Chapter 8: General discussion

8.1. General discussion

In this thesis several tick-borne pathogens that occur in dogs, particularly in South Africa, are identified and characterized. Parasite occurrence, clinical relevance, molecular diagnosis and, to some extent, the genetic diversity of a number of protozoan tick-borne pathogens was investigated. In this concluding chapter, the most important findings of the preceding chapters are summarized and discussed. Specific recommendations for the control of tick-borne diseases in dogs are given and, finally, opportunities for future research are suggested.

8.1.1. Babesia rossi

The most common haemoparasite species detected in domestic dogs in all areas sampled was *B. rossi* (Chapter 3). Interestingly, there appeared to be a correlation between the clinical manifestation of the disease and the *B. rossi* genotype based on the *BrEMA*1 gene. In particular, genotype11, 19 and 28/29 were mostly associated with complications leading to death as a result of solid-organ complications. The analysis indicated that the *BrEMA*1 gene may be a suitable genetic marker for surveying *B. rossi* infections in South Africa, especially since *B. vogeli* and *B. canis* isolates were shown not to have the *BrEMA*1 gene. Early detection of virulent genotypes may alert clinicians to be especially vigilant for early signs of complications.

8.1.2. Babesia vogeli

The discovery of *Babesia vogeli* in dogs for the first time in South Africa (Chapter 2) implied that there are currently two species of *Babesia* implicated as the cause of canine babesiosis. This may explain why canine babesiosis has such varied clinical manifestations, which range from a very mild to a fatal infection. Some of these differences may be due to an excessive inflammatory response rather than due to the parasite itself. We conclude, however that the presence of both *B. rossi* and *B. vogeli* contributes partially to the varied clinical manifestations that are typical of the disease to South Africa. Thus some non-complicated cases of canine babesisois may be as a result of *B. vogeli* infections whereas severe to complicated cases may be due to *B. rossi* infections.

In general, however, Uilenberg, Franssen, Perie, and Spanjer (1989) suggested that *B. vogeli* parasites may occur in large parts of tropical and subtropical regions on all continents, coinciding with the global distribution of *R. sanguineus* ticks. Our finding confirmed this suggestions since both *H. elliptica* (regarded as a synonym of *Haemaphysalis leachi*; Apanaskevic, Horak and Camica, 2007) and *R. sanguineus* have been collected from dogs presented with babesiosis at the Onderstepoort Veterinary Academic Hospital (Horak, 1995).

8.1.3. Babesia gibsoni

Babesia gibsoni, another tick-borne pathogen causing canine babesiosis, was diagnosed in a three-month-old pit-bull pup imported into South Africa during a routine clinical

examination (Chapter 7). Diagnosis was confirmed by way of blood smear examination, PCR/RLB and sequence analysis. Prior to the blood samples being sent for PCR/RLB test it was not clear which piroplasm was parasitizing the erythrocytes. Although the dog had no obvious signs of clinical babesiosis, microscopic examination of the capillary blood smears revealed the presence of piroplasms in the erythrocytes. Prior to the atovaquone / azithromycin therapy, infected erythrocytes could still be observed on capillary blood smears on all five occasions during which the dog was examined. Treatment of canine babesiosis is effective when the disease is treated at an early stage with diminazene aceturate (Berenil RTU®) and imidocarb diproprionate (Forray-65®) singly or in combination. However, *B. gibsoni* infections will only respond to a combination drug therapy of atovaquone and azithromycin. There is a potential risk of *B. gibsoni* becoming established in South Africa, especially since the possible tick vector, *R. sanguineus*, is present in dog populations.

Although transmission experiments have not been done to demonstrate which tick species is the vector for *B. gibsoni*, both *H. leachi* and *R. sanguineus* have been incriminated in the transmission of the parasite (Kjemtrup, Kocan, Whitworth, Meinkoth, Birkenheuer, Cummings, Boudreaux, Stockham, Irizarry-Rovira and Conrad, 2000; Wozniak, Barr, Thomford, Yamane, McDonough, Moore, Naydan, Robinson and Conrad, 1997).

