AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
0187

1. SUBJECT CLASSIFICATION

A. PRIMARY: Agriculture — AL72-0000-0000

B. SECONDARY: Pests of animals

2. TITLE AND SUBTITLE

Serial passage of an attenuated Anaplasma marginale in splenectomized calves

3. AUTHOR(S)

Kuttler,K.L.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1969	6p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Tex.A&M

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)

(In Proc.,73d annual meeting of U.S.Animal Health Assn.)

9. ABSTRACT

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-RAA- 136	

12. DESCRIPTORS

Anaplasma
Complement fixation
Protozoan infections

13. PROJECT NUMBER

14. CONTRACT NUMBER

CSD-1947 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

7

Reprinted from proceedings of 73rd Annual
Meeting of U. S. Animal Health Association 1969

SERIAL PASSAGE OF AN ATTENUATED *ANAPLASMA MARGINALE* IN SPLENECTOMIZED CALVES

K. L. Kuttler, DVM

The description of an attenuated *Anaplasma marginale* by Ristic *et al*[4] and Welter and Woods[5], suggests that an effective, safe premunization program for the control of bovine anaplasmosis might now be possible. Since a parasitemia does occur following the inoculation of this organism, the possibility of cow to cow transfer by natural vectors cannot be entirely eliminated. For this reason 12 serial passages have been made in splenectomized calves to determine if any evidence of altered virulence could be detected by this procedure.

Materials and Methods

Attenuated *A. marginale* infections were serially induced in each of 11 spenectomized, male, Holstein-Friesian calves averaging 202 ± 49 days of age. The first passage was made from vaccine obtained from the producing laboratory*. This vaccine had been stored several months at -40°C before use. A total of 10 ml. of this material was inoculated intravenously (I.V.) and 10 ml. subcutaneously (SC). A second passage was made from the infected blood of the first passage calf. This process of serially inoculating calves was continued until an eleventh passage was completed. The size, route of inoculation, and parasitemias of each inoculum is presented in Table 1.

A second phase of this study consisted of comparing the relative severity produced by twelfth and second passage attenuated *Anaplasma*. Group 1 consisted of 4 splenectomized, male, Holstein calves with an average age of 94 ± 5 days. They were inoculated with infected blood showing a 1% parasitemia from an ascending infection occurring in the eleventh passage calf (2468). The inoculum consisted of 5 ml. of a 30% washed red blood cell suspension in 0.85% NaCl, injected SC.

Group 2 consisted of 4 spenectomized, male, Holstein calves with an average age of 102 ± 4 days. They were inoculated with infected bovine blood showing a 0.5% parasitemia from an ascending infection occurring in a first calf passage. The inoculum consisted of 5 ml. of a 60% washed RBC suspension in 0.85% NaCl injected SC. This first passage calf had been infected with material obtained from Mexico City+ and held frozen for 5 months at -40°C.

Each of the 8 calves used in this trial were given 5 ml. Spirotripan Fuerte++ I.V. at the time infected red cells were injected, to minimize the possibility of *Eperythrozoon* infection which might alter the course and severity of the induced *A. marginale* infection.[2]

---

* Diamond Laboratories, Des Moines, Iowa – Lot 8E.

** From The Institute of Tropical Veterinary Medicine, College of Veterinary Medicine, Texas A&M University, College Station, Texas.

\+ Diamond Laboratories, Lot unknown.

++ Farberkw Hoechse Ag., Germany.
[(sodium 2-di (B, y-dihydroxypropyl) - animo-phenol (4 arseno-5) B- (benzoxazolyl-(2) - Mercaptol) - propionate]

In the first phase of 11 serial passages blood samples were collected twice weekly to monitor the course of infection. During the second phase, blood samples were collected three times a week until a parasitemia occurred and then daily until recovery or death.

