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EPERYTHROZOONOSIS IN CATTLE AND SHEEP OF LOUISIANA, PRELIMINARY REPORT

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INTRODUCTION

While studying anaplasmosis in splenectomized cattle, beginning September, 1942, an extraneous organism has been encountered which parasitizes the erythrocytes and which tends to complicate anaplasmosis under experimental conditions. This organism is tentatively identified as *Eperythrozoon Wenyoni* which has been described from the blood of cattle by Adler and Ellenbogen[1] in Palestine and by Neitz[2] of South Africa. In the region of Beltsville, Lotz and Yiengst[3] observed eperythrozoon organisms in the blood of a calf that had been inoculated with blood from an animal which originated in Louisiana. Obviously it was not known if this donor animal had become infected while in Louisiana or whether infection occurred subsequent to leaving the state. While studying anaplasmosis in Louisiana, Dikmans[4] in 1932 observed blood from splenectomized cattle that was heavily infected with small organisms, which were interpreted as being possible developmental forms of anaplasma. It is reasonable to assume that he saw eperythrozoa. Since an ovine species, *Eperythrozoon ovis,* has also been described by Neitz, Alexander, and Du Toit[5] from sheep of South Africa, it was considered worthwhile to determine if the ovine strain were also present in native sheep of Louisiana. With a minimum of survey work, the ovine strain was located. Both bovine and ovine strains were compared morphologically with *Eperythrozoon coccoides, Haemobartonella muris,* and *Bartonella bacilliformis,* each the type species of its respective genus, as supplied by Dr. David Weinman of Harvard University Medical School. Although not proved, it is reasonably assumed that these organisms occurring in sheep and cattle of Louisiana are similar if not identical to those of Africa; namely,

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* Presented at the annual meeting of the Louisiana Veterinary Medical Association February 11, 1943.

[1] Adler, S. and Ellenbogen, V. A note on two new blood parasites of cattle, *Eperythrozoon* and *Bartonella.* Journ. Comp. Path. and Therap., Vol. 47, No. 3, pp. 219-221, Sept., 1934.

[2] Neitz, W. O. Eperythrozoonosis in cattle. Onderstepoort Journ. of Vet. Sc. and An. Ind., Vol. 14, No. 1, pp. 9-30, 1940.

[3] Lotz, J. C. and Yiengst, M. J. "Eperythrozoonosis" in cattle in United States. N. A. V., Vol. 22, No. 6, June, 1941.

[4] Dikmans, G. VI The morphology of anaplasma. Journ. A. V. M. A., Vol. LXXXIII, N. S., Vol. 37, No. 2, pp. 203-213, Aug. 1933.

[5] Neitz, W. O.; Alexander, R. A.; and Du Toit, P. J. *Eperythrozoon ovis* (sp. nov.) infection in sheep. Onderstepoort Journ. of Vet. Sc. and An. Ind., Vol. 3, No. 2, Oct., 1934.

Eperythrozoon Weynoni of cattle, and *Eperythrozoon ovis* of sheep. Dr. David Weinman concurs in the identification.

To date all observations on the bovine strain have been made on splenectomized cattle in which the disease was contracted naturally and by blood inoculation. No opportunity has arisen to study the disease in pure form in unoperated cattle. Observations on the ovine strain have been made on cases induced by blood inoculations in unoperated feeder lambs one year of age.

The importance of this disease under field conditions has not been determined, but it does have considerable significance in experimental pathology.

MATERIALS AND METHODS

Cattle used in this investigation were native to Louisiana. Calves were of dairy and beef breeds obtained from local dealers or, more frequently, from the university herds. These calves were maintained in small feed lots until approximately three months of age when they were splenectomized. Inoculation was within one month following operation or as soon as recovery had occurred. Calves were splenectomized for the purpose of obtaining animals uniformly susceptible to anaplasmosis.

The source of inoculum from which the bovine strain of eperythrozoa was originally obtained was two aged steers, one eight years of age and the other seven years of age, that had been born and raised at the university pastures. Both of these animals were known to have recovered from anaplasmosis. 10 cc. of their blood were inoculated into a splenectomized calf. Eperythrozoonosis developed during the incubation period of anaplasmosis. Both eperythrozoa and anaplasma were propagated by subinoculations.

