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Review article

Persistent human babesiosis with low-grade parasitemia, challenges for clinical diagnosis and management

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ABSTRACT

Human babesiosis, caused by several *Babesia* parasites and transmitted by tick bites and other blood-associated containments, has emerged as a major public health threat around the world. In the absence of readily discernible clinical manifestations, the diagnosis of human babesiosis has been contingent upon the identification of *Babesia* parasites through the utilization of detection arrays. Nevertheless, cases of persistent and relapsing babesiosis with low-grade parasitemia have been sporadically observed in patients with and without immunosuppression, prompting a challenge to the reliability of routine clinical laboratory tests and efficient anti-babesial therapy. In such instances, it is essential to implement repeated and prolonged monitoring until complete eradication of the parasites is achieved. This review presents an overview of the epidemiology of persistent and relapsing human babesiosis, current diagnostic techniques, the mechanism of persistent relapse and practical clinical management strategies. In order to respond effectively to the challenge of low-grade parasitemia infection in the aforementioned patients, it is essential to prioritize the development and validation of rapid, sensitive, and cost-effective point-of-care diagnostic techniques, as well as the development of novel pharmaceutical agents and their combinations.

1. Epidemiology of human babesiosis

Babesiosis represents a disease caused by intraerythrocytic protozoa belonging to the phylum Apicomplexa, transmitted by diverse Ixodid ticks. It is rarely transmitted by red blood cells (RBCs) transfusion, transplacentally from mother to fetus, and by organ transplantation [1–3]. Since its first description in Romanian cattle by Victor Babes in 1888, more than 100 *Babesia* species have been described [4,5]. Babesiosis was initially recognized as a disease harmful to livestock [6]. Specifically, six *Babesia* species have been found to be infectious to humans, including *B. microti*, *B. duncani*, *B. divergens*, *B. motasi* (KO-1) and *B. crassa*-like agent as well as

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B. venatorum (formerly *Babesia* sp. EU1) [3]. Meanwhile, two genetically related Babesiidae sub-strains, *B. divergens*-like MO1 and *B. microti*-like protozoa, have also been documented to infect humans [3]. Historically, the first human case of babesiosis was described by Skrabalo and Deanovic in Yugoslavia (now Croatia) in 1957, and the causative agent *B. divergens* was identified several years later [7]. Until the 1970s, a resident of Nantucket Island, Massachusetts, was literally described as the first clinical case of human babesiosis in the United States [8].

Human babesiosis has posed a significant health burden on public since its discovery [9]. In the United States, human babesiosis is listed as one of 120 nationally notifiable diseases, and surveillance data based on a standard case definition are reported annually to CDC through the National Notifiable Diseases Surveillance System (NNDSS) using a specific babesiosis case report form (CRF) approved by the Office of Management and Budget in 2011 [10]. Currently, over 3000 cases from 14 states account for the vast majority of babesiosis cases in the United States each year. Furthermore, the overwhelming majority of these cases are caused by *B. microti*, whose manifestations range from subclinical appearance to fulminating disease with a high mortality rate [11]. *B. duncani* infection presents with viral-like symptoms and causes a markedly severe illness, even fatal results in infected individuals [12]. While in Europe, quantitation of true babesiosis incidence remains a challenge as many countries has not listed it as a notifiable disease and asymptomatic manifestation are common in immunocompetent individuals [13]. Furthermore, *B. divergens*, the primary causative agent, usually causes fulminant disease in humans, and all but a few cases have been reported in patients with asplenia. The predisposing risk factors for *B. divergens* infection are largely attributed to extreme age and immunocompromised status, which may ultimately lead to mortality from multi-organ failure [14]. In Asia, human cases of babesiosis have been reported in China, India, Japan, Korea and Mongolia [3]. However, it is still unavailable to take a panoramic view of human babesiosis in Asia because much research has focused on the discovery of additional *Babesia* species and new endemic areas, rather than on comprehensive interpretation of the epidemiological studies of *Babesia* infections. Indeed, human babesiosis has emerged as a public health threat in China and both of *B. divergens* and *B. venatorum* frequently result in asymptomatic infections in individuals with intact immune systems [15,16]. In the latter, the manifestations are comparatively less severe [17]. Consequently, the actual prevalence of human babesiosis in China is likely to be on the rise [18,19]. Similarly, cases of autochthonous and imported human babesiosis have also been diagnosed in Australia and Africa (Egypt, Mozambique, South Africa, Cameroon, and Equatorial Guinea) [3]. The increase in human cases of babesiosis and the expansion of its geographic ranges are largely attributed to ecological factors, including the creation of adaptive niches for host (rodents, deer) and tick (*Ixodes persulcatus* complex) populations, increased human exposure to infected ticks or host animals, and social factors, with increased awareness of the disease and the improved diagnostic technologies [20].

2. Current diagnostic techniques for human babesiosis

Early diagnosis of symptomatic patients hastens appropriate anti-parasite therapy, which typically reduces the severity and duration of symptoms and helps prevent complications [21]. Typical clinical manifestations of human babesiosis include fever, fatigue, chills, sweats, headache, and anorexia [22], which are not easily recognized clinical features, such as the erythema migrant skin lesion of Lyme disease. Furthermore the symptoms have also been reported in other diseases caused by *Plasmodium* sp., bacteria, viruses, and other pathogens. Consequently, the diagnosis of babesiosis is typically considered in a suspected patient who experiences clinical symptoms consistent with documented babesiosis or has characteristic laboratory test abnormalities, coupled with a substantial exposure to an endemic area or tick bites, blood transfusions or organs transplanting operation [23]. Characteristic abnormalities on routine laboratory tests include anemia, thrombocytopenia, elevated liver enzymes (aspartate aminotransferase [AST], alanine transaminase [ALT], alkaline phosphatase [ALP]), and/or evidence of intravascular hemolysis (elevated lactate dehydrogenase [LDH], elevated total and indirect bilirubin levels, reduced haptoglobin) [24]. Following the Clinical Practice Guidelines (CPG), the diagnosis should be confirmed with the identification of *Babesia* parasites by microscopic evaluation of blood smears or amplification of *Babesia* DNA using a polymerase chain reaction (PCR) assay as described in the guidance of CDC-10473 [25]. Parasitemia is usually high enough for blood smear and PCR performance in patients with symptomatic babesiosis. Microscopic evaluation of *Babesia* parasites in blood smears requires skilled technicians and the result is highly dependent on the parasite load, microscopic fields examined and the experience of the technicians. The interpretation of these parasites can be challenging, particularly given their rarity in organized tetrads (Maltese cross forms), which are pathognomonic for babesiosis. If blood smears are negative and babesiosis is still suspected, a PCR test capable of detecting all *Babesia* species (a pan-*Babesia* PCR test) is recommended, as the species-specific PCR tests (e.g. *B. microti*) offered by most clinical laboratories may not be sufficient to make a correct diagnosis of different *Babesia* species [25]. In addition, another DNA molecular analysis developed by some commercial laboratories and parasitic disease referral units would be clinically validated and adopted [25].

