Comparison of Babesia rossi and Babesia canis isolates with emphasis on effects of vaccination with soluble parasite antigens: a review

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ABSTRACT

Babesia canis and B. rossi are large Babesia species that infect dogs and cause clinical disease. The spectrum of disease is highly diverse with either parasite, but upon evaluation of field cases it has been suggested that in general B. rossi is more virulent than B. canis. This difference was also found in experimental infections using B. canis and B. rossi isolates and appeared to be related to a difference in parasitaemia. Whether this difference reflects the essential difference between B. canis and B. rossi species in general, or merely reflects the variability in virulence of individual isolates cannot be discerned. Comparative in vitro and in vivo studies revealed a number of qualitative differences between the B. canis and B. rossi isolates studied; however, more research is required to determine any causal relationship between in vitro and in vivo characteristics. Vaccination with a bivalent vaccine (containing soluble parasite antigen [SPA] from supernatants of in vitro cultures of B. canis and B. rossi) induced protection against clinical babesiosis upon challenge infection with either parasite. The dynamics of parasitaemia upon challenge infection of vaccinated animals indicated a biological difference between the B. canis and B. rossi isolates studied. Vaccinated dogs that were challenged with B. rossi parasites (2 isolates tested) effectively controlled parasitaemia. By contrast, in vaccinated dogs that were challenged with B. canis isolates (2 isolates tested) there was little or no effect on parasitaemia but levels of SPA in plasma were reduced. Apparently the nature of vaccine-induced immunity differs with respect to the challenge species.

Keywords: Babesia canis, Babesia rossi, immunity, pathogenesis, vaccine.


INTRODUCTION

Babesiosis is a disease caused by unicellular Babesia parasites that infect vertebrates. The parasites are transmitted by arthropod vectors and invade and proliferate in red blood cells. In almost every host species a large and a small Babesia species has been found and formerly, in host species a large and a small Babesia species was reported in dogs only a single small Babesia (B. gibsoni) and a single large Babesia (B. canis) were described\textsuperscript{12}. With the advent of molecular biological techniques, however, the number of small and large Babesia species has increased and presently at least 3 different small canine Babesia species are recognised and 4 large canine Babesia species\textsuperscript{2} are described. Two of the large species, B. canis and B. rossi, have been subject to scientific research for almost a century and biological differences have been described. This review aims to bring together data obtained for the respective species in order to shed light on differences and similarities between these parasites with emphasis on the impact of this on vaccine-induced immunity in dogs.

LARGE BABESIA SPECIES THAT INFECT DOGS

Babesia canis and B. rossi are the large parasites that cause most of the clinical problems in dogs in Europe\textsuperscript{3} and South Africa\textsuperscript{11} respectively. There have been scattered reports on clinical cases due to B. vogeli, and now it seems that this parasite is more globally distributed, with reports coming from Australia, Asia, America, South Africa, Brazil and Europe (reviewed\textsuperscript{13}). Geographical distribution and vector specificity (each of these 3 large Babesia species appears to be transmitted by its ‘own’ tick vector) argue in favour of a taxonomic differentiation at the species level. However, cross-protection studies have lent support to a subspecies classification\textsuperscript{12}. It has now become clear that the 3 parasites are clearly distinct at the genomic level. Firstly, the chromosomal organisation of B. canis isolates is clearly different from that of B. rossi isolates (2 isolates of each species were analysed\textsuperscript{4,5,35}); the genome size of B. canis is c.14.5 Mbp and that of B. rossi 16 Mbp. Secondly, analyses of the small subunit ribosomal RNA genes of isolates of the 3 parasite groups revealed a clear classification into 3 species: B. rossi, B. canis and B. vogeli\textsuperscript{4,12,26}.

VACCINES CONTAINING SOLUBLE ANTIGENS OF BABESIA PARASITES

Based on soluble parasite antigens (SPA) that are produced by Babesia parasites, vaccines have been developed against B. boris, B. bigemina, B. divergens, and B. canis (reviewed\textsuperscript{14}). Although it is claimed that vaccines that contain SPA derived from serum of infected animals provide a broad spectrum of immunity\textsuperscript{23}, this was not obtained with vaccines containing SPA derived from in vitro cultures. With the latter vaccines, protective immunity against homologous challenge was obtained, but less so against heterologous challenge\textsuperscript{23}.

