Host-Parasite Interactions in Vector-borne Protozoan Infections

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Protists embrace many species, some of which may be either occasional or permanent parasites of vertebrate animals. Between the parasite species, several of medical and veterinary importance are vector-transmitted. The ecology and epidemiology of vector-borne parasitoses, including babesiosis, leishmaniasis and malaria, are particularly complex, as they are influenced by many factors, such as vector reproductive efficiency and geographical spread, vectorial capacity, host immunity, travel and human behaviour and climatic factors. Transmission dynamics are determined by the interactions between pathogen, vector, host and environmental factors and, given their complexity, many different types of mathematical models have been developed to understand them. A good basic knowledge of vector-pathogen relationships and transmission dynamics is thus essential for disease surveillance and control interventions and may help in understanding the spread of epidemics and be useful for public health planning.

1. *Babesiosis and Babesia spp. of Domestic Dogs – Where do they Fit in the Piroplasmid World?*

Babesia are tick-borne protozoan parasites from the phylum Apicomplexa, class Piroplasmea and order Piroplasmida which infect erythrocytes of animals and humans. The main clinical manifestations of babesiosis in animals and humans are associated

with hemolysis, anemia, tissue anoxia, hemolytic toxins and inflammatory mediators produced during infection (Irwin, 2009; Lempereur et al., 2017). Babesiosis is a medically important tick-borne disease of humans in several areas of the world and it also has a major economic impact to livestock and agriculture (Lempereur et al., 2017; Wagner et al., 2019). *Babesia* spp. are an important threat in human and veterinary medicine as they are transmitted via blood transfusion from donors to recipients (Wagner et al., 2019; Wardrop et al., 2016). The genera *Babesia*, *Theileria* and *Cytauxzoon* are genetically related to each other and are referred to collectively as piroplasmids. Parasites belonging to these three genera infect erythrocytes, however, while the *Theileria* and *Cytauxzoon* have schizont stages that infect other types of cells, *Babesia* spp. infect only erythrocytes. There is currently a motion to reorganize parasites belonging to the Piroplasmida into several genetically distinct clades and possible future different genera dividing also the *Babesia* into more than one genus (Jalovecka et al., 2019). The currently known *Babesia* spp. which infect dogs are a good example of a group of parasites that often have similar characteristics, however they differ genetically and are likely to be assigned to different and diverging genera in the future (Baneth, 2011; Irwin, 2009; Solano-Gallego and Baneth, 2011).

The canine babesiae are divided into several lineages as assessed by *18S* RNA gene phylogeny (Jalovecka et al., 2019). The babesial spp. that infect dogs can also be separated into those that present with a large merozoite stage in erythrocytes (approximately 5 x 2 µm), termed “large” canine *Babesia*, which belong to the *Babesia* sensu stricto and those which have distinctly smaller merozoites (approximately 0.3 x 3 µm) termed “small” and classified mostly as *Babesia* sensu lato (Baneth, 2011, 2018; Jalovecka et al., 2019; Solano-Gallego and Baneth, 2011). The clinical manifestations of canine babesiosis are mainly dependant on the infecting species and host-related

factors. *Babesia rossi, B. canis* and *B. vogeli* have large merozoites and are identical morphologically but differ in the severity of clinical manifestations which they cause, their tick vectors, genetic characteristics, and geographic distributions (Baneth, 2011, 2018; Irwin, 2009; Solano-Gallego and Baneth, 2011). Another yet unnamed large *Babesia* sp. most closely related to *B. bigemina* of cattle infects immunocompromised dogs in North America (Sikorski et al., 2010). The small *Babesia* spp. that infect dogs include *B. gibsoni*, *B. conradae* described from California, *B. vulpes* which primarily infects foxes and *B. negevi* described from Israel (Baneth, 2011, 2018; Baneth et al., 2020; Irwin, 2009; Solano-Gallego and Baneth, 2011). None of the *Babesia* spp. that infect dogs is known to be zoonotic. Although the transmission of canine babesiae occurs through the bite of a vector tick, infection has also been demonstrated to be transmitted via blood transfusion and transplacentally for some species (Baneth, 2011; Irwin, 2009; Solano-Gallego and Baneth, 2011; Wardrop et al., 2016). Furthermore, several studies have provided evidence that *B. gibsoni* is likely transmitted directly from dog to dog via bite wounds, saliva, or ingested blood (Birkenheuer et al., 2005; Jefferies et al., 2007). As *Babesia* spp. are transmitted by blood product transfusions, it is recommended to screen canine blood donors for infection (Wardrop et al., 2016). Non-vectorial dog to dog transmission of different *Babesia* spp. by fighting and blood transfusion can be responsible for the spread of babesiosis into non-endemic areas where their tick vectors are not present. The differences between *Babesia* spp. that infect dogs are also reflected in their susceptibility to drugs and therefore accurate detection and species recognition are important for the selection of the correct therapy and predicting the course of disease. While large *Babesia* spp. are usually susceptible to certain drugs such as imidcorab dipropionate and diminazene aceturate, small

