Observations on Trypanosoma theileri Infection in Cattle

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SUMMARY

Naturally occurring Trypanosoma theileri infection was studied in two cattle herds. Herd A was a dairy herd of approximately 250. Herd B was an isolated herd of 32 and contained both dairy and beef breeds. Blood samples were collected from all animals in Herd A during July and August on two successive years. Samples were collected from Herd B at monthly intervals. Total leukocyte and differential counts packed cell volume determinations, and trypanosome cultures were made on each sample.

Infection was detected in all age groups between seven months and fifteen years but it was rare in calves. Infected animals were not consistently positive for trypanosomes on consecutive blood cultures and there was considerable variation between infected individuals. Positive cultures were usually obtained from some animals while others were positive intermittently. No correlation was found between trypanosome isolations and the season of the year.

A correlation was found between trypanosome isolation and lymphocytosis. Of the 920 blood samples examined, approximately one in every five trypanosome positive samples had lymphocyte levels in the Bendixen positive range. Approximately one in every twenty trypanosome negative samples had lymphocyte numbers in the Bendixen positive range. Evidence indicated that trypanosome isolation from animals with lymphocytosis was not caused by increased numbers of infected buffy coat cells in the inoculum cultured.

Eight calves were inoculated intravenously with trypanosome-infected blood. Lymphocyte numbers increased an average of 3549 per cu mm above pre-inoculation levels in seven and remained essentially unchanged in one. Prior to inoculation with infective blood, two of the calves were intravenously inoculated with trypanosome-infected blood that had been frozen and thawed to kill the trypanosomes contained in it. Neither developed lymphocytosis following this inoculation.

No clinical disease problems which could be attributed to trypanosome infection were found.

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RÉSUMÉ

On étudia une infection naturelle par Trypanosoma theileri chez deux troupeaux de bétail. Le troupeau A se composait d'animaux laitiers au nombre d'environ 250 têtes. Le troupeau B comprenait 32 bêtes laitières et de boucherie, isolées. Au cours des mois de juillet et d'août de deux années successives, on préleva des échantillons de sang chez tous les animaux du troupeau A. Des échantillons furent prélevés mensuellement dans le troupeau B. Sur chaque échantillon, on effectua un comptage leucocytaire total, un comptage différentiel, une détermination du volume cellulaire et des cultures de trypanosomes.

On décela l'infection chez des animaux de tout âge, entre sept mois et 15 ans, mais elle était rare chez les veaux. Des cultures consécutives d'échantillons sur gélose au sang, provenant d'animaux infectés ne permettant pas régulièrement le développement de trypanosomes; on releva des variations importantes dans l'isolation de trypanosomes chez les animaux infectés. Certains animaux se révélèrent habituellement positifs, alors que d'autres le furent de façon intermittente. On ne put établir de corrélation entre l'isolation des trypanosomes et la période de l'année.

On trouva cependant une corrélation entre l'isolation des trypanosomes et la lymphocytose. Sur 920 échantillons de sang examinés, approximativement un échantillon sur cinq positifs aux trypanosomes présentait des lymphocytes à des taux correspondants à la marge positive de Bendixen. Environ un échantillon sur 20 négatifs contenait des lymphocytes en

nombre correspondant. Les résultats indiquent que l'augmentation du nombre des trypanosomes isolés des animaux manifestant de la lymphocytes n'était pas causée par l'augmentation du nombre de cellules utilisées dans la contamination du milieu de culture.

On injecta, par voie intra-veineuse, du sang infecté de trypanosomes à huit veaux. Leur nombre de lymphocytes dépassa, en moyenne, de 3549 par cc le niveau atteint avant l'inoculation chez sept animaux. Chez un veau, ce nombre demeura essentiellement inchangé. Avant l'inoculation avec du sang infecté, deux veaux furent inoculés par voie intra-veineuse avec du sang infecté préalablement gelé et dégelé afin de détruire les trypanosomes. Aucune lymphocytose ne se manifesta après cette inoculation.

On ne découvrit pas de symptôme clinique qui pourrait être attribué à l'infection par les trypanosomes.

INTRODUCTION

Trypanosoma theileri has been described as a truly cosmopolitan parasite of cattle which has been reported from cattle in every country that they inhabit (3). It has been found in a variety of clinical conditions (3, 4) and has been associated with lymphocytosis (1, 2). The purpose of this project was to investigate the nature of *T. theileri* infection and its association with lymphocytosis in naturally and experimentally infected cattle.

