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9. ABSTRACT

Attempts at Anaplasma premunization in varying age groups have been reported using a virulent A. marginale of Texas origin, a virulent A. marginale of Colombian origin, an attenuated A. marginale, and A. centrale. Results of premunization response and the response to field and artificial challenge have been reported. Premunization is a practical approach for the prevention of clinical losses associated with anaplasmosis, but a series of variables must be considered if success is to be achieved. Some of these factors are: (1) age of the animals being premunized, (2) virulence, potency, and size of the premunizing inoculum, and (3) the strain, or size, of the expected challenge exposure. In some instances, the use of a highly virulent A. marginale in adult cattle resulted in overly severe reactions even with treatment. Gloxazone (356C61) and Imidocarb (4A65) were superior to oxytetracycline in moderating the premunizing infection. Attenuated strains of A. marginale when used in young intact calves failed to produce the desired premunizing effect; however, attenuated strains were very effective in adult cattle. Premunization is dependent on establishing an active infection, and in the absence of such infection, susceptibility to field or experimental challenge occurs. Successful premunization resulted in almost complete protection following challenge with antigenically similar A. marginale isolates. Protection was only partial, however, in instances where heterologous challenge was encountered.

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Techniques of Premunization for the Control of Anaplasmosis

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Anaplasmosis premunition, or co-infectious immunity, represents an immune or resistant state in the absence of clinical evidence of disease brought about by the presence of a chronic or carrier infection. This immunity persists as long as the infectious organism remains present. The use of premunization for the prevention of acute episodes of anaplasmosis has been known for almost as long as the disease has been recognized. Theiler (11) in 1911, only a year after his first publication concerning *Anaplasma marginale* (10), described the use of *Anaplasma centrale* to induce premunition and a resistance to *A. marginale.* The subsequent use of *A. centrale* as a premunizing vaccine was widespread throughout much of Africa and Asia. Ristic (7) suggests that premunition is the only effective means for producing a practical and effective immunity to anaplasmosis.

In 1937, Schmidt (8) described the use of field isolates of *Anaplasma* in the U.S. for premunizing cattle against anaplasmosis, which consisted of injecting blood from known carriers into susceptible cattle. He reported a 1.3% mortality from this procedure in over a thousand cattle. These infections were induced during the cool season and in cattle less than 15 months of age.

Franklin and Huff (3) proposed a method of premunization with small inocula in which only moderate response occurred but protection was induced. Protection from subsequent exposure is generally good against antigenically similar anaplasms. The existence of antigenically different organisms in South America has been suggested by Kuttler and Zaraza (5). In these instances, however, premunition does appear to produce a degree of cross protection.

Premunition is contraindicated in areas where the disease is sporadic and treatment or management programs offer the hope of control and/or eradication. In endemic areas of the tropics, premunization is often considered the only effective means for control. Field premunition by natural vectors is known to occur at an early age in many areas (4). Because of the innate resistance of young animals, recovery from such infection is usual, with the resultant premunition preventing future acute infections. The introduction of *Anaplasma*-free adult cattle into such areas is often associated with severe losses, unless some form of immunization or treatment is practiced.

During the past few years, experimental data has been accumulated from Africa, South America, and the U.S. on the premunization of cattle of various age groups, using several different techniques. Generally these practices have been successful, but experience shows the continued need for care in this procedure. The intent of this paper is to review and report data involving premunization of both calves and adult cattle and the response of these animals to challenge with virulent *Anaplasma.*

Materials and Methods

Premunization response using varying methods was measured in 129 cattle. A total of 78 adult cattle 18 months of age and older of varying breeds, predominantly European type (Bos Taurus), were premunized, using four different *Anaplasma* isolates in selected groups (Tables 1 and 2). Two of these anaplasms represent attenuated organisms and two are fully virulent field isolates. The fully virulent organisms were used with and without

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Table 1

Premunization of Adult Cattle with Two Virulent *Anaplasma* Isolates Modified by Treatment

