



Case Report

First report of *Babesia divergens* infection in an HIV patient

Luis M. González^{a,g}, Emma Castro^{b,g}, Cheryl A. Lobo^c, Alberto Richart^d, Raquel Ramiro^a, Fernando González-Camacho^e, Daniel Luque^e, Aurelio C. Velasco^f, Estrella Montero^{a,*}

^a Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

^b Cruz Roja Española, Centro de Transfusión, Madrid, Spain

^c Blood Borne Parasites Department, Lindsley Kimball Research Institute New York Blood Center, New York, New York, USA

^d Centro de Transfusión de la Comunidad de Madrid, Madrid, Spain

^e Unidad de Microscopía Electrónica y Confocal, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

^f Departamento de Microbiología, Hospital Universitario San Carlos, Madrid, Spain

ARTICLE INFO

Article history:

Received 10 December 2014

Received in revised form 30 January 2015

Accepted 3 February 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:

Babesia divergens

patient

HIV

babesiosis

blood transfusion

transmission route

ABSTRACT

Human babesiosis is a zoonosis primarily transmitted through *Ixodes* ticks and alternatively by routes such as blood transfusions from asymptomatic donors. We report the first case of human babesiosis caused by *Babesia divergens* in a patient with HIV. This study also focuses on elucidating the possible transmission route of infection in this patient, who received numerous blood transfusions but showed patent symptoms only after splenectomy. A battery of detection tools along with a novel Western-Blot Assay and Enzyme Linked Immunosorbent Assay using the major surface protein of *B. divergens* (Bd37) as a target were used to evaluate the presence of *B. divergens* or antibodies against the parasite in samples from the patient and the blood donors involved in this case. A retrospective study of the humoral status against the parasite revealed *B. divergens* IgG antibodies in one of the implicated donors, but also showed that the patient had been already exposed to the parasite before any transfusion. Thus, this analysis of natural and transfusion transmission routes suggests a pre-existing subclinical babesiosis in the patient. © 2015 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Human babesiosis is an expanding zoonosis transmitted through ticks. *Babesia microti*, *B. divergens*, *B. duncani*, *B. venatorum*, and *B. divergens*-like organisms (1) are responsible for the human disease, causing a febrile illness similar to malaria. Transmission of *B. microti* and *B. duncani* via blood transfusion from infected asymptomatic donors has also been reported. The spread of *Babesia* through transfusions is increasingly a problem and is one of the most commonly reported transfusion-transmitted infections in the USA. *B. divergens* is considered the main agent of human babesiosis in Europe (1). Although *B. divergens* also meets the requirements for potential transmission through transfusion of

blood components, no transfusion transmission has been reported yet. We report the first case of babesiosis caused by *B. divergens* in an immunocompromised patient with HIV.

1. The patient

A 37-year-old man from Spain, newly diagnosed with HIV infection, showed plasma HIV RNA levels of 277,000 copies/mL and a CD4+ T cell count of 178/μL at diagnosis. The patient was admitted to a tertiary University Hospital in Madrid, Spain. Two weeks after starting the antiretroviral therapy, a hemophagocytic syndrome developed in the patient, possibly as part of an immune restoration syndrome, and he received multiple blood transfusions. To control his progressive deterioration, the patient underwent an elective splenectomy. After a slow recovery, the patient showed a new decline in his general condition, without relevant microbiological findings. About three weeks later, the patient was admitted to the ICU with acute respiratory distress syndrome, transfused again and treated empirically with multiple broad-spectrum antibiotics, antifungals and appropriate drugs for *Mycobacterium tuberculosis* and *M. avium-intracellulare*, without

* Corresponding author. Estrella Montero, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Carretera Majadahonda-Pozuelo, Km 2.2, Majadahonda, Madrid, 28220, Spain. Tel.: +34 606 275315; Fax: +34 91 5097034.

E-mail addresses: lmgonzal@isciii.es (L.M. González), emmacastroizaguirre@gmail.com (E. Castro), CLobo@NYBloodCenter.org (C.A. Lobo), alberto.richart@salud.madrid.org (A. Richart), rramiro@isciii.es (R. Ramiro), fgonzalez@isciii.es (F. González-Camacho), dluque@isciii.es (D. Luque), aurelio.velasco@salud.madrid.org (A.C. Velasco), estrella.montero@isciii.es (E. Montero).

[‡] These authors contributed equally to this article.

any improvement. On the 3rd day after admission into the ICU, a Giemsa-stained thin film from peripheral blood showed a few intraerythrocytic parasites compatible with *Babesia* spp. A 156 bp fragment encoded by the variable region V4 of the *B. divergens* 18S rRNA gene (from thymine 609 to guanine 764) was amplified from the patient's blood by PCR by using F4Bd (5'-ttgctggtgtaatat-3') and R1 Mx (5'-ccaacaaatagaaccaagtc-3') primers. The fragment showed 100% identity to *B. divergens* and differed to *B. divergens* like (BLASTN 2.2.30) confirming the presence of *B. divergens* parasites. Specific treatment for *Babesia* was started immediately with quinine plus clindamycin, later changed to azithromycin and atovaquone for lower toxicity. The patient's condition showed a clear improvement and he was discharged from the ICU two weeks later. The patient was found free of parasites by PCR in subsequent follow up visits.

