

Dermatological symptoms in tropical theileriosis (*Theileria annulata* infection), a review

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Summary

Bovine tropical theileriosis (*Theileria annulata* infection) is an important tick-borne disease in several regions of the world. This paper describes two clinical cases of tropical theileriosis in Northern Tunisia with the uncommon symptom of skin nodules. The density of nodules was estimated at 15–20 per 10 cm²; they were distributed over the animal's body and were 0.5–2 cm in diameter.

Microscopic examination of the skin nodules 18 days after treatment onset showed a perivascular infiltrate of lymphocytes, macrophages and eosinophils. Nevertheless, no schizonts were observed. Appropriate treatment led to the recovery of one of the two animals with total disappearance of the skin lesions; the other animal died. A

review of clinical cases of tropical theileriosis (*T. annulata* infection) with cutaneous lesions is presented. Various dermatological symptoms are described in the literature: skin nodules, haemorrhagic lesions, cutaneous gangrene, etc. Most authors reported the presence of schizonts in the nodular lesions. Dermatological involvement in bovine tropical theileriosis should be considered by field veterinarians and should be differentiated from other diseases with dermatological symptoms.

Keywords

Cattle – Nodule – *Theileria annulata* – Tunisia.

Introduction

Bovine tropical theileriosis (*Theileria annulata* infection) is a protozoan disease transmitted by several tick species all belonging to the genus *Hyalomma* (1). It is an important tick-borne disease in several countries. The protozoan has a wide geographical distribution including North Africa, Southern Europe and a large part of Asia. Because the disease is associated with high economic losses and some mortality, the identification of clinical cases of tropical theileriosis by field veterinarians represents a cornerstone in controlling this disease and reducing its economic impact. This requires an excellent and exhaustive knowledge of all the symptoms that may occur (2). The symptoms of tropical theileriosis are polymorphic and expressed at different frequencies, which may lead to misdiagnosis and an increase of the impact of the disease. Among these symptoms, several authors have reported cutaneous lesions due to *T. annulata* infection, including nodular, haemorrhagic and/or necrotic lesions. Skin lesions have been reported in different regions of the world in cattle and buffaloes (3).

Two clinical cases of tropical theileriosis in cattle that expressed skin lesions in Northern Tunisia are reported herein. In the second part of this paper, most of the published cases of dermatological lesions due to tropical theileriosis are reviewed.

Case report

Symptoms

Two Holstein–Friesian cattle from a farm consisting of seven animals in the North of Tunisia (Governorate of Ariana) presented symptoms of tropical theileriosis (fever, lymph node enlargement and hyporexia) with skin lesions in August 2015. The barn contained cracks and crevices favourable for the development of the tick vector *Hyalomma scupense* (1). The animals were examined and blood samples were collected in ethylenediamine tetraacetic acid (EDTA). Giemsa-stained blood smears showed the presence of *T. annulata* piroplasms, with a percentage parasitaemia of 70% and 20% in the affected cow and the heifer, respectively. The five-year-old cow presented fever, an increase in heart and respiratory rates, salivation, and was in poor physical condition, with oedema of the jaw and unilateral oedema of an eyelid (Table I). The vulva, udder and medial thighs showed haemorrhagic suffusion. The cow aborted at seven months of pregnancy (Figs 1, 2 and 3) and died despite treatment.

Insert Table I and Figs 1, 2 and 3

The heifer was two years old and 2.5 months pregnant; she presented the same symptoms but they were much milder (Table I), except for the ocular symptoms. The oedema of the jaw was also less severe. There was a severe anaemia, but of different intensity, in both animals (Table II).

Insert Table II

The anaemia of the cow was severe, with low levels of erythrocytes ($3.6 \times 10^{12}/l$ [reference range: $5-10.10 \times 10^{12}/l$]) and haemoglobinaemia (7 g/dl [reference range: 9–13.9 g/dl]); there was also a slight increase in granulocytes (70.8% [reference range: 30–65%]). Blood samples were collected from the animals into EDTA tubes; the blood smears were stained with Giemsa and examined under a microscope at $\times 1,000$ magnification. The blood samples showed the presence of *T. annulata* piroplasms.

