BOVINE EPERYTHROZOONOSIS IN COLOMBIA *

L. GARRY ADAMS, THOMAS M. CRAIG,

KENNETH B. PLATT y JOHN WYSS. **

BOVINE EPERYTHROZOONOSIS IN COLOMBIA

L. GARRY ADAMS, THOMAS M. CRAIG,
KENNETH B. PLATT, and JOHN H. WYSS.**

* Contribution of the Institute of Tropical Veterinary Medicine of Texas A & M University (College Station, Texas), United States Agency for International Development, Rockefeller Foundation, Centro Internacional de Agricultura Tropical (Cali, Colombia) and the Laboratorio de Investigaciones Médicas Veterinarias of the Instituto Colombiano Agropecuario (Bogotá, Colombia).

** Adams, Associate Professor, Department of Veterinary Pathology, Craig, Platt and Wyss, Research Associates, Department of Veterinary Microbiology are members of the Institute of Tropical Veterinary Medicine, College of Veterinary Medicine, Texas A & M University, College Station, Texas whose present addresses are: CIAT, Apartado Aéreo 67-13, Cali, Colombia.

INTRODUCTION

Bovine eperythrozoonosis in Palestine was first described in 1934 by Adler and Ellenbogen (1). Since then, it has been diagnosed by several investigators (2, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 25, 28, 29, 32, 32). Bovine eperythrozoonosis was reviewed by Weinman (30), Sommer (23) and Kreier (13).

Eperythrozoon wenyoni (Erythrocytozoon bovis) (18), stained by the Giemsa method, was described as reddish-purple discoid, thread-shaped, coccoid, rod-shaped or ring-shaped organisms (0.3 - 1.5 microns in diameter) found on the surface of erythrocytes and platelets or free in the plasma. The pleomorphic organisms may completely cover the periphery of the erythrocytes (2, 6, 14, 15, 17, 18, 21, 25, 28). Fluorescent antibody-stained E. wenyoni are similar to Giemsa-stained organisms (17), but acridine orange-stained E wenyoni appear as coccoids or chains of coccoids, 0.5 microns in diameter (18). Eperythrozoon wenyoni observed with phase contrast microscope appear as spheres (17). Ultrastructural studies of E. wenyoni revealed it to be a short rod or disk with granular masses in the cytoplasm, and it more closely resembled rickettsiae or bacteria than protozoa (17).

Eperythrozoon wenyoni is species specific (16, 17, 18, 22, 30) and possibly may be transmitted by nymphs of Ixodes ricinus (30), by Dermacentor marginatus and Rhipicephalus bursa (12) or by Hyalomma sp. (3). Eperythrozoon wenyoni exists in Japan (15), Madagascar (28), Iran (5), East Africa (2), Algeria (8), Australia (14), South Africa (21), Iran, Jerusalem, France (30), United States (4, 7, 15, 18, 20), Russia (12), Holland (31), Finland (25),

and possibly Venezuela (11). Concurrent infections of E. wenyoni with Anaplasma marginale (10) or Babesia bigemina (13) were shown to interfere with one another in the development of parasitemia. Furthermore, cross-reactions between E. wenyoni and A. marginale were demonstrated by the complement fixation and direct fluorescent antibody tests (17), but not by the passive hemagglutination test (9).

Eperythrozoon wenyoni exhibits a variable parasitemia which causes a mild anemia with little or no clinical manifestations in intact cattle, however, Crocker and Sutter (4) reported a case that apparently was related to E. wenyoni, causing clinical manifestations and mortality of Hereford cattle in the United States. Increased body temperature (1, 2, 10, 14, 15, 16, 25, 30), anorexia (25), moderate anemia (10, 14, 15, 16, 17, 25, 30), icterus (21, 25, 31), debilitation (25, 31), lacrimation and hyperemia of the conjuntivae (15) were observed in splenectomized cattle, Clinical pathological findings in splenectomized cattle were described as: decreased packed cell volumes (10, 15, 17, 25), hemoglobin concentrations (15, 17, 25), erythrocyte counts (17, 25) and serum globulin concentrations (7); increased leukocyte counts and sedimentation rates (15); unchanged mean corpuscular volumes and mean corpuscular hemoglobin contents (17). The mild anemia caused by E. wenyoni was described as normochromic-and normocytic, which suggests an anemia due to erythrocytic destruction (17).

