



Scientific contribution

# Clinical-Hematological Examination of Cattle Affected by Theileriosis

Nebieridze Shota <sup>1</sup>, Kereselidze Maia <sup>1\*</sup>, Beruashvili Maia <sup>1</sup>, Kvachrelishvili Vazha <sup>1</sup>, Matskepladze Marine<sup>1</sup>

<sup>1</sup>. European University, Tbilisi, Georgia

\* Correspondence: Maia Kereselidze; [maia.kereselidze@eu.edu.ge](mailto:maia.kereselidze@eu.edu.ge)

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## Abstract:

Theileriosis is the most severe transmissible disease among the piroplasmid diseases of ruminants, which is widespread all over the world, especially in the countries with a hot climate in the south, it is also found in Georgia. The source of infection is a sick animal, and the carrier is the pasture ticks *Hyalomma Detricum* and *Hyalomma anatolicum anatolicum*. The aim of the study was to investigate the dynamics of the clinical course and solution of the disease in the case of Theileriosis in cattle, for which nine 6-month-old calves were naturally infected with ticks of the *Ixodidae* species carrying the field strain of *Theileria annulata*. Three calves were selected as a control group. During the trial, clinical observation was carried out of infected animals: morning and evening temperature control. Blood was taken from the jugular vein for hematological and serological examination. Blood smears were stained by the Romanovsky-Giemsa method. The degree of infection of erythrocytes by Theilers was determined. Serological examination of blood was performed by prolonged complement fixation reaction to detect specific antibodies.

**Keywords:** Theileriosis, Theileria annulate, schizont-infected cells, cattle, Infection, Hyalomma anatolicum anatolicum,



### 1. Introduction

Theileriosis is the most severe transmissible disease among the piroplasmid diseases of ruminants, which is widespread all over the world, especially in the south countries with a hot climate, it is also found in Georgia.

Theileriosis is caused by the unpigmented protozoa - from the genus Theileridae. The agent of theileriosis in different species of animals is specific. It parasitizes in cattle in the form of *Theileria Annulata*, *Theileria Sergenti*, *Theileria Mutans*; In sheep and goats – *Theileria Ovis*, in Northern Deer – *Theileria Tarandirangiferis*. The source of infection is a sick animal, and the carrier is the pasture ticks *Hyalomma Detricum* and *Hyalomma anatolicum anatolicum*. These ticks also harbour in livestock holdings. Theileria that invades into the body of cattle goes through two stages of development: (i) Multiple dividing – schizogony, which is characterized by formation of schizont-infected cells ("pomegranatelike body") in lymph nodes, spleen, bone marrow; (ii) Simple dividing (into two, four parts) formation of erythrocyte form of *Theilers* in erythrocytes of the host. They will undergo further development in the body of *Ixodidae* ticks. The disease develops when ticks infected with *Theileria* attack livestock and, during blood-sucking, inoculate the *Theileria* into the host's blood and lymph along with saliva. (Fedorov, 1956; Nebieridze, 2009a; Nebieridze, 2009b). The damage caused by Theileriosis is determined primarily by high mortality (30-90%) with abortions, by losing weight and decreasing the quality of meat (Nebieridze, 2009a; Stepanova et al., 1987; Zablotsky, 1985).

Theileriosis was diagnosed by epizootological, clinical and laboratory tests, during which erythrocyte forms of theileriosis are seen in blood smears and macro schizonts or "pomegranatelike bodies" in the material taken from the damaged lymph nodes and spleen. The disease proceeds in an acute and subacute form, it is rarely possible to turn into a chronic form (Fedorov, 1956; Kamanov et al., 2007).

The objective of present study was to investigate the dynamics of the clinical course and solution of the disease in the case of Theileriosis in cattle, for which nine 6-month-old calves were naturally infected with *Th. annulata* field strain carrier by ticks of the *Ixodidae* species. We included 3 calves for control.

### 2. Material and methods

The experiment was carried out with the informed consent of the owners of naturally infected animals, which was approved by the Bioethics Committee of the Faculty of Veterinary Medicine of the European University on April 5, 2022, registration N74817. During the trial clinical observation of the sick animals, hematological and serological examinations and temperature control was performed in the morning and in the evening. Blood was taken from the jugular vein for hematological and serological examinations. Blood smears were stained by the Romanovsky-Giemsa method. The number of erythrocytes and leukocytes was determined, as well as the hemoglobin content and the rate of infection of erythrocytes by *Theileria annulata*. Serological examination of blood was performed by prolonged complement fixation reaction to detect specific antibodies.

### 3. Results and discussion

The disease had subacute development while some authors (Fedorov, 1956; Kamanov et al., 2007; Lal et al., 1985) indicate the acute development of the disease. The incubation period lasted for 9-10 days. The body temperature in infected animals varied within the norm (39.3 °C - 39.4 °C). Hemoglobin content was within 100.27 - 90.75 g/l; The number of erythrocytes in 1 mm<sup>3</sup> of blood was 7.5 - 6.7 millions, and of leukocytes was 7.4 - 6.6 thousand. The erythrocyte forms of theilers were not yet observed in the blood.

