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Extraordinary high level of propagation of *Babesia divergens* in severe human babesiosis

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Research Article

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Abstract

Babesias are obligate apicomplexan parasites that affect the red blood cells (RBCs) of animals. Humans can serve as accidental hosts for them. Asexual reproduction of a parasite occurs in a vertebrate host through asynchronous binary fission, yielding a complex pleomorphic population of intraerythrocytic forms. In natural hosts (*Bos taurus*), paired pyriforms ('figure 8') of *Babesia divergens* are usual, but tetrads ('Maltese Cross') are very rare (only in 0.02% infected erythrocytes); in humans, however, up to 5% of infected erythrocytes show tetrads. The current study shows that *B. divergens* proliferating in an accidental human host can promote extraordinarily high level of fission. This phenomenon is expressed as the simultaneous division of the parasite into 6 and possibly a greater number of merozoites, forming a 'daisy head' (*vs* the usual 2, less often 4 merozoites). Reproduction is possible without egressing merozoites from the erythrocyte, which results in multi-occupancy of an RBC (≥5 parasites per RBC). An unusually high polyparasitism – up to 14 parasites developed in the affected erythrocytes – was observed. This phenomenon is rare in natural hosts (usually ≤5), but when *B. divergens* is cultured *in vitro* it can be 10–12.

Introduction

Human babesiosis is a vector-borne disease transmitted by ticks; the frequency of occurrence and geography of its cases have been increasing in recent decades (Yabsley and Shock, 2013; Hildebrandt *et al.*, 2021). The causative agent of babesiosis is the protozoan blood parasites *Babesia* spp. (Apicomplexa: Babesiidae). Babesial parasites require both a competent vertebrate and invertebrate host to maintain transmission cycles and 2 types of reproduction: asexual and sexual. Sexual reproduction (sporogony) occurs in the invertebrate host and consists of fusion of gametes and formation of zygotes in the gut of the vector, followed by multiple fissions in various tick tissues, and culminating in the development of infective stages in the salivary glands. Infective sporozoites are injected into the bloodstream of a vertebrate host during tick feeding and invade red blood cells (RBCs), where they initiate cycles of asexual reproduction (merogony) by asynchronous binary fission, yielding a complex pleomorphic population of intraerythrocytic (IE) parasites (Sevilla *et al.*, 2018; Gray *et al.*, 2019; Conesa *et al.*, 2020). Asexual stages cause clinical manifestations of the disease in the vertebrate host. In addition to merogony, gametogenesis occurs in the blood of the vertebrate host. The formation of gametocytes is a mandatory stage in the apicomplexan life cycle. It is necessary for further sporogony in invertebrate hosts.

From the first reported case of human babesiosis in 1956 (Skrabalo and Deanovic, 1957) to the present, about 60 cases have been published in 19 European countries (Hildebrandt *et al.*, 2013, 2021). Almost all cases of human babesiosis in Europe were caused by *Babesia divergens* and were observed in patients who had been splenectomized prior to infection (Centeno-Lima *et al.*, 2003; Corpelet *et al.*, 2005; Mørch *et al.*, 2015; Asensi *et al.*, 2018; Kukina *et al.*, 2018). These cases are often fatal. However, in Europe, sporadic cases of babesiosis have been diagnosed in patients with an intact spleen (Martinot *et al.*, 2011; Gonzalez *et al.*, 2014; O'Connell *et al.*, 2017; Kukina *et al.*, 2019).

Babesia divergens is primarily specific to bovines and widespread throughout Europe with *Ixodes ricinus* as the vector. People are not natural hosts for *B. divergens* but can serve as accidental hosts. The trophozoite size, position inside the erythrocyte and morphological detail are dependent on the host species (Krylov, 1996; Zintl *et al.*, 2003). In recent years, significant progress has been made in our understanding of the asexual reproduction of *Babesia* based on studying this process in human RBCs cultured *in vitro* (Rossouw *et al.*, 2015; Cursino-Santos *et al.*, 2016; Conesa *et al.*, 2020). However, the development of the parasite *in vitro* and *in vivo* may vary somewhat, and some aspects of this process may be studied by using microscopy blood smears from patients. Unfortunately, the appearance of gametocytes in the blood is a crucial event that is still not fully understood. The characteristics of asexual reproduction of *B. divergens* in a splenectomized patient and a patient with an intact spleen are compared with a description of the division stages of parasites that had not been reported previously in infected humans.

