Experimental and Applied Acarology **34:** 375–382, 2004.

Integrated control of *Boophilus microplus* ticks in Cuba based on vaccination with the anti-tick vaccine Gavac ™

MANUEL RODRIGUEZ VALLE[1,*], LUIS MÈNDEZ[2], MARIO VALDEZ[2], MIGUEL REDONDO[1], CARLOS MONTERO ESPINOSA[1], MILAGRO VARGAS[1], RICARDO LLEONART CRUZ[1], HUMBERTO PEREZ BARRIOS[2], GUILLERMO SEOANE[2], EMERIO SERRANO RAMIREZ[2], OSCAR BOUE[1], JORGE LODOS VIGIL[1], HÉCTOR MACHADO[1], CARLOS BORROTO NORDELO[1] and MARISDANIA JOGLAR PIÑEIRO[1]

*[1]Animal Biotechnology Division, Centro de Ingeniería Genéticay Biotecnología, Ave. 31; 159 & 190, Cubanacan, Playa, P.O. box 6162, Havana, Cuba; [2]Instituto Nacional de Medicina Veterinaria, Havana, Cuba; *Author for correspondence (e-mail: mrodriguez@cigb.edu.cu; manuel_rodriguezvalle@yahoo.es)*

Received 22 January 2004; accepted in revised form 10 July 2004

Key words: *Boophilus microplus* ticks, Cuba, Gavac™, Vaccine

Abstract. *Boophilus microplus* has developed resistance against a range of chemical acaricides which has stimulated the development of alternative methods such as vaccination against ticks. In Cuba, the Bm86-based recombinant vaccine Gavac™ has been successfully used in a number of controlled laboratory and field trials in cattle against *B. microplus*. In this paper, we have evaluated Gavac™ in a large scale field trial wherein 588,573 dairy cattle were vaccinated with the aim to reduce the number of acaricidal treatments. It was found that the number of acaricidal treatments could be reduced by 87% over a period of 8 years (1995–2003). Prior to the introduction of the vaccine, 54 clinical cases of babesiosis and six fatal cases were reported per 1000 animals. Six years later, the incidence of babesiosis was reduced to 1.9 cases per 1000 cattle and mortality reduced to 0.18 per 1000. The national consumption of acaricides in Cuba could be reduced by 82% after the implementation of the integrated anti-*B. microplus* control program.

Introduction

Tick infestation due to *Boophilus microplus* constitutes a major problem for cattle in tropical and subtropical regions, affecting productivity and includes the risk to contract babesiosis and anaplasmosis. The conventional control method is the use of chemical acaricides that has only partially been successful, due to the development of resistance and chemical residues in milk and meat. These drawbacks have lead to alternative approaches to tick control, such as the use of host resistance and vaccine development to induce an immunological response against tick infestation (Johnston et al. 1986; Willadsen and Jongejan 1999; Mulenga et al. 2000).

A protective concealed antigen Bm86 has been identified from the gut of semi-engorged adult female ticks (Willadsen and Kemp 1988; Willadsen et al. 1989) and produced by recombinant DNA technology (Rand et al. 1989; Turnbull et al. 1990; Rodriguez et al. 1994). Two vaccines, Gavac^{TM} and TickGARD^{TM}, containing the recombinant Bm86 antigen have been registered. Gavac^{TM} was released in 1993 in Cuba and subsequently in several Latin American countries (Rodriguez et al. 1995a, b), whereas TickGARD^{TM} was released in 1994 on the Australian market (Willadsen 1995). Several field trials conducted in Cuba, Colombia and Mexico were successful in controlling tick populations using Gavac^{TM} against *Boophilus* ticks (Rodríguez et al. 1995a, b; de la Fuente et al. 1998; Fragoso et al. 1998; Redondo et al. 1999; de la Fuente et al. 2000).

