Clinical, haematologic and pathologic aspects of experimental ovine babesiosis in Iran

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Summary

Studies on the pathogenesis of Babesia ovis infection following blood transfusion of infected blood to sheep with intact spleen and splenectomised sheep showed that all animals developed fever concurrent with a parasitaemia that were occurred within 2-4 days post-inoculation (dpi), clinical signs of disease were severe and included varying degrees of anorexia, listlessness, anaemia, moderate jaundice and haemoglobinuria. In intact animals, the hyperthermia returned to normal on the fourth day after the peak pyrexia and parasitaemia was eliminated within the course of the disease in four cases. However, other cases which had severe clinical signs of the disease were died. The parasitaemia reached a maximum of 7% in splenectomised sheep 7-8 dpi; in animals with intact spleen, the parasitaemia was much lower and reached to a maximum of 1%. In both of the infected groups, the red cell counts, haematocrit and haemoglobin concentration fell soon after the appearance of parasitaemia, reaching their lowest levels simultaneously with the peak parasitaemia. The total leukocyte counts were significantly decreased. The total serum bilirubin levels of the infected group rose above the normal and peaked on 14-16 dpi; the rise in AST, BUN and creatinine levels were slight. The kidneys and lungs were the organs most severely affected by experimental infection with B. ovis. Acute alveolar oedema and infiltration of neutrophils and macrophages in interstitial tissue were present, acute diffuse proliferative glomerulitis, congestion and stasis in glomerular capillaries and acute tubular necrosis were also present.

Key words: Babesia ovis, Ovine babesiosis, Sheep, Haematology, Pathology

Introduction

Babesia spp. are tick-borne apicomplexan parasites which infect a wide range of vertebrate hosts. Delpy (1936) reported Babesia ovis to be the organism causing sheep babesiosis in Iran. Rafyi and Maghami (1966) showed that the mixed infection with B. ovis and B. motasi is a highly-pathogenic disease syndrome in sheep. Anwar (1974) considered B. ovis as a highly pathogen organism which covered most parts of sheep ranges in Iran. Morphologic and serologic comparison of ovine Babesia strains from Bulgaria and Iran has been done by Khalacheva and Kyartov (1981). Tavasoli and Rahbari (1998) have conducted a seroepidemiologic survey on B. ovis associated with the occurrence of infection in different geographic areas of Iran. Razmi et al. (2003) determined the high prevalence of B. ovis in sheep and goats in north-east of Iran. Little conclusive information is available regarding the pathogenesis of B. ovis infection in sheep. Alani and Herbert (1988) described haematologic and biochemical changes in splenectomised sheep experimentally infected with B. motasi. Studies on the lesions produced by B. ovis infection seem to be limited to those of Suteu et al. (1975), Halacheva and Paulov (1977), Habella et al. (1991) and Yeruham et al. (1998). The objective of this study was to determine the clinical, haematologic and histopathologic changes in sheep experimentally infected...
Materials and Methods

Eighteen male 4-6-month local breed sheep prepared from Animal Breed Research Institute of Tehran University. A week prior to initiation of the study, blood samples were taken from all animals. The test results for *B. ovis* in Giemsa-stained blood films were negative. Six splenectomised sheep and eight sheep with intact spleen received $5.2 \times 10^8$ of *B. ovis* infected erythrocytes which were isolated from West Azerbaijan, north-west of Iran (Tavasoli and Rahbari, 1998). The remaining animals were kept as control group. All splenectomised animals were inoculated two weeks after splenectomy. Giemsa-stained smears were also used to determine the infection rate of erythrocytes.

Blood sample collection

To obtain a complete blood count (CBC) and measure the serum biochemical parameters, blood samples were collected from the jugular vein into EDTA tubes for all sheep prior to the inoculation and every two days on post-inoculation for two weeks.

Clinical study of disease

Daily physical examination was performed on each animal. The parasitaemia and clinical signs including rectal temperature, prepatent period, colour of the mucous membranes (e.g., conjunctival, oral and vaginal), pulse rate, respiratory rate, anorexia and time of death were recorded.

Haematologic and serum biochemical changes

Biochemical parameters were determined according to Burtis and Ashwood (1999). The values of glucose (glucose oxidase), creatinine (Jaffe), BUN (urease), AST (Carman), ALT (Ritman and Frankel), total bilirubin (Vandenberg), total protein (Biuret), fibrinogen (refractometry) and urinalysis were measured in this study. Haematologic parameters were estimated as described by Meyer and Harvey (2004). Data were compared between the control and the infected animals according to Sendocoro and Cochran (1980).

