

| | |
|---|-------------------------|
| AGENCY FOR INTERNATIONAL DEVELOPMENT WASHINGTON, D. C. 20523 BIBLIOGRAPHIC INPUT SHEET | FOR AID USE ONLY |
|---|-------------------------|

| | | |
|---------------------------|--------------|---------------------|
| 1. SUBJECT CLASSIFICATION | A. PRIMARY | Agriculture |
| | B. SECONDARY | Veterinary Medicine |

2. TITLE AND SUBTITLE
 Isolation of a Bovine Theileria

3. AUTHOR(S)
 Kuttler, K.L. and Craig, T.M.

| | | |
|--------------------------------|---------------------------|----------------------------------|
| 4. DOCUMENT DATE March 1975 | 5. NUMBER OF PAGES 3p. | 6. ARC NUMBER ARC 636.2-K 97e |
|--------------------------------|---------------------------|----------------------------------|

7. REFERENCE ORGANIZATION NAME AND ADDRESS
 Texas A&M University
 Institute of Tropical Veterinary Medicine
 College Station, Texas 77843

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)
 Published in Am. J. Vet. Res., Vol, 36, no.3

9. ABSTRACT
 Dual infections of Anaplasma marginale and a Theileria, resembling Theileria mutans, occurred in splenectomized calves inoculated with pooled blood samples from eastern Texas cattle. Theileria was obtained in pure form by treating dually infected cattle with gloxazone and imidocarb which selectively eliminated Anaplasma. These theileria infections were responsible for mild, transient reductions in packed red blood cell volume (PCV).

| | |
|--|---|
| 10. CONTROL NUMBER PN-AAB- 114 | 11. PRICE OF DOCUMENT |
| 12. DESCRIPTORS Splenectomized Calves, Inoculation, Treatment, Gloxazone, Imidocarb, Anaplasma, Red Blood Cell Volume | 13. PROJECT NUMBER 931-17-130-475 |
| | 14. CONTRACT NUMBER AID/CSD-1947 |
| | 15. TYPE OF DOCUMENT Research Pamphlet |

Isolation of a Bovine *Theileria*

K. L. Kuttler, DVM, PhD, and T. M. Craig, DVM, MS

SUMMARY

Dual infections of *Anaplasma marginale* and a *Theileria*, resembling *Theileria mutans*, occurred in splenectomized calves inoculated with pooled blood samples from eastern Texas cattle. *Theileria* was obtained in pure form by treating dually infected cattle with gloxazone and imidocarb which selectively eliminated *Anaplasma*. These theileria infections were responsible for mild, transient reductions in packed red blood cell volume (PCV).

Theileria mutans has been described as a nonpathogenic or mildly pathogenic, small pleomorphic intraerythrocytic piroplasm in cattle, which is morphologically indistinguishable from the pathogenic organism *T parva*^{4,5,10} and is similar in size to *Babesia argentina*. A lymphocytic schizogonous phase has been described for this organism.¹⁰ Splitter¹⁴ in 1950 described the blood forms of *T mutans* in cattle from Kansas, but little is known about its prevalence or distribution in the United States. He did not report a schizogonous phase.

A morphologically similar *Theileria*, *T cervi*, has been described in white-tailed deer (*Odocoileus virginianus*) in the United States and appears to be widespread.^{2,7,11,12} Antigens prepared from *T cervi*

have been reported to cross-react with serums from sheep and cattle infected with *Theileria*.¹³ Ease of transmission by blood inoculation, morphologic features of the intraerythrocytic forms, frequent absence of schizogonous forms, and benign nature of *T cervi* infections in deer closely resemble the pattern of *T mutans* infection observed in the United States and Europe.^{4,7,9-11,14}

Even though *T mutans* is generally considered unimportant as a disease agent, under certain circumstances it has been found to be pathogenic in cattle.^{3,5,6,12} Possibly, under certain circumstances *T mutans* may undergo changes in pathogenicity to resemble *T parva*.³ The possibility that concurrent *T mutans* infections might influence the pathogenicity of other hemotropic disease agents has not been thoroughly investigated.

Theileria parva is most commonly transmitted transstadially by the 3-host tick *Rhipicephalus appendiculatus*.^{5,10} It has been shown that *C cervi* is transmitted trans-

stadially by the 3-host tick *Amblyomma americanum*.^{2,8} Even though the vector or vectors for the bovine *Theileria* occurring in the United States are unidentified, such vectors that do exist might be capable of transmitting other more pathogenic *Theileria* if they were inadvertently introduced.

Associated with studies of anaplasmosis, dual infections of *Anaplasma marginale* and a bovine *Theileria* resembling *T mutans* have been observed in eastern Texas cattle. Experimental dual and pure theileria infections have been produced in splenectomized calves by blood inoculation and are described in the present report.

