PRELIMINARY STUDY ON THE SAFETY OF A NEW VACCINE AGAINST CANINE BABESIOSIS CONTAINING SOLUBLE PARASITIC ANTIGEN (SPA)

ŁUKASZ ADASZEK, ROMANA WERNICKA-FURMAGA, AND STANISŁAW WINIARCZYK

Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences, 20-612 Lublin, Poland
ukaszek0@wp.pl

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Abstract

The aim of this study was to assess the safety of a new vaccine, containing soluble parasitic antigen (SPA), against canine babesiosis. Fifteen dogs were included in the experiment. Five controls received only the adjuvant and 10 dogs were vaccinated with Babesia canis canis SPA twice, at a two weeks interval. For the whole period of the study all animals were under constant clinical observation. Haematological and biochemical tests were performed. Flu-like symptoms and local reactions at the injection site were observed in three animals from the vaccinated group and in two dogs from the control group. These events were transient, receded spontaneously and did not require any appropriate treatment. In 50% of the vaccinated dogs, a slight and spontaneously receding thrombocytopenia developed. However, in none of the animals used in the experiment shock symptoms were observed. Administration of the SPA did not affect the functions of internal organs, which was confirmed by normal results of biochemical tests. The obtained Babesia canis SPA can be considered safe and well tolerated by dogs, and therefore it can be used in further studies on the immunisation of animals against babesiosis.

Key words: dogs, Babesia canis, babesiosis, soluble parasitic antigen, vaccination.

Canine babesiosis is a common and clinically significant tick-borne disease caused by hematozoan parasites of the genus Babesia (1). The classification of Babesia sp. places them in the order Piroplasmida within the phylum Apicomplexa. Two morphologically distinct forms of the erythrocytic stage in the canine host were recognised in early studies, which have led to naming the larger form B. canis, measuring approximately 3-5 μm, and the smaller (1-3 μm) B. gibsoni. On the basis of cross-immunity, serological testing, vector specificity, and molecular phylogeny, Babesia canis was reclassified into three sub-species: B. canis canis, B. canis rossi, and B. canis vogeli. All of them are now considered to be separate species (8, 22). So far, only Babesia canis canis has been found in dogs in Poland (1-3, 5, 23, 24). These parasites are also the most common aetiological factor of babesiosis in dogs in other parts of Europe (6, 7, 19).

Clinically, these pathogens cause remittent fever, progressive anaemia, haemoglobinuria, and marked splenomegaly and hepatomegaly in dogs and, in some cases, death of infected animals (3, 10, 25). Prevention of the disease is based on avoiding sites where ticks live and using effective prophylaxis against ectoparasites. Attempts to obtain the vaccine, which would effectively protect dogs against the development of babesiosis, have been ongoing for several years (15, 16). They resulted in the development of a new product Nobivac Piro available also on the Polish market (5).

In previous studies, a soluble parasitic antigen (SPA) from the native Polish strains of Babesia canis was obtained. It has been used to vaccinate dogs against babesiosis (4).

The purpose of this study was to assess the safety of the new vaccine containing the SPA obtained from the in vitro Babesia canis culture in dogs.

Material and Methods

Preparation of the SPA. The process for determining and breeding Babesia canis strains 18S RNA-A and 18S RNA-B was presented in an earlier study (4). Briefly, supernatant media from in vitro culture were collected and mixed when parasitemias reached the level of 0.6%. The obtained supernatant media mixture was centrifuged at 700 g for 30 min in order to eliminate the red globules and possible parasites as well as their remains. Then, the medium was subjected to sterilising filtration on a 0.22 μ membrane and concentrated by ultra-filtration on a membrane of 20 000 Daltons. The concentrate was treated with formol in a proportion of 0.12 mg/mL for a night at 4°C, then lyophilised and divided into doses.
administration to the dogs the lyophilised antigen was dissolved in a solution of saponin (adjuvant) at 0.5 mg/mL in pyrogenic sterile water (9).