Merino sheep used in this investigation were also native to Louisiana and were obtained from the university flock. Except for the original isolation of the ovine strain, all observations were made on unoperated feeder lambs approximately one year of age.

After finding eperythrozoa in cattle, the plan was to survey the sheep population to determine the presence or absence of the ovine strain. This was to be accomplished by injecting composite samples of mature sheep blood into susceptible sheep until the organism was located or until several flocks of sheep had been sampled and found negative. Two animals known to be susceptible were obtained by splenectomizing two yearling sheep. Stained blood smears from these two sheep were carefully examined at two-day intervals. Thirty days following the operation, eperythrozoa had been observed at no time; it was then concluded that the splenectomized sheep were not in a state of premunition and, therefore, were susceptible. The first composite sample of blood taken from

ten mature ewes from the university flock and inoculated into the splenectomized sheep proved to be infective. This isolated ovine strain was then propagated by subinoculations into normal, unoperated yearling feeder lambs and used in all later studies on sheep.

Blood smears, erythrocyte counts, and temperature readings were made at two- and three-day intervals preceding and following inoculations. During active infections these were made daily. Numerous stains were tried. The best differentiation was obtained with Giemsa stain. The routine technique employed consisted of fixation of air dried blood smears in methyl alcohol, or equal parts of methyl alcohol and ether for ten minutes, or in May-Grunwald fixing agent for three minutes. Staining was accomplished by submerging smears for thirty minutes in Giemsa solution buffered to pH 7.0-7.2.

Diagnosis was made by demonstrating the organism in stained blood smears.

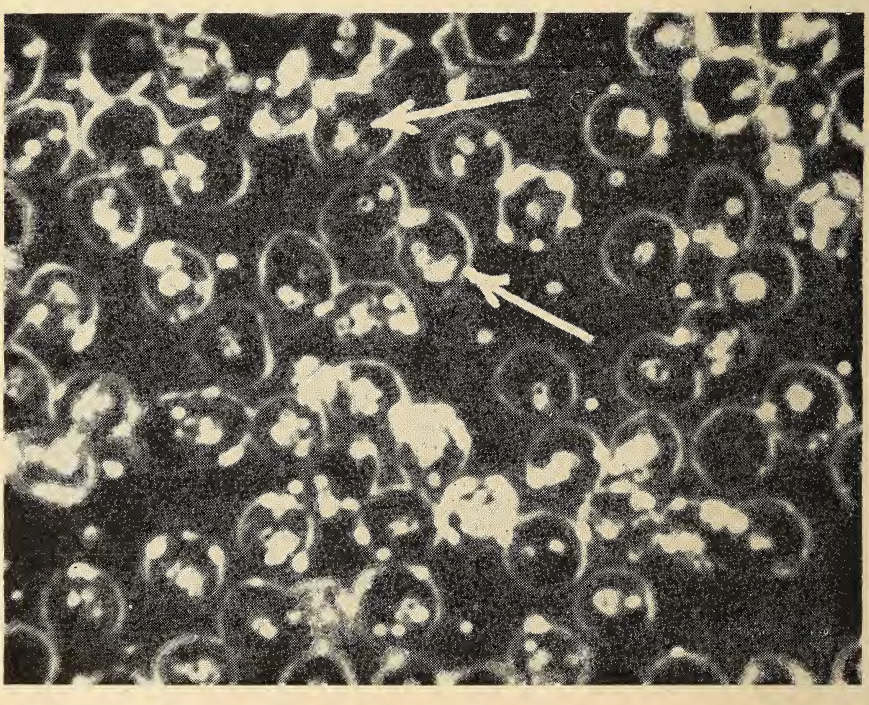

PLATE I

Photomicrograph with darkfield illumination of eperythrozoa in blood of unoperated sheep. x 950 Arrows point to organisms situated on erythrocytes.