Babesia bovis is the main cause of bovine babesiosis in Cuba and is transmitted by *B. microplus*. Although live attenuated *Babesia* strains can be used for vaccination (Callow 1977), this method has not been implemented in Cuba. As a result, the main method to control babesiosis has been the use of chemical acaricides. However, in an integrated control strategy, recombinant vaccines against ticks can be combined with chemical tick control to minimize the number of chemical treatments.

In this paper, we present the results obtained with the long-term application of an integrated strategy to control *B. microplus* ticks on cattle in Cuba and the reduction in the incidence of bovine babesiosis.

Materials and methods

Experimental design

A total of 588,573 Friesian-s-Holstein cattle distributed over a large number of dairy farms located in 14 provinces of Cuba were included in the integrated control program using Gavac^{TM}. More than 3 million head of cattle, mainly crossbred *Bos indicus* cattle, were not vaccinated. The predominantly Friesian cattle were chosen for their relatively high susceptibility to tick infestation and tick-borne pathogens. All dairy farms were maintained under standard production conditions, whereby cattle were grazing on pasture and provided with supplementary feeding. Morbidity and mortality due to babesiosis was recorded during 5 years (1989–1994) prior to the onset of the vaccination program and continued for 9 years (1995–2003) when Gavac^{TM} was administered. Clinical diagnosis of *B. bovis* was performed as described in the FAO Practical Field Manual for Tick and Tick Borne disease control (FAO 1984).

Acaricides

Organophosphorate (Esteladon, CIBA–Geigy, Switzerland), Amidine (Bobitraz, Bayer, Germany) or Pyrethroid (Bayticol, Bayer, Germany) were used to treat the animals. The animals were treated if the infestation level was higher than 10 adult ticks per animal. The number of acaricidal treatments was recorded at each farm by veterinary assistants. The average period between chemical acaricidal treatments was calculated before and after the application of the integrated control program with Gavac™.

Vaccination

Vaccination against ticks was performed using Gavac™ (Heber Biotech S.A., Cuba). Animals older then 1 month of age were vaccinated according to the instructions from the producer. The vaccination protocol consisted of three injections: one in the first week, another 4 weeks later and the third one during week seven. Animals received a booster inoculation every 6 months (Rodríguez, et al. 1994, 1995a, b).

Ticks

Tick infestation rates were determined by counting adult female *Boophilus* ticks every 7 days on 20% of the animals randomly selected on each farm where the integrated control program was implemented (Rodriguez et al. 1995a; Redondo et al. 1999).

Serology

Sera from 20% of vaccinated cattle in randomized farms were collected before each immunization. Sera were stored at −20 °C until tested. The level of antibodies against Bm86 antigen was determined by ELISA as described by Triguero et al. (1999).

Statistical analysis

The frequency of acaricidal treatment before and after the introduction of the integrated control with Gavac™ against *B. microplus* was compared using Student's *t*-test. The mortality and morbidity caused by *B. bovis* recorded before and after the introduction of the vaccine was determined by Wilcoxon signed-ranks test.

Results and discussion

The application of integrated control with Gavac^{TM} in different farms distributed throughout the country has significantly increased the interval between acaricide treatments by an average of 171 ± 75 days (Table 1). The number of acaricide treatments was reduced by 87% after introduction of the integrated control with Gavac^{TM} in this period (Table 1).

In farms with more resistant *Bos indicus* type cattle, control of *B. microplus* without Gavac^{TM} was used (applying treatments only when tick counts were higher than 10 fully engorged females per animal). This strategy also resulted in an increase in interval between acaricidal treatments by an average of 71 days and therefore, acaricidal usage in these farms was decreased by 68%. However, the frequency of acaricidal treatments in farms with the integrated control was significantly lower as compared with farms without Gavac^{TM} ($p < 0.001$). In Table 1 examples are given where the increase in the interval between treatments were dramatic (Sancti Spiritus, 362 days; Camaguey, 266 days; just to mention a few). The effect of Gavac^{TM} on the reproduction capacity of *B. microplus* had been demonstrated by some authors (Rand et al. 1989; Rodriguez et al. 1994; Redondo et al. 1999; Willadsen and Jongejan 1999). This effect of Gavac^{TM} can significantly reduce the *B. microplus* populations on the pastures. Therefore, in Gavac^{TM} integrated control areas the interval of the

Table 1. Average period between chemical acaricide treatments (days) with or without the use of Gavac.