On the 11th day after infection, the infected animals started to have a sharp increase in temperature. Constant type of fever lasted for 12 - 16 days and reached 41,5 °C, sometimes the temperature reached up to 42 °C; Hemoglobin content decreased to 80.1 g/l;

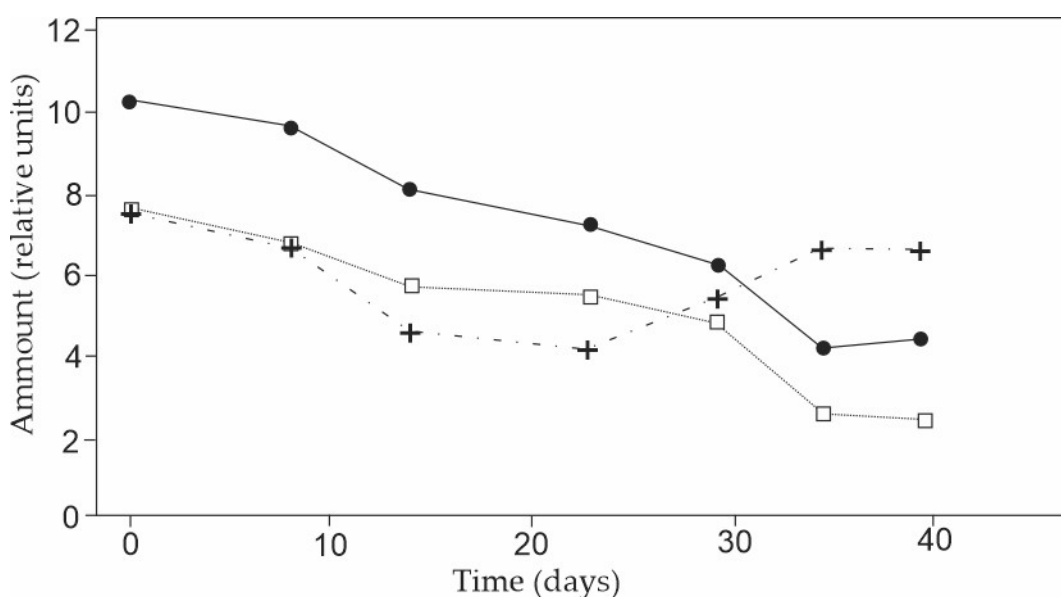


The number of erythrocytes and leukocytes is 5.6 millions, respectively-decreased to 4.5 thousand. Parasitemia in the blood was 44%.

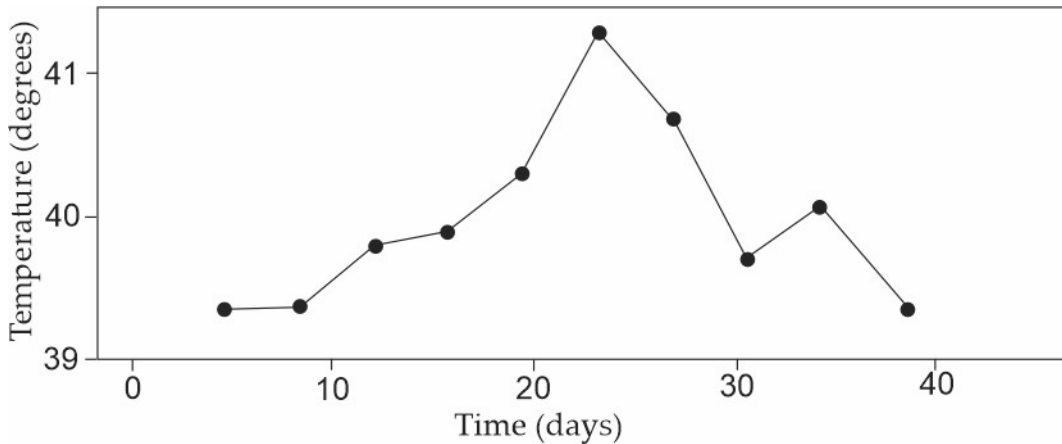
On the 14th day after infection, a pea-sized dense nodules with a dark red center, surrounded by a relatively pale rash were observed on the skin at the site of attachment of the tick. The superficial lymph nodes near the site of invasion (groin, knee fold) were enlarged and painful to the touch.

