

## Prevalence and chemotherapy of babesiosis among Lohi sheep in the Livestock Experiment Station, Qadirabad, Pakistan, and environs

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**Abstract:** A total of 310 blood smears were collected from sheep of the Livestock Experiment Station, Qadirabad, Sahiwal district, Pakistan, and surrounding areas. The samples were examined microscopically and 30 (9.67%) were positive for babesiosis. The animals were divided into two groups (A and B) for chemotherapy. Group A sheep were treated with diminazene diaceturate while group B animals received imidocarb dipropionate. Drug efficacy was determined by negative blood smear examination. Diminazene diaceturate effectiveness against babesiosis was 80% while that of imidocarb dipropionate was 100%. Hematological studies revealed a significant decrease in hemoglobin concentrations and hematocrit values for *Babesia*-positive animals compared to healthy controls.

**Key words:** babesiosis, sheep, hemoglobin, hematocrit, diminazene, imidocarb, Pakistan.

### INTRODUCTION

The livestock sector plays an important role in the economy of Pakistan. It contributed with about 51.8 percent of the agriculture value and 11.3 percent of the national gross domestic product during 2008 and 2009. The gross value addition of livestock at the current cost factor has increased from Rs. 1052 billion to Rs. 1287 billion, showing a 22.3% increase (1). Sheep give rise to four major products: meat, wool/hair, milk and skins. In many parts of the world, particularly the temperate regions, meat is the major product and the importance of its production has increased worldwide (2).

Babesiosis is the third most important disease of sheep in Pakistan from an epidemiological point of view (3). The economic losses in small ruminant production are significant in the tropical and subtropical regions of the world (4).

Animals develop fever concurrent with parasitemia within 2 to 4 days; the clinical signs of the disease are severe and include varying

degrees of anorexia, listlessness, anemia, moderate jaundice and hemoglobinuria. In intact animals, hyperthermia returned to normal on the fourth day after the peak pyrexia, and parasitemia was eliminated within the course of the disease (5). Chronically infected sheep are usually symptomless, except for parasitemia and unthriftiness (6). In the acute phase, diagnosis of small ruminant piroplasmiasis is mainly based on the microscopic examination of Giemsa-stained blood smears and clinical symptoms (7).

During ovine babesiosis, red blood cell count, hematocrit and hemoglobin concentration decrease and neutrophils increase, compared to reference values (8, 9). The decrease in the packed cell volume ranges from 30 to 40% (10). A variety of drugs were used to treat babesiosis in the past, but only diminazene aceturate and imidocarb dipropionate are in common use nowadays (11). Imidocarb dipropionate (IMDP) is of therapeutic and prophylactic value against babesial infections (12-14). The therapeutic efficacy of IMDP against *Babesia ovis* has been well documented (15-18).

The present study describes the prevalence and chemotherapy of babesiosis among Lohi sheep in and around the Livestock Experiment Station, Qadirabad, Sahiwal, Pakistan, and focuses on the examination of selected hematological parameters.

## MATERIALS AND METHODS

### Field Study Area

The study was done in and around (Chak 62/4R, Chak 63/4R, Chak 75/5R, Chak 76/4R and Chak 77/4R) the Livestock Experiment Station (LES), Qadirabad, in Sahiwal, which is an important livestock production region in Pakistan (Figure 1). Ecologically, this area is classified as a centrally mixed zone. Its climate is extreme, reaching 52°C in the summer and -5°C in the winter. There are also agricultural lands in the area.

### Sample Size

Due to the lack of information about the prevalence of ovine babesiosis in these areas, 310 blood smears were tested. From each flock, five to seven animals were randomly sampled (19).

### Blood Smear Examination

Thin blood smears were prepared immediately after the collection of blood samples and marked in the field. After returning to the laboratory, they were fixed in methanol for five minutes and stained in Giemsa stain diluted at 10% with buffer solution for 30 minutes; then, they were examined

with an oil immersion lens at a total magnification of 1000x for the presence of *Babesia* piroplasms. The smears were recorded as negative for *Babesia* if no parasites were found in the oil-immersion field.

### Drugs

Diminazene diacetate and imidocarb dipropionate were purchased commercially. Doses were calculated on the basis of the active principle ingredient of 35% w/v diminazene diacetate in Diminazene® (Star Lab. Ltd., Pakistan) and 12% w/v imidocarb dipropionate in Imizol® (ICI Pakistan Ltd).

### Treatment Schedule

Thirty sheep positive for babesiosis were divided into three equal groups, each consisting of ten animals. The animals of group A were injected with Diminazene® at a dose rate of 3.5 mg/kg body weight intramuscularly. In group B, animals were injected with Imizol® intramuscularly at a dose rate of 2 mg/kg body weight. The animals of group C served as *Babesia*-infected control.

### Hematological Examination

A volume of 10 mL blood was collected from the jugular vein of each infected and healthy sheep into labeled tubes containing anti-coagulant. Hematological examination of blood samples from all sheep groups was carried out at day zero (pre-medication) and then at days 3, 7 and 10 post-medication. The effect of babesiosis on hemoglobin and hematocrit was determined by adopting the procedure described by Coles (20).