8.1.4. Babesia canis

Babesia canis has never been reported in South Africa and given its tick specificity is unlikely to ever be reported as an autochthonous case. An outbreak of canine babesiosis

in the Netherlands in dogs that had never been outside the country is described in chapter 5. In the spring of 2004, pathogen DNA isolated from 18 cases (including four fatalities) was found positive for *B. canis,* whereas other haemoparasites could be excluded. According to the literature, a single dose (7.5 mg/kg) of imidocarb dipropionate (Penzhorn, Lewis, De Waal and Lopez Rebollar, 1995; Boozer and MacIntire, 2003) or two doses (7mg/kg) with a 14-day interval (Brandao, Hagiwara and Myiashiro, 2003) will sterilize the infection. In addition to the life-saving ability of the drug, sterilizing the infection in dogs in the Netherlands would render the dogs non-infective for ticks. Moreover, it would also ease matters with respect to blood-component therapy, which has become more accessible in veterinary practice over recent years. Vaccination, which sufficiently reduces clinical symptoms but does not prevent infection, would be the preferred preventive measure in an endemic area (Schetters, Kleuskens, Scholtes, Pasman and Goovaerts, 1997). A survey to identify ticks and tick-borne pathogens in dogs presented at veterinary clinics in the Netherlands was initiated after the fatal cases had occurred in 2004. The results of the survey were recently published (Nijhof, Bodaan, Postigo, Nieuwenhuijs, Opsteegh, Franssen, Jebbink and Jongejan, 2007) wherein it was shown that *Dermacentor* ticks have established themselves at several locations in the Netherlands in large numbers. *Dermacentor reticulatus* adults and nymphs were found in six localities, all with apparently suitable habitats (Nijhof et al., 2007). With *D. reticulatus* seemingly becoming established in the Netherlands, it is anticipated that *B. canis*-related canine babesiosis will be an endemic disease in the Netherlands.

8.1.5. Theileria sp.

In Chapter 6, a novel *Theileria* species that was detected in domestic dogs from Pietermaritzburg in the KwaZulu-Natal province and from the Onderstepoort Veterinary Academic Hospital is reported. Although the pathophysiology of the detected *Theileria* sp. in dogs is unknown, it is apparent from the few cases described here that haemolysis and an immune-mediated syndrome may be associated with this organism. Similar clinical findings have been reported for *Theileria annae* infected dogs (Garcia, 2006). Phylogenetic analysis showed a close similarity with one to four base pair difference with *Theileria* sp. (sable), the species that causes mortalities in sable antelopes (Nijhof, Pillay, Steyl, Prozesky, Stoltsz, Lawrence, Penzhorn and Jongejan, 2005). To our knowledge, none of the dogs from which the *Theileria s*p. was isolated, died as a result of the infection. This finding adds a *Theileria* species to the list of haemoprotozoan parasites infecting dogs in South Africa. Isolation of the parasite and subsequent tick-transmission studies will be required in order to determine the importance of this *Theileria* species.

8.1.6. Ehrlichia / Anaplasma species

Currently the two tick-transmitted *Ehrlichia* / *Anaplasma* species known to cause human disease are *E. chaffeensis* and *Anaplasma phagocytophilum*. Although we had anticipated detecting some of these zoonotic *Ehrlichia* and *Anaplasma* species, our results were negative for the known zoonotic species. The reported detection of *E. chaffeensis* in dogs and in a human being from Bloemfontein, Free State Province ((Pretorius, Venter, Ryst and Kelly, 1999), was based on serological assay and not on the detection of parasite DNA. Cross-reactivity between *Ehrlichia* infections are known to

occur (Parola, Inokuma, Camicas, Brouqui and Raoult, 2001), which suggests that molecular assays should be used to support serological evidence. On the other hand, a species closely related to *A. phagocytophilum* has been identified from 3 dog samples in South Africa (Inokuma, Oyamada, Kelly, Jacobson, Fournier, Itamoto, Okuda, and Brouqui, 2005). A more directed study considering *Ehrlichia*-infected dogs only may elucidate the existence of potentially zoonotic *Ehrlichia* species.

Our current results indicate that the abundance of tick vectors, on domestic and wild canine hosts, encourages the cyclical transmission of tick-borne pathogens in the country. Although tick vectors of *B. vogeli* and *E. canis* (*R. sanguineus*), and *B. rossi* (*H. elliptica*) have overlapping distribution and have been isolated on the same host, dual infections of blood parasites seems to be rare in South Africa. Molecular diagnostic techniques allow previously unknown species to be identified. There is no doubt that if the current momentum of research is maintained, various other important pathogens will be discovered, which will in return influence our understanding of the epidemiology, management and treatment of tick-borne pathogens of domestic dogs.

