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FIELD EVALUATION OF AN EXOANTIGEN-CONTAINING BABESIA VACCINE IN VENEZUELA

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Bovine babesiosis is endemic in Venezuela, causing significant losses in highly susceptible imported cattle. Current immunoprophylactic methods include the less desirable use of live parasites. Inactivated vaccines derived from exoantigen-containing supernatant fluids of in vitro Babesia bovis *and* B. bigemina *cultures have been developed and constitute a major improvement in vaccine safety, stability and ease of handling. Vaccination trials conducted under field conditions provide the final evalutation of a culture-derived* B. bovis-B. bigemina *vaccine. During a 5-year period, approximately 8,000 cattle were vaccinated and 16 clinical trials carried out in 7 states of Venezuela. Clinical, serologic and parasitologic data were collected monthly from 10% of the animals over a 2-year period. Data were also collected from a similar number of nonvaccinated control cattle. Analysis of results from these trials demonstrated a reduction in the incidence of clinical disease among vaccinated animals and complete protection against mortality caused by babesiosis. Vaccine efficacy was measured calculating the incidence rates of disease and mortality among vaccinated and nonvaccinated cattle. Use of this inactivated vaccine offers the best combination of safety, potency and efficacy for the effective immunoprophylactic control of bovine babesiosis.*

Key words: *Babesia bovis* – *Babesia bigemina* – babesiosis vaccine – immunoprophylaxis – field trials – exoantigen

Bovine babesiosis caused by *Babesia bovis* and *B. bigemina* remains one of the major obstacles in the development of the livestock industry in most countries of Latin America. The causative hemoparasites are transmitted by the tick *Boophilus microplus* and generally coexist in warm, humid climates which favor tick development (Callow, 1974). The problem of bovine babesiosis persists in most tropical and semitropical regions of the world where approximately 1 billion cattle are at risk (McKosker, 1981).

In most countries, control of babesiosis has mainly relied on the use of chemoprophylactic and chemotherapeutic drugs. In many instances these control measures have become expensive, difficult and less practical with range-raised cattle (Pipano & Hadani, 1984).

At present, only live vaccines are available in a limited number of countries. Serious disadvantages such as limited shelf life, strict dependency on a cold chain, variable infectivity and morbidity and risk of contamination by other pathogens have precluded use in most Latin America countries (Lora, 1981).

The lack of an entirely satisfactory vaccine against babesiosis has placed a high priority on the development of safe and effective vaccines. Such vaccines would prevent losses due to the disease and could even allow expansion of the livestock industry in endemic areas. Two main approaches are being followed towards this goal: development of improved conventional, inactivated vaccines, namely, a culture-derived, organism-free, exoantigen-containing vaccine (Smith et al., 1981; Kuttler et al., 1982, 1983; Montenegro-James et al., 1985, 1987, 1989) and, use of recombinant DNA technology for expression of selected antigens (Hines et al., 1989; Timms et al., 1989; Figueroa & Buening, 1991; Wright, 1991). Considerable basic research remains to be conducted, especially in the definition of protective antigenic epitopes and in the selection of effective adjuvant and delivery systems.

We have extensively studied the effectiveness of a culture-derived *B. bovis-B. bigemina* exoantigen-containing vaccine and have found it to possess the following characteristics: (1) safe; (2) stable (lyophilized, over 2 years at 4 °C); (3) two doses at 4-6 week interval provide protective immunity for at least 14 months; (3) good degree of heterologous cross-protection; (5) saponin (Quil A®) adjuvant at 3 mg/dose is safe and effectively elicits strong humoral and CMI responses. These characteristics were demonstrated during 11 laboratory trials performed at the Veterinary Research Institute in Maracay, Venezuela (Montenegro-James, 1989).

The logistics of vaccine development and its subsequent application require extensive testing in order to evaluate the safety and efficacy under field conditions. To comply with Phase III of vaccine development, controlled field vaccination trials were carried out in Venezuela during a 5-year period. In this report an evaluation of the performance of an inactivated *B. bovis-B. bigemina* vaccine under field conditions is presented.

