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PREMUNIZATION WITH AN ATTENUATED *ANAPLASMA MARGINALE*

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Anaplasmosis immunization attempts, using killed antigens, have been shown to reduce the severity of subsequent infections, but generally incapable of preventing infection when exposure occurs[1,2,4] The absence of a complete, lasting, sterile immunity suggests that a premunizing approach might be indicated in enzootic areas where more complete protection is needed

Theiler[7], as early as 1911, observed that infection with *Anaplasma centrale* produced a degree of resisitance to virulent anaplasmosis Schmidt[6], in 1937, described a technique of premunition using virulent *A marginale* He recognized age and ambient temperature as factors influencing the successful premunization of cattle Over 1000 cattle were premunized with an estimated loss of only 1.3%. Kuttler[3] reported the use of an *A marginale* isolate equally as mild as *A centrale*, which resulted in complete protection against virulent challenge, whereas *A centrale* merely reduced the severity of a virulent challenge

Ristic *et al*[5] and Welter and Woods[8] reported the development of an attenuated *A marginale* by serial passage of irradiated organisms in abnormal hosts. It was reported that virulence was lost for aged cattle and splenectomized calves

This report deals with the attenuated *A marginale* and its use in Columbia, with the object of characterizing animal response to premunition as well as to evaluate its efficacy in preventing acute, severe anaplasmosis following challenge.

Materials and Methods

Infection with the attenuated *A marginale* was induced in 21 Holstein-Friesian calves with an average age of 9 3 months, by the subcutaneous inoculation of 5 ml of infected blood no more than 2 passages removed from the vaccine* Twelve similar calves with an average age of 3 7 months were infected with virulent *A marginale* Animal responses to these infections were compared (Table 1)

Infection with the attenuated *A marginale* was induced in 12 mature cattle of mixed breeding Seven animals received 1 ml of infected blood, and the remaining 5 were given 5 ml All inoculations were made subcutaneously Five mature cows were similarly infected by injecting 1 ml of blood containing a virulent *A. marginale* Animal responses to these infections were compared (Table 2)

The comparative severity of animal response at 2 climatic zones to the attenuated *A marginale* was determined (Table 3) Fifteen Holstein-Friesian calves at Bogota, and 10 similar calves at Palmira, with a mean temperature of 14°C and 24°C respectively, were infected with the attenuated *A marginale* by the subcutaneous inoculation of 5 ml infected blood not more than 2 passages removed from the vaccine.

Comparisons in Tables 1, 2 and 3 include low packed cell volume (PCV), high

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

* Obtained from Diamond Laboratories, Des Moines, Iowa.

parasitemia and high complement-fixation (CF) titers for each group. An analysis of variance was conducted in each instance to determine the probability of error in mean differences

The PCV response of vaccinated and non-vaccinated cattle to both artificial and natural challenge is presented in Figures 1, 2 and 3. Seven vaccinated and 5 non-vaccinated mature cattle were challenged with 1 ml of a 0 75% suspension in 0 85% NaCl of washed red blood cells showing a 4% ascending *A marginale* parasitemia (Fig 1) The challenging organism was isolated in Texas, and administered 50 days after the premunizing infection

Two splenectomized calves, previously premunized with the attenuated *A. marginale* were challenged with 20 ml packed red blood cells showing a 0 2% parasitemia from a calf with virulent *A marginale* isolated in Texas Two additional splenectomized calves, previously premunized with the attenuated *A marginale* were challenged with 5 ml of blood showing a 0 2% parasitemia from a calf with a virulent *A marginale* isolated in Colombia (Figure 2) All 4 calves had been premunized about 4 months prior to challenge

A total of 10 Holstein Friesian calves, 5 vaccinated and 5 unvaccinated, were placed on known *Anaplasma* infected premises and naturally exposed to anaplasmosis The day on which the low PCV occured, if associated with other evidence of anaplasmosis, was designated Day O The PCVs were charted 42 days before and after Day O for evidence of significant fluctuation

Results

Calves inoculated with the attenuated *A marginale* showed a significantly milder response when compared to reactions produced by the fully virulent organism (Table 1) Mature cattle infected with virulent *A marginale* developed an average low PCV of 12 4% (Table 2) Mature cattle infected with the attenuated organism developed an average low PCV of 30 0% One death occurred among the 5 adults receiving the virulent organism No deaths occurred among the 12 animals infected with the attenuated organism

