

UNUSUAL CASES OF THEILERIA ANNULATA INFECTION IN CALVES.

BY:

BAHARSEFAT, M., AMJADI, A.R., HASHEMI-FESHARKI,
R., AHOURAI, P., AND ARBABI, I.

SUMMARY

Unusual cases of *Theileria annulata* infection were observed in two calves, in different farms. The calves were 45 and 90 days old. The enlargement of superficial lymph-nodes, presence of numerous subcutaneous and intermuscular nodules which scattered throughout the body, also sharp bilateral exophthalmia in both animals reminded the cases of lymphosarcoma.

Gross and histopathological changes, examination of the smears which were prepared from various organs and cross reaction tests indicated *T. annulata* infection.

INTRODUCTION

Theileriosis is a protozoan disease of the blood and lymphoid tissues of cattle and sheep caused by different species of parasites of family *Theileridae* genus *Theileria* (1-2, 17 & 21).

Theileriosis in cattle caused by *T. annulata* which is transmitted by species of the ticks, *Hyalomma* (2-3 & 4), is common in Iran and causes a severe disease course especially in susceptible pure breeds. The mortality rate in susceptible pure imported breeds ranges from 70-80 % of infected cattle (2).

Since 1935 extensive works have been done on the disease concerning diagnosis, treatment, transmission, induced immunity, vaccine production as well as preservation of the different strains of *T. annulata* at -70°C (1-5, 11-15 & 19).

Vaccination of calves and dairy cattle with tissue culture vaccine has been a very effective method for control of the disease (11).

The pathological changes in *T. annulata* infection whether natural or experimentally produced have not been described as fully as, those in *T. parva* infection (6-9 & 17).

Rafiyi et al, (18), Maghami (16) and Sergent et al (22) observed various skin lesions in calves which correlated with severe *T. annulata* infection. According to Gremoret (10) there might be some relation between the cutaneous and intestinal lesions which are pronounced in acute *theileriosis*. Tsur et al (23) described cutaneous lesions in two cattle which were affected with acute Theileriosis due to *T. annulata*. They believed that in severe cases of Theileriosis, schizonts might have multiplied rapidly and invaded the peripheral blood vessels where their appearance indicated an unfavourable prognosis. Rhahfa et al (20) reported a case of *T. annulata* infection with ulceration of the conjunctiva and numerous hyperplastic red circular lesions in the wall of the intestine.

The object of this communication is to report *T. annulata* infection in calves with unusual lesions.

THE SUBJECT:

Two indigeneous calves, 45 and 90 days old, were sick and were sent to the Pathology Dept., Razi State Institute, for diagnosis. The calves were from different farms and the disease was not seen in any other animals, within two months, in either place. The animals were kept for clinical examination but both became moribund and were slaughtered and post mortem examination was performed immediately. Tissues from various organs throughout the body were collected for histopathological studies. The tissues were embedded in paraffin wax and two sets of sections were prepared; one was stained by H. & E. and the other was stained with Giemsa's staining method.

CLINICAL MANIFESTATION:

The calves were dull, depressed and weak. The rectal temperature ranged between 38.5 and 39.5°C . The animals refused all food. The prescapular lymph-nodes were slightly enlarged. Numerous subcutaneous nodules measuring $0.5-3\text{cm}$. in diameter were noted throughout the skin. The nodules that were located around lips, neck, shoulder, chest and back of animals were quite prominent, (Fig. 1). Both animals were exophthalmic, (Fig. 2). The eyelids and

conjunctiva were swollen, oedematous and covered with a dirty yellowish, sticky exudate. The corneas showed slight opacity and keratitis, (Fig. 3).

