COMPARISON OF CATTLE RESPONSES TO MONO-SPECIFIC OR COMBINED INOCULATIONS WITH *BABESIA BIGEMINA* AND *BABESIA BOVIS* VACCINE STRAINS

VISERAS J.*, LUGARESI C.I.***, VOLPOGNI M.M.** & GUGLIELMONE A.A.**

Summary :

Combined inoculation of cattle with vaccine strains of *Babesia bigemina* and *Babesia bovis* induced lower antibody titers to *B. bigemina* than to *B. bovis* (previous study). Three groups of heifers were used to detect if the low antibody level was due to competition between *Babesia* species: individuals of G1 and G2 were inoculated with 10 million *B. bigemina* and *B. bovis*, respectively, and those of G3 with 10 million of each parasite. The prepatent periods, maximum parasitaemias and antibody titers (indirect immunofluorescent antibody test) were evaluated. The mean prepatent periods (days) for *B. bigemina* was of 5.6 (G1) and 5.2 (G3) and 7.0 (G2) and 6.7 (G3) for *B. bovis* ($P > 0.05$, "t" test). No differences were found in the parasitaemias. The only difference was found in the antibody titers to *B. bovis*, that were lower ($P < 0.05$ "t" test) from week 7 onwards when *B. bovis* was used in combination. The biological significance of this difference is unclear.

KEY WORDS : *Babesia bovis*, *Babesia bigemina*, vaccinal strains, cattle immune response.

Résumé : COMPARAISON DE LA RÉPONSE DES BOVINS À L'INOCULATION MONO-SPÉCIFIQUE OU CONJOINTE DES SOUCHES VACCINALES DE *BABESIA BIGEMINA* ET DE *BABESIA BOVIS*

L'inoculation conjointe de souches vaccinales de *Babesia bigemina* et de *Babesia bovis* chez les bovins produit des titres d'anticorps plus bas pour *B. bigemina* que pour *B. bovis* (étude préalable). Pour déceler si ce bas niveau d'anticorps est dû à une compétition entre les espèces de *Babesia*, trois groupes (G) de génisses ont été utilisés : les individus des groupes G1 et G2 ont été inoculés avec dix millions de *B. bigemina* et de *B. bovis*, respectivement, tandis que ceux du groupe G3 ont reçu dix millions de chaque espèce de *Babesia*. Les périodes pré-patentes ainsi que les parasitémies maximales ont été déterminées ; les titres d'anticorps ont été évalués par immunofluorescence indirecte.

Les moyennes des périodes pré-patentes (jours) ont été de 5,6 (G1) et de 5,2 (G3) pour *B. bigemina*, tandis que pour *B. bovis* elles ont été de 7,0 (G2) et de 6,7 (G3) ($P > 0,05$, « t » test). On n'a pas observé de différences dans les parasitémies. L'unique différence a été trouvée dans les titres d'anticorps pour *B. bovis*, lesquels ont été plus bas dans le groupe G3 que dans le groupe G2 ($P < 0,05$, « t » test) à partir de la 7^e semaine. Il est difficile de spéculer sur la signification biologique de cette observation.

MOTS CLÉS : *Babesia bovis*, *Babesia bigemina*, souches vaccinales, réponse immune des bovins.

Babesia bovis and *B. bigemina* are tick-borne hemoprotozoa which are widespread throughout the world causing economic losses to the cattle industry. Both parasites are mainly transmitted by *Boophilus* ticks and mortality rates higher than 50% can occur in susceptible cattle, mainly due to *B. bovis* (Wright, 1991); live vaccines remain the principal method to prevent cattle babesiosis (Callow *et al.*, 1997). A previous study with Argentinian vaccine strains showed that cattle inoculated simultaneously with both *Babesia* species had *B. bigemina* antibody titers lagging behind titers to *B. bovis*, being uncertain

if this was a consequence of a normal characteristic of the strains or the result of competition between the two parasites (Guglielmone *et al.*, 1997). To obtain more information on this subject, we observed the responses of cattle inoculated with *B. bigemina* and *B. bovis* alone or combined. The results are presented in this article.

MATERIALS AND METHODS

Thirty Holstein (12-14 months-old) heifers born and raised in the area of Argentina free of *B. microplus* ticks and negative in the immunofluorescent antibody (IFA) test to *B. bovis* and *B. bigemina* were allocated into three groups (G) of 10 heifers each according to their body weights: G1) heifer weight average of 263.6 ± 39.0 kg, inoculation (subcutaneous) of 10 million *B. bigemina* attenuated strain S1A per heifer; G2) heifer weight average of 262.6 ± 33.6 kg, inoculation of 10 million *B. bovis* atte-

* Departamento de Parasitología, Universidad de Granada, Campus Universitario de Cartuja, 18071 Granada, Spain.

** Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, CC 22, CP 2300 Rafaela (Santa Fe), Argentina.

Correspondence: AA Guglielmone.

Tel: 54 492 41121-5 – Fax: 54 492 40114-24147.

E-mail: aguglielmone@inta.gov.ar

nuated strain R1A per heifer; G3) heifer weight average of 263.8 ± 32.1 kg, simultaneous inoculation of 10 million *B. bigemina* S1A and 10 million *B. bovis* R1A. The protozoan were derived from the commercial batch of chilled vaccine No 50 (6000 doses) produced on February 18, 1997 in INTA Rafaela (Santa Fe, Argentina). *B. bovis* R1A strain was attenuated after rapid passages in splenectomized calves as described by Callow *et al.* (1979), while *B. bigemina* S1A was attenuated after two passages with six months-interval in splenectomized calves (Aguirre *et al.*, 1989).

Tail tip thick and thin blood smears were taken daily between 08 am and 12 am from all heifers on days 4 to 15 after vaccination. The smears were stained with Giemsa, inspected using light microscopy under oil immersion and the parasitaemia of *B. bovis* and *B. bigemina* expressed using the score provided by Callow & Pepper (1974). Serum samples from all heifers were obtained once a week for nine weeks after inoculation and analysed using an IFA test with antigens derived from *in vitro* culture of both *Babesia* species and locally made fluorescein-labelled rabbit anti-bovine IgG, as described previously (Guglielmone *et al.*, 1997). The prepatent period (period from inoculation to first detection of parasites in blood smears), maximum parasitaemia and weekly mean log 10 antibody titer were used to compare the reactions of heifers to monospecific or combined inoculations of *B. bovis* and *B. bigemina*. All results were analysed by using the "t" test. Negative IFA results were excluded from the analysis (Thrusfield, 1996).

RESULTS

No statistically significant differences were found in the mean prepatent period and mean maximum parasitaemia after mono-specific or combined inoculations of *B. bigemina* (G1 versus G3)

Group of heifers	Prepatent period		Maximum parasitaemia	
	Mean	sd	Mean	sd
			<i>B. bigemina</i> infections	
Mono-specific (G1)	5.6	1.84	3.2	1.55
Combined (G3)	5.2	0.93	3.2	2.78
			<i>B. bovis</i> infections	
Mono-specific (G2)	7.0	0.81	1.6	0.52
Combined (G3)	6.7	0.95	1.9	0.32

p > 0.05 in all cases ("t" test)

Table I. – Mean and standard deviation (sd) of prepatent periods (days) and maximum parasitaemia (score) in groups of 10 heifers each, inoculated with vaccinal strains of *Babesia bigemina*, *Babesia bovis* or a combination of both.

Weeks after inoculation	<i>B. bigemina</i> infections		<i>B. bovis</i> infections	
	Mono-specific (G1)	Combined (G3)	Mono-specific (G2)	Combined (G3)
2	2.71±0.28 ^a	2.53±0.28 ^a	2.74±0.24 ^a	2.56±0.35 ^a
3	3.04±0.42 ^a	2.91±0.42 ^a	3.01±0.17 ^a	3.16±0.32 ^a
4	3.15±0.16 ^a	3.04±0.28 ^a	3.52±0.19 ^a	3.40±0.29 ^a
5	3.16±0.42 ^a	3.22±0.24 ^a	3.40±0.16 ^a	3.22±0.24 ^a
6	3.13±0.45 ^a	3.22±0.24 ^a	3.43±0.29 ^a	3.43±0.33 ^a
7	3.18±0.15 ^a	3.10±0.16 ^a	3.46±0.16 ^a	3.25±0.22 ^b
8	2.89±0.35 ^a	2.92±0.19 ^a	3.40±0.16 ^a	3.22±0.13 ^b
9	2.77±0.20 ^a	2.62±0.13 ^a	3.28±0.00 ^{**}	2.92±0.32

* P obtained by using the "t" test; means followed with different letters are significantly different (P < 0.05).

** "t" test not applicable due to invariable titers in heifers with mono-specific *B. bovis* infection.

Table II. – Mean and standard deviation (sd) and statistical significance* of log 10 titers (IFA test with antigens derived from *in vitro* culture of both *Babesia* species) from week 2 to week 9 after inoculation of three groups of ten heifers each with *Babesia bigemina*, *Babesia bovis* vaccinal strains or a combination of both.

or *B. bovis* (G2 versus G3) (Table I). Sero-conversion started in week 2 after inoculation and all heifers were positive to the corresponding *Babesia* spp. in week 3. The mean log 10 antibody titers to *B. bovis* and *B. bigemina* from week 2 to week 9 after inoculation of each group of heifers are presented in Table II. No significant differences were found between *B. bigemina* titers from heifers of G1 and heifers of G3. For *B. bovis*, the antibody titers after mono-specific vaccination (G2) were statistically higher than titers from heifers of G3 from week 7 post-inoculation.