Anaplasma Isolate Injected	No. of Animals	Est. Avg. Age	Low PCV % of Normal	Avg. High Paras.	Avg. High CF Response	Persistence of Anemia	Treatment
		years				days	
TAM	9*	7.3	42 ± 10	14 ± 8	1:508	33 ± 16	No Treatment
TAM	6	1.5	63 ± 5	2 ± 1	1:202	10 ± 4	4A65(1)
TAM	6	3.8	64 ± 15	8 ± 6	1:452	11 ± 8	356C61(2)
Significance			P< 0.01	P< 0.01	NS	P< 0.05	
DRS			14.6	8.6		18.2	
NCAM	13	1.5	43 ± 11	6 ± 5	1:465	30 ± 15	Oxytet.(3)
NCAM	5	1.5	71 ± 7	.4 ± .2	1:1280	6 ± 6	356C61(4)
NCAM	5	1.5	72 ± 6	.4 ± .1	1:139	13 ± 14	356C61(5)
Significance			P< 0.01	P< 0.05	P< 0.05	P< 0.01	
DRS			13.4	5.2	1:139 lower than 1:1280 only	19.0	

TAM – virulent *Anaplasma*, Texas isolate.
NCAM – virulent *Anaplasma*, north coast of Colombia isolate.
(1) 4A65: 4 mg/kg given I/M 31 days after exposure.
(2) 356C61: 5 mg/kg given I/V 25 days after exposure.
(3) Oxytetracycline: 12 mg/kg given 9 times on days 21, 41, 45, 46, 47, 48, 49, 50, 51.
(4) 356C61: 5 mg/kg given I/V 7 days after exposure.
(5) 356C61: 5 mg/kg given I/V 7, 28, 29, and 30 days after exposure.
DRS – Difference Required for Significance.
NS – Not Significant
*1 cow premunized without treatment died.
±: Standard Deviation.

Table 2

Premunization of Adult Cattle With a Virulent and Two Attenuated Anaplasmas Not Modified By Treatment, But Exposed to Heterologous Challenge

Anaplasma Isolate	No. of Animals	Est. Avg. Age	Low PCV % of Normal	Avg. High Paras.	Avg. High CF Response	Persistence of Anemia	Response to Challenge NR	MR	SR
		years				days			
TAM	9*	7.3	42 ± 10	14 ± 8	1:508	33 ± 16	–	–	–
AC	14	2.0	72 ± 14	4 ± 4	1:131	8 ± 9	11	3	0
AAM	15	5.6	74 ± 16	3 ± 4	1:305	8 ± 12	15	0	0
AAM	5	1.5	76 ± 4	1.5 ± 0.8	1:907	2 ± 3	1**	1**	3**
Significance			P<0.01	P<0.01	P<0.01	P<0.01			
DRS			16.0	6.0	1:131 lower than 1:508 1:907	14.5			

TAM – virulent *Anaplasma*, Texas isolate.
AC – *Anaplasma centrale.*
AAM -- attenuated *Anaplasma marginale* (Diamond Laboratories).
NR – no response to challenge – the low PCV percent of normal being > 75%.
MR – moderate response to challenge – the low PCV percent of normal being 50-75%.
SR – severe response to challenge – the low PCV percent of normal being < 50%.
*1 of the 9 animals died of acute anaplasmosis.
**subjected to field challenge on the north coast of Colombia.
±: Standard Deviation.

treatment. Treatment consisted of 4A65* (Imidocarb), 356C61** (Gloxazone), and oxytetracycline*** (Liquamycin). Premunization consisted of injecting subcutaneously (S/C) 2-5 ml fresh blood from carrier animals, except where the attenuated *A. marginale* (AAM)**** (7,12) was used and in most instances this organism was obtained from frozen inocula which was thawed and injected in 5 ml amounts S/C.