FIELD EVALUATION OF EXOANTIGEN-CONTAINING VACCINE

Vaccination trials conducted under field conditions provide the final evaluation of the safety and efficacy of culture-derived *Babesia* vaccines. Seroepidemiological studies to monitor the prevalence of antibodies to *Babesia* in major cattle regions of Venezuela indicated that babesiosis is endemic with a prevalence rate of approximately 50% (Fig. 1). Venezuela is particularly suitable for immunization studies because of its varied epidemiological conditions. First, highly suscep-

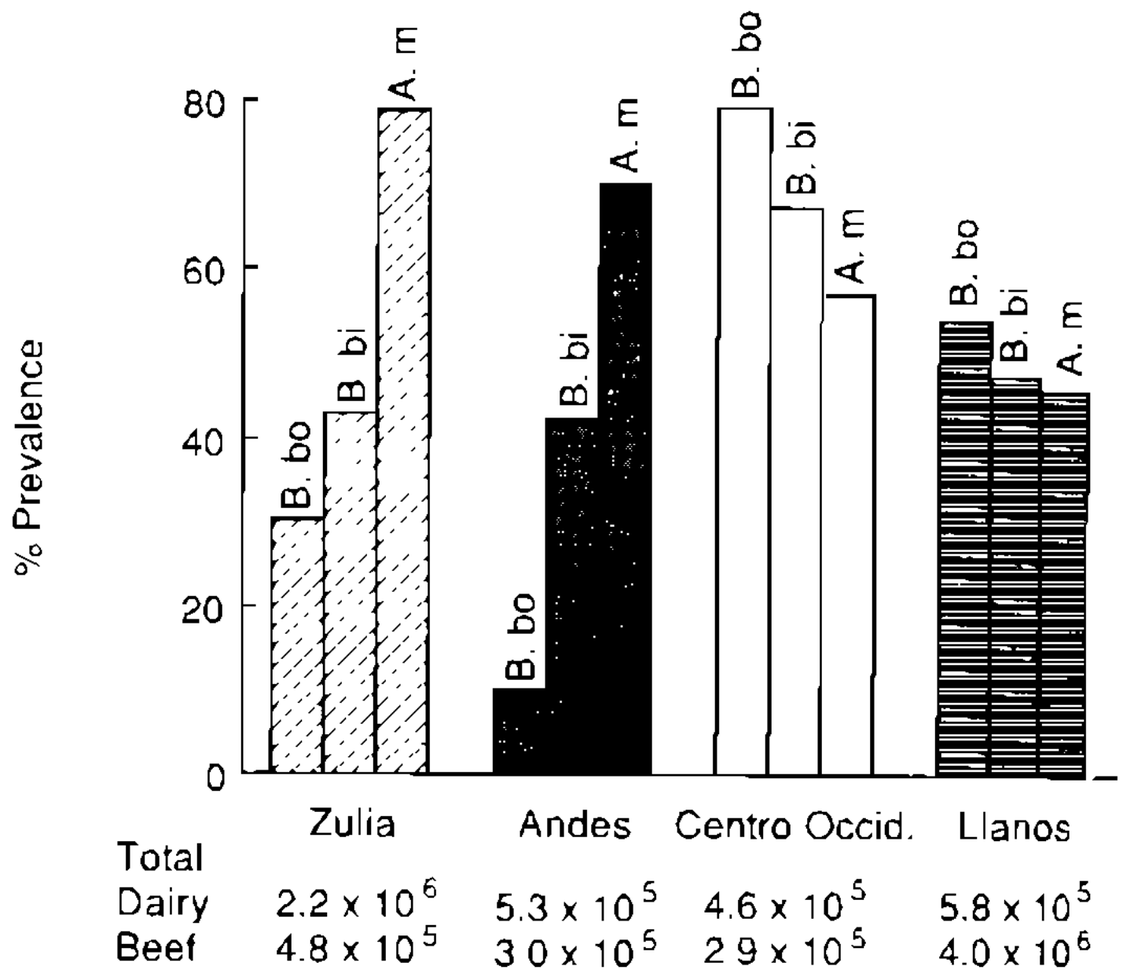

Fig. 1: seroprevalence (%) of hemoparasites in major cattle-producing regions of Venezuela.