In general, the nature of immunity obtained by vaccination with plasma/serum-derived SPA is reflected in shorter duration of patent parasitaemia and reduced peak parasitaemia in the circulation\textsuperscript{11}. However, results from studies with SPA from supernatants of in vitro cultures of B. canis showed that protection against clinical disease can be induced without an apparent effect on parasite proliferation\textsuperscript{11}. This suggests that Babesia parasites release molecules that are pathogenic, and that this activity can be diminished by vaccine-induced antibodies that neutralise pathogenic molecules and/or vaccine-induced anti-parasite activity that reduces the production of such pathogenic molecules. The protective activity of SPA derived from B. canis, demonstrated in the 1980s, led to the development of a commercially available vaccine in Europe\textsuperscript{28}. Preliminary experiments using SPA of B. rossi to vaccinate dogs against challenge infection were disappointing\textsuperscript{32}. Additional studies confirmed these results\textsuperscript{32}. A deeper understanding of the differences between B. canis and B. rossi could give insight into
the nature of vaccine-induced immunity and explain variable results obtained with SPA-based vaccines.

**COMPARISON OF B. CANIS AND B. ROSSI ISOLATES**

Characterisation of infected erythrocytes in vitro

*Babesia canis* and *B. rossi* cannot be distinguished by examination of Giemsa-stained blood smears; both are large parasites and the morphology (ring, trophozoites, merozoite) is comparable (Fig. 1).

Using atomic-force microscopy, structural alterations of the erythrocyte surface were observed in bovine erythrocytes that were infected with *B. bovis* parasites.

These structures had been demonstrated before using electron microscopy and are called knob-like protrusions. They are considered to be analogous to the knobs on *Plasmodium falciparum*-infected erythrocytes and possibly play a crucial role in sequestration of infected erythrocytes in the microvasculature.

Structural alterations such as knob-like protrusions could not be detected by atomic-force microscopy on canine red blood cells that were infected with mature dividing forms of either *B. canis* or *B. rossi* isolates (Fig. 1). Any difference in sequestration profile of *B. canis* and *B. rossi* (see below) therefore cannot be simply related to the presence of knob-like structures.

By contrast, canine red blood cells that were infected with *B. canis* parasites showed biochemical differences when compared with red blood cells infected with *B. rossi* parasites; *B. canis*-infected red blood cells could be partially purified over Plasmagel whereas *B. rossi*-infected erythrocytes could not.

It was also noted that *B. canis*-infected erythrocytes tended to osteoglutinate in vitro whereas *B. rossi*-infected erythrocytes did not. It is also unknown whether this putative molecule is parasite-derived or a host molecule, produced perhaps during the inflammatory response, as has been suggested in *B. bovis* infections.

There is ample evidence of an inflammatory response in animals that are infected with *Babesia* parasites, which is associated with increased levels of fibrinogen (in dogs known as an acute-phase protein).

It has been suggested that erythrocytes may become coated with fibrinogen (or fibrinogen-like molecules), which increases the propensity to autoagglutinate. In-saline autoagglutination, which is observed in a proportion of clinical cases, could reflect such coating of *B. rossi*-infected erythrocytes, but this has not been proven yet.

Antigenic differences between *B. canis* and *B. rossi* isolates have been shown using immunofluorescence on fixed blood smears and immunoblotting. Using immunofluorescence (in which mainly merosites become fluorescent), there was considerable cross-reactivity between the 2 isolates, but titres against homologous antigens were the highest.

An alternative immunofluorescence test in which infected red blood cells were not fixed prior to incubation with specific antisera (which focuses on reactivity against the infected erythrocyte membrane) revealed a more distinct pattern: *B. rossi*-infected erythrocytes reacted only with *B. rossi*-specific antisemur and not with an antiserum that stained all *B. canis*-infected red blood cells and vice versa (Table 1). It was concluded that *B. canis* and *B. rossi* infected erythrocytes expose species-specific epitopes at the cell surface.