When infected animals were judged to be close to death, they were slaughtered and necropsied. The animals of the control group were slaughtered simultaneously. Tissue samples were collected in 10% neutral buffered formalin, embedded in paraffin, prepared as 5-micron sections and stained with haematoxylin and eosin and Giemsa Gener’s for histopathologic studies, histological sections of kidney were prepared and stained with periodic acid schiff (PAS) and Jones. Sections of lymph nodes and spleen stained with Perls (Luna, 1968).

Statistical analysis

Two tailed Student’s t-test was used to compare means between two groups.

Results

Clinical and haematologic changes

All animals developed fever concurrent with a parasitaemia that were occurred within two dpi in splenectomised sheep. During the pyrexic phase, clinical signs of disease like varying degrees of anorexia, listlessness, anaemia and moderate jaundice became severe. In some cases, unexpectedly, haemoglobinuria was not observed. The reaction of sheep following infection with *B. ovis* is shown in Figs. 1a and b and Figs. 2a, b, c, d and e. As the animals approached death, its body temperature was decreased to normal level, the intact animals showed parasitaemia and hyperthermia (Figs. 1a and b) from 4 dpi. The hyperthermia returned to normal on the fourth day after the peak pyrexia and parasitaemia was eliminated within the course of the disease in four cases. Other cases with severe clinical signs ended to death. The parasitaemia reached a maximum of 7% in splenectomised sheep on day 7-8 post-inoculation, but parasitaemia in intact lambs was very low (Max. 1%). During the experimental period, all inoculated animals from splenectomised group died between 9 and 10 dpi, whereas four intact animals survived and were killed within 13-14 dpi. Polypnoea and tachycardia up to almost double the normal rate, were noticed in all infected animals. No clinical
Fig. 1: The course of parasitaemia (Fig. 1a) and body temperature changes (Fig. 1b) in splenectomised and intact sheep infected with *B. ovis*

Fig. 2: changes in PCV (Fig. 2a), MCV (Fig. 2b), MCHC (Fig. 2c), leukocytes (Fig. 2d) and lymphocytes (Fig. 2e) in splenectomised and intact sheep infected with *B. ovis*

...or pathologic abnormalities were observed in control animals. The red cell counts, haematocrit and haemoglobin concentration fell soon after the appearance of parasitaemia, reaching their lowest levels coincidentally with peak parasitaemia. The red cell counts, haematocrit and haemoglobin values started to rise by day 12 post-inoculation; MCHC and MCV values also changed in infected animals and were significantly (P<0.05) different from those of the control (Figs. 2a, b and c). The total
leukocyte count in both infected groups was significantly (P<0.05) decreased (Fig. 2d). Nevertheless, lymphocyte count in both groups was higher than those of normal, reached the peak on days 8 and 10 post-inoculation (Fig. 2e); neutrophil count was decreased.

Biochemical changes
The total serum bilirubin level of the infected group rose above normal to peak on 14-16 dpi; there was significant differences between the control and infected groups (P<0.05). Moreover, the rise in AST, ALT, BUN and creatinine levels were slight. Haemoglobinuria was occurred during 4-10 dpi in splenectomised sheep and sheep with intact spleen, respectively. There were no considerable changes in other biochemical parameters in infected animals.

Histopathologic changes
Histopathologic examination revealed focal necrosis, lymphohistiocytic, pericholangitis and cholangiohepatitis and canalicular cholestasis in the liver. Severe oedema, mild lymphocytolysis and haemorrhagic lymphadenitis were also present. Pathologic examinations of the tissues indicated that the kidneys and lungs were the organs most severely affected by experimental infection with *B. ovis*. Acute alveolar oedema and infiltration of neutrophils and macrophages in interstitial were present. Acute diffuse proliferative glomerulitis, congestion and stasis in glomerular capillaries and acute tubular necrosis were also present.

Discussion
The results of epidemiologic studies on ovine babesiosis in Iran showed that the level of infection with *B. ovis* is extremely variable between and within sheep ranging area, with the highest infection rate (58.8%) in mountainous area and lowest level (12%) in areas surrounding deserts (Tavasoli and Rahbarghi, 1998). Razmi et al. (2003) indicated that sheep were highly susceptible to *B. ovis* than goats. The course of parasitaemia, clinical data and time to death were reported by Habella et al. (1990).