Babesia spp. are often resistant and treatment requires the use of other drugs and their combinations such as atovaquone with azithromycin (Baneth, 2018).

In conclusion, the variety of *Babesia* spp. which infect dogs represent a microcosm of the total variety of species which infect wildlife, domestic animals and humans. The similarities and differences between the diseases caused by these agents and the precautions taken to prevent transmission of babesiosis by blood transfusion in dogs are also applicable to human medicine where the same problem occurs.

2. *New Leishmania Parasites and New Vectors.*

Leishmania are protozoan parasites that are transmitted by the bites of phlebotomine sand flies, small blood-feeding dipteran insects (Bates, 2007). In their vertebrate hosts *Leishmania* live as intracellular parasites inside various cells, typically macrophages, residing within phagolysosomes as amastigote life-cycle stages. Female sand flies, when seeking a blood meal, will cause damage to the skin of the vertebrate host, causing the release of infected cells containing amastigotes into the wound, which are then taken up with the blood meal. In the midgut of the sand fly amastigotes transform into promastigotes, elongated and motile forms that go through various life-cycle stages to complete their development in the vector (Bates, 2018). The final stage in the sand fly host are the metacyclic promastigotes, infective forms that are transmitted by bite when the sand fly takes another blood meal.

Over 30 species of *Leishmania* have been described, and of these 11 are of major biomedical importance: *L. donovani* and *L. infantum*, which cause visceral leishmaniasis; *L. major*, *L. tropica* and *L. aethiopica*, which cause cutaneous leishmaniasis in Europe, African and Asia; *L. mexicana*, *L. amazonensis*, *L. panamensis*, *L. guyanensis* and *L. peruviana*, which cause cutaneous leishmaniasis in

Central and South America; and *L. braziliensis*, which cause cutaneous and mucocutaneous leishmaniasis in Central and South America (Alvar et al., 2012). These 11 species cause the majority of the symptomatic human cases of leishmaniasis in the world and, therefore, have been the most intensively studied to date.

Leishmania spp. can infect a wide variety of vertebrate hosts depending on the particular species of parasite, and these hosts can include various mammals such as domestic and wild canines (*Dusicyon thous*, *Vulpes vulpes*), rodents (*Ototylomys phyllotis*, *Proechimys guyanensis*, *Psammomys obesus*, *Rhombomys opimus*), hyraxes (*Heterohyrax brucei*, *Procavia capensis*), sloths (*Choloepus didactylus*, *C. hoffmani*), opossums (*Didelphis marsupialis*) and anteaters (*Tamandua tetradactyla*), as well various lizards (Ashford, 2000). Some of these are dead end hosts, but others are true intermediate hosts acting as a potential source of parasites for feeding sand flies and continuing the life-cycle. Similarly there are many different species of sand fly involved in the transmission of leishmaniasis, with approximately 70 identified as vectors (Maroli et al., 2013). There are specific associations between vectors and the parasites they transmit, such that each parasite is transmitted by a small number of vector species, but each vector can only transmit one species of parasite. Transmission occurs in a wide variety of environments around the world including semi-arid through to tropical rainforest environments, and of increasing concern also in urban environments.