*4A65: Imidocarb – 3,3'-Bis-(2-imidazolin-2-yl)-carbanilide dihydrochloride (or dipropionate) - Burroughs Wellcome Co., Inc.
**365C61: Gloxazone - *Alpha*-Ethoxyethylglyoxal Dithiosemicarbazone - Burroughs Wellcome Co., Inc.
***oxytetracycline: Liquamycin - Chas. Pfizer & Co., Inc.
****Diamond Laboratories

Table 3

Premunization of Intact Calves With a Virulent and Two Attenuated *Anaplasma* Isolates Followed By Challenge

Anaplasma Isolate	No. of Animals	Est. Avg. Age	Low PCV % of Normal	Avg. High Paras.	Avg. High CF Response	Persistence of Anemia	Response to Challenge NR	MR	SR
		mos.				days			
TAM	12	3.5	63 ± 11	1.0 ± .5	1:214	28 ± 30	2*	3*	0
AC	6	2.3	67 ± 21	6 ± 8	1:320	5 ± 8	4	1	1
AAM	11	5.2	72 ± 8	1.4 ± 2.0	1:220	6 ± 6	3	2*	0
Significance			NS	NS	NS	P < 0.05			
DRS						22			

TAM – virulent *Anaplasma*, Texas isolate.
AC – *Anaplasma centrale*
AAM – attenuated *Anaplasma marginale* (Diamond Laboratories).
NR: no response to challenge – the low PCV percent of normal being >75%.
MR: moderate response to challenge – the low PCV percent of normal being 50-75%.
SR: severe response to challenge – the low PCV percent of normal being < 50%.
NS – Not Significant
*field challenge on north coast of Colombia
all others are needle challenges with virulent *A. marginale*
±: Standard Deviation.

Nine cattle averaging 7.3 years of age were inoculated with virulent A. marginale isolated in Texas (TAM). This organism has been repeatedly passaged in experimental cattle. These nine cattle were not treated in any way to moderate infection. Six young adults averaging 1.5 years of age were premunized with TAM and then treated with 4A65 at the level of 4 mg/kg administered S/C 31 days after exposure. Six cattle averaging 3.8 years of age were premunized with TAM and then treated with 356C61 at the level of 5 mg/kg given I/V 25 days after exposure (Table 1).

A group of 23 cattle from *Anaplasma*-free areas, averaging 1.5 years of age, were each premunized with a virulent *A. marginale* isolate from the north coast of Colombia (Table 1) (NCAM). Thirteen of the 23 were treated with 12 mg/kg of oxytetracycline nine times beginning 21 days after exposure and being repeated on days 41, 45, 46, 47, 48, 49, 50, and 51. Five were treated with 5 mg/kg 356C61 given seven days after exposure. An additional five were treated with 5 mg/kg 356C61 given 7, 28, 29, and 30 days after exposure.

A regression correlation analysis was made of 20 adult cattle listed in Table 1 on which exact ages were available to determine the possible influence of age on the severity of infection in these particular animals. Even though a range in age from one year to 13 was observed, no statistically significant correlation between the percent of normal PCV at the height of infection and age was detected.

In Table 2 the effects and efficacy of premunizing 34 cattle with attenuated anaplasms is compared with the group of nine TAM infected cattle previously listed in Table 1. Fourteen cattle averaging two years of age were premunized with *A. centrale* (AC). No treatment was given to moderate the resulting infection. All cattle were subsequently exposed within three months of premunization by the inoculation of blood containing a virulent *A. marginale*. Fifteen cattle averaging 5.6 years of age were premunized with AAM. No treatment was given after premunization. All cattle were subsequently challenged within three months of premunition by the inoculation of 5 ml blood containing a virulent *A. marginale*. An additional five cattle averaging 1.5 years of age were premunized with AAM and exposed to a field challenge on the north coast of Colombia.

A group of 29 young intact calves was premunized with three different *Anaplasma* isolates (Table 3). No treatment was used in these premunization attempts. Twelve calves averaging 3.5 months of age were premunized with TAM, and five were later subjected to field challenge on the north coast of Colombia. Six calves averaging 2.3 months of age were premunized with AC, and all were later challenged by the inoculation of blood containing virulent *A. marginale*. Eleven calves averaging 5.2 months of age were premunized with AAM, and two were later exposed to field challenge, and three were inoculated with blood containing virulent *A. marginale*. A total of 17 calves were actually injected with the AAM infected blood which had been frozen, but only 11 or 65% showed evidence of a premunizing infection. Six or 35% failed to develop evidence of infection as characterized by a complement fixation response and/or an *Anaplasma* para-