Of 15 calves inoculated at Bogota with the attenuated organism, only 7 reacted with diagnostic evidence of *Anaplasma* infection (Table 3) The subsequent reactions observed in these 7 animals at Bogota were compared to similarly infected animals at Palmira Significant differences in the severity of response failed to occur in these comparisons There was a trend toward a milder response at Bogota, but the relative PCV (percent of normal) and parasitemia differences failed to reach significance A significantly higher CF titer was recorded among animals quartered at Palmira

The challenge results on 7 vaccinated and 5 non vaccinated mature cattle inoculated with virulent *A marginale* of Texas origin is presented in Figure 1 The non-vaccinated cattle developed acute anaplasmosis with low PCV values and demonstrable parasitemias One death occurred in this group No apparent change in PCV values or other evidence of anaplasmosis was noted among the vaccinated animals

The PCV responses and parasitemia of vaccinated, splenectomized calves, following exposure to two different *A marginale* field isolates, are presented in Figure 2. A Texas isolate did not produce a characteristic drop in PCV, although

considerable fluctuation occurred. The Colombian isolate produced a marked reduction in PCV at a time compatible with a challenge infection.

No appreciable differences could be detected between 5 vaccinated and 5 non-vaccinated calves receiving a field exposure to *A. marginale* in Colombia (Figure 3). The drop in PCV for both groups as illustrated in Figure 3 was also accompanied by an increase in the anaplasmosis CF titer .

Discussion and Conclusions

Infections produced by the attenuated *A. marginale* organism in calves and adult nonlactating cattle were less severe than infections resulting from virulent *A. marginale*

Environmental factors are apparently involved in the course of premunizing infections with the attenuated *A marginale* This is best illustrated by a failure of known infective inoculums to produce a demonstrable *A marginale* parasitemia in 8 of 15 calves, at Bogota The remaining 7 calves developed evidence of infection which was slightly less severe although not significantly less than infections seen among 10 calves at Palmira

Premunition or vaccinating infections in adult cattle with the attenuated *A marginale* resulted in complete protection from a Texas isolate Splenectomized and intact calves presumed to be carrying the attenuated organism reacted to challenge when exposed to a Colombian isolate by developing signs of acute anaplasmosis

Mott[4] observed that in some instances heavy challenge of carrier animals could result in a mild exacerbation of anaplasmosis signs He and others[3] have also noted that considerable variation in virulence exists among different *A marginale* isolates The results of these trials indicate that either the Colombian isolate is more virulent, resulting in a more pronounced secondary response following challenge, or that it represents an immunologically distinct strain Differences between the Texas and Colombian isolates were not detected with the routine CF test, or by change in morphology of the *Anaplasma* body as seen with Giemsa stain

Summary

An attenuated *Anaplasma marginale* infection has been established in 21 calves and 12 mature cattle The resulting infections were found to be significantly less severe than virulent *A marginale* in 12 calves and 5 mature cattle A slightly milder response to the attenuated *A marginale* occurred in calves at Bogota with a mean temperature of 14° C when compared to calves similarily infected at Palmira with a mean temperature of 24°C.

Calves and mature cattle previously premunized with the attenuated organism appeared to be immune to virulent challenge using a Texas isolate of *A marginale.* Experimental and natural challenge with a Colombian isolate resulted in evidence of acute anaplasmosis in both vaccinated and non vaccinated animals

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TABLE 1

Comparative Response of Young Calves to Attenuated and Virulent *A. marginale.*

	No. of Animals	Avg. Age Months	Avg. Pre-Infection PCV %	Avg. Low PCV %	Avg. High Parasitemia %	High CF Titer	Deaths
Attenuated A. marginale	21	9.3	29.5	21.8	1.3	1:525	0
Virulent A. marginale	12	3.7	30.3	17.1	5.2	1:498	0
Significance			N.S.	P<0.01	P<0.01	N.S.	