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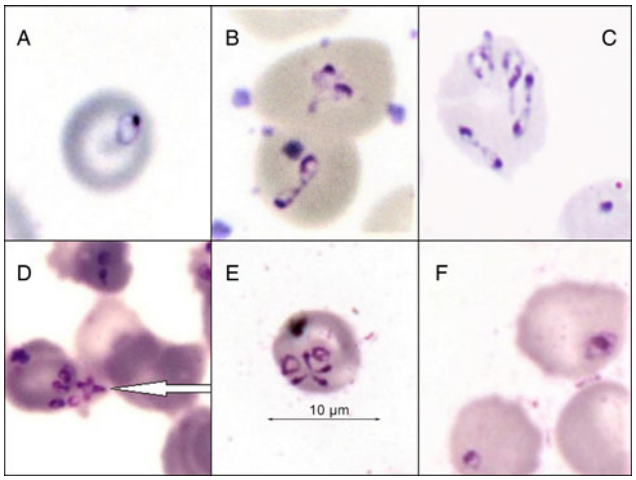

Fig. 1. *Babesia divergens.* Romanovsky-stained thin blood smear. Ring form (A); the paired forms 'figure 8s' diverge at a wide angle (up to 180°); club-shaped forms (B); double paired pyriforms (2 sets of paired sister cells) (C); simultaneous division into 4 daughter nuclei (D); tetrads or Maltese Crosses (4 attached sister cells) and 2 separated mononucleated rounded sister trophozoites (E); a presumable gametocyte (F).

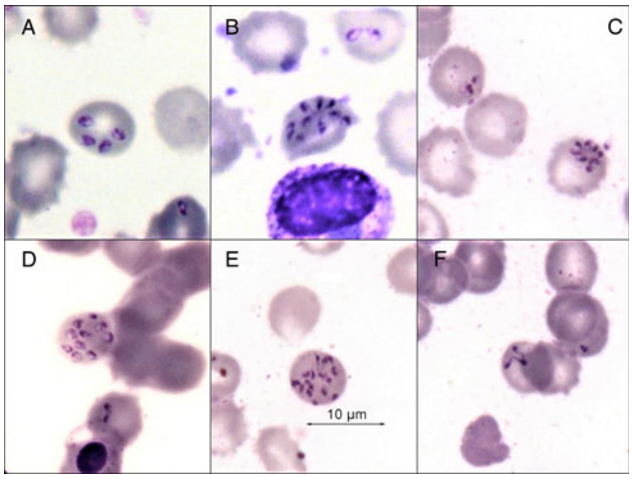

Fig. 2. *Babesia divergens.* Romanovsky-stained thin blood smear. Four sister trophozoites (A); super multiple fission – formation of a sextet and possibly more merozoites ('daisy head') (B, C); multiple invasions (D, E); filamentous forms, noted as crisis forms (F).

Materials and methods

Patient 1 was a 58-year-old man, a huntsman. Post-traumatic splenectomy had been performed 12 years ago. Babesiosis was diagnosed only on the 7th day of illness, and therapy with clindamycin was started. Multisystem failure increased progressively and the patient died on day 10 of illness (Kukina *et al.*, 2018).

Patient 2 was a 74-year-old female whose spleen was intact; she was hospitalized after 10 days of fever. She developed jaundice, dyspnoea, fatigue and a decrease in diuresis. The patient was diagnosed with a severe course of babesiosis with multisystem failure. Therapy was started with quinine orally (650 mg per 8 h), clindamycin intravenously (1800 mg day^{-1}), intubation, dialysis and plasmapheresis. The parasitaemia diminished gradually and resolved 12 days later. However, the patient developed a systemic inflammatory syndrome of an infectious nature, and after 3 months, she died. The cause of death was pneumonia (Kukina *et al.*, 2019).

In both cases, the diagnosis was obtained from blood smears. The morphological features of the parasites were described before the start of specific treatment. Numerous IE parasites were found, which were initially falsely identified as *Plasmodium falciparum*. Babesiosis was diagnosed only on days 7 and 10 for patients 1 and 2, respectively. Complex morphological characteristics [absence of haemozoin, pear-shaped trophozoites, paired pyriforms and tetrad forms ('Maltese Cross')] allowed us to identify the parasites as *Babesia* sp. The paired forms diverged at a wide angle (up to 180°), which is a characteristic feature of *B. divergens*. The piroplasm 18S ribosomal RNA sequence (MK510929, GenBank) from patient 2 was identical to *B. divergens* EU lineage (99.5–100% identity).

