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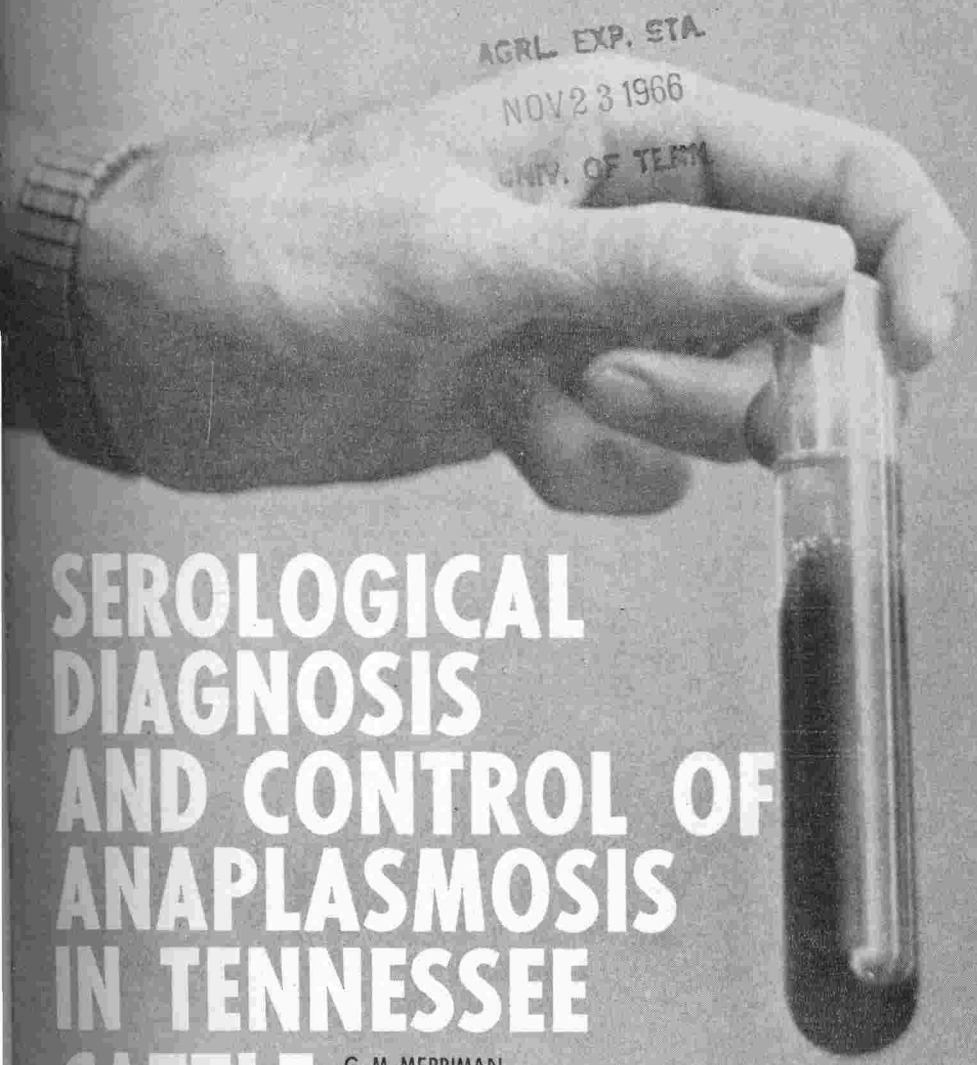

SEROLOGICAL DIAGNOSIS AND CONTROL OF ANAPLASMOSIS IN TENNESSEE CATTLE

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Serological Diagnosis and Control of Anaplasmosis in Tennessee Cattle

by

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INTRODUCTION AND REVIEW OF LITERATURE

ANAPLASMOSIS is an infectious, parasitic disease of the erythrocytes (red blood cells) of cattle and deer. It is caused by an organism of uncertain zoological classification identified as *Anaplasma marginale.* Ristic (1962) has shown that the organism is harbored in blood platelets as well as in erythrocytes. The nature and symptoms of the disease and nature of the causative agent have been described by a number of workers, including Mott (1957), Carricaburu (1957), Ristic (1960 and 1962), Foote, Geer, and Stick (1958), Espana and Espana (1962), Gates and Ritchie (1962), and Brock (1962).

Mott (1957) has given information on the behavior and appearance of the parasite in the bovine erythrocyte during the course of the disease and on the development of the normal-appearing, recovered anaplasmosis carrier. These carriers serve as reservoirs from which the infection is spread and they must be detected if bovine anaplasmosis is to be controlled. The anaplasma or marginal bodies, assumed to be *A. marginale,* can be seen in microscopic preparations of the erythrocytes from the infected animal during the late part of the incubation period, and, in large numbers, during the acute, symptomatic stage of the disease. As symptoms abate, the number of parasitized erythrocytes gradually decreases over a period of weeks or months until, with complete recovery of the animal, no parasitized cells can be demonstrated

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by ordinary microscopic technique. Despite the apparent disappearance of *A. marginale* from the blood, the recovered animal becomes a normal-appearing carrier of anaplasmosis. Blood from this carrier, transferred to anaplasmosis-susceptible cattle by ticks, horseflies, other biting or sucking insects, or by such equipment and instruments as bleeding needles, castrating knives, and tattooing instruments, can cause a new case of anaplasmosis. While the carrier animal is usually assumed to be one recovered from noticeable clinical signs of the disease, the carrier state also appears to develop as a result of mild, unnoticed cases among younger animals. Therefore, a farmer may have in a herd one or more cattle which are carriers of anaplasmosis but which have never shown signs of illness. The recovered animals may remain carriers for life.

