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COMPARATIVE RESPONSE TO PREMUNIZATION USING ATTENUATED *ANAPLASMA MARGINALE*, VIRULENT *A. MARGINALE* AND *A. CENTRALE* IN DIFFERENT AGE GROUPS

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SUMMARY

Premunizing infections using virulent Anaplasma marginale (*VAM*), *attenuated* A. marginale (*AAM*) *and* A. centrale (*AC*) *have been induced in* 46 *mature cattle,* 33 *intact calves, and* 38 *splenectomized calves, for the purpose of comparing the relative response to these infections.*

The VAM produced significantly more severe reactions in adult cattle, and splenectomized calves, and a slightly more severe response in intact calves; however, these animals were relatively more resistant to all three infections. There was no detectable difference between the reactions caused by AAM and AC when measured in adult cattle and intact calves. Among splenectomized calves, however, the AAM infections resulted in a milder response as measured by the relative drop in packed cell volume and per cent parasitaemia. The CF response was significantly lower in the AC infection.

INTRODUCTION

Anaplasmosis immunization, in areas where this disease is endemic, has for years been accomplished by the technique of premunization. In young animals, because of natural resistance, it is possible to use fully virulent *Anaplasma marginale* to induce active infections and premunization under some conditions (Schmidt, 1937). In many endemic zones unintentional premunization is known to occur in a high percentage of calves as the result of transmission by natural vectors (Kuttler & Bohman, 1960). Even though such calves generally become resistant to future acute infections they remain carriers of the infection, thus perpetuating the disease in a given area. A second possible hazard associated with either intentional or unintentional premunization with virulent *Anaplasma* is the mild, transient, but sometimes persistent anaemia associated with such infection. It is not known to what extent this stress factor may influence the animal's ability to cope with other hemoparasitic diseases and internal or external parasitic infestations, which contribute an added burden on the hemopoietic system. The disease hazard of premunization with virulent *A. marginale* is increased in older animals, and is generally not recommended except where the disease can be moderated by specific drug therapy.

Theiler (1911), in his first description of *A. centrale*, suggested that premunization with this organism might be useful in preventing the more severe disease caused by *A. marginale*. Walker (1915) reported on the use of *A. centrale* as a vaccine for anaplasmosis in South Africa. He reported that a prior infection of *A. centrale* reduced the severity of a later *A. marginale* infection. The serologic and immunologic relationships of *A. marginale* and *A. centrale* were described in greater detail by Kuttler (1967a, b).

There appeared to be common antigens present in the two organisms, which were characterized by cross reactions with the complement-fixation test. Animals premunized with either *A. centrale* or *A. marginale* resisted a later challenge by either the homologous or heterologous organism. The resistance to a homologous challenge was complete whereas the degree of resistance to the heterologous challenge was incomplete. This prompted the observation that an attenuated or mild *A. marginale* would offer better protection under those situations where premunition was indicated.

Ristic, Sibinovic and Welter (1968) described the attenuation of *A. marginale* for cattle by numerous serial passages through sheep. The resulting organism was described as producing a mild infection, with few if any clinical manifestations of anaplasmosis, followed by complete protection against a virulent *A. marginale* (Welter & Woods, 1968). It was presumed that the attenuated organism would establish a carrier infection, which would result in a safe, long-lasting immunity.

Kuttler, Zaraza and Roberts (1968) basically confirmed the earlier reports of the mild nature of the attenuated organism, and the solid immunity to needle challenge using a virulent *A. marginale* of Texas origin. Field challenge on the north coast of Colombia, however, resulted in marked infections among calves vaccinated with the attenuated organism, suggesting the presence of an antigenic variant of *A. marginale* in this area (Zaraza & Kuttler, 1971).

The value of the attenuated *A. marginale* against antigenically similar, virulent organisms strongly suggests the use of this agent in areas where premunition is the method of choice for anaplasmosis control. The severity of the premunizing infection assumes importance in the selection of an *Anaplasma* strain for this purpose. This study attempts to compare the relative response of adult cattle, splenectomized calves, and intact calves to premunizing *Anaplasma* infections induced by fully virulent *A. marginale*, the attenuated *A. marginale* and *A. centrale*.