**Animals.** The study included 15 dogs (seven males and eight females), aged 20-27 months, divided into two groups. Dogs from group 1 (n=5, no. 1-5) were used as negative control and received only adjuvant. Dogs from group 2 (n=10, no. 6-15) were vaccinated with SPA. All animals used in the experiment were kept for experimental purposes in the Clinic of Infectious Diseases at the Faculty of Veterinary Medicine in Lublin, and had no contact with the *Babesia* protozoans. Before the beginning of the study, all animals used in the experiment were tested by PCR and IF methods for the presence of piroplasm genetic material or *Babesia canis* antibodies. The tests gave negative results.

**Vaccination schedule.** The antigen in group 2 (1 ml) and adjuvant in control group were administered subcutaneously in the subscapular region. The vaccination and application of adjuvant comprised two injections, administered at two weeks interval (days 0 and 14 of the study).

**Clinical observations.** For the whole period of the study all animals were under constant clinical observation (21 d). In vaccinated and in control dogs, internal body temperature was measured daily. Haematological and biochemical tests were performed one day before the first injection, on days 0-7, and then on days 14-21 of the study.

Local reaction at the site of injection with SPA or adjuvant was monitored for 3 d after each injection. It was expressed as the diameter of affected surface area in millimetres.

### Results

One day after the first administration of the tested antigen in group 2, and the adjuvant in the control group, it was noted that dogs no. 2, 5, 6, 7, and 9 had increased internal body temperature (39.8-41.3°C), persisting for 1-2 d. All of these animals developed flu-like symptoms such as apathy, lack of appetite, and serous nasal discharge. These symptoms receded spontaneously within 5-6 d. Painful inflammatory infiltrates (5-20 mm in diameter) that developed at the injection site and withdrew spontaneously within 3 d were observed in all of these dogs. The second administration of the antigen and adjuvant on the 14th d of the experiment also resulted in inflammatory infiltrates in these animals at the injection site. However, this time they were bigger and their diameter reached 10-50 mm (Figure 1, Table 1). They withdrew spontaneously within 2-3 d.

A haematological test on day 1, after the first vaccination, showed mild anaemia lasting 1-2 d (dog 6: RBC = 5.63 x 10¹²/l, Ht 36.6%, dog 7: RBC = 5.13 x 10¹²/l, Ht 33.6%, dog 9: RBC = 4.95 x 10¹²/l, Ht 32.1%) and leukopenia (dog 6: WBC = 5.7 x 10⁹/l, dog 7: WBC = 5.5 x 10⁹/l, dog 9: WBC = 6.5 x 10⁹/l).

Furthermore, in dogs no. 6, 7, 9, 12, and 15, the development of a mild thrombocytopenia was observed (140-180 x 10⁹/L) (Table 1). In all animals with this anomaly, a gradual decline in the platelet count was observed during the 2-3 d after the vaccination. Then the platelet count increased, reaching a correct value after the next 2-3 d.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of dog</th>
<th>Local reaction at the injection site</th>
<th>Flu-like symptoms</th>
<th>Anaemia</th>
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By the end of the experiment no more clinical disorders or changes in the haematological parameters were observed in any of the dogs used in the study. Throughout the research period, the results of the biochemical tests (the activity of AST, ALT, AP, and the concentration of urea, total bilirubin, creatinine, and glucose, as well as the level of Ca, P, and Mg) remained also within the normal physiological ranges in all animals of both groups.

Fig. 1. Inflammatory infiltrates at the injection site after second application of SPA (the arrow shows the site of the vaccine application).

Discussion

SPA plays an important role in the aetiology of canine babesiosis. It appears that SPA activates coagulation system. Earlier results by Schetters et al. (14) have shown that in canine babesiosis, which is characterised by erythrocyte retention, the disease is correlated with the effects on the coagulation system, in contrast to infections characterised by exponentially developing parasitaemia, where the disease is correlated with the peripheral parasitaemia. Vaccination appears to prime Th-cells that assist in the generation of antibodies against SPA, which interfere with the trigger of this pathological process (14). The clinical picture of babesiosis largely depends on the systems and organs within which SPA-induced pathological reactions take place (11, 15-17). Initially, a drop in haematocrit and swellings of the lymph nodes and spleen were observed in dogs, which were vaccinated and then infected with virulent protozoa. As antibodies specific for SPA appeared in the serum of the studied animals, the number of erythrocytes increased, the swellings of the lymph nodes and spleen withdrew, and the blood flow improved (12). These results clearly show that the SPA antibodies, formed as a consequence of antigen stimulation, prevent the development or exacerbation of the pathological processes presented above. Vaccination with SPA also stops the chain of disorders leading to a circulatory shock (17). Numerous clinical observations confirm the efficacy of SPA in the prevention of piroplasmosis in animals. This antigen was first used by Sibinovic et al. (18) to prevent piroplasmosis.