Travel within and movement of animals into South Africa has increased the possibility of new tick-borne pathogens being introduced to non-endemic areas. The risk of *B. gibsoni* becoming established in South Africa is limited, due to the requirements of pre-import testing of all dogs from countries that are not free of *B. gibsoni*. It is also crucial that blood testing should always include serological and molecular testing. Without vigilant surveillance and stringent import control, the presence of potential tick vectors increases the risk of this pathogen becoming established in South Africa.

8.1.7. Multiple infections

Dual infections of *B. rossi* and *E. canis* were also detected in all our sampled areas except in Free State and Eastern Cape provinces. These could indicate that *H. elliptica* and *R. sanguineus* have overlapping distributions and also feed on the same hosts in those areas.

Therefore there exists a wide variety of tick-borne pathogen in dog populations, in so far as we can generalize:

- *Babesia rossi* is probably the most prevalent tick-born pathogen of dogs in South Africa.
- *Babesia vogeli* occurs in South Africa, but it is not as prevalent as *B. rossi*.
- There is a novel *Theileria* species infecting dogs, which may be linked to anaemia (possibly haemolytic) and a possible immune-mediated syndrome.
- *Ehrlichia canis* is present in dog populations and it is usually found in dual infections with *B. rossi*, *B. vogeli* or the novel *Theileria* sp. (dog).
- Mixed infections of *B. rossi* and *B. vogeli* and of *B. rossi, B. vogeli* and *E. canis* occurring simultaneously in dogs are rare.

We are also able to conclude the following:

- Although the occurrence of *B. vogeli* has been confirmed, its clinical importance in South Africa is regarded as less than that of *B. rossi*.
- There are several *B. rossi* genotypes, based on the *BrEMA*1 gene.
- There may be a correlation between *B. rossi* genotypes and canine babesiosis phenotypes.

- *BrEMA1* gene might be a good genetic marker for surveying *B. rossi* infections in South Africa.
- Virulent *B. rossi* genotypes could possibly cause differing host responses to infection.
- *Babesia vogeli* and *B. canis* parasites do not have the gene *BrEMA*1.

There is evidence to suggest that tick-borne pathogens have been introduced in previously non-endemic areas based on the following:

- *Babesia canis* is becoming endemic in pockets of dog populations in the Netherlands.
- There are localized populations of *Dermacentor reticulatus* in the Netherlands.
- A previously unknown *Theileria* sp. occurs in dogs in South Africa.
- *Babesia gibsoni* infections can be misdiagnosed if diagnosis is based only on smear examination without the use of molecular diagnostics.
- Diminazene aceturate (Berenil RTU®) and imidocarb diproprionate (Forray-65®) are only effective against the large *Babesia* of dog infections.
- A 10-day course of combination drug therapy of atovaquone and azithromycin is effective against *B. gibsoni* infections.

8.2. Conclusion

In this thesis we were able to identify the most prevalent tick-borne pathogens infecting domestic dogs in the areas studied. Species of organisms responsible for canine babesiosis and ehrlichiosis were isolated and characterized. Although canine babesiosis

is an endemic disease in South Africa, it has only been associated with *B. rossi*. We discovered that two species of *Babesia* were responsible for canine babesiosis in South Africa. Canine babesiosis associated with *B. canis* infections was found to be the only cause of out-breaks in the Netherlands. *Babesia vogeli* was identified for the first time in South Africa, but clinical canine babesiosis is mostly associated with *B. rossi* infections. Characterization of the *B. rossi (BrEMA*1) revealed several genotypes. These genotypes seem to differ in virulence. It was found that only genotypes 11, 19 and 28/29 were associated with solid-organ complications. Although genotype19 induced around twice as many solid-organ complications and death than genotype 28/29, genotype 28/29 showed the same severity in causing complicated disease and subsequent death.

The only *Theileria* species known to cause clinical disease in dogs is *Theileria annae*, which is endemic only in Spain. We detected for the first time a novel *Theileria* species of dogs in South Africa. The *Theileria* species described seems to be associated with haemolysis and an immune-mediated syndrome in infected dogs. This species was also found concurrently with *E. canis* infections. Although the tick vector of this species is unknown, it is suspected that *R. sanguineus* may play a role in the transmission of the *Theileria* species.