MATERIALS AND METHODS

Experimental Animals

Two herds were used for the study of the naturally occurring infection. Herd A contained approximately 250 dairy cattle and Herd B comprised 32 animals of both dairy and beef breeding. Herd B was maintained in open shed-type housing isolated from other cattle. Both herds were under veterinary supervision and health records were available.

Eight calves of both dairy and beef breeds were used for the study of the artificially induced infection. These calves were maintained in separate isolation facilities and were between eight and 12 months of age at the time of experimental infection.

Sample Collection and Examination

Blood samples were collected from Herd A in July and August on two successive years. Blood samples were collected from Herd B at monthly intervals. Blood samples were collected from the experimentally infected calves twice a week for a period of at least two weeks before and four weeks after each experimental inoculation.

Total and differential leukocyte counts, packed cell volume determinations (PCV), and trypanosome cultures were made on each sample. An electronic particle counter[1] was used for the leukocyte counts, the microhematocrit method was used for the PCV, and Splitter's or lactalbumin hydrolysate medium was used for culturing the trypanosomes.

The Splitter's medium was made as previously described (1) but the heparin was omitted. The lactalbumin hydrolysate medium was composed of 0.5% lactalbumin hydrolysate[2] in Hanks' balanced salt solution plus 15% bovine serum. Phenol red was used as an indicator and the pH was adjusted 7.4. Both mediums contained 200 units of procaine-penicillin G and 250 mg of dihydrostreptomycin sulfate per ml. The mediums gave comparable isolation results. They were dispensed in 4 ml amounts in 15 X 150 mm screw-cap vials and stored at −25°C until needed. Routine cultures were made by adding 1 ml of freshly collected blood to one tube of freshly thawed medium. The inoculated tubes were incubated at 37°C and examined for trypanosomes after seven and fourteen days. One series of cultures from an experimentally infected calf was made by collecting larger amounts of blood and inoculating multiple tubes of medium.

Experimental Infection

Each of the eight calves was intravenously inoculated with 10 ml of citrated blood collected from a known infected cow. The presence of trypanosomes in each inoculum was demonstrated by culturing 1 ml amounts in each of three tubes of medium.

Prior to inoculation with infective blood, two of the eight calves were inoculated with blood that had been frozen and thawed one time. The blood was positive for trypanosomes when cultured before freezing and negative when cultured after freezing and thawing. One of these calves was given a second inoculation of frozen and thawed blood seven weeks after inoculation with infective blood.

[1]Coulter Counter, Coulter Electronics, Chicago, Ill.
[2]Difco Laboratories, Detroit, Mich.

MEANS OF EVALUATION

In order to determine the prevalence of trypanosome infection in various age groups, infected animals in Herd A were ranked according to their age when infection was first detected.

The monthly culture results from Herd B were compiled to detect seasonal fluctuations and month to month individual variation in trypanosome isolations. Because none of the infected cattle were consistently positive on consecutive blood cultures, one of the experimentally infected calves was selected for a series of cultures using larger volumes of blood. The first series of cultures, in which 1 ml of blood was used as a standard inoculum, had been completed. This initial series covered the first 43 days post-inoculation. The second series was made by obtaining 5 or 10 ml of blood at each collection and distributing it, in 1 ml amounts, into five or ten tubes of medium. The second series covered a period of 48 days. Results from the two series were compared.

The Bendixen leukosis key (7) was used as a means of measuring lymphocytosis. The Bendixen classification was determined for each sample and compared with the trypanosome culture results from the same sample. In Herd B, the lymphocyte numbers in the trypanosome positive and negative samples cultured from each animal during the period of known infection were compared. The period of known infection was defined as the period between the initial and final isolation.

Isolates from both herds and isolates used for experimental infection were classified as *Trypanosoma theileri* according to established criteria (4).

Mold contamination was an occasional problem and all contaminated samples were excluded from the results.

RESULTS

Natural infection by *T. theileri* was found in cattle of all age groups but it was rare in animals under one year of age. The age distribution in Herd A is given in Table I.

Infected animals were not consistently positive on consecutive blood cultures and there was considerable variation between infected individuals. Some animals usually had positive cultures, others rarely had positive cultures. The monthly culture results from all known infected animals in Herd B over a period of one year are shown in Table II. Isolations also occurred at irregular intervals in the experimentally infected calves. Increasing the volume of blood cultured increased the number of isolations but did not eliminate periods of negative results. This is illustrated in Table III.

