annulata* under a microscope. In all cases, there was an average of 1-4 piroplasmic forms in the red blood cells, with a range of 10–45% parasitemia.

Before the first disease season began in March, Sayin *et al.* (2003) performed blood smear and serological examination on the 198 cattle. *T. annulata* seroprevalence was 10.6% (21 out of 198), and piroplasmosis prevalence was 11.1% (22 out of 198). By microscopic analysis, Dumanli *et al.* (2005) revealed a 19.7% (293/1483) prevalence of *Theileria annulata*. Aktas *et al.* (2006) tested 252 blood samples, 41 of which (16.26%) tested positive for piroplasms under a microscope. Ananda *et al.* (2009) used Giemsa's stain to screen 132 clinically suspected blood samples from cross-bred cattle; of these, 57 (43.18 percent) animals tested positive for hemoprotozoan

parasites. 41 instances (31.06%) out of the 57 positive cases tested positive for *Theileria annulata* alone. In order to test for the existence of hemoprotozoans in cattle, Durrani *et al.* (2010) obtained blood samples from three districts in Pakistan's Punjab region. The results of their microscopy revealed a 6.8% prevalence of *Theileria* parasite. In Southern Punjab (Pakistan), Shahnawaz *et al.* (2011) found that *Theileria annulata* was present in 3% of large ruminant animals. Ninety-five blood samples were studied by Khattak *et al.* (2012) from two areas in Southern Punjab. Out of 95 blood samples, only five (5.2%) tested positive for parasites when Giemsa-stained blood smears were examined under a microscope. In comparison to Peshawar, the Kohat district had a considerably (P = 0.053) higher prevalence of *T. annulata*. After microscopically examining 150 smears, Saeid *et al.* (2013) found that 16 of them (10.66%) had *Theileria annulata* piroplasmic forms. According to Ariyaratne *et al.* (2014), thin blood smears examined under a light microscope revealed a prevalence of *Theileria* infection of 7.31% (3/41). According to Kohli *et al.* (2014), prevalence of theileriosis by blood smear analysis was 27.2 percent. In a study on the assessment of clinical markers for the diagnosis of bovine theileriosis, Singh *et al.* (2014) reported that a blood smear examination showed that only schizonts were present in 14.29 percent (3/21) of the mononuclear cells and only piroplasms were present in 42.86 percent (9/21) of the RBCs of the samples.117 cows were examined by Modi *et al.* (2015) for *Theileria annulata* infection; of them, 20 (17.09%) were determined to be positive for the infection due to cytoplasmic inclusions in the peripheral blood smear examination stained with Giemsa. In all, 1278 blood samples from 20 districts spread across Punjab's five main agroclimatic zones were gathered by Tuli *et al.* (2015). 118 samples (9.23%) of which underwent a Giemsa-stained blood smear (GSTBS) testing and tested positive for *Theileria* spp.

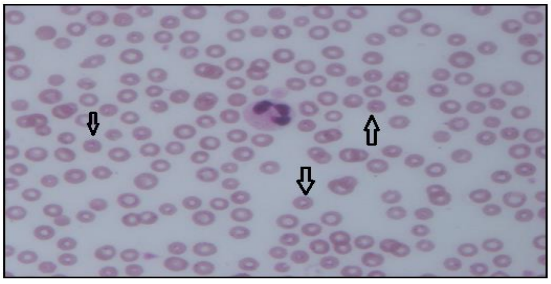

Fig. 1. Ring shaped piroplasms of *T. annulata* (100X).

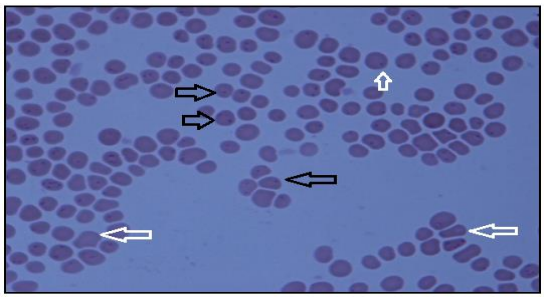

Fig. 2. Dot shaped piroplasms of *Theileria annulata*as Black arrows, anisocytosis and poikilocytosis as White arrows in Giemsa stained blood smear (100X)