3. Persistent relapsing babesiosis and its mechanisms

In addition to symptomatic babesiosis, a significant proportion of cases are asymptomatic, with patients either immunocompetent or immunocompromised [17]. Asymptomatic *Babesia* infection may persist for months or years in previously healthy patients, especially if the infection is not diagnosed and treated promptly [26]. Most immunocompetent patients infected with *Babesia* parasites usually resolve most symptoms of babesiosis and clear the infection with or without anti-babesial therapy. Their blood smears usually become negative during the 7 to 10-day course of standard anti-babesial therapy, but PCR may remain positive for months, even more than one or two years after completion of standard treatment [27]. The remaining few patients may endure persistent and relapsing babesiosis for months or years. Persistent babesiosis was first reported in an immunocompetent 59-year-old woman who experienced babesiosis symptoms for three weeks and had *B. microti* parasites in her blood smear for more than four months after symptoms had

resolved [8]. Peter J. Krause conducted the first retrospective study focused on 46 persistent babesiosis patients in southern New England [26]. In his study, a few patients experienced asymptomatic and low grade of parasitemia that lasted more than one year despite the administration of clindamycin and quinine. In patients with mild infection or subclinical manifestation, parasitemia of *B. microti* persisted for a period exceeding two years. This is in line with the findings in 56 asymptomatically infected blood donors [27]. In the immunocompromised patients, *Babesia* parasite infection tends to persistent and relapsing disease with severe complications. In a retrospective case-control study performed in southern New England, New York, and Wisconsin, Peter J. Krause et al. (2008) reported a series of immunocompromised patients who experienced persistent and relapsing symptomatic babesiosis despite the use of standard anti-babesial therapy. Their findings conclusively demonstrated that the impaired anti-babesial antibody response, in the context of overall host immunosuppression, is the primary factor preventing the clearance of *Babesia* infection [28]. In 2015, Raffalli and Wormser reported that a patient treated with rituximab for rheumatoid arthritis had *B. microti* infection for 26 months despite prolonged anti-babesial therapy. They attributed the persistence of *Babesia* infection to the long-term immunosuppressive effects of rituximab [29]. This is clearly demonstrated by the persistence of seronegative antibodies for more than a year after the onset of symptoms or clinical signs. Individuals who have experienced asplenia [30], or HIV/AIDS [31], or malignancy, or B cell lymphoma [32], or chemotherapeutic agents, or others, may easily be in a state of immunosuppression with impaired immunological functions of their own and eventually prolong the process of anti-babesial therapy. In comparison, the peak parasite load, the days of babesiosis-related hospitalization, the frequency of complications, and fatal outcome of the immunocompromised patients were generally greater or worse than those of immunocompetent patients. Physicians should continue to raise awareness of the detrimental effects of immunosuppressive status on clearance of *Babesia* infections and balance the benefits of anti-babesial therapy and immunosuppressive admission to minimize the health burden of persistent and recrudescent babesiosis with a longer course ($\geq$6 weeks) of a first-line treatment regimen [25,33].

Currently, there is limited understanding of the immune mechanisms that attenuate the severity and duration of *Babesia* infection. Existing data suggest that innate and adaptive immune mechanisms are able to clear *Babesia* infection in the majority of immunocompetent individuals, even in the absence of anti-*Babesia* therapy [23,24,34]. As suggested in numerous literatures, both protective and deleterious inflammatory responses to *Babesia* infection are coordinated in the host spleen, contributing to cellular and humoral parasite clearance, and the spleen is recognized as the first immunological barrier for *Babesia* to systematically infect hosts. As the largest secondary lymphoid organ in the body, the spleen has a wide range of immunological functions in addition to its role in haematopoiesis and erythropoiesis. Recognition of *Babesia* infection in the specialized splenic architecture activates a plethora of pattern recognition receptors (PRRs) on myeloid cells, which in turn induce the necessary T cell activation signals on antigen presenting cells (APCs), cytokine secretion, and pathogen clearance in phagocytes [35]. Thus, the splenic red pulp (RP) is equipped to extract aged, dead or infected red blood cells (RBCs) from the circulation through percolation and filtration of the RP cords in the sinuses, where RBCs must traverse tortuous venous sinusoids to re-enter the circulation and pathogens opsonized by antibody or complement are simultaneously removed by RP macrophages. While the splenic white pulp (WP) acts as a conduit for chemokine chemokine-driven cellular trafficking, naive and central memory T cells are activated to produce cytokines such as gamma interferon (IFN-γ), which enhance macrophage destruction of *Babesia* and activate B cells to secrete *Babesia*-specific antibodies. At the RP-WP interface, a unique type of innate-like B cells, marginal zone B cells (MZBs), resides in marginal zone (mouse) or perifollicular zone (PFZ in humans) where specialized leukocytes including dendritic cells (DCs) and MZBs capture and transport RBC antigens to the WP for surveillance by T and B cells [36–38]. It is also noted that protozoa, such as *Plasmodium* or *Babesia*, require repeated or prolonged infection before protective humoral immunity is established [39]. This may attribute to the severe disruption of the splenic architecture induced by parasite infection, including loss of the MZ or PFZ, blurring of the RP-WP distinction, and disruption of T-dependent B cell germinal center [40,41]. It appears to be a practical tactic for *Babesia* parasites to employ series of evasive measures, such as cyto-adhesion/sequestration, host-protein binding, induction of immunosuppression, and medullary dyserythropoiesis, to avoid attack by immunological and macrophagic stress [42,43]. Thus, asplenia hosts or hosts with hypofunctional spleens generally experienced the severe complication of persistent *Babesia* infection, which is associated with high patient mortality rates [23,25], prompting a comprehensive diagnostic evaluation and evidence-based analysis of the unexplained outcome. These accumulated asplenia deaths eventually led to the discovery of *Babesia* parasites by microscopic evaluation of thin blood smears. Detailed investigations also explained the close association between the severe complications and immunocompromised conditions in patients with non-exclusive defenses, including humoral and/or cellular immunity [44]. In addition, antibody is also an important factor in limiting and clearing *Babesia* infection, at least in immunocompromised individuals. Antibodies clear infection through neutralization by blocking pathogen entry into erythrocytes, enhance opsonization of parasites by macrophages and neutrophils, eradicate parasites by antibody-dependent cytotoxicity of natural killer cells, and activate complement. Patients with B-cell lymphoma and/or rituximab therapy have impaired antibody responses, and a prolonged relapsing clinical course has been reported despite anti-babesial therapy [29]. It is clear that cytokines play a vital role in protecting the host against *Babesia* infection. They regulate cell differentiation and proliferation, cell activation, cell migration and cell survival, which is critical for normal immune function and other biological processes. The release of pro-inflammatory cytokines helps to activate the immune response to eradicate *Babesia* in host animals [45,46]. An impaired cytokine response would likely facilitate the persistence or relapse of *Babesia* infection, although there is no solid evidence to support this possibility. Paradoxically, an excessive cytokine response is thought to increase disease severity and contribute to complications and death [47]. An apparent cytokine-induced pulmonary oedema and death observed in hamsters infected with *B. duncani* is paradigmatically illustrated and explained by elevated pulmonary concentrations of TNFα and IFNγ [48].