A correlation existed between trypanosome isolations and lymphocytosis. Approximately one in every five trypanosome-infected samples was also Bendixen positive while only one in every twenty trypanosome-negative samples was Bendixen positive. The comparison between trypanosome isolations and Bendixen classifications is shown in Table IV.

Approximately the same number of lymphocytes were present in trypanosome positive and trypanosome negative blood samples obtained from any single infected individual in Herd B during the period of known infection. This comparison is shown in Table V.

Lymphocyte counts from seven of the eight experimentally infected calves rose

TABLE I. Age Distribution of Trypanosoma theileri Infection in a Dairy Herd

Age (years)	Initial Test		Final Test[a]	
	Cows in Group	No. Infected	Cows in Group	No. Infected
0-1	51	0	45	1[b]
1-2	40	9	36	3
2-3	39	2	15	2
3-4	25	2	25	3
4-5	24	2	18	2
5-6	18	4	13	2
6-7	8	2	15	2
7-8	13	2	3	0
8-15	10	0	14	2

[a]The interval between tests was one year.
[b]This animal was seven months old.

TABLE II. Results of Monthly Blood Cultures of Trypanosoma theileri Infected Cattle in Herd B Over a Period of One Year[a]

Cow	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept[b]	Oct	Nov	Dec
1...	−[c]	−	−	−	−	−	+	−	+	+	−	−
23...	+[d]	+	+	+	−	+	−	+	+	−	−	+
25...	+	−	+	−	−	−	−	−	−	−	−	−
27...	−	−	−	−	+	−	−	−	+	−	−	+
30...	−	−	−	−	−	−	+	−	−	−	−	−
31...	−	−	−	−	−	−	−	−	−	−	−	+
148...	−	−	−	−	−	−	−	−	+	−	−	+
1647...	+	−	+	+	−	−	−	−	−	−	−	−
1735...	+	−	+	+	+	+	+	−	+	−	−	−
1736...	−	−	−	−	−	−	+	−	+	−	−	−
1739...	−	−	−	−	−	−	−	−	+	−	−	−
1809...	−	−	−	−	−	−	+	−	−	−	−	−
1815...	−	−	+	−	−	−	−	−	+	−	−	+

[a]A total of 32 cows were in the herd.
[b]2 ml samples of blood were cultured this month. 1 ml samples were cultured all other months.
[c] − = Trypanosomes not isolated
[d] + = Trypanosomes isolated

TABLE III. Results of Single and Multiple Cultures of Blood from a Trypanosoma theileri Infected Calf

Single Culture[a]		Multiple Cultures		
Days PI[b]	Results	Days PI	Aliquots Cultured	No. Positive
3	−[c]	72	10	0
7	−	78	10	0
10	+[d]	80	5	3
15	−	86	5	1
17	−	88	5	5
21	−	91	5	1
24	−	94	5	2
28	−	99	5	5
30	−	106	5	1
35	+	113	5	2
47	+	116	5	5
43	+	120	5	0

[a]1 ml of whole blood was used as the inoculum for the single cultures and for each aliquot of the multiple cultures
[b]Days PI = Days post-inoculation with infective blood
[c] − = Trypanosomes not isolated
[d] + = Trypanosomes isolated

TABLE IV. Comparison of Trypanosoma theileri Isolations with Bendixen Leukosis Key Reactions in Bovine Blood Samples

	Herd A[a]		Herd B[b]	
Trypanosome positive samples	41		62	
Bendixen positive samples	9	(21.9%)	13	(20.9%)
Bendixen doubtful samples	8	(19.5%)	3	(4.8%)
Bendixen negative samples	24	(58.5%)	46	(74.19%)
Trypanosome negative samples	372		445	
Bendixen positive samples	25	(6.7%)	19	(4.3%)
Bendixen doubtful samples	43	(11.5%)	30	(6.7%)
Bendixen negative samples	304	(81.7%)	396	(88.98%)
Total samples	413		507	

[a]Sum of two annual herd tests.
[b]Sum of 18 monthly herd tests.