MATERIALS AND METHODS

Infections using field isolates of fully virulent *Anaplasma marginale* (VAM), attenuated *A. marginale* (AAM) and *A. centrale* (AC) were induced in 46 mature cattle, 33 intact and 38 splenectomized calves.

The average age of 46 adult cattle used in these trials was six years (±2·9). They were European type cattle of mixed breeding. A total of 14 adult cattle received the attenuated *A. marginale* (AAM). Seven were injected subcutaneously (s/c) with 5 ml of vaccine* (AAM), which had been stored frozen and was thawed immediately prior to use. The remaining cattle each were inoculated with 1 ml s/c of freshly collected whole blood from a splenectomized calf carrying a first bovine passage of the AAM organism. Fourteen cattle were injected s/c with 5 ml of blood freshly collected from a splenectomized calf carrying an AC infection.† A VAM infection was induced in 18 cattle. Six cattle were infected by the s/c injection of 5 ml of whole blood freshly collected from a splenectomized carrier calf. The remaining 12 cattle in this group were injected s/c with 1 ml each of whole blood from a splenectomized carrier calf.

The average age of the 33 intact calves used in these trials was 3·6 months (±2·3). These calves were European dairy type cattle of mixed breeding. Ten calves were injected s/c with 5 ml of AAM, which had been stored frozen, and thawed immediately prior to use. Six calves were injected s/c with 5 ml of whole blood freshly collected from a splenectomized calf carrying an AC infection. A VAM infection was induced in 17 intact calves by the s/c

* Diamond Laboratories, Des Moines, Iowa.

† Obtained from the Kenya Veterinary Department, Veterinary Laboratory at Kabete. They in turn had received the organisms from South Africa.

injection of 1 to 5 ml whole blood per calf, freshly collected from a splenectomized carrier calf.

The 38 splenectomized calves used in these trials were approximately 60 days older than the intact calves, and were European dairy type of mixed breeding. Thirteen calves were infected with the AAM. Of this number four each received 5 ml of the AAM, which had been frozen, and thawed immediately prior to use. The remaining nine each received 5 to 10 ml s/c, of freshly drawn blood from a first or second passage AAM splenectomized carrier calf. Nine calves were infected with AC. In every instance the infected inoculum consisted of 5–10 ml of freshly drawn blood from an AC, splenectomized carrier calf. Sixteen calves were infected with VAM by the s/c injection of 5–10 ml freshly drawn blood from a splenectomized carrier calf.

Following exposure, blood samples were taken from each animal twice weekly at 3 and 4 day intervals for a minimum of 60 days, and longer if required to cover the entire period of primary reaction. Serum samples were tested for evidence of specific complement-fixing antibodies, using modifications of the USDA (1958) technique. A balanced oxalate salt was used as the anticoagulant for a second blood sample used to determine packed cell volume (PCV), and the *Anaplasma* parasitaemia. Parasitaemias are expressed as the per cent erythrocytes showing identifiable *Anaplasma* bodies, as seen on Giemsa-stained blood slides.

The incubation period was measured in days from the time of exposure to the development of *Anaplasma* infection as characterized by a 4+ complement-fixation reaction or a 1 per cent *Anaplasma* parasitaemia. The duration of anaemia was measured, in days, as that period when the PCV was 75 per cent or less of the normal pre-infection PCV. The per cent of normal PCV measures the relative drop in PCV as the result of *Anaplasma* infection taking into consideration the pre-infection PCV. The low PCV, high parasitaemia, and the high CF titre observed during the course of infection are recorded in the tables of results.

RESULTS

The response of adult cattle premunized with AAM, AC and VAM, in the absence of any treatment, is presented in Table I. In nearly every category significantly more severe reactions occurred in animals infected with VAM, as compared to AAM or AC. There were no significant differences in the severity of infections caused by AAM and AC. A significantly lower CF titre was detected in those cattle having AC infections. No deaths occurred among the AAM and AC groups, but two cattle died in the VAM group.