GROSS PATHOLOGICAL FINDINGS :

The mucous membranes and subcutaneous tissues were pale and icteric. The striking lesions consisted of many reddish nodules in the subcutis, skeletal muscles, tongue, on the sternal and costal periosteae, in the myocardium, endocardium, omentum, abomasum, intestinal walls and the adipose tissue of the kidneys, (Fig 4-5 & 6). The nodules varied in size from 0.5 to 3cm. in diameters. The sciatic and brachial nerves were swollen and thickened, (Fig. 4). Keratitis was present in both eyes. Enlargement of the eye balls and some nodular new growths in ocular muscles and retrobulbar fats in the both eyes caused the exophthalmos. Lymph-nodes were enlarged liver was swollen and hard in consistency. Spleen was rather normal.

Blood smears prepared from peripheral vessels of sick animals revealed 2 % red blood cells with intra-erythrocytic piroplasms. Annular forms were predominant. While biopsy smears prepared from conjunctiva and subcutaneous nodules manifested more than 20 schizonts per microscopic field, biopsy smears prepared from prescapular lymph-nodes showed only a few schizonts in each microscopic fields. Schizonts in smears prepared from spleen, liver and abomasum were also less than 5 per microscopic field, while smears prepared from mesenteric lymph-nodes and intra muscular nodules showed 20 schizonts per microscopic field.

HISTOPATHOLOGICAL CHANGES:

The histopathological changes in different organs were as follow:

a. Lymph-nodes: The capillaries were engorged and filled with red blood cells with some focal hemorrhages. The lymphatic follicles showed hyperplasia. The intermediate as well as marginal sinuses were heavily distended and filled with enormous amount of immature lymphocytes of which many showed necrosis with nuclear debris. Some of the lymphocytes contained schizonts. The lymphatic capsules also were invaded and there was infiltration of young lymphocytes. In some lymph-nodes, the germinal centre of most of the follicles was depleted and necrotic. The follicles did not show any infiltration of young lymphocytes.

b. Spleen: There were congestion and hemorrhages in the red pulp with enormous amounts of hemosiderin scattered throughout. The white pulp was surrounded by irregular wide eosinophilic zones, while the germinal centres showed some degree of necrosis. Severe infiltration of immature lymphocytes, some of them with schizonts, was noted in non follicular areas.

c. Liver: The hepatic cells showed moderate fatty changes. Some mid-zonal necrosis and hemorrhages were scattered throughout. Focal disseminated hemorrhages were present under the capsule. The sinusoids were distended and filled with young lymphocytes, some of them were necrotic and contained schizonts.

d. Kidneys: There was some infiltration of mononuclear cells between the urinary tubules, probably indicative of a nonsuppurative interstitial nephritis. The cells were predominantly immature lymphocytes and some of these contained schizonts. The urinary tubules and glomeruli were intact except for some homogenous eosinophilic material in the Bowman's capsules and in the urinary ducts (Fig. 7).

e. Heart: The heart showed some intermuscular nodules that consisted of young lymphocytes (Fig. 8 & 9). The same lymphocytic nodules were scattered in the skeletal muscles and the tongue (Fig. 10 & 11).

f. Abomasum: Hemorrhagic ulceration was seen in the abomasal mucosa extending to the lamina propria and the muscularis mucosa layers. Some ulceration with extensive hemorrhages in the muscularis mucosa and muscle layers was present.

g. Intestine: Marked infiltration of immature lymphocytes was present between the glandular tissues of the intestine. The infiltrated cells extended deep down and invaded the submucosa, muscularis mucosa and in some instances to ring muscles. Occasionally these lymphocytes were accompanied by focal hemorrhages in muscular areas.

h. Brain: Characteristic changes were found in the brain stem. Blood vessels were engorged, small capillaries showed fibrinoid degeneration in white substance of the cerebellum. The Virchow-Robin's spaces were distended and filled with homogeneous pinkish materials indicating severe oedema. Some of the capillaries presented mild perivascular cuffing, and the meninges also were moderately infiltrated with cells which were mainly immature lymphocytes.