On the 18th day after the infection (7th day after the rise in temperature), the animals were in agony, depressed, with a severe course of the disease: the hair was disheveled, multiple firm granulomas with a dark red concave center and raised edges on the surface of the skin were observed around the anus. The conjunctiva was wet and hyperemic, the mucous membrane of the nose was covered with mucus, there were pinpoint and petechiae hemorrhages. There are similar bruises on the skin of the abdomen and other bright areas of the body. Superficial lymph nodes were enlarged, hardened and painful on palpation. Sick animals stopped receiving food and water; animals stopped rumination and developed atony, constipation or diarrhea alternate; the pulse were rare - heart impulses were strengthened. Schizonts were found in the punctate smear obtained from the lymph nodes - the so-called "Pomegranatelike bodies". The same type of changes were described by other researchers (Fedorov, 1956; Nebieridze, 2009a). Sick animals with clinical signs had persistent fever (41.5 °C - 42 °C), hemodynamic disturbances, a sharp drop in hemoglobin content to 70.27 g/l, while the number of erythrocytes and leukocytes was approximately the same as on the 14th day after infection. 67% of erythrocytes were infected with theilers. N. Kamalov et al. (2007) investigated 172 cattle in southern Tajikistan, where parasitemia reached to 70-75%. Schizont-infected cells found in punctate smear obtained from the lymph nodes of infected calves on 18th day (Figure 1).

On the 23rd day after infection (13-15 days after the fever), a constant type of fever was still maintained, although a tendency to decrease in temperature (41.2 °C - 40.7 °C) was observed. Hemoglobin content further decreased (6.37%). The number of erythrocytes decreased to 4.8 millions. The number of leukocytes increased to 5.4 thousands, which indicated the strengthening of the defense mechanism. Parasitemia in the blood was slightly reduced to 65%.



**Figure 1:** Hematological changes in infected calves in dependence on time. Hemoglobin concentration (circles) in mg/l, erythrocyte number density (squares) in millions and leukocyte number density in thousands per mm<sup>3</sup> of blood.



**Figure 2:** Temperature in infected calves in dependence on time.

In the mentioned period, progressive cachexia and weight loss of sick animals were noted. Anemia of the conjunctiva and mucous membrane of the mouth was noted together with hemorrhages. On the 23rd day after infection, the condition of four infected animals worsened, they had complete anorexia; They were in agony and culled. The carcasses and offal (internal organs) were destroyed due to pathological anatomical abrupt changes. We share the opinion of Fedorov (1956) and Lal et al. (1985) that during Theileriosis there is an activation of autoimmune reactions during which the cells of the macrophage system phagocytize not only the Theileriasis-infected, but also normal erythrocytes, which contributes to the development of anemia and, as a result, lack of oxygen in tissues and organs. In addition, an important role in the development of anemia is played by the toxins released during the activity of parasites, as well as toxic substances produced by the destruction of tissues, which enter the organs through the blood, including the bone marrow, and cause the blocking of the blood formation process. These indications agree with our results. By serological examination, specific antibodies were detected for the first time on the 23rd day after infection, the titer of which was 1:40. The antibody titer reached its peak 1:160 in the recovery stage - on the 39th day which indicates the formation of non-sterile immunity.

#### 4. Conclusion:

On the 39th day after infection (recovery stage), normalization of temperature was observed - 39.4 °C. Hemoglobin content and the number of erythrocytes were still low against the background of an increase in the number of leukocytes. Parasitemia and general intoxication of the body slowed down, hemorrhagic diathesis weakened. The action of compensatory recovery mechanisms of the body was strengthened and the animals begun to recover clinically. Thus, as a result of our investigations, it is possible to make the following conclusions:

4.1 Incubation period of cattle theileriosis caused by *Theileria annulata* is 9 - 10 days. The disease has subacute development.

4.2 Permanent fever begins on the 10-11th day after infection and lasts for 12-28 days, which in our opinion depends on the individual condition of the animal and the virulence of the parasite.

4.3 The disease is characterized by hemorrhagic diathesis - Petechiae on visible mucous membranes and tender, visible areas of the skin.



4.4 Specific formations (granulomas) characteristic of Theileriosis in the smears made from the punctate of the lymph nodes of a diseased animal – schizont-infected cells (so-called “Pomegranatelike body” ) were discovered on the 3rd-4th day after the incubation period.

4.5 Theileria settle in erythrocytes, causing their lysis, which results in a sharp decrease in hemoglobin, lack of oxygen in the tissues and the development of severe anemia.

4.6 As a result of activity of theileria, toxins produced by the destruction of such tissues cause hemodynamic disorders and blocking of the blood formation process.

4.7 With the progression of the disease, on the 18th-23rd day after infection, part of the animals stopped taking water and feed, complete anorexia developed, and if the compensatory mechanisms for their protection were weak and medical intervention was not performed in time, the animals died due to a sharp heart failure. Only two from infected calves were recovered on the 39th day, in which titre of specific antibodies reached a peak of 1:160 which indicated formation of nonsterile immunity.

**Conflicts of Interest:** The authors declare no conflict of interest.

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