Locations	Integrated control + Gavac^{TM}	Acaricide treatments reduction (%)	Acaricides only**	Acaricide treatments reduction (%)	Total no. of vaccinated cattle
Pinar del Río	203	91	86	79	20,000
C. Habana	130	86	–	–	15,000
La Habana	70	74	–	–	108,665
Matanzas	204	91	73	75	26,000
Cienfuegos	112	84	95	81	24,000
Villa Clara	130	86	40	54	10,000
Sancti piritus	362	95	122	85	6000
Ciego de Avila	106	83	91	80	8000
Camagüey	266	93	52	65	222,818
Las Tunas	248	93	65	72	62,000
Holguín	160	89	70	74	25,500
Granma	144	87	91	80	21,000
Santiago de Cuba	163	89	87	79	25,891
Guantánamo	120	85	25	27	11,073
Isla de la Juventud	145	87	30	39	2626
Mean ± SD	$171 \pm 75^{a*}$	87	71 ± 28^{b}	68	
Total					588,573

*A different letter indicates a statistical significance ($p < 0.001$).

**More than 3 million head of cattle, mainly crossbred *Bos indicus* cattle, were not vaccinated.

chemical acaricide treatments was increased significantly with respect to areas without the vaccine (Table 1).

Before the implantation of the integrated program, tick control was aimed at total eradication of *B. microplus*. Therefore, the average period between chemical acaricide treatments was 18 days. The introduction of integrated control in 1995 with the criteria of treating cattle when the infestation level was

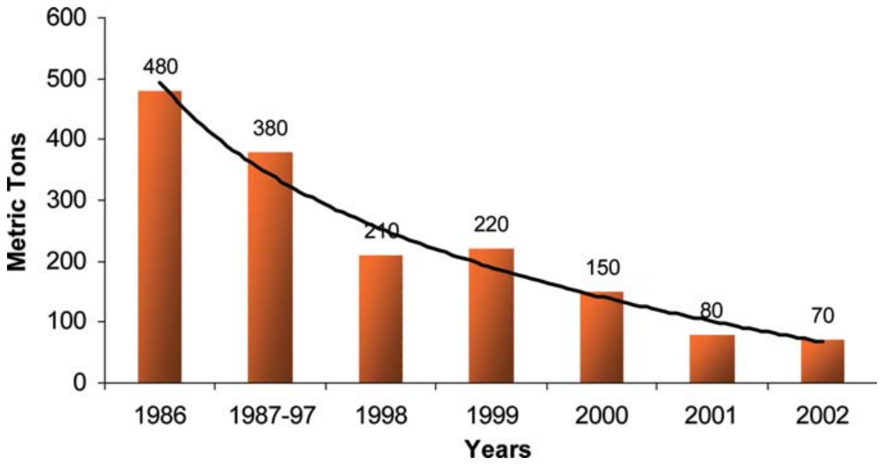

Figure 1. The reduction of the national acaricide consumption in metric tons before 1986 and after implementation of the integrated control program in 1995 against *B. microplus*.