MATERIALS AND METHODS

Bovine tropical theileriosis screening was done on one hundred cow calves, regardless of breed, age, or sex, who were transported to the Teaching Veterinary Clinical Complex of the College of Veterinary and Animal Science, Bikaner for treatment. Blood smears were made from the suspected cow calves' ear veins by adopting aseptic procedures. The superficial lymph nodes that were swollen and infected were used to obtain lymph node aspiration fluid. For this use, a sterile 22-gauze needle was employed. The needle was allowed to enter the afflicted lymph node and then pushed forward and backward in the lymph node tissue after being grasped between the thumb and index finger. The fluid was then aspirated in little amounts using a syringe. Smears were made on spotless, clean glass slides and allowed to air dry immediately. According to the method outlined by Soulsby (1982), smears were stained with Giemsa's stain and evaluated while submerged in oil.

RESULTS AND DISCUSSION

Study revealed presence of intra-erythrocytic piroplasms of *Theileria annulata* when examined underoil immersion lens using Giemsa's-stained blood smears. The piroplasms mostly had an oval or ring form with a little amount of spots. The structure of the erythrocytes showed anisocytosis and poikilocytosis, and the infected erythrocytes appeared as echinocytes (Fig. 1-3). Al-Emarah *et al.* (2012); Khan *et al.* (2011) have both published findings that are similar. Durrani and Kamal (2008) also noted anomalies in the structure of erythrocytes, such as anisocytosis, poikilocytosis, basophilic stippling, and the presence of reticulocytes.

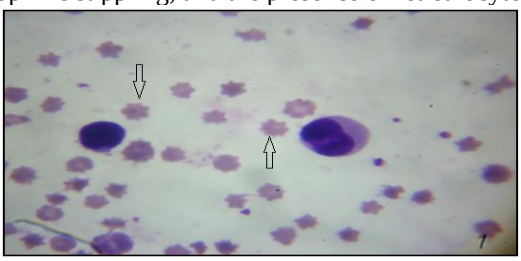

Fig. 3. Echinocytes (Under 100X).

Giemsa-stained lymph node aspirate smears examined under an oil immersion lens showed presence of schizonts (Koch's blue bodies) inside or outside of lymphocytes (3.2% cases), and only one case had merozoites releasing from the infected cell (Fig. 4-6).

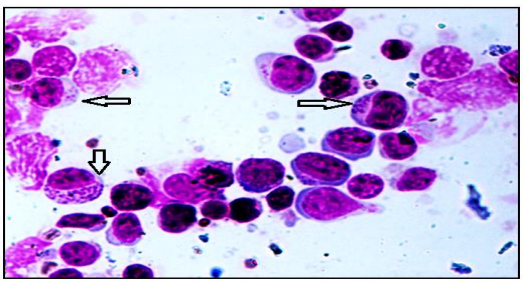

Fig. 4. Koch's blue bodies (Under 100X).

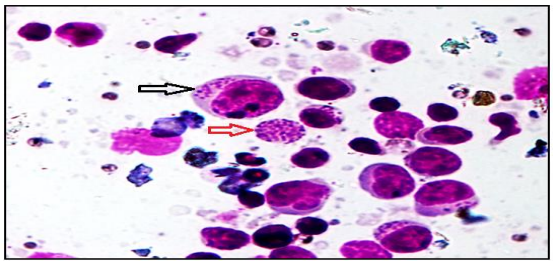

Fig. 5. Extracellular schizonts outside the lymphocytes as in red arrowand intracellular schizonts in infectedlymphocytes as in black arrowin Giemsa stained lymph node aspirate smear (Under 100X)

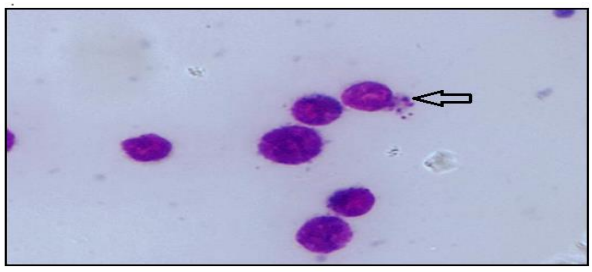

Fig. 6. Merozoites releasing from the infected cell(Under 100X).