On a morphological basis only, Eperythrozoon teganodes (14, 18, 19) and E. tuomii (3, 24, 26, 27, 28, 29, 32) were also described in cattle. Giemsa stained Eperythrozoon teganodes were described as occurring free in the plasma (i.e. not attached to erythrocytes) as reddish-purple rings (0.4 - 1.2 microns in diameter), rods or threads, and rings with rods (0.2 - 0.3 microns X 0.3 -

3.5 microns) attached (14, 28). The pathogenecity of E. tegnodes induced infections in splenectomized calves was not different from E. wenyoni infections (14, 28, 29). Giemsa stained Eperythrozoon tuomii were described as reddish-purple small rings or ovals (0.3 - 1.0 microns) adhered to the periphery of thrombocytes with occasional free rings forms (24, 26, 27, 28, 29, 32). Eperythrozoon tuomii completely encircled thrombocytes, forming a dark purple corona around them as E. wenyoni does erythrocytes (29). Eperythrozoon tuomii caused only moderate body temperature elevations and was incapable of producing anemia (29, 32).

The following describes the first cases of bovine eperythrozoonosis reported in Colombia.

HISTORY

During bovine hemoparasitic disease investigation, 37 male Holstein-Freisian, 4 to 11-month-old cattle, were splenectomized within 3 weeks to 3 months after their arrival at the research facilities. Three groups of calves came from one farm in the northern part of the Sabana de Bogotá and one group of calves came from another farm in the Central Sabana de Bogotá. The two farms were tick free; however, Linognathus vituli (the long nosed cattle louse) occurred frequently on both farms. Twenty calves were splenectomized and housed in an open barn at Bogotá, 18 calves were splenectomized and housed in a tick proofed, screened barn at Palmira, Valle.

Each of the 4 groups of splenectomized calves was housed in separate pens and, in an adjacent pen, 6 other splenectomized calves were housed for a period of 3 weeks prior and 4 months after splenectomy. Biting flies were occasionally

observed in small numbers, but ticks were never seen in either of the barns. The calves were each fed 1.5 kg of the concentrate ration (Table I) plus 1.0 kg of ground corn cobs per day.. Blood samples for packed cell volumes and Giemsa stained blood smears were collected weekly before and twice after splenectomy. Rectal temperatures were taken every second day. Body weights were determined monthly.

CLINICAL SIGNS

Eight calves had sub-clinical infections, and 6 other infected calves exhibited depression and anorexia. All of the calves had elevated rectal temperature (Fig. I). Two calves had serous conjunctivitis with excessive lacrimation. Of the six calves showing clinical signs, 2 had infectious pododermatitis. The 14 affected splenectomized calves gained 177 g/day while the 6 unaffected splenectomized calves gained 277 g/day for the 31 days.

MICROSCOPIC AND CLINICAL PATHOLOGICAL FINDINGS

Microscopic examination of Giemsa-stained, Ph 7.05 blood smears from 14 (37.8%) of the 37 splenectomized calves revealed variable parasitemias of Eperythrozoon sp. Predominantly curved filamentous rods (0.3 X 1.5 microns), coccoids (0.2 - 0.4 microns in diameter) and, less commonly, rings (0.4 - 1.2 microns in diameter) were found to be located upon the surface of erythrocytes, and occasionally free in the plasma (Figs. 2 & 3) of 11 of the 14 affected calves. The pleomorphic organisms stained pinkish-purple and were determined to be E. wenyoni morphologically.

TABLE I

MEZCLA DE CONCENTRADOS

Maíz desgranado	430 kg
Torta de Algodón o Soya	300 kg
Salvado de Trigo	230 kg
Sal Yodada	10 kg
Harina de Hueso	20 kg
*Premezcla de Vitaminas	10 kg
**Premezcla de Minerales	10 kg
	1.000 kg

*PREMEZCLA DE VITAMINAS

Salvado de Trigo	10 kg
Vit A 750,000 i.u./gm y Vit. D 150,000 i.u./gm	8 kg
	10 kg

** PREMEZCLA DE MINERALES

Sulfato de Cobre	1.95 kg
Sulfato de Hierro	7.47 kg
Oxido de Zinc	1.24 kg
Sulfato de Magnesio	3.09 kg
Sulfato de Cobalto	0.20 kg
Yoduro de Potasio	0.07 kg
Salvado de Trigo	85.98 kg
	100.00 kg

Blood smears of 2 of the 14 affected calves revealed large numbers reddish-purple filamentous rods of threads (0.3 X 3.0 microns) with much fewer numbers of rings and rings with rods attached (0.4 - 1.3 microns in diameter) occurring free in the plasma, which were considered morphologically to be E. teranodes (Fig. 4). Also observed in the blood of these two calves was a low level of E. wenyoni affected erythrocytes.