The response of intact calves, premunized with AAM, AC and VAM, in the absence of treatment, is presented in Table II. A more severe response to VAM infection was observed, but with the exception of the duration of anaemia these differences were not significant at the 0·05 level. Calves inoculated with AAM had a longer incubation time than in the other two groups. The duration of anaemia, while showing considerable animal variation was significantly longer in VAM infections.

The response of splenectomized calves premunized with AAM, AC and VAM, in the absence of treatment, is presented in Table III. The reactions observed in these animals resembled more nearly the response of adult cattle. In nearly all categories significantly more severe reactions occurred in animals infected with VAM as compared to AAM or AC. Comparisons between AAM and AC showed a more severe reaction occurring in calves infected with AC. Calves infected with AC had significantly higher parasitaemias, and a relatively lower PCV. The incubation time of calves infected with AAM was significantly longer than experienced with either AC or VAM.

DISCUSSION AND CONCLUSIONS

The longer incubation time observed in mature cattle and calves inoculated with AAM probably reflects the inoculum used to induce infection. The AAM inoculum

TABLE I

Mean response of adult cattle to premunition with attenuated A. marginale, A. centrale *and virulent* A. marginale

	No. of animals	Pre-infect. PCV (%)	Incub. time (days)	Low PCV (%)	Per cent. of normal PCV ↑ (%)	High paras. (%)	High CF serum titres	Duration of anaemia (days)	Deaths
Attenuated *A. marginale*	14	34·4	25·1	24·9	72	3·40	1:390	8·6	0
A. centrale	14	36·5	17·1	26·5	73	4·04	1:131*	8·3	0
Virulent *A. marginale*	18	32·9	22·6	14·5	44	12·10	1:470	26·6	2
Significance‡		NS	NS	P<0·01	P<0·01	P<0·01	P<0·01	P<0·01	
DRS				5·4	12	5·0	*Lower than 1:470	11·6	

NS Not significant.
DRS Difference required for significance.
↑ These values represent the relative drop in PCV due to infection or low PCV %/Pre-infection PCV % = Per cent of normal PCV, example: 24·9/34·4 = 72%.
‡ An analysis of variance was conducted on values obtained for the three *Anaplasma* groups (AAM, AC, VAM).

TABLE II

Mean response of intact calves to premunition with attenuated A. marginale, A. centrale *and virulent* A. marginale

	No. of animals	Age (months)	Pre-infect. PCV (%)	Incub. time (days)	Low PCV (%)	Per cent of normal PCV† (%)	High paras. (%)	High CF serum titres	Duration of anaemia (days)
Attenuated *A. marginale*	10	5·1	31·0	29·3	22·0	71	1·56	1:211	6·4
A. centrale	6	2·3	34·8	10·8	23·5	67	5·60	1:320	5·0
Virulent *A. marginale*	17	3·2	33·1	15·4	19·5	59	7·10	1:295	25·5
Significance‡		NS	NS	P<0·01	NS	NS	NS	NS	P<0·05
DRS				6·7					19·0

NS Not significant.
DRS Difference required for significance.
† These values represent the relative drop in PCV due to infection or low PCV %/Pre-infection PCV % = Per cent of normal PCV, example: 22·0/31·0 = 71%.
‡ An analysis of variance was conducted on values obtained for the three *Anaplasma* groups (AAM, AC, VAM).

TABLE III

Mean response of splenectomized calves to premunition with attenuated A. marginale, A. centrale *and virulent* A. marginale

	No. of animals	Pre-infect. PCV (%)	Incub. time (days)	Low PCV (%)	Per cent of normal PCV (%)	High paras. (%)	High CF serum titres	Duration of anaemia (days)	Deaths
Attenuated *A. marginale*	13	34·2	27·4	17·8	52	7·3	1:675	21·0	0
A. centrale	9	36·4	18·7	15·8	43	35·8	1:204*	14·6	0
Virulent *A. marginale*	16	36·8	20·8	8·5	23	63·0	1:364	50·7	12
Significance‡		NS	P<0·05	P<0·01	P<0·01	P<0·01	P<0·01	P<0·01	
DRS			8·6	2·5	8	20·3	*Lower than 1:675	11·6	

NS Not significant.
DRS Difference required for significance.
† These values represent the relative drop in PCV due to infection or low PCV %/Pre-infection PCV % = Per cent of normal PCV, example: 17·8/34·2 = 52%.
‡ An analysis of variance was conducted on values obtained for the three *Anaplasma* groups (AAM, AC, VAM).

in most cases, particularly in calves where differences reach significance, had been frozen. Bedell and Dimopoullos (1962) demonstrated an increased incubation time following freezing, but no significant alteration in severity of infection as manifested by parasitaemia. Lotze (1947) and Kuttler (1966) made the observation that the number of infective particles or the inoculum size influenced the incubation time more markedly than the actual severity of infection.