Either acutely infected or carrier animals are the source of new infection to susceptible herds or individuals. The disease is spread by transfer of blood from infected or carrier cattle to susceptible cattle (Mott, 1957; Howell, 1957). While the disease has been identified in certain deer and has been transmitted from deer to cattle by ticks and by deliberate blood transfer (Osebold, Douglas, and Christian, 1962; Osebold, 1962; Roberts and Lancaster, 1963), we have no knowledge at this time that Tennessee deer represent a problem in this respect.

Anaplasmosis is currently less of a hazard to cattle in most of Tennessee than it is in states immediately adjoining Tennessee on the east, south, and west (Saulmon, 1962). However, the definite presence of anaplasmosis in Tennessee (Table 1, Tennessee Department of Public Health, 1956-1964) and in neighboring states, the presence in the state of ticks and horseflies which could serve to transmit the infection from carriers, and the likelihood of unknowingly importing carriers into areas of the state currently free of the disease—all these make anaplasmosis a disease which Tennessee cattlemen must guard against.

Table 1. Numbers of bovine cases of anaplasmosis reported annually by veterinarians to the Tennessee Department of Public Health

Year	No. of cases	No. of farms	Year	No. of cases	No. of farms
1956	31	23	1961	170	94
1957	300	31	1962	136	91
1958	116	65	1963	136	104
1959	107	39	1964	137	79
1960	111	69			

Detection of anaplasmosis carriers, unlike the identification of the acutely-infected animals, cannot be accomplished by physical examination of the animal or by microscopic examination of its erythrocytes. In cattle, detection of carrier animals is accomplished by identification of antibodies which the infected animal has developed specifically against *A. marginale* due to infection. The antibodies are assumed to persist throughout the carrier state.

One method of detecting serum antibodies against *A. marginale* has been the use of the complement-fixation (CF) test. Blood serum, carefully collected and processed, is mixed with antigen prepared from *A. marginale* and with a carefully measured amount of complement. The complement, secured from the blood of guinea pigs, is a material which facilitates a reaction between an antibody and its specific antigen. This mixture is incubated and then mixed with red blood cells of sheep which already carry an antibody produced against them in rabbits. If the blood serum in the original mixture came from a cow which had been exposed to *A. marginale,* it would contain antibodies against the organism, which would have united with the complement during incubation and the added red cells would have undergone no change. If the blood serum was from an animal without antibodies against *A. marginale*—one which had never had anaplasmosis—the complement would be unchanged during the incubation. When the red cells and their antibodies were added to the mixture, the erythrocytes would have been destroyed. The resulting loss of hemoglobin or coloring matter into the mixture would indicate that the blood serum came from a non-infected animal. All ingredients for the test must not only be carefully prepared but must be accurately standardized and measured so that they will give reliable, repeatable results.

The application of the CF test to the detection of anaplasmosis carriers has been reported by many workers including Roby (1957); Mott, *et al.* (1959); Riemenschneider *et al.* (1959); Saulman (1957); Merriman and Hobbs (1961); Merriman, Buckner, and Hobbs (1962); and Heck, Franklin, and Huff (1962). This test, though somewhat complicated and having slight inherent inaccuracies, has been a useful tool in anaplasmosis control and research. It has been used to successfully eradicate anaplasmosis from at least one state (Willers, 1957).

A less complex method of detecting serum antibodies against *A. marginale,* the capillary-agglutination (CA) test, has been developed (Ristic, 1962; Welter and Zuschek, 1962; Merriman, Owens and Chung, 1964). In the CA test for anaplasmosis a small

amount of antigen is drawn into a capillary tube. The remainder of the capillary tube is filled with the blood serum to be tested. The tube is sealed and inserted into a bar of clay so that it remains upright. If the animal from which the serum was collected had no exposure to anaplasmosis and thus had produced no antibodies, there will be no change. If however, antibodies against *A. marginale* had been developed because of an earlier infection, clumping (agglutination) of the antigen will take place and can be easily observed with the unaided eye. While the agglutination often takes place promptly, the final reading of the test is not made until the antigen-serum mixture has been in the capillary tube for 24 hours. Technical advantages of the CA test over the CF test are primarily that it can be conducted rapidly without highly trained personnel or special laboratory equipment.

OBJECTIVES AND SCOPE

Objectives

The original objectives of this experiment were as follows:

1) Study the effectiveness, as measured by the CF test, of controlling anaplasmosis by retention of positive reactors, slaughter of positive reactors or segregation of positive reactors from non-reactor cattle.
2) Determine, by repeated testing of breeding herds on Tennessee experiment stations and by calf inoculations, the accuracy of the CF test in detecting anaplasmosis carriers.

Modifications of these objectives were necessitated by 1) transient problems with the CF test; 2) the low incidence of anaplasmosis in experiment station herds; and 3) the availability, late in the experiment, of the newly-developed CA antigen. The ultimate objectives, then, can be stated as follows:

1) Evaluate the efficiency of controlling anaplasmosis by segregating positive CF reactors from the herd.
2) Evaluate the accuracy of the CF test as stated in the original objectives.
3) Compare the accuracies of the CF and CA tests in non-infected, naturally-infected, and artificially-infected cattle.

Scope

The project was begun in 1956 and was continued, in part, into 1963. The testing of the breeding herds at Ames Plantation and of the herds at seven additional research centers of the Ten-

nessee Agricultural Experiment Station involved collection of more than 8,000 blood serum samples which were submitted to the Animal Disease and Parasite Research Branch, USDA, for CF testing. Blood serum samples from 33 mature cattle were tested at the Animal Husbandry—Veterinary Science laboratories with CA antigen.