INVESTIGATION OF THE DISEASE:

a. *To reproduce the disease:* 100 ml of blood was collected from the 2nd sick calf and inoculated subcutaneously into one healthy calf, which splenectomized 25 days previously, and maintained in a tick-free stable. On the 17th day post-inoculation the calf revealed acute theileriosis with maximum intra-erythrocytic piroplasms. Forty four per cent of the erythrocytes were infected and more than 20 schizonts were seen in each microscopic field in biopsy smears of prescapular lymph-nodes. The animal died on the 24th day after inoculation. The post-mortem examination of animal showed typical *T. annulata* infection.

b. *Cross reaction examination*: 240 ml of blood of the infected splenectomized animal was collected when the animal had high parasitaemia and maximum numbers of schizonts. The infected blood was inoculated subcutaneously into three calves aged 4–5 months. Two out of the three calves which had been vaccinated with *T. annulata* vaccine two months previously, recovered, but the non vaccinated one that was considered as control in this experiment, suffered severe Theileriosis and died 24 days after inoculation.

DISCUSSION

According to the results of microscopic examination of blood smears of infected calves, that indicated, intra-erythrocytic piroplasms which were predominantly annular and round form, also due to test animals regarding their clinical manifestations, pathological alteration and cross-reaction tests, it has been considered that the isolated strains were *T. annulata* and they differed from *T. parva* and *T. mutans*, two other essential species of Theileria (17).

Clinical manifestation and also gross pathological changes of infected calves revealed that the course of the disease was not the same as that appears in typical theileriosis. The pathognomic changes of typical *T. annulata* infection, that is, ulceration of the abomasum, splenomegaly and enlargement of liver and superficial lymph-nodes, either, were not observed or were scarcely prominent in discussed cases.

Subcutaneous and intramuscular nodules and also ocular lesions consisting of exophthalmos, lachrimation, swollen eyelids with exudate excretion and opacity of the corneas were specific lesions in two affected calves while none of these lesions have ever been observed in typical *T. annulata* infection, (2, 17, 22). The infestation of erythrocytes in peripheral blood vessels were very low (app. 2%). Numerous schizonts could be found in prepared smears from nodules and mesentric lymph-nodes, while they were very low in number in spleen and consequently the cases considered as *T. annulata* infection with unusual lesions.

ACKNOWLEDGMENT

Authors wish to thank Dr. M. Kaveh, General Director, Dr. G. Magami Head of Parasitology Department and Dr. N. Syadat from Razi State Institute for their helpful advice and Dr. J.K.H. Wilde, Head of Protozoology Department in Center for Tropical veterinary Medicine, Scotland for his valuable advice and proof reading of the manuscript.

REFERENCES

1. Delpy, L.P. (1936), Bull. Soc. Path. exot, 29, 157-161.
2. Delpy, L.P. (1937), Arch. Inst. Past. Algerie, 5, 225-264.
3. Delpy, L.P. (1946), Arch. Inst. Hesarak., 4, 79-107.
4. Delpy, L.P. (1947), Arch. Inst. Hesarak., 5, 14-53.
5. Delpy, L.P. (1949), Bull. Soc. Path. exot, 42, 285-294.
6. De Koch, G. (1957), Onder. J. Vet. Res., 27, 431-452.
7. Demartin, J., & al., (1973), J. Comp. Path., 83, 281-298.
8. Demartin, J., & al., (1973), J. Comp. Path., 83, 299-306.
9. Flangan, H.O., & al., (1957), J. Comp. Path: 83, 299, 306.
10. Gremort, N.J., (1953), Bull. Acad. Vet. Fr., 26, 535 -537.
11. Hashemi-Fesharki, R., & al., (1973), Am.J. Vet. Res, 34, No. 11, 1465-1467.
12. Hashemi-Fesharki, R., & al., (1973), Vet. Rec, 92, No. 6, 150-151.
13. Hashemi - Fesharki, R., & al., (1974), Trop. Anim. Hlth. Prod, 6, 119-121.
14. Hooshmand-Rad, P., & al., (1968), Arch. Razi. Inst., 20, 85-89.
15. Hooshmand-Rad, P., & al., (1971), Br. Vet. J., 127, 244-250.
16. Maghami, G., (1960), Personal Communication.
17. Neiz, W.O., (1957), Onder. J. Vet. Res, 27, No. 3, 275-430.
18. Rafyi, A., & al., (1962), Bull. Off. Inter, epiz, 58, 119-146.
19. Rafyi, A., & al., (1965), Bull. Off. Inter, epiz, 46, 431-446.
20. Rhahfa, R., & al., (1967), Vet. Rec., 81, 76-78.
21. Sergeant, E., & al., (1927), Ann. Inst. Past., 41, 721-785, 1175-1185.
22. Sergeant, E., & al., (1932), Arch. Inst. Past. Algerie, 10, 294-295.
23. Tsur, I., & al., (1960), Refuah. Vet., 17, 99-100.