TABLE V. The Number of Lymphocytes in Trypanosome Positive and Negative Blood Samples Taken at Monthly Intervals During a Period of Known Trypanosome Infection[a]

Cow	Lym/+[b]	Lym/−[c]
1	5615 (7)	6069 (4)
22	5288 (4)	11502 (2)
23	4671 (12)	4358 (7)
25	15568 (2)	12263 (1)
26	5062 (2)	5972 (4)
27	4913 (4)	4606 (8)
31	6360 (2)	6242 (1)
148	3725 (4)	3863 (6)
1647	5938 (4)	5624 (13)
1735	11867 (12)	10829 (6)
1736	6683 (4)	7682 (6)
1739	4191 (2)	4627 (7)
1815	3802 (3)	3347 (7)

[a]The period of known trypanosome infection was defined as the period between the initial and final isolation.
[b]Lym/+ = Mean absolute lymphocyte count of tryapanosome positive blood cultures. Parenthesis indicates the number of samples.
[c]Lym/− = Mean absolute lymphocyte count of trypanosome negative blood cultures.

above pre-inoculation levels and one remained essentially unchanged. The increase occurred between the second and fourth week post-inoculation and ranged from 2000 to 6316 with a mean increase of 3549 lymphocytes per cu mm. Lymphocyte numbers receded from the peak but never returned to pre-inoculation levels. Only one calf reached a Bendixen positive level and this did not persist. Trypanosomes were not recovered from any calf before inoculation and were isolated from every calf following inoculation.

Intravenous inoculation of infected blood that had been frozen and thawed to kill the trypanosomes contained in it caused no change in the lymphocyte count of one calf (Fig. 1) but caused a temporary decrease in the lymphocyte count of the other (Fig. 2). This temporary reduction in lymphocyte numbers also occurred after inoculation with infective blood and again following re-inoculation with frozen blood.

DISCUSSION

Although *T. theileri* has been associated with various clinical syndromes (3, 4), no signs which could be attributed to trypanosome infection were found in any of the naturally occurring cases and the single change detected in the experimentally infected calves was elevation of lymphocyte numbers above pre-inoculation levels.

In both herds where the naturally occurring infection was studied, trypanosome positive blood samples had lymphocyte numbers in the Bendixen positive range approximately four times as often as the trypanosome negative samples. Because trypanosomes are known to infect buffy coat cells (1, 5, 6) the correlation between trypanosome isolation and lymphocytosis might be explained as resulting from the larger number of infected buffy coat cells in the inoculum. Comparing trypanosome isolations with lymphocyte counts of samples from individual animals during periods of known infection indicated that trypanosome isolation was not dependent on the

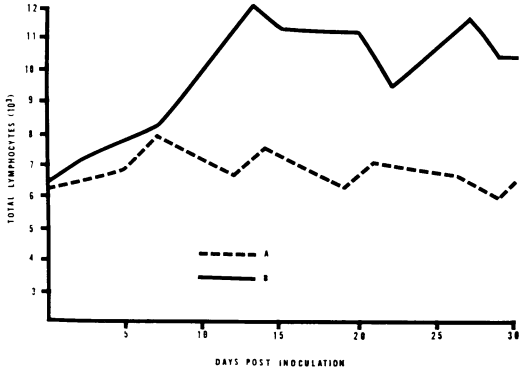

Fig. 1. Total lymphocyte values in a calf after intravenous inoculation of bovine blood containing dead trypanosomes and following subsequent inoculation with blood containing live trypanosomes.
A. Frozen and thawed blood given before infection.
B. Whole blood producing infection.

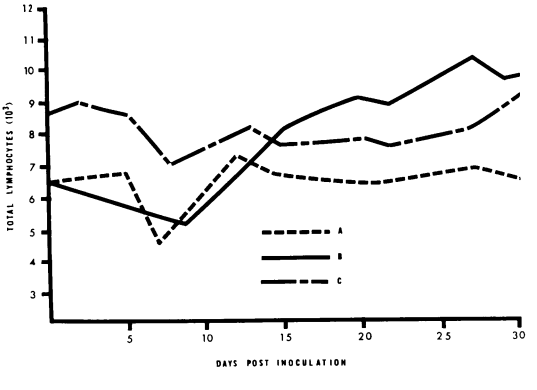

Fig. 2. Total lymphocyte values in a calf intravenously inoculated with bovine blood containing dead trypanosomes both before and after infection with trypanosomes.
A. Frozen and thawed blood given before infection.
B. Whole blood producing infection.
C. Frozen and thawed blood given after infection.

number of buffy coat cells in the inoculum. Monocytes varied in parallel with the lymphocytes.

The possibility that trypanosomes act as a vector for some other agent which causes lymphocytosis has not been excluded but no such effect was demonstrated by inoculation of two calves with infected blood that had been frozen and thawed to kill the trypanosomes contained in it. The temporary depression of lymphocyte numbers seen in one of these calves has also been noticed in other calves following intravenous inoculation of foreign proteins.

Because infected animals are not consistently positive on consecutive blood cultures, it is probable that the true number of *T. theileri* infected animals is higher than published surveys would indicate.

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