In addition to cattle used in breeding herds, a total of 8 splenectomized calves and 12 intact calves were used in separate experiments. Blood serum samples for 167 CF tests and 144 CA tests were collected from these animals.

PROCEDURE

For Objective 1. Anaplasmosis Control by Complement-Fixation Testing and Separation of Positive Reactors

This phase was conducted from 1956 through 1961 at the Ames Plantation Field Station in West Tennessee. The field station, occupying approximately 18,000 acres in parts of Fayette and Hardeman counties, has been used several years for research and teaching purposes by the University of Tennessee under terms of the will of the late Julia C. Ames. The Department of Animal Husbandry—Veterinary Science assumed responsibility for the cattle operation in 1955 and retained many of the purebred Angus cattle then present. The commercial cattle were generally disposed of by 1956 or 1957. A herd of 100 breeding-age Angus, purchased in East Tennessee, was added in September, 1955. Very few additional cattle were purchased during the experiment.

Anaplasmosis may have existed in the Ames cattle before 1955. Reports of clinical cases before then are largely unconfirmed. However, CF tests of the cattle in June, 1955, revealed evidence of a rather low incidence of earlier infection. There were 45 (8.5%) reactors among 529 cattle tested.

Consultations of University representatives with personnel of the Tennessee Department of Agriculture and the Animal Disease and Parasite Research and Animal Disease Eradication Branches of the USDA resulted in the development of the following program to be put in effect and evaluated as a means of practical anaplasmosis control:

1. Annual CF-testing of the breeding herds.
2. Removal of CF-positive cattle either for slaughter or to be placed in a "reactor herd."

Methods for collection of blood and serum, CF testing, and

management of the cattle necessitated by this program and some technical problems are described in the following paragraphs:

Collection of blood and serum: Thirty milliliters (ml.) or more of blood was drawn from the jugular vein through a sterile needle into a dry tube for each sample. Serum was removed within 2 to 12 hours by decanting or centrifugation. A 5% solution of phenol was added to each sample of serum to obtain a final phenol concentration of 0.5%. Blood collections were made by U.T. personnel in 1956 and by veterinarians in private practice in that area in later years. In 1956-1959, serum was processed by persons collecting the blood. Following 1959, blood samples for processing were taken to the State Serologist at Dresden, Tennessee. All sampling was done in winter.

CF testing: Phenolized serum samples were sent directly to the laboratories of the Animal Disease and Parasite Research Branch, USDA, for CF testing.

Management of cattle: During the experiment, the breeding cattle were kept in various large herds dispersed throughout Ames Plantation. All CF-positive cattle were removed as soon as test results were known. Cattle placed in the reactor herd were never returned to the other herds. While occasionally only a fence separated the reactor herd from other groups, this herd was usually completely isolated from other animals.

Usual management precautions were taken against transmission of anaplasmosis among cattle. Ticks were never abundant. Lice were controlled by spraying with various insecticides. Horseflies, perhaps the most common potential vector, were partly controlled whenever they became a problem by the addition of synergized pyrethrins (Gulfspray) to the backrubbers in each affected pasture. Instruments used in vaccination, blood collecting, tattooing, and castrating were disinfected to minimize mechanical transfer of infection.

Technical problems: The remote location of the cattle from the offices and laboratories of the research personnel caused no serious difficulty. Occasional trouble, when the herds were first being assembled, was experienced in identification of individual animals. As record systems became established and all cattle were hip-branded, this difficulty lessened.

Incomplete data in various years, due to unsatisfactory or broken samples, was the greatest single problem and might be similar in extent to that experienced in a program of anaplasmosis control with similar area and cattle numbers involved. Several

days to a few weeks would elapse before the identities of unsatisfactory samples and samples broken in shipping would be learned. This made it impractical to bring the cattle from the widely-dispersed herds back for resampling. The percentage of samples which could not be tested for any 1 year ranged from 0 to 0.36 for 1956 and 1959-1962, but was 15.5 and 12.0 for 1957 and 1958, respectively.

Small numbers of tenant-owned cattle and a few deer were scattered about the plantation. These posed a potential anaplasmosis threat which was minimized by good fences and by relatively small numbers of ticks and other vectors.

For Objective 2. Evaluate Accuracy of Complement-Fixation Test

Beef cattle at seven locations were used in this phase of the research. Data on breed, numbers of samples, and frequency of sampling are shown in Table 2. In all, 4,178 samples were collected and processed by University of Tennessee personnel and sent to the Animal Disease and Parasite Research Branch, USDA, for CF testing.

Table 2. Complement fixation testing at seven research centers, 1956-1961

Station	Breed	Cattle tested		Station	Breed	Cattle tested	
		Year	Number			Year	Number
Alcoa	Hereford	1956	181	Tobacco	Hereford	1956	46
Farms,		1957	229	Experiment		1959	92
Knoxville		1958	235	Station,		1959	82
		1959	223	Greeneville			
Blount Farm,	Angus &	1957	71	Plateau	Angus	1959	131
Main Station,	Hereford	1958	119	Experiment		1959	129
Knoxville		1959	111	Station,		1959	3
				Crossville		1960	122
Highland Rim	Hereford	1957	45			1960	6
Experiment		1958	34			1960	126
Station,		1959	36			1961	208
Springfield		1960	51				
Middle Tenn.	Hereford	1957	62	UT-AEC	Hereford	1956	79
Experiment		1958	48	Agric. Res.		1957	1083
Station,		1959	67	Lab., Oak		1958	232
Spring Hill		1960	68	Ridge		1959	258

There were no histories of clinical disease resembling anaplasmosis in cattle of the following Experiment Stations: Blount Farm (BF), Alcoa Farms (AF), Highland Rim (HRES), Middle Tennessee (MTES), Plateau (PES), and Tobacco (TES). Anaplasmosis had been clinically and serologically diagnosed in cattle at the University of Tennessee—Atomic Energy Commission Agri-

cultural Research Laboratory (UT-AEC), but no clinical cases had been reported in recent years.