DISCUSSION

As expected, the prepatent periods and maximum parasitaemia of *B. bovis* R1A and *B. bigemina* S1A were similar to results of previous studies using refrigerated inocula of the same strains (Anziani *et al.*, 1993; Mangold *et al.*, 1996) and antibody titers to *B. bigemina* were consistently lower than titers to *B. bovis* in the heifers inoculated with both *Babesia* (Guglielmone *et al.*, 1997).

The only difference between the strains was the significantly lower titers against *B. bovis* from week 7 after inoculation onwards in heifers inoculated with both *Babesia* in comparison to heifers inoculated with *B. bovis* alone. This was unexpected since we speculated that *B. bigemina* may be affected by the simultaneous inoculation to cattle with *B. bovis*. Nevertheless, the maximum difference between mean log titers was 0.36 being difficult to infer about the biological significance of this difference, and surely it cannot be

considered as an evidence of competition between the strains.

Only one reference was found of a comparison of response of cattle following inoculation with individual or combined vaccine strains of *B. bovis* and *B. bigemina* (Bessenger & Schoeman, 1983). These authors also detected higher *B. bovis* antibody titers when this parasite was inoculated alone than when it was inoculated simultaneously with *B. bigemina*. These authors also found a marked difference in the *B. bovis* prepatent period (7.2 days when inoculated alone versus 17.5 days in cattle also inoculated with *B. bigemina*). The authors concluded that a concurrent *B. bigemina* infection had a suppressive effect on the development of *B. bovis*. The prepatent periods of Argentinian vaccine strains were not altered by monospecific or combined babesial infection, indicating that the outcome can be substantially different with strains from different origins.

ACKNOWLEDGEMENTS

We thank V. Vanzini, O. Warnke, A. Ibarreche and S. Arias for laboratory and field assistance, the INTA (Argentina) and INIA (Spain) Postdoctoral Research Fellowship (J. Viseras) financial support.

REFERENCES

- AGUIRRE D.H., MANGOLD A.J., GAIDO A.B., RÍOS L.G. DE & GUGLIELMONE A.A. Evaluación de la infectividad de una vacuna congelada en nitrógeno líquido elaborada en base a *Babesia bigemina* atenuada. *Revista de Medicina Veterinaria (Buenos Aires)*, 1989, 70, 142-145.
- ANZIANI O.S., GUGLIELMONE A.A., ABDALA A.A., AGUIRRE, D.H. & MANGOLD A.J. Protección conferida por *Babesia bovis* vacunal en novillos Holando Argentino. *Revista de Medicina Veterinaria (Buenos Aires)*, 1993, 74, 47-49.
- BESSENGER R. & SCHOEMAN J.H. Serological response of cattle to infection with *Babesia bigemina* and *Babesia bovis* in southern Africa. *Onderstepoort Journal of Veterinary Research*, 1983, 50, 115-117.
- CALLOW L.L. & PEPPER P.M. Measurement of and correlations between fever, changes in the packed cell volume and parasitaemia in the evaluation of the susceptibility of cattle to infection with *Babesia argentina*. *Australian Veterinary Journal*, 1974, 50, 1-5.
- CALLOW L.L., MELLORS R.T. & MC GREGOR W. Reduction in virulence of *Babesia bovis* due to rapid passage in splenectomized cattle. *International Journal of Parasitology*, 1979, 9, 333-338.
- CALLOW L.L., DALGLIESH R.J. & VOS A.J. DE. Development of effective living vaccines against bovine babesiosis. The longest field trial? *International Journal of Parasitology*, 1997, 27, 747-767.
- GUGLIELMONE A.A., LUGARESI C.I., VOLPOGNI M.M., ANZIANI O.S. & VANZINI V.R. Babesial antibody dynamics after cattle immunisation with live vaccines, measured with and indirect immunofluorescence test. *Veterinary Parasitology*, 1997, 70, 33-39.
- MANGOLD A.J., GUGLIELMONE A.A., ECHAIDE I.E., VOLPOGNI M.M., ECHAIDE S.T. DE & ANZIANI O.S. Efectos de diferentes períodos de refrigeración sobre la viabilidad de cepas vacunales de *Babesia bigemina* y *Anaplasma centrale*. *Revista de Investigaciones Agropecuarias*, 1996, 26, 123-126.
- THRUSFIELD M. *Veterinary Epidemiology*. Butterworth, London, 1986, 280 pp.
- WRIGHT I.G. Towards a synthetic *Babesia* vaccine. *International Journal of Parasitology*, 1991, 21, 156-159.

Reçu le 21 juillet 1998

Accepté le 17 décembre 1998