Studies on its application in the prevention of canine babesiosis have been carried out for years (12, 14-16). So far, there are no reports showing the degree of tolerance to SPA by the organism of the vaccinated animals. Aside from definite protective effects, this antigen can also cause some adverse reactions. In our studies, local and general reactions following administration of SPA were observed in three out of 10 dogs in the vaccinated group. Similar observations on the occurrence of local reactions after application of the antigen in dogs were made by Schetters et al. (12). In the experiments conducted by these authors on the efficacy of SPA in the prevention of Babesia canis infection, in all animals, which received the antigen, a local reaction was observed at the injection site, in some cases associated with pain. This reaction was visible as a nodule for 1-2 d. We hypothesise that this reaction and flu-like symptoms observed in dogs used in our study were caused by the used adjuvant, as they were also found in animals receiving adjuvant alone. To minimise the adverse reaction upon vaccination, studies to determine the lowest saponin dose necessary to obtain a good adjuvant response are required.

The reason of the decline in the number of platelets in five out of 10 dogs vaccinated with the SPA is not entirely clear. So far, this irregularity has not been reported in animals immunised against babesiosis, but the fact that it was found in 50% of the test animals in our studies cannot be coincidental. In the clinical course of babesiosis, thrombocytopenia is one of the most characteristic haematological disorders (3, 14). According to Zyger et al. (23) reports, it can be found in 99.5% of patients with this disease and it develops before anaemia and leukopenia (21).

In dogs suffering from babesiosis, the influence of the SPA leads to vasodilatation, hypotension, and blood stasis in the vessels. These disorders predispose to the deposition of parasite-infected erythrocytes on the vascular endothelia, which is accompanied by coagulation disorders and symptoms of intravascular coagulation with thrombocytopenia (11). We hypothesise that similar, but much less intense phenomena may accompany the application of the SPA isolated from cell cultures of Babesia canis, which would explain the origin of a transient decrease in the number of thrombocytes in the dogs vaccinated with this antigen.

Hypotension and stagnation of blood in the vessels may also be the factors, which lead to a short-term development of anaemia after the administration of the SPA. This antigen can indirectly increase the adhesiveness and agglutination tendency of red blood cells (12). In order to be able to have a definite confirmation for these hypotheses, it is necessary to conduct further studies in this matter.

The transient leukopenia present in three dogs after the application of the SPA is a common phenomenon observed after the immunisation of animals. A decline in T-cell-mediated immunity and transient state of immunosuppression after immunisation have
been reported in dogs. Nevertheless, dogs are still routinely vaccinated with mono- and polyvalent live and inactivated vaccines and severe disease does not generally occur (20).

In conclusion, it should be noted that despite the relatively frequent adverse reactions recorded in dogs following the administration of the SPA of *Babesia canis*, this antigen can be considered relatively safe. Although the flu-like symptoms and local reactions at the injection site were observed in three out of 10 vaccinated animals, they were transient and did not require any appropriate treatment. Thrombocytopenia observed in 50% of the tested dogs was also minor and receded spontaneously within 4-5 d after the vaccination. In none of the animals used in the experiment, the symptoms of shock, which are sometimes seen in experiments conducted in dogs with the SPA (12), were observed. It should be underlined that the administration of SPA did not affect the functions of internal organs, as confirmed by the normal results of the biochemical tests. Therefore, the *Babesia canis* SPA obtained in our studies can be considered safe and well tolerated by dogs. In addition, the fact that it stimulates the development of antibodies in the organisms of vaccinated animals, which protect against the development of the disease (manuscript in preparation), makes it suitable for further studies on the immunisation of dogs against babesiosis.

References