A Vaccine for Anaplasmosis

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Anaplasmosis is an infectious disease of cattle caused by *Anaplasma marginale* and characterized by anemia. Recovered animals remain carriers and reservoirs of infection, usually for life. No completely effective treatment has been developed for the acute stages of the disease. Present control methods are prophylactic antibiotic therapy (1) and removal of carrier cattle after serologic identification (2). A vaccine which would prevent infection by *Anaplasma marginale* would be the ideal method of control.

With this ideal in mind, we have, during the past eighteen years, tested many types of vaccine preparations (3), but were never able to prevent anaplasma infection. Mott and Gates (4) and Kuttler (5) showed that some resistance to clinical anaplasmosis was produced by antigens obtained from whole blood infected with *Anaplasma marginale*. Since complete protection did not seem feasible in the near future, we have attempted to develop a vaccine which would provide sufficient resistance to prevent the appearance of the clinical disease. With this aim in mind, several antigenic preparations were tested for the degree of resistance they produced to the mortality and morbidity of anaplasmosis.

Materials and Methods

The purified antigen used in the vaccine for all experiments was prepared from anaplasma infected bovine blood by several steps designed to remove most of the plasma and cellular material. The remaining antigenic material was freeze-dried. Just prior to injection into cattle the desired amount of antigen was suspended in 5 ml of an adjuvant.

One to two-year-old Hereford cattle which were negative to the C-F test for anaplasmosis were used in the experiments. Animals of this age usually exhibit obvious clinical disease with but minimal mortality.

Antibody response of the animals to the vaccine and to the infections following challenge was measured by the highest complement-fixing

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(C-F) titer giving a 4+ reaction. An estimation of resistance in vaccinated and unvaccinated animals was determined by the degree of anemia as measured by packed cell volume (PCV), and the percentage of anaplasma infected erythrocytes appearing in the cattle subsequent to challenge infection. The cattle were observed daily during the patent phase of the disease for evidence of illness and clinical signs of anaplasmosis.

Each animal in the experiments was challenged with 0.01 or 0.10 ml of anaplasmosis carrier blood from the same carrier animal. All cattle in each experiment were challenged with blood drawn from the carrier at one time. The blood was diluted with anaplasmosis negative bovine serum so that 1 ml of the diluted blood contained the desired challenge dose.

Comparison of Adjuvants

In earlier experiments at this laboratory, the antigen was suspended in light mineral oil (6). Since other adjuvants have been developed which are easier to use and perhaps more efficient than mineral oil, two such adjuvants were tested with the anaplasma antigen.

The antigen, immediately prior to use, was suspended in 5 ml of light mineral oil adjuvant, F-3* adjuvant, or F-5* adjuvant. The last two adjuvants are essentially emulsions of water and oil. Three groups of five cattle per group were vaccinated with two doses at a 12-week interval using the antigen in the three adjuvants. A fourth group was not vaccinated. Each animal in the four groups was challenged with 0.10 ml of carrier blood two months after the last injection of vaccine.

The results of this experiment are summarized in Table 1. The anemia and the percentage of anaplasma infected erythrocytes are very significantly less in the three vaccinated groups than in the unvaccinated group. Although the average values for the F-5 group appear to indicate some advantage for this adjuvant, there are no significant differences between the various adjuvants.

This experiment shows that the antigen suspended in the two emulsion adjuvants provides an effect equal to that achieved with the oil adjuvant. A readily obtained fine homogeneous dispersion of the antigen in the emulsion adjuvants is their principal advantage over the oil adjuvant.

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*Supplied by Ft. Dodge Laboratories, Fort Dodge, Iowa.

Table 1 — Comparison of Adjuvants

Adjuvant Groups	Minimum %PCV		Maximum % Infected RBC		Maximum CF Titer Vaccine		Maximum CF Titer Infection	
	Mean	SD	Mean	SD	Mean	Range	Mean	Range
Oil	25.2	7.1	1.6	1.9	1:211	1:40-1:640	1:7745	1:5120-1:20480
F3	27.2	6.4	2.0	3.1	1:968	1:320-1:1280	1:2938	1:40-1:20480
F5	33.6	2.6	0.6	0.1	1:242	1:80-1:640	1:1469	1:10-1:20480
Control	11.6	2.1	34	9.5			1:4456	1:2560-1:20480

Comparison of Dosage Intervals

In previous work two injections of the antigen 12 and 19 weeks apart were successfully used (6). In an effort to determine if shorter intervals were satisfactory two-week and six-week intervals were compared with a 12-week interval.