Table 4

Comparative Response to AAM Premunition of Adult Cattle and Calves Located in Colombia and in Texas A&M

Adult Cattle	No. of Cattle	Avg. Age (yrs.)	Avg. Ambient Temp.	PCV Response (% of Normal)	Duration of Anemia
					days
Colombia	7	3.6 ± 2	13 C	83.3 ± 7	0
Texas A&M	8	7.2 ± 2.0	29.4 C	66.1 ± 18	15.2 ± 13
		P<0.01		P<0.05	
Intact Calves		(mos.)			
Colombia	4	3.0 ± 2	13 C	77.8 ± 7.9	4.5 ± 3.0
Texas A&M	7	6.4 ± 3	29.4 C	68.6 ± 6.0	7.7 ± 6.1
		P<0.01		NS	NS

NS – Not Significant
±: Standard Deviation

sitemia. These calves were not included in our tables because in effect no premunization occurred.

Schmidt (8) recommended premunization in the winter and early spring, during cool weather. The suggestion was made that *Anaplasma* reactions were less severe at this time. Franklin (2) observed greater parasitemias in antigen production cows when the temperature was increased. During the course of our studies we have had occasion to premunize adult cattle and intact calves at both Texas and Colombia when there was considerable variation in mean ambient temperature. Table 4 presents a comparison of the reactions produced by AAM in adult cattle in Texas and those in Colombia. The source of the AAM in both instances was Diamond Laboratories. Seven cattle with an average age of 3.6 ± two years were premunized on the Bogota savannah where the mean temperature was 13°C. Eight cattle with an average age of 7.2 ± two years were premunized at College Station in August when the mean temperature was 29.4°C. In addition, four intact calves were premunized in Colombia and seven in Texas (Table 4).

In an effort to further characterize the apparent temperature response on the course of anaplasmosis, the course of infection was carefully followed in 14 splenectomized calves, one-half of which were exposed to summer heat and the other half maintained in an air-conditioned room. The mean temperature of those exposed to summer conditions was 30.0°C; whereas, the mean temperature in the air-conditioned stall was 18.5°C. All calves were inoculated with the TAM organism and the course of infection observed for a period of over 60 days (Table 5).

A group of 12 intact calves averaging 5.2 months of age were premunized with TAM and

Table 5

Influence of Temperature on PCV Following Infection of Splenectomized Calves With *A. marginale* (TAM)

	No. of Animals	Low PCV Avg. Temp.	% of Normal
Outside	7	30.0 C	30.2%
Air-conditioned room	7	18.5 C	33.5%

No Significant Different

used to monitor the effect of oxytetracycline treatments in moderating the premunizing infection. Four calves were treated with oxytetracycline at the rate of 5 mg/lbs I/M 14 days after TAM exposure. Four calves were treated in the same manner 21 days after exposure, and four remained as untreated controls (Table 6).

The comparative response to virulent *A. marginale* challenge imposed on 11 splenectomized calves previously premunized with an *A. marginale* isolate of reduced virulence (not AAM)* and *A. centrale* was measured and recorded in Table 7. Seven splenectomized calves premunized with *A. centrale* and four splenectomized calves premunized with a mild *A. marginale* were challenged with virulent *A. marginale* by the inoculation of 5 ml infected blood. Response to this challenge is recorded in Table 7.

Packed cell volume (PCV), parasitemia, complement fixation (CF) response, and the duration of immunity were the parameters recorded in tables to measure the severity of infection. The PCV is expressed as the low percent of normal occurring during the primary infection. If the pre-infection PCV normal was 32% and the low PCV during the primary *Anaplasma* infection was 12% then the

*Obtained in 1964 from T. O. Roby, Beltsville, Maryland.