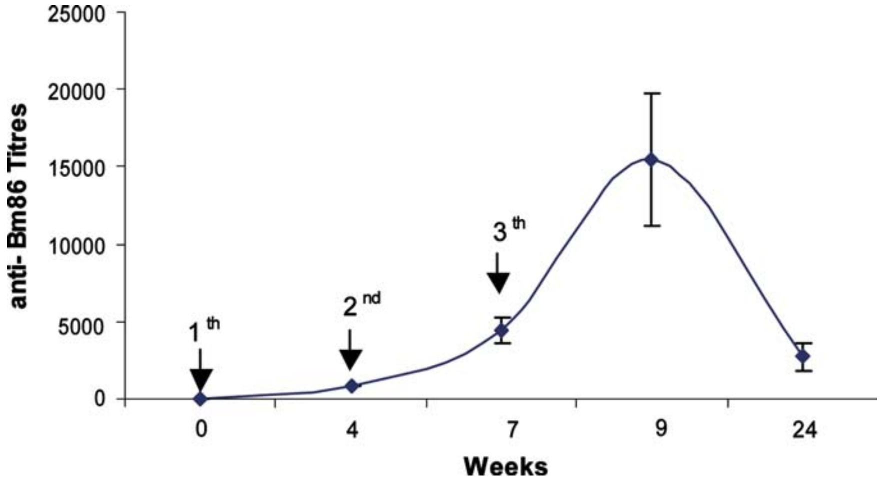

Figure 2. Arithmetic average of serum anti-Bm86 titers (mean ± SE) of vaccinated bovine from eight dairy farms in Habana province. Serum anti-Bm86 titers were determined in the animals employing an ELISA test (Triguero et al. 1999). The time of the three vaccination shuts are indicated by arrows (↓).

higher than 10 adult ticks per animal allowed the reduction of the number of chemical acaricide treatments per year.

The national consumption of chemical acaricide was reduced gradually after the introduction of the integrated program from 380 metric tons (1987–1997) to less than 70 metric tons (2002, Figure 1).