Two yearling Hereford cattle, each were given two doses of the antigen in mineral oil at a two-week interval. To evaluate a six-week interval between vaccine doses, four cattle were used; two were given antigen in mineral oil, one animal the antigen in F-3 adjuvant and one the antigen in F-5 adjuvant. A third group of five cattle was inoculated with two doses of the antigen in F-3 adjuvant at a 12-week interval. All cattle in the experiment were challenged with 0.01 ml of anaplasma carrier blood one-month after the last dose.

Since the previous experiments indicated no difference between these adjuvants the four cattle receiving two doses at six-week intervals were used for comparison with the two-week and the 12-week interval groups.

While the data from the two-week interval trial should not be compared statistically with that of the six-week or 12-week interval groups, the results show that both cattle in the two-week interval trial developed lower PCV and higher percentages of infected erythrocytes than any cattle in the other groups. It is therefore probable that two doses of vaccine at two-week intervals are not as effective as at six or 12 weeks. Comparison of the results summarized in Table 2 indicate that there is no significant difference produced in the resistance to anaplasmosis between the vaccine given at six week or at 12 week intervals.

Table 2 — Comparison of Dosage Intervals

Interval Groups	% PCV		Maximum % Infected RBC		Maximum CF Titer Vaccine		Maximum CF Titer Infection	
	Mean	SD	Mean	SD	Mean	Range	Mean	Range
2 Wks (2 Animals)	12		20		<1:10	Neg-1:10	1:5120	1:2560-1:10240
6 Wks (4 Animals)	29	2.8	<1	<1	1:160	1:80-1:320	1:2560	1:40-1:20480
12 Wks (5 Animals)	29.2	2.1	<1	<1	1:92	1:20-1:320	1:2103	1:10-1:20480

Infectivity of the Vaccine

Although evidence in these and previous experiments indicate that the vaccine will not cause anaplasma infection (6,7), a specific test of the infectivity of the vaccine was designed.

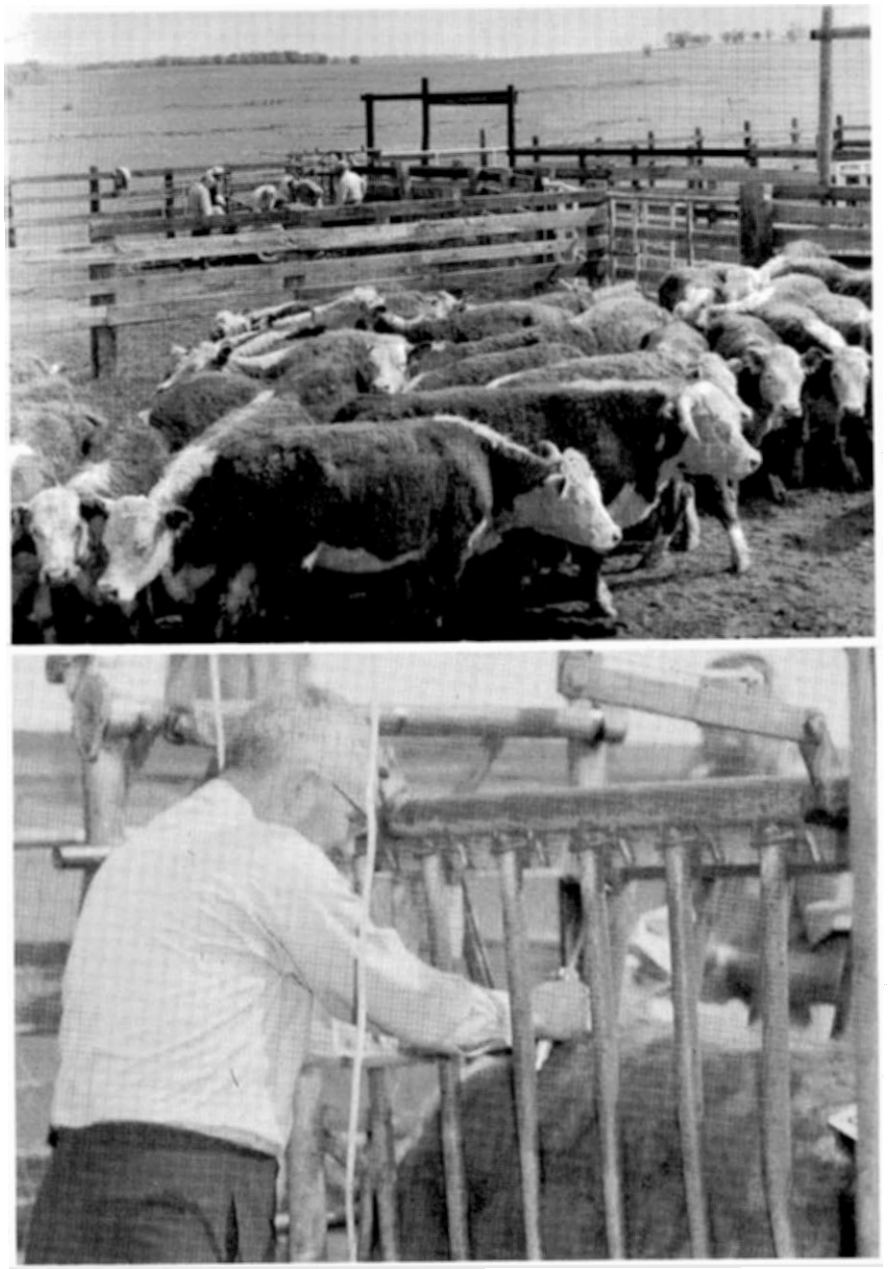
Twenty doses of vaccine from three different batches were pooled to give a total of 60 doses. The pooled vaccine was inoculated subcutaneously into a splenectomized calf. At 12 and at 26 days after inoculation, 750 ml of blood was taken from the calf and injected intravenously into a second splenectomized calf. Both calves were examined for evidence of anaplasma infection by the complement-fixation test, development of anemia and the presence of anaplasma bodies in Wright's stained blood smears.