The *Theileria* observed and a highly pathogenic babesia organism are shown (Fig 1). This *Babesia*, tentatively identified as *Babesia argentina*, was isolated from a naturally occurring infection in cattle on the Mexican border. It is somewhat similar in size and shape to the *Theileria* encountered in eastern Texas. Infections resulting from this *Babesia*, although associated

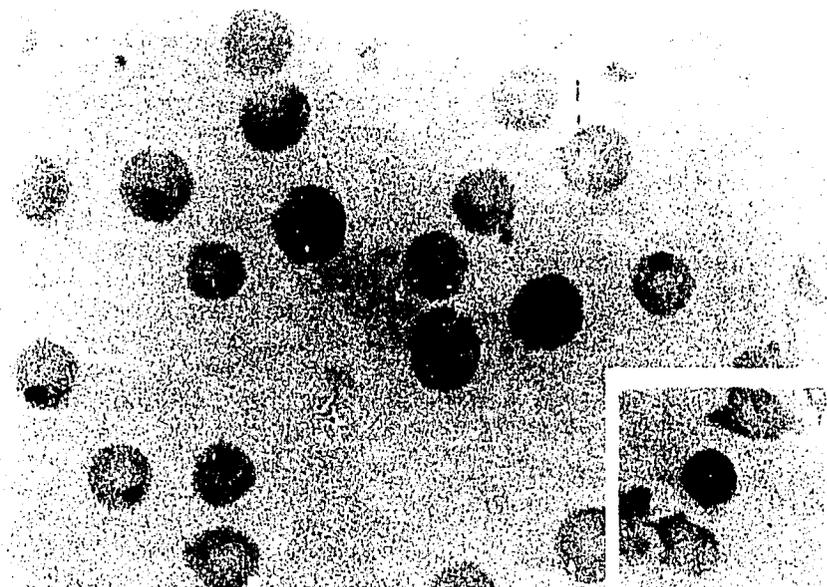


Fig 1—A benign bovine *Theileria*; the insert in lower right is *Babesia argentina*. Notice the difference in sizes. Giemsa stain; $\times 1,000$.

Received for publication June 10, 1974.

From the Institute of Tropical Veterinary Medicine, Texas A&M University, College Station, TX 77843.

Sponsored by a grant from the US Agency for International Development.

¹ Schaeffer, W. F.: *Theileria* in White-Tailed Deer in the United States (abstr). *J Protozool*, 8, suppl 10 (1961): 30.

with relatively low parasitemias, were very pathogenic, producing almost 100% mortality in splenectomized calves. *Theileria mutans*, on the other hand, has been reported to be benign or only mildly pathogenic even in splenectomized calves.^{4,14}

Materials and Methods

Laboratory observations to confirm the presence of the disease agents and to follow their pathogenesis included examining thin Giemsa-stained blood smears, performing the anaplasmosis complement-fixation (CF) test (using US Department of Agriculture antigen) according to standardized procedures,¹ and determining packed red blood cell volume (using a microhematocrit centrifuge).

Needle biopsies of the prescapular lymph glands were made twice a week on 2 splenectomized calves after their exposure to *Theileria* and for 2 weeks after the development of theileria parasitemia. Smears were made from the glandular material, stained with Giemsa, and examined for evidence of lymphocytic schizonts. On another occasion, a calf was killed at a time when a theileria parasitemia was occurring and stained smears from several lymph glands (prescapular, prefemoral, parotid, and mesenteric) were examined for the presence of schizonts.

A serologic survey (CF test) of a herd of 430 cattle in eastern Texas was made to determine the prevalence of anaplasmosis before a control program based on treatment of serologic reactors or infected cattle was started. To confirm the presence of anaplasmosis, 50-ml samples of blood (citrate, as anticoagulant) were collected from 8 seropositive (CF test) cows and injected into 2 splenectomized calves. Calf 1 was inoculated intravenously (iv) with 200 ml of blood collected from 4 of the cows, and calf 2 was given a similar inoculum from the 4 remaining cows.

The 1st attempt to obtain a pure theileria infection consisted of iv inoculating a noninfected, splenectomized calf (calf 3) with 10 ml of whole blood containing both *Anaplasma* and *Theileria*. Calf 3 was given (iv) 5 mg of gloxazone^b/kg of body weight on the 1st day after the calf was exposed (to inhibit the *Anaplasma*). A subinoculation of 30 ml

of blood from calf 3 was given to calf 4 on day 21 when only *Theileria* was seen on thin blood smear. Calf 4 was observed for evidence of infection for 121 days.

Calf 4 subsequently developed a dual infection and was then treated with 3 mg of imidocarb^b/kg and 3 mg of gloxazone/kg on postinfection days (PID) 127 and 141. Subinoculation was not made from this calf. Evidence of posttreatment infection was based on presence of the hemoparasites as seen on stained blood smears.