Leishmania spp. have, until recently, been placed into one of three subgenera: *Leishmania*, *Viannia* and *Sauroleishmania*. However, recent work has provided evidence for a new phylogenetic grouping of *Leishmania* parasites, placed in the new subgenus *Mundinia* (Espinosa et al., 2018). Species in the *Mundinia* include *L. enriettii* from guinea pigs, *Cavia porcellus*, in Brazil; (Lainson, 1997), *L. martiniquensis* from humans in Martinique, but now known to have a wider distribution; (Pothirat et al.,

2014), *L. macropodum* from kangaroos in Australia; (Rose et al., 2004), *L. orientalis* from humans in Thailand; (Jariyapan et al., 2018), and two as yet unnamed *Leishmania* species from Ghana and Namibia. Some of these parasites are known human pathogens, whilst others appear to be confined to wild animals. The very wide geographical spread of the *Mundinia*, wider than any of the other subgenera, together with their branching from the base of the *Leishmania* phylogenetic tree, both suggest an early branching and ancient lineage (Pothirat et al., 2014). The insect vectors of the *Mundinia* have not been established with certainty for any member.

L. enriettii is the best known of these species, first isolated in 1948 (Lainson, 1997). It has been found only in domestic guinea pigs in Curitiba and Sao Paulo, but assumed to occur in related wild rodents. *L. enriettii* causes cutaneous leishmaniasis in guinea pigs, but it is not pathogenic to humans. *L. martiniquensis* has been isolated from humans in Martinique and Thailand, but with evidence that it also occurs in both Europe and the USA in horses and cattle (Pothirat et al., 2014). In both of these human foci the majority of cases present as disseminated or visceral leishmaniasis in immunocompromised (HIV-infected) patients. *L. orientalis* from Thailand causes cutaneous leishmaniasis, but again can occur as disseminated or visceral forms in HIV-infected individuals (Jariyapan et al., 2018). The currently un-named *Leishmania* from Ghana causes typical cutaneous leishmaniasis in humans living in the Volta region, the infections are usually self-healing but can leave unsightly scars (Kwakye-Nuako et al., 2015). The *Leishmania* from Namibia were originally isolated in the 1970s by Grové and colleagues. DNA sequencing showed that the human and sand fly Namibian isolates were *L. tropica*, but an isolate from a rock hyrax is another member of the *Mundinia* (Bates, unpublished data). *L. macropodum* has been found in a variety of marsupials in northern Australia, causing cutaneous leishmaniasis in these animals, but does not

appear to be pathogenic in humans. This *Mundinia* species is the only one for which there is field evidence of the vector identity to date, and interestingly, the evidence to date supports the vectors of *L. macropodum* to be day-biting midges of the genus *Forcipmyia* (Dougall et al., 2011). However, this is not conclusive evidence as transmission by midge-bite has not been demonstrated.

So far other efforts to identify the vectors of *Mundinia* in the field have proved inconclusive. However, various laboratory investigations have been performed using model vectors. For example, *L. enriettii* is unable to establish mature infections in *Lutzomyia longipalpis*, a widely used permissive sand fly host. However, *Leishmania* from Ghana, Thailand and *L. enriettii* are all able to develop better in experimental midge vectors (*Culicoides*) than in *Lu. longipalpis* under laboratory conditions, supporting that the midges might be vectors (Chanmol et al., 2019; Seblova et al., 2015). On the other hand some species of *Sergentomyia* sand flies have been found PCR-positive in Ghana and Thailand. Therefore, although there is still uncertainty around the vectors for *Mundinia* species, the current evidence indicates the *Leishmania* (*Mundinia*) have non-conventional vectors. Their vectors appear unlikely to be *Lutzomyia* or *Phlebotomus* spp., the usual vectors. Further, it appears that some of the *Leishmania* (*Mundinia*) may even have non-sand fly vectors. This remains to be proven or disproven, however, if true, this would require a re-definition of the genus *Leishmania* to include non-sand fly transmission or the creation of a new genus to accomodate the non-sand fly transmitted parasites.

In conclusion, the discovery of a new group of *Leishmania* species, now placed in the subgenus *Mundinia*, was something of a surprise. The number of clinical cases remains small in comparison to the 11 well known species listed above. However, in addition to their intrinsic biological interest there are important reasons to study these species

from public health and biomedical perspectives. First, it is clear that in HIV-immunocompromised individuals they can cause serious and fatal infections. Second, it is likely that there is potentially widespread subclinical infection with these species in humans, which could have either beneficial or detrimental effects if a subsequent infection with a more pathogenic species occurs. Finally, the *Mundinia* present a good opportunity to use comparative genomics to explore the basis of pathogenicity, as the group contains species that are pathogenic and non-pathogenic to humans (Butenko et al., 2019).