CLINICAL MANIFESTATIONS IN CATTLE AND SHEEP

In naturally infected splenectomized calves, the infection increased rapidly in intensity, as determined by the number of infected erythrocytes, until after four to five days essentially all erythrocytes were heavily infected. During the infection, the temperature fluctuated between normal and 106° F. The erythrocyte count descended rapidly from 8,000,000 per cmm. to as low as 1,500,000 per cmm. Accordingly, the visible membranes were pale and respiration was accelerated. No fatalities have been observed. Recovery was gradual, but relapses occurred. Several cases, resulting from inoculation of splenectomized calves with blood from animals recovered from anaplasmosis, have been observed in which eperythrozoa occurred in the blood during the incubation period of anaplasmosis. In mixed infections anaplasma rapidly displaced the eperythrozoa. No opportunity has yet arisen to study uncomplicated cases in unoperated calves or adult cattle.

In sheep all clinical observations were made on unoperated feeder lambs approximately one year of age in which the infection had been induced by intravenous inoculation of heavily infected blood. In one experiment six yearling lambs, selected at random from a feed lot, were inoculated intravenously each with 10 cc. of heavily infected blood. After an incubation period which varied from 3–21 days all six lambs developed the disease. The degree of infection varied from mild to severe and symptoms varied accordingly. Anemia ranged from mild to grave. In the most severe case, the erythrocyte count descended rapidly from 9,000,000 per cmm. to 2,500,000 per cmm. Visible membranes were pale and slightly icteric. Respiration was accelerated. During the first part of the heavy infection, the temperature curve was characterized by extreme fluctuation. All six animals recovered.

The degree of infection, temperature, and erythrocyte count for the most severely infected sheep, No. 253, is shown in curves in Figure 1. Table 1 is a tabulation of the data pertaining to the six individual sheep during the infection. This experiment was repeated on another lot of six unoperated feeder lambs in which the disease was manifested by a similar range of severity, and by similar symptoms and blood changes.

TABLE I. Individual Records of Six Unoperated Sheep Inoculated Intravenously with Eperythrozoa.

Sheep No.	Incubation Period (Days)	Preinfection R. B. C. Count	Lowest R. B. C. Level Reached	Duration of Demonstrable Infection (Days)	Highest Infection Temperature
253	3	9,000,000	2,500,000	65	105.7
257	3	10,000,000	6,000,000	16	103.9
274	4	10,500,000	6,000.000	18	106.1
277	8	8,500,000	6,000,000	15	104.8
280	3	10,500,000	4,500,000	27	105.6
286	17	8,000,000	6,500.000	6	104.0

THERMAL DEATH POINT FOR EPERYTHROZOA

In experimental anaplasmosis, it is desirable to have a source of inoculum that is uncomplicated with other organisms, including eperythrozoa. An attempt was, therefore, made to separate the two. Rees,[6] attempting to establish the thermal death point for anaplasma, obtained variable results. In one trial the anaplasma survived 60° C. for ten minutes; in another trial they were killed at 48° C; and in still others they survived at 48° C. Thus, the range for anaplasma appears to be wide.