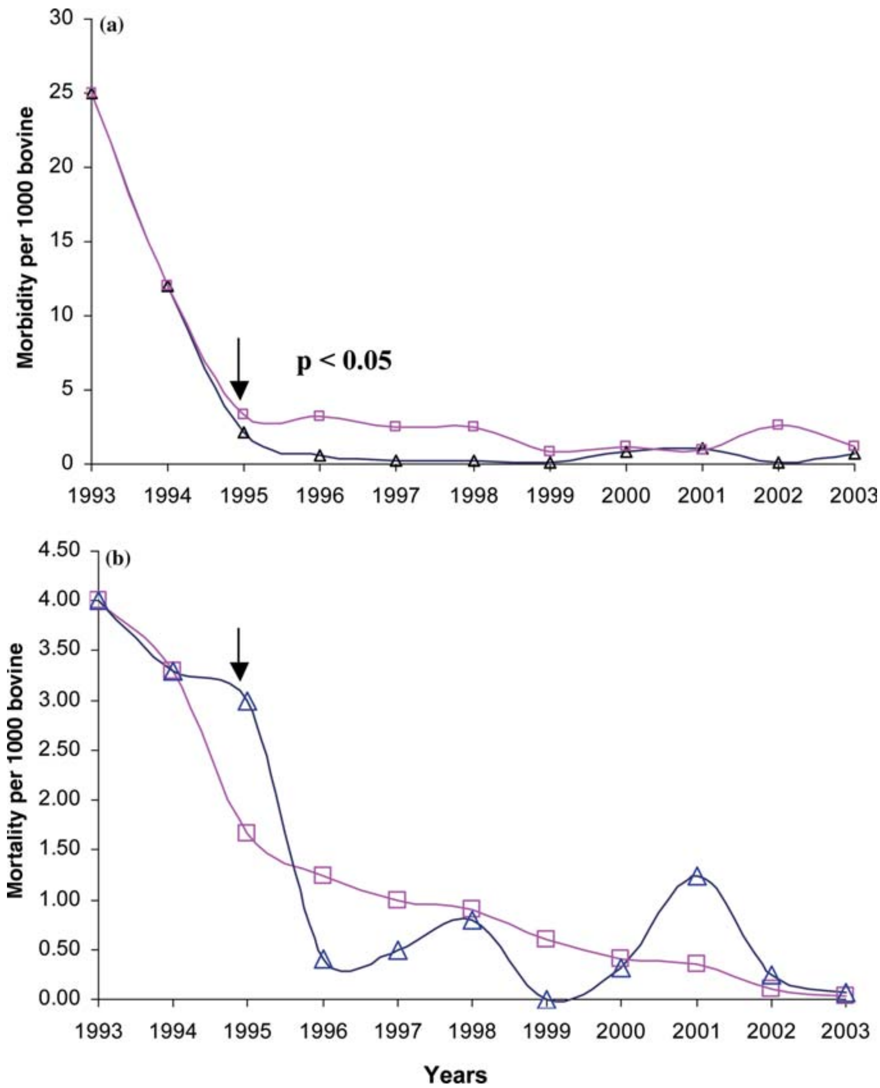

Figure 3. Morbidity (a) and mortality (b) due to babesiosis before and after the integrated control program for *B. microplus* was implemented. Triangles represent area with Gavac and the square represent area with chemical acaricide treatment only. Arrow indicated the initiation of the integrated control program.

ELISA was used to confirm that cattle had responded to the vaccine (Triguero et al. 1999). In all cases a typical antibody response against Bm86 antigen was found as reported elsewhere (Rodriguez et al. 1995; de la Fuente et al. 1998; Redondo et al. 1999). For example, antibody titers of cattle in Holguin province after the third vaccination were 6747 ± 259. Although, antibody titers were variable, all animals tested responded to the vaccine. The antibody titers of vaccinated bovine showed an antibody kinetic against Bm86 like the anti-Bm86 kinetics reported by others (Rodriguez et al. 1994, 1995a, b; Redondo et al. 1999) of Gavac™ vaccination (Figure 2).

The morbidity caused by *B. bovis* was significantly reduced after the introduction of the integrated program (Figure 3). In areas with Gavac™ the morbidity was significantly reduced ($p < 0.05$) as compared with the regions with acaricide only (Figure 3). Apparently, the integrated control program correlated with a reduction in the number of cases due to babesiosis. A possible explanation is that before vaccination was introduced there was a strict acaricide application protocol. By doing so, an endemically unstable situation was created because too few ticks were allowed to ensure transmission of babesia. An increase in acaricide interval most likely led to a larger number of ticks surviving to ensure transmission of the parasite.

In conclusion, the effect of Gavac™ on the control of ticks in successive generations and a reduction in *Babesia* infection after field use of Gavac™ as reported elsewhere (Rodriguez et al. 1995; de la Fuente et al. 1998) is confirmed in the present study. The integrated approach of acaricides and Gavac™ contributed to improved animal health, reduction of costs and diminished the potential negative impact of acaricides on the environment.

Acknowledgements

The authors would like to acknowledge the contributions of researchers and technicians from the tick research groups of the Center for Genetic Engineering and Biotechnology, from the National Parasitology Station, the Institute for Veterinary Medicine, as well as the Provincial Veterinary and Animal Production Administrations in Cuba.

References

Callow L.L. 1977. Vaccination against bovine babesiosis. Adv. Exp. Med. Biol. 93: 121–149.

de la Fuente J., Rodríguez M., Redondo M., Montero C., García-García J.C., Méndez L., Serrano E., Valdés M., Enrique A., Canales M., Ramos E., Boué O., Machado H., Lleonart R., de Armas C.A., Rey S., Rodríguez J.L., Artiles M. and García L. 1998. Field studies and cost-effectiveness analysis of vaccination with Gavac™ against the cattle tick *B. microplus*. Vaccine 16(4): 365–374.

de la Fuente J., Rodríguez M. and Garcia-Garcia J.C. 2000. Immunological control of ticks through vaccination with *Boophilus microplus* gut antigens. Ann. NY Acad. Sci. 916: 617–621.

FAO 1984. Ticks and Tick – Borne Disease Control. A Practical Field Manual. Rome, Vol. I and II.

Fragoso H., Hoshman Rad P., Ortiz M., Rodriguez M., Redondo M., Herrera L. and de la Fuente J. 1998. Protection against *B. annulatus* infestations in cattle vaccinated with the *B microplus* vaccine Gavac. Vaccine 16(20): 1990–1992.

