

## Cutaneous and ocular signs in a calf infected with *Theileria annulata* - case report

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**Abstract:** Tropical theileriosis is an important tick-borne disease in cattle that is caused by an obligate intracellular protozoan, *Theileria annulata* (*T. annulata*). In addition to the common clinical signs of tropical theileriosis such as pyrexia, lymph node enlargement, conjunctival petechiae, and anemia, veterinary practitioners may encounter unexpected clinical signs such as skin nodules and bilateral exophthalmos due to conjunctival edema. The main purpose of this report is to present clinical manifestations and histopathological findings of extensive cutaneous nodules and conjunctival edema in a calf infected with *T. annulata*.

**Keywords:** Calf, conjunctival edema, exophthalmos, skin nodules, tropical theileriosis

### **Theileria annulata ile enfekte bir buzağıda kutanöz ve oküler bulgular**

**Özet:** Tropikal theileriosis, obligat intrasellüler bir protozoon olan *Theileria annulata* (*T. annulata*)'nın neden olduğu sığırların kene kaynaklı önemli bir hastalığıdır. Ateş, lenf yumrularında büyüme, konjunktivada peteşi ve anemi gibi klasik bulgulara ilaveten, klinisyenler deride nodül oluşumu ve konjunktiva ödeminde kaynaklanan bilateral ekzoftalmus gibi nadir gözlemlenen klinik bulgularla karşılaşabilir. Bu raporda *T. annulata* ile enfekte bir buzağıda yaygın deri nodülleri ve konjunktiva ödeminin klinik görünümü ve deri lezyonlarından alınan örneklerin histopatolojik bulguların sunulması konu edilmiştir.

**Anahtar Kelimeler:** Buzağı, konjunktiva ödemi, ekzoftalmus, deri nodülleri, tropikal theileriosis

### **Introduction**

Tropical theileriosis is an important tick-borne disease in cattle that is caused by an obligate intracellular protozoan, *Theileria annulata* (*T. annulata*) (Brown, 1990). It is transmitted to cattle by Hyalomma ticks and particularly affects nonimmunized cattle. The disease is responsible for significant economic losses in the livestock industry in tropical and subtropical regions of the world, including Turkey (Uilenberg, 1995).

The clinical presentation of tropical theileriosis may vary from subacute to chronic disease depending on the host susceptibility, the quantity of the inoculated sporozoites, and the damaging effect of the pathogen on lymphoid tissue ( Gill et al., 1977; Altay & Aktas, 2004). The common clinical signs are pyrexia, enlargement of superficial lymph nodes, lacrimation, nasal discharge, conjunctival petechia, and anemia (Gill et al., 1977; Aulakh & Singla, 2006). In addition to these classic signs of tropical theileriosis, the conjunctival edema and formation of nodular lesions on the skin in tropical theileriosis have been reported in a limited number of cases ( Muhammad et al., 1999; Branco et al., 2010; Oryan et al., 2013; Gharbi et al., 2017). To the best of our knowledge, there is no report about the detailed presentation of conjunctival edema and cutaneous nodular lesions in Tropical Theileriosis in Turkey.

The main purpose of this report is to present clinical manifestations and histopathological findings of extensive cutaneous nodules and conjunctival edema in a calf infected with *T. annulata*.

### **Case Description**

A 1-month-old calf was presented to Firat University Veterinary Teaching Hospital. The history revealed that the calf had dyspnea, inappetence, and weakness for 5 days. Physical examination findings included increased lung sounds, tachypnea (120 breaths/minute), tachycardia (180 beats/minute), increased body temperature (40.8 °C), bilateral enlargement of the superficial cervical lymph nodes, petechia on the muzzle, bilateral exophthalmos, conjunctival edema and icterus (Figure 1B-C). In addition, extensive cutaneous hemorrhagic nodules ranging in diameter from 0.5 to 2.0 cm were palpated on the skin over the entire body surface (Figure 1A).



Figure 1: Cutaneous hemorrhagic nodules on the skin (A), Bilateral exophthalmos due to conjunctival edema and petechia on the muzzle (B), Conjunctival edema and icterus (C)

To examine the hematological changes, a 2 mL blood sample was collected from the jugular vein into the EDTA-containing tube and analyzed with an autoanalyzer (Prokan PE-6800 Vet, China). The peripheral blood smear was prepared from an ear tip blood sample and stained with Giemsa to examine the presence of blood parasites. In addition, two different skin nodules were excised for the histological examination. The nodule specimens were fixed in the formalin solution, embedded in paraffin, sectioned at 3  $\mu$ m, stained with hematoxylin&eosin and examined using a light microscope (Olympus BX43, Tokyo, Japan). Histological examination showed hyperkeratosis, excessive thinning of the squamous epithelium layer, granuloma formations, lymphangitis, and severe lymphohistiocytic inflammatory cell infiltrations in the epidermis, dermis, subcutaneous muscle layer, and around hair follicles (Figure 2A-F).



