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ORIGINAL ARTICLE

Hepatic tissue damage induced in *Meriones ungliculatus* due to infection with *Babesia divergens*-infected erythrocytes

M.A. Dkhil [a,b,*], S. AL-Quraishy [a], A.S. Abdel-Baki [a,c]

[a] *Zoology Department, College of Science, King Saud University, Saudi Arabia*
[b] *Zoology and Entomology Department, Faculty of Science, Helwan University, Egypt*
[c] *Zoology Department, Faculty of Science, Beni-Suef University, Egypt*

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KEYWORDS

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Abstract *Babesia divergens* is an intraerythrocytic parasite which is capable of infecting a wide range of vertebrates causing huge economic losses.

Histopathological, hematological and biochemical changes during *B. divergens* infection in female *Meriones ungliculatus* were reported. Animals were challenged with 5×10^6 *B. divergens*-infected erythrocytes. Parasitemia were maximum at day 5 postinfection where all gerbils died. Infection of gerbils with *Babesia* induced a significant decrease in erythrocytic count as well as the hemoglobin concentration and hematocrit percentage but leucocytes were increased significantly when compared to uninfected gerbils. Liver enzymes aspartate aminotransferase (AST) and aniline aminotransferase (ALT) were significantly increased while albumin and total bilirubin were significantly decreased at day 5 postinfection with *B. divergens*-infected erythrocytes. Histopathological scores of inflammation after infection of gerbils were done using Ischak's activity index and indicated that the liver was severely affected. In conclusion, the study indicated that the course of infection by *B. divergens*-induced alternations in hematology, biochemistry and histopathology of the hepatic tissue.

1. Introduction

Babesiosis is due to various tick-borne intraerythrocytic parasites of the protozoan genus *Babesia*. These blood parasites infect many domestic and wild animals throughout the world (Kuttler, 1988). *Babesia divergens* is an intraerythrocytic parasite which is capable of infecting a wide range of vertebrates and it is responsible for important economic losses. It is the most pathogenic and widespread *Babesia* in northern temperate areas (L'Hostis and Chauvin, 1999). Human infections have also been observed, especially in splenectomized patients, and a high mortality rate has been reported (Gorenflot, 1988;

* Corresponding author. Address: Zoology Department, College of Science, King Saud University, Saudi Arabia.
E-mail address: mohameddkhil@yahoo.com (M.A. Dkhil).

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Spach et al., 1998; Walker et al., 1996). It is believed that the tick responsible for transmission of *B. divergens* to humans is *Ixodes ricinus* (Gorenflot et al., 1998; Telford and Spielman, 1998). Main clinical manifestations of human babesiosis include intravascular hemolysis with hemoglobinuria and jaundice. Persistent high fever, shaking chills, sweating, headache, myalgia, and lumbar and abdominal pain are concomitant symptoms (Marion et al., 2008).

Histological activity index is an accurate analysis of the tissue sections to determine the severity of the pathological change in organs due to infection. Modified quantitative Ishak's scoring was used (Ishak et al., 1995).

The study aims to estimate the histological and biochemical changes induced in hepatic tissue of gerbils due to infection with *B. divergens*.

2. Materials and methods

2.1. Infection of gerbils

Female *Meriones unguiculatus* aged from 12 to 15 weeks old were used. They were bred under specific pathogen-free conditions in the animal facilities of King Saud University, Riyadh, Saudi Arabia. They were housed in plastic cages and fed on standard diet and given water *ad libitum*. Animals were challenged with 5×10^6 *B. divergens*-infected erythrocytes. Parasitemia was evaluated in Giemsa stained blood smears from the tail veins (Krücken et al., 2009). The experiments were approved by the state authorities and followed Saudi Arabian law on animal protection.

2.2. Hematological and biochemical analysis

The blood was obtained by cardiac puncture, allowed to clot for 30 min at 4 °C, and centrifuged at 3000*g* for 3 min. Sera were collected and stored at −20 °C. Serum aspartate aminotransferase (AST), aniline aminotransferase (ALT), albumin and total bilirubin (Biosystems, Spain) levels were determined using commercial kits on a Shimadzu-UV 1230 model spectrophotometer.

