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Severe babesiosis caused by *Babesia divergens* in a host with intact spleen, Russia, 2018

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ABSTRACT

We report a case of severe babesiosis caused by the bovine pathogen *Babesia divergens* with the development of multisystem failure in a splenic host. Immunosuppression other than splenectomy can also predispose people to *B. divergens*. There was heavy multiple invasion of up to 14 parasites inside the erythrocyte, which had not been previously observed even in asplenic hosts. The piroplasm 18S rRNA sequence from our patient was identical *B. divergens* EU lineage with identity 99.5–100%.

1. Introduction

Babesia divergens, a protozoan blood parasite (Apicomplexa: Babesiidae) is primarily specific to bovines. This parasite is widespread throughout Europe within the vector *Ixodes ricinus*. The distribution area and abundance of *I. ricinus* increased considerably during the last decades (Jaenson et al., 2012; Vasil'eva et al., 2013). Almost all cases of human babesiosis in Europe are caused by *B. divergens* and observed in patients who have been splenectomised prior to infection (Rabinovich et al., 1978; Centeno-Lima et al., 2003; Corpelet et al., 2005; Mørch et al., 2015; Kukina et al., 2018). These cases are often fatal. However, in Europe, sporadic cases of babesiosis have also been diagnosed in patients with intact spleens (Gonzalez et al., 2011; Martinot et al., 2011; O'Connell et al., 2017). We report the fifth case of severe human babesiosis, caused by *B. divergens* in a patient with an intact spleen.

2. Case summary

The patient, a 74-year old female pensioner, was living in Moscow. On August 6, 2018 she developed an influenza-like syndrome with a severe fever of 38.5 °C and tussis. She received antipyretic self-treatment for one week. On the tenth day after infection, she developed jaundice, dyspnoea, fatigue, and a decrease of diuresis and was brought to the hospital on August 15. Her condition was extremely critical. Selected laboratory signs at admission are shown in Table 1.

Leucocyte left shift with immature neutrophils, signs of dyserythropoiesis, anisocytosis, and poikilocytosis were seen on the peripheral smear. Numerous intra-erythrocytic parasites were found, which were initially falsely identified as *Plasmodium falciparum*. The patient was transferred to a specialist infectious disease hospital.

The patient was living in a temperate region where falciparum malaria is absent, and she had not travelled to an endemic area. Three weeks before her illness she went to the Tver region, Northwest Russia, where she stayed for one week at a summer house and noticed an attached tick. She did not remove it, instead taping the tick with a band aid, allowing it to feed completely. Thus, the duration of tick feeding was sufficient to complete sporogony. Identification of the tick species and stage was not carried out.

The blood smear was re-examined in the reference laboratory. Parasites were small, with great variation in their forms. Complex morphological characteristics (absence of haemozoin, pear-shaped trophozoites, paired pyriforms and tetrad forms «Maltese Cross») allowed us to speciate the parasites as *Babesia* sp. The paired forms diverged at a wide-angle (up to 180°), which is a characteristic feature of *B. divergens* (Fig. 1).

Many red blood cells had multiple invasion, with as many as 10–14 trophozoites inside one host cell (Fig. 2).

We extracted DNA from 10 µl of blood, using a commercial kit (AmpliSens RiboPrep Kit (Central Research Institute of Epidemiology, Moscow, Russia)), following the manufacturer's instructions. Samples

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Table 1
Selected laboratory signs at admission (12th day of illness).

Haematology			Biochemistry		
HGB	6.5	11.7–18.0 g/dl	UA	801.5	143–417 μmol/L
HCT	19.1	35–52%	TBi	69.5	3.1–16.9 μmol/L
RBC	2.03	3.8–6.1 × 10^{12}/L	DBi	35.0	0.78–4.23 μmol/L
RBCN	89	–	LDG	4074.4	135–240 U/L
PLT	150.0	150–450 × 10^{9}/L	CRP	222.12	0–0.5 mg/L
WBC	4.4	4–11 × 10^{9}/L			
IRBC	17	–			
HJB	+	–			
TC	+	–			
SCHC	15	0.2%			

HGB: haemoglobin, HCT: haematocrit, RBC: total red blood count, RBCN: normoblasts per 100 leukocytes, PLT: total platelet count, WBC: white blood cell, IRBC: % invaded erythrocytes, HJB: Howell-Jolly bodies, TC: target cells, SCHC: schistocytes, UA: urea acid, TBi: total bilirubin, DBi: conjugated bilirubin, LDG: lactate dehydrogenase, CRP: C-reactive protein.

