Trop. Anim. Hlth Prod. (1971) 3, 77–82

COMPARATIVE EFFICACY OF DIFFERENT IMMUNIZATION SYSTEMS AGAINST ANAPLASMOSIS

H. ZARAZA* and K. L. KUTTLER†

SUMMARY

Animal response to anaplasmosis vaccination was measured using an attenuated organism, a killed adjuvant vaccine, and a virulent *Anaplasma marginale*. A total of 7 calves (2–4 months of age) and 5 heifers (18 months of age) received the attenuated organism; 8 calves were given the adjuvant vaccine; 7 calves were premunized with virulent *A. marginale*; and 7 calves remained as non-vaccinated controls. The animals were vaccinated at Tibaitata on the Bogota Savannah, and later moved to the north coast of Colombia, an anaplasmosis enzootic area.

All vaccination methods produced positive CF results. The live agents resulted in low parasitaemias in most instances, although the attenuated organism was particularly mild in the younger animals.

Protection from field challenge was observed in all calves premunized with virulent organism, and in two of five heifers premunized with the attenuated organism. All other vaccinated animals developed anaplasmosis which was equally as severe as seen in the non-vaccinated controls.

INTRODUCTION

Previous studies, as well as personal communication with veterinary clinicians confirm the presence of large anaplasmosis enzootic areas in Colombia. A preliminary survey (Kuttler, Adams & Zaraza, 1969) suggested that ranching areas less than 1,524 m elevation should generally be classified as anaplasmosis enzootic zones. Anaplasmosis is recognized as a serious clinical problem among previously clean cattle introduced into these areas. The extent of losses in native cattle is not fully known but is probably significant.

Mott & Gates (1948) showed that some resistance to anaplasmosis was produced by antigens obtained from whole blood infected with *Anaplasma marginale*. Kuttler (1961) described a significant degree of protection using oil adjuvants with similar antigens. Brock (1965) described a similar vaccine which when given in two inoculations spaced at 4 to 6 week intervals gave sufficient protection to warrant commercial production.

Ristic, Sibinovic & Welter (1968) and Welter & Woods (1968) described an attenuated *Anaplasma* which would produce a mild reaction in susceptible animals, rendering them immune to future *A. marginale* exposure. This vaccine is somewhat similar to premunization work done by Schmidt (1947) except that an attenuated organism is used to replace the virulent *A. marginale*.

This study compares the safety and efficacy of these immunologic approaches

* Present address: Laboratorio Investigactiones Medicas (Veterinarias), Bogota, Colombia. Apartado Aereo 7984.
† Institute of Tropical Veterinary Medicine, Texas A. & M. University, College Station, Texas.

when animals are vaccinated on the Bogota Savannah (altitude 2,600 m) and later moved to Turipana on the north cost of Colombia (altitude 13 m), an anaplasmosis enzootic area.

MATERIALS AND METHODS

A total of 29 Holstein Friesian calves, 2–4 months of age were divided into four groups. An additional group of five 18 month old Holstein Friesian heifers were used.

Group I consisted of 7 calves. On day 0 they were injected subcutaneously with 5 ml of attenuated *A. marginale* vaccine*. Group I-A, consisting of five 18 month old heifers, were injected subcutaneously on day 0 with 5 ml of the attenuated *A. marginale* vaccine.

Group II consisted of 8 calves. On day 0 and 28 they were injected with 2 ml of the reconstituted adjuvant vaccine†. Group III consisted of 7 calves. On day 0 they were injected with 2 ml whole blood from a splenectomized calf carrying a fully virulent *A. marginale* infection. The donor calf while having fewer than 0·1 per cent parasitized cells was showing a 1:40 anaplasmosis complement-fixation titre. The 7 calves of Group IV remained as non-vaccinated controls.

All animals were bled twice weekly from day 0, date of vaccination, until day 74 when they were transported by air to Turipana. During this time packed cell volume (PCV) determination, anaplasmosis complement-fixation (CF) tests, and Giemsa-stained blood smears were examined, to monitor animal response to the immunologic procedures performed.

The same observations were made twice weekly following arrival at Turipana for the next 150 days. One animal died of undetermined causes at Bogota and several others of babesiosis following arrival at Turipana. Observations at Turipana were therefore limited to 5 calves in Group I, 5 in I-A, 5 in II, 4 in III, and 6 in IV.

The CF test was performed using the basic technique described by the USDA (1958) with serum titre determined by a micro technique similar to that described by Hidalgo & Dimopoullos (1967). An analysis of variance, was performed to determine significant differences. A logarithmic transformation was made of the serum dilution factors for statistical analysis.