In one of the 14 affected calves, blood smears demonstrated very small reddish-purple rings or coccoids (0.3 - 0.8 microns) which adhered to and completely encircled the thrombocytes (Fig. 5). In addition, small numbers of rings (0.4 - 1.0 microns) were free in the plasma. Based upon the morphology of the organism, this calf was determined to have a E. tuomii infection. Therefore, 11 calves had E. wenyoni infections only, 2 had a dual infection of E. wenyoni and E. teranodes, and one calf had an E. tuomii infection only. The parasitemias varied from 2 or 3% to 80 or 90% infected erythrocytes. Prior and during the onset of parasitemia, thrombocytes noticeable increased in Giemsa-stained blood smears although no specific counts were made. The results of the packed cell volume determination are given in Fig. 1. The distribution of the first onset of parasitemias after splenectomy are given in Fig. 6. The average incubation period and standard deviation was 14.9 ± 3.5 days post-splenectomy with a range of 9 to 22 days.

CONTROL

Twelve of 14 affected splenectomized calves were treated once intramuscularly with 29 mg/kg of 2-di-(Beta, gamma-dioxipropil)-aminofenol-(4 arseno 5)-(benzoxazolil)-mercaptol-proprionato de sodio*, an average and standard deviation

* Spirotrypan-Fuerte, Farbwerke Hoechst AG, Frankfurt, Germany

of 1.2 ± 1 day following the onset of parasitemia. One day following treatment, no parasitemias were demonstrable.

DISCUSSION

The 14 affected calves were most likely infected prior to arrival at the research facilities, because the calves exhibited parasitemia within 22 days following splenectomy; 29 other splenectomized calves in contact with them did not have demonstrable parasitemias. Because the cattle were not exposed to ticks, but were exposed to lice and biting flies prior to leaving the farms, it is suggested that lice and/or biting flies may have been the vectors in this case, in addition to possible mechanical means of transmission.

The clinical signs and microscopic findings of the affected calves correspond to those described by other investigators (1, 2, 4, 6, 8, 14, 15, 16, 17, 18, 19, 21, 25, 28, 29, 30, 31, 32). The morphological characteristics of the organisms found in the blood of the 14 affected calves were strikingly similar to the description of E. wenyoni (1, 4, 6, 14, 15, 17, 21, 25, 28,30), E. taganodes (14, 18, 19) and E. tuomii (24, 26, 29, 32). The average incubation period and standard deviation of 14.9 ± 3.5 days and the distribution of the onset of parasitemia post-splenectomy (Fig. 6) is generally in agreement with that reported by other investigators (1, 4, 6, 14, 15, 17, 20, 25, 28, 29, 31, 32). The clinical manifestations were mild, but 6 unaffected splenectomized infected calves gained an average of 100 g/day more than the calves with eperythrozoonosis. As indicated in Fig. 1, the packed cell volumes increased following splenectomy and decreased following the onset of parasitemia. The temperature increased sharply with the onset of parasitemia and decreased

as the parasitemia became undemonstrable. Following the intramuscular injection of 2-di-(Beta, gamma-dioxipropiol)-aminofenol-(4 arsono 3)-Beta-(benzoxazolil-(2)-mercaptol)-propionato de sodio at 29 mg/kg, the parasitemia became undemonstrable within 24 hours with further recrudescence occurring in 2 of 12 calves 35-40 days following treatment. The 2 untreated infected calves both had low percentages of infected erythrocytes and one calf had a slightly decreased packed cell volume which was followed by a recrudescence of parasitemia in 14 days.