The relatively mild response of intact calves to all three anaplasms was anticipated and confirms the belief that young animals have a natural resistance to this organism. Of possible significance, however, is the persistence of the anaemia resulting from VAM infections in all three age groups. In the absence of regular blood examinations, many of these cattle would have appeared normal, but in reality the PCV remained markedly below normal for an extended period of time. No attempts were made in these studies to determine the influence of this low PCV on weight gain, productivity, or susceptibility to other diseases, but it would appear that consideration should be given to these factors. Natural premunition with virulent field strains is probably followed by a period of depressed PCVs.

The significantly lower CF titres associated with AC infection is probably a reflection of serologic differences between *A. centrale* and *A. marginale* as described by Kuttler (1967a).

There is little doubt that the results presented in this study show an attenuated reaction following AC and AAM infections as compared with VAM. Among intact calves and adult cattle there appeared to be no detectable difference between AC and AAM, however in splenectomized calves the response to AC infection was more severe than that produced in AAM.

It would appear that AAM is at least equally as mild in its premunizing infection as is AC. The antigenic relationship of AAM to VAM is probably closer than that of AC to VAM. For this reason there would appear to be some marked advantages to using an AAM when premunization appears to be the method of choice in preventing clinical anaplasmosis.

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Responses comparees dans la premunition a l'aide d'anaplasma marginale atténué, A. Marginale virulent et A. centrale dans differents groups d'age.

Résumé—Des infections prémunisantes à l'aide d' *Anaplasma marginale* virulent, d' *A. marginale* atténué et d'*A. centrale* ont été effectuées chez 46 bovins adultes, 33 veaux intacts et 38 veaux splénectomisés, afin de comparer leurs réponses relatives à ces infections.

A. marginale virulent produit des réactions nettement graves chez les adultes et chez les veaux splénectomisés, et une réponse légèrement grave chez les veaux intacts; toutefois, ces animaux étaient relativement résistants aux trois infections. Il n'y a pas eu de différences notables entre les réactions causées par *A. marginale* atténué et par *A. centrale* chez les bovins adultes et chez les veaux intacts. Chez les veaux splénectomisés en revanche, les infections à *A. marginale* atténué ont donné des réponses bénignes, mesurées selon la chute relative du volume des hématies et selon le pourcentage de parasitémie. La réponse à la fixation du complément était significativement plus faible dans les infections à *A. centrale*.

Respuesta comparativa de premunisacion usando *anaplasma marginale* atenuado, *A. marginale* virulento y *A. centrale* virulento en grupos de diferente edad.

Sumario—Infecciones premunisantes usando *Anaplasma marginale* virulento (VAM), *A. marginale* atenuado (AAM) y *A. centrale* (AC) han sido inducidas en 46 bovinos adultos, 33 terneros enteros y en 38 terneros esplenectomizados con el proposito de comparar la respuesta relativa a estas infecciones.

El VAM produjo en bovinos adultos reacciones significativamente mas severas, lo mismo que en terneros esplenectomizados, y una respuesta ligeramente mas severa en terneros enteros; sin embargo, estos animales fueron relativamente mas resistentes a las tres infecciones. No hubo ninguna diferencia detectable entre las reacciones causadas por AAM y AC tanto en bovinos adultos como en terneros enteros. En los terneros esplenectomizados, sin embargo, las infecciones con AAM resultaron en una respuesta mas ligera evidenciada por la caida relativa del volumen de celulas rojas y porcentaje de parasitemia. La respuesta CF fue significativamente menor en la infeccion AC.