TABLE I

Characteristics of ranches selected to evaluate efficacy of an inactivated *Babesia* vaccine under field conditions

Trial	State	No. head	Breed	Age (Mo).	Management	% Seroprevalence *B. bov.*	% Seroprevalence *B. big.*
1	Zulia	2,000	Holstein	24-36	C/SD	1.2	45.0
2	Guarico	1,500	Brahman	7-8	P/OD	0.0	0.0
3	Guarico	400	MxHols.	10-12	P/OD	5.0	13.3
4	Miranda	400	B. Swiss	8-12	S/SD	6.2	53.1
5	Carabobo	400	MxHols/BS	12-36	S/SD	0.0	2.2
6	Falcon	300	MxHols/BS	12-24	P/OD	62.9	83.1
7	Zulia	3,000	MxHols/BS	24-36	P/OD	45.2	60.7
8	Portuguesa	400	MxHols/BS	8-18	S/SD	24.6	42.4
9	Zulia	1,400	Holstein	7-8	C/SD	21.8	34.5
10	Guarico	400	Brahm/BS	8-10	P/OD	20.0	40.0
11	Miranda	1,000	Holstein	24-36	C/SD	0.0	0.0
12	Guarico	800	Holstein	18-24	C/SD	9.6	0.0
13	Zulia	2,000	Holstein	24-36	C/SD	26.8	9.7
14	Zulia	400	MxCreole/BS	24-48	S/SD	10.0	21.6
15	Zulia	1,500	MxCreole	12-36	P/OD	26.8	26.8
16	Falcon	600	MxZebu	12-24	P/OD	12.5	29.1

C: confinement; P: pasture; S: semiconfinement; SD: systematic tick dipping; OD: occasional tick dipping; Mx: mixed.

TABLE II

Evaluation of field vaccination trials (1988-1990). Group 1. Dairy farms, imported cattle from USA and Canada, unstable epidemiologic status (7,200 hd)

		Seroconversion[a]					Vaccine efficacy		
		Post-vacc.		Post-exp.					
Trial	Group	*B. bov.*	*B. big.*	*B. bov.*	*B. big.*	Morb. rate	% Red. morb.	Mort. rate	% Red. mort.
1	Vacc.	485	640	1810	2152	20	50.0	0	100
	Nonvacc.	(–)	(–)	335	597	40		10	
9	Vacc.	80	160	54	444	5	75.0	0	NC
	Nonvacc.	(–)	(–)	28	171	20		0	
11	Vacc.	160	320	223	20	NA	NA	0	NC
	Nonvacc.	(–)	(–)	89	20	NA		0	
12	Vacc.	80	261	244	80	NA		0	100
	Nonvacc.	(–)	(–)	50	50	NA		10	
13	Vacc.	80	360	640	1167	NA	NA	0	100
	Nonvacc.	(–)	(–)	226	309	NA		10	

a: reciprocal IFA Titer; NA: not available; NC: not calculated (no morbidity or mortality records from vaccinated and nonvaccinated control cattle); exp.: tick exposure.