Complement-fixation (CF) tests were conducted weekly using the basic procedures outlined by the USDA[1], but employing a microtechnique for titrations[3]. Packed cell volume determinations were made on all samples, using a micro-hematocrit centrifuge. Giemsa stained blood smears were used to determine parasitemias.

An analysis of variance was used for the determination of statistical differences. Averages and statistical evaluation of complement-fixation serum titers were made on logarithmic transformations of the dilution factors.

Results

The results of 11 serial passages are tabulated in Table 1. The incubation time is recorded as days required for the development of diagnostic evidence of anaplasmosis. The first passage, using vaccine material which had been frozen several months, produced infection after a prolonged incubation time of 47 days. Thereafter the incubation was much shorter. No significant correlation between passage level and high parasitemias or low PCV was evident. The seventh passage calf died of acute anaplasmosis, but the ninth passage calf developed a relatively mild reaction with a low PCV of only 17%.

Comparisons between groups 1 and 2 of phase 2 are presented in Table 2. The average incubation time was almost identical between the 2 groups. No significant difference in pre-infection PCV was noted. The average parasitemia of 36.8% observed in group 1 was significantly greater than the average of 3.6% observed in group 2. The average low PCV of 9.5% observed in group 1 was significantly lower than the 19.5% observed in group 2. There was no significant difference in CF titers. One animal in group 1 died of acute anaplasmosis. No deaths occurred in group 2.

Discussion and Conclusions

A second rather than a first passage was compared to the twelfth in order to standardize the inoculum used to induce infection. The vaccine material had been frozen, the parasitemia was unknown, and was from a non-bovine host. It would have been difficult and probably impossible to have standardized the vaccine as obtained with the twelfth passage material, to give a comparable inoculum.

Even though no clear pattern of reversion could be detected in the 11 serial passages, a comparison of reactions produced by twelfth and second passage material in 2 groups of 4 animals each clearly shows a difference. A reversion to virulence is indicated by the increase in parasitemia and the lower PCV observed in calves at the twelfth passage level.

This experiment does not prove that such a reversion would occur following 12 serial passages in intact calves under natural conditions. It does indicate a potential for reversion.

The number of susceptible cattle in contact with vaccinated animals, their age, and possibly breed, the type and number of vectors present, and general

management practices are all factors which might influence the serial passage of this attenuated organism in nature, and the rate of reversion to virulence.

In enzootic areas where premunization is the method of choice, the return to virulence is mostly of academic interest. When faced with the decision of whether to depend on premunition with a virulent organism by natural means on a sporadic basis or to vaccinate all cavles with an attenuated organism, the choice is clearly in favor of the latter.

A potential for reversion does exist, and in areas of low anaplasmosis incidence, the introduction of even an attenuated agent might be hazardous when suitable vectors are present to produce serial transmission. The indiscriminate use of such a vaccine in some areas could establish infection where it previously had not existed.

Summary

Twelve serial passages of an attenuated *Anaplasma marginale* were made in splenectomized calves by blood inoculation. The severity of infection produced at the twelfth passage level in 4 splenectomized calves was compared to the infection occurring in 4 similar calves at a second passage level. Significantly higher parasitemias and lower packed cell volumes occurred in the twelfth passage group, suggesting an increased virulence. No deaths occurred among animals of the second passage group whereas 1 of 4 died in the twelfth passage group.

ACKNOWLEDGEMENTS

This work was supported by grants from The Rockefeller Foundation and The U.S. Agency for International Development to The Institute of Tropical Veterinary Medicine, Texas A&M University. The Instituto Colombiano Agropecuario, Laboratorio de Investigaciones Medicas Veterinarias, where the work was conducted contributed space, laboratores and use of equipment.

REFERENCES

1. Anon: United States Department of Agriculture: A Manual of Conducting the Complement-Fixation Test for Anaplasmosis, August, 1958.

2. Foote, L. E., Levy, H. E., Torbert, B. J. and Oglesby, W. T.: Interference Between Anaplasmosis and Eperythrozoonosis in Splenectomized Calves, Am. J. Vet. Res. *18* (July, 1957) 556-559.

