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BLOOD, BULL TERRIERS AND BABESIOSIS: A REVIEW OF CANINE BABESIOSIS

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Taxonomy and Molecular Phylogeny

The classification of *Babesia* spp. places them in order *Piroplasmida*, within the phylum *Apicomplexa*. *Babesia* spp. are often referred to as piroplasms, a collective term for morphologically similar protozoan parasites that utilize mammalian erythrocytes in their life cycle. Piroplasms encompass two main genera, *Babesia* and *Theileria*; which are currently the subject of intense research interest and molecular-based (re-)characterization. Since babesiosis is an emerging disease in many parts of the world, it is very important to determine the precise species of the parasite causing the clinical illness and the isolate(s) normally present in any given geographical location. The success of treatment may depend on such information since most anti-babesial drugs have limited efficacy against different *Babesia* spp. Similarly, inclusion of appropriate antigens into serologic tests is necessary to reduce the risk of inaccurate results during screening for diagnostic or epidemiological purposes. The 18S rRNA gene has been favoured for molecular phylogenetic studies to date, but others regions of the small subunit ribosomal RNA gene, including the first and second transcribed spacers (ITS1 & ITS2) and 5.8S rRNA gene, and the heat shock protein genes (HSP 70 and HSP 90) have also been used, or show promise for future taxonomic studies.

Until recently only two *Babesia* parasites were thought to occur in dogs; the relatively large intra-erythrocytic piroplasm referred to as *Babesia canis* and a smaller parasite, known predominantly as the cause of canine babesiosis in Asia, *Babesia gibsoni*. Since the late 1980's "*B. canis*" has been reclassified into three separate species (*B. canis*, *B. rossi* and *B. vogeli*) on the basis of cross-immunity, serological testing, vector specificity and molecular phylogeny and a new 'large' *Babesia* sp. has been recently described in a dog in North Carolina (1).

Molecular characterization of small canine piroplasms has also shed light on the classification of these parasites. Studies, predominantly utilizing the 18S rRNA gene locus have revealed that, in general, small canine piroplasms are more closely related to *Theileria* than to *Babesia*. These two genera were previously separated on the basis of certain life cycle features and transovarial passage within the tick vector. However such historical definitions need revisiting in the light of these molecular studies. To date at least 4 genetically and clinically distinct small piroplasms affect dogs which include: *Babesia gibsoni* – originally described in India nearly a century ago (2) and now occurring sporadically in other parts of the world including the Australia; *Babesia conradae*, a piroplasm that occasionally infects dogs in California (3); *Theileria annae*, a *Babesia microti*-like parasite that has so far been reported only in northwest Spain, transmitted by *Ixodes hexagonus*

(4,5); and a fourth small piroplasm, *B.* (=*T.*) *equi* has also been reported in dogs in Spain (6).

Epidemiology of Babesiosis

Babesia Transmission

It has long been recognised that *Babesia* spp. can be transmitted by the needle passage of infected blood, inadvertently in the case of blood transfusions or deliberately during experimental studies. In general however, babesiosis is considered to be a tick-transmitted disease and there have been many experiments over the years to elucidate the various tick species that fulfil this epidemiological role under natural conditions. Recent studies involving *Babesia gibsoni* have started to raise some intriguing questions about this parasite's natural mode of transmission.

It is assumed that the initial reports of *B. gibsoni* infection in countries outside Asia (e.g. Australia & USA) were the result of its introduction by (usually asymptomatic) carrier dogs travelling from endemic regions. Recent surveys in the USA and our studies in Australia have revealed a high proportion of American Staffordshire/Pit Bull Terriers among those dogs with confirmed *B. gibsoni* infection. In one region of Victoria, 17.5% APBT tested were positive for *B. gibsoni*. Furthermore, these *B. gibsoni*-positive APBT did not harbour any ticks. Analogous findings have recently been reported in thrombocytopenic and anaemic Tosa dogs in northeast Japan, a region that is also climatically unfavourable for ticks (7). The higher prevalence of *B. gibsoni* infection among these particular breeds, renowned for inter-dog aggression, suggests that blood exchange, not vector transmission, is the main mode of dissemination of *B. gibsoni* in the USA, Australia and certain areas of Japan. Our epidemiological studies in Victoria revealed that male APBT, and those individuals with a history of having bitten, or been bitten by other APBT were more likely to have babesiosis. It is presumed that the exposed surfaces of open lacerations in both combatant dogs (infected 'donor' and 'recipient') need to come into intimate contact with each other, and this is most likely to occur with injuries inflicted to the head and neck in these individuals.

Observations made of these *B. gibsoni* epizootics may also have implications for our understanding of the epidemiology of *B. gibsoni* infection in endemic regions. Coincidentally, many parts of Asia have large populations of stray dogs that roam unhindered throughout urban and rural localities and fighting among quarrelsome dogs is inevitable. Direct dog-to-dog transmission may be an important route of infection of *B. gibsoni* in all localities where it is found. This 'continual passage' would limit the opportunities for exchange of genetic material for the parasite, since this only occurs during sporogony within the tick vector, and the remarkable absence of genetic variation that is found between *B. gibsoni* isolates as geographically distant from each other as Sri Lanka, Oklahoma, Malaysia and Okinawa provides further evidence that this may indeed be occurring.

Host Specificity

Molecular studies of the piroplasms infecting dogs have challenged the perception that *Babesia* spp. are host specific. It appears that closely related piroplasms are capable of infecting several mammalian hosts, a finding that may have clinical implications with regard to pathogenicity, diagnosis and treatment. *Babesia* (=*Theileria*) *equi* DNA has been amplified in dogs (6) and the *Babesia* sp. described in North Carolina (1) is closely related to the ungulate *Babesia* species. The dog from which it was isolated was undergoing chemotherapy which suggests that this infection might be opportunistic. It is currently unknown whether this isolate represents a true species of dogs, or whether the dog is just an accidental host.