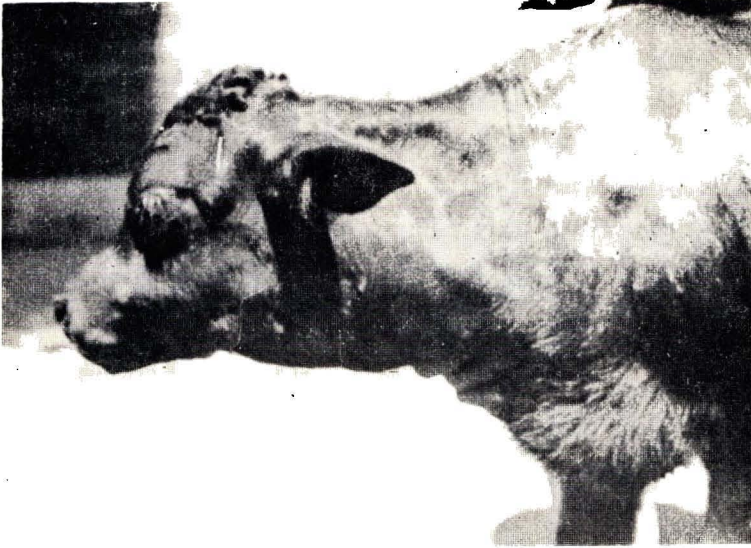


Fig. 1. Note nodules around neck and face.



Fig. 2. The calf with exophthalmos, note the nodules around the lower lip.



Fig. 3. The calf with keratitis and sticky exudate in the eyelids.



Fig. 4. Nodular formation in the skeletal muscles, note the thickness of sciatic nerve.



Fig. 5. Note nodules in the intestinal wall, omentum, adipose tissues around kidneys and peritoneum.

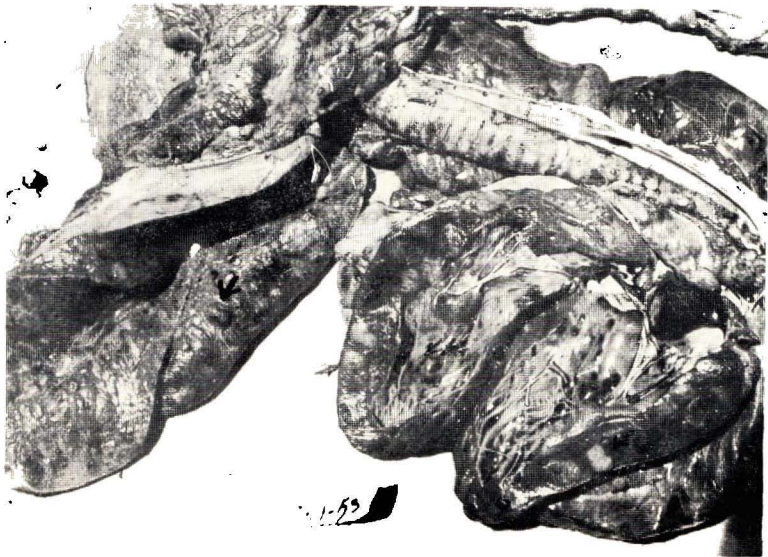


Fig. 6. Note nodules in myocardium, endocardium and tongue muscles.