8.2.1. Control measures

Soluble parasite antigens (SPA) derived from serum of *Babesia*-infected animals or supernatants of *in vitro* culture of *Babesia* parasites have been shown to confer protection against challenge infection when used as a vaccine (Schetters and Montenegro-James,

1995). Initial attempts to produce such a vaccine against *Babesia rossi* infection using SPA from *B. rossi* culture supernatants were not or were only partially successful. Recent reports have shown that a vaccine containing a mixture of SPA obtained from *in vitro* cultures of *B. rossi* and *B. canis* induces protection in dogs against heterologous challenge infection with *B. canis* (Schetters, Kleuskens, Scholtes, Gorenflot, Moubri and Vermeulen, 2001) and heterologous challenge infection with *B. rossi* (Schetters, Strydom, Crafford, Kleuskens, Van de Crommert, and Vermeulen, 2007). Although this improved vaccine (Nobivac Piro, Intervet) (Schetters, Kleuskens, Carcy, Gorenflot and Vermeulen, 2007) is available commercially in Europe, it is not yet available in South Africa. Chemotherapy is still the method of choice for treatment and control of *Babesia* infections in dogs in South Africa. Treatment of canine babesiosis is effective when the disease is treated at an early stage with diminazene aceturate (Berenil RTU®) and/or imidocarb diproprionate (Forray-65®). However, *B. gibsoni* infections will only respond to a combination drug therapy of atovaquone and azithromycin. Since *B. gibsoni* is not endemic in South Africa, this treatment regimen is often not used. Dogs infected with *Ehrlichia* often recover from infection after treatment with tetracyclines. Alternatively, prophylactic use of impregnated dog-collars seems to be effective in preventing tick-infestations and ultimately the transmission of tick-borne pathogens (Last, Hill, Matjila and Reme, 2007). The implications are that if clinical diagnosis is not accompanied by molecular confirmation, then chances of the wrong treatment being administered are high. Also, abundance of different tick species in endemic areas further validates the use of molecular techniques as a tool in ensuring that the correct treatment is used for the specific pathogen infection.

8.2.2. Scope for future research

The indication of a possible correlation of *B. rossi* genotypes to disease phenotype was one of the interesting findings of this thesis. The significant suggestion of this work is that different parasite genotypes may cause differing host responses to infection (i.e. there could be a relationship between parasite genotypes and disease pathogenesis). Changing disease outcome through treatment depends heavily on understanding of disease pathogenesis. We have identified *BrEMA*1 as a valuable genetic marker for the diagnosis of virulent genotypes. The currently available test to date is however, a double PCR (18S rDNA PCR first, followed by *BrEMA*1 PCR). Thus, a real-time PCR-based test will have the advantages of being more sensitive, rapid and less time-consuming than our current double PCR test.

Furthermore, the *BrEMA*1 is located on the cytoplasmic side of red blood cell-membrane under an insoluble phosphoprotein. A soluble antigen found in the supernatant of *B. rossi in vitro* culture is also present, which is immunogenic to the hyperimmune serum from *B. rossi* infected dogs. Moreover, like many repetitive regions of parasitic antigens, the recombinant repetitive region of *BrEMA*1 is highly immunogenic. Currently, there is no ELISA test available to evaluate the incidence of babesiosis in South Africa at the immunological level. The recombinant *BrEMA*1 is a valuable antigen for the development of such test.

The relationship between virulence and sequestration (by molecular and cellular analysis of the surface of the infected erythrocytes and by identifying parasite proteins present on

the membrane of the infected erythrocyte by electron microscopic analysis of the surface modifications) needs to be investigated. To date, available data concerning the function of adhesive antigens, located on the extracellular side of infected red blood cell-membrane, in the pathogenicity of apicomplexan haemoparasites is mostly derived from the human malaria parasite *P. falciparum* and, to a lesser extent, from the bovine babesiosis parasite *B. bovis.* Adhesive surface antigens from the extracellular side of the sequestering canine *Babesia* species *B. rossi* are unknown. This antigen needs to be characterized, not only for a better understanding of mechanisms governing the pathogenicity of *B. rossi* in dogs, but also of the genus *Babesia* in general.

