SOME CHARACTERISTICS OF A STRAIN OF THEILERIA MUTANS (THEILER, 1906) ISOLATED FROM CATTLE IN THE COUNTY OF KENT, ENGLAND, AND MAINTAINED IN SPLENECTOMIZED CALVES

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Abstract

BROCKLESBY D. W., SELLWOOD S. A. and HARNESS E., 1972. Some characteristics of a strain of Theileria mutans (Theiler, 1906) isolated from cattle in the County of Kent, England and maintained in splenectomized calves. International Journal for Parasitology, 2: 265-271. A strain of T. mutans was isolated from British cattle and maintained by blood inoculation in splenectomized calves. The strain was avirulent but caused a haemolytic anaemia with a tendency towards macrocytosis. Sixteen passages were made with blood taken at or near peak parasitaemia but this did not result in any change in the behaviour or virulence of the parasite. Maximum parasitaemias varied between 5/1000 R.B.C. and 131/1000 R.B.C. and were achieved between the 8th and 31st day after infection.

Attempts to transmit the parasite with three tick species were not successful.

INDEX KEY WORDS: Theileria mutans; Ixodes ricinus; Haemaphysalis punctata; Rhipicephalus appendiculatus; piroplasm of cattle; haematology; splenectomy.

INTRODUCTION

Theileria mutans is a cosmopolitan piroplasm of cattle which is particularly common in tropical and sub-tropical areas. Reports of disease being associated with infection are scanty (Neitz, 1957; Flanagan & Le Roux, 1957) and, in general, it is accepted that the organism is benign in non-splenectomized cattle. Infection with T. mutans does not confer an immunity to subsequent challenge with Theileria parva, the cause of East Coast Fever, and this character is the main differential diagnostic criterion for the two species; they are morphologically similar but can be distinguished serologically (J. P. J. Ross, personal communication). Brocklesby (1969) described a single passage line in which a parasite diagnosed as being T. mutans became transformed so that it became indistinguishable from T. parva; he proposed that the explanation for this may have been provided by Jarrett, Crighton & Pirie (1969) who indicated that the standard methods (Bailey, 1960) used to maintain T. parva via the tick Rhipicephalus appendiculatus might result in a selection of clones with a rapid replication characteristic. This interpretation has recently been challenged by Radley (1970, and personal communication) who, since he was able to show that the growth rate of T. parva in the bovine host was directly related to the dose of infected material, suggested that the transformations mentioned by Brocklesby (1969) could be explained by postulating an increasing efficiency in tick transmission; this could lead to an increased dose rate of infective particles and a consequent change in the character of the infection.

The records suggest that T. mutans is a rare parasite in Britain, for there are only three instances of infection reported in the literature. The parasite was first detected by Hignett (1953) in a splenectomized cow at Frant in Sussex; the origin of this strain could not be determined but there were some grounds for suggesting that the source of infection was a suspension of ground-up Ixodes ricinus ticks that had been collected from a case of redwater in
Hampshire. The second isolation was made by Dr. H. M. D. Hoyte of the University of Queensland in 1968 (personal communication) and this has been referred to by Brocklesby, Irvin & MacMillan (1969). Dr. Hoyte obtained this strain by the inoculation of blood collected from a group of 8 cattle, slaughtered at Hastings abattoir, that had been grazing on Romney Marsh in Kent. Two calves became infected with *T. mutans* and it is with this isolate that this paper is concerned: the strain will be referred to as *T. mutans* (Hoyte strain).

Recently Barnett & Brocklesby (1971) described the isolation of a large *Babesia* species from a group of 20 cattle in Kent and they noted that every animal was infected with *T. mutans*. This seemed to suggest that the parasite might not be uncommon in certain areas of Britain.

We have been concerned to attempt the establishment of *T. mutans* (Hoyte strain) in a tick-transmitted situation with the idea that continued passage might result in interesting changes in the character of the parasite and the infection in cattle.