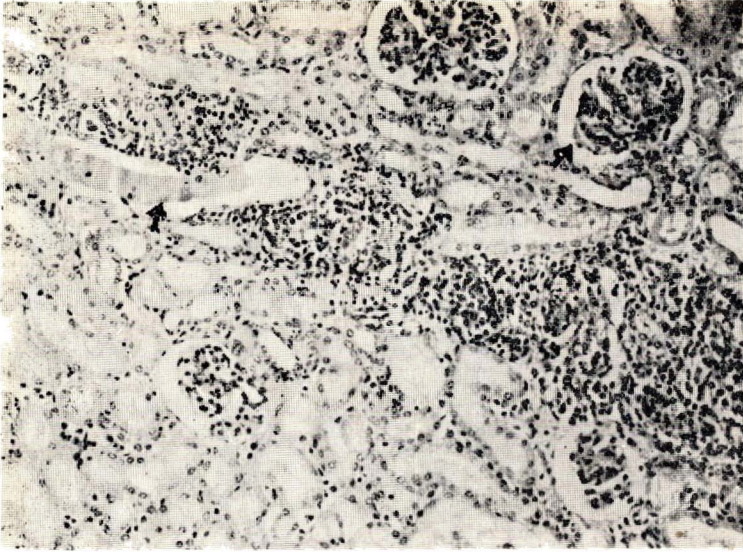


Fig. 7. X 125. Non suppurative interstitial nephritis. Note the homogeneous eosinophilic materials in the urinary tubules and Bowman's capsules.

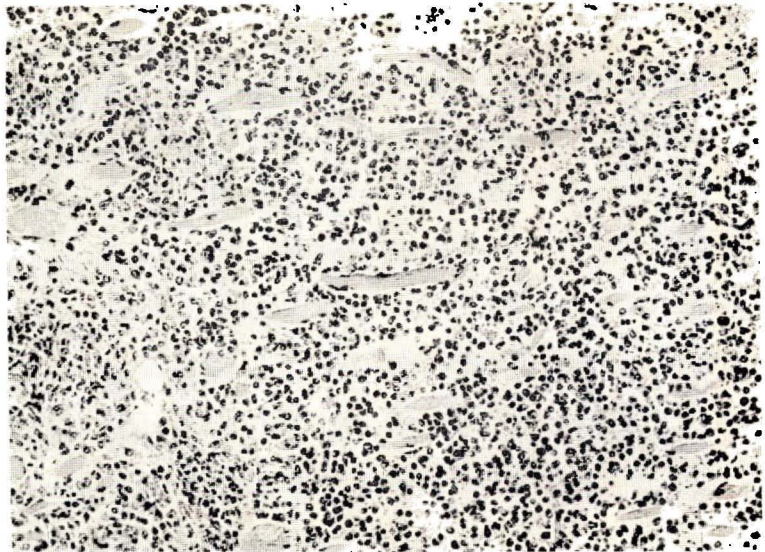


Fig. 8. X 125. Heavy intramuscular infiltration of young lymphocytes in myocardium.