Blood and serum collecting procedures were similar to those used at Ames Plantation (see procedure for Objective 1). Blood was usually collected before or after the season of greatest activity of insect vectors. Ticks were not a problem in these herds. Lice and horseflies were generally controlled as they were at Ames Plantation. No segregation of CF-positive cattle was made and reactors were retained in the herds until culled for reasons other then CF reaction.

Fresh citrated whole blood from the 3 CF-positive cows at PES was inoculated intravenously into 2 splenectomized Holstein-Friesian bull calves. The blood from 1 cow was administered to 1 calf and a mixture of blood from the other 2 cows was given to the second calf. Clinical examinations of the calves were made daily or twice daily. Blood for CF testing and hematologic examination (hemaglobin level, packed cell volume, and examination of stained erythrocytes) was collected twice weekly. Details of these examinations and procedures have been reported (Merriman, Buckner, and Hobbs, 1962).

Later studies comparing CF and CA tests were completed which gave supplemental information on the CF test results with different classes of cattle.

For Objective 3. Comparison of the Complement-Fixation and Capillary-Agglutination Test in an Anaplasmosis Infected Herd

At Ames a herd of grade and purebred Angus was maintained on a separate unit known as the Demonstration Farm. Some of these cows were CF-positive reactors removed from breeding groups on the Plantation. Thus, a known CF history was available on many of these cows. The 32 cattle in this herd which were 2 years or more of age were used in 1963 for this phase of the project.

Field testing. Blood collected on April 4, 1963 from each of the 32 Demonstration Farm cattle was returned to the Animal Husbandry—Veterinary Science (AH-VS) laboratories at Knoxville for processing. One-half of the serum from each was phenolized and forwarded for CF testing to the Animal Disease and Parasite Research Branch of USDA.

CA tests on the remaining half of each sample were conducted at the AH-VS laboratories. The methods of Ristic (1962) and of Welter and Zuschek (1962) were used.

Calf inoculations. On April 29, aseptically-collected, citrated

blood from CA- and CF-tested cows at the Demonstration Farm was flown to Knoxville and intravenously administered to Holstein-Friesian bull calves splenectomized 21 to 28 days earlier. Each calf received 150 to 200 ml. of blood. Inoculations were completed not more than 9 hours after collection of blood. Donors used were selected primarily on the basis of the CF and CA reactions of their blood serum collected on April 4. The April 4 sampling test results for the 5 individual donors were CA-, CF-positive in 2 cases; CA-, CF-suspicious in 1; CA-, CF-negative in 1; and in the last case, CF-positive and CA-suspicious. Blood from each of these donors was administered to a single randomly-selected calf. All 5 cows had been CF-positive on one or more previous tests. The CA and CF tests were repeated April 29 on all cows that had positive or suspicious reactions on April 4. The 6th calf was inoculated with a composite of blood from 6 cows CF- and CA-negative on the April 4 samples. Daily or twice-daily clinical examinations were made of the calves until the animals died or until 60 or more days had elapsed after inoculation. Blood for CA and CF testing and for hematologic studies of packed cell volume, and for erythrocyte appearance was collected twice weekly during the pre- and post-inoculation phases of the trial. The CA tests, conducted according to the method of Ristic (1962), were considered "suspect" if a 1 + reaction was observed and "positive" if 2 + or 3 + reactions occurred. A necropsy was made on the 1 calf which succumbed. These procedures were reported in detail in earlier papers (Merriman, Buckner, and Hobbs, 1962; and Merriman, Owens, and Chung, 1964).

Studies with intact, non-infected calves: Blood from 12 intact Hereford calves from an anaplasmosis-free herd was collected for CF and CA testing and for examination of erythrocytes.

Table 3. Complement-fixation status of cattle in central-unit herds, Ames Field Station, 1956-62

Year	Samples collected No.	Results: Passed No.	Positive No.	Positive Percent	Suspect No.	Unusable Samples: A.C.[a] No.	Other No.
1956	693	674	14[b]	2.0	3	0	2
1957	584	484	8	1.3	1	0	91
1958	685	603	0	0.0	0	0	82
1959	586	546	16	2.7	23	0	1
1960	542	532	3	0.5	4	1	2
1961	559	553	0	0.0	6	0	0
1962	504	500	1[c]	0.2	3	0	0

[a] Anti-complementary.
[b] 10 were already in an isolated unit based on CF results, 1955.
[c] A 5-month-old bull calf.

Results and Discussion

For Objective 1. Anaplasmosis Control by Complement-Fixation Testing and Removal of Positive Reactors

The results of the annual CF tests for anaplasmosis for all cattle of the breeding herds of the Ames Plantation's central unit are shown in Table 3. In studying these data, one should consider that there had been a CF test of the cattle which were present when the University of Tennessee entered into the management of the Plantation. This 1955 test was not part of the experiment. Ten reactors identified in 1955 were kept and were placed in a small, isolated herd made up primarily of commercial cows. The remaining reactors were sold for slaughter.

The numbers of CF-positive animals each year, 1956 through 1959, varied erratically with a high of 16 in 1959. After this, however, there were but 3 new CF-positive animals in 1960, none in 1961 and 1, a 5-month-old calf from a CF-negative dam, in 1962. It is possible that there were some anaplasmosis carriers among the large number of cows from which unsatisfactory samples were collected in 1957 and 1958. Other explanations, such as changes in numbers of insect vectors or in the numbers of anaplasmosis-infected deer in the area, do not appear to be substantiated by available evidence.