Preliminary evidence presented in this thesis indicated the presence of a *Theileria* species which may be linked to a haemolytic and immune-mediated syndrome. Although the parasite was detected from a number of samples, only three samples were from dogs showing clinical signs. The clinical significance of this *Theileria* species needs to be investigated. All the *Theileria*-positive dogs had a common feature in that they each had thrombocytopenia in the absence of *Ehrlichia* infection. Dogs that are presented with an unexplained thrombocytopenia should be selected and sampled. Attempts should be made to isolate the parasite and adapt it to *in vitro* cultures. Experimental tick-transmission trials should be undertaken, to establish the tick vector. Additionally, the life cycle of this *Theileria* species could be investigated by conducting experimental infections in dogs.

Although there is no evidence to link *B. vogeli* to severe clinical disease in South Africa, it has been linked to significant clinical disease in the USA and Australia, areas from which *B. rossi* infections are known not to occur. The hypothesis is that in the absence of *B. rossi*, *B. vogeli* can cause a significant clinical disease. There is a need to compare the different *B. vogeli* isolates from different regions, especially those areas free of *B. rossi*. This will provide information on genetic diversity. Correlations could also be made between virulent genotypes and clinical manifestations.

Finally, the work done in this thesis has shown that populations of dogs that live in tick-endemic areas are exposed to single or multiple tick-borne pathogens. These pathogens continue to cause morbidity and mortality in susceptible dogs. Correct diagnosis (supported by molecular diagnostic tools) followed by appropriate treatment offers a better understanding and management of these tick-borne pathogens. Preventative measures either by vaccination or transmission-blocking acaricidal treatments should be fully evaluated and applied to prevent these tick-borne pathogens from adversely affecting the canine population in South Africa and elsewhere.

8.3. References

Apanaskevich, D.A., Horak, I.G., Camica J.-L., 2007. Redescription of *Haemaphysalis* (*Rhipistoma*) *elliptica* (Koch, 1844), an old taxon of the *Haemaphysalis* (*Rhipistoma*) *leachi* group from East and Southern Africa, and of *Haemaphysalis* (*Rhipistoma*) *leachi* (Audouin, 1826) (Ixodida, Ixodidae). Onderstepoort Journal of Veterinary Research 74, 181-208.

Boozer, A.L., MacIntire, D.K., 2003. Canine babesiosis. Veterinary Clinics of the North American Small Animal Practice 33, 885-904.

Brandao, L.P., Hagiwara, M.K., Myiashiro, S.I., 2003. Humoral immunity and re-infection resistance in dogs experimentally inoculated with *Babesia canis* and either treated or untreated with imidocarb dipropionate. Veterinary Parasitology 114, 253-265.

Garcia, A.T.C., 2006. Piroplasma-infection in dogs in northern Spain. Veterinary Parasitology 138, 97-102.

Horak, I.G., 1995. Ixodid ticks collected at the Faculty of Veterinary Science, Onderstepoort, from dogs diagnosed with *Babesia canis* infection. Journal of the South African Veterinary Association 66, 170-171.

Inokuma, H., Oyamada, M., Kelly, P.J., Jacobson, L.A., Fournier, P.E., Itamoto, K., Okuda, M., Brouqui, P., 2005. Molecular detection of a new *Anaplasma* species closely related to *Anaplasma phagocytophilum* in canine blood from South Africa. Journal of Clinical Microbiology 43, 2934-2937.

Kjemtrup, A.M., Kocan, A.A., Whitworth, L., Meinkoth, J., Birkenheuer, A.J., Cummings, J., Boudreaux, M.K., Stockham, S.L., Irizarry-Rovira, A., Conrad, P.A.,

2000. There are at least three genetically distinct small piroplasms from dogs. International Journal of Parasitology 30, 1501-1505.

Last, R.D., Hill, J.M., Matjila, P.T., Reme, C.A., 2007. A field trial evaluation of the prophylactic efficacy of amitraz-impregnated collars against canine babesiosis (*Babesia canis rossi*) in South Africa. Journal of the South African Veterinary Association 78, 63-65.

Nijhof, A.M., Bodaan, C., Postigo, M., Nieuwenhuijs, H., Opsteegh, M., Franssen, L., Jebbink, F., Jongejan, F., 2007. Ticks and associated pathogens collected from domestic animals in the Netherlands. Vector-Borne and Zoonotic Diseases 7, 585-596.