Polymerase chain reaction

The DNA was extracted from blood using a Genomic DNA Prep Kit (Biobasic Inc, Ontario, Canada) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed with a set of primers (N517: 5'-GTTACGAACATGGGTTT-3' and N516: 3'-GTAACCTTTAAAAACGT-5') (Biobasic, Canada) that amplify a 721 bp (base pair) region of the *T. annulata* merozoite gene sequence (Tams-1) (5, 6).

The polymerase chain reaction (PCR) mix consisted of 2 mM of each dinucleotide triphosphate (dNTP), 0.5 μ M of each primer, 0.05 U/ μ l Taq polymerase (Vivantis, United States of America), 1 \times Taq buffer (10 \times), 3 mM of MgCl₂ (25 mM), 3 μ l of DNA template and distilled water to a total volume of 25 μ l. The cycling conditions consisted of an initial denaturation step for 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 1 min, followed by a final elongation at 72°C for 10 min. The amplified PCR products were electrophoresed in 1.5% agarose gels and visualised by staining with ethidium bromide under an ultraviolet (UV) transilluminator using a Biospectrum® AC Imaging System. The electrophoresis showed specific positive blood samples.

Histopathology of nodules

The two cattle presented several skin nodules (Table III, Fig. 4). Samples from the nodules were examined directly and after Giemsa staining. Schizonts of *T. annulata* were not observed, nor were *Demodex* mites as reported by Uilenberg and Zwart (3) in cases of East Coast fever. Tissue samples from nodules were surgically excised, fixed in 10% formalin, embedded in paraffin, sectioned at 3 μ m, stained with haematoxylin and eosin and examined by light microscopy. There was a perivascular infiltrate of lymphocytes, macrophages and eosinophils. Nevertheless, no schizonts were observed (Fig. 5). This could be explained by the fact that, owing to practical constraints, the biopsy was taken 18 days after buparvaquone treatment.

Insert Table III and Figs 4 and 5

Treatment and outcome

Treatment of both animals involved intramuscular injection of buparvaquone (Teldex, Médivet) at the conventional dose of 2.5 mg/kg, oxytetracycline (Engemycine 10%, MSD Santé Animale) at 10 mg/kg, flunixin meglumine (Fluxydin, Médivet) at 2.2 mg/kg, a cocktail containing vitamin B12 (Hémathopan B12, Merial) (20 ml/animal) and a stimulant of rumen function (Rumactyl, Médivet). The animals were treated against ticks with deltamethrin (Vectocid 50 ND, Céva) at a concentration of 50 ppm twice a month. After two days, the cow died and the heifer recovered totally. At day 18, all the nodules except one had disappeared from the skin of the recovered animal (Figs 6 and 7).

Insert Figs 6 and 7

Review of skin lesions due to *Theileria annulata* infection

***Theileria annulata* induced skin lesions**

Bovine dermatology is a neglected speciality in bovine pathology; this could be due to:

- the low level of care given by farmers to the aesthetic aspects of cattle compared with pet animals
- the possibility of culling diseased animals
- the fact that dermatology is a difficult field and needs specific skills.

Some dermatological symptoms are the expression of severe systemic diseases, which should be diagnosed and rapidly treated. In this paper, tropical theileriosis (*T. annulata* infection) is discussed; in this disease cutaneous symptoms appear occasionally subsequent to the acute phase (7). Indeed, several scattered publications have reported cutaneous symptoms due to tropical theileriosis. As reported above, two cases of cutaneous expression of bovine tropical theileriosis were

found in Tunisia, and it is believed that an overview of the majority of clinical cases reported in the world literature will be very useful. These skin lesions have been reported for both *T. annulata* and *T. parva* infections (3). Most authors describe the symptoms of tropical theileriosis without reporting any skin lesions; hence, a synoptic review of all reported dermatological lesions in cattle with tropical theileriosis symptoms is presented in Table IV. The country of origin is included in the table, because there are genetic differences among *T. annulata* populations (8).