Another attempt to remove *Anaplasma* selectively involved the use of chemotherapeutic compounds on 2 additional splenectomized calves having dual infections. The first of these, calf 1, was treated with 3 mg of imidocarb/kg, given subcutaneously (sc), and 3 mg of gloxazone/kg, given iv, on PID 17, 20, and 22. Splenectomized calf 5 was inoculated iv with 35 ml of washed, packed erythrocytes from calf 1 at 60 days after the latter was treated (to determine the presence of *Anaplasma*, *Theileria*, or both).

Calf 2 was treated with 3 mg of imidocarb/kg (sc) and 3 mg of gloxazone/kg (iv) on PID 52 and 66. Calf 6 was iv inoculated with 200 ml of blood collected from calf 2 at 134 days after the latter was treated (to determine the type of infection present in calf 2).

Results

Calf 1 inoculated with 200 ml of whole blood pooled from 4 seropositive (anaplasmosis CF test) cows developed a theileria parasitemia (< 0.1%) 6 days after exposure. On day 9, this parasitemia had increased to 1%. On day 13, 0.5% *A marginale* parasitemia was observed, and the *Theileria* was present at the level of 0.1%. During the next few days, the *A marginale* parasitemia predominated, and few theilerias were observed. A low PCV (6%) occurred in association with high *Anaplasma* parasitemia, before the calf recovered. Both anaplasma and theileria bodies disappeared after the calf was treated with both imidocarb and gloxazone, starting on day 17. On PID 72, low-level theileria parasitemia was observed (0.1%). The *Anaplasma* CF test was negative on PID 82 or 60 days after the calf was treated. *Anaplasma* was not observed after treatment, even though the thei-

leria parasitemia persisted at levels ranging from 0.1 to 4.0% after day 72.

Calf 2 developed anaplasma parasitemia 17 days after inoculation and underwent a response characterized by a 16% parasitemia associated with an 8% PCV. Treatment for anaplasmosis was given on PID 52 and 66. *Anaplasma* was not seen after treatment, and the calf was seronegative (CF test) on day 126 (59 days after treatment). On PID 161, calf 2 developed theileria parasitemia (0.3%). This parasitemia progressed to 13% on day 175 and gradually became inapparent by day 192. The calf was killed shortly after this time.

The attempt to retard and eliminate *Anaplasma* in calf 3 by treating with gloxazone on the 1st day after the inoculation of blood containing both *Anaplasma* and *Theileria* was only partially successful. On PID 21 only *Theileria* was detected. On PID 32, anaplasma bodies were seen, and 7 days later calf 3 died of acute anaplasmosis. *Theileria* parasitemia appeared to regress with the development of *Anaplasma*. On day 21, a transfer was made of 30 ml of blood from calf 3 to calf 4 to determine if the *Theileria* might be recovered in the absence of *Anaplasma*. Calf 4 first showed *Anaplasma* parasitemia on PID 14, which proceeded to develop into typical anaplasmosis. On PID 18, theileria parasitemia developed, which persisted in low levels (< 1.0%) during the acute anaplasma infection. Treatment of calf 3 with gloxazone, using a dosage below that needed to eliminate *Anaplasma*, did not influence the theileria parasitemia, even though it apparently did temporarily retard detectable anaplasma parasitemia.

Calf 4 temporarily lost its anaplasma parasitemia after its treatment with gloxazone and imidocarb, but the theileria parasitemia persisted, with no apparent change that could be related to treatment. Treatment in this instance was unsuccessful in eliminating anaplasma infection, because parasitemia recurred 42 days after treatment. In this calf, a fluctuating theileria parasitemia averaging 1.2% ± 1.7 (between 0 and 4.0%) was seen over a 150-day period. Of 44 ex-

^b Gloxazone: alpha-ethoxyethylglyoxal dithiosemicarbazone; imidocarb: imizol (3,3'-bis-[2-imidazolyl]-carbanilide dihydrochloride/dipropionate, Burroughs Wellcome Company, Research Triangle Park, NC (experimental drugs only).

aminations for *Theileria* during this period, 32 were found positive.

The use of imidocarb and gloxazone in large enough doses to eliminate *Anaplasma* failed in every instance to eliminate *Theileria*, although it did appear to suppress this parasite temporarily. *Anaplasma* was eliminated in calf 1, and the subinoculation of blood from calf 2 into calf 5 produced an apparent pure infection of *Theileria*. Calf 5 failed to show either serologic or other evidence of anaplasma infection. *Theileria* parasitemia occurred in calf 5 at PID 22.

The inoculation of calf 6 with 200 ml of blood from calf 2 after the latter had been treated with gloxazone and imidocarb resulted in an apparent pure theileria parasitemia 13 days later. Evidence of anaplasma infection did not occur. The *Theileria* persisted in levels ranging from 0.1 to 3.0% for about 20 days and then regressed below detectable levels.