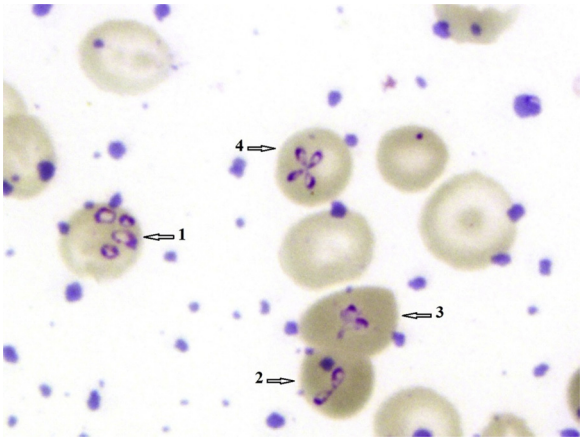

Fig. 1. *Babesia divergens.* Romanovsky-stained thin blood smear. Diversity forms of trophozoites: ring forms (1), the paired forms «Figure 8s» diverging at a wide-angle (up to 180°) (2), pear-shaped trophozoites (3), tetrad forms «Maltese Cross» (4). (Original), (x1000).

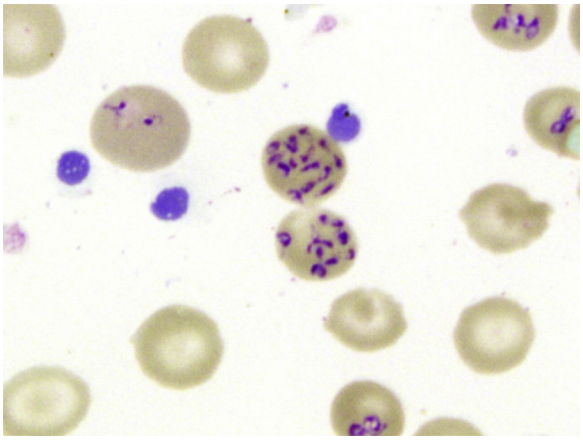

Fig. 2. *Babesia divergens.* Romanovsky-stained thin blood smear. Multiple invasion (up to 14 parasites). (Original), (x1000).

were screened for *B. divergens* and *Babesia venatorum* (EU1) by PCR, using the following primers and probes: Bab di hsp70F CTCATTGGTG ACGCCGCTA, Bab di hsp70R CTCCTCCCGATAAGCCTCTT, Bab di hsp70P R6G-AGAACCAGGAGGCCCGTAACCCAGA-BQH1, and Bab EU RNA18S F GCGCGCTACACTGATGCATT, Bab EU RNA18S R CAAAAA TCAATCCCCGTCACG, Bab EU RNA18S P FAM-CATCGAGTTTAATCCT GTCCCGAAAGG-BQH1 (Michelet et al., 2014). Sequencing analysis was carried out using BS4 AGGGACGTAGTCGGCACGAG and BS5 CGAGG CAGCAACGGGTAACG primers (Rar et al., 2011) on ABI PRISM 3500 (Applied Biosystems, United States) and analysed with the BLASTN (http://www.ncbi.nlm.nih.gov/BLAST).

The 18S rRNA gene was partially sequenced (MK510929, GenBank) and aligned with closely related sequences available from GenBank. The *B. divergens/capreoli* group consisted of 5 or more lineages, correlating with geographical origin. The piroplasm 18S rRNA sequence from our patient was close to *B. divergens* sequences with identity between it and *B. divergens* EU lineage – 99.5–100%; with *B. divergens* US lineage – 99.8%; with *B. capreoli* lineage – 99.8–99.7%; with *B. divergens* Asian lineage – 99.3–99.1%; with *B. divergens* Portugal- 98.8%; with *B. venatorum* – 98.0%, and with *Babesia microti* – 96.0%.

The *Babesia* spp. 18S rRNA gene has a hypervariable region between 626–666 positions (*B. divergens*, FJ944826), which can be used for differentiation of all these lineages. Our isolate has substitutions at positions 626, 627, 629, 632, 633, 638, 639, 640 that are common only to *B. divergens* EU lineage. Phylogenetic tree of partial 18S rRNA gene (881 bp) was constructed using the neighbor-joining method, Tamura-Nei model with 1,000 bootstrap replications (Fig. 3).

The patient was diagnosed with severe course babesiosis with multisystem failure. Therapy was started with quinine orally (650 mg/8 h), clindamycin intravenously (1800 mg/d), intubation, dialysis, and plasmapheresis.