3. *Plasmodium development in the mosquito midgut. A molecular and cellular view.*

Malaria is a devastating disease caused by unicellular protozoa, belonging to the genus *Plasmodium*. In 2018, WHO reported 220 million malaria cases globally and 405 thousand lethal outcomes, mainly in children under 5 years of age (WHO, 2018). Even though between years 2000 and 2015, the global number of malaria cases significantly decreased and the number of malaria deaths had a decline of 60%, no significant progress in reducing global malaria cases was made for the period 2015–2018. This is due to several factors, such as low efficiency of the available malaria vaccine, spread of insecticide resistant mosquito vectors and drug resistant parasite strains, and inadequate monitoring of asymptomatic cases. Some high-burden countries even reported an increase in the number of cases between 2016 and 2017: of these, Nigeria, Madagascar and the Democratic Republic of the Congo had an estimated increase greater than half a million cases (WHO, 2018). One of the main cause of this surge is that malaria is concentrated in countries with the least resourced health systems. Democratic Republic of the Congo and Nigeria, both between the lowest income

countries in Africa, together account for more than 35% of the total malaria deaths (WHO, 2018).

It is now established that malaria long-term elimination cannot be achieved without controlling parasite transmission to mosquitoes and that we cannot rely only on vector control (Baird, 2019; Cohen et al., 2012). The discovery of new drugs to block malaria transmission is considered essential in the fight against the disease. In addition, drugs targeting transmission have the dual advantage of lowering parasite transmission and preventing the spread of drug-resistant parasites (Lu et al., 2018).

Plasmodium asexual stages are responsible for the clinical manifestations of the disease, while the sexual stages, called gametocytes, are essential for parasite transmission to the mosquito vector. Malaria transmission is highly efficient: individuals with very low gametocyte densities, even undetectable by microscopy, can be infectious to mosquitoes (Bousema and Drakeley, 2011; Schneider et al., 2007). Monitoring individuals able to transmit the disease is thus difficult, in particular in countries where molecular diagnostic methods are not available. Malaria transmission dynamics are difficult to predict, since they are affected by many factors, such as climate (temperature, humidity and rainfalls), parasite and mosquito strains present in an area and different susceptibility to infection of human populations. Moreover, most of the available antimalarial drugs do not kill mature transmission stages and rather increase gametocyte production (Adjalley et al., 2011; Bousema and Drakeley, 2011; Plouffe et al., 2016). This, together with the fact that treated patients remain infectious for weeks after clinical symptoms disappearance (Bousema and Drakeley, 2011), makes malaria transmission control difficult to achieve.

When ingested by mosquitoes, parasites differentiate into gametes and egress from the host cell to mate and form a zygote. During this whole process, parasites will have to

survive outside the host cell in the mosquito midgut lumen for about 24 hours and defend themselves against the human immune system, the microbial flora and innate immune system of the insect. This exposure leads to an approximate 300-fold loss of parasite abundance, representing a bottleneck in the parasite life cycle (Smith et al., 2014). In this context, even a slight reduction in efficiency may dramatically affect parasite survival. The midgut stages are thus viewed as prime targets for transmission-blocking interventions. For mating to occur, gametes must egress from the host cell. This process takes place by successive inside-out rupture of the two membranes surrounding the parasite, the parasitophorous vacuole membrane and the host cell membrane (Andreadaki et al., 2018). A few minutes before egressing, some parasite secretory organelles, the osmiophilic bodies (OBs), migrate to the cell periphery and release their content in the parasitophorous vacuole lumen (Olivieri et al., 2015). We studied these secretory organelles involved in egress and contributed to their characterization in previous studies on *Plasmodium berghei* gametocytes. In male gemetocytes, OBs are club-shaped vesicles, smaller than the oval-shaped female OBs. We named these vescicles *male osmiophilic bodies* (MOBs). Upon gametocyte activation, OBs accumulate at the parasite plasma membrane in multiple foci prior to secreting their contents into the parasitophorous vacuole. In contrast, MOBs cluster together to form larger structures and then release their contents at only a few focal points (Olivieri et al., 2015). These different behaviors likely reflect specific mechanisms of vesicle discharge.

