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 dogs only a single small *Babesia* (*B. gibsoni*) and a single large *Babesia* (*B. canis*) were described[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[32]. 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[3] and South Africa[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[33]). 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[32]. 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[6]); 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*[4,5,35].

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. bovis, B. bigemina, B. divergens,* and *B. canis* (reviewed[22]). Although it is claimed that vaccines that contain SPA derived from serum of infected animals provide a broad spectrum of immunity[31], 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[22].

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[31]. 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[23]. 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[18]. Preliminary experiments using SPA of *B. rossi* to vaccinate dogs against challenge infection were disappointing[13]. Additional studies confirmed these results[29]. A deeper understanding of the differences between *B. canis* and *B. rossi* could give insight into

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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[9]. These structures had been demonstrated before using electron microscopy and are called knob-like protrusions[10]. 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[2,26]. 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[24]. It was also noted that *B. canis*-infected erythrocytes tended to autoagglutinate *in vitro* whereas *B. rossi*-infected erythrocytes did not (K. Moubri, pers. comm.). Nothing is known about the nature of this difference, and 2 possibilities are considered: either there is a real biochemical difference in the molecules that make up the erythrocyte membrane, or the infected erythrocyte is coated with an as yet unidentified molecule (or molecules). 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[34]. 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[7,16,20]). It has been suggested that erythrocytes may become coated with fibrinogen (or fibrinogen-like molecules),
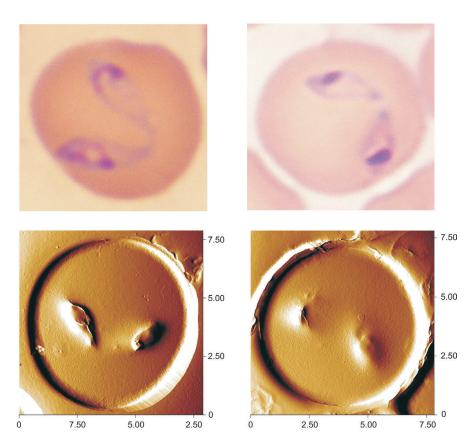

Fig. 1: **Morphology of *Babesia canis* and *B. rossi* parasites. Upper panel, photographs of Giemsa-stained blood smears from *B. canis*** (upper left) **and *B. rossi*** (upper right) **in *in vitro* cultures. Lower panel, photographs of *B. canis*** (lower left) **and *B. rossi*** (lower right) **infected erythrocytes from *in vitro* cultures visualised by atomic force microscopy.** (Courtesy of B Cooke.)

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[11].

Antigenic differences between *B. canis* and *B. rossi* isolates have been shown using immunofluorescence on fixed blood smears[32] and immunoblotting[8]. Using immunofluorescence (in which mainly merozoites become fluorescent), there was considerable cross-reactivity between the 2 isolates, but titres against homologous antigens were the highest[32]. 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[1]) revealed a more distinct pattern: *B. rossi*-infected erythrocytes reacted only with *B. rossi*-specific antiserum and

not with an antiserum that stained all *B. canis*-infected red blood cells and *vice versa*[24] (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[14] (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*[19]. 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*.[24].**

Erythrocytes infected with:	*B. rossi*	*B. canis*
Anti-dog immunoglobulin	20–30	0
Anti-*B. canis* serum	100	0
Anti-*B. rossi* serum	20–30	100

indicated that breed, but not age or sex, was specifically associated with clinical disease[15]. 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[20]. 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[32]. 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[24]. 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[24]. Apparently, other mechanisms are involved. Since a proportion of the infected erythrocytes sequesters in the microvasculature in both *B. canis* and *B. rossi* infections[11], a difference in the tendency of infected erythrocytes to sequester in the microvasculature may be associated with virulence. The mechanisms involved in such sequestration are not clear, but do not seem to require the presence of knob-like structures as seen in *B. bovis* infections in cattle (see above). It has been hypothesised that this difference in virulence is a qualitative difference, *i.e.* infection with *B. rossi* triggers different reactions in the host compared with *B. canis,* for instance the induction of fibrinogen[25]. Fibrinogen increase was higher in *B. canis* infection than in *B. rossi* infection and, if coating of infected erythrocytes with fibrinogen predisposes sequestration, this could offer an explanation for the difference in parasitaemia[24].

VACCINATION AGAINST *B. CANIS* AND *B. ROSSI* USING SPA

Soluble parasite antigens of supernatants from *in vitro* cultures have been successfully used to vaccinate animals against challenge with homologous *Babesia* parasites, including vaccination of dogs against *B. canis*[22]. Vaccination against the South African *B. rossi* parasite has been less successful[13] and even repeated vaccination with SPA did not improve vaccine efficacy[29]. This does not necessarily mean that clinical disease caused by *B. rossi* infection is not due to reactions triggered by SPA; it shows that vaccination with SPA in combination with a saponin adjuvant is not able to induce a protective immune response. These results indicate that indeed *B. rossi* differs from *B. canis* immunobiologically, which could be reflected in (immuno-) biochemical differences found in *in vitro* assays (see above)[24].

In the case of *B. canis* infection, the spectrum of immunity obtained after vaccination with SPA of *B. canis* could be broadened by adding SPA of *B. rossi*[27]. Dogs that were vaccinated with the bivalent vaccine preparation showed significant protection upon challenge with a heterologous *B. canis* parasite (vaccine production has been described in detail previously[30]). As for the monovalent vaccine, the nature of immunity appeared to be anti-disease *i.e.* protection could be shown in dogs without an apparent effect on parasitaemia[28]. This could be explained assuming that the vaccinated animals produced neutralising antibodies against the putative toxic component(s) of SPA upon experimental challenge infection. Although not completely elucidated, the result suggested that this bivalent antigen preparation stimulates immune responsiveness against cross-reactive epitopes that are shared between *B. canis* and *B. rossi* SPA, thus transcending the species specificity[27]. This posed the question whether this vaccine preparation could also induce immunity against *B. rossi* challenge infection. Indeed, although the monovalent vaccine based on *B. rossi* SPA only did not induce protection, the bivalent preparation was effective against a homologous as well as heterologous *B. rossi* challenge infection[29]. Surprisingly, the protective effect that was expressed as reduced clinical signs correlated with a reduction of parasitaemia; in dogs that had received 3 vaccinations not a single infected erythrocyte was detected in blood smears taken after challenge infection. The level of SPA in plasma after challenge infection was reduced likewise and probably the result of reduced parasitaemia (less production) and/or antibody reactivity against SPA (neutralisation)[29]. This leaves, amongst others, the possibility that SPA also induces pathology in *B. rossi* infection. SPA preparations are complex mixtures of parasite and host molecules that are poorly characterised and little is known at the molecular level[17,21]. Clearly more research is needed to explain the results obtained so far.

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 parasitaemia 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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