Johnston L.A.Y., Kemp D.H. and Person R.D. 1986. Immunization of cattle against *B. microplus* using extract derived from adult female ticks: effects of induced immunity on tick populations. Int. J. Parasitol. 16: 27–34.

Mulenga A., Sugimoto C. and Onuma M. 2000. Issues in tick vaccine development: identification and characterization of potential antigens. Microb. Infect. 2: 1353–1361.

Rand K.N., Moore T., Sriskantha A., Spring K., Tellam R., Willadsen P. and Cobon G. 1989. Cloning and expression of a protective antigen from the cattle tick *Boophilus microplus*. Proc. Natl. Acad. Sci. 86: 9657–9661.

Redondo M., Fragoso H., Ortiz M., Montero C., Lona J., Medellín A. J., Fría R., Hernández V., Franco R., Machado H., Rodríguez M. and de la Fuente J. 1999. Integrated control of acaricide-resistant *B. microplus* populations on grazing cattle in Mexico using vaccination with Gavac^{TM} and amidine treatments. Exp. Appl. Acarol. 23: 841–849.

Rodríguez M., Massard C.L., da Fonseca A.H., Ramos N.F., Machado H., Labarta V. and de la Fuente J. 1995b. Effect of vaccination with a recombinant Bm86 antigen preparation on natural infestation of *B. microplus* in grazing dairy and beef puree and cross-bred cattle in Brazil. Vaccine 13(18): 1804–1808.

Rodríguez M., Montero C., Labarta V. and de la Fuente J. 1995. Effect of vaccination with Gavac^{TM} on the incidence of *Babesia bovis* infestations and the reduction in the number and frequency of acaricide treatments in cattle under production conditions in Cuba. In: J. de la Fuente (ed.), Recombinant Vaccine for the Control of Cattle Tick, Elfos Scientiae, Havana, Cuba, pp. 187–194.

Rodríguez M., Penichet M.L., Mouris A.E., Labarta V., Lorenzo Luaces L., Rubiera R., Cordobés C., Sánchez P.A., Ramos E., Sotom A., Canales M., Palenzuela D., Triguero A., Lleonart R., Herrera L. and de la Fuente J. 1995a. Control of *B. microplus* populations in grazing cattle vaccinated with a recombinant Bm86 antigen preparation. Vet. Parasitol. 57: 339–349.

Rodríguez M., Rubiera R. and Penichet M. et al. 1994. High level expression of the *B. microplus* Bm86 antigen in the yeast *P. pastoris* forming highly immunogenic particles for cattle. J. Biotechnol. 33: 135–146.

Triguero A., Blanco R., Machado H., Rodríguez M. and de la Fuente J. 1999. Development of enzyme linked immunosorbent assays to measure Bm86 antigen of *B. microplus* (cattle tick) and to detect anti-Bm86 antibodies in serum samples. Biotechnol. Techn. 13: 119–125.

Turnbull I.F., Smith D.R.J., Sharp P.J., Cobon G.S. and Hynes M.J. 1990. Expression and secretion in *Aspergillus nidulans* and *Aspergillus niger* of a cell surface glycoprotein from the cattle tick, *Boophilus microplus*, by using the fungal *amds* promoter system. Appl. Environ. Microbiol. 56: 2847–2852.

Willadsen P., Bird P., Cobon G.S. and Hungerford J. 1995. Commercialization of a recombinant vaccine against *Boophilus microplus*. Parasitology 110: S43–50.

Willadsen P. and Jongejan F. 1999. Immunology of the Tick–Host interaction and the control of ticks and tick borne diseases. Parasitol. Today 15(7): 258–62.

Willadsen P. and Kemp D.H. 1988. Vaccination with concealed antigens for tick control. Parasitol. Today 4: 196–198.

Willadsen P., Riding G.A. and Mc Kenna R.V. et al. 1989. Immunological control of a parasitic arthropod: identification of a protective antigen against *Boophilus microplus*. J. Immunol. 143: 1346.