**MATERIALS AND METHODS**

**Cattle**

The experimental calves were Friesian/Ayrshire bull calves born and reared at Compton. All calves were weaned at 8-9 weeks and were splenectomized at about that time.

**The parasite**

*T. mutans* was passaged from calf to calf by the intravenous injection of 100 ml of blood in acid–citrate–dextrose solution (A.C.D.) except when stated otherwise. Thin blood films were prepared from blood collected from a jugular vein into a tube containing di-potassium EDTA to give a concentration of 2-4 mg/ml. The same blood sample, when required, was used for haematological observations. Blood smears and lymph node biopsy smears were fixed with methanol and stained with 10 per cent Giemsa (pH 7·2) for 1 h: parasitaemia was expressed as the number of infected erythrocytes per 1000. A weekly blood count for each calf was estimated from the mean of three counts taken each week.

**Haematology**

Haemoglobin was measured as oxyhaemoglobin in a spectrophotometer (EEL, Spectra) at 540 nm, standardized against a similarly treated blood of known haemoglobin content: packed cell volume (P.C.V.) was measured in duplicate in a Hawksley microhaematocrit centrifuge.

**Ticks**

The ticks were reared and maintained by methods based on those of Bailey (1960) and Irvin & Brocklesby (1970). *Rhipicephalus appendiculatus* was obtained from the East African Veterinary Research Organization; *Haemaphysalis punctata* and *Ixodes ricinus* were kindly supplied by Dr. S. F. Barnett.

**RESULTS**

*Attempts to transmit T. mutans (Hoyte strain) with ticks*

The two originally infected calves were known as H1 and H2: the latter was used to attempt the infection of *R. appendiculatus*. Nymphal ticks were allowed to engorge on the ears of H2 2 months after it was originally infected with the blood of the cattle from Kent and they fed during a period when the parasitaemia fluctuated between 34 and 58/1000
R.B.C. After moulting to adults the ticks were allowed to feed on the ears of 2 splenectomized calves, Nos. B680 and B699: 42 ticks engorged on B680 and 93 ticks on B699. Blood smears were examined daily for 21 days and then 3 times weekly. No infection resulted and when the calves were challenged with infected blood 6 months later they were both found to be susceptible.

Blood from calf H2 was injected into a splenectomized rabbit on which nymphal and larval *R. appendiculatus* were engorging: 10 ml were given intravenously and 20 ml intraperitoneally on 2 successive days. A low parasitaemia (<1/1000 R.B.C.) was achieved in the rabbit during the time that the ticks detached. When they had moulted to the next stage the ticks were allowed to feed on 3 splenectomized calves as follows: 27 adults engorged on calf A677; 44 nymphs on A101 and 81 nymphs on B700. None of the calves became infected with *T. mutans* and they were all susceptible when they were challenged with infected blood at a later date. The nymphs that failed to transmit to calves A101 and A677 were allowed to moult to adults when they were placed on 2 fresh splenectomized calves: 13 adults fed on one calf and 7 on the second but no transmission of *T. mutans* took place.

After 15 blood passages a further attempt at transmission with *R. appendiculatus* was made. Larvae engorged on an infected calf when the parasitaemia was between 132 and 144/1000 R.B.C. On moulting to the nympha! stage about 700 engorged on calf C410 but again no transmission was effected. This calf was later challenged with a new strain of *T. mutans* from Essex (Barnett & Brocklesby, unpublished) and was found to be susceptible.

Similar attempts at tick transmission were made with *Haemaphysalis punctata* and with *Ixodes ricinus*. Larvae of *H. punctata* were placed on calf A63 (which had been infected by blood inoculation from calf H2) and they dropped engorged when the parasitaemia was between <1 and 2/1000 R.B.C. After moulting to nymphs 35 were allowed to feed on each of 2 fresh splenectomized calves: 12 engorged on one calf and 14 on the second but neither animal became infected with *T. mutans* and both were later shown to be susceptible. The same donor calf (A63) was used to feed larvae of *I. ricinus* at the same time: the resultant nymphs were fed on 3 splenectomized calves, 34 fed on one calf, 41 on the second and 44 on the third. None of the calves became infected with *T. mutans* and all were later shown to be susceptible when challenged by blood inoculation.