RESUMEN

Eperythrozoon wenyoni, E. teganodes y E. tuomii fueron diagnosticados en 14 de 37 terneros de raza Holstein-Freisian de 4 a 11 meses de edad, traídos de la Sabana de Bogotá. Once terneros mostraron infecciones puras de E. wenyoni, dos terneros mostraron infecciones simultáneas de E. wenyoni y E. teganodes y un ternero mostró una infección pura de E. tuomii. El diagnóstico fué determinado por medio de muestras de sangre con tinción por el método Giemsa; también, se hizo una descripción morfológica de Eperythrozoon sp. Seis terneros esplenectomizados mostraron depresión y anorexia, sin embargo, todos los 14 terneros tenían elevada temperatura rectal. Dos terneros mostraron conjuntivitis serosa con lacrimación excesiva. El aumento de la temperatura coincidió con el primer ataque de parasitemia mientras que el hematocrito disminuyó después del ataque de parasitemia. El promedio y desviación estandard del periodo de incubación fué de 14.9 ± 3. 5 días después de la esplenectomía. El tratamiento con 2-

di-(Beta, gama-dioxipropil)-aminofenol-(4 arseno 5)-Beta-(benzoxazolil-(2)-mercaptol)-proprionato de sodio, a razón de 29 mg/kg intramuscular, causó la parasitemia la cual se hizo indemostrable en 24 horas con una recrudecimientos dentro de seis semanas. Este es el primer informe sobre eperythrozoonosis en Colombia debido a Eperythrozoon wenyoni, E. teganodes y E. tuomii.

SUMMARY

Eperythrozoon wenyoni, E. teganodes and E. tuomii were diagnosed in 14 of 37 splenectomized, Holstein-Freisian, 4 to 11-month-old calves that originated from the Sabana de Bogotá. Eleven calves had pure infections of E. wenyoni, 2 calves had dual infections of E. wenyoni and E. teganodes, and one calf had a pure infection of E. tuomii. The diagnosis was determined on Giemsa-stained blood smears, and morphological descriptions of the Eperythrozoon sp. were given. Six splenectomized calves exhibited depression and anorexia, but all 14 calves had elevated rectal temperatures. Two calves had serous conjunctivitis with excessive lacrimation. The increase in rectal temperature coincided with the onset of parasitemia while the packed cell volume decreased after the onset of parasitemia. The average incubation period and standard deviation was 14.9 ± 3.5 days post-splenectomy. Treatment with 2-di-(Beta, gamma-dioxipropil)-aminofenol)-(4 arseno 5)-Beta-(benzoxazolil-(2)-mercaptol-proprionato de sodio at 29 mg/kg intramuscularly caused the parasitemia to become undemonstrable within 24 hours with further recrudescence

occurring within 6 weeks. This is the first report of bovine eperythrozoonosis due to *Eperythrozoon wenyoni*, *E. tegnodes* and *E. suomii* in Colombia!

REFERENCES

1. ADLER, S, and ELLENBOGEN, V. 1934. A Note on Two New Blood Parasites of Cattle, Eperythrozoon and Bartonella. J. Comp. Path. & Therapy. 47: 219-221.

2. BROCKLESBY, D. W. 1958. The Occurrence of Eperythrozoon wenyoni, Bartonella bovis and Anaplasma marginale in an Ox in Kenya, J. Parasitol. 44: 51.

3. BROCKLESBY, D. W., PURNELL, R. E., and SELLWOOD, Sylvia A. 1972. The Effect of Irradiation on Intraerythrocytic Stages of Babesia major. Brit. Vet. J. 128: 3-5.

4. CROCKER, K. W. and SUTTER, M. D. 1954. Bovine Eperythrozoonosis. Vet. Med. 49: 305-306.

5. DELPY, L. 1936. Agents Pathogénes Observés en Iran dans le sang des Animaux Domestiques. Bull. Soc. Path. Exot. 29: 157-161.

6. DELPY, L. and RAFYI, A. 1938. Sur la Morphologie, l'evolution et la différenciation d'Eperythrozoon wenyoni (Adler, 1934). Bull. Acad. Vét. Fr. 11: 203-208.

7. DIMOPOULLOS, G. T., SCHRADER, G. T. and FOOTE, L. E. 1959. Electrophoretic Studies of Bovine Serum. II. Concurrent Hypoglobulinemia and Natural Infections of Eperythrozoonosis. Proc. Soc. Exp. Biol. 100: 55-58.

8. DONATIEN, A, and LESTOQUARD, F. 1937. Transmission Naturelle d'Eperythrozoon wenyoni par une Tique au Genre Hyalomma. Bull. Soc. Path. Exot. 30: 459-460.

9. FINERTY, J. E., HIDALGO, R. J., and DIMOPOULLOS, G. T. 1969. A Passive Hemagglutination Procedure for the Detection of Eperythrozoon Infection in Calves. Am. J. Vet. Res. 30: 43-45.