The parasitaemia diminished gradually and resolved 12 days later. After three months, the patient died. The cause of death was pneumonia. A pathological condition that can lead to the above cause of death is systemic inflammatory response syndrome infectious origin with multisystem failure.

3. Discussion

Falciparum malaria was falsely diagnosed due to the presence of small ring forms in the blood smear. The differential diagnosis is essential to determine whether the causative agent of disease is *Babesia* or *Plasmodium*, as the therapy is different for these two diseases.

Multiple invasion of *Babesia* (unlike *Plasmodium*) can be due to a repeated multiplication of the organisms in the host cell and non-efficient egress. Polyparasitism seems to be infrequent in the bovine host but is common in humans and in culture. We point out the unusual hyperparasitism of erythrocytes of up to 14 parasites. Such a heavy invasion of the erythrocyte had not been previously observed in even an asplenic host (usually up to 4–6, but not very frequently up to 8) (Zintl et al., 2003; Kukina et al., 2018).

The patient had a history of sigmoid colon cancer, with a left-side hemicolectomy in 2004, autoimmune thyroiditis, and hypothyroidism. This case was complicated by a late request for care that caused delayed diagnosis and the late initiation of appropriate therapy on the twelfth day after infection. Although this patient had an intact spleen, the detection of Howell-Jolly bodies and 15% schistocytes in the peripheral blood smear may indicate hyposplenism.

The patient did not deal with agriculture or animals, making her distinct from a huntsman (Kukina et al., 2018) or livestock farmer (Rabinovich et al., 1978), who are frequently exposed to the bites of ticks. She was likely exposed within a garden plot. Many elderly live in their garden plots in the summer, so the probability of infection with babesiosis must be determined.

About 100 cases of cattle babesiosis were registered in the Central region of Russia in 2017 (Unpublished data of Ministry of Agriculture). Both *I. ricinus* and *Ixodes persulcatus* occur in the Tver region (Vasil'eva et al., 2013), but only *I. ricinus* (probably all active stages) is a vector of *B. divergens* (Zintl et al., 2003; Gray et al., 2010). Unfortunately, to date, little is known about the occurrence of *Babesia* in ticks in forested, urban environments in Eastern Europe, including Russia. The infection rates of *I. ricinus* with *B. divergens* collected in mixed beech and oak forests was relatively low in Hungary (0.5%) (Egyed et al., 2012), in