4. Diagnosis strategies recommended for persistent relapsing babesiosis

Microscopic examination of blood smears for the presence of *Babesia* remains the gold standard for laboratory diagnosis of human babesiosis. However, the sensitivity and specificity of parasite identification are dependent on the experience and proficiency of the examiner [21]. Microscopic examination, conducted on at least 300 fields for each specimen under oil immersion, is a widely recommended approach for diagnosing persistent relapsing human babesiosis, given the low cost and ready availability of the method in standard laboratories [25]. However, it should be noted that there is no standardized number of fields that is universally accepted in this context [49]. The examination of thick blood smears may result in increased sensitivity, albeit at the expense of specificity, as a large number of red blood cells are examined. Nevertheless, the laborious and tedious microscopic examination is suboptimal for monitoring *Babesia* parasitemia, particularly in the early and/or long-term chronic stages of infection when low-grade parasitemia persists [26]. Recently, the exceptional accuracy and efficiency in identifying and categorizing *Babesia* protozoan in blood smears had been demonstrated through the application of deep learning models in conjunction with meticulous image processing techniques. The deep learning-based microscopic *Babesia* diagnosis has demonstrated consistent values of approximately 0.99 for precision, recall, and F1 score, respectively [50]. This represents an automatable and promising technology with the capacity to significantly reduce the incidence of misdiagnosis and missed diagnoses, as well as instances of drug abuse. Moreover, for diagnostic purposes, in vitro culture and xenodiagnosis using animal inoculation had also been employed in the detection of *Babesia* infection. The isolation and maintenance of various *Babesia* species in vitro has been accomplished through the utilization of microaerophilous stationary-phase systems. The systems are characterized by reduced O_2 tension in the atmosphere, and a static layer of erythrocytes settled at the bottom of the culture unit [51]. Whilst inoculation of susceptible animals with whole blood from a suspected case is also employed under the auspices of ethical permission to facilitate the diagnosis of human babesiosis [16]. Nevertheless, the two aforementioned procedures are employed with less frequency in contemporary laboratory practices due to their diminished sensitivity, increased time requirements, and higher costs.

Notwithstanding these conventional diagnostic procedures, the molecular detection methodology, based on the analysis of nucleic acids, proteins and other molecular markers, has reached the most advanced stage of development and is currently the most widely implemented method. For the purpose of the security of blood donation, the US Federal Drug Administration (FDA) had licensed some nucleic acid-detection based assays (NAT) across 15 high-risk states, including Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, Wisconsin, and Washington DC [52]. Various NATs, including routine PCR, real-time PCR high-resolution melting analysis (qPCR- HRM) [53], and transcription-mediated amplification (TMA) have been developed and validated with claimed limits of detection (Lod) for *Babesia* as low as two or three parasites/mL [54]. As an example, the automated Cobas 6800/8800 systems (Roche Molecular Systems, Inc.) demonstrated excellent sensitivity and specificity in screening for all four pathogenic *Babesia* species (*B. microti*, *B. duncani*, *B. divergens* and *B. venatorum*) in blood donors in the US. The claimed detection limit of the Cobas *Babesia* system was reported as 6.1 infected red blood cells (iRBC)/mL for *B. microti,* 26.1 iRBC/mL for *B. divergens,* 40.0 and 50.2 iRBC/mL for *B. venatorum* and *B. duncani* respectively [55]. The notable advantage of the Cobas *Babesia* System is that it uses a whole blood sample for NAT by adding a proprietary chaotropic reagent that lyses red blood cells and preserves DNA and RNA in the pre-analytical stage, reducing the possibility of contamination and minimizing occupational exposure to blood borne pathogens [55]. Moreover, additional technological advances such as sample concentration (selective pathogen-DNA enrichment by restriction enzyme digestion on host DNA prior to PCR, UPDx) [56], fluorescence in situ hybridization [57], high copy detection targets such as BMN multigene family members [58], reverse line blot hybridization assay [59], and bead-based target capture [60] had proven to efficiently increase the sensitivity of NAT assays and conveniently complement to the existing diagnostic tests (Fig. 2). Among them, the detection of *Babesia* antigen(s) [61] and subsequent high-throughput assays [62] should be promoted as a priority for diagnostic and blood donor screening purposes. Besides these above, two remarkable technologies have been applied to *Babesia* detection or monitoring. The metagenomic next-generation sequencing (mNGS) and CRISPR-based diagnostics, such as SHERLOCK CRISPR collateral cleavage-based diagnostics, have been demonstrated as rapid, low-cost deployable assays for *Babesia* infection, species differentiation and drug-resistance genotyping [63] (Fig. 2). In the near future, clinical validation of these assays is expected. On the other hand, the major limitation of antibody-based assays is that a single positive antibody usually cannot distinguish between current (active) and past (resolved) infection, although a *Babesia* IgG antibody titer of $\geq$1:1024 or the presence of IgM antibodies has been accepted by the CDC [25]. Identification of antigens that could discriminate between active and resolved infections could greatly enhance the utility of antibody-based assays to develop more rapid, sensitive, specific and cost-effective tests, such as point-of-care diagnostic technique, validated for *Babesia* diagnosis in the near future.

5. Management for persistent relapsing babesiosis

The current arsenal for the management of human babesiosis is therefore primarily based on *Babesia* detection (especially for parasitemia monitoring) and highly promising anti-babesial therapies. In patients with severe symptoms of babesiosis, such as severe fever, chills, extreme fatigue and severe anemia, the disease is usually correctly diagnosed and promptly treated, but in those with immunocompromised or subtle symptoms, low-grade parasitemia or unfamiliar *Babesia* species infections, correct diagnosis and subsequent necessary monitoring appear to be much more difficult. These difficulties also affect subsequent treatment regimens, although clinical practice guidelines (CPG) recommend a combination of atovaquone and azithromycin because of better compliance and comparable efficacy to clindamycin + quinine with fewer side effects [24,25]. Prolonged anti-babesial therapy is mandatory for patients with immunocompromised status [30,31] or receiving immunosuppressive therapy [24,32]. These patients are at high risk of persistent relapsing disease with severe complications including severe hemolytic anemia, acute respiratory distress syndrome,

congestive heart failure, renal impairment, shock, disseminated intravascular coagulation, warm autoimmune hemolytic anemia, relapse, hemophagocytic lymphohistiocytosis and/or fatal outcome despite standard anti-babesial therapy [1,30]. In these immunocompromised patients, prolonged monitoring for >3 months is recommended, even after apparent cure [23]. Practical management of human babesiosis depends heavily on the examination of multiple thin blood smear fields in the clinical laboratory. If positive blood smears are observed or patients present with symptoms suggestive of babesiosis, indicating an episode of recrudescent babesiosis, a necessary surveillance procedure should be initiated in a timely manner. While in immunocompetent individuals, monitoring for persistent *Babesia* parasitemia is not usually necessary after completion of the recommended course of chemotherapy. However, to avoid the risk of transmitting *Babesia* parasites through blood contamination, these patients are excluded as blood or solid organ donors, as the silent infection can persist for many months or even years and the apparently benign condition can relapse [23].