While one cannot state that anaplasmosis was eliminated from Ames Plantation by this program, it is true that the incidence was reduced to a practical level.

During the experiment, horseflies were the only plentiful insects on the plantation which are generally acknowledged to transmit anaplasmosis. These were quite well controlled. Ticks were uncommon. If large populations of horseflies or, especially, ticks had been present, it is likely that CF testing and the removal of CF-positive cattle would not have controlled anaplasmosis until the vectors had been controlled.

The CF-positive cattle were removed from the central unit herds after each annual test and placed in an isolated herd consisting partly of anaplasmosis-free animals. The positive cattle were kept in this herd until, for reasons other than anaplasmosis status, they were sold for slaughter. The results of several CF tests of this herd are shown in Table 4. The significance in the CF status of this group is the fact that a herd with 2 or more anaplasmosis carriers was known to be present on the plantation during the entire experiment.

For Objective 2. Evaluate Accuracy of Complement-Fixation Test

During the period 1956-1960, 4,178 serum samples were submitted from cattle at seven research centers in the Tennessee Experiment Station for CF testing by the laboratories of the USDA's Animal Diseases and Parasite Research Branch. There was no clinical evidence of anaplasmosis in any of the herds. The disease had been known to exist only in the herd of the UT-AEC Agricultural Research Laboratory. In this herd, the disease had not been clinically evident for 2 or more years before the start of this experiment. Thus, the herds were considered anaplasmosis-free on the basis of clinical history.

Table 4. Complement-fixation status of isolated herd, Ames Plantation, 1958-1962

Year	Month	Samples collected No.	Results			Unusable Samples	
			Passed No.	Reactor No.	Suspect No.	A.C.[a] No.	Other No.
1958	Jan.	36	27	8	1	0	0
1958	June	31	29	2	0	0	0
1959	Oct.	36	30	6	0	0	0
1960	June	35	20	11	3	1	0
1960	Oct.	56	52	4	0	0	0
1961	Oct.	67	65	2	0	0	0
1962	Nov.	44	37	6	1	0	0

[a] Anti-complementary

As shown in Table 5, 4,070 (97.4%) of the samples submitted gave negative results and only 17 (0.4%) gave positive results. While it is necessary to recognize that 1% of the samples could not, for various reasons, be tested, the inaccuracy of the CF test during this period, as reflected by the appearance of "false positive reactions," would approximate only 0.4%.

CF-reaction histories on the 14 cows which accounted for all 17 CF-positive tests are shown in Table 6. Eleven of these cows were detected in 1957 and were at the MTES, HRES, and UT-AEC Experiment Stations. Three, all at UT-AEC, had been tested in 1956 and were negative. Eight of the 11 were present for retest in 1958, 7 in 1959, and 5 in 1960. All retests were negative and included all three herds in 1958 and 1959, and the MTES and HRES herds in 1960. No additional CF-positive reactors were detected in these herds after 1957.

The 3 CF-positive cows at PES were detected in November, 1959, and were positive again in January, 1960. All 3 were CF-negative in tests conducted in September and November, 1960. The

inoculation during November, 1960 of blood from the 3 CF-positive reactors into splenectomized calves did not produce hematological, serological, or clinical evidence of anaplasmosis in the calves. Detailed results of these studies have been reported (Merriman, Buckner, and Hobbs, 1962).

The CF-positive reactions in the four herds described above may be assumed to be false reactions. It would be preferred that such reactions did not occur. Yet, the removal by test and slaughter of a comparable percentage of non-infected cows from infected herds such as those at Ames Plantation would be a relatively low cost for control of a disease as serious as anaplasmosis.

Table 5. Results of repeated complement fixation tests in herds having no clinical evidence of anaplasmosis

Herd location	Date of test	Samples collected No.	Results			Samples not usable	
			CF-negative No.	CF-positive No.	CF-suspect No.	AC No.	Other No.
Alcoa Farm,	Oct. 1956	181	178	0	3		0
Main Station,	Sept. & Oct. 1957	229	225	0	0		4
Knoxville	Oct. & Nov. 1958	235	223	0	2		10
	Sept. & Oct. 1959	223	217	0	2		4
Blount Farm,	April 1957	71	71	0	0		0
Main Station,	Dec. 1958	119	116	0	1		2
Knoxville	Oct. 1959	112	109	0	2		1
Highland Rim	April 1957	45	38	5	1		1
Experiment	Oct. 1958	34	32	0	2		0
Station,	Oct. 1959	36	31	0	3		2
Springfield	Nov. 1960	51	49	0	1		1
Middle Tennessee	April 1957	62	57	3	2		0
Experiment	Oct. 1958	48	43	0	3		2[a]
Station,	Oct. 1959	67	65	0	1		1
Spring Hill	Oct. 1960	68	67	0	0		1
Tobacco	Nov. 1956	46	45	0	0		1
Experiment	May 1959	92	90	0	2		0
Station,	Oct. 1959	82	82	0	0		0
Greeneville							
Plateau	May 1959	131	131	0	0		0
Experiment	Oct. 1959	129	124	3	2		0
Station,	Dec. 1959	3[b]	0	3	0		0
Crossville	May 1960	122	122	0	0		0
	Sept. 1960	6[b]	6	0	0		0
	Nov. 1960	126	125	0	1		0
	May 1961	208[b]	206	0	2		0
UT-AEC Agric.	Nov. 1956	79	77	0	2		0
Res. Lab.,	Jan.-April 1957	667	660	0	3	1	3
Oak Ridge	Nov. 1957	416	399	3	8	5	1
	Dec. 1958	232	230	0	1		1
	Oct. 1959	258	252	0	3		3
Grand Total		4,178	4,070	17	47	6	38
Percentages of Total Submitted			97.4	00.4	01.10	00.14	00.9

[a] Both negative, 1957.