Diagnosis of Babesiosis

Whilst observation of the parasite(s) by light microscopy has long been the gold standard test, the genotype of piroplasms cannot be determined by their phenotype. Microscopy is still the only viable option available to veterinarians in many parts of the world where babesiosis is endemic but significant limitations in its sensitivity and specificity are well recognized. The parasitaemia associated with *Babesia* is often very low, especially during chronic infection, and is easily overlooked. Failure to detect *Babesia* parasites in animals with haemolytic anaemia or thrombocytopenia has led to an incorrect diagnosis in documented cases, often when the clinical suspicion of babesiosis was also low. Given the possibility of direct horizontal transmission of canine piroplasms, veterinary clinicians should always ascertain whether the patient has been bitten by any other dog in the preceding 4-8 weeks, irrespective of its breed.

It is clear that the introduction of PCR has greatly increased the sensitivity of parasite detection, yet from a global perspective the routine testing for canine babesiosis in this manner is still restricted to very few laboratories. In very early infections, when small numbers of parasites remain at the site of inoculation, detection in peripheral blood even by PCR may be unrewarding. Of greater clinical significance would be the failure of PCR to detect infection in chronic cases. These carrier dogs are potential sources of infection, especially if the right epidemiological conditions exist for transmission. Following experimental infection with *B. gibsoni* we monitored clinical parameters, haematology, serologic titre (by IFAT) and the presence of *Babesia* DNA on a daily basis. All dogs made a full clinical recovery, as judged by normal clinical signs, absence of splenic enlargement, a normal haemogram and absence of piroplasms on microscopic examination, by 30-50 days after peak parasitaemia (unpublished results). During this period of clinical normality babesial DNA was inconsistently detected during first-round PCR. Second round PCR improved detection rates but each dog was still intermittently negative, yet serological titre was consistently positive. This suggests a very low, fluctuating parasitaemia in these dogs analogous to chronic, asymptomatic natural infection. Serological testing should be used concurrently for diagnosis in these dogs but discordant results between PCR and IFAT still frustrate efforts to find a perfect testing protocol (8).

The clinical consequences of chronic babesial infection are unclear and while most dogs appear to tolerate this state of premunity with few ill effects in our experience, theoretically they remain at risk of developing immune-mediated complications and recrudescence of clinical disease (and parasitaemia) if immunocompromised at a later time. Chronic infection may be inconsequential in some dogs and may be even beneficial for hosts living in endemic regions by protecting them from further disease (9), but is an unacceptable situation in blood donors or in dogs that are to be exported to *Babesia*-free countries such as New Zealand. In these latter cases the testing protocol must be capable of detecting the carriers with 100% certainty, a utopian goal that has yet to be achieved.

Immunofluorescent antibody testing is currently the preferred method of serological diagnosis for babesiosis. As with any serologic assay its major diagnostic limitation is the inability to differentiate acute from chronic infection. This is of particular relevance to clinicians working in regions that are endemic for babesiosis. In contrast, for *B. gibsoni* infections in the USA and Australia, where the prevalence is generally low in the dog population, the IFAT is a useful tool for detection of infected dogs. Here its application can be extended to screening purposes for blood donors and for pre-export testing of dogs destined for *Babesia*-free countries. Despite the occasional discordant result referred to earlier, the combination of IFAT and an appropriately controlled PCR seems to hold most promise for achieving optimal diagnostic accuracy.

Treatment of Babesiosis

Until recently there has been little change in the options available to veterinarians for the treatment of babesiosis in dogs and cats. Imidocarb dipropionate (at 5-6mg/kg IM once, repeated 2 weeks later) and diminazine aceturate (3.5mg/kg IN once) have been used extensively in dogs around the world in the therapy of large and small babesial infections respectively. National therapeutics registration authorities have restricted access to some of these drugs in certain countries, including the USA, and some, notably the diamidine derivative diminazine, are associated with a high rate of side-effects. Other drugs such as doxycycline, clindamycin, quinuronium sulphate, trypan blue, pentamidine, phenamidine and parvaquone have all been reported with variable degrees of clinical success. Most, if not all of the drugs that have been used to treat babesiosis result in amelioration of clinical signs at best and rarely achieve true sterilization of the infection.

The successful treatment of the small piroplasm infections, notably *B. gibsoni*, has been especially challenging. Based on the close phylogenetic relationship between these parasites and *Theileria* spp., the successful therapeutic outcome of a macrolide antibiotic combined with an antiprotozoal has been noted in rodent models. The combined use of azithromycin (10mg/kg q24h PO for 10 days) and atovaquone (13.3mg/kg q8h PO for 10 days) for treating *B. gibsoni* in dogs is a significant breakthrough that combines real clinical efficacy with great safety (10). Unfortunately the expense of the hydroxynapthoquinone component (atovaquone) will reduce

widespread acceptance of this therapy where it is most needed, in Asia, unless the costs can be reduced. A cheaper formulation of atovaquone with proguanil causes an unacceptably high incidence of gastrointestinal side-effects in dogs. Despite the undoubted benefits of the atovaquone and azithromycin combination for treating *B. gibsoni* infections, further research is needed to determine whether the parasite is truly eliminated. Data from experimental infections in our laboratory and from Japan (11) suggest that a low parasitaemia persists as indicated by persistent detection of parasite DNA in the blood of some dogs following treatment. The search for better antiprotozoal agents has recently led to the investigation of plant extracts from the rainforests of Indonesia (12).

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