Therefore, in Bikaner, Rajasthan, the hospital prevalence of *Theileria annulata* infection in cattle-calves was found to be 7.5% and 3.2%, respectively, by blood smear and lymph node aspirate smear testing. Many researchers have reported the prevalence of *Theileria annulata* infection by microscopic examination, these include 62.26% by Martin-Sanchez *et al.* (1999); 15.4% by Omer *et al.* (2002); 11.1% by Sayin *et al.* (2003);19.7% by Dumanli *et al.* (2005); 16.26% by Aktas *et al.* (2006); 31.06% by Ananda *et al.* (2009); 6.8% by Durrani *et al.* (2010); 3% by Shahnawaz *et al.* (2011); 5.2% by Khattak *et al.* (2012), 10.66% by Saeid *et al.* (2013), 7.31% by Ariyaratne *et al.* (2014), 27.20% by Kohli *et al.* (2014), 14.29% by Singh *et al.* (2014), 42.86% in piroplasmic form and 17.9% in schizont form by Modi *et al.* (2015), 9.23% by Tuli *et al.* (2015), 12.8% by Ullah *et al.*(2021) and 10.8% by Valente *et al.* (2023).

According to studies by Stockham *et al.* (2000); Singh *et al.* (2001), the primary causes of the aberrant erythrocyte shape are the toxic effect of parasites on the erythrocytes, erythrocyte oxidation, and immune-mediated processes. The analysis of lymph node aspirate smears and thin blood stained with Giemsa is required for the conventional diagnosis of tropical theileriosis. According to studies by Stockham *et al.* (2000); Singh *et al.* (2001), the primary causes of the aberrant erythrocyte shape are the toxic effect of parasites on the erythrocytes, erythrocyte oxidation, and immune-mediated processes.

CONCLUSIONS

The prevalence of *Theileria annulata* in cattle-calves was determined by the examination of Giemsa-stained blood smears and lymph node aspirate smears and it was found 7.5% and 3.2%, respectively. The analysis of lymph node aspirate smears and thin blood stained with Giemsa is required for the conventional diagnosis of tropical theileriosis. This technique is only effective during the acute phase of the illness, when the parasitemia is high enough to be seen under a microscope. The degree of parasitemia is typically below the threshold for microscopical detection during the chronic and carrier stages.

FUTURE SCOPE

Due to low sensitivity of the conventional methods, new alternative methods such as molecular detection will be carried out to find out even a very low infection and also the carrier animals.

Acknowledgements. The College of Veterinary and Animal Science, Bikaner, Rajasthan's Dr. S.K. Kashyap, Professor and Head of the Department of Microbiology and Biotechnology, and Dr. G.C. Gahlot, Professor and Head of the Department of Animal Genetics and Breeding, are greatly appreciated by the authors for providing the facilities needed to conduct this study.

Conflict of Interest. None.

REFERENCES

Aktas, M., Altay, K. and Dumanli, N. (2006). A molecular survey of bovine *Theileria* parasites among apparently healthy cattle and with a note on the distribution of ticks in eastern Turkey. *Veterinary Parasitology, 138*, 179–185.

Al-Emarah, G. Y. A., Khudor, M. H. and Daham, H. R. (2012). Clinical, haematological and biochemical study to cattle naturally infected with *Theileria annulata* in north of Basrah province, AL-Qadisiya. *Journal of Vet. Med. Sci., 11*(1), 54-62.

Ananda, K. J., D'Souza, P. E. and Puttalakshmamma, G. C. (2009). Prevalence of haemoprotozoan diseases in cross-bred cattle in Bangalore North. *Veterinary World, 2*(1), 15–16.

Ariyaratne, M. E. S. A. De S., Gothami, W. S. and Rajapakse, R. V. P. J. (2014). Application of PCR technique on confirming *Theileria* Infection in Cattle and Buffaloes with determining the relationship between Animals' PCV and WBC Count with the Infection. *International Journal of Scientific and Research Publications, 4*(7), 2250-3153.