In the search for novel potential drug target to block transmission, we analysed by proteomic analysis the proteins released by *P. berghei* gametes upon egress from the host cell. Between the proteins identified, we focused our attention on SUB1, a subtilisin-like serine protease known to be essential for egress of parasite asexual stages

from the host cell (Yeoh et al., 2007). SUB1 subcellular localization, function and potential substrates in the sexual stages were unknown.

We then characterized the expression profile and subcellular localization of SUB1 in *P. berghei* sexual stages. We showed that the protease is selectively expressed in mature male gametocytes and localizes to the secretory organelles involved in gamete egress, the osmiophilic bodies (Pace et al., 2019). We have investigated SUB1 function in the sexual stages by generating *P. berghei* transgenic lines deficient in SUB1 expression or enzyme activity in gametocytes and demonstrated that SUB1 plays an important role in male gamete egress (Pace et al., 2019). We also show for the first time that the SUB1 substrate PbSERA3 is expressed in gametocytes and processed by SUB1 also in these stages, upon gametocyte activation (Pace et al., 2019).

Taken together, our results strongly suggest that SUB1 is not only a promising drug target for asexual stages, but can also be considered an attractive malaria transmission-blocking target.

Conclusions:

This review aimed at elaborating on different aspects of three vector-borne parasitoses, babesiosis, leishmaniaisis and malaria, with a focus on the relationships between the parasites causing these diseases and their different vectors.

We discussed the variety of *Babesia* spp. that infect dogs and represent a microcosm of the total variety of species which infect humans, domestic and wildlife animals. The similarities and differences between the diseases caused by piroplasmid agents in dogs and the precautions taken to prevent their transmission by blood transfusion can provide useful information also for human medicine.

We also described a new group of *Leishmania* parasites recently discovered, forming the new subgenus *Mundinia*. These include both human pathogens and non-pathogenic species and raise interest because of their unusual insect vectors.

Finally, we discussed some critical aspects of malaria transmission, with a specific focus on molecular mechanisms of *P. berghei* transmission to mosquito vectors.

Information, education and public awareness are crucial elements in reducing the burden of vector-borne parasitoses. A lack of knowledge, both among patients and physicians, can increase transmission levels and lead to misdiagnosis and improper treatment. Therefore, a deep understanding of host-parasite interactions is essential to elucidate the complex dynamics of vector-borne parasite transmission.

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References

Adjalley, S.H., Johnston, G.L., Li, T., Eastman, R.T., Ekland, E.H., Eappen, A.G., Richman, A., Sim, B.K., Lee, M.C., Hoffman, S.L., *et al.* (2011). Quantitative assessment of *Plasmodium falciparum* sexual development reveals potent transmission-blocking activity by methylene blue. Proc Natl Acad Sci U S A *108*, E1214-1223.

Alvar, J., Vélez, I.D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., den Boer, M., and Team, W.L.C. (2012). Leishmaniasis worldwide and global estimates of its incidence. PLoS One *7*, e35671.

Andreadaki, M., Hanssen, E., Deligianni, E., Claudet, C., Wengelnik, K., Mollard, V., McFadden, G.I., Abkarian, M., Braun-Breton, C., and Siden-Kiamos, I. (2018). Sequential Membrane Rupture and Vesiculation during *Plasmodium berghei* Gametocyte Egress from the Red Blood Cell. Sci Rep *8*, 3543.

Ashford, R.W. (2000). The leishmaniases as emerging and reemerging zoonoses. Int J Parasitol *30*, 1269-1281.

Baird, J.K. (2019). Essential guidance on malaria elimination in its history. J Vector Borne Dis *56*, 11-14.

Baneth, G. (2011). Perspectives on canine and feline hepatozoonosis. Vet Parasitol *181*, 3-11.

Baneth, G. (2018). Antiprotozoal treatment of canine babesiosis. Vet Parasitol *254*, 58-63.

Baneth, G., Nachum-Biala, Y., Birkenheuer, A.J., Schreeg, M.E., Prince, H., Florin-Christensen, M., Schnittger, L., and Aroch, I. (2020). A new piroplasmid species infecting dogs: morphological and molecular characterization and pathogeny of *Babesia negevi* n. sp. Parasit Vectors *13*, 130.

Bates, P.A. (2007). Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. Int J Parasitol *37*, 1097-1106.

Bates, P.A. (2018). Revising *Leishmania*'s life cycle. Nat Microbiol *3*, 529-530.