The other critical aspect of human babesiosis management highly dependent on well-designed therapeutic strategies, which include the duration of treatment regimens, the dose of anti-babesial drugs and their combination, hospitalization admission, and alternative compounds with promising anti-*Babesia* efficacy (Fig. 1), even partial or complete RBC exchange transfusion indicated in patients presenting with a parasitemia of at least 10 % and anemia with hemoglobin of <10 g/dL [21]. The current recommended treatment regime for human babesiosis is based on the combinations of atovaquone and azithromycin or clindamycin and quinine,

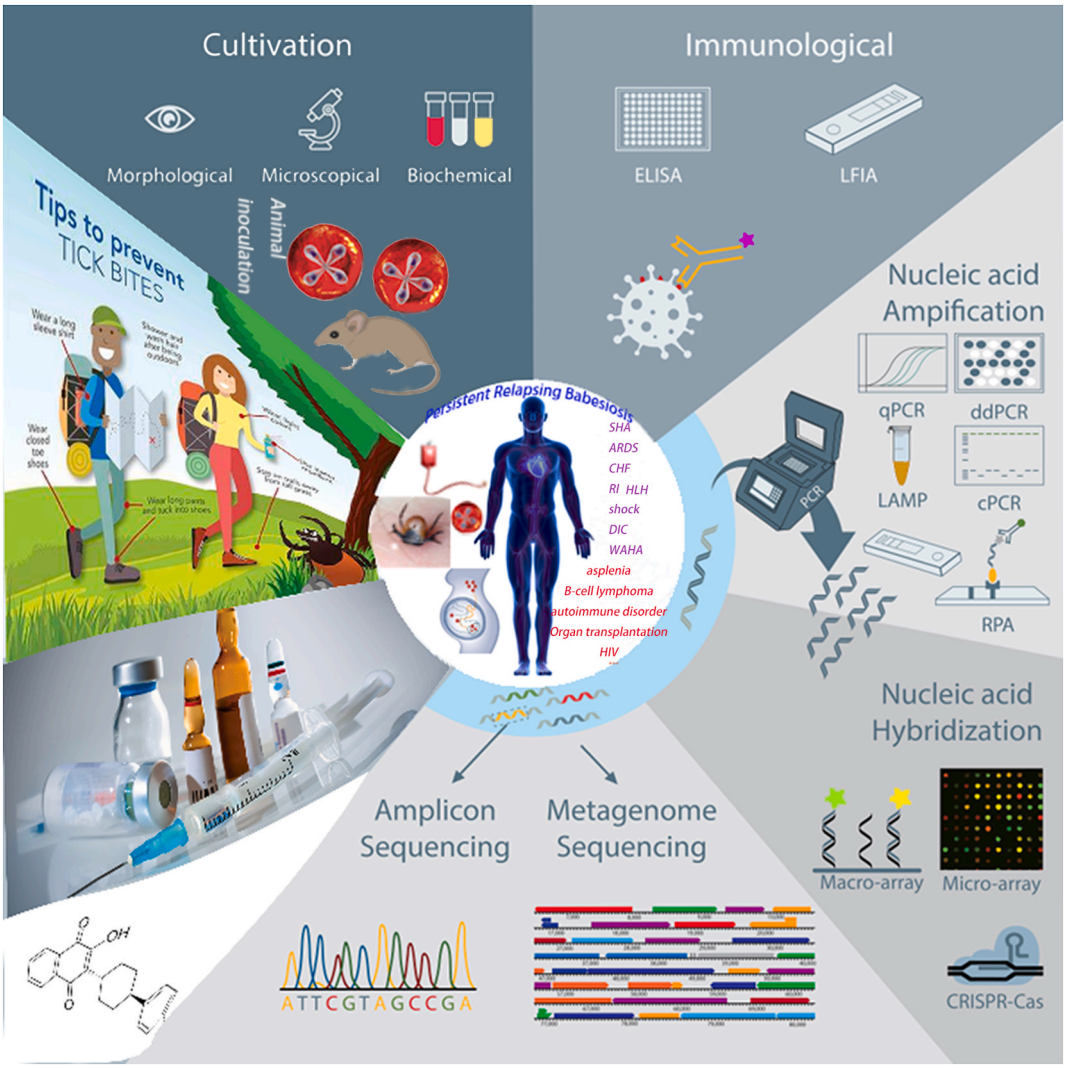

Fig. 1. Schematic flowchart for persisting relapse human babesiosis management.
The flowchart was developed following the pathogen determination, diagnosis and medicine administration and prevention procedure.
Note: ELISA, enzyme-linked immune-sorbent assay; LFIA, lateral flow immunochromatography assay; qPCR, Quantitative Real-time PCR; ddPCR, Digital Droplet PCR; LAMP, Loop-mediated Isothermal Amplification; cPCR, convective PCR; CRISPR-Cas, clustered regularly interspaced short palindromic repeats CRISPR-associated genes. SHA, severe hemolytic anemia; ARDS, acute respiratory distress syndrome; CHF, congestive heart failure; RI, renal impairment; DIC disseminated intravascular coagulation; WAHA warm autoimmune hemolytic anemia; HLH, Hemophagocytic lymphohistiocytosis.

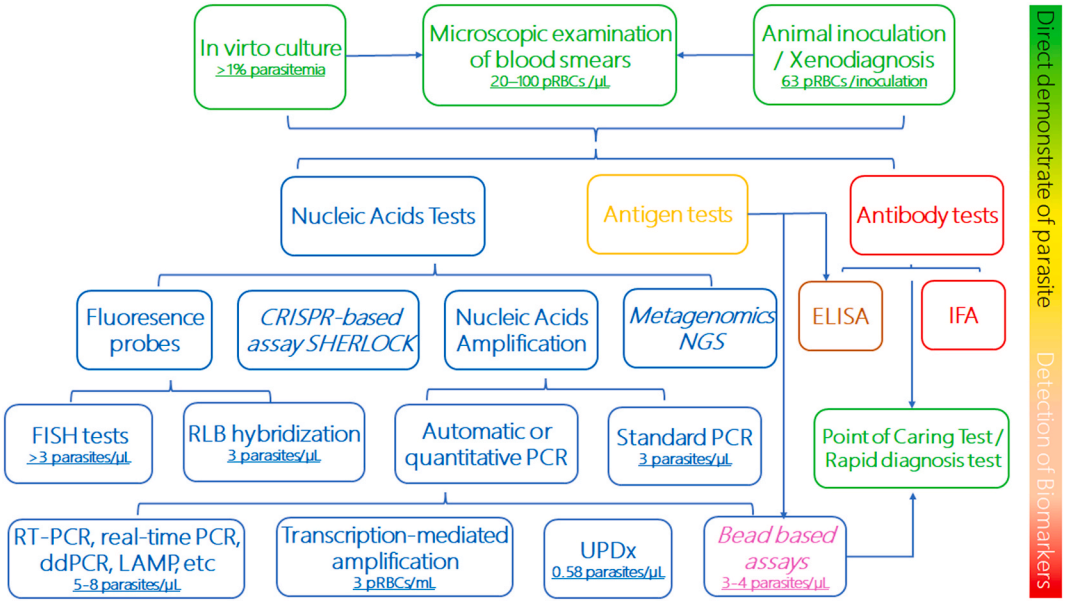

Fig. 2. Diagram depicting developments in *Babesia* diagnosis technology.
Biomarkers for diagnosis detection are classified according to the type of specimens and biomarker: blood smears(green), nucleic acid (blue), antibody (red), or antigen (orange). Bead-based methods (purple) can be adapted for detection of either nucleic acid or antibody, while ELISA (brown) can be used to detect antibody or antigen. Limit of detection for direct observation and molecular detection were also shown underlined below. Technologies in italics are proposed for detection of *Babesia* parasites but have not yet been effectively adapted.