Table 6

Moderation of Premunizing *A. marginale* (TAM) Infection in Calves by the Use of Oxytetracycline

No. of Calves	Avg. Age	PCV % of Normal	Time Required After Exposure to Reach Low PCV	Paras.	CF Response	Treatment
	mos.		days			
4	5.2	56.8 ± 8	24.2 ± 3	0.55 ± .4	1:190	Oxytetracycline 11 mg/kg I/M on day 14
4	5.5	60.5 ± 7	21.8 ± 7	0.42 ± 0.05	1:270	Oxytetracycline 11 mg/kg I/M on day 21
4	5.0	54.8 ± 3 NS	23.5 ± 7 NS	0.58 ± .30 NS	1:270 NS	No Treatment

NS – Not Significant
±: Standard Deviation

Table 7

Comparative Response to Virulent *A. marginale* Challenge Imposed on Splenectomized Calves Premunized With a Non-Virulent *A. marginale* and *A. centrale*

Premunizing Infection	No. of Animals	Low PCV % of Normal Associated With Premunition	Challenge With Virulent *A. marginale* NR	MR	SR
AC	7	44 ± 8	1	1	5
MAM	4	40 ± 11 NS	2	2	0

AC – *Anaplasma centrale*
MAM – a mild *A. marginale* isolate (obtained in 1964 from Dr. T. O. Roby, Beltsville, Maryland).
NR: No Response
MR: Moderate Response
SR: Severe Response
NS – Not Significant
±: Standard Deviation.

percent of normal would be 12/32 or 37.5%. Persistence of anemia represents the time in days that the PCV remains lower than 75% of the pre-infection normal. The response to challenge was expressed as a severe response (SR) where the low PCV was 50% or less of normal, a moderate response (MR) where the low PCV was 50-75% of normal, or no response (NR) where the low PCV was 75-100% of normal.

Serum samples were tested for evidence of specific complement-fixing antibodies, using modification of the USDA (1958) technique (1). Average CF titers were obtained using a logrithmic transformation of the dilution factor. An analysis of variance was conducted for the establishment of significance (9).

Results

Results tabulated in Table 1 show relatively severe reactions occurred in adult cattle premunized with TAM, with one of nine cattle dying. Treatment with 4A65 and 356C61 were generally successful in moderating the severity of infection. The PCV, parasitemia, and persistence of anemia were significantly different reflecting the value of treatment. Premunization of young adult cattle with NCAM produced a severe response when attempts were made to moderate infection with oxytetracycline. The other two groups in which 356C61 was used showed significantly more moderate infections.

The attenuated anaplasms tabulated in Table 2 resulted in moderate infections which were significantly milder than the control TAM infections. No significant difference could be detected between AC and AAM infections, except for the lower CF titers with AC infections. Challenge infection induced on the AC carrier animals resulted in three moderate reactions, but 11 showed no response. A TAM challenge imposed on 15 AAM premunized cattle failed to produce any evidence of renewed *Anaplasma* reaction. Animals with AAM infections exposed to a field challenge on the north coast of Colombia developed three severe reactions, one moderate response, and one showing no response.

A similar comparison of TAM, AC, and AAM infections induced in intact calves is recorded in Table 3. A trend toward more moderate reactions with AC and AAM was noted but the only significance was in the persistence of anemia. The anemia persisted significantly longer following TAM premunization than was observed in AC infections. An *A. marginale* challenge given six AC premunized animals resulted in four showing no response, one a moderate response, and one a severe response. A similar challenge administered to three calves having an AAM infection failed to produce any signs of a challenge response. Five TAM premunized calves when exposed to a north coast (Colombia) field challenge showed a moderate response in three and no response in two.

A similar challenge of two AAM premunized calves resulted in two moderate reactions.

Significant differences in the reaction of adult cattle premunized with AAM at Bogota, Colombia, and College Station, Texas, was detected. There was a time lapse of about two years between the time this work was done in Colombia and when it was done in Texas, but the organism used had the same origin. The cattle in Colombia were mostly Angus while in Texas they were predominantly grade Hereford cattle. The cattle used in Texas were somewhat older but correlation regression analysis plotting the low PCV response against age failed to show a significant correlation. The two most outstanding differences are the altitude and temperature. Premunization was carried out in Texas in August at about sea level when the mean temperature was 29.4°C. In Bogota, with an altitude of 2,600 M., the mean temperature was only 13°C. A significantly more severe response occurred in Texas even though the average reactions were considered moderate. All 15 cattle as seen in Table 3 were solidly immune to TAM challenge. Similar differences in response were noted among intact calves but they were not so marked and failed to reach significance.