RESULTS

Animal response to vaccination is tabulated and compared to non-vaccinated controls in Table I. Prior to vaccination PCV values were essentially the same for all groups. Among Group I only 5 of 7 calves responded with evidence of *Anaplasma* infection. All animals of Groups I-A and III showed evidence of infection as anticipated. The incubation time in those animals receiving and responding to the live vaccines was 45 days in Group I, 28 days in Group I-A, and 20 days in Group III. The 45 day incubation seen in Group I was significantly greater than the others. The average low PCV occurring during a 74 day period following vaccination was significantly lower in Group III. A marked CF response was observed in all animals in the vaccinated groups with the exception of 2 calves in Group I. Group I (five calves) had an average maximum titre of 1:61, I-A 1:970, II 1:110, and III 1:177. The CF titre occurring in Group I-A was significantly higher than seen in Groups I, II and III, although no other significant differences were detected. Parasitaemias, however, were generally very low in all groups. Group I showed an average maximum parasitaemia of only 0·32 per cent among the five animals responding to the attenuated organism.

* An attenuated *A. marginale* vaccine—Diamond Laboratories, Des Moines, Iowa. The vaccine was hand-carried in dry ice from Mexico City and used the day of arrival in Bogota.

† "Anaplaz"—Fort Dodge Laboratories, Ft. Dodge, Iowa.

TABLE I

Response to Anaplasma *Vaccines (Tibaitata)*

	Groups					
	I	I-A	II	III	IV	Significance
Number of animals	7	5	8	7	7	
Number of animals responding to live agents	5	5	—	7	—	
Average pre-vaccination PCV	37	37	35	36	37	NS
Average incubation time in days	45*	28	—	20	—	P < 0·01 DRS = 16·0
Average low PCV during the 74-day period at Tibaitata	28*	27	28	25	28	P < 0·01 DRS = 3·0
Average high CF titre	1:61*	1:970†	1:110	1:177	—	P < 0·01
High parasitaemia	0·32*	1·12	—	1·18	—	NS

Group I—2–4 month old calves vaccinated with an attenuated *A. marginale*.
Group I-A—18 month old heifers vaccinated with an attenuated *A. marginale* (Diamond Laboratories).
Group II—2–4 month old calves vaccinated with a killed adjuvant vaccine (Anaplaz, Ft. Dodge).
Group III—2–4 month old calves premunised with virulent *Anaplasma*.
Group IV—2–4 month old calves, non-vaccinated controls.
DRS—Difference required for significance.
* Calculated only for those five calves showing a response to vaccination.
† The titre 1:970 was significantly higher than all others but differences among groups I, II and III were not significant.

TABLE II

Response of vaccinated and non-vaccinated cattle to naturally occurring field challenge at Turipana

	Groups					
	I	I-A	II	III	IV	Significance
Number of animals	5	5	5	4	6	
Average PCV before arrival at Turipana	31%	31%	32%	32%	32%	NS
Number of animals showing evidence of challenge infection	5	3	5	0	6	
Average time, in days, required for low (*Anaplasma* induced) PCV	76	63	78	0	74	NS
Average low PCV	15%	11%	12%	18%	13%	P < 0·05*
Average CF titre before arrival at Turipana	1:5	1:80	1:5	1:80	0	P < 0·01
Average high CF titre after arrival at Turipana	1:640	1:422	1:1100	1:190	1:806	P < 0·05†
Deaths attributed to anaplasmosis	0	0	1	0	0	
Average weight gain (kg) 150 days after arrival at Turipana	26	NT	27	34	16	NS

Group I—Calves previously vaccinated with the attenuated organism.
Group I-A—18 month old heifers previously vaccinated with the attenuated organism.
Group II—Calves vaccinated with killed adjuvant vaccine ("Anaplaz").
Group III—Calves premunised with virulent *A. marginale*.
Group IV—Non-vaccinated controls.
* The average low PCV in Group III although not due to anaplasmosis was 18 per cent. This was significantly higher than seen in Groups II, IV and I-A. No other significant differences were detected.
† Group III titres were significantly lower than titres in Groups II and IV.
NT—Not tested.
NS—Not significant.

The responses of vaccinated and non-vaccinated cattle to naturally occurring field challenge at Turipana are recorded in Table II. The average PCV 74 days after vaccination, but prior to arrival at Turipana was essentially the same in all groups notwithstanding the temporary low seen in Group III after vaccination. All animals of Groups I, II and IV, plus 3 of 5 in Group I-A, developed signs of anaplasmosis after arrival at Turipana, as evidenced by an *A. marginale* parasitaemia, drop in PCV, and in increase in CF titre. No clear cut evidence of anaplasmosis occurred in Group III. Of those animals showing signs of anaplasmosis, the time required for a low PCV to occur after arrival at Turipana was very nearly the same in all groups. Calves of Group III showed significantly higher PCV during a comparable period of time when compared to reacting animals of Groups I, I-A, II and IV.