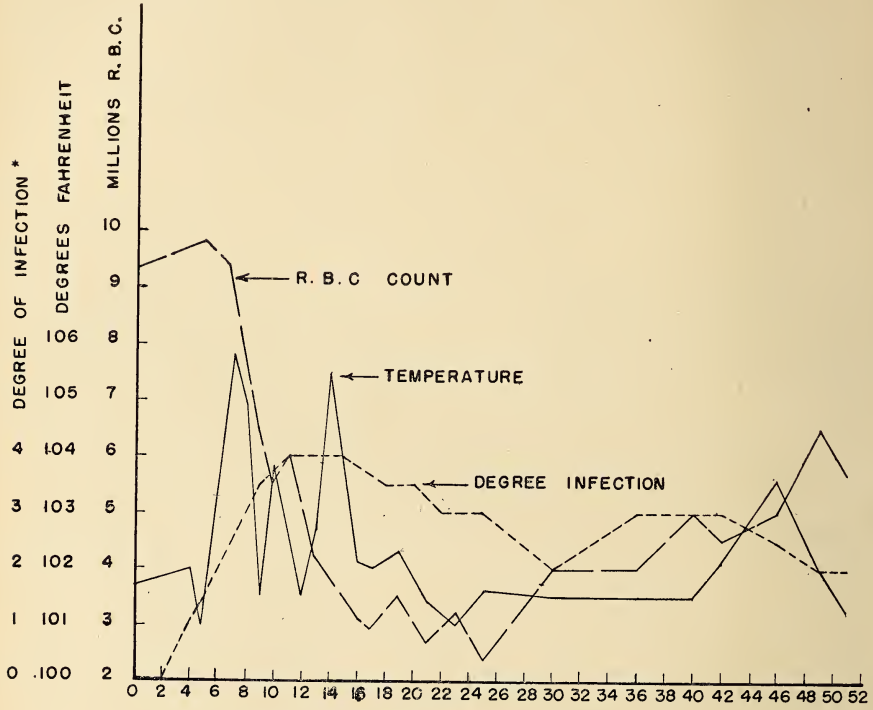

FIGURE 1.

An experiment was designed on this basis to determine the thermal death point of eperythrozoa. The sheep strain was employed because sheep are more available as experimental animals. The blood was heated in 2 cc. quantities in test tubes submerged in a Wasserman bath. Great care was exercised to insure that all blood to be used in each inoculum was heated to the required temperature. The blood was introduced into test tubes by means of suitable pipettes. The top of each tube, which

[6] Rees, C. W. The effect of exposure to different degrees of temperature on the etiological agent of bovine anaplasmosis and piroplasmosis. Journ. Parasit., Vol. 23, No. 2, April, 1937.

projected above the water bath, was flamed to prevent mixture of unheated blood with heated portions. By previous trials it was determined that two minutes were required to bring the 2 cc. quantity of blood to the temperature of the water bath. The blood samples, therefore, were in the water bath for a total of twelve full minutes. Heated and control samples were injected intravenously. Data of this experiment are given in Table II.

TABLE II

Sheep No.	Temperature Degrees C.	Time Heated Minutes	CC. of Inoculum	Reaction*
225	45	10	10	Infected 4/1/43
260	48	10	10	Infected 4/8/43
265	50	10	10	Not infected
283	54	10	10	Not infected
285	room	..	10	Infected 3/29/43
295	room	..	10	Infected 3/25/43

*Inoculations made 3/24/43.

From this limited number of trials, it is seen that the thermal death point for ovine eperythrozoa is greater than 48° C. and less than or equal to 50° C. when heated for ten minutes which is within the same range as established by Rees for bovine anaplasma. Therefore, the application of heat *in vitro* to inoculum containing anaplasma and eperythrozoa probably cannot be used to separate either infection from the other.

CROSS INOCULATION TESTS

Two splenectomized calves known to be susceptible to the bovine strain were inoculated intravenously each with 10 cc. of defibrinated blood from an infected sheep. Daily examinations were made of them for a period of thirty days. At no time did eperythrozoa appear in blood smear preparations. Subsequently, both calves were inoculated with the bovine strain and both contracted the disease.

Likewise, two sheep were inoculated with infected calf blood. During a period of sixty days no eperythrozoa were demonstrated in blood smears. Subsequently, these two sheep were inoculated with the sheep strain and both developed an infection. However, a calf inoculated with blood from these two sheep developed eperythrozoonosis after an incubation period of eighteen days. Thus, the bovine strain was maintained in a viable condition in the sheep. The above tests indicate an interspecial non-susceptibility which is the basis for establishing separate species for cattle and sheep.

TRANSMISSION

Previous investigators 1, 2, 3, 4, 5, 6, have suspected natural transmission to be accomplished by arthropod vectors. By none has this been proved. In the present studies all artificially induced cases were by blood inoculations. Natural spread of the disease has been observed frequently among calves. During fly season the infection spread rapidly when non-infected calves were maintained in pens with infected or carrier calves. Biting flies were abundant. A few lice were observed. No insect transmission studies were attempted.

CONCLUSIONS

1. Eperythrozoonosis exists in native cattle and sheep of Louisiana.

2. In unoperated sheep the disease is characterized by a varying degree of anemia and high, but fluctuating, temperature.

3. The thermal death point of eperythrozoa of sheep is greater than 48° C. and less than or equal to 50° C. when heated for ten minutes.

4. Cattle are not susceptible to eperythrozoa of sheep; and sheep are not susceptible to eperythrozoa of cattle. However, the bovine strain is maintained in sheep when placed there by artificial inoculation.