Our experiment indicated that the splenectomised and intact animals showed parasitaemia and hyperthermia between two and four dpi, respectively. We showed peripheral blood parasitaemia in intact and splenectomised animals. This observation was in agreement with that described by Habella et al. (1990), and in contrary to findings of Alani and Herbert (1988) who could not find parasitaemia in *B. motasi* infected intact animals; they showed a very low parasitaemia in splenectomised animal. In this study, in intact animals, the hyperthermia returned to normal on the fourth day after the peak of pyrexia. Parasitaemia was eliminated within the course of the disease in four cases; other cases that had severe signs ended to death. Halacheva and Pavlov (1977) documented that death occurred during the experimental period of ovine babesiosis due to *B. ovis*. The parasitaemia reached a maximum of 7% in splenectomised sheep 7-8 dpi. However, the level of parasitaemia was very low (Max. 1%) in animals with intact spleen. The results of the current experiment indicated that anaemia consistently became apparent on day four after infection; it reached almost invariably its maximal level between day 7 and 8. The lowest erythrocyte counts in *B. ovis*-infected animals coincided with the highest parasitaemia. This is in agreement with other reports (Koch, 1968; Wright, 1973). In some cases, short period of clinical signs masked the appearance of jaundice and haemoglobinuria. Habella et al. (1991) reported that splenectomised animals developed more pronounced lesions than intact animals, particularly in certain cases in which parasitaemia rose up to 70%.

In comparison with the control group, haematologic values in animals infected with *B. ovis* were in most cases significantly different. The values for RBCs, haematocrit and haemoglobin clearly suggested that anaemia was an almost constant characteristic of the infection; it was found that microcytic hypochromic anaemia in intact and macrocytic hypochromic anaemia in splenectomised sheep occurred during the course of the disease. Alani and Herbert (1988) described macrocytic hypochromic anaemia in splenectomised sheep following infection with *B. motasi*. The anaemia following *Babesia* infections was attributed
to mechanical damage (Callow and Pepper, 1974), autoimmune phenomena (Argon, 1976) and erythrophagocytosis. Habella et al. (1991) included both non-parasitized and parasitized animals in their study.

Koch (1968) reported one peak of leukocytosis during B. ovis infection, but we found leukopenia which seems to be due to the extended tissue damage.

The significant hyperbilirubinaemia in infected sheep as compared to controls further supported the presence of haemolytic anaemia (Hailat et al., 1997; Camacho et al., 2005). Erythrocytes contain AST, therefore, increased serum AST activity may occur in haemolysis (Latimer et al., 2003) as it was shown in our study. AST often is the enzyme of choice for the routine detection of hepatocellular injury in ruminants. In this species, increased serum AST activity can result from liver diseases (Thrall, 2004). Although, in histopathologic study liver damage was detected, changes in AST activity were slight which was in keeping with another report (Thrall, 2004). Histopathologic observation revealed focal necrosis, lymphohistiocytic, pericholangitis and canaliculcholestasis in liver.

Studies on the lesion produced by B. ovis infection seem to be limited to those of Koch (1968), Suteu et al. (1975) and Halacheva and Paulov (1977). Little is known of the pathogenic mechanisms of B. ovis, but the Habella et al. (1991) indicated that they are broadly similar to those of B. bovis in that both provoke lesions characteristics for hypovolaemic shock. It should be pointed out, however, that high levels of parasitaemia detected in splenectomised animals may enhance the pathogenicity of B. ovis. Lesions observed generally arise from vascular alterations such as vasodilation and vascular stasis leading to tissue hypoxia. These alterations give rise to the degenerative processes in the central nervous system, which are also described by Hildebrandt (1981) in B. bovis-infected cattle. It appears that B. ovis has little capacity for colonizing the brain circulation, because only occasional accumulations of endothelial cells were observed and both parasitized and non-parasitized erythrocytes were involved. According to Hildebrandt (1981), this mechanism seems to be more frequent in B. bovis infection, causing nervous signs. Pathogenesis in lung was due to vascular origin. The acute pulmonary oedema observed was also reported by Hildebrandt (1981). The lesions in the kidney showed the most marked evidence of severe haemolysis. This condition was aggravated by colonization of B. ovis in the renal circulation and the alterations are therefore clearly vascular in origin (Habella et al., 1991). It seems that slight increase in BUN and creatinine is results of kidney injuries.

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