The average CF response of vaccinated animals at the time of movement to Turipana was highest in Groups I-A and III, each being 1:80. Groups I and II each showed an average CF titre of 1:5 at this time. Following natural *A. marginale* challenge at Turipana an average high CF titre of 1:640 occurred in Group I, 1:422 in Group I-A, 1:1100 in Group II, 1:190 in Group III, and 1:806 for Group IV. Group III CF titres were significantly lower than titres in Groups II and IV.

Weight gains at the end of a 150 day period at Turipana were highest in Group III and lowest in Group IV, but the differences did not reach significance.

DISCUSSION AND CONCLUSIONS

The results of premunition with attenuated vaccine suggest an age variation in susceptibility which may well affect the success or failure of this vaccine. The older heifers developed a markedly higher CF titre, and higher average parasitaemia in a shorter period of time than did the younger calves following vaccination. In young calves, the mildness of this attenuated organism is a more severe limiting factor to its use than the hazards from over reactions. Among these young calves, inoculated at Bogota, two of seven failed to show any reaction. Among the remaining five calves the response was so mild in some animals that the only evidence of infection was the development of CF titres, and these for the most part were low. It is questionable if a persistent *A. marginale* carrier status was actually achieved in all instances following inoculation with the attenuated organism. The older heifers, vaccinated with the attenuated organism showed evidence of active infection as demonstrated by the appearance of a parasitaemia, a high CF response, and in most instances a slight drop in PCV.

Premunition with a supposedly virulent *A. marginale* at Bogota and in young calves produced a surprisingly mild response. The response was sufficient, however, to clearly identify the premunizing infection.

Vaccination with the killed adjuvant vaccine produced a characteristic low level CF response, with no parasitaemia, and no reduction in PCV.

Following arrival at Turipana difficulties were encountered with *Babesia*, resulting in some death losses. The surviving animals were observed for a 150 day period during which time *A. marginale* challenge occurred. Table II reports only those animals surviving the initial *Babesia* challenge. As reported previously (Kuttler *et al.*, 1968), an unexplained reduction in PCV occurred in all animals following arrival at Turipana, which did not return to pre-Turipana norms even after apparent recovery from *Babesia* and *Anaplasma* infections.

No evidence of protection was detected as a result of vaccination with the adjuvant

vaccine. The only death loss that could be attributed primarily to anaplasmosis occurred in this group.

Young calves receiving an attenuated vaccine were susceptible to naturally occurring field *Anaplasma* challenge. All five developed evidence of anaplasmosis. The average low PCV resulting from anaplasmosis was 15 per cent as compared to 13 per cent in the controls, but this difference was not statistically significant. The susceptibility to *Anaplasma* might have been expected in these young calves, since their response to the attenuated vaccination had been minimal. On the other hand the five older heifers gave a good response to the vaccinating inoculum, and three of this number developed marked evidence of anaplasmosis as the result of natural challenge. In view of 100 per cent infection occurring in Groups I, II and IV it would appear that vaccination in the older animals was at least partially effective. Premunition with virulent *A. marginale* in young calves, even though characterised by mild initial response was sufficient to prevent anaplasmosis.

In all groups a marked increase in CF titres occurred, after arrival at Turipana, probably reflecting an exposure to *A. marginale* via naturally occurring vectors. It is suspected that in some instances these natural challenges become extremely heavy, and while not overwhelming the premunized animals were capable of stimulating antibody response.

Theoretically, calves infected with the attenuated organism should follow a pattern similar to those premunized with virulent organisms, but this did not occur. The inoculum may not have been sufficiently virulent to induce a persistent infection in the normally resistant calf. A second possible explanation is that antigenic variations are present in the causative organism at Turipana on the north coast of Colombia. If adequate infections can be induced, with the attenuated organism, a degree of protection should occur even though antigenic variants are present. This type respor . occurs with *A. centrale* in Africa, which produces a partial protection characterised by milder, less severe reactions to virulent *A. marginale* challenge (Kuttler, 1967).

These results while inconclusive with regards to the attenuated organism as a vaccine, do show the value of premunition with virulent *A. marginale*. The killed adjuvant vaccine was not effective.

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, laboratories and use of equipment.

Accepted for publication December, 1970

REFERENCES

BROCK, W. E., KLIEWER, I. O. & PEARSON, C. C. (1965). 'A vaccine for anaplasmosis'. *Journal of the American Veterinary Medical Association*, 147, 948–951.