TABLE III

Evaluation of field vaccination trials (1988-1990).
Group 2. Dairy farms, crossbred cattle, stable and unstable epidemiologic status (1,600 hd)

		Seroconversion[a]					Vaccine efficacy		
		Post-vacc.		Post-exp.					
Trial	Group	*B. bov.*	*B. big.*	*B. bov.*	*B big.*	Morb. rate	% Red. morb.	Mort. rate	% Red. mort.
4	Vacc.	144	110	640	320	NA	NA	0	100
	Nonvacc.	(–)	(–)	160	80	NA		40	
5	Vacc.	168	76	95	80	0	NC	0	NC
	Nonvacc.	(–)	(–)	40	80	0		0	
8	Vacc.	82	70	1576	640	5	83.3	0	NC
	Nonvacc.	40	40	686	437	30		0	
14	Vacc.	160	160	453	735	0	NC	0	NC
	Nonvacc.	40	80	394	453	0		0	

a: reciprocal IFA Titer; NA: not available; NC: not calculated (no morbidity or mortality records from vaccinated and nonvaccinated control cattle); exp.: tick exposure.

TABLE IV

Evaluation of field vaccination trials (1988-1990). Group 3. Dual purpose (DP) or beef (B) farms, zebu and crossbred cattle, stable and unstable epidemiologic status (7,700 hd)

		Seroconversion[a]				Vaccine efficacy				
		Post-vacc.		Post-exp.						
Trial	Group	*B. bov.*	*B. big.*	*B. bov.*	B. *big.*	Morb. rate	% Red. morb.	Mort. rate	% Red. mort.	Wt gain (kg)
2 (B)	Vacc.	87	167	320	640	6	66.6	0	0	12.2
	Nonvacc.	(–)	(–)	67	80	18		0		4.5
3 (DP)	Vacc.	320	640	532	2265	11	68.6	0	100	13.5
	Nonvacc.	(–)	(–)	277	320	35		25		8.5
6 (DP)	Vacc.	160	320	194	533	5	0.0	0	NC	4.3
	Nonvacc.	40	80	230	461	5		0		5.6
7 (DP)	Vacc.	40	80	50	123	NA	NA	0	NC	NA
	Nonvacc.	23	26	40	40	NA		0		NA
10 (B)	Vacc.	60	120	80	226	12	20.0	0	NC	5.2
	Nonvacc.	(–)	(–)	35	96	15		0		5.7
15 (DP)	Vacc.	80	320	254	453	5	37.5	0	NC	NA
	Nonvacc.	35	40	101	127	8		0		NA
16 (B)	Vacc.	80	160	160	263	7	65.0	0	NC	NA
	Nonvacc.	(–)	(–)	44	74	20		0		NA

a: reciprocal IFA Titer; NA: not available; NC: not calculated (no morbidity or mortality records from vaccinated and nonvaccinated control cattle); exp: tick exposure.

tible, purebred cattle are regularly imported from the U.S. and Canada, and second, native crossbred animals affect varying degrees of enzootic stability for *Babesia* infections.

Dairy cattle were mainly selected for field evaluation of the exoantigen-containing vaccine because most of the valuable, *Babesia*-susceptible animals are raised for milk production. Furthermore, diversity in management practices, geographical characteristics, and farm size offered the opportunity for analysis within a wide range of epidemiological conditions.

Assessment of clinical safety under field conditions was determined in a total of 7,390 animals of all ages and physiological conditions. No side effects were observed after vaccination in any of the cattle.

For field evaluation of vaccine efficacy, private and government-owned ranches were selected in areas where large-scale dairy and beef production is crucial for the livestock industry. The selection was based on the following criteria: (1) high risk cattle: herds maintained in enzootic unstability, or highly susceptible purebred and imported cattle; (2) knowledge of local seroprevalence and management (tick control measures); (3) geographic location and herd size; (4) ease of follow-up and sample collection of animals under study.