3. Hidalgo, R. J. and Dimopoullos, G. T.: Complement-Fixation Micro Procedures for Anaplasmosis. Am. J. Vet. Res., *28* (Jan., 1967) 245.

4. Ristic, M., Sibinovic, S. and Welter, J. C.: An Attenuated *Anaplasma marginale* Vaccine. Proceedings. 72nd Annual Mtg. USLSA, (1968) New Orleans, La., 56-59.

5. Welter, J. C. and Woods, R. D.: Preliminary Evaluation of an Attenuated *Anaplasma marginale* Vaccine in Cattle. Vet. Med./Sm. Anim. Clin., *63* (August, 1968) 798-802.

Table I

Serial Passage of Attenuated *Anaplasma* in Splenectomized Calves

Passage Level	Animal No.	Size, Date, and Route of Inoculum	Parasitemia Of Inoculum %	Incubation (Days)	Pre-Infect. Normal PCV %	High Paras.%	High CF	Low PCV %
1	093	10 ml. I.V., 10 ml. S.C. Lot 8E Diamond Vaccine (7/5/67)		47	35	4	1:2560	16
2	18	2 ml. S.C. blood from 093 (8/25/67)	3	17	30	7	1:1280	23
3	511	10 ml. I.V. blood from 18 (2/14/68)	1	12	40	15	1:640	15
4	513	10 ml. I.V. blood from 511 (3/8/68)	8	10	44	50	1:1280	12
5	514	10 ml. I.V. blood from 513 (3/23/68)	26	10	33	10	1:640	12
6	518	10 ml. I.V. blood from 514 (4/8/68)	8	14	36	5	1:320	20
7	522	10 ml. I.V. blood from 518 (4/27/68)	2	16	30	30	1:320 died	9
8	34	10 ml. I.V. blood from 522 (5/17/68)	12	10	34	30	1:2560	11
9	35	10 ml. I.V. blood from 34 (5/31/68)	22	11	38	2	1:640	17
10	30	10 ml. I.V. blood from 35 (6/14/68)	0.5	27	39	32	1:1280	11
11	2468	10 ml. I.V. blood from 30 (9/14/68)	1.0	23	30	10	1:1280	13

Table II

Comparative Reaction of 12th and 2nd Passage Attenuated *Anaplasma*

In Splenectomized Calves

Passage Level	Animal No.	Parasitemia of Inoculum %	Incubation (Days)	Pre-Infect. Normal PCV %	High Paras. %	High CF	Low PCV %
12	2568	1.0	13	31	50	1:1280	8
12	3368	1.0	20	36	28	1:1280	10
12	3568	1.0	15	44	65	1:80	9 died
12	3668	1.0	15	38	4	1:1280	11
Averages			15.75	37.25	36.8	1:640	9.5
2	3068	0.5	14	37	7.0	1:1280	17
2	3168	0.5	20	38	3.0	1:640	17
2	3268	0.5	14	36	1.2	1:1280	24
2	3868	0.5	14	45	3.0	1:640	20
Averages			15.5	39.0	3.6	1:905	19.5
Significance			NS	NS	$P<0.05$	NS	$P<0.01$

Inoculum:

Calves 2568, 3368, 3568 and 3668 at the 12th passage level received 5 ml. subcutaneously washed erythrocytes as a 30% suspension in 0.85% NaCl. These RBCs showed a 1% parasitemia from an ascending *Anaplasma* infection in animal 2468.

Calves 3068, 3168, 3268 and 3868 at the 2nd passage level received an equivalent inoculum consisting of 5 ml. S.C. washed erythrocytes as a 60% suspension in 0.85% NaCl. These RBCs showed a 0.5% parasitemia from an ascending *Anaplasma* infection. 5 ml. Spirotripan-Fuerce was injected I.V. in each of the 8 calves at the time they received the infecting inoculum.