Bakheit, M. A., Schnittger, J., Salih, D. A., Boguslawski, K., Beyer, D., Fadl, M. and Ahmed, J. S. (2004). Application of the recombinant *Theileria annulata* surface protein in an indirect ELISA for the diagnosis of tropical theileriosis. *Parasitology Research, 92*, 299–302.

Branco, S., Orvalho, J., Leitao, A., Pereira, I., Malta, M., Mariano, I., Carvalho, T., Baptista, R., Shiels, B.R. and Peleteiro, M. C. (2010). Fatal cases of *Theileria annulata* infection in calves in Portugal associated with neoplastic-like lymphoid cell proliferation. *Journal of Veterinary Science*, *11*(1), 27-34.

Dumanli, N., Aktas, M., Cetinkaya, B., Cakmak, A., Koroglu, E., Saki, C. E., Erdogmus, Z., Nalbantoglu, S., Ongor, H., Simsek, S., Karahan, M. and Altay, K. (2005). Prevalence and distribution of tropical theileriosis in Eastern Turkey. *Veterinary Parasitology, 127*, 9–15.

Durrani, A. Z. and Kamal, N. (2008). Identification of ticks and detection of blood protozoa in Friesian cattle by polmerase chain react on test and estimation of blood parameters in district Kasur, Pakistan. *Trop. Anim. Health Prod., 40*, 441–447.

Durrani, A. Z., Mahmood, N. and Shakoori, A. R. (2010). Comparison of three diagnostic methods for *Theileria*

annulata in Sahiwal and Friesian cattle in Pakistan. *Pak. J. Zool.*, *42*(4), 467–472.

Durrani, A. Z., Shakoori, A. R. and Kamal, N. 2008. Bionomics of *Hyalomma* ticks in three districts of Punjab. *Pakistan J. Anim. Plant Sci.*, *18*(1), 20–23.

Gill, B.S., Bhattacharyulu, Y. and Kaur, D. (1977). Symptoms and pathology of experimental bovine tropical theileriosis (*Theileria annulata* infection). *Annales de Parasitologie Humaine et Comparee, 52*,597-608.

Hasanpour, A., Sabegh, Y. G. and Sadeghi-nasab, A. (2013). Assessment of serum antioxidant enzymes activity in cattle suffering from theileriosis. *European Journal of Experimental Biology*, *3*(1), 493–496.

Khan, I.A., Khan, A., Hussein, A., Riaz, A. and Aziz, A. (2011). Haemato-biochemical alterations in cross-bred cattle affected with bovine theileriosis in Semi Arid Zone. *Pak. Vet. Journal, 31*(2), 137-140.

Khattak, R. M., Rabib, M., Khan, Z., Ishaq, M., Hameed, H., Taqddus, A., Faryal, M., Durranis, S., Gillani, Q. U. A., Allahyar, R., Shaikh, R. S., Khan, M. A., Ali, M. and Iqbal, F. (2012). A comparison of two different techniques for the detection of blood parasite, *Theileria annulata*, in cattle from two districts in Khyber Pukhtoon Khwa province (Pakistan). http://www.parasite-journal.org or http://dx.doi. org/10.1051/ parasite/2012 191091, 91-95.

Kohli, S., Atheya, U. K. and Thapliyal, A. (2014). Prevalence of theileriosis in cross-bred cattle: its detection through blood smear examination and polymerase chain reaction in Dehradun district, Uttarakhand, India. *Veterinary World, 7*(3), 168-171.

Ma, Q., Liu, J., Li, Z., Xiang,Q., Wang, J., Liu, A., Li, Y., Yin, H., Guan, G. and Luo, J. (2020). Clinical and Pathological Studies on Cattle Experimentally Infected with *Theileria annulata* in China. *Pathogens, 9*(9), 727.

Martin-Sanchez, J., Viseras, J., Adroher, F.J. and Garcia-Fernandez, P. (1999). Nested polymerase chain reaction for detection of *Theileria annulata* and comparison with conventional diagnostic techniques: its use in epidemiological studies. *Parasitology Research, 85*, 243-245.

Modi, D. V., Bhadesiya, C. M. and Mandali, G. C. (2015). Haemato-biochemical Changes in Cross-bred Cattle Infected with *Theileria annulata* in Banaskantha District of Gujarat. *International Journal of Scientific and Research Publications, 5*(1), 1-4.