There was no evidence of anaplamsa infection in either calf. The complement-fixation test showed transient positive titers following injection of the 60 doses of vaccine to the 34th day when the test became negative. The blood smears were negative for *Anaplasma marginale* throughout the trial. The calf which received blood from the vaccinate remained negative to all tests during the trial.

Field Trial

In order to test the effectiveness of the vaccine when used under ranch conditions 200 grade Hereford cattle on a ranch in Osage County, Oklahoma, were vaccinated. The cattle were kept in six pastures with 21 to 55 animals per pasture. In previous years, it is estimated that 5 to 8% of the cattle in this herd died of anaplasmosis each year.

Immediately prior to vaccination on May 1, the cattle were tested for anaplasmosis using the complement-fixation test. Two doses of vac-



Effectiveness of the vaccine was tested on 200 grade Hereford cattle on a ranch in Osage County, Oklahoma. Mortality decreased from 5-8 percent to 0 percent, and only one mild case of clinical anaplasmosis developed.

cine were given with a six weeks interval between doses. Carrier cattle, as identified by the C-F test, were adjusted between pastures so that each pasture contained at least 10 percent anaplasmosis carrier cattle. Two weeks following the second dose of vaccine C-F titers were run on 67 cattle from the vaccinated herd in order to determine the reaction to the vaccine (Table 3).

Rather than depend upon the carrier cattle within the herd for challenge to the vaccine, 12 additional cattle were infected with anaplasmosis and added to the herd, six in August, and six in September. All of the artificially infected cattle became acutely ill with signs of anaplasmosis while in the vaccinated herd. Nine of the 12 cattle died from the disease.

The cattle in the six pastures were observed and counted almost daily. Animals sick from any cause were reported to the Veterinary Research Station at Pawhuska. C-F tests, blood smears and PCV were run on all of the reported cattle.

One year after vaccination, the number of carrier cattle in the herd was again determined by the C-F test. Previous work has indicated that reaction to the C-F test due to vaccination disappears within one year after vaccination (7). The difference between additional carriers and the number of clinical cases observed in the herd after one year, therefore, may be attributed to vaccinated cattle which became infected but did not show clinical signs of the disease.

A comparison of the prevaccination and the 12 months postvaccination C-F tests of the herd is shown in Table 4. The difference in the numbers of cattle between 1964 and 1965 is due to two cattle being missed on the 1964 test in pasture #1 and one animal in pasture #5. These cattle were included in the 1965 test but six other cattle in pastures 2, 3, and six were not tested in 1965. There is an overall increase of 19 cattle or 10.5 percent that are positive to the C-F test in 1965 over 1964 if the suspects are included as positive. One animal showed mild clinical signs of anaplasmosis. There were no deaths during the year. When these

Table 3

No. Animals Tested	Pasture	Age Class	Average C-F Titer
43	1	Heifers	1:92
24	2	Old Cows	1:11