The infection seen in the original calves

Blood smears from the 2 original calves were examined, usually daily, for 14 weeks and then at less frequent intervals until September 1970 when the animals were killed. Five weeks after inoculation *T. mutans* disappeared from calf H1 but the parasite reappeared 4 weeks later and from then on it was consistently detectable. The parasitaemia fluctuated between <1/1000 R.B.C. and 103/1000 R.B.C. but was usually about 15/1000 R.B.C. Six months and 1 yr after infection blood from calf H1 was shown to be infective by being inoculated into splenectomized calves.

The parasite was consistently present in calf H2, the parasitaemia varying between <1/1000 R.B.C. and 58/1000 R.B.C. but was usually about 20/1000 R.B.C.

Both calves became infected with *Haemobartonella bovis* and this was the first record of this parasite in Britain (Brocklesby, 1970); they were also found to be infected with Eperythrozoon species from time to time.

*T. mutans* was still easily found in both animals 2 yr after their original infection.

Morphology of the intra-erythrocytic piroplasms

The piroplasms seen in erythrocytes in Giemsa-stained thin blood films were extremely variable in size and shape. The common forms seen are illustrated in Fig. 1; the types most
frequently seen are shown in the top two rows of infected erythrocytes in the diagram. 'Cross' or 'nutlet' configurations were not uncommon: these consisted of four anaplasmoid organisms, sometimes with a delicate wisp of cytoplasm, arranged rather regularly at the corners of a square. It could not be determined whether these forms did divide into four healthy merozoites and in fact, erythrocytes containing four similar piroplasms were observed only rarely. Rod-shaped piroplasms with two dense nuclei arranged in tandem at one pole were seen frequently. Apart from this the morphology did not differ from the descriptions in the literature (Theiler, 1906; Neitz, 1957; Hignett, 1953).

Lymphoid cells were examined extensively in all smears but no schizonts were detected.

**Passage of T. mutans (Hoyte strain) through splenectomized calves**

The parasite has been carried through 16 passages during which 32 splenectomized calves have been infected. The intravenous injection of 100 ml of infected blood always resulted in infection.
In the early stages, before supplies of splenectomized calves were arranged, it was necessary to passage the organism slowly. Later a short passage line, involving 3 calves, was made with blood taken early in the course of the infection; latterly a long passage line, involving 25 calves, has been made with blood collected at or near to peak parasitaemia. None of these manoeuvres resulted in any significant change in the behaviour of the parasite. At the 13th passage a group of 5 calves was infected and these cases will be described in some detail later.

There was usually a gradual increase in parasitaemia with maximum counts varying between 5/1000 R.B.C. and 120/1000 R.B.C., being achieved between the 8th and 31st day after infection. No animals died and after the primary parasitaemia parasites remained easy to detect in thin blood films; parasitaemia in carrier calves fluctuated rather widely. Anaemic changes were usually minor and temperature changes insignificant.

Haematology of five calves infected at the 13th passage

Three weeks after splenectomy 5 calves were infected with $4.5 \times 10^6$ infected erythrocytes (100 ml infected blood in A.C.D. injected intravenously). Blood smears were examined daily.
and haematological observations were carried out thrice weekly. Three uninfected splenectomized control calves were included to monitor the effects of splenectomy alone. Details of parasitaemia and the changes that occurred in haemoglobin and packed cell volume values are shown in Fig. 2.

Parasites were seen by the third day after infection in thin blood smears taken from all 5 calves. The numbers of parasites increased rather rapidly to reach peak levels of 64–131/1000 R.B.C. between days 11 and 21. This was followed by decreasing levels of parasitaemia and although slight to moderate recrudescences did occur in most calves, blood smears taken at 8 weeks following peak parasitaemia varied from 'no parasites observed' to 4/1000 R.B.C.