Insert Table IV

Differential diagnosis of skin lesions of tropical theileriosis

Table V contains a list of diseases giving similar cutaneous lesions. Although the cutaneous form of tropical theileriosis is easily differentiated from dermal diseases associated with pruritus (lice, flea infestations, etc.), the association of tropical theileriosis and any ectoparasite is of course possible. For example, Uilenberg and Zwart (3) reported one case of cutaneous nodules in which both *T. parva* schizonts and *Demodex bovis* were found. Multiplication of *Demodex* parasites was attributed to the immunosuppression caused by East Coast fever.

Insert Table V

Conclusion

Dermatological symptoms in bovine tropical theileriosis are considered rare. Scattered clinical cases have been reported in several enzootic regions of the world. The presence of skin nodules may indicate a bad prognosis (14); in Tunisia the majority of these animals die as a consequence of late diagnosis. Veterinarians in enzootic regions should be aware of this type of symptom, which is expressed by an unknown number of cattle. Adequate preventive measures should be implemented to avoid complications (bacterial infections and myiases) of these skin lesions.

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Table I
Comparison of the bovine tropical theileriosis symptoms before and after treatment

Abnormal findings are indicated in bold characters

Clinical indicator [reference values]	Cow (animal 1) ^(a)		Heifer (animal 2)	
	Before treatment		Before treatment	Four days after treatment
Age (years)	5		2	
Eyelids	Unilateral oedema		Normal	Normal
Lymph nodes	Enlarged		Normal	Normal
Temperature (°C) [38 to 39]	40.5		39	38.8
Heart rate (bpm) [65 to 80]	122		71	65
Respiratory rate (cpm) [15 to 35]	75		36	32
Mucosae	Pale		Slightly pale	Normal
Parasitaemia	70		20	ND

(a) The cow died after treatment

ND: not done

bpm: beats per minute

cpm: cycles per minute

Table II**Haematological results of the diseased cattle**

Reference values were adapted from Blood & Radostits (4)

Indicator	Diseased cow	Diseased heifer	Reference values
Leucocytes ($10^9/l$)	7.2	12.8 (H)	4–12
Lymphocytes ($10^9/l$)	1.4 (L)	6	2.0–7.0
Monocytes ($10^9/l$)	0.7	1 (H)	0–0.8
Granulocytes ($10^9/l$)	5.1	5.8	2.3–9.1
Lymphocytes (%)	19.4 (L)	46.9	20–60.3
Monocytes (%)	9.8	8.1	4–12.1
Granulocytes (%)	70.8 (H)	45	30–65
Erythrocytes ($10^{12}/l$)	3.60 (L)	5.16	5.0–10.0
Haemoglobin (g/dl)	7 (L)	8.4	8.0–15.0

L: Low

H: High

Table III**Characteristics of the skin nodules associated with *Theileria annulata* infection in the two reported clinical cases**

Location	Diameter (cm)	Approximate density/10 cm ²
Neck	0.5–1.5	18–22
Abdomen, chest and thoracolumbar region	1–2	15–20

Table IV**A review of reported dermatological lesions in clinical cases of tropical theileriosis (*Theileria annulata* infection) throughout the world**

To give an exact clinical and microscopic description, in some parts of the table, the text was transcribed as reported by the authors and should not be considered as plagiarism