Table 4 — Field Trial

Pasture	No. Animals		No. Positive		No. Suspect		No. Negative		% Reactors	
	1964	1965	1964	1965	1964	1965	1964	1965	1964	1965
#1 East	42	44	1	12	1	2	40	30	4.8	31.8
#2 School	24	21	1	4	1	1	22	16	8.3	23.8
#3 E. Katy	30	29	1	5	1	1	28	23	6.7	20.7
#4 Lookout	26	26	4	4	2	2	20	20	23.1	23.1
#5 Kiheka	20	21	2	5	0	2	18	14	10.0	33.3
#6 W. Katy	55	50	8	11	10	2	37	37	32.7	26.0
Totals	197	191	17	41	15	10	165	140		
Mean									16.2	26.7

results are compared with the herd history of 5 to 8 percent mortality per year due to anaplasmosis, there is indication that a relatively high degree of resistance to the clinical disease and death from anaplasmosis is conferred by the vaccine under commercial ranch conditions.

Vaccine Experiments

Table 5 shows the anemia and clinical signs of anaplasmosis developed in all the cattle in our experiments which received two doses of vaccine at six-and 12-week intervals. The cattle were challenged with 0.1 or 0.01 ml of carrier blood one to three months after the last dose of vaccine. One animal became slightly ill when the PCV declined to 16 percent. The other 44 cattle were equally divided between slight anemia with no clinical signs of anaplasmosis and no development of anemia.

Discussion

The results obtained in these experiments indicate that a vaccine prepared from anaplasma infected bovine whole blood will increase the resistance of anaplasma infected cattle to clinical anaplasmosis and death. While all cattle in these experiments became anaplasmosis carriers following challenge, only one of the 45 cattle which received the vaccine developed mild clinical disease for one day. Twenty-two of the vaccinated cattle developed slight anemia while the other 22 did not become anemic. The average minimum PCV of the vaccinated cattle is 27.8 percent with a standard deviation of 5.7.

The PCV of the 10 unvaccinated animals dropped below one-half normal in all cases. The average minimum PCV for the unvaccinated cattle was 11.8 percent with a standard deviation of 2.0.

Table 5 — Summary of Anemia in Vaccinated Cattle

Groups	Anemia Clinical (PCV)	Non Clinical Anemia (PCV)	No Anemia (PCV)
Vaccinated (45 head)	16	22	33
		22	35
		26	34
		25	37
		19	30
		21	32
		23	35
		26	36
		24	35
		18	34
		22	31
		22	31
		19	30
		19	32
		25	32
		28	34
		29	35
		20	31
		29	31
		25	31
		26	31
		25	30
Unvaccinated (10 head)	9		
	11		
	16		
	11		
	11		
	10		
	13		
	14		
	12		
	11		

Vaccinated cattle mean = 27.8, SD = 5.7
Unvaccinated cattle mean = 11.8, SD = 2.0

These experiments indicate that both mineral oil or oil emulsion adjuvants can be used as vehicles for the antigen. However, for practical purposes the powdered antigen suspends more readily in the emulsified adjuvants.

Experiments concerning the time intervals between doses of vaccine show that the two-week interval is not effective. The six-week interval seems to be equal in vaccination response to the 12-week interval and in some cases, more practical.

No animal in these or earlier experiments has been infected with anaplasmosis as a result of the vaccine and the experiment using much greater doses of vaccine than practical has failed to produce anaplasmosis.

It therefore appears that this vaccine contains killed organisms which will not produce anaplasmosis.

The results from testing the vaccine under ranch conditions appears to confirm the results obtained in more closely controlled experiments.

Summary

These experiments show the development of a vaccine which will reduce the mortality and aid in the control of bovine anaplasmosis. Vaccinated cattle become carriers when challenged with either 0.1 or 0.01 ml of anaplasmosis carrier blood. The best adjuvant to use as a vehicle for the antigen appears to be an oil and water emulsion. The vaccine should be administered in two doses separated by an interval of about six weeks. Field trial of the vaccine under a commercial ranching operation resulted in a decrease in mortality from 5-8 percent to 0 and only one mild case of clinical anaplasmosis.

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Oklahoma's Wealth in Agriculture

Agriculture is Oklahoma's number one industry. It has more capital invested and employs more people than any other industry in the state. Farms and ranches alone represent a capital investment of four billion dollars—three billion in land and buildings, one-half billion in machinery and one-half billion in livestock.

Farm income currently amounts to more than $700,000,000 annually. The value added by manufacture of farm products adds another $130,000,000 annually.

Some 175,000 Oklahomans manage and operate its nearly 100,000 farms and ranches. Another 14,000 workers are required to keep farmers supplied with production items. Approximately 300,000 full-time employees are engaged by the firms that market and process Oklahoma farm products.

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