Birkenheuer, A.J., Correa, M.T., Levy, M.G., and Breitschwerdt, E.B. (2005). Geographic distribution of babesiosis among dogs in the United States and association with dog bites: 150 cases (2000-2003). J Am Vet Med Assoc *227*, 942-947.

Bousema, T., and Drakeley, C. (2011). Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clin Microbiol Rev *24*, 377-410.

Butenko, A., Kostygov, A.Y., Sádlová, J., Kleschenko, Y., Bečvář, T., Podešvová, L., Macedo, D.H., Žihala, D., Lukeš, J., Bates, P.A., *et al.* (2019). Comparative genomics of *Leishmania* (*Mundinia*). BMC Genomics *20*, 726.

Chanmol, W., Jariyapan, N., Somboon, P., Bates, M.D., and Bates, P.A. (2019). Development of *Leishmania orientalis* in the sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae) and the biting midge *Culicoides soronensis* (Diptera: Ceratopogonidae). Acta Trop *199*, 105157.

Cohen, J.M., Smith, D.L., Cotter, C., Ward, A., Yamey, G., Sabot, O.J., and Moonen, B. (2012). Malaria resurgence: a systematic review and assessment of its causes. Malar J *11*, 122.

Dougall, A.M., Alexander, B., Holt, D.C., Harris, T., Sultan, A.H., Bates, P.A., Rose, K., and Walton, S.F. (2011). Evidence incriminating midges (Diptera: Ceratopogonidae) as potential vectors of *Leishmania* in Australia. Int J Parasitol *41*, 571-579.

Espinosa, O.A., Serrano, M.G., Camargo, E.P., Teixeira, M.M.G., and Shaw, J.J. (2018). An appraisal of the taxonomy and nomenclature of trypanosomatids presently classified as *Leishmania* and *Endotrypanum*. Parasitology *145*, 430-442.

Irwin, P.J. (2009). Canine babesiosis: from molecular taxonomy to control. Parasit Vectors *2 Suppl 1*, S4.

Jalovecka, M., Sojka, D., Ascencio, M., and Schnittger, L. (2019). *Babesia* Life Cycle - When Phylogeny Meets Biology. Trends Parasitol *35*, 356-368.

Jariyapan, N., Daroontum, T., Jaiwong, K., Chanmol, W., Intakhan, N., Sor-Suwan, S., Siriyasatien, P., Somboon, P., Bates, M.D., and Bates, P.A. (2018). *Leishmania* (*Mundinia*) *orientalis* n. sp. (Trypanosomatidae), a parasite from Thailand responsible for localised cutaneous leishmaniasis. Parasit Vectors *11*, 351.

Jefferies, R., Ryan, U.M., Jardine, J., Broughton, D.K., Robertson, I.D., and Irwin, P.J. (2007). Blood, Bull Terriers and Babesiosis: further evidence for direct transmission of *Babesia gibsoni* in dogs. Aust Vet J *85*, 459-463.

Kwakye-Nuako, G., Mosore, M.T., Duplessis, C., Bates, M.D., Puplampu, N., Mensah-Attipoe, I., Desewu, K., Afegbe, G., Asmah, R.H., Jamjoom, M.B., *et al.* (2015). First isolation of a new species of *Leishmania* responsible for human cutaneous leishmaniasis in Ghana and classification in the *Leishmania enriettii* complex. Int J Parasitol *45*, 679-684.

Lainson, R. (1997). On *Leishmania enriettii* and other enigmatic *Leishmania* species of the Neotropics. Mem Inst Oswaldo Cruz *92*, 377-387.

Lempereur, L., Beck, R., Fonseca, I., Marques, C., Duarte, A., Santos, M., Zúquete, S., Gomes, J., Walder, G., Domingos, A., *et al.* (2017). Guidelines for the Detection of *Babesia* and *Theileria* Parasites. Vector Borne Zoonotic Dis *17*, 51-65.

Lu, G., Nagbanshi, M., Goldau, N., Mendes Jorge, M., Meissner, P., Jahn, A., Mockenhaupt, F.P., and Müller, O. (2018). Efficacy and safety of methylene blue in the treatment of malaria: a systematic review. BMC Med *16*, 59.

Maroli, M., Feliciangeli, M.D., Bichaud, L., Charrel, R.N., and Gradoni, L. (2013). Phlebotomine sandflies and the spreading of leishmaniases and other diseases of public health concern. Med Vet Entomol *27*, 123-147.