which were developed based on their well-established efficacy against other apicomplexan parasites rather than *Babesia* species [33]. In clinical practice, atovaquone has been shown to have 50 % inhibitory concentration (IC_{50}) values in the low nanomolar range against *B. divergens* and *B. duncani* by targeting their cytochrome *bc*1 complex of the mitochondrial electron transport chain. And azithromycin was found to have a “delayed death" effect on the apicoplast of the protozoan *Babesiidae*, in which parasite division produces viable daughter cells that are subsequently unable to divide in the next cycle [64]. Although atovaquone + azithromycin is now the preferred course of treatment for severe babesiosis, the standard 7-10-day treatment regimen of oral atovaquone + azithromycin is usually not enough, and higher doses of drugs administration or longer treatment duration and in some cases intravenous administration, are frequently required to eliminate *Babesia* infection. It should also be noted that the use of immunosuppressive agents such as Rituximab to treat prior diseases (B cell lymphoid malignancies, rheumatoid arthritis, *etc*.) may lead to babesiosis relapse and prolonged persistence of *Babesia* parasites [32,65] (Fig. 1). A disadvantage of a prolonged treatment regimen and dose escalation is the risk of developing drug resistance. For example, the emergence of mutations (methionine-to-isoleucine mutation at position 134, M134I and tyrosine-to-cysteine mutation at position 272, Y272C in Cyt-b) in the highly conserved Q_0 site (atovaquone-binding site) of *Babesia microti* cytochrome *b* (BmCytb) has been recorded in humans following treatment with atovaquone [65,66]. These mutations were closely associated with reduced sensitivity to atovaquone and its combinations via the atovaquone-binding domains. Alternative management strategies for human babesiosis in the case of persistent relapse include the use of different drug combinations such as atovaquone + azithromycin + clindamycin, atovaquone + clindamycin, atovaquone + proguanil, or atovaquone + azithromycin + clindamycin + quinine [33]. The addition of other drugs such as doxycycline, moxifloxacin, pentamidine, trimethoprim-sulfamethoxazole or artemisinin to the standard regimens had also been reported in some clinical practices [67]. Recently, efforts to develop new therapeutics for the treatment of human babesiosis have mostly focused on screening of the Pathogen Box (approximately 400 compounds active against neglected diseases) and its derivatives [68]. Among these, tafenoquine, clofazimine, and endochin-like quinolones (ELQs) are probably the most promising drugs with potent anti-*Babesia* efficacy. Based on their potency, selectivity, and ability to eliminate *Babesia* infection when combined with atovaquone, ELQs appear to be the most promising candidates for radical cure of human babesiosis without recrudescence, highlighting the need for new therapeutic strategies that are specifically tailored to *Babesia* parasites [33]. In one case series study on relapsing human babesiosis, the combination of Tafenoquine-Atovaquone achieved radical cure [69], and unexpectedly conferred sterile immunity against future babesiosis infections in human [70]. Nevertheless, a consensus protocol agreed upon by members of the community and standard methods for long term monitoring of parasitemia and promised therapies are warranted for the future management of human babesiosis.

6. Conclusions

The occurrence of persistent relapsing human babesiosis is greatly facilitated by the evasion strategies employed by the parasite *Babesia*, as well as the impaired host immune function. The health burden of persistent and relapsing babesiosis could be mitigated

through the validation and implementation of novel diagnostic and therapeutic measures. The advancement of sensitive and precise point-of-care diagnostic techniques, in conjunction with the introduction of novel promising anti-babesial drugs, would enable a proficient response to the challenge of persistent relapsing human babesiosis in the future.

CRediT authorship contribution statement

Fei Chen: Writing – original draft, Resources, Data curation. **Shuhong Fu:** Writing – original draft, Resources, Data curation. **Jia-fu Jiang:** Writing – review & editing, Conceptualization. **Hao Feng:** Writing – original draft, Investigation, Data curation. **Zhitong Liu:** Writing – original draft, Visualization, Investigation. **Yi Sun:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Mianyang Li:** Writing – review & editing, Resources, Data curation, Conceptualization.

Data availability statement

No new data were created. The information presented in this review article are from published reports available in public databases.

Consent for publication

All authors gave their consent for publication.

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Declaration of competing interest

The authors declare no conflict of interests existed involved during the study and the manuscript prepared.