Country (host species)	Number of skin cases/number of diseased	Description of symptoms	Microscopic description	References
Algeria (<i>Bos taurus</i>)	4	Only calves presented these symptoms, they appeared during acute phase then disappeared when the animals recovered. Two types of skin lesion were reported: - Localised dry gangrene on the internal face of the thighs and hock. When the skin is eliminated, bedsores and ulcerations are slow to resolve - Lenticular haemorrhagic eruptions, sometimes with nodules or vesicles affecting mucosae (scleral, pituitary, internal face of the lips and dental pad)	Periarterial lesions with schizonts more numerous than in spleen	(9)
Country not mentioned (<i>Bos taurus</i>)	NR	Observed in cattle with brown colour, exclusively observed in pie coloured animals Extensive severe skin inflammation. Dry loss of large parts of skin then replaced by new skin Constant association with digestive symptoms	ND	(10)
Israel (<i>Bos taurus</i>)	2	Case 1 (bull): erythematous eruption on the skin with numerous papules and pustules, particularly in the non-pigmented areas. The bull recovered Case 2 (cow): erythematous skin with haemorrhages and papular eruptions	Infiltration with lymphatic cells Numerous intra- and extracellular schizonts	(7)
India (<i>Bos taurus</i>)		Cross-bred calves Haemorrhagic spots or patches in the skin of ear, medial thighs, axilla, perineal region, around anus and caudal fold Extensive haemorrhage in the subcutis in the axial and perineal region	Focal haemorrhages with parasitised lymphocytes packed in these areas	(11)
India (<i>Bos taurus</i>)	2	36 days, and a 1.5-month-old cross-bred calf Papules (2–4 mm in diameter) over the dorsal surface up to thoracic region and on	Infiltration of lymphoid cells, mononuclear cells	(12)

		the sides of neck and shoulder. When pressed: small amount of reddish exudate	and macrophages in the dermis and in such places the epidermis had thinned out. Several macroshizonts (3–4 to 16 chromatin granules) and few microsizonts	
Pakistan (<i>Bos taurus</i>)	3/4	Haemorrhages (extensive in two calves) in the subcutaneous tissues in calves aged 4, 12 and 16 days	NR	(13)
India (<i>B. taurus</i> × <i>B. indicus</i>)	3/3	3-month-old calves Nodules 0.1–0.5 cm in diameter, haemorrhagic in the centre, on the face, neck and shoulder, extending to other parts of the body Severe itching; died after 2–3 days	Denuded epidermis, presence of schizonts Infiltrated by inflammatory cells and neutrophils Macrophages and rare lymphocytes Necrosis in the centre of the nodule	(14)
Iran (<i>Bos taurus</i>)	NR	On the whole body surface: numerous subcutaneous or intradermal nodules (0.3–1 cm in diameter), some of them quite prominent	Focal haemorrhages and aggregates of parasitised lymphocytes	(15)
Iran (<i>Bos taurus</i>)	NR	Nodules only in calves less than 2 months old	>15–20 schizonts per microscopic field	(16)
China (<i>Bos taurus</i>)	NR	Papular urticaria of skin lesions	Presence of schizonts	(17)
India (<i>Bos taurus</i>)	NR	Red papular cutaneous lesions 0.5–1 cm in diameter mainly in the skin of neck and perineal region Intense itching and scratching of the body	Large number of the theilerial schizonts in lymphoid cells	(18)
Pakistan (<i>Bos taurus</i>)	11/112	Innumerable small (0.5–1 cm in diameter) swellings under the skin and muscles	NR	(19)
Egypt (<i>Bubalus bubalis</i>)	2/68	Not described by the authors	NR	(20)
Portugal (<i>Bos taurus</i>)	15	<i>Symptoms:</i> Multifocal to coalescent white nodular skin lesions (0.2–3 cm diameter), similar to multicentric malignant lymphoma (sometimes haemorrhagic or with a haemorrhagic halo)	<i>Microscopy:</i> large, round, lymphoblastoid neoplastic-like cells <i>Immunohistochemistry:</i> mostly CD3 positive	(21)

Necropsy: haemorrhagic nodules and nodules with a haemorrhagic halo, particularly in the skin, subcutaneous tissue, skeletal and cardiac muscles, pharynx, trachea and intestinal serosa