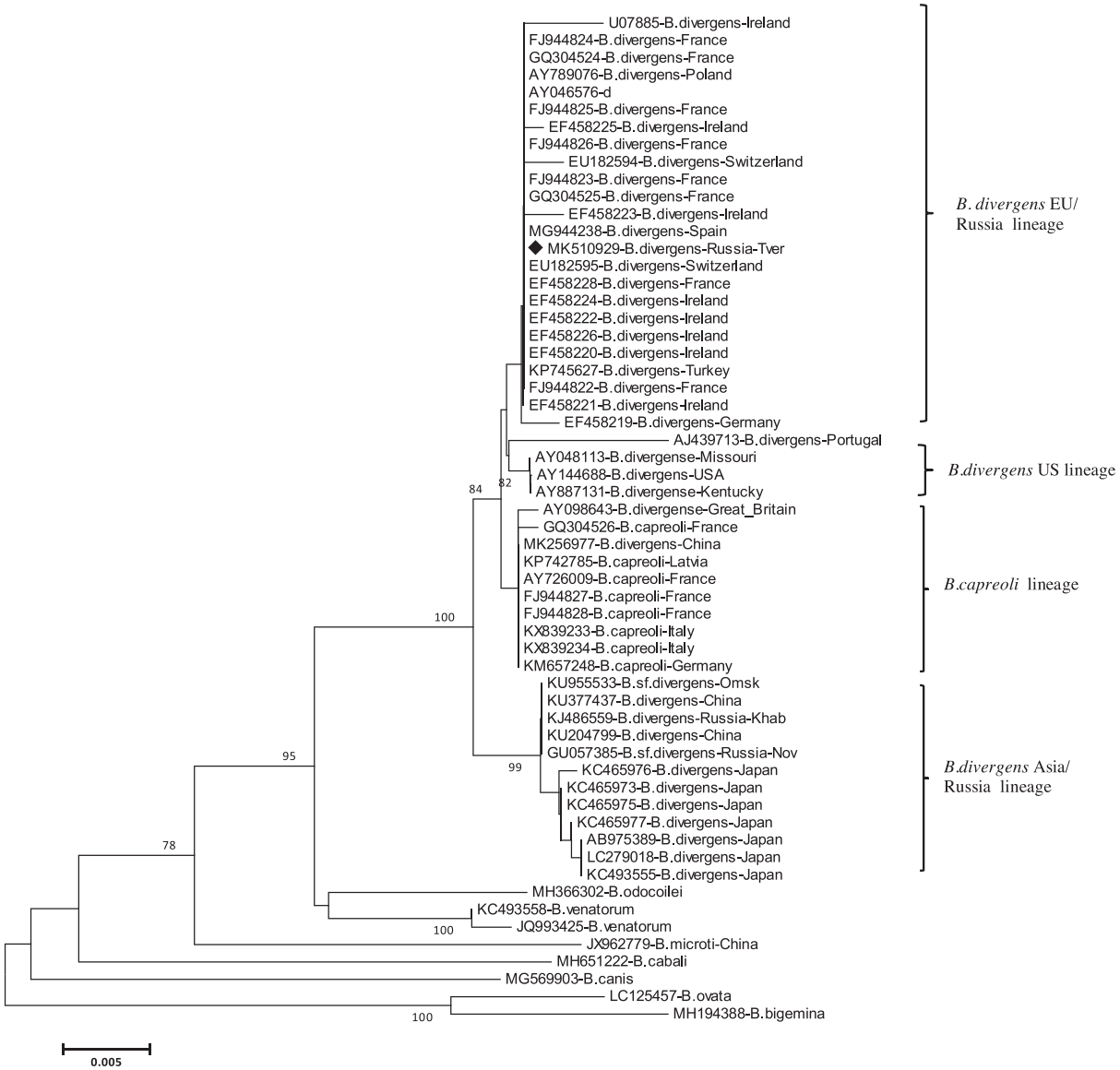

Fig. 3. Phylogenetic tree of partial 18S rRNA gene (881 bp) was constructed using the neighbor-joining method, Tamura-Nei model with 1,000 bootstrap replications.

various habitats such as grassland, bushy areas, and deciduous woodland in Latvia (0.2%) (Capligina et al., 2016) and 0.1% in Estonia (no date about habitats) (Katargina et al., 2011). *B. divergens* was found in one of eleven adult *I. ricinus* ticks from Bratislava and in one of 37 *I. ricinus* nymphs from the surrounding forests (Hamšíková et al., 2016).

In addition to the low occurrence of *Babesia* in ticks, actual disease only seems to occur in immunocompromised patients (sometimes just with splenectomy). This alone reduces incidence of the disease but also probably infection.

4. Conclusion

Human babesiosis is a rare but life-threatening disease.

Physicians should be alert for additional cases. Babesiosis is a seasonal disease, which depends on the activity of *I. ricinus,* which occurs from May to October in temperate regions. Risk factors include accommodation in or travel to endemic areas, splenectomy, hyposplenism, and other immunocompromising factors, with old age possibly being an additional predisposing factor.

Patients should understand that attached ticks need to be removed as soon as possible.

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References

Capligina, V., Berzina, I., Bormane, A., Salmane, I., Vilks, K., Kazarina, A., Bandere, D., Baumanis, V., Ranka, R., 2016. Prevalence and phylogenetic analysis of *Babesia* spp. In *Ixodes ricinus* and *Ixodes persulcatus* ticks in Latvia. Exp. Appl. Acarol. 68 (3), 325–336. https://doi.org/10.1007/s10493-015-9978-0.

Centeno-Lima, S., Do Rosário, V., Parreira, R., Maia, A.J., Freudenthal, A.M., Nijhof, A.M., Jongejan, F., 2003. A fatal case of human babesiosis in Portugal: molecular and phylogenetic analysis. Trop. Med. Int. Health 8, 760–764. https://doi.org/10.1046/j.1365-3156.2003.01074.x.

Corpelet, C., Vacher, P., Coudore, F., Laurichesse, H., Conort, N., Souweine, B., 2005. Role of quinine in life-threatening *Babesia divergens* infection successfully treated with clindamycin. Eur. J. Clin. Microbiol. Infect. Dis. 24, 74–75. https://doi.org/10.1007/s10096-004-1270-x.

Egyed, L., Elő, P., Sréter-Lancz, Z., Széll, Z., Balogh, Z., Sréter, T., 2012. Seasonal activity and tick-borne pathogen infection rates of *Ixodes ricinus* ticks in Hungary. Ticks Tick. Dis. 3, 90–94. https://doi.org/10.1016/j.ttbdis.2012.01.002.