[b] Includes the 3 CF-positive cows indicated in the October test.

Table 6. Anaplasmosis complement-fixation test status by years of all cows positive on one or more tests

Herd location	Cow No.	Complement-fixation status								
		1956	1957	1958	1959		1960			
					June	Nov.	Jan.	May	Sept.	Nov.
Middle Tennessee	212	—	Pos.	Sold[a]						
Experiment Station,	83	—	Pos.	Neg.	—	Neg.	—	—	—	Sold
Spring Hill	16	—	Pos.	Neg.	—	Neg.	—	—	—	Neg.
Highland Rim	H176	—	Pos.	Neg.	—	Neg.	—	—	—	Neg.
Experiment Station,	H226	—	Pos.	Neg.	—	Neg.	—	—	—	Neg.
Springfield	H166	—	Pos.	Neg.	—	Neg.	—	—	—	Neg.
	H945	—	Pos.	Neg.	—	Sold[a]	—	—	—	Sold
	H814	—	Pos.	Neg.	—	Neg.	—	—	—	Neg.
UT-AEC Agric.	-660	Neg.	Pos.	Sold[a]						
Res. Lab.	1518	Neg.	Pos.	Neg.	—	Neg.	—	—	—	—
	726	Neg.	Pos.	Sold[a]						
Plateau Experiment	64	—	—	—	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.
Station,	P416	—	—	—	Neg.	Pos.	Pos.	Neg.	Neg.	Neg.
Crossville	P517	—	—	—		Pos.	Pos.	Neg.	Neg.	Neg.

[a] Animals were sold for reasons other than their anaplasmosis test results.

Table 7. Complement-fixation test status by years of all cows with suspect results on one or more tests

Substation herd	Cow No.	Complement-fixation status									
		1956		1957		1958	1959		1960		1961
		Fall	Jan.	Spring	Fall	Fall	Spring	Fall	Spring	Fall	June
Middle Tennessee	256	—	—	2+	—	—	—	—	—	—	—
Experiment Station,	133	—	—	2+	—	Neg.	—	Neg.	—	Neg.	—
Spring Hill	021	—	—	Neg.	—	1+	—	Neg.	—	Neg.	—
	237	—	—	—	—	2+	—	Neg.	—	Neg.	—
	407	—	—	—	—	2+	—	Neg.	—	Neg.	—
	333	—	—	Neg.	—	—	—	1+	—	Neg.	—
Highland Rim	1	—	—	2+	—	Neg.	—	Neg.	—	Neg.	—
Experiment	527	—	—	—	—	1+	—	—	—	Neg.	—
Station,	965	—	—	Neg.	—	2+	—	2+	—	Neg.	—
Springfield	854	—	—	Neg.	—	Neg.	—	2+	—	1+	—
	908	—	—	—	—	—	—	3+	—	Neg.	—
UT-AEC Agric. Res.	357	1+	—	—	Neg.	—	—	—	—	—	—
Lab.,	726	2+	Neg.	—	Positive	—	—	—	—	—	—
Oak Ridge	853	Neg.	2+	—	Neg.	—	—	—	—	—	—
	1211	—	2+	—	Neg.	Neg.	—	Neg.	—	—	—
	1693	—	2+	—	—	—	—	—	—	—	—
	1313	—	Neg.	—	2+	—	—	—	—	—	—
	1180	—	—	—	1+	—	—	—	—	—	—
	1300	—	—	—	1+	—	—	—	—	—	—
	1040	—	Neg.	—	2+	Neg.	—	Neg.	—	—	—
	1283	—	Neg.	—	—	1+	—	1+	—	—	—
	39-	—	—	—	Neg.	Neg.	—	2+	—	—	—
	-966	—	Neg.	—	Neg.	Neg.	—	—	—	—	—
	-58	—	—	—	—	—	—	1+	—	—	—
	651	Neg.	—	—	3+	—	—	—	—	—	—
	843	Neg.	—	—	1+	—	—	—	—	—	—
	659	Neg.	Neg.	—	1+	—	—	—	—	—	—
	840	Neg.	Neg.	—	2+	—	—	—	—	—	—

Table 7. Complement-fixation test status by years of all cows with suspect results on one or more tests (Continued)

Substation herd	Cow No.	Complement-fixation status									
		1956		1957		1958	1959		1960		1961
		Fall	Jan.	Spring	Fall	Fall	Spring	Fall	Spring	Fall	June
Middle Tennessee	16	—	—	—	—	—	Neg.	1+	Neg.	Neg.	Neg.
Experiment Station,	188	—	—	—	—	—	—	1+	Neg.	Neg.	Neg.
Spring Hill	185	—	—	—	—	—	Neg.	Neg.	Neg.	1+	Neg.
	NC239	—	—	—	—	—	—	—	—	—	1+
	G37	—	—	—	—	—	—	—	—	—	1+
Tobacco Experiment	877	—	—	—	—	—	1+	Neg.	—	—	—
Station,	55	Neg.	—	—	—	—	3+	Neg.	—	—	—
Greeneville											
Blount Farm,	358	—	—	—	—	—	—	3+	Neg.	—	—
Knoxville	368	—	—	—	—	—	—	1+	—	—	—
Alcoa Farm,	A471	3+	—	—	Neg.	—	—	—	—	—	—
Knoxville	A378	2+	—	—	—	Neg.	—	Neg.	—	—	—
	G277	3+	—	—	Neg.	Neg.	—	Neg.	—	—	—
	G554	Neg.	—	—	Neg.	1+	—	—	—	—	—
	A458	Neg.	—	—	Neg.	1+	—	—	—	—	—
	G494	Neg.	—	—	Neg.	1+	—	—	—	—	—
	204	Neg.	—	—	Neg.	Neg.	—	1+	—	—	—
	A483	Neg.	—	—	Neg.	Neg.	—	2+	—	—	—