Compared with normal intact calves of similar breed and age (Harness, Fitzsimmons & Sellwood, 1970) there was surprisingly little change in the blood picture of the splenectomized uninfected calves. Following splenectomy there was a tendency for both haemoglobin and packed cell volume values to rise slightly over the next 2 months but abnormal values were not recorded. In infected calves, however, reductions in haemoglobin and packed cell volume were noted between the first and second week after infection with *T. mutans* and these values continued to decrease as the parasitaemia increased, reaching their lowest levels about 1 week after peak parasitaemia. About the time of peak parasitaemia abnormalities were seen amongst the uninfected erythrocytes and this was heralded by the appearance of anisocytosis and slight polychromasia. Within a few days these changes had developed to marked anisocytosis with polychromatic macrocytes showing severe stippling; in films from 4 of the calves nucleated erythrocytes were common. Usually within 1 week of these gross manifestations of active haemopoiesis the only abnormality to be seen in the stained blood films was the presence of moderate numbers of macrocytes and at this time there was an evident reversion of the haemoglobin and packed cell volume towards normal values. During periods of parasite recrudescence it was not uncommon to find an occasional polychromatic or stippled cell and in 3 of the 5 calves a slight to moderate degree of anisocytosis persisted for 2 months following infection.

Mean corpuscular haemoglobin concentration remained within normal limits throughout the infection period, but during the third and fourth week after infection, values were depressed to the lower limits of normality. No changes were observed outside normal limits in total or differential leucocyte counts.

DISCUSSION

Our attempts to transmit *T. mutans* (Hoyte strain) with ticks were completely unsuccessful. This experience is in line with that of Purnell, Branagan & Brown (1970) who failed to transmit an East African strain of *T. mutans* with *R. appendiculatus* even though this tick is listed as a known vector by Neitz (1957). Purnell *et al.* (1970), however, believe that the experimental evidence upon which Neitz's citation is based is rather slim and it may well be that *R. appendiculatus* is not an efficient vector of this piroplasm in Africa.

What tick, then, is the vector in Britain? It seemed likely that *Haemaphysalis punctata* would be shown to be the carrier as this tick was recorded as the vector of *T. mutans* in the U.S.S.R. (Markov, 1957), *Haemaphysalis bispinosa* was reported by Riek (1966) to be the vector in certain parts of Australia and a third member of the genus, *Haemaphysalis neumanni* was reported by Ishihara (1968) to be capable of transmitting a similar bovine Theileria species in Japan.

The effect of rapid blood transfers on piroplasms is unpredictable. Sergent *et al.* (1924) found that *T. mutans* infections became weaker when the parasite was serially transmitted:
in none of our passage lines, however, was there any significant change in the behaviour or pathogenicity of the parasite.

There is no evidence that *T. mutans* causes any disease in British cattle but such a possibility should be entertained: we were somewhat surprised at the relatively severe haemolytic anaemia that was induced in the 5 calves infected at the 13th passage. These were, admittedly, splenectomized animals but the results do indicate that British strains of *T. mutans* may not be completely benign. About the time of maximum parasitaemia there was a marked haemopoietic response and a tendency towards a macrocytic anaemia although the mean corpuscular haemoglobin concentration did in fact remain just within normal limits. It is of interest to note (Fig. 2) that the graph line of the P.C.V. from each infected calf crossed the line of the haemoglobin concentration while that from the uninfected control calves always remained below and almost parallel to the haemoglobin values.

Efforts to examine the hypothesis put forward by Brocklesby (1969) must await the establishment of *T. mutans* in laboratory-reared ticks. That this may be achieved seems likely since Barnett & Brocklesby (unpublished) have infected splenectomized calves with *T. mutans* by allowing field collections of *H. punctata* to feed upon them.

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REFERENCES