T lymphocytes and MAC387-positive macrophages

Presence of *T. annulata* schizonts

Iran (<i>Bos taurus</i>)	11	Severe petechial and ecchymotic haemorrhages throughout the skin, particularly in hairless regions	Multifocal necrotic areas in the epidermis (22) Dermatitis in the dermis beneath the necrotic foci Presence of <i>T. annulata</i> schizonts in the cytoplasm of the lymphocytes and macrophages
Tunisia (<i>Bos taurus</i>)	2/2	15–22 cutaneous nodules/10 cm ²	Perivascular infiltrate of lymphocytes, macrophages and eosinophils. No schizonts were observed Present study

NR: not reported

Table V
Skin diseases to be differentiated from skin forms of tropical theileriosis (*Theileria annulata* infection)

Disease	Chronic evolution	Presence of other symptoms	Contagiousness	Impact on the general condition	Severity
Demodicosis (<i>Demodex bovis</i> infection)	+++	0	0	+	-
Warbles (<i>Hypoderma</i> spp. infestation)	+++	Possible	0	Possible	Generally 0
Besnoitiosis (<i>Besnoitia besnoiti</i> infection)	+++	0	0	++	++
Lumpy skin disease	++	Possible	+	Variable	Variable
Abscess	+++ or 0	Possible	-	Variable	Variable
Bovine leucosis	++	Possible	+	Variable	Variable
Allergy	0	0	0	Possible	Variable
Cutaneous tuberculosis (<i>Mycobacterium bovis</i> infection)	+++	Possible	+	Variable	Variable
Leptospirosis (<i>Leptospira</i> spp.)	+	+++	+	Variable	Variable

0: Absent

+ Rare

++ Moderately frequent

+++ Very frequent



Fig. 1
Oedema of the jaw in a cow with bovine tropical theileriosis

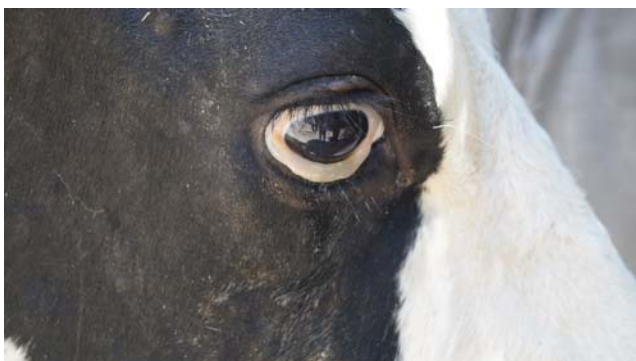


Fig. 2
Ocular oedema in a cow with bovine tropical theileriosis



Fig. 3
Haemorrhagic and ulcerative lesions in a cow with bovine tropical theileriosis



Fig. 4
Numerous skin nodules on the neck and shoulders of a cow with bovine tropical theileriosis

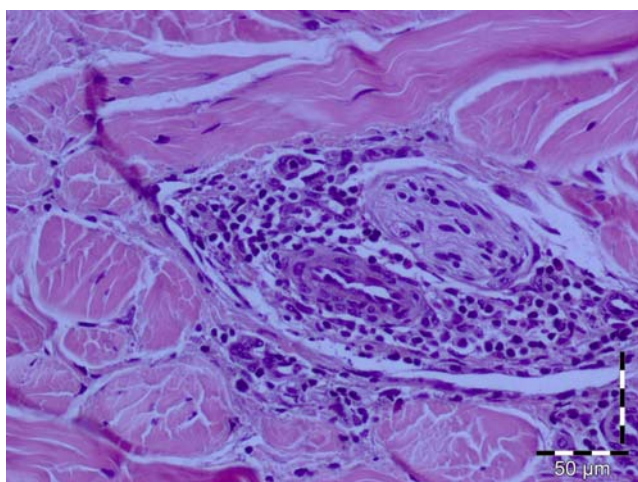


Fig. 5
Perivascular inflammatory infiltrate (lymphocytes, macrophages and eosinophils) (haematoxylin and eosin stain, ×400)



Fig. 6
Skin of a heifer with bovine tropical theileriosis four days after treatment

Note that a small nodule is still present on the left shoulder of the animal



Fig. 7
Nodules on medial thighs of a heifer with bovine tropical theileriosis four days after treatment