**Analysis of *B. canis* and *B. rossi* infections in dogs**

Apart from the fact that there may be differences in virulence depending on the particular isolate studied (P T Matjila et al., in prep., Faculty of Veterinary Science, University of Pretoria), it has been suggested that in general there is a difference in the clinical picture seen in South African babesiosis caused by *B. rossi* and European babesiosis caused by *B. canis*.

It is, however, difficult to draw conclusions from such field observations as a multitude of factors play a role in the outcome of infection. An epidemiological study of *B. canis* infection in France.

**Table 1: Fluorescence of infected erythrocytes upon incubation with different antisera. Results are expressed as percentage of fluorescent cells. Adapted from Schetters et al.**

<table>
<thead>
<tr>
<th>Erythrocytes infected with:</th>
<th><em>B. rossi</em></th>
<th><em>B. canis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dog immunoglobulin</td>
<td>20–30</td>
<td>0</td>
</tr>
<tr>
<td>Anti-<em>B. canis</em> serum</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Anti-<em>B. rossi</em> serum</td>
<td>20–30</td>
<td>100</td>
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indicated that breed, but not age or sex, was specifically associated with clinical disease. By contrast, in a large-scale survey of clinically affected dogs in South Africa, it was shown that age had a significant effect on the main clinico-pathological findings. In order to compare the virulence of different Babesia parasites in dogs, controlled experimental infections in age-matched animals of a single breed are indispensable. In a small-scale study, Uilenberg showed that the B. rossi isolate was highly pathogenic and irrespective of whether the dogs had survived infection with B. canis, they all had to be treated to prevent them from succumbing to the infection. By contrast, dogs that were infected with B. canis appeared less affected. Additional studies using the same parasite isolates and age-matched experimental groups also indicated that the B. rossi isolate was more virulent than the B. canis isolate, in that B. rossi-infected dogs developed progressive parasitaemia that required early treatment (at day 7 after infection), whereas B. canis-infected dogs showed a limited transient parasitaemia that did not require early treatment. The simplest explanation is that dogs that are infected with B. canis effectively control the parasite, whereas dogs infected with B. rossi do not. If this were the case one would expect to find a correlation between the time point of apparent control of parasitaemia and recovery from clinical signs. This has not been found in experimental B. canis infections. Apparently, other mechanisms are involved. Since a proportion of the infected erythrocytes sequesters in the microvasculature may be sequestered in the microvasculature may be the putative toxic component of SPA on parasitaemia shown in dogs without an apparent effect on parasitaemia. This could be explained assuming that the vaccinated animals produced neutralising antibodies against the nature of immunity appeared to be anti-disease i.e. protection could be shown in dogs without an apparent effect on parasitaemia. This could be explained assuming that the vaccinated animals produced neutralising antibodies against the nature of immunity appeared to be anti-disease i.e. protection could be shown in dogs without an apparent effect on parasitaemia. This could be explained assuming that the vaccinated animals produced neutralising antibodies against the nature of immunity appeared to be anti-disease i.e. protection could be shown in dogs without an apparent effect on parasitaemia. This could be explained assuming that the vaccinated animals produced neutralising antibodies against the nature of immunity appeared to be anti-disease i.e. protection could be shown in dogs without an apparent effect on parasitaemia.

CONCLUSIONS

The clinical picture of dogs infected with either of the 2 most virulent large Babesia species that infect dogs, B. canis and B. rossi, is highly diverse. Data from the field and from experimental studies suggest that B. rossi is more virulent than B. canis; however, some of the data can be explained by the variability in virulence of individual isolates. Vaccination with a bivalent vaccine containing soluble parasite antigen (SPA) from supernatants of in vitro cultures of B. canis and B. rossi induced protection against clinical babesiosis upon challenge infection with either parasite. The dynamics of parasitemia upon challenge infection of vaccinated animals indicated a biological difference between the B. canis and B. rossi isolates studied. Apparently the nature of vaccine-induced immunity differs with respect to the challenge species. In order to fully understand the protective mechanisms, more detailed characterisation of SPA preparations and analysis of the immunoprotective effects of specific components need to be addressed.

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