N.S. - Not Significant

TABLE 2

Comparative Response of Mature Cattle to Attenuated and Virulent *A marginale*

	No. of Animals	Avg. Age Months	Avg. Pre-Infection PCV %	Avg. Low PCV %	Avg. High Parasitemia %	Avg. High CF	Deaths
Attenuated A. marginale	12	37 ±22	37	30.0	0.9	1:640	0
Virulent A marginale	5	79 ±50	36	12.4	20.8	1:1930	1
Significance			N.S.	P<0.01	P<0.01	P<0.01	

N.S. - Not Significant

TABLE 3

Comparative Response of Intact Calves to Attenuated *A. marginale* at Bogota (14°C) and Palmira (24°C)

	No. of Animals	No. of Animals Reacting	Avg. Pre-Infection PCV %	Avg. Low PCV %	Percent of Normal PCV	Avg. High Paras. %	Avg. High CF	Deaths
Bogota	15	7	36.9	29.3	79.4	0.69	1:290	0
Palmira	10	10	29.5	21.1	71.5	1.89	1:788	0
Significance			P<0.01	P<0.01	N.S.	N.S.	P<0.01	

N.S. - Not Significant

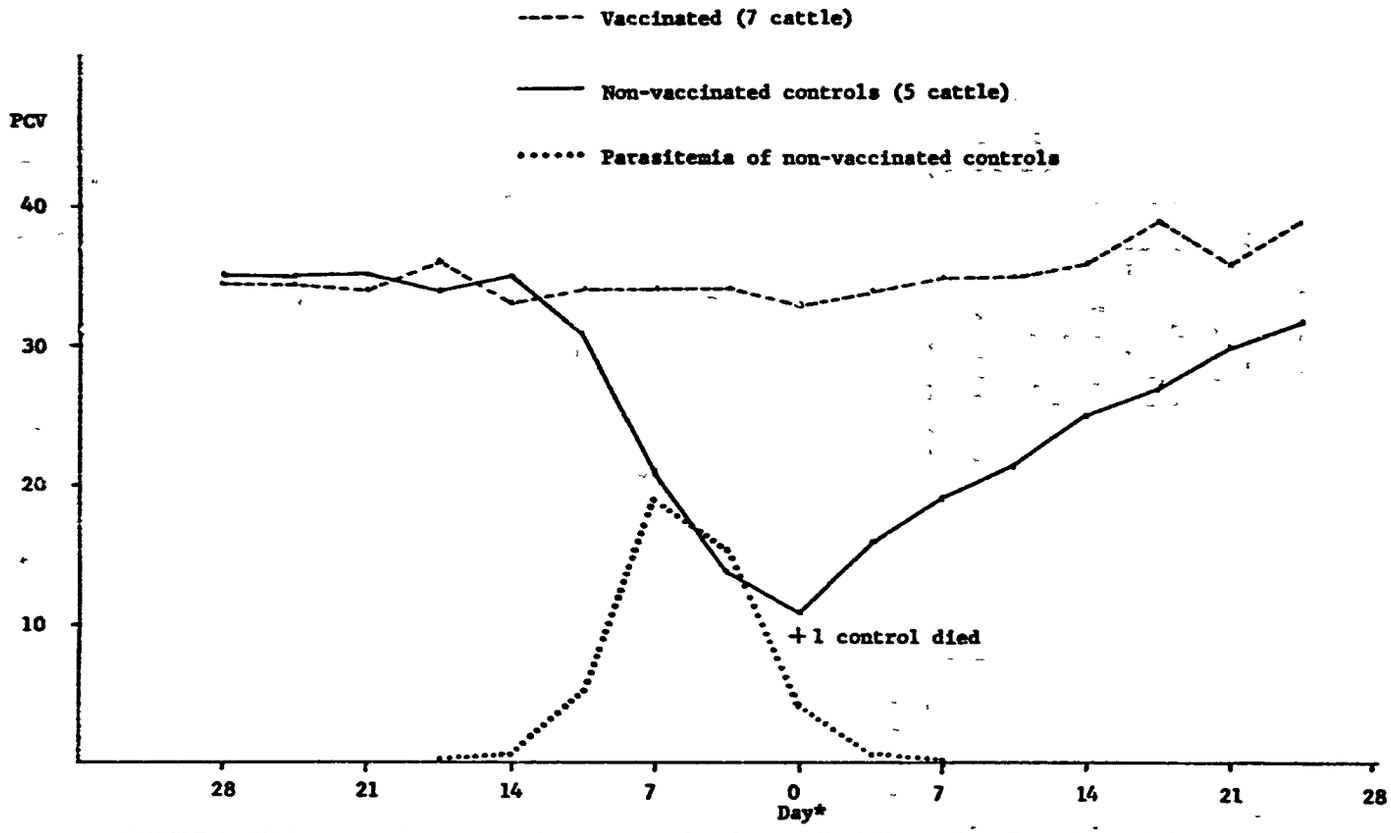

FIGURE 1. PCV response of vaccinated and non-vaccinated cattle to needle challenge using a Texas *A. marginale* isolate.
* The day on which the low PCV occurred due to anaplasmosis (non-vaccinated).

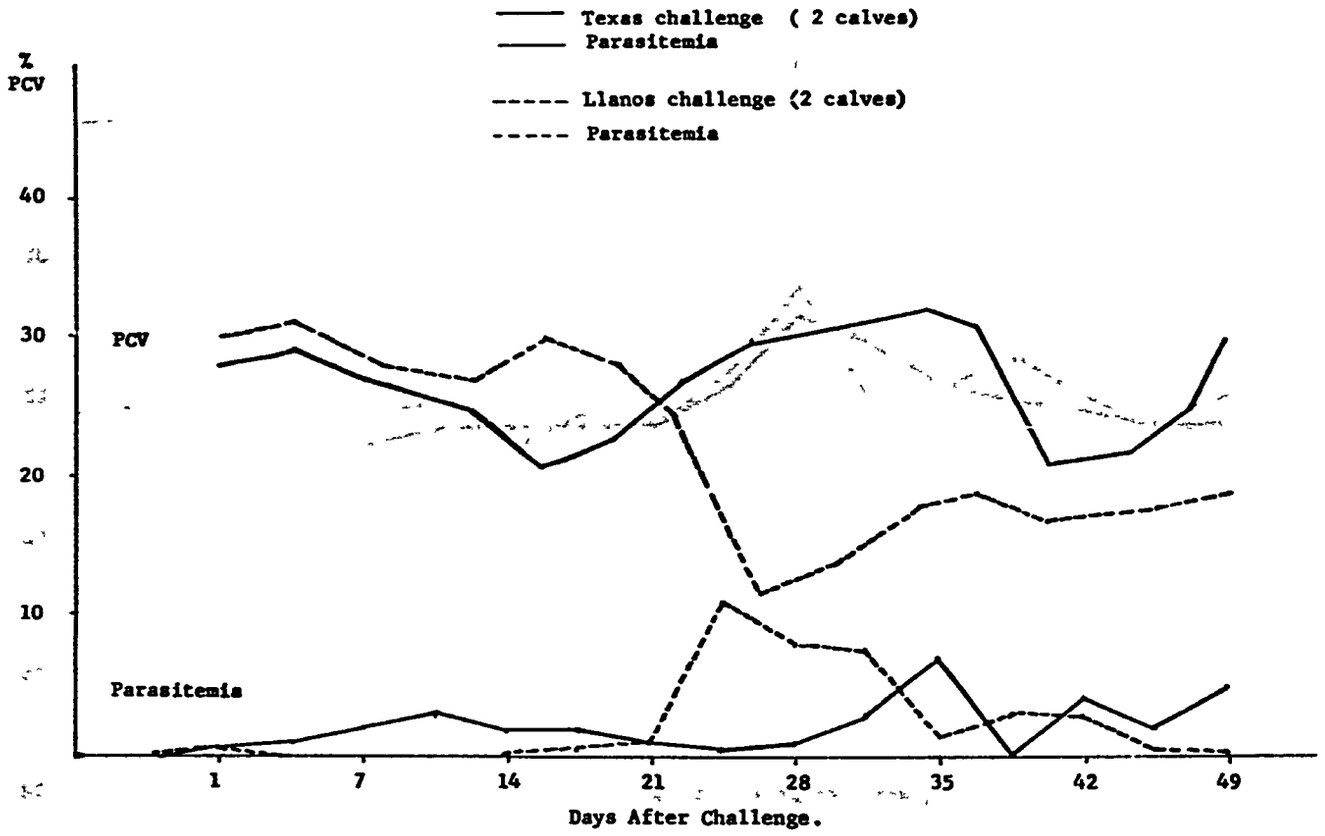

FIGURE 2. PCV and parasitemia response of vaccinated splenectomized calves challenged with Texas and Colombian isolates.