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References

[1] K. Wudhikarn, E.H. Perry, M. Kemperman, K.A. Jensen, S.E. Kline, Transfusion transmitted babesiosis in an immunocompromised patient: a case report and review, Am. J. Med. 124 (2011) 800–805, https://doi.org/10.1016/j.amjmed.2011.03.009.
[2] A.S. Handel, J. Krugman, S. Hymes, S. Inkeles, C.A. Beneri, A case of relapsed vertically transmitted babesiosis, Journal of the Pediatric Infectious Diseases Society 10 (2021) 386–388, https://doi.org/10.1093/jpids/piaa104.
[3] A. Kumar, J. O'Bryan, P.J. Krause, The global emergence of human babesiosis, Pathogens 10 (2021) 1447, https://doi.org/10.3390/pathogens10111447.
[4] V. Babes, On bacterial haemoglobinuria in cattle, 1888, Comptes Rendus l'Acad. Sci. 107 (2013) 692–694.
[5] L. Schnittger, A.E. Rodriguez, M. Florin-Christensen, D.A. Morrison, Babesia: a world emerging, Infect. Genet. Evol. 12 (2012) 1788–1809, https://doi.org/10.1016/j.meegid.2012.07.004.
[6] B.L. Penzhorn, Babesiosis of wild carnivores and ungulates, Vet. Parasitol. 138 (1–2) (2006) 11–21, https://doi.org/10.1016/j.vetpar.2006.01.036.
[7] Z. Skrabalo, Z. Deanovic, Piroplasmosis in man; report of a case, Doc. Med. Geogr. Trop. 9 (1957) 11–16.
[8] K.A. Western, G.D. Benson, N.N. Gleason, G.R. Healy, M.G. Schultz, Babesiosis in a Massachusetts resident, N. Engl. J. Med. 283 (1970) 854–856, https://doi.org/10.1056/NEJM197010152831607.
[9] E.B. Gray, B.L. Herwaldt, Babesiosis surveillance - United States, 2011–2015, MMWR Surveill Summ 68 (2019) 1–11.
[10] CDC, Babesiosis surveillance -18 states, 2011, MMWR (Morb. Mortal. Wkly. Rep.) 61 (27) (2012) 505–509.
[11] M. Menis, B.I. Whitaker, M. Wernecke, Y. Jiao, A. Eder, S. Kumar, W. Xu, J. Liao, Y. Wei, T.E. MaCurdy, Babesiosis occurrence among United States Medicare Beneficiaries, ages 65 and older, during 2006–2017: overall and by state and county of residence, Open Forum Infect. Dis. 8 (2) (2021) ofaa608, https://doi.org/10.1093/ofid/ofaa608.
[12] J.D. Scott, C.M. Scott, Human babesiosis caused by *Babesia duncani* has widespread distribution across Canada, Healthcare (Basel) 6 (2) (2018) 49, https://doi.org/10.3390/healthcare6020049.
[13] J.S. Gray, Identity of the causal agents of human babesiosis in Europe, Int J Med Microbiol 296 (Suppl. 40) (2006) 131–136, https://doi.org/10.1016/J.IJMM.2006.01.029.
[14] K.P. Hunfeld, A. Hildebrandt, J.S. Gray, Babesiosis: recent insights into an ancient disease, Int. J. Parasitol. 38 (2008) 1219–1237, https://doi.org/10.1016/j.ijpara.2008.03.001.
[15] E. Vannier, P.J. Krause, Babesiosis in China, an emerging threat, Lancet Infect. Dis. 15 (2015) 137–139, https://doi.org/10.1016/S1473-3099(14)71062-X.
[16] N. Jia, Y.C. Zheng, J.F. Jiang, R. Jiang, B. Jiang, R. Wei, H. Liu, Q. Huo, Y. Sun, Y. Chu, H. Fan, Q. Chang, N. Yao, W. Zhang, H. Wang, D. Guo, X. Fu, Y. Wang, P. J. Krause, J. Song, W. Cao, Human babesiosis caused by a *Babesia crassa*-like pathogen: a case series, Clin. Infect. Dis. 67 (2018) 1110–1119, https://doi.org/10.1093/cid/ciy212.
[17] J.F. Jiang, Y.C. Zheng, R.R. Jiang, H. Li, Q. Huo, B. Jiang, Y. Sun, N. Jia, Y. Wang, L. Ma, H. Liu, Y. Chu, X. Ni, K. Liu, Y. Song, N. Yao, H. Wang, T. Sun, W. Cao, Epidemiological, clinical, and laboratory characteristics of 48 cases of "*Babesia venatorum*" infection in China: a descriptive study, Lancet Infect. Dis. 15 (2015) 196–203, https://doi.org/10.1016/S1473-3099(14)71046-1.

[18] Z. Chen, H. Li, X. Gao, A. Bian, H. Yan, D. Kong, X. Liu, Human babesiosis in China: a systematic review, Parasitol. Res. 118 (2019) 1103–1112, https://doi.org/10.1007/s00436-019-06250-9.

[19] L. Zhao, R. Jiang, N. Jia, N. Ning, Y. Zheng, Q. Huo, Y. Sun, T. Yuan, B. Jiang, T. Li, H. Liu, X. Liu, Y. Chu, R. Wei, C. Bian, H. Wang, J. Song, H. Wang, J. Jiang, W. Cao, Human case infected with *Babesia venatorum*: a 5-year follow-up study, Open Forum Infect. Dis. 7 (2020) 1–9, https://doi.org/10.1093/ofid/ofaa062.

[20] L.Q. Fang, K. Liu, X.L. Li, S. Liang, Y. Yang, H.W. Yao, R.X. Sun, Y. Sun, W.J. Chen, S.Q. Zuo, Emerging tick-borne infections in mainland China: an increasing public health threat, Lancet Infect. Dis. 15 (2015) 1467–1479, https://doi.org/10.1016/S1473-3099(15)00177-2.

[21] R.P. Smith, K.P. Hunfeld, P.J. Krause, Management strategies for human babesiosis, Expert Rev. Anti-infect. Ther. 18 (2020) 625–636, https://doi.org/10.1080/14787210.2020.1752193.

[22] P.J. Krause, Human babesiosis, Int. J. Parasitol. 49 (2) (2019) 165–174, https://doi.org/10.1016/j.ijpara.2018.11.07.

[23] P.J. Krause, T. Lepore, V.K. Sikand, J.J. Gadbaw, G.S. Burke, S. Telford, P. Brassard, D. Pearl, J. Azlanzadeh, D. Christianson, D. Mcgrath, A. Spielman, Atovaquone and azithromycin for the treatment of babesiosis, N. Engl. J. Med. 343 (2000) 1, https://doi.org/10.1056/NEJM200011163432004.

[24] F. Rosner, M.H. Zarrabi, J.L. Benach, G.S. Habicht, Babesiosis in splenectomies adults. Review of 22 reported cases, Am. J. Med. 76 (1984) 696–701, https://doi.org/10.1016/0002-9343(84)90298-5.

[25] P.J. Krause, P. Auwaerter, R. Bannuru, J.A. Branda, Y.T. Falck-Ytter, P.M. Lantos, V. Lavergne, H.C. Meissner, M.C. Osani, J.G. Rips, S.K. Sood, E. Vannier, E. E Vaysbrof, G.P. Wormser, Clincal practice guidelines by the infectious diseases society of American (IDSA), Guideline on diagnosis and management of Babesiosis. Clin. Infect. Dis. 72 (2021) 185–189, https://doi.org/10.1093/cid/ciab050.

[26] P.J. Krause, A. Spielman, S.R. Telford III, V.K. Sikand, K. McKay, D. Christianson, Persistent parasitemia after acute babesiosis, N. Engl. J. Med. 339 (1998) 160–165, 10.1056/NEJM199807163390304.

[27] E. Moritz, C.S. Winton, L. Tonnetti, R.L. Townsend, V.P. Berardi, M.E. Hewins, K.E. Weeks, R.Y. Dodd, S.L. Stramer, Screening for *Babesia microti* in the U.S. blood supply, N. Engl. J. Med. 375 (2016) 2236–2245, https://doi.org/10.1056/NEJMOA1600897.

[28] P.J. Krause, B.E. Gewurz, D. Hill, Persistent and relapsing babesiosis in immunocompromised patients, Clin. Infect. Dis. 46 (2008) 370–376, https://doi.org/10.1086/525852.

[29] J. Raffalli, G.P. Wormser, Persistence of babesiosis for >2 years in a patient on rituximab for rheumatoid arthritis, Diagn. Microbiol. Infect. Dis. 85 (2016) 231–232, 10.1016/j.diagmicrobio. 2016.02.016.