Some blood was collected into tubes with ethylene diamin tetra acetic acid for the determination of some hematological parameters (total erythrocytes count, total Leucocytic count, hemoglobin contents and hematocrit) using an automatic counter (VET-530 CA Medonic; Medonic, Stockholm, Sweden).

2.3. Histopathology

Small pieces of liver were fixed in 10% neutral buffered formalin, processed for light microscopic examination, and then 5–7 μm paraffin sections were cut and stained with Hematoxylin and Eosin for histological study. Modified quantitative Ishak scoring system (Ishak et al., 1995) were used; scores of 1–3 were assigned to cases of minimal liver damage, scores of 4–8 to mild, scores of 9–12 to moderate and scores of 13–18 to severe cases.

2.4. Statistical analysis

Statistical analyses were performed using an unpaired Student's *t*-test. The data were analyzed by using Excel 2003 (Microsoft, USA), and Sigma Plot 2001 (SPSS, USA).

3. Results

3.1. Characteristics of B. divergens infection

Gerbils were fully susceptible to *B. divergens*. Challenge with 5×10^6 parasitized erythrocytes resulted in a lethal outcome of the infection. Parasitemia was about 1% on day one postinfection, then it jumps to 5% on day 2 p.i. The parasitemia rises until reaching about 35% on day 5 p.i. (Fig. 1). No gerbils had been survived after day 5 p.i. (Fig. 1).

Symptoms of babesiosis clearly appeared on day 5 postinfection with shivering, fever and bloody urine. There was a

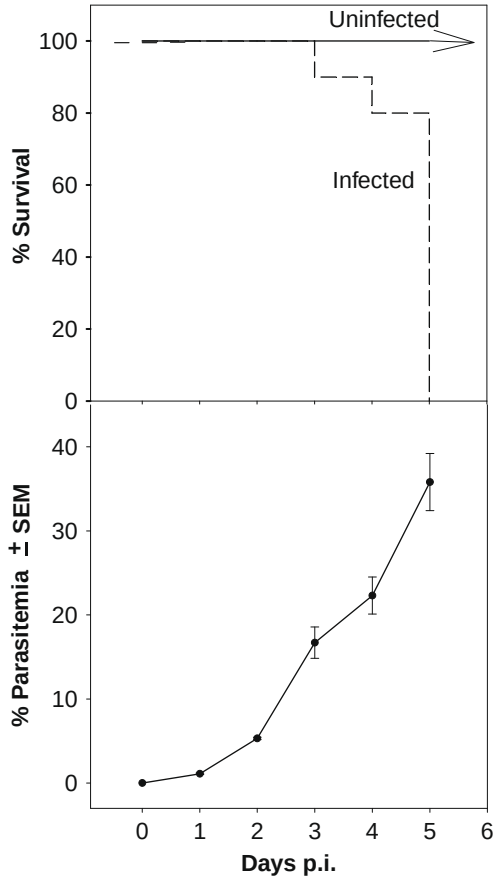

Figure 1 Characteristics of *B. divergens* infection in *Meriones unguiculatus* challenged with 5×10^6 parasitized erythrocytes. Parasitemia with survival ($n = 10$). (All values are mean ± SEM.)

Table 1 Histopathological scores of inflammation after infection of gerbils with *B. divergens*-parasitized erythrocytes.

Liver parameters	Uninfected gerbils ($n = 5$)	Infected gerbils ($n = 8$)
Histological activity index[a]	1–3	12–14
Sinusoid dilatation	+	+ + +
Cytoplasmic vacuolization	+	+ + +
Binucleated cells	+	+ +
Cell swelling	No	+ +
Hyperplasia of Kupffer cells	+	+ + +

[a] Modified according to Ishak et al. (1995). *Score:* 1–3, minimal; 4–8, mild; 9–12, moderate; 13–18, severe.