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ISOLATION OF TRYPANOSOMA THEILERI FROM THE BLOOD OF TWO COWS, ONE LEUKOTIC, ONE EXHIBITING LYMPHOCYTOSIS*

By

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STRANDSTRÖM, HELENA, PIRJO VEIJALAINEN, R. BERGER and J. TUOMI: *Isolation of Trypanosoma theileri from the blood of two cows, one leukotic, one exhibiting lymphocytosis.* Acta vet. scand. 1972, *13*, 332—339. — Trypanosoma theileri was isolated by the blood culture method from a leukotic cow in extremis. The parasite could be maintained along with leukocytes with weekly changes of culture medium up to 6 months. In connection with a subsequent transmission experiment a cow, not inoculated with trypanosomes but kept in the same shelter as the inoculated one, commenced to be positive for T. theileri in its blood cultures. This cow had a long history of lymphocytosis but showed no signs of leukosis when slaughtered. The significance of the findings is discussed from the point of view of false positive diagnosis of bovine leukosis and the possible role of trypanosome in the etiology of leukosis.

Trypanosoma; Trypanosoma theileri; lymphocytosis; leukosis.

The literature of Trypanosoma theileri has been extensively reviewed by *Herbert* (1964). The organism has been described as a truly cosmopolitan parasite of cattle (*Ewing & Carnahan* 1967, *Lamy & Bouley* 1967, *Wells et al.* 1968). The only previous

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report of the occurrence of T. theileri in Scandinavian countries is by the German workers *Knuth & Behr* (1911), recording its presence in Denmark and Sweden. The parasite has occasionally been found in a variety of clinical conditions (e.g. *Carmichael* 1939, *Dikmans et al.* 1957, *Ristic & Trager* 1958), but generally been considered as nonpathogenic. Interestingly, T. theileri has been detected in association with lymphocytosis (*Cross et al.* 1971, *Miller et al.* 1969), and it has also been reported as a contaminant in tissue cultures of fetal bovine origin (*Lundholm et al.* 1959) and in leukocyte cultures from cattle, some of them cases of bovine leukosis (*Malmquist* 1965, *Miller et al.* 1969).

In an attempt to cultivate in long term cultures bovine leukocytes from a leukosis case, some of the cultures were found to be contaminated with trypanosomes. In connection with a subsequent transmission experiment trypanosomes were found in leukocyte cultures from another cow, which on hematological grounds was suspected to be leukotic. This cow was, however, not directly involved in the transmission experiment.

MATERIAL AND METHODS

The three year old Ayrshire cow from which the primary isolation of the trypanosome was made (cow 1) originated from Pohjanmaa, Northeastern Finland, and had been transferred in an advanced state of leukosis during the summer of 1970 to the Clinics of the Veterinary College. A few days after securing a blood sample for leukocyte cultures the cow was exterminated. At this phase another blood specimen and samples from tumorous lymph nodes were obtained and attempts of long term cultures commenced.

The blood for leukocyte cultures was collected according to normal procedure. Approx. 50—100 ml blood was collected aseptically into sterile vessels containing EDTA or heparin as anticoagulant. The blood was added to 0.83 % ammoniumchloride in proportions of 1:3 and allowed to stand at room temperature for five min. and then centrifuged for five min. at 1.000 r.p.m. The supernatant was aspirated, and the leukocytes resuspended in phosphate-buffered-saline (PBS). The cell suspension was centrifuged at 1.000 r.p.m. for ten min. The washing procedure

was repeated once. The remaining leukocytes were then resuspended in tissue culture medium consisting of 30 % newborn calf serum and 70 % McCoy medium to a concentration of 5—7 $\times$ 10^6 cells/ml. To the medium penicillin and streptomycin were added. Five ml of this cell suspension was inoculated into tissue culture bottles (Falcon 25 cm^2) and incubated at 37°C in a humidified atmosphere containing 5 % CO_2.

For more detailed microscopic examination of the parasite a small drop of the culture fluid was placed on a microscope slide, allowed to dry at room temperature and stained with May-Grünwald and Giemsa.