Results

Different morphological IE stages of asexual parasites were found in thin blood smears, namely a single ring trophozoite (Fig. 1A), paired pyriforms (club-shaped and 2 attached pear-shaped sister cells) (Fig. 1B), double trophozoites (2 round unattached cells) (Fig. 1E) and double paired pyriforms (2 sets of paired sister cells) (Fig. 1C). Less commonly, we observed tetrads or 'Maltese Crosses' (4 attached sister cells) (Fig. 1E), 4 trophozoites (4 round unattached cells) (Fig. 2A) and filamentous shapes (Fig. 2F). Moreover, in the blood smear of patient 2, we found parasites that divided into 6 and more merozoites; they are typically piriform and joined by their pointed ends, forming a 'daisy head' (Fig. 2B and C).

Both patients showed a similar per cent of paired pyriforms and tetrad forms considering all infected erythrocytes (iRBCs) (Table 1). However, the total number of trophozoites in the erythrocytes of patient 2 was higher than that of patient 1 (2.9 parasites per iRBC and 1.8 parasites per iRBC, respectively), due to multi-occupancy with practically equal parasitaemia (i.e. the per cent of iRBCs). Multi-occupancy was found in 22.8% of iRBC, with up to 14 merozoites inside the erythrocyte of patient 2 (Fig. 2D and E).

Discussion

Unlike *P. falciparum*, *Babesia* sporozoites, which enter the bloodstream of the vertebrate host after a tick bite, immediately penetrate RBCs, develop into trophozoites and begin to divide (Krylov, 1996; Hunfeld *et al.*, 2008). Based on *in vitro* studies, there are various pathways by which IE stages could develop (Cursino-Santos *et al.*, 2016). In the first way, inside the host erythrocytes, a sporozoite becomes a ring trophozoite, proliferates (mononucleated rounded/ovoid trophozoite) and divides by binary fission (budding) (Fig. 1A–C). This process produces 2 daughter merozoites. Typically, all merozoites of *B. divergens* parasites are piriform. Finally, they egress from the RBC and invade intact erythrocytes, become ring trophozoites and initiate new cycles of infection. *In vitro*, the cycle of asexual reproduction from the merozoite penetration of the cell to the release of the next generation of parasites takes 4–6 h (Rossouw *et al.*, 2015; Conesa *et al.*, 2020). In the second way, daughter merozoites do not leave the host cell. They become ring trophozoites and begin the next cycle of asexual reproduction, sharing the same host cytoplasm as sister cells, a phenomenon that eventually results in multi-occupancy of the erythrocyte (Fig. 2A, D and E).

According to previous views, multi-occupancy is limited to 10–12 parasites per iRBC when *B. divergens* is cultured *in vitro* (Cursino-Santos *et al.*, 2016). In natural hosts (*B. taurus*), multi-occupancy of an RBC is very rare: no more than 4 parasites and tetrads are very rare (only in 0.02% of infected erythrocytes). By contrast, in humans up to 5% of infected erythrocytes contain tetrads (Gorenflot *et al.*, 1991; Krylov, 1996; Zintl *et al.*, 2003). The latter is undoubtedly consistent with the data obtained in both patients. Among the published cases of human babesiosis in

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Table 1. Characteristics of patients by the level of infected erythrocytes

Patient number	Parasitaemia level (%)	Polyparasitism (number of parasites per erythrocyte)	Paired pyriforms[a]	Tetrad forms[a]	Multiple parasites (>4 parasites)[a]
1	23	Up to 8	24	4	0.01
2	17	Up to 14	26	5	22.8

[a]Per cent of paired pyriforms/tetrad stages/multiple parasites in all infected erythrocytes.

Europe, there are no mentions of the extraordinarily high level of propagation of *B. divergens* (>8 IE parasites). The number of parasites in 1 erythrocyte did not exceed 8 on the presented photos of thin blood smears (Martinot *et al.*, 2011; Gonzalez *et al.*, 2014; Mørch *et al.*, 2015; O'Connell *et al.*, 2017). Even in fatal cases of patients who had undergone splenectomy, including patient 1, most iRBC did not have more than 8 parasites (Fig. 1C) (Centeno-Lima *et al.*, 2003; Asensi *et al.*, 2018; Kukina *et al.*, 2018). A very high infection of erythrocytes in patient 2 (22.8% of iRBC contained 5–14 parasites) is described for the first time. Such an extraordinarily high rate of parasite reproduction had been unknown for *B. divergens* in either natural or accidental hosts (Fig. 2D and E). The same intensity of the division of rabbit *Babesia* sp. (strain NR774) was noted in an *in vitro* culture of human RBCs (Spencer *et al.*, 2006).