Natural (tick) and transfusion-transmission sources of *Babesia* were considered in an effort to trace the possible route of infection. Cattle and wild mammals are hosts for *B. divergens*¹. The patient lived in Spain, and was the owner of a cat but did not have contact with dogs or other domestic and farm animals. The medical history of the patient included frequent travel to various European countries. In particular, a few weeks before his medical crisis, the patient had travelled to Berlin and visited public green parks, but no pasture or wooded areas. Of note, ticks infected with *B. microti*, *B. venatorum* and *B. divergens* have been recently observed in some urban areas from Germany², but the patient did not recall recent tick bites.

Because the patient's route of infection did not seem to follow the natural tick-mammal route for acquisition of *B. divergens*¹, we investigated transfusion-transmitted babesiosis (TTB) as an alternative route. During his hospital stay, the patient received numerous RBC and platelets transfusions. To find a potential source of infection, repository sample sera from 27 blood donors involved in erythrocyte transfusions to the patient were tested retrospectively.

The sequence encoding Asn²⁸ to Phe³⁴¹ of the major surface antigen of *B. divergens*, Bd37³, were cloned into the expression vector pGEX-4T (GE Healthcare Bio-sciences AB, Uppsala, Sweden), expressed in *E. coli* BL21 (DE) and purified as recombinant Bd37-GST (Glutathione S-Transferase) fusion protein following manufacturer's instructions. This protein was then used as a target substrate for both Western blot assay and Enzyme Linked Immunosorbent Assay (ELISA)⁴ to assess the presence of antibodies in the patient and donors. Indirect Immunofluorescence Assay (IFA) on smears of parasitized RBCs was also performed to detect anti-parasite antibodies. The tests revealed the presence of specific antibodies against *B. divergens* by IFA and specific anti-Bd37 IgG antibodies by WB and ELISA in just one serum sample of a blood donor from a rural area of Spain (Table 1, Supplementary files S1 and S2). Two days before the *Babesia* detection by microscopy, the patient had received a packed erythrocytes concentrate from this positive donor, who therefore could be considered as a possible source of infection in our case. Unfortunately, this donor could not be located, preventing us from performing further analysis such as genotyping the infecting strain. A retrospective study was also performed with six pre-transfusion and three post-transfusion sera samples from the patient. Surprisingly, these samples showed the presence of anti-*B. divergens* IgG by IFA and anti-Bd37 IgG antibodies detected by WB and ELISA in as early as the first blood sample collected at the beginning of his hospital admission. As a consequence, we could not confirm TTB leading to patient's sero-conversion (Supplementary files S1 and S2). Since the patient was *Babesia*-seropositive before any transfusion, natural acquisition of the infection and the recent visits of the patient to urban green areas were reconsidered. To find out whether the patient had acquired the *Babesia* infection

Table 1

Detection of *B. divergens* and anti-parasite antibodies at diagnosis and follow up of the HIV-patient using microscopy, PCR, Western blot, ELISA and IFA assays. (PC) A serum sample of a patient who suffered a severe babesiosis caused by *B. divergens* was kindly provided by Dr. Lappalainen⁵ and used as a positive control. (NC) A sera pool from humans negative for malaria, toxoplasmosis, leishmaniasis, filariasis, and some others was kindly provided by the Unit of Serologic Diagnosis of Parasitic Diseases CNM, Spain, and used as a negative control.

	Time (mo)	Parasite detection in peripheral blood		Retrospective antibody detection				
		Optical Microscopy	PCR	WB		ELISA		IFA
				IgG	IgM	IgG	IgM	
Patient	1	Not done ^a	Not done ^a	+ ^c	- ^c	+ ^c	- ^c	+ ^c
	2	+	+	+ ^c	- ^c	+ ^c	- ^c	+ ^c
	3	-	-	+ ^c	- ^c	+ ^c	- ^c	+ ^c
	4	-	-	+ ^d	- ^d	+ ^d	- ^d	+ ^d
Donor		Not done ^b	Not done ^b	+	-	+	-	+
PC				+	+	+	+	+
NC				-	-	-	-	-

(a) No specific studies were done for parasites. (b) In spite of an active search the donor was not found. (c) Pre-transfusion patient's sera samples and (d) Post-transfusion patient's sera samples collected during 4 months.

recently, serologic testing for specific IgM was performed by WB (Supplementary file S2) and ELISA. *B. divergens* IgM antibodies could not be detected in sera samples from the patient. This could indicate that the *B. divergens* infection was not recently acquired, but rather was a pre-existing subclinical babesiosis with a low-grade parasitemia that flourished following splenectomy and the immune decline of the patient.