The CF history of each CF-suspicious animal is shown in Table 7. The suspicious reactions, reported as 1 +, 2 +, or 3 +, appeared not to be repeatable from year to year nor from season to season. The CF-suspicious reaction did not accurately warn of developing infection. Of 46 CF-suspect reactions, only 1 was from a cow (Cow 726, UT-AEC) which subsequently became a CF-positive reactor.

Forty-four of the 4,178 samples collected could not be tested. Six (0.14% of all samples) possessed anticomplementary (AC) activity. Five of these were in the November, 1957, samples from UT-AEC. Thus AC reactions were only a minor problem. The remaining 38 unusable samples contained insufficient serum or had gross contamination or were broken during shipment.

For Objective 3. Comparison of the Complement-Fixation and Capillary Agglutination Tests in an Anaplasmosis-Infected Herd

There was general agreement between results of the CA tests and results of the CF tests as applied to the demonstration farm herd in 1963 (Table 8).

Annual CF reactions of all cattle in this herd with 1 or more positive reactions from 1959 through 1962 were recorded (Table 9). There were minor fluctuations in CF results in some individuals over a period of years. There was agreement in 1963 between the results of the two types of tests for the 6 remaining cows with previous CF-positive reactions. However, on the basis of calf-inoculation results (Table 9), the CF reactions more accurately reflected the status of *Anaplasma* infection in 1 cow (029) than did the CA reactions.

Results of CF and CA tests for the splenectomized calves before they were administered blood from Ames Plantation cows are

Table 8. Comparison of complement-fixation and capillary-agglutination test results for adult cattle in demonstration farm herd, Ames Plantation Field Station, 1963

Type of test	Date tested	Number tested	Number negative	Number suspects	Number of positive reactors
CF	4-4-63	33	29	1[a]	3[b]
CA	4-4-63	33	29	2[c]	2
CF	4-29-63	4	1[a]	0	3[b]
CA	4-29-63	4	0	1[a]	3[b]

[a] Cow 029 was CF-positive 1960-62 and 1+ on CF test of 4-4-63.
[b] Cows *536*, 3085, and 1741.
[c] Cows 029 and 3085.

Table 9. Complement-fixation and capillary-agglutination test histories on adult cattle

Animal No.	Type of test	Test Results 1959	1960 (June)	1960 (Fall)	1961	1962	1963 (Apr. 4)	1963 (Apr. 29)	Calf inoculation results
536	CF	Pos.	Pos.	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.
	CA	—	—	—	—	—	Pos.	Pos.	—
547	CF	Pos.	Sold						
422	CF	Pos.	Sus.	Neg.	Neg.	Neg.	Neg.	—	—
	CA	—	—	—	—	—	Neg.	—	—
77	CF	Pos.	—	Neg.	Neg.	Neg.	—	—	—
	CA	—	—	—	—	—	—	—	—
400	CF	Pos.	Pos.	Sold					
427	CF	Pos.	Pos.	Pos.	Pos.	Sold			
029	CF	Neg.	Pos.	Pos.	Pos.	Pos.	Sus.	Neg.	Neg.
	CA	—	—	—	—	—	Sus.	Sus.	—
382	CF	Neg.	—	Pos.	Neg.	Pos.	Sold		
3085	CF	—	—	—	—	Pos.	Pos.	Pos.	Pos.
	CA	—	—	—	—	—	Sus.	Pos.	—
81	CF	—	—	Neg.	Neg.	Pos.	Neg.	—	Neg.
	CA	—	—	—	—	—	Neg.	—	—
1741	CF	—	—	—	—	Pos.	Pos.	Pos.	Pos.
	CA	—	—	—	—	—	Pos.	Pos.	—

shown in Table 10. All 36 CF tests were negative. There were 41 CA tests, of which 5 (12%) were positive and 21 (51%) were suspicious. Among the suspicious reactions were included many of 1 + magnitude, which by interpretation of others (Ristic, 1962), would have been called positive reactions.

Brandly (1964) points out that the injection of large amounts of blood into cattle will increase certain isoagglutinins and other antibodies which might cause non-specific serologic reactions. The suspicious and positive reactions reported here, however, were observed prior to inoculation of the blood. Eperythrozoa were present in erythrocytes of all 6 calves.

Anaplasmosis, as determined by clinical and hematological studies reported in detail by Merriman, Owens, and Chung (1964), developed in 3 of the 6 splenectomized calves (Tables 9 to 11). In all 3 positive cases, the donor's blood was CF- and CA-positive when collected for calf inoculation. Signs of anaplasmosis were prominent in all 3 calves. The 1 receiving blood from Cow 3085 died on the 24th post-inoculation (PI) day. Illness was severe in the other 2 but recovery occurred.

Two of the 3 inoculated calves which did not become infected received blood from individual donors. Cow 81 was CF- and CA-negative 25 days before additional blood was collected for calf inoculation, but her serum was not retested on the inoculation day.

