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Title: First report of Babesia divergens infection in an HIV patient

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Highlights

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Transfusion transmission and natural routes of infection were evaluated

*Babesia* specific antibodies were detected by IFA/WesternBlot/ELISA in the patient and in one donor

The patient's humoral status showed exposure to the parasite before any transfusion

Sensitive tests are needed to fully recognize the status of babesiosis in Europe
First report of *Babesia divergens* infection in an HIV patient

Authors

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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 Immuno Sorbent 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.

**Keywords:** *Babesia divergens*, patient, HIV, babesiosis, blood transfusion, transmission route.
Highlights

1. First case of babesiosis caused by *Babesia divergens* in a patient with HIV
2. Transfusion transmission and natural routes of infection were evaluated
3. *Babesia* specific antibodies were detected by IFA/WesternBlot /ELISA in the patient and in one donor
4. The patient’s humoral status showed exposure to the parasite before any transfusion
5. Sensitive tests are needed to fully recognize the status of babesiosis in Europe
6. Human babesiosis is an expanding zoonosis transmitted through ticks. *Babesia microti*, *B. divergens*, *B. duncani*, *B. venatorum*, and *B. divergens*-like (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.

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
topatient underwent an elective splenectomy. After a slow recovery, the patient showed a
new decline of 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 any improvement. On the 3\(^{rd}\) 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'-ttgcgtggttaatat-3') and
R1Mx (5'-ccaacaaaatagaaccaaagtcc-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 experienced a clear improvement
and 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 Germany (2), 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 Asn^{28} to Phe^{341} of the major surface antigen of B. divergens, Bd37 (3), were cloned into the expression vector pGEX-4T (GE Healthcare Biosciences 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 of the patient. Surprisingly, these samples showed
the presence of anti-\textit{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 \textit{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 \textit{Babesia} infection recently, serologic testing for specific
\textit{IgM} was performed by WB (Supplementary file S2) and ELISA. \textit{B. divergens} \textit{IgM}
antibodies could not be detected in sera samples of the patient. This could indicate that
the \textit{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.

\textbf{Discussion}

We have reported, what we think is the first case of a patient coinfected with HIV and
\textit{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 \textit{Babesia} infection to the patient.
The use of the recombinant Bd37 protein in WB and ELISA along with IFA allowed us
to identify \textit{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 \textit{B. divergens} IgM antibodies in sera samples of 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 \textit{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 Europe 
(1) although only around 50 cases of *Babesia* infections have been reported in the 
continent so far (1). 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.

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**References**

1. Hildebrandt A, Gray JS, Hunfeld KP. Human babesiosis in Europe: what clinicians 
   need to know. Infection 2013; 41:1057-72.


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. Lappalainen (5) 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.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time (mo)</th>
<th>Parasite detection in peripheral blood</th>
<th>Retrospective antibody detection</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Optical Microscopy</td>
<td>PCR</td>
</tr>
<tr>
<td>1</td>
<td>Not done</td>
<td>Not done</td>
<td>IgG</td>
</tr>
<tr>
<td>2</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
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</tr>
<tr>
<td>PC</td>
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<td></td>
<td>+</td>
</tr>
<tr>
<td>NC</td>
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</tbody>
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(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.