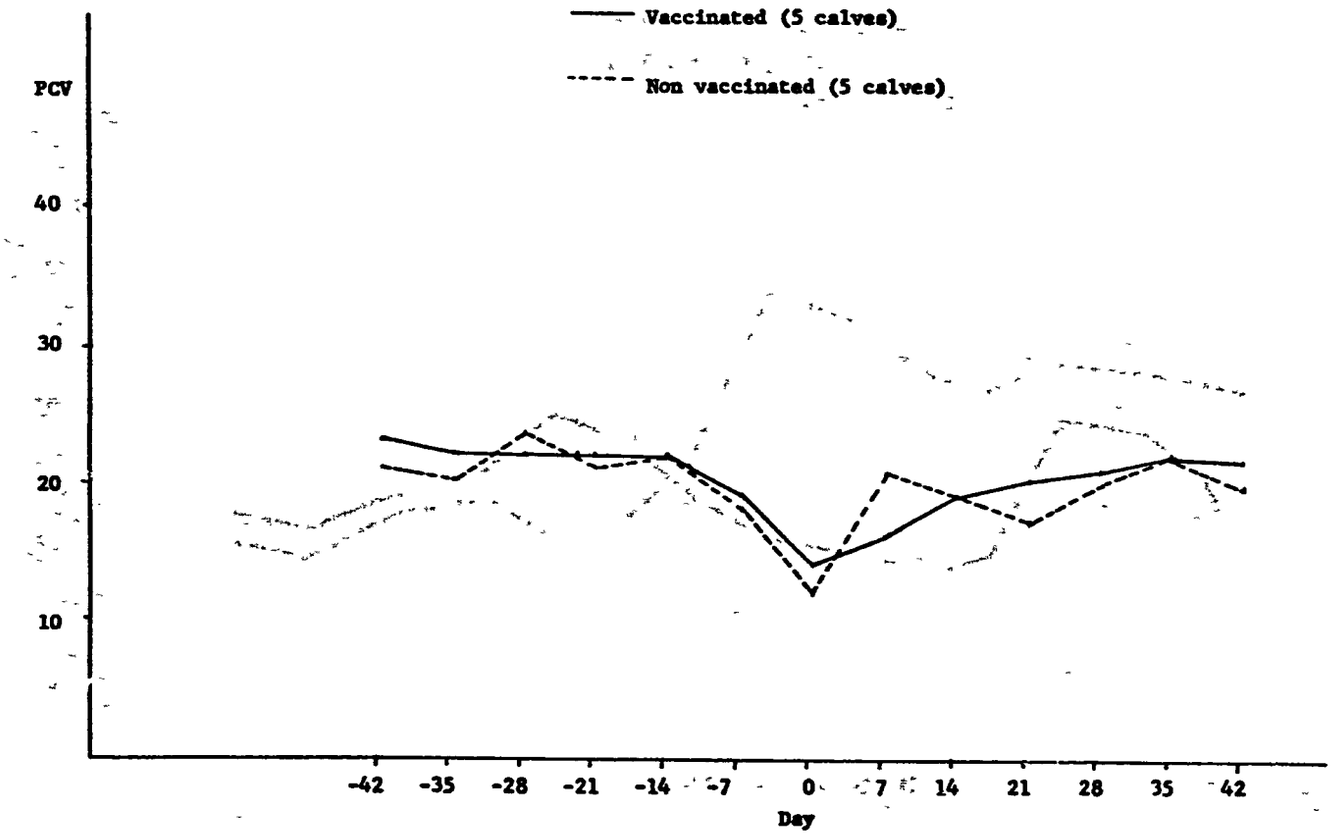

FIGURE 3. PCV response of vaccinated and non-vaccinated calves to natural *Anaplasma* challenge in Colombia.

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