10. FOOTE, L. E., LEVY. H. E., TORBERT, B. J., and OGLESBY, W. T. 1957. Interference between Anaplasmosis and Eperythrozoonosis in Splenectomized Cattle. Am.J. Vet. Res. 18: 556-559.

11. GALLO, P. Y., VOGELSANG, E. G. 1940. La Anaplasmosis en Venezuela. Presencia de la Babesia bovis, Grahamia y Eperythrozoon (?) en Bovinos Rev. Med. Vet. Parasit., Caracas. 2: 3-21.

12. GROBOV, O. F. 1964. Eperythrozoonosis in Cattle: Case Report. Veterinariya Moscow, 41: 53-55. Vet. Bull. 34 (1964): 586.

13. HOYTE, H. M. D. 1961. Initial Development of Infections with Babesia bigemina. J. Prot. 8: 462-466.

14. HOYTE, H. M. D. 1962. Eperythrozoon teganodes sp. nov. (Rickettsiales). Parasitic in Cattle. Parasitology. 52: 527-532.

15. ISHIHARA, T. 1962. Eperythrozoonosis in Cattle in Japan. Nat. Inst. Animal Hlth. Quart., Tokyo. 2: 21-30.

16. JENSEN, R. 1943. Eperythrozoonosis in Cattle and Sheep of Louisiana, Preliminary Report, Bull. La. Agric. Exp. Sta., No. 366, p. 8 Vet. Bull. 25 (1945):. 291.

17. KREIER, J. P. and RISTIC, M. 1963. Morphologic, Antigenic and Pathogenic Characteristics of Eperythrozoon ovia and Eperythrozoon wenyoni Am. J. Vet. Res. 24: 488-500.

18. KREIER, J. P. and RISTIC, M. 1968. Infectious Diseases of Man and Animals. Ed. Weinan, D., and Ristic, M. Vol. II. Academic Press New York, pp. 453-459.

19. LESTOQUARD, F., and DONATIEN, A. 1937. Sur Deux Parasites des Hématies du Mouton et du Boeuf d'un Genre Nouveau: Eperythrocytozoon N. G. Bull. Soc. Pathol. Exotique. 30: 454-459.

20. LOTZE, J. C. and YIENGST, M. J. 1941. "Eperythrozoonosis" in Cattle in the United States. N. Amer. Vet. 22: 345-346.

21. NEITZ, W. O. 1940. Eperythrozoonosis in Cattle. Odestepoort J. Vet. Sci. & Anim. Indust. 14: 9-28.

22. RICHARDSON, V. F., and KENDALL, S. B. 1963. Veterinary Protozoology. Oliver and Boyd, Edinburgh, pp 205-207.

23. SEAMER, J. 1959. Eperythrozoon and Haemobartonella. Vet. Rec. 71: 437-438.

24. TUOMI, J. 1966. A Microorganism Affecting Bovine Platelets. Experientia. 22: 458-459.

25. TUOMI, J. 1966. Studies on Eperythrozoon wenyoni. Infection of Cattle. Nord. Vet. Med. 18: 555-564.

26. TUOMI, J. and von BONSDORFF, C. H. Some Characteristics of a Bovine Infection Affecting Platelets and of its Causative Agent. Scand. J. Clin. Lab. Invest. 19 (Suppl. 95) 98.

27. TUOMI, J. and VON BONSDORFF, C. H., 1967. Ultrastructure of a Microorganism Associated with Bovine Platelets. Experientia 23: 111-112.

28. UILENBERG, G. 1965. Notes sur les Eperythrozoon de Bovins a Madagascar. Rev. Elev. Méd. Vet. Pays Trop. 18: 73-81.

29. UILENBERG, G. 1967. Eperythrozoon tuomii, n. sp. (Rickettsiales), troisième espèce d'Eperythrozoon des Bovins à Madagascar. Rev. Elev. Méd. Pays Trop. 20: 563-569.

30. WEINMAN, D. 1944. Infectious Anemias Due to Bartonella and Related Red Cell Parasites. Trans, Am. Phil. Soc. 33: 243-345.

31. WILSON, S. G., ZWART, D., [illegible], [illegible], and PERIE, N. M. 1967. Eperythrozoon Infection in Cattle in the Netherlands. Tijdsch. Diergeneesk. 92: 209-221.

32. ZWART, D., LEEFRANG, P., and van VORSTENBOSCH. C. J. A. H. V. 1969. Studies on An Eperythrozoon Associated with Bovine Thrombocytes. Zentbl. Bakt. Parisitkde. I. 210: 82-105.