Experimental transmission of the cultivated trypanosome was attempted by inoculating s.c. 1 ml of culture fluid, containing large numbers of trypanosomes, into a 3-year old cow of Finncattle race (cow 2). Cow 2 was kept in shelters, quite separately from those where cow 1 had been located; even the personnel taking care of the animals was different. In the same shelter as cow 2, a 6-year old Ayrshire cow (cow 3) was kept in a separate box. This cow was, on hematological grounds, suspected to be leukotic. Leukocyte cultures from cow 3 had been initiated on three different occasions within a period of five months preceding the arrival of cow 1 to the Veterinary College. These cultures never showed the presence of trypanosomes. After the commencement of the transmission experiment, cattle in this shelter were bled twice a week or more frequently, cow 2 daily, for a month. Cow 2 was always bled prior to cow 3 and during the process all the cattle were immobilized with the same nose pressing device of iron. Slight injuries and bleeding of nasal mucosa frequently resulted from the procedure. No blood sucking insects were observed in the shelters during the experimental period.

Blood smears from cow 2, prepared daily, were stained with Giemsa and examined microscopically for the presence of trypanosomes (about 5 min. screening). After the appearance of trypanosomes in the leukocyte cultures from the blood of cow 3, also Giemsa-stained smears were prepared occasionally from this cow and examined for the presence of trypanosomes.

Blood from cows 2 and 3 was also examined occasionally by staining thick films with Giemsa according to the method described by *Killick-Kendrick* (1968).

RESULTS

At the first occasion trypanosomes appeared only in one out of the four tissue culture bottles inoculated with the leukocytes from the blood of cow 1. From the specimens secured at the cow's death 10 out of 18 leukocyte cultures and five out of six lymph node cultures turned out to be positive in regard to trypanosomes. When present, the trypanosomes usually appeared in the cultures after four-five days of incubation. Microscopic examinations of these culture bottles revealed trypanosomes moving vigorously around or attached to certain cells. The May-Grünwald or Giemsa-stained preparations of the same culture fluids showed numerous trypanosomes gathered in groups or single representing different forms, from typically trypanosomal to those with more or less roundish cell bodies. Morphologically we considered the organism to fit the descriptions of Trypanosoma theileri (*Levine* 1961). Fig. 1 shows the appearance of an individual trypanosome.

By weekly passaging of the cell suspension into half the

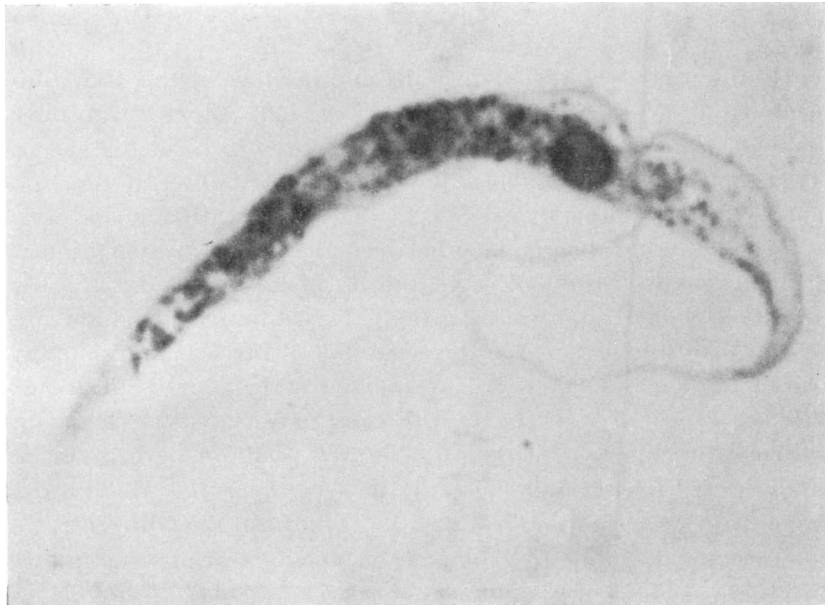

Figure 1. Culture form of Trypanosoma theileri grown at 37°C in tissue culture media containing bovine lymphocytes. Giemsa stained, × 900.

volume of the fresh tissue culture medium the trypanosomes as well as the cells could be kept alive for up to six months.

No trypanosomes were detected in the Giemsa-stained blood smears of cow 2.

After 12 weeks had passed from the time of inoculation of cow 2 with cultured trypanosomes, trypanosomes were also isolated from the blood of cow 3. Reisolations of trypanosomes from cow 3 were successful on three different occasions, succeeding the primary isolation by one, two and three weeks. At the initial isolation nine out of 17 were positive for trypanosomes, the reisolations gave three out of four, two out of two and three out of five positive, respectively. In none of the ten leukocyte cultures started 20 weeks later, trypanosomes could be observed. In routine examination of the Giemsa-stained blood smears trypanosomes, however, were not detected.