[30] M. Ivancich, L. Lutwick, F.N.U. Shweta, Trust the process: prolonged *Babesia* parasitemia in patient with asplenia from the American midwest, Am J Case Rep 23 (2022) e936326, https://doi.org/10.12659/AJCR.936326.

[31] J.M. Vyas, S.R. Telford, G.K. Robbins, Treatment of refractory *Babesia microti* infection with atovaquone-proguanil in an HIV-infected patient: case report, Clin. Infect. Dis. 45 (12) (2007) 1588–1590, https://doi.org/10.1086/523731.

[32] A.S. Lubin, D.R. Snydman, K.B. Miller, Persistent babesiosis in a stem cell transplant recipient, Leuk. Res. 35 (6) (2011) e77–e78, https://doi.org/10.1016/j.leukres.2010.11.029.

[33] I. Renard, C. Ben Mamoun, Treatment of human babesiosis: then and now, Pathogens 10 (2021) 1120, https://doi.org/10.3390/pathogens10091120.

[34] E. Vannier, I. Borggraefe, S.R. Telford, S. Menon, T. Brauns, A. Spielman, J.A. Gelfand, H.H. Wortis, Age-associated decline in resistance to *Babesia microti* is genetically determined, J. Infect. Dis. 189 (2004) 1721–1728, https://doi.org/10.1086/382965.

[35] V. Djokic, L. Akoolo, N. Parveen, *Babesia microti* infection changes host spleen architecture and is cleared by a Th1 immune response, Front. Microbiol. 9 (2018) 85, https://doi.org/10.3389/fmicb.2018.00085.

[36] S. Calabro, D. Liu, A.E. Gallman, M.S. Nascimento, Z. Yu, T. Zhang, P. Chen, B. Zhang, L. Xu, U. Gowthaman, J.K. Krishnaswamy, A.M. Haberman, A. Williams, S.C. Eisenbarth, Differential intrasplenic migration of dendritic cell subsets tailors adaptive immunity, Cell Rep. 16 (2016) 2472–2485, https://doi.org/10.1016/j.celrep.2016.07.076.

[37] T.I. Arnon, R.M. Horton, I.L. Grigorova, J.G. Cyster, Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress, Nature 493 (2013) 684–688, https://doi.org/10.1038/nature11738.

[38] C. Qiu, Y. Miyake, H. Kaise, H. Kitamura, O. Ohara, M. Tanaka, Novel subset of CD8α^{+} dendritic cells localized in the marginal zone is responsible for tolerance to cell-associated antigens, J. Immunol. 182 (2009) 4127–4136, https://doi.org/10.4049/jimmunol.0803364.

[39] G.E. Weiss, B. Traore, K. Kayentao, A. Ongoiba, S.N. Doumbo, D. Doumtabe, Y. Koné, S. Dia, A.M. Guindo, A. Traoré, C. Huang, K. Miura, M. Mircetic, S. Li, A. Baughman, D.L. Narum, L.H. Miller, O.K. Doumbo, S.K. Pierce, P.D. Crompton, The *Plasmodium falciparum*-specific human memory B cell compartment expands gradually with repeated malaria infections, PLoS Pathog. 6 (2010) e1000912, 10.1371/journal. ppat.1000912.

[40] W.O. Hahn, N.S. Butler, S.E. Lindner, H.M. Akilesh, D.N. Sather, S.H. Kappe, J.A. Hamerman, M. Gale, W.C. Liles, M. Pepper, cGAS-mediated control of blood-stage malaria promotes *Plasmodium*-specific germinal center responses, JCI Insight 3 (1) (2018) e94142, 10.1172/jci.insight. 94142.

[41] L.J. Carvalho, M.F. Ferreira-da-Cruz, C.T. Daniel-Ribeiro, M. Pelajo-Machado, H.L. Lenzi, Germinal center architecture disturbance during *Plasmodium berghei* ANKA infection in CBA mice, Malar. J. 6 (2007) 59, https://doi.org/10.1186/1475-2875-6-59.

[42] H. Hakimi, J. Yamagishi, S. Kawazu, M. Asada, Advances in understanding red blood cell modifications by *Babesia*, PLoS Pathog. 18 (9) (2022) e1010770, https://doi.org/10.1371/journal.ppat.1010770.

[43] I. Farooq, T.J. Moriarty, The impact of tick-borne diseases on the bone, Microorganisms 9 (2021) 663, https://doi.org/10.3390/microorganisms9030663.

[44] A. Kumar, J. O'Bryan, P.J. Krause, The global emergence of human babesiosis, Pathogens 10 (2021) 1447, https://doi.org/10.3390/pathogens10111447.

[45] M. Shaio, P.R. Lin, A case study of cytokine profiles in acute human babesiosis, Am. J. Trop. Med. Hyg. 58 (1998) 335–337, https://doi.org/10.4269/AJTMH.1998.58.335.

[46] I.A. Clark, L.M. Alleva, A.C. Mills, W.B. Cowden, Pathogenesis of malaria and clinically similar conditions, Clin. Microbiol. Rev. 17 (2004) 509–539, https://doi.org/10.1128/CMR.17.3.509-539.2004.

[47] R.M. Hemmer, D.A. Ferrick, P.A. Conrad, Up-regulation of tumor necrosis factor-alpha and interferon-gamma expression in the spleen and lungs of mice infected with the human *Babesia* isolate WA1, Parasitol. Res. 86 (2000) 121–128, https://doi.org/10.1007/s004360050021.

[48] R.M. Hemmer, E.J. Wozniak, L.J. Lowenstine, C.G. Plopper, V.J. Wong, P.A. Conrad, Endothelial cell changes are associated with pulmonary edema and respiratory distress in mice infected with the WA1 human *Babesia* parasite, J Parasit 85 (3) (1999) 479–489, https://doi.org/10.2307/3285783.

[49] B.S. Pritt, *Plasmodium* and *Babesia*, in: J.H. Jorgensen, K.C. Carroll, G. Funke, M.A. Pfaller, M.L. Landry, S.S. Richter, D.W. Warnock (Eds.), Manual of Clinical Microbiology, ASM Press, Washington, DC, 2019.

[50] Y. Kumar, P. Garg, M.R. Moudgil, R. Singh, M. Woźniak, J. Shafi, M.F. Ijaz, Enhancing parasitic organism detection in microscopy images through deep learning and fine-tuned optimizer, Sci. Rep. 14 (1) (2024) 5753, https://doi.org/10.1038/s41598-024-56323-8.

[51] Y. Sun, B. Jiang, W. Zheng, H. Wang, R. Jiang, X. Wang, N. Jia, F. Yang, H. Chen, J. Jiang, W. Cao, Isolation and in vitro cultivation of *Babesia venatorum* (Apicomplexa: Babesiidae), a zoonotic hemoprotozoan from *Ixodes persulcatus* ticks in China, Biosafety and Health 3 (4) (2021) 210–216, 10.1016/j.bsheal.2021. 06.004.

[52] US Food and Drug Administration, Recommendations for Reducing the Risk of Transfusion-Transmitted Babesiosis, U.S. Department of Health and Human Services, Silver Spring, MD, 2019. CBER: Office of Communication OCOD.