Nijhof, A.M., Pillay, V., Steyl, J., Prozesky, L., Stoltsz, W.H., Lawrence, J.A., Penzhorn, B.L., Jongejan, F., 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. J. Clin. Microbiol. 43, 5907-5911.

Parola, P., Inokuma, H., Camicas, J.L., Brouqui, P., Raoult, D., 2001. Detection and identification of spotted fever group Rickettsiae and Ehrlichiae in African ticks. Emerging Infectious Diseases 7, 1014-1017.

Penzhorn, B.L., Lewis, B.D., De Waal, D.T., Lopez Rebollar, L.M., 1995. Sterilisation of *Babesia canis* infections by imidocarb alone or in combination with diminazene. Journal of the South African Veterinary Association 66, 157-159.

Pretorius, A.M., Venter, T.P., Ryst, E.V.D., Kelly, P.J., 1999. Possible human ehrlichiosis in the Free State. South African Medical Journal 89, 961-961.

Schetters, T.P., Kleuskens, J., Carcy, B., Gorenflot, A., Vermeulen, A., 2007. Vaccination against large *Babesia* species from dogs. Parassitologia 49, 13-19.

Schetters, T.P., Kleuskens, J.A., Scholtes, N.C., Gorenflot, A., Moubri, K., Vermeulen, A.N., 2001. Vaccination of dogs against heterologous *Babesia canis* infection using antigens from culture supernatants. Veterinary Parasitology 100, 75-86.

Schetters, T.P.M., Kleuskens, J.A.G.M., Scholtes, N.C., Pasman, J.W., Goovaerts, D., 1997. Vaccination of dogs against *Babesia canis* infection. Veterinary Parasitology 73, 35-41.

Schetters, T.P., Montenegro-James, S., 1995. Vaccines against babesiosis using soluble parasite antigens. Parasitology Today 11, 456-462.

Schetters, T., Strydom, T., Crafford, D., Kleuskens, J.A.G.M., Van de Crommert, J., Vermeulen, A.N., 2007. Immunity against *Babesia rossi* infection in dogs vaccinated with antigens from culture supernatants. Veterinary Parasitology 144, 10-19.

Uilenberg, G., Franssen, F.F., Perie, N.M., Spanjer, A.A., 1989. Three groups of *Babesia canis* distinguished and a proposal for nomenclature. Veterinary Quarterly 11, 33-40.

Wozniak, E.J., Barr, B.C., Thomford, J.W., Yamane, I., McDonough, S.P., Moore, P.F., Naydan, D., Robinson, T.W., Conrad, P.A., 1997. Clinical, anatomic, and immunopathologic characterization of *Babesia gibsoni* infection in the domestic dog (*Canis familiaris*). Journal of Parasitology 83, 692-699.

Scientific publications connected with this thesis

Matjila, P.T., Penzhorn, B.L., Bekker, C.P., Nijhof, A.M., Jongejan, F., 2004. Confirmation of occurrence of *Babesia canis vogeli* in domestic dogs in South Africa. Veterinary Parasitology 122, 119-125.

Matjila, T.P., Nijhof, A.M., Taoufik, A., Houwers, D., Teske, E., Penzhorn, B.L., De Lange, T.D., Jongejan, F., 2005. Autochthonous canine babesiosis in The Netherlands. Veterinary Parasitology 131, 23-29.

Matjila, P.T., Penzhorn, B.L., Leisewitz, A.L., Bhoora, R., Barker, R., 2007. Molecular characterization of *Babesia gibsoni* infection from a pit-bull terrier pup recently imported into South Africa. Journal of the South African Veterinary Association 78, 2-5.

Matjila, P.T., Leisewitz A.L., Jongejan, F., Penzhorn, B.L., 2008. Molecular detection of tick-borne protozoal and ehrlichial infections in domestic dogs in South Africa. Veterinary Parasitology 155, 152-157.

Matjila, P.T., Leisewitz A.L., Oosthuizen, M.C., Jongejan, F., Penzhorn, B.L., 2008. Detection of a *Theileria* species in dogs in South Africa. Veterinary Parasitology 157, 34-40.

Matjila, P.T., Carcy, B., Leisewitz A.L., Schetters, T., Jongejan, F., Penzhorn, B.L., 2008. Correlation between *Babesia rossi* genotypes and clinical canine babesiosis (Submitted) Journal of Clinical Microbiology.