The principal characteristics of ranches se-

lected to evaluate efficacy of the inactivated *Babesia* vaccine under field conditions in Venezuela are presented in Table I. The ranches encompassed a total cattle population of 16,500 of which approximately 3,000 were vaccinated with the combined *B. bovis-B. bigemina* vaccine in a regime of 2 subcutaneous inoculations at a 4-week interval. To calculate vaccine efficacy, vaccinated and unvaccinated animals were followed prospectively to determine the morbidity and mortality rates in both groups. With the collaboration of local veterinarians, clinical, serologic, parasitologic and hematologic data were collected on a monthly basis from 10% of the animals. Data were also collected fom a similar number of unvaccinated control cattle. Most animals also received other vaccinations recommended for dairy cattle in Venezuela, e.g., immunizations against foot-and-mouth disease, brucellosis, and a bacterin containing a combination of *Clostridium* spp. and *Pasteurella* spp. In 75% of the trials, tick exposure occurred between 0 and 4 months after vaccination. The most frequent concurrent infection was *Anaplasma marginale* (69% of the ranches). Sporadic outbreaks due to rabies, brucellosis and trypanosomiasis (*Trypanosoma vivax*) were also recorded.

The method used to evaluate vaccine efficacy was adapted from that of Orenstein et al. (1985). According to the recommended method, vaccine efficacy was measured by calculating the incidence rates of disease (morbidity rates) and mortality among vaccinated and nonvaccinated cattle. Essentially, the percent reduction in the incidence rate of disease and mortality among vaccinated animals was compared to that of the unvaccinated group. The basic formula is:

$$VE = \frac{IRU\text{-}IRV}{IRU} \times 100$$

where VE = vaccine efficacy, IRU = incidence rate of disease in the unvaccinated population, and IRV = incidence rate of disease in the vaccinated population.

Babesiosis cases were defined on the basis of 40% (or greater) reduction in packed cell volume (PCV), clinical diagnoses by local veterinarians, and laboratory confirmation of cases. Parasitemias (thin blood films), PCV, antibody responses (indirect fluorescent antibody test) and body weights (when possible) were monitored throughout the follow-up period.

Data collected from 16 vaccination trials during a 2-year monitoring period following vaccination and natural tick exposure are presented in Tables II, III and IV. The capacity of the vaccine to induce an immune response under field conditions was demonstrated by the good degree of seroconversion observed among vaccinated cattle. Analysis of vaccine efficacy indicated a considerable reduction in the incidence of babesiosis, and more importantly, no deaths were recorded among vaccinated cattle. A lower percent reduction in disease was observed in ranches that presented characteristics of enzootic stability, suggesting that the lower prevalence to babesiosis was due to naturally-acquired immunity (trials No. 6, 7, 10). In Trial No. 3, vaccinated cattle were protected against death (up to 25% of nonvaccinated cattle succumbed to babesiosis) and showed a 68.0% reduction in disease (Table IV). In that particud deaht (up to 25% of nonvaccinated cattle succumbed to babesiosis) and showed a 68.0% reduction in disease (Table IV). In that particular trial, the vaccine was effective even under unfavorable conditions of concurrent outbreaks of anaplasmosis, rabies and trypanosomiasis that occurred during the follow-up period (Fig. 2).

The results obtained from field evaluation of the exoantigen-containing *Babesia* vaccine in Venezuela are encouraging and demonstrate the