[53] Y. Wang, S. Zhang, X. Li, Y. Nian, X. Liu, J. Liu, H. Yin, G. Guan, J. Wang, A high-resolution melting approach for the simultaneous differentiation of five human babesiosis-causing *Babesia* species, Parasit Vectors 16 (1) (2023) 299, https://doi.org/10.1186/s13071-023-05839-5.

[54] M.P. Busch, E.M. Bloch, S. Kleinman, Prevention of transfusion-transmitted infections, Blood 133 (2019) 1854–1864, https://doi.org/10.1182/blood-2018-11-833996.

[55] J. Stanley, S.L. Stramer, Y. Erickson, J.L. Cruz, J.B. Gorlin, M. Janzen, S.N. Rossmann, T. Straus, P. Albrecht, L.L. Pate, S.A. Galel, Detection of *Babesia* RNA and DNA in whole blood samples from US blood donations, Transfusion 61 (2021) 2969–2980, https://doi.org/10.1111/trf.16617.

[56] B.R. Flaherty, J.L. Barratt, M. Lane, E. Talundzic, R.S. Bradbury, Sensitive universal detection of blood parasites by selective pathogen-DNA enrichment and deep amplicon sequencing, Microbiome 9 (2021) 1–19, https://doi.org/10.1186/s40168-020-00939-1.

[57] J.S. Shah, R. Ramasamy, Fluorescence in situ hybridization (FISH) tests for identifying protozoan and bacterial pathogens in infectious diseases, Diagnostics 12 (2022) 1286, 10.3390/diagnostics 12051286.
[58] B. Grabias, J. Clement, P.J. Krause, T. Lepore, S. Kumar, Superior real-time polymerase chain reaction detection of *Babesia microti* parasites in whole blood utilizing high-copy BMN antigens as amplification targets, Transfusion 58 (2018) 1924–1932, https://doi.org/10.1111/trf.14642.
[59] M.S. Paoletta, L. López Arias, S. de la Fournière, E.C. Guillemi, C.A. Luciani, N.F. Sarmiento, J. Mosqueda, M.D. Farber, S.E. Wilkowsky, Epidemiology of *Babesia, Anaplasma* and *Trypanosoma* species using a new expanded reverse line blot hybridization assay, Ticks and Tick-Borne Diseases 9 (2) (2017) 155–163, https://doi.org/10.1016/j.ttbdis.2017.08.011.
[60] J. Liu, B. Allo, M.A. Winnik, Development of multiplexed bead-based immunoassays for profiling soluble cytokines and CD163 using mass cytometers, ACS Measurement Science Au 2 (2022) 629–640, https://doi.org/10.1021/acsmeasuresciau.2c00038.
[61] J. Thekkiniath, S. Mootien, L. Lawres, B.A. Perrin, M.A. Gewirtz, P.J. Krause, S.C. Williams, J.S. Doggett, M. Ledizet, C. Ben Mamoun, BmGPAC, an antigen capture assay for detection of active *Babesia microti* infection, J. Clin. Microbiol. 56 (2018), https://doi.org/10.1128/JCM.00067-18, 00067–18.
[62] J. Capelli-Peixoto, P. Saelao, W. Johnson, L. Kappmeyer, K.E. Reif, H.E. Masterson, N.S. Taus, C.E. Suárez, K.A. Brayton, M.W. Ueti, Comparison of high throughput RNA sequences between *Babesia bigemina* and *Babesia bovis* revealed consistent differential gene expression that is required for the *Babesia* life cycle in the vertebrate and invertebrate hosts, Front. Cell. Infect. Microbiol. 12 (2022) 1093338, https://doi.org/10.3389/fcimb.2022.1093338.
[63] S. Meredith, M. Oakley, S. Kumar, Technologies for detection of *Babesia microti*: advances and challenges, Pathogens 10 (2021) 1563, 10.3390/pathogens10121563.
[64] A.B.S. Sidhu, Q.G. Sun, L.J. Nkrumah, M.W. Dunne, J.C. Sacchettini, D.A. Fidock, In vitro efficacy, resistance selection, and structural modeling studies implicate the malarial parasite apicoplast as the target of azithromycin, J. Biol. Chem. 282 (2007) 2494–2504, https://doi.org/10.1074/jbc.M608615200.
[65] M.S. Simon, L.F. Westblade, A. Dziedziech, J.E. Visone, R.R. Furman, S.G. Jenkins, A.N. Schuetz, L.A. Kirkman, Clinical and molecular evidence of atovaquone and azithromycin resistance in relapsed *Babesia microti* infection associated with rituximab and chronic lymphocytic leukemia, Clin. Infect. Dis. 65 (2017) 1222–1225, https://doi.org/10.1093/cid/cix477.
[66] J.E. Lemieux, A.D. Tran, L. Freimark, S.F. Schaffner, H. Goethert, K.G. Andersen, S. Bazner, A. Li, G. McGrath, L. Sloan, A global map of genetic diversity in *Babesia microti* reveals strong population structure and identifies variants associated with clinical relapse, Nat Microbiol 1 (2016) 7, 10.1038/nmicrobiol.2016.79.
[67] L. Huang, Y. Sun, D.D. Huo, M. Xu, L.Y. Xia, N. Yang, W. Hong, L. Huang, W.M. Nie, R.H. Liao, M.Z. Zhang, D.Y. Zhu, Y. Li, H.C. Ma, X. Zhang, Y.G. Li, X. A. Huang, J.Y. Wang, W.C. Cao, F.S. Wang, J.F. Jiang, Successful treatment with doxycycline monotherapy for human infection with *Babesia venatorum* (Babesiidae, Sporozoa) in China: a case report and proposal for a clinical regimen, Infect Dis Poverty 12 (1) (2023) 67, https://doi.org/10.1186/s40249-023-01111-1.
[68] A.B. Nugraha, B. Tuvshintulga, A. Guswanto, D.S. Tayebwa, M.A. Rizk, S. Gantuya, G.E. Batiha, A.M. Beshbishy, T. Sivakumar, N. Yokoyama, Screening the medicines for malaria venture pathogen box against piroplasm parasites, Int J Parasitol Drugs Drug Resist 10 (2019) 84–90, https://doi.org/10.1016/j.ijpddr.2019.06.004.
[69] P.J. Krause, R. Rogers, M.K. Shah, H. Kang, J. Parsonnet, R. Kodama, E. Vannier, Tafenoquine for relapsing babesiosis: a case series, Clin. Infect. Dis. 79 (1) (2024) 130–137, https://doi.org/10.1093/cid/ciae238.
[70] Pratap Vydyam, Anasuya C. Pal, Isaline Renard, Meenal Chand, Vandana Kumari, Joseph C. Gennaro, Choukri Ben Mamoun, Tafenoquine-atovaquone combination achieves radical cure and confers sterile immunity in experimental models of human babesiosis, J. Infect. Dis. 229 (1) (2024) 161–172, https://doi.org/10.1093/infdis/jiad315.