The attempt to duplicate this finding using 14 splenectomized calves was generally unsuccessful as indicated in Table 5. There were no significant differences between PCV response to TAM infection of calves kept at 18.5°C and those held at approximately 30°C. A trend of more severe response was noted in those calves held at 30°C but the differences were not significant.

The administration to intact calves of 11 mg/kg oxytetracycline 14 and 21 days after exposure to virulent *Anaplasma* failed to significantly alter the course of infection (Table 6), which was extremely mild in the controls.

A virulent *A. marginale* challenge of seven splenectomized calves premunized with *A. centrale* resulted in five showing a severe response, one a moderate response, and one no response. Similar challenge of four calves premunized with a mild *A. marginale* resulted in two showing a moderate response, with two showing no response to challenge (Table 7).

Conclusion and Discussion

Premunization of adult cattle with fully virulent *A. marginale* is feasible when the infection is moderated by appropriate drug therapy. Both 4A65 (Imidocarb) and 356C61 (Gloxazone) have proven effective. Under the conditions of these trials oxytetracycline was relatively less effective than Imidocarb and Gloxazone for this purpose; however, only two trials with oxytetracycline were attempted.

From a time and economy point of view, the attenuated anaplasms offer more promise as premunizing agents. Needle challenge with a virulent Texas isolate (TAM) showed complete protection in cattle previously premunized with the AAM strain. Protection against field challenge was not complete, even though a degree of protection was apparent. It is recommended that controlled studies be made to characterize possible antigenic differences between the organism being used for premunization and the locally occurring *A. marginale* isolate, whenever possible.

Premunization of calves less than six months of age can probably best be accomplished using a virulent *A. marginale* which has first been characterized as to its virulence. An isolate such as is present at Texas (TAM) produced only a moderate response in intact calves. It is probable that some field isolates might be excessively virulent for use even in young animals. The AAM organism that has been frozen is often too mild or too low in titer to produce consistent premunization in intact calves. This observation was prompted by a failure of 6 of 17 calves (35%) to develop evidence of premunizing infection following their inoculation with this material. The injection of freshly collected blood from carrier animals infected with either AC or AAM appeared more satisfactory for premunizing purposes.

The observations of Schmidt (8) and Franklin (3) relative to the influence of ambient temperature on the severity of infection appeared to be confirmed when reactions produced by AAM in Colombia were compared to reactions in Texas. Our attempts to reproduce this effect in splenectomized calves was unsuccessful but an insufficient range of temperature differences may not have been present. Three factors which could contribute to the differences noted between Colombia and Texas are altitude, temperature, and possible strain (AAM) differences occurring during the time interval between trials.

The greater antigenic similarity of a mild *A. marginale* (MAM) to virulent *A. marginale* than *A. centrale* is quite obvious in the results of Table 6. For this reason attenuated or mild *A. marginale* isolates would have distinct advantages over *A. centrale* as premunizing vaccines.

Summary

Attempts at *Anaplasma* premunization in varying age groups have been reported using a virulent

A. marginale of Texas origin, a virulent *A. marginale* of Colombian origin, an attentuated *A. marginale*, and *A. centrale*. Results of premunization response and the response to field and artificial challenge have been reported.

Premunization is a practical approach for the prevention of clinical losses associated with anaplasmosis, but a series of variables must be considered if success is to be achieved. Some of these factors are: (1) age of the animals being premunized, (2) virulence, potency, and size of the premunizing inoculum, and (3) the strain, or size, of the expected challenge exposure. In some instances, the use of a highly virulent *A. marginale* in adult cattle resulted in overly severe reactions even with treatment. Gloxazone (356C61) and Imidocarb (4A65) were superior to oxytetracycline in moderating the premunizing infection. Attenuated strains of *A. marginale* when used in young intact calves failed to produce the desired premunizing effect; however, they were very effective in adult cattle. Premunization is dependent on establishing an active infection, and in the absence of such infection, susceptibility to field or experimental challenge occurs.

Successful premunization resulted in almost complete protection following challenge with antigenically similar *A. marginale* isolates. Protection was only partial, however, in instances where heterologous challenge was encountered.

Acknowledgements

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