2. Discussion

We have reported what we think is the first case of a patient coinfecting with HIV and *B. divergens*. HIV-derived immunosuppression and the splenectomy probably co-contributed to the clinical flourishing of the babesiosis. The report also focuses on elucidating the transmission route of the *Babesia* infection to the patient.

The use of the recombinant Bd37 protein in WB and ELISA along with IFA allowed us to identify *B. divergens* IgG antibodies in one of the implicated blood donors, but also revealed that the patient had been already exposed to the parasite before transfusions. Additional evidence that the infection was not recently acquired was obtained from the lack of *B. divergens* IgM antibodies in sera samples from the patient.

As for most emerging infectious diseases, many epidemiological parameters remain unknown: the proportion of undetected babesiosis cases, the prevalence of babesiosis in blood donors, and the risk of getting a *Babesia* infection following blood transfusion is not well established. *Babesia* infected ticks and their vertebrate hosts are present in urban, rural and natural areas of Europe. Infection by *B. divergens* and *B. venatorum* may follow a protracted course, representing a threat to the blood supply which could lead to TTB cases. Significant seroprevalence rates have been documented in Europe¹ although only around 50 cases of *Babesia* infections have been reported in the continent so far¹. This low number of cases invites us to think about asymptomatic carriers going undetected or to misdiagnosis of symptomatic patients. Thus, to fully recognize the status of babesiosis in Europe, sensitive and specific assays capable of detecting the infection are needed to provide robust data for diagnosis, track alternative transmission routes of infection, determine seroprevalence rates in the blood donor population, and thus improve the safety of the blood supply.

Supplementary file S1. The IFA test revealed specific anti-*B. divergens* (Bd Rouen 1987 strain) antibodies in sera of the patient

and the positive donor. *B. divergens* smears were performed using intra-erythrocytic forms (A and D) and free parasites (B, C, and E) prepared from *in vitro* human erythrocyte cultures. All sera samples were diluted at 1:128 and the IgG specific antibodies were detected with goat anti-human IgG antibodies conjugated to fluorescein isothiocyanate (FITC). A and B: serum sample of the patient collected at the beginning of hospital admission. C: serum sample of the positive donor. D and E: negative control (NC). Column 1: Infected blood cells or free merozoites captured by differential interference contrast (DIC). Column 2: DAPI-stained nucleic acids (shown in blue). Column 3: Fluorescing intraerythrocytic parasites or isolated free extracellular merozoites probed with IgG antibodies from the sera samples. Column 4 shows all images overlaid.

Supplementary file S2. Serological detection of specific anti-*B. divergens* IgG and IgM antibodies. WB and ELISA were prepared using recombinant Bd37-GST (59.5 kDa), and GST fusion partner for Bd37 (26 kDa). Specificity for the patient's and donor's sera against recombinant Bd37 from *B. divergens* was observed by WB. Sera samples were diluted and the specific cutoff was applied at 1:200 for WB by using 150 ng of each of Bd37 and GST recombinant proteins and probing with IgG (A and B) and IgM (C and D). Bd37 (Panel A) and GST (Panel B) recombinant proteins subjected to SDS-PAGE. Lane 1: positive control (PC). Lane 2: the serum sample of the patient collected at the beginning of hospital admission (early January). Lane 3: the serum sample of the positive donor. Lane 4: One of the 26 sera samples from negative donors. Lane 5: negative control (NC). Lane 6 a pool of donors infected by *B. microti* (kindly provided by LFKRI New York Blood Center, New York, NY) did not recognize the recombinant Bd37 protein. None of the sera reacted with the recombinant GST protein. E. Detection of *B. divergens* IgG antibodies by ELISA using the positive control (PC), the negative control (NC) and the positive donor sera samples (symbols and lines in black), six pre-transfusion sera samples from the patient collected between early January to mid-February (symbols and lines in grey) and three post-transfusion sera samples collected between late February to early May (symbols and dotted lines in grey). Samples were diluted 1:100 to 1:800.

Although all infected sera showed higher OD values than the NC sample in a dilution-dependent manner at dilutions 1:100, 1:200 and 1:400, no significant differences in reactivity between pre-transfusion and post-transfusion sera were observed.

Acknowledgments

Blood donors' information was provided by Centro de Transfusión de la Comunidad de Madrid, Madrid, Spain, Dr. Angeles Andreu (Cruz Roja Española, Centro de Transfusión, Madrid, Spain) and María Pirón (Banco de Sangre y Tejidos de Barcelona, Barcelona, Spain). The pre-analytical phase of the donor samples was conducted by Giovanni Fedele (Centro Nacional de Microbiología (CNM), Madrid, Spain). This work is funded by the Surveillance Program of CNM and the grants from Ministerio de Economía y Competitividad from Spain (AGL2010-21774) to EM and PI10/00895 to DL and Instituto de Salud Carlos III (MPY1411/09) to EM. Work in CAL's laboratory is funded by the NIH (HL105694).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijid.2015.02.005>.

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