Observations in *in vitro* culture have shown that the tetrad forms ('Maltese Crosses') are formed from paired pyriform ('figure 8') by sequential division (Cursino-Santos *et al.*, 2016; Conesa *et al.*, 2020). It has previously been assumed that a tetrad of parasites is, in some, perhaps a simple superposition of 2 pairs of dividing individuals on top of each other. However, in Fig. 1D, it is clear that the division into 4 daughter nuclei occurred simultaneously. The discovery of the formation of a 'daisy head' as a result of simultaneous division of the parasite into 6 and possibly more merozoites all the more disproves the simple overlap of individuals.

There is a lack of data on gametogenesis because previous work has often been carried out on long-term cultures of parasites, which lose the ability to gametogenesis. The capacity of *B. divergens* to produce gametocytes has been shown (measured by expression of the *bdccp* genes) in several bovine strains after short-term cultivation (Jalovecka *et al.*, 2016). We did not find data on the morphology of babesial gametocytes in blood smears stained by the Romanovsky–Giemsa method. Perhaps by analogy with *Plasmodium*, gametocytes could be single, large, vacuole-free cells with a large centrally located nucleus (Fig. 1F).

Babesia reproduction occurs asynchronously, so all developmental stages of the parasite may be seen simultaneously. RBCs can bear multiple parasites that may not necessarily be in the same step in the division's process (Fig. 1C and E). A very high infection of erythrocytes is evidenced by the presence of filamentous forms, noted as crisis forms during *in vitro B. divergens* cultivation when the culture medium is exhausted (Fig. 2F) (Gorenflot *et al.*, 1991). It can be assumed that the reason for the extraordinarily high level of multi-occupancy iRBCs and the formation of crisis forms of the parasite, in the described case, was an adverse condition, namely an increase in the concentration of metabolites in the blood due to the development of acute renal failure in the patient (Fig. 2B–F).

In conclusion, it is shown that in *Babesia*, in addition to simple binary fission, there are probably more complex variants of asexual reproduction. Simultaneous division of the parasite into 6 and, possibly, more merozoites (*vs* the usual 2, less often 4 merozoites), was found. This phenomenon had been unknown in both natural and accidental hosts. Under certain conditions, fission of parasite reproduction is possible without egressing the erythrocyte. This phenomenon results in unusually high multi-occupancy – up to 14 parasites developed in the affected erythrocytes. Some RBCs were so filled with trophozoites that they could not be counted.

Data. All research data are presented in the article. Photos of Romanovsky-stained thin blood smears can be obtained from the author for correspondence.

Author contributions. The authors contributed equally to the conceptualization, investigation (data collection), writing and editing of this article.

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Ethical standards. Not applicable.

References

Asensi V, González LM, Fernández-Suárez J, Sevilla E, Navascués RÁ, Suárez ML, Lauret ME, Bernardo A, Carton JA and Montero E (2018) A fatal case of *Babesia divergens* infection in Northwestern Spain. *Ticks and Tick-Borne Diseases* **9**, 730–734.

Centeno-Lima S, do Rosário V, Parreira R, Maia AJ, Freudenthal AM, Nijhof AM and Jongejan F (2003) A fatal case of human babesiosis in Portugal: molecular and phylogenetic analysis. *Tropical Medicine and International Health* **8**, 760–764.

Conesa JJ, Sevilla E, Terrón MC, González LM, Gray J, Pérez-Berná AJ, Carrascosa JL, Pereiro E, Chichón FJ, Luque D and Montero E (2020) Four-dimensional characterization of the *Babesia divergens* asexual life cycle, from the trophozoite to the multiparasite stage. *mSphere* **5**, e00928-20.

Corpelet C, Vacher P, Coudore F, Laurichesse H, Conort N and Souweine B (2005) Role of quinine in life-threatening *Babesia divergens* infection successfully treated with clindamycin. *European Journal of Clinical Microbiology and Infectious Diseases* **24**, 74–75.

Cursino-Santos JR, Singh M, Pham P, Rodriguez M and Lobo CA (2016) *Babesia divergens* builds a complex population structure composed of specific ratios of infected cells to ensure a prompt response to changing environmental conditions. *Cellular Microbiology* **18**, 859–874.