Microscopic examinations of thick films from cows 2 and 3 remained negative.

Cow 3 was slaughtered in April 1971. No macroscopic or microscopic signs of leukotic tumorous growths could be detected.

DISCUSSION

Both on morphological grounds and because of the nonpathogenicity as demonstrated in this experiment, the trypanosomes described in the present study are considered to be of the species Trypanosoma theileri. The known wide distribution of this species (*Herbert* 1964) also supports the identification made.

The apparent discrepancy between the successful isolation of trypanosomes by the leukocyte culture method, and the negative results obtained by examining the Giemsa-stained blood smears, are probably explaned by the scarcity of the trypanosomes in the blood of cows 1 and 3. The method of leukocyte culture including repeated washing of the cells may, however, have decreased the chance of isolation. The low grade of parasitemia is underscored by the fact that only part of the leukocyte cultures from the same sample resulted in isolation. The difficulty of microscopic detection of T. theileri in blood smears is well known (*Herbert*).

Whether cow 3 became infected by transmission of T. theileri from the possibly inapparently infected cow 2 by the nose holding device, or whether cow 3 experienced a relapse, an activation of

a latent T. theileri-infection, remains unsettled. In support for the first possibility would seem to speak the fact that such a type of transmission obviously operates in case of some other blood parasites (e.g. Eperythrozoon tuomii (*Tuomi,* unpublished data)), for the latter the scarcity of the trypanosomes, if at all present, in the blood of cow 2. Furthermore, lymphocytosis of cow 3, intermittenly present at least during the last three years, can also fit into a picture of prolonged latent infection by T. theileri (*Cross et al.* 1968). Activation of a latency is a phenomenon frequently encountered among infections by protozoan parasites.

Of great interest in the present observations, according to our judgment, is, however, the association of T. theileri infection on one hand with a case of verified leukosis, on the other hand with a cow exhibiting lymphocytosis, but lacking confirmative evidence of leukosis when slaughtered. It must be emphasized, however, that in the present study only two cows suspected or verified to be leukotic were studied; and thus whether "controls" also would turn out to be infected by T. theileri in substantial proportion remains unknown. The reports by *Malmquist* (1965), *Cross et al.* (1968) and *Miller et al.* (1969), already cited, support the theory of some degree of association of T. theileri with bovine leukosis and with lymphocytosis.

If trypanosomes may cause, as seems possible, lymphocytosis not related to leukosis, this source of false positive diagnosis should be recognized when control measures against bovine leukosis are carried out. On the other hand, there are some suggestions that trypanosomes may play a role in the etiology of bovine leukosis, or more generally, of protozoan parasites in some types of malignant growth. In addition of causing lymphocytosis in vivo (*Cross et al.* 1968), trypanosomes are suspected to induce cell devision in short term cultures of bovine peripheral blood, without the presence of phytohemagglutinin (*Marshak & Abt* 1968). The changes might be steps in a possible way leading to leukosis. *Marshak & Abt* suggest that, for normal cells to be transformed into cancer cells, they must be in a special state of receptivity or competence and that cattle with lymphocytosis may be more likely to develop leukosis, because they have more competent cells to be transformed.

In regard to the occurrence of Burkitt's lymphoma in Africa, association of its high incidence with that of malaria has been

pointed out (*Burkitt* 1963, 1969 and 1970, *Stewart* 1970). The relationship between protozoan and malignant growth has also been reported in connection with the use of certain lots of vaccine against bovine piroplasmosis and subsequent appearance of leukosis (*Olson* 1961). The finding that the vaccine, containing live parasites, apparently had effected the spreading of bovine leukosis might be worth while considering even from the point of view that protozoan had some part in the causation of malignancy.

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SAMMANFATTNING

Isolering av Trypanosoma theileri ur blodet från en leukotisk ko, samt från en ko med lymfosytos.

I blododlingar från en leukotisk ko in extremis påvisades förekomsten av Trypanosoma theileri. Parasiten jämte bovina leukocyter kunde bevaras ända upp till sex månader, med byte av näringslösning varje vecka. I samband med ett senare överföringsexperiment, isolerades Trypanosoma theileri från en annan ko. Denna ko hölls i samma stall, som den trypanosom inokulerade kon, och hade en lång historia av lymfosytos, men visade vid slaktningen inga tecken på leukos. Värdet av dessa observationer har diskuterats med tanke på felaktiga positiva bovin leukos diagnoser, samt trypanosomernas eventuella roll i leukosens etiologi.

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