Table 10. Preinoculation capillary-agglutination and complement-fixation results for calves

Test calf No.	CF tests prior to splenectomy		CA tests prior to splenectomy				CF tests following splenectomy		CA tests following splenectomy			
	Total	Negative	Total	Negative	Suspicious	Positive	Total	Negative	Total	Negative	Suspicious	Positive
233	1	1	2	1	1	0	5	5	5	2	3	0
232	1	1	2	1	1	0	5	5	5	1	4	0
228	1	1	2	1	0	1	5	5	5	2	3	0
227	1	1	2	1	1	0	5	5	5	3	2	0
240	1	1	1	0	1	0	5	5	5	2	3	0
229	1	1	2	1	0	1	5	5	5	0	2	3

Table 11. Response of calves to inoculation with 150 to 200 ml. of citrated blood from cows in an anaplasmosis-infected herd

Test calf No.	Donor cow No.	Donor cow Test results[a] CF	Donor cow Test results[a] CA	CF test results Total	CF test results Negative	CF test results Suspect	CF test results AC	CF test results Positive	CA test results Total	CA test results Negative	CA test results Suspect	CA test results Positive	Anaplasmosis status of calves
233	**536**	P	P	15[b]	4	1	0	10	17	4	3	10	Infected
232	3085	P	P	6[c]	4	1	0	1	6	1	3	2	Infected
228	1741	P	P	16[b]	3	0	2	11	17	2	5	10	Infected
227	029	N	S	16[b]	11	2	3	0	17	2	13	2	Negative
240	81	N	N	16[b]	15	0	0	1[d]	17	2	14	1	Negative
229	Neg. cows	N	N	17	15	2	0	0	17	3	10	4	Negative

[a] Status at time inoculation sample was obtained, N = negative; S = suspect; P = positive; AC = anti-complementary.

[b] Samples broken during shipping.

[c] Died on post inoculation (PI) day 24.

[d] PI day 24, followed by 10 negative tests.

Cow 029 had, in the past, been CF-positive on repeated tests. Twenty-five days before the inoculation studies began, blood serum from this cow gave suspicious reactions with each antigen while on inoculation day, her serum was CF-negative and CA-suspicious.

The 6th splenectomized calf, inoculated with pooled blood from cows which gave CF- and CA-negative reactions, did not develop anaplasmosis.

Thus, results with 5 of the 6 splenectomized calves confirmed the CA and CF test results for the donor cows. The remaining calf, receiving blood from Cow 029, gave results which confirmed the CF test but not the CA test of the donor cows made the day blood was collected for inoculation.

Blood serum from those inoculated calves which developed anaplasmosis gave satisfactory test results with both antigens (Table 11). However, as shown in the same table, calves inoculated with non-infective blood gave a preponderance of CA-suspected reactions (73%) and a smaller number (14%) of positive reactions.

Anticomplementary CF reactions appeared in blood of 2 calves following inoculation. One of these had received non-infective blood while the other had received infective blood.

Both antigens performed well in sera from non-infected, intact, beef calves. Eleven of these calves showed hematologic evidence of infection with *Eperythrozoon sp.* (Table 12).

Table 12. Serologic and hematologic findings in intact calves

No. of calves	No. with epery-throzoon	Av. hemato-crit	Range of hemato-crit	CF results		CA results	
				Total	Neg.	Total	Neg.
12	11[a]	44.75	39-49	9[b]	9	12	12

[a] One slide unsatisfactory.
[b] 3 tubes broken.

SUMMARY

BEEF cattle at eight research centers of the Tennessee Agricultural Experiment Station and splenectomized calves were used to evaluate 1) removal of CF-positive cattle as a method of anaplasmosis control, 2) the accuracy of the CF test, and 3) the accuracy of the CA test in detecting carriers of anaplasmosis.

1. **Anaplasmosis Control:** In a herd in which the proportion of CF-positive animals never exceeded 15.5% and in which ticks were not a problem, a marked reduction in incidence of anaplasmosis was achieved over a 7-year period by removing CF-positive cattle at the end of each vector season. The removed cattle were either slaughtered or placed in an isolated herd. In the early years of the study, incomplete sampling caused by collecting some unsatisfactory samples appeared to hinder the detection of CF-positive cattle. In the last 3 years of the study, however, the incidence of CF-positive cattle remained consistently low, with rates of 0.5%, 0.0%, and 0.2% per year, respectively.

2. **CF-Test Accuracy:** Repeated annual CF tests on anaplasmosis-free cattle at seven research centers of the Tennessee Agricultural Experiment Station and inoculation studies with 2 splenectomized calves revealed about 0.4% false CF-positive reactions in 4,178 samples during 1956 through 1961.

3. **Comparison of CA and CF Tests for Anaplasmosis:** CF and CA antigens gave comparable accuracies when used with sera from a herd of beef cattle that had an approximate infection rate of 9.0%. All CF- and CA-positive cattle were proved infected by calf inoculation tests. Blood from one CF-negative, CA-suspicious reactor proved non-infective. Pooled blood from 6 CA- ,CF-negative cows was not infective. All of the 36 samples of serum from the 6 non-infected, non-inoculated splenectomized calves gave negative CF results, while 41 CA tests from these same non-inoculated calves yielded 12% positive and 51% suspect reactions. Both CF and CA antigens performed well with serum from infected calves. However, in 51 CA tests with serum from calves inoculated with non-infective blood, 73% of the reactions were suspicious and 14% positive. In 49 CF tests with sera from these same inoculated, non-infected animals, 84% were negative. Both antigens performed well in serum from intact, non-infected calves.

The CA antigen can, in states where its use is permitted, be used in conducting the test rapidly and easily in the laboratory of the practicing veterinarian.

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