Gonzalez LM, Rojo S, Gonzalez-Camacho F, Luque D, Lobo CA and Montero E (2014) Severe babesiosis in immunocompetent man, Spain, 2011. *Emerging Infectious Diseases* **20**, 724–726.

Gorenflot A, Brasseur P, Precigout E, L'Hostis M, Marchand A and Schrevel J (1991) Cytological and immunological responses to *Babesia divergens* in different hosts: ox, gerbil, man. *Parasitology Research* **77**, 3–12.

Gray JS, Estrada-Peña A and Zintl A (2019) Vectors of babesiosis. *Annual Review of Entomology* **64**, 149–165.

Hildebrandt A, Gray JS and Hunfeld K-P (2013) Human babesiosis in Europe: what clinicians need to know. *Infection* **41**, 1057–1072.

Hildebrandt A, Zintl A, Montero E, Hunfeld K-P and Gray J (2021) Human babesiosis in Europe pathogens **10**, 1165.

Hunfeld K-P, Hildebrandt A and Gray JS (2008) Babesiosis: recent insights into an ancient disease. *International Journal for Parasitology* **38**, 1219–1237.

Jalovecka M, Bonsergent C, Hajdusek O, Kopacek P and Malandrin L (2016) Stimulation and quantification of *Babesia divergens* gametocytogenesis. *Parasites and Vectors* **9**, 439.

Krylov MV (1996) *Key to Protozoan Parasites (of Man, Domestic Animals and Agricultural Plants)*. St. Petersburg: Russian Academy of Sciences. Zoological Institute.

Kukina IV, Guzeeva TM, Zelya OP and Ganushkina LA (2018) Fatal human babesiosis caused by *Babesia divergens* in an asplenic host. *IDCases* **13**, e00414.

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Kukina IV, Zelya OP, Guzeeva TM, Karan LS, Perkovskaya IA, Tymoshenko NI and Guzeeva MV (2019) Severe babesiosis caused by *Babesia divergens* in a host with intact spleen, Russia, 2018. *Ticks and Tick-Borne Diseases* **10**, 101262.

Martinot M, Zadeh MM, Hansmann Y, Grawey I, Christmann D, Aguillon S, Jouglin M, Chauvin A and DeBriel D (2011) Babesiosis in immunocompetent patients, Europe. *Emerging Infectious Diseases* **17**, 114–116.

Mørch K, Holmaas G, Frolander PS and Kristoffersen EK (2015) Severe human *Babesia divergens* infection in Norway. *International Journal of Infectious Diseases* **33**, 37–38.

O'Connell S, Lyons C, Abdou M, Patowary R, Aslam S, Kinsella N, Zintl A, Hunfeld K-P, Wormser GP, Gray J, Merry C and Alizadeh H (2017) Splenic dysfunction from celiac disease resulting in severe babesiosis. *Ticks and Tick-Borne Diseases* **8**, 537–539.

Rossouw I, Maritz-Olivier C, Niemand J, van Biljon R, Smit A, Olivier NA and Birkholtz L-M (2015) Morphological and molecular descriptors of the developmental cycle of *Babesia divergens* parasites in human erythrocytes. *PLoS Neglected Tropical Diseases* **9**, e0003711.

Sevilla E, González LM, Luque D, Gray J and Montero E (2018) Kinetics of the invasion and egress processes of *Babesia divergens*, observed by time-lapse video microscopy. *Scientific Reports* **8**, 14116.

Skrabalo Z and Deanovic Z (1957) Piroplasmosis in man; report of a case. *Documenta de medicina geographica et tropica* **9**, 11–16.

Spencer AM, Goethert HK, Telford 3rd SR and Holman PJ (2006) In vitro host erythrocyte specificity and differential morphology of *Babesia divergens* and a zoonotic *Babesia* sp. from eastern cottontail rabbits (*Sylvilagus floridanus*). *Journal of Parasitology* **92**, 333–340.

Yabsley MJ and Shock BC (2013) Natural history of zoonotic *Babesia*: role of wildlife reservoirs. *International Journal for Parasitology: Parasites and Wildlife* **22**, 18–31.

Zintl A, Mulcahy G, Skerrett HE, Taylor SM and Gray J (2003) *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. *Clinical Microbiology Reviews* **16**, 622–636.

https://doi.org/10.1017/S0031182022000439 Published online by Cambridge University Press