



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating: 4.61

VET 2025; 10(6): 235-236

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www.veterinarypaper.com

Received: 06-04-2025

Accepted: 09-05-2025

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Molecular detection of *theileria annulata* and evaluation of haematobiochemical parameters in case of theileriosis

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Abstract

The present investigation was carried out to evaluate haematobiochemical changes in case of theileriosis and PCR based detection of *Theileria annulata*. A case of buffalo having clinical signs of haemoprotozoan infection was presented at Veterinary Clinical Complex, Junagadh. Blood smear examination and PCR was carried out to identify *Theileria annulata*. Haematological analysis revealed low haemoglobin, low PCV and lower erythrocyte count. Biochemical analysis revealed hyperproteinemia and hypoalbuminemia.

Keywords: Haematology, PCR, *Theileria annulata*, hypoalbuminemia, theileriosis, present investigation

Introduction

Numerous tick-borne illnesses, such as anaplasmosis, bovine babesiosis, and tropical theileriosis, lead to significant health and management issues and lower productivity and financial losses in domestic animal production systems across the globe (Bilgic *et al.*, 2013) [2]. Potential vectors develop in the hot, humid atmosphere, which also serves as a continual source of infection for susceptible animals. (Sharma *et al.*, 2017) [6].

The protozoan parasites *Theileria annulata* and *Theileria parva* are the cause of bovine theileriosis. The illness is recognized as one of the most devastating hurdles to the production of animals (Durani *et al.*, 2008) [3]. Pyrexia, anorexia, enlargement of superficial lymph nodes, mild nasal and ocular discharges with conjunctival congestion, and salivation are clinical indicators of infected animals (Islam *et al.*, 2017) [4].

Clinical observations and microscopic analysis of thin blood smears stained with Giemsa are the primary methods used to diagnose theileriosis. Since the PCR was developed, molecular diagnostic techniques that are sensitive and specific have been developed to identify and describe the organisms that cause theileriosis (Sharma *et al.*, 2017) [6]. Molecular detection of *Theileria annulata* and monitoring of the haemato-biochemical alterations in case of theileriosis were the objectives of the current study.

Materials and Methods

- **History and clinical observations:** A 4 year buffalo with history of high fever, anorexia and tick infestation was presented at Veterinary Clinical Complex, Veterinary College, Junagadh. Clinical examination reveals pale mucous membrane, rough body coat and enlarged superficial lymph nodes.
- **Haemato-biochemical analysis:** Approximately 4 ml blood was drawn from animal and hematological analysis was performed by BC 2800 Vet auto hematology analyzer. Various parameters i.e. hemoglobin (HB), Total Erythrocyte Count (TEC) and packed cell volume (PCV), were recorded. Biochemical analysis was performed by biochemical analyzing reagents kits (Randox laboratories, Northern Ireland) with the use of Microlab 300 semi-automatic biochemical analyzer. Various biochemical parameters i.e. alanine amino-transferase (ALT), total protein and albumin were recorded.

- **Blood Smear Examination:** Thin blood smear was prepared from collected blood and stained by the giemsa stain and observed under oil immersion in microscope to identify *Theileria annulata*.
- **Molecular detection of *Theileria annulata*:** For molecular detection PCR was carried out using primers (forward 5'-ACT TTG GCC GTA ATG TTA AAC and reverse 5'-CTC TGG ACC AAC TGT TTGG) as per cycling conditions described by Bilgic *et al.* 2013 [2].

Results

Giemsa-stained blood smear analysis under an oil immersion revealed signet ring-shaped *Theileria* spp. in erythrocytes (Figure 1). The result of PCR shows amplification of targeting 312 bp amplicon confirmed the presence of *Theileria annulata* (Figure 2).

Haematological investigation revealed hemoglobin 7.2 g/dl, packed cell volume 26%, and red blood cells count $4.42 \times 10^6/\mu\text{l}$, indicating anemia in the affected animal. Furthermore, the results of bio-chemical analysis revealed total protein 5.1 g/dl, albumin 2.13 g/dl and ALT 46 IU/L.

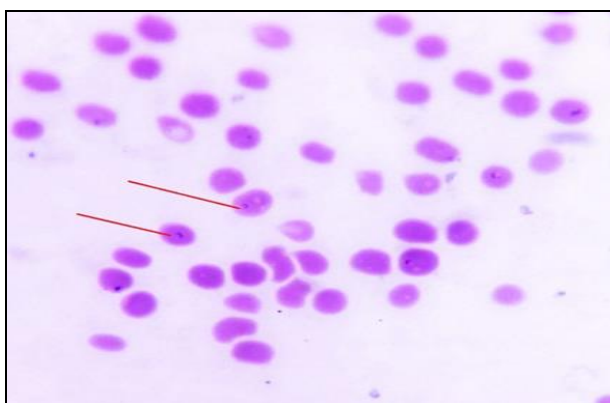


Fig 1: Blood smear shows *Theileria* spp.

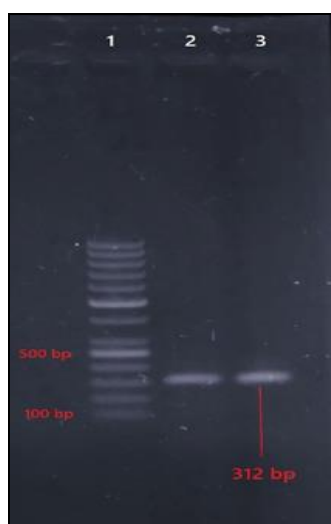


Fig 2: PCR detection shows 312 bp amplicon of *Theileria annulata*

Discussion

Haematological levels declined, indicating anemia, which could be caused by macrophages in the spleen, lymph nodes, and other reticuloendothelial system organs destroying erythrocytes (Kaur *et al.*, 2020) [5]. The hyperproteinemia and hypoalbuminemia is possibly due to the harmful effect of toxic metabolites of *Theileria* and due to liver failure (Al-Emarah *et al.*, 2012) [1]. PCR-based detection can accurately

diagnose hemoprotozoa at the species level, it has an advantage over blood smear screening.

Conclusion

The current study highlights the significant impact of *Theileria annulata* on bovine health, evidenced by clinical signs, hematological disturbances, and molecular confirmation. Molecular techniques like PCR offer highly sensitive and specific diagnosis, surpassing traditional blood smear methods. Theileriosis causes anemia, hyperproteinemia, and liver dysfunction, posing a major threat to animal productivity and management. Early detection and accurate diagnosis are crucial for effective control and treatment strategies. Understanding the disease's pathology and employing advanced diagnostic tools can help mitigate economic losses and improve animal health outcomes in endemic regions.

Conflict of Interest: Not available

Financial Support: Not available

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How to Cite This Article

Gundaliya KG, Fefar DT, Kachara SH, Parasana DK. Molecular detection of *theileria annulata* and evaluation of haematobiochemical parameters in case of theileriosis. *International Journal of Veterinary Sciences and Animal Husbandry.* 2025;10(6):235-236.

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