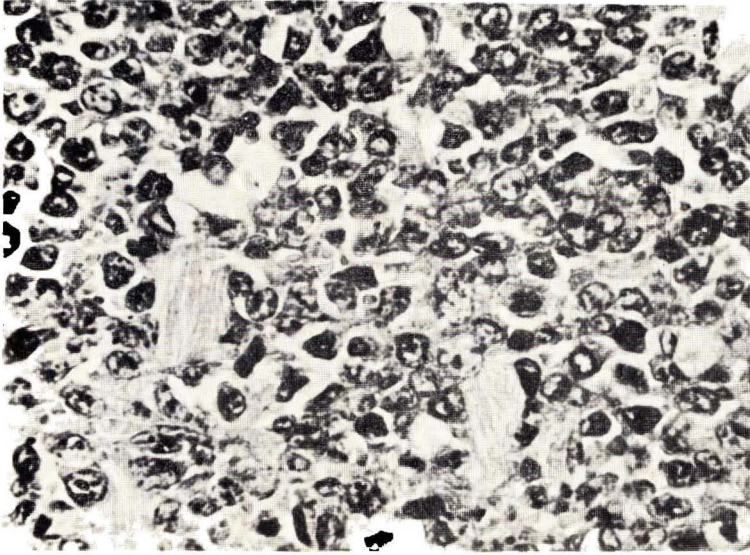


Fig. 9. X 1560. Higher magnification of fig. 8

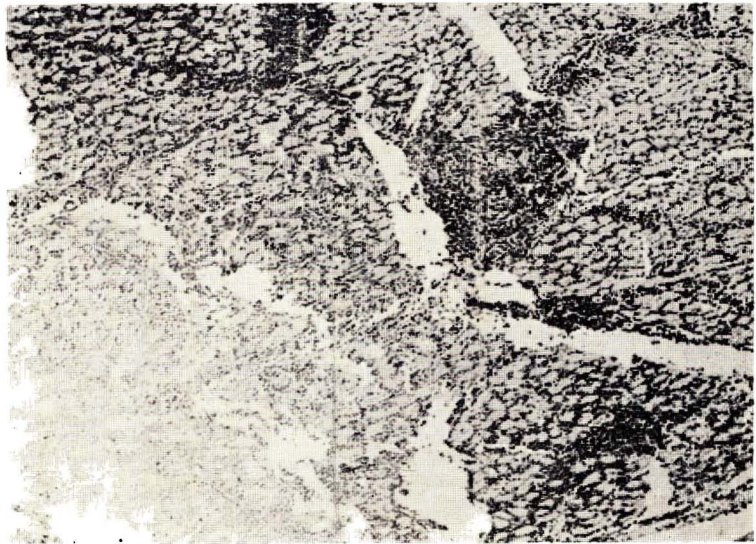


Fig. 10. X 60. Skeletal muscle, note heavy young lymphocytic infiltration.

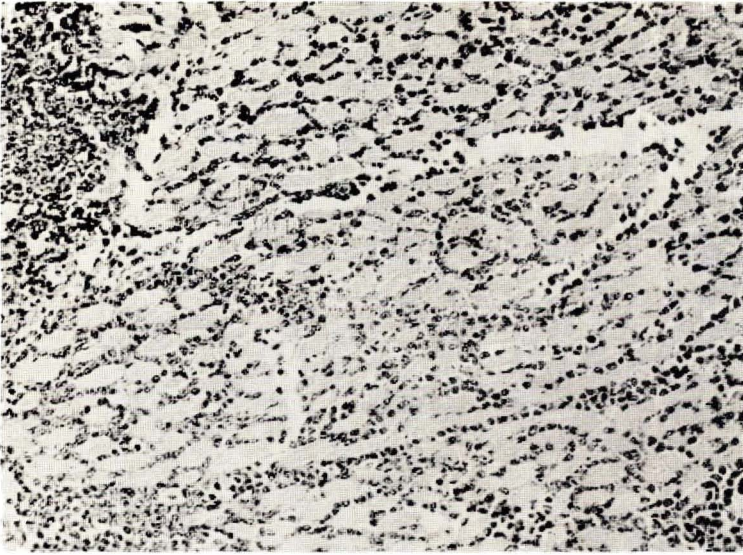


Fig. 11. X 125. Higher magnification of fig 10.