Omer, O. H., El-Malik, K. H., Mahmoud, O. M., Haroun, E. M., Hawas, A., Sweeney, D. and Magzoub, M. (2002). Haematological profiles in pure bred cattle naturally infected with *Theileria annulata* in Saudi Arabia. *Veterinary Parasitology, 107*, 161–168.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W. and Constable, P. D. (2007). Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats, tenth ed. Elsevier, Philadelphia.

Saeid, R. N. F., Khalili, M. and Ghalekhani, N. (2013). Detection of *Theileria annulata* in blood samples of native cattle by PCR and smear method in Southeast of Iran. *Journal of Parasitic Disease*.

Sayin, F., Karaer, Z., Dincer, S., Cakmak, A., Inci, A., Yukari, B. A., Eren, H., Vatansever, Z., Nalbantoglu, S. and Melrose, T. R. (2003). A comparison of susceptibilities to infection of four species of *Hyalomma* ticks with *Theileria annulata*. *Veterinary Parasitology, 113*(2), 115–121.

Shahnawaz, S., Ali, M., Aslam, M. A., Fatima, R., Chaudhary, Z. I., Hassan, M. U., Ali, M. and Iqbal, F. (2011). A study on the prevalence of a tick transmitted pathogen, *Theileria annulata*, the haematological profile of cattle from Southern Punjab (Pakistan). *Parasitology Research, 109*, 1155–1160.

Singh, A., Singh, J., Grewal, A. S. and Brar, R. S. (2001). Study on some blood parameters of cross-bred calves with experimental *Theileria annulata* infections. *Veterinary research communications, 25*, 289-300.

Singh, S. K., Sudan, V., Singh, A. P. and Yadav, B. K. (2014). Evaluation of Clinical Markers for Diagnosis of Bovine Theileriosis: A Study of 21 Calves. *Intas Polivet, 15*(1), 91-95.

Soulsby, E. J. L. (1982). Helmints, arthropods and protozoa of domesticated animals, seventh ed. Baillier, Tindall and Cassel Ltd., London.

Stockham, S., Kjemtrup, A., Conrad, P., Schmit, D., Scott, M., Robinson, T., Tyler, J., Jonson, G., Carson, C. and Cuddihee, P. (2000). Theileriosis in a Missouri beef herd caused by *Theileria buffeli*: case report, herd investigation, ultrastructure, phylogenetic analysis, and experimental transmission. *Veterinary Pathology, 37*, 11-21.

Tanwar, R. K., Gahlot, A. K., Yadav, J. S., Sharma, S. N. and Lodha, K. R. (1984). Theileriosis (*Theileria annulata*) in indigenous Rathi calves. *Indian Journal of Animal Science, 54*, 118-120.

Tuli, A., Singla, L.D., Sharma, A., Bal, M. S., Filia, G. and Kaur, P. (2015). Molecular epidemiology, risk factors and haematochemical alterations induced by *Theileria annulata* in bovines of Punjab (India). *Acta Parasitologica, 60*(3), 378–390.

Ullah, R., Shams, S., Khan, M. A., Ayaz, S., Akbar, N. U., Din, Q. U., Khan, A., Leon, R. and Zeb, J. (2021). Epidemiology and molecular characterization of *Theileria annulata* in cattle from central Khyber Pakhtunkhwa, Pakistan. *PLoS ONE*, *16*(9), e0249417.

Valente, D., Dutra, A.P., Carolino, N., Gomes, J., Coelho, A.C., Espadinha, P., Pais, j. and Carolino, I. (2023). Prevalence and Risk Factors Associated with *Theileria annulata* Infection in Two Bovine Portuguese Autochthonous Breeds. *Pathogens*, *12*, 669.

Zeb, J., Shams, S., Din, I. U., Ayaz, S., Khan, A., Nasreen, N. (2020). Molecular epidemiology and associated risk factors of *Anaplasma marginale* and *Theileria annulata* in cattle from North-western Pakistan. *Veterinary parasitology*, *279*, 109044.

How to cite this article: Pavan Goyal (2024). Hospital Based Prevalence of *Theileria annulata* in Cattle-calves in Bikaner District of Rajasthan. *Biological Forum – An International Journal, 16*(1): 132-135.