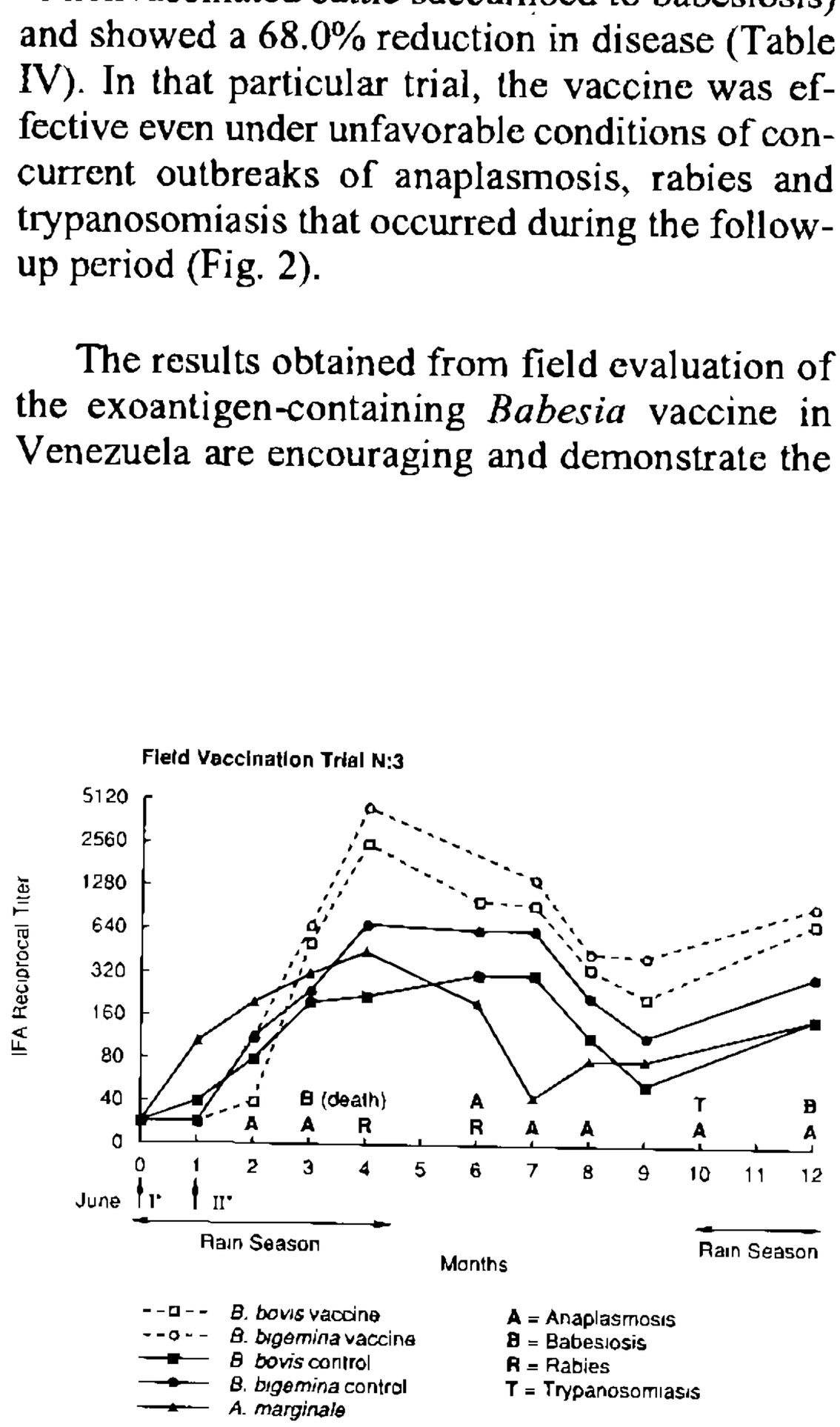

Fig. 2: serological responses of cattle in Field Vaccination Trial Nº 3 (12-month observation period).

considerable value of this type of vaccine for the immunoprophylactic control of bovine babesiosis.

The mode of action of such exoantigen-based vaccines has been proposed by Playfair et al. (1990). The basis of such a vaccine is to inhibit or minimize the pathological consequences of infection and to generate anti-disease immunity, probably by reduction of cytokine production. The effectiveness of a *B. canis* exoantigen vaccine (Pirodog®) has been reported to confer between 70-100% protection (Moreau et al., 1988). More recently, further promising results with a similar *B. divergens* immunogen have been published (Gorenflot et al., 1990; Precigout et al., 1991). In conclusion, an effective means of immunizing against bovine babesiosis has become available. Although the prospects of developing genetically-engineered vaccines are good, it will be several years before recombinant vaccines are ready for use. Meanwhile, the exoantigen-containing *Babesia* vaccine is a feasible and immediate alternative.

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