Gonzalez, L.M., Rojo, S., Gonzalez-Camacho, F., Luque, D., Lobo, C.A., Montero, E., 2011. Severe babesiosis in immunocompetent man, Spain, 2011. Emerg. Infect. Dis. 20, 724–726. https://doi.org/10.3201/eid2004.131409.

Gray, J.S., Zintl, A., Hildebrandt, A., Hunfeld, K.P., Weiss, L., 2010. Zoonotic babesiosis:

overview of the disease and novel aspects of pathogen identity. Ticks Tick. Dis. 1, 3–10. https://doi.org/10.1016/j.ttbdis.2009.11.003.

Hamšíková, Z., Kazimírová, M., Haruštiaková, D., Mahríková, L., Slovák, M., Berthová, L., Kocianová, E., Schnittger, L., 2016. *Babesia* spp. in ticks and wildlife in different habitat types of Slovakia. Parasit. Vectors 9, 292. https://doi.org/10.1186/s13071-016-1560-z.

Jaenson, T.G., Jaenson, D.G., Eisen, L., Petersson, E., Lindgren, E., 2012. Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. Parasit. Vectors 5, 8. https://doi.org/10.1186/1756-3305-5-8.

Katargina, O., Geller, J., Vasilenko, V., Kuznetsova, T., Järvekülg, L., Vene, S., Lundkvist, Å, Golovljova, I., 2011. Detection and characterization of *Babesia* species in *Ixodes* ticks in Estonia. Vector Borne Zoonotic Dis. 11, 923–928. https://doi.org/10.1089/vbz.2010.0199.

Kukina, I.V., Guzeeva, T.M., Zelya, O.P., Ganushkina, L.A., 2018. Fatal human babesiosis caused by *Babesia divergens* in an asplenic host. IDCases 13, e00414. https://doi.org/10.1016/j.idcr.2018.e00414.

Martinot, M., Zadeh, M.M., Hansmann, Y., Grawey, I., Christmann, D., Aguillon, S., Jouglin, M., Chauvin, A., De Briel, D., 2011. Babesiosis in immunocompetent patients, Europe. Emerg. Infect. Dis. 17, 114–116. https://doi.org/10.3201/eid1701.100737.

Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., Bødker, R., Fach, P., Moutailler, S., 2014. High-throughput screening of tick-borne pathogens in Europe. Front. Cell. Infect. Microbiol. 4, 103. https://doi.org/10.3389/fcimb.2014.00103.

Mørch, K., Holmaas, G., Frolander, P.S., Kristoffersen, E.K., 2015. Severe human *Babesia divergens* infection in Norway. Int. J. Infect. Dis. 33, 37–38. https://doi.org/10.1016/j.ijid.2014.12.034.

O'Connell, S., Lyons, C., Abdou, M., Patowary, R., Aslam, S., Kinsella, N., Zintl, A., Hunfeld, K.P., Wormser, G.P., Gray, J., Merry, C., Alizadeh, H., 2017. Splenic dysfunction from celiac disease resulting in severe babesiosis. Ticks Tick. Dis. 8, 537–539. https://doi.org/10.1016/j.ttbdis.2017.02.016.

Rabinovich, S.A., Voronina, Z.K., Stepanova, N.I., Maruashvili, G.M., Bakradze, T.L., 1978. 1st detection of human babesiosis in the USSR and a short analysis of the cases described in literature (in Russian). Med. Parazitol. (Mosk) 47, 97–107 PubMed PMID:149906.

Rar, V.A., Epikhina, T.I., Livanova, N.N., Panov, V.V., 2011. Genetic diversity of *Babesia* in *Ixodes persulcatus* and small mammals from North Ural and West Siberia, Russia. Parasitology 138, 175–182. https://doi.org/10.1017/S0031182010001162.

Vasil'eva, I.S., Ganushkina, L.A., Gutova, V.P., Litvinov, S.K., 2013. The impact of climatic changes on *Ixodes* (Ixodidae) ticks and their related natural and focal infections (In Russian). Med. Parazitol. (Mosk) (3), 55–63 PubMed PMID: 25924291.

Zintl, A., Mulcahy, G., Skerrett, H.E., Taylor, S.M., Gray, J.S., 2003. *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. Clin. Microbiol. Rev. 16, 622–636. https://doi.org/10.1128/CMR.16.4.622-636.