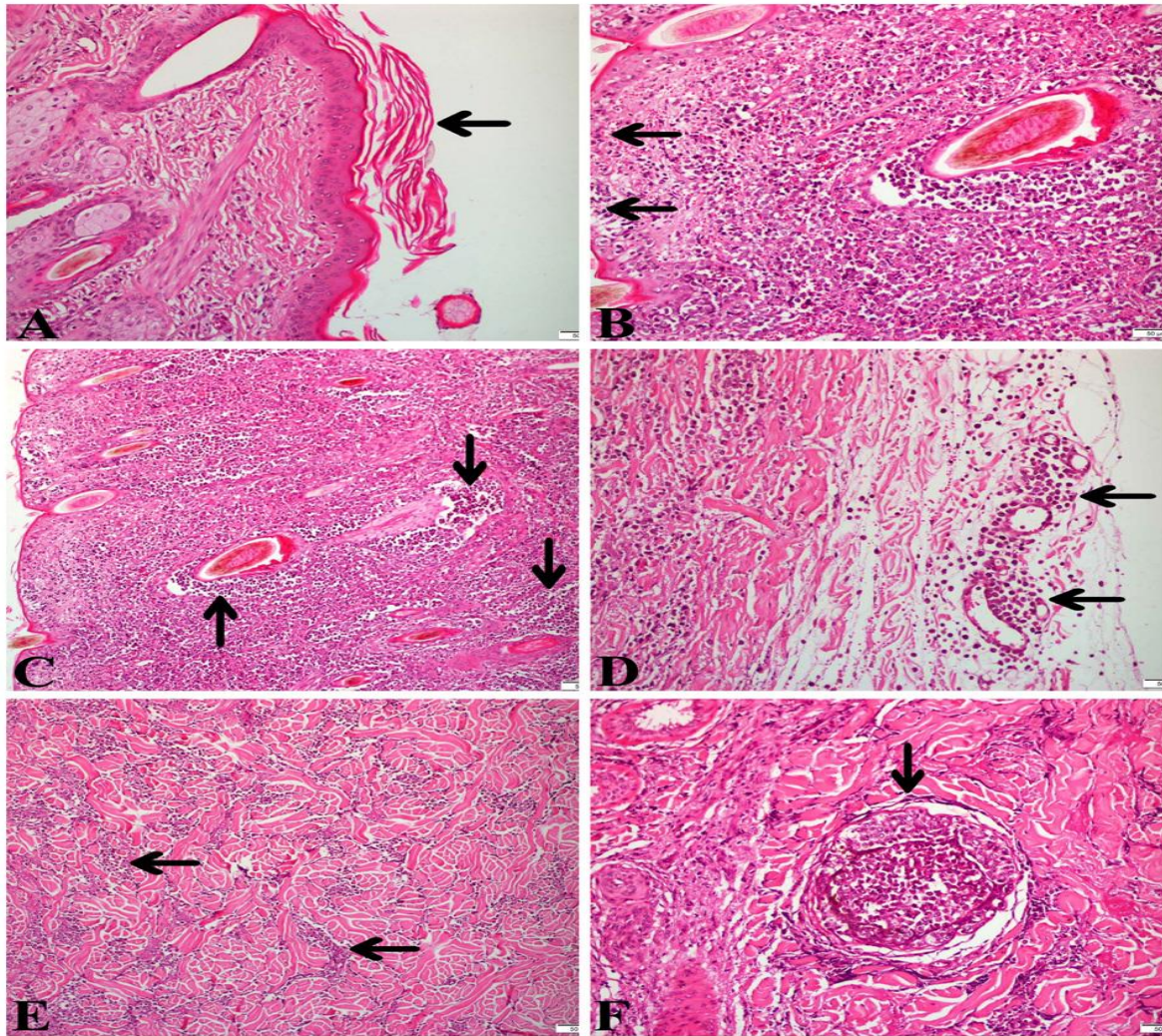


Figure 2: Hyperkeratosis (A), thinned epithelium layer (arrows) (B), severe inflammation in epidermis and dermis, particularly around the hair follicles (arrows) (C), lymphangitis, with mononuclear cells (arrows) (D), lymphohistiocytic inflammatory cells between the muscle fibers (arrows) (E) and a granuloma formation in muscle tissue (arrow) (F).

Hematological examination results revealed moderate anemia (Table). The peripheral blood smear was examined using a light microscope at 100x magnification and *T. annulata* piroplasms were detected within erythrocytes (Figure 3).

