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Onset and duration of immunity against *Babesia canis* infection in dogs vaccinated with antigens from culture supernatants

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Abstract

It has previously been shown that dogs can be vaccinated against heterologous *Babesia canis* infection using a vaccine containing soluble parasite antigens (SPA) from in vitro cultures of *B. canis* and *B. rossi* that are adjuvanted with saponin. In the present study the onset and duration of immunity of vaccinated dogs were studied. Results showed that 3–26 weeks after initial vaccination, dogs effectively limit the level of SPA in plasma upon challenge infection, which was reflected in limited duration and extent of clinical manifestations. There was no statistically significant effect of vaccination on the parasite load in the circulation, which was determined from blood smears. It was further shown that the level of immunity of primary vaccinated dogs (priming and booster vaccination with a 6-week interval) and that of repeatedly vaccinated dogs (a single additional vaccination 6 months after primary vaccination) is comparable. From this study it is concluded that vaccination with this preparation induces protective immunity against clinical babesiosis from 3 weeks after booster vaccination onwards, and remains effective for a period of at least another 6 months. A single booster vaccination is sufficient to maintain immunity for at least another 6 months.

Keywords: *Babesia canis*; Vaccine; Dogs; Soluble parasite antigens; Immunity; Nobivac® Piro

1. Introduction

Dogs can be successfully immunised against the sequelae of a *Babesia canis* challenge infection using antigens from in vitro cultures of *Babesia* parasites (Schetters et al., 2001). Protection against challenge infection is achieved using a cocktail vaccine containing antigens from *B. canis* and *B. rossi* parasites. In France, canine babesiosis occurs seasonally; one peak in spring and another peak in autumn (Martinod and Gilot, 1991). Hence the minimal period of protective immunity after vaccination should be 4–5 months to provide protection during the subsequent tick season. In a vaccinated population two possible situations are

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envisaged: animals that are primary vaccinated (a priming and a booster vaccination with 3–6-week interval) and animals that have received a re-vaccination (a single dose 6 months after primary vaccination). The objective of this experiment was to determine whether such dogs resist a challenge infection with virulent *B. canis* parasites 6 months after the last vaccination. In addition, the effect of vaccination on parasite proliferation and levels of soluble parasite antigen (SPA) in plasma were measured after heterologous challenge infection to determine whether protective immunity was anti-parasite or anti-disease (Playfair et al., 1990).

2. Materials and methods

2.1. Animals

In total 30 Beagle dogs of either sex of approximately 6 months of age were used in the experiments. They were obtained from a commercial breeder (Harlan CPB, Zeist, The Netherlands). The animals were clinically healthy, and had no history of babesiosis. The animals were identified by a unique number that was applied as a tattoo in the ear. Animals were housed as pairs and acclimatised for 1 week. The animals received standard dog feed daily. Drinking water was supplied ad libitum.

2.2. Vaccination

A single dose of vaccine consisted of the soluble parasite antigens produced by 5×10^7 infected erythrocytes of each vaccine strain (*B. canis* and *B. rossi*, respectively; Schetters et al., 2001). The antigen was aliquoted into 3-ml vials and freeze-dried (one dose per vial). Immediately prior to use, one dose of freeze-dried antigen was reconstituted with 1-ml of freshly prepared, saponin solution (250 μg/ml Quil A in distilled water, Superfos-Biosector, Denmark). The vaccine was injected subcutaneously at the subscapular region. Six groups of five animals each were formed assuring an even distribution of age and litter. The animals were treated according to the schedule below (Table 1).

For the initial vaccination, each animal received two injections at a 3- or 6-week interval. For re-vaccination 6 months after primary vaccination, animals received a single dose of vaccine. Control animals did not receive any injections.

Table 1
Treatment schedule duration of immunity study

Group[a]	Priming	Booster	Challenge	Re-vaccination	Challenge
1	Day 0	Week 3	Week 6		
2	Control	Control	Week 6		
3	Day 0	Week 6	Week 26		
4	Control	Control	Week 26		
5	Day 0	Week 6		Week 32	Week 58
6	Control	Control		Control	Week 58

[a] Treatment schedule of groups of five animals. Control animals did not receive any injections.

2.3. Challenge

Strain B of *B. canis*, which is antigenically different from the vaccine strains (Schetters et al., 1995, 2001) was used for challenge infections. These parasite strains were described by Uilenberg et al. (1989) and obtained from Prof. Dr. F. Jongejan (University of Utrecht, The Netherlands). The parasites were stored as stabilates in liquid nitrogen, and were passed through a splenectomised dog to obtain challenge parasites (Schetters et al., 1992). Briefly, infected blood was taken by venapuncture from the *vena jugularis*, using heparin (5–10 U/ml, Leo) to prevent clotting. Blood was washed with *Babesia* medium. The amount of blood containing $(1–2) \times 10^6$ parasitised erythrocytes was injected intravenously in experimental animals.

2.4. Observations and clinical examinations

After challenge infection animals were examined daily for clinical signs of babesiosis. Special attention was given to behaviour, spleen size, size of lymph nodes, and colour of the mucous membranes of mouth and eye-lid, and the capillary refill time. Clinical observations were scored as a numeric value (Schetters et al., 1994). From these scores the total clinical score value was calculated for each day. Of these, the maximal clinical score value during the observation period was determined. As some animals were treated, an additional parameter, mean clinical score per day, was calculated (i.e. total clinical score