Pure theileria infections were associated with slight but temporary decreases in PCV. In calf 2, a 26% reduction in PCV occurred after the 13% theileria parasitemia, which was the highest observed among any of the experimentally infected splenectomized calves. Calf 5 had a 19% decrease in PCV persisting over a period of about 30 days, during which a 1 to 2% theileria parasitemia was observed. A low-level theileria parasitemia (between 0.1 and 1.0%) persisted for about 3 months beyond this time, which apparently did not adversely affect the PCV. Calf 6, while developing a theileria parasitemia, failed to show decreased PCV. This parasitemia was relatively brief, being first

observed 13 days after exposure and persisting only 25 days beyond this time.

Morphologically, these organisms closely resembled the description given by Brocklesby et al⁴ for *T. mutans* occurring in England. Most of the erythrocytic forms reported by Brocklesby et al have been seen. Schizonts were not observed in lymph gland smears collected from affected calves.

Discussion

The unexpected occurrence of a *Theileria* in splenectomized calves inoculated with large volumes of blood from anaplasma-seropositive (CF test) cattle confirms a previous report of the presence of this hemoparasite in the United States.¹⁴ Evidence of pathogenicity was not observed in the cattle from which the organism was recovered, and it proved only mildly pathogenic in splenectomized calves. Dual infections of *Anaplasma* and *Theileria* do occur; however, *Anaplasma* will overshadow the milder theileria infection.

Theileria does not appear to be responsive to imidocarb and gloxazone therapy. Means of eliminating theileria infections by chemotherapy were not observed in this study. Low-level theileria parasitemias appear to persist in splenectomized calves for extended periods, and it is suspected that nondetectable levels of infection persist even longer.

References

1. Anon.: A Manual for Conducting the Complement-Fixation Test for Anaplasmosis. ARS, US Department of Agriculture, Washington, DC, 1958.

2. Barker, R. W., Hoch, A. L., Buckner, R. G., and Hair, J. A.: Hematological Changes in White-Tailed Deer Fawns, *Odocoileus virginianus*, Infected with *Theileria* Infected Lone Star Ticks. *J Parasitol*, 59, (Dec, 1973): 1091-1098.

3. Brocklesby, D. W.: The Lability of a Bovine *Theileria* Species. *Exp Parasitol*, 25, (1969): 258-264.

4. Brocklesby, D. W., Sellwood, S. A., and Harness, E.: Some Characteristics of a Strain of *Theileria mutans* (Theiler, 1906) Isolated from Cattle in the County of Kent, England, and Maintained in Splenectomized Calves. *Int J Parasitol*, 2, (1972): 265-271.

5. DeKoch, G., Van Heerden, C. J., Dutoit, R., and Neitz, W. O.: Bovine Theileriosis in South Africa with Special Reference to *Theileria mutans*. *Onderstepoort J Vet Sci and Anim Indust*, 8, (Jan, 1937): 9-123.

6. Flanagan, H. O., and Le Roux, J. M. W.: Bovine Cerebral Theileriosis. A Report on Two Cases Occurring in the Union. *Onderstepoort J Vet Res*, 27, (Dec, 1957): 453-461.

7. Krier, J. P., Ristic, M., and Watrach, A. M.: *Theileria* Sp. in a Deer in the United States. *Am J Vet Res*, 23, (May, 1962): 657-662.

8. Kuttler, K. L., Robinson, R. M., and Bell, R. R.: Tick Transmission of Theileriosis in a White-Tailed Deer. *Bull Wildl Dis Assoc*, 3, (Oct, 1967): 182-183.

9. Kuttler, K. L., Robinson, R. M., and Rogers, W. P.: Exacerbation of Latent Erythrocytic Infections in Deer Following Splenectomy. *Can J Comp Med*, 31, (Dec, 1967): 317-319.

10. Neitz, W. O.: Theileriosis, Gonderiosis, and Cytauxzoonosis: A Review. *Onderstepoort J Vet Res*, 27, (Dec, 1957): 275-430.

11. Robinson, R. M., Kuttler, K. L., Thomas, J. W., and Marburger, R. G.: Theileriosis in Texas White-Tailed Deer. *J Wildl Management*, 31, (July, 1967): 455-459.

12. Rogers, R. J., and Callow, L. L.: Three Fatal Cases of *Theileria mutans* Infection. *Aust Vet J*, 42, (Feb, 1966): 42-46.

13. Schaeffler, W. F.: Serologic Tests for *Theileria cervi* in White-Tailed Deer and for Other Species of *Theileria* in Cattle and Sheep. *Am J Vet Res*, 24, (July, 1963): 784-791.

14. Splitter, E. J.: *Theileria mutans* Associated with Bovine Anaplasmosis in the United States. *JAVMA*, 117, (Aug, 1950): 134-135.