Table: The results of the hematological analysis

Parameters	Results	Reference*
WBC count (x 10 <sup>3</sup> /μL)	6.7	4-12
RBC count (x 10 <sup>6</sup> /μL)	4.45	5-10
Hemoglobin (g/dL)	6.2	8-15
Hematocrit (%)	20	24-46
MCV (fL)	52.18	40-60
MCHC (g/dL)	26.8	30-36
Thrombocytes (x 10 <sup>9</sup> /μL)	309	100-800

WBC: white blood cell; RBC: red blood cell; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; \*(Radostits et al., 2007)

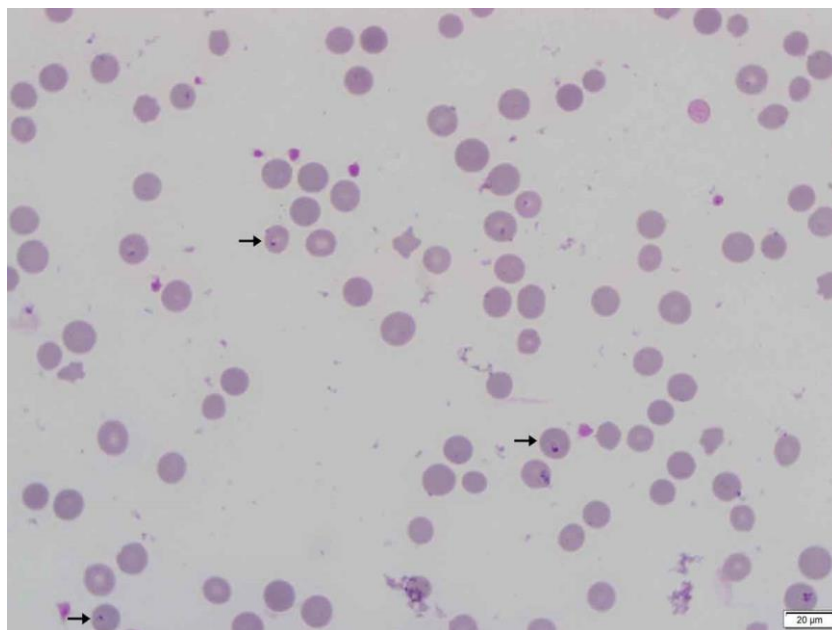


Figure 3: *Theileria annulata* piroplasms within erythrocytes (arrows)

Based on the clinical findings and the results of the hematology and blood smear, the calf was tentatively diagnosed with Tropical Theileriosis. To confirm the presence of *T. annulata*, nested PCR was performed. The primers Nbab1F (5'-AAGCCATGCATGTCTAAGTATAAGCTTTT-3') and Nbab1R (5'-CCTCTCCTTCCTTTAAGTGATAAGGTTTAC-3') were used for first amplification of an approximately 1600 bp fragment of the 18S rRNA gene in *Theileria* and *Babesia* spp. (Oosthuizen et al., 2009). The nested amplification performed using the reverse line blotting

(RLB) primers RLB-F2 (5'-GACACAGGGAGGTAGTGACAAG-3') and RLB-R2 (5'-biotin CTAAGAATTTACCTCTGACAGT-3') generated 492–498 bp-long fragment from the V4 hypervariable region of the 18S ribosomal RNA (rRNA) gene (Georges et al., 2001). According to the RLB assay, the sample was found to be infected with *T. annulata*.

The calf was treated with a single injection of buparvaquone (Butalex, MSD Animal Health, Turkey) at a dose of 2.5 mg/kg and a daily injection of oxytetracycline (Primamycin LA, Zoetis, USA) at a dose of 20 mg/kg. The calf gradually recovered, and cutaneous nodules and conjunctival edema completely disappeared after 25 days of the treatment.

### Discussion

Conjunctival edema and the formation of nodular lesions on the skin are unusual clinical findings in Tropical Theileriosis. A previous study performed on 112 cattle with Tropical Theileriosis revealed that only 9.8% and 14.3% of the clinical cases showed nodular lesions on the skin and conjunctival edema, respectively (Muhammad et al., 1999). Whereas, in the same study, the distribution of classical signs such as pyrexia, enlargement of superficial lymph nodes, lacrimation, and anemia were found 100%, 88.4%, 71.4%, and 42.7%, respectively. Because of the low frequency of dermatological and ocular findings, veterinary practitioners may overlook these clinical signs unless they are seen in conjunction with classic findings such as fever, lacrimation, enlargement of superficial lymph nodes, and anemia.

The results of the histopathological examination of nodule specimens revealed hyperkeratosis, excessive thinning of the squamous epithelium layer, granuloma formations, lymphangitis, and severe lymphohistiocytic inflammatory cell infiltrations in the epidermis, dermis, subcutaneous muscle layer and around the hair follicles. In a previous report, the researchers stated that pathological lesions that occurred in different organs and tissues are caused by the proliferation of the infected macrophages (Forsyth et al., 1999). In another study, uninfected mature T lymphoid cells and macrophages were detected in different organs (Branco et al., 2010). In that study, the authors also noted that pathological lesions may be associated with the overproduction of interferon gamma (IFN- $\gamma$ ) and (interleukin) IL-2 by naïve T cells, leading to the proliferation of infected and noninfected macrophages. Some researchers showed the presence of intra- and extracellular schizonts with immunohistochemistry of the nodules by labeling tissue sections with an antibody (Mab 1C7) that reacts with schizonts in the tissue sections (Oryan et al., 2013; Branco et al., 2010).

## Conclusion

Veterinary practitioners should consider Tropical theileriosis in the differential diagnosis when they encounter the signs such as skin nodules and conjunctival edema.

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## Ethical Statement

This study does not present any ethical concerns.

## Conflict of Interest

The authors declared that there is no conflict of interest.

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