Olivieri, A., Bertuccini, L., Deligianni, E., Franke-Fayard, B., Curra, C., Siden-Kiamos, I., Hanssen, E., Grasso, F., Superti, F., Pace, T., *et al.* (2015). Distinct properties of the egress-related osmiophilic bodies in male and female gametocytes of the rodent malaria parasite *Plasmodium berghei*. Cell Microbiol *17*, 355-368.

Pace, T., Grasso, F., Camarda, G., Suarez, C., Blackman, M.J., Ponzi, M., and Olivieri, A. (2019). The *Plasmodium berghei* serine protease PbSUB1 plays an important role in male gamete egress. Cell Microbiol *21*, e13028.

Plouffe, D.M., Wree, M., Du, A.Y., Meister, S., Li, F., Patra, K., Lubar, A., Okitsu, S.L., Flannery, E.L., Kato, N., *et al.* (2016). High-Throughput Assay and Discovery of Small Molecules that Interrupt Malaria Transmission. Cell Host Microbe *19*, 114-126.

Pothirat, T., Tantiworawit, A., Chaiwarith, R., Jariyapan, N., Wannasan, A., Siriyasatien, P., Supparatpinyo, K., Bates, M.D., Kwakye-Nuako, G., and Bates, P.A. (2014). First isolation of *Leishmania* from Northern Thailand: case report, identification as *Leishmania martiniquensis* and phylogenetic position within the *Leishmania enriettii* complex. PLoS Negl Trop Dis *8*, e3339.

Rose, K., Curtis, J., Baldwin, T., Mathis, A., Kumar, B., Sakthianandeswaren, A., Spurck, T., Low Choy, J., and Handman, E. (2004). Cutaneous leishmaniasis in red kangaroos: isolation and characterisation of the causative organisms. Int J Parasitol *34*, 655-664.

Schneider, P., Bousema, J.T., Gouagna, L.C., Otieno, S., van de Vegte-Bolmer, M., Omar, S.A., and Sauerwein, R.W. (2007). Submicroscopic *Plasmodium falciparum* gametocyte densities frequently result in mosquito infection. Am J Trop Med Hyg *76*, 470-474.

Seblova, V., Sadlova, J., Vojtkova, B., Votypka, J., Carpenter, S., Bates, P.A., and Volf, P. (2015). The Biting Midge *Culicoides sonorensis* (Diptera: Ceratopogonidae) Is Capable of Developing Late Stage Infections of *Leishmania enriettii*. PLoS Negl Trop Dis *9*, e0004060.

Sikorski, L.E., Birkenheuer, A.J., Holowaychuk, M.K., McCleary-Wheeler, A.L., Davis, J.M., and Littman, M.P. (2010). Babesiosis caused by a large *Babesia* species in 7 immunocompromised dogs. J Vet Intern Med *24*, 127-131.

Smith, R.C., Vega-Rodríguez, J., and Jacobs-Lorena, M. (2014). The *Plasmodium* bottleneck: malaria parasite losses in the mosquito vector. Mem Inst Oswaldo Cruz *109*, 644-661.

Solano-Gallego, L., and Baneth, G. (2011). Babesiosis in dogs and cats--expanding parasitological and clinical spectra. Vet Parasitol *181*, 48-60.

Wagner, S.J., Leiby, D.A., and Roback, J.D. (2019). Existing and Emerging Blood-Borne Pathogens: Impact on the Safety of Blood Transfusion for the Hematology/Oncology Patient. Hematol Oncol Clin North Am *33*, 739-748.

Wardrop, K.J., Birkenheuer, A., Blais, M.C., Callan, M.B., Kohn, B., Lappin, M.R., and Sykes, J. (2016). Update on Canine and Feline Blood Donor Screening for Blood-Borne Pathogens. J Vet Intern Med *30*, 15-35.

WHO (2018). World Malaria Report 2018.

Yeoh, S., O'Donnell, R.A., Koussis, K., Dluzewski, A.R., Ansell, K.H., Osborne, S.A., Hackett, F., Withers-Martinez, C., Mitchell, G.H., Bannister, L.H., *et al.* (2007). Subcellular discharge of a serine protease mediates release of invasive malaria parasites from host erythrocytes. Cell *131*, 1072-1083.