HIDALGO, R. T. & DIMOPOULLOS, G. T. (1967). 'Complement-fixation micro procedures for anaplasmosis'. *American Journal of Veterinary Research*, 28, 245–251.

KUTTLER, K. L. (1961). 'Anaplasmosis immunization studies'. *65th Annual Proceedings, United States Livestock Sanitation Association*, 79–87.

KUTTLER, K. L. (1967). 'A study of the immunological relationship of *Anaplasma marginale* and *Anaplasma centrale*'. *Research in Veterinary Science*, 8, 467–471.

KUTTLER, K. L., ADAMS, L. G. & ZARAZA, H. (1969). 'An epidemiologic and geographic survey of *Anaplasma marginale* and *Trypanosoma theileri* in Colombia'. *Journal of the American Veterinary Medical Association*, 154, 1398.

KUTTLER, K. L., ZARAZA, H. & ROBERTS, E. D. (1968). 'Hematologic and clinical response to anaplasmosis vaccines and the comparative efficacy of these vaccines, as measured by field and experimental challenge'. *Proceedings of the 5th National Anaplasmosis Conference*, 39–49.

MOTT, L. O. & GATES, D. W. (1948). 'Anaplasmosis immunization studies'. *Report of the First National Anaplasmosis Conference*.

RISTIC, M., SHIMOVIC, S. & WELTER, C. J. (1968). 'An attenuated *Anaplasma marginale* vaccine'. *Proceedings of the 72nd Annual Meeting of the United States Livestock Sanitation Association*, 56–69.

SCHMIDT, H. (1947). 'Anaplasmosis in cattle'. *Journal of the American Veterinary Medical Association*, 90, 723–736.

U.S.D.A. (1958). 'A manual of conducting the complement-fixation test for Anaplasmosis'.

WELTER, C. J. & WOODS, R. D. (1968). 'Preliminary evaluation of an attenuated *Anaplasma marginale* vaccine in cattle'. *Veterinary Medicine/Small Animal Clinician*, 63, 798–802.

Efficacite comparée de differentes méthodes d'immunisation contre l'anaplasmose.

Résumé—La réponse à la vaccination contre l'anaplasmose, utilisant un organisme atténué, un vaccin tué contenant des adjuvants et des *Anaplasma marginale* virulents, a été évalué. Au total 7 veaux (âgés de 2 à 4 mois) et 5 génisses (âgés de 18 mois) ont reçu l'organisme atténué; 8 veaux ont reçu le vaccin avec adjuvants; 7 veaux ont été prémunis avec l'*A. marginale* virulent; et 7 veaux non-vaccinés, ont servi de témoins. Les animaux ont été vaccinés à Tibaitata sur la savane de Bogota, et ont été transportés par la suite sur la côte nord de la Colombie, région où l'anaplasmose est enzootique. Toutes les méthodes de vaccination ont produit des réactions positives de fixation du complément. Les organismes vivants ont le plus souvent causé une faible parasitémie, bien que le germe atténué ait été particulièrement bénin pour les animaux les plus jeunes.

Une protection contre l'épreuve naturelle à été observée chez tous les veaux prémunis avec des organismes virulents, et chez 2 génisses sur 5 prémunies avec l'organisme atténué. Tous les autres animaux vaccinés ont contracté une anaplasmose aussi sévère que celle observée chez les témoins non-vaccinés.

Sumario—La respuesta de los animales a la vacuna contra anaplasmosis se midió usando organismos atenuados, vacuna adyuvante muerta y una cepa virulenta de *Anaplasma marginale*. Un total de 7 terneros (2–4 meses de edad) y 5 novillas (18 meses de edad) recibieron Anaplasma atenuado; 8 terneros recibieron vacuna muerta; 7 terneros fueron premunizados con *A. marginale* virulento; 7 terneros no vacunados se usaron como controles. Los animales se vacunaron en Tibaitatá, localizado en la Sabana de Bogotá y más tarde se llevaron a la Costa Norte de Colombia, la cual es un área enzoótica de anaplasmosis.

Todos los métodos de vacunación usados produjeron resultados positivos a la fijación del complemento (CF).

Los grupos de animales que recibieron organismos vivos resultaron con baja parasitemia en muchos casos, aunque los animales que recibieron anaplasma atenuado, la parasitemia resultó particularmente moderada especialmente en los animales más jóvenes.

La protección a la infección natural fue observada en todos los animales premunizados con organismos virulentos, y en 2 de las 5 terneras premunizadas con organismo atenuado. Los demás animales vacunados desarrollaron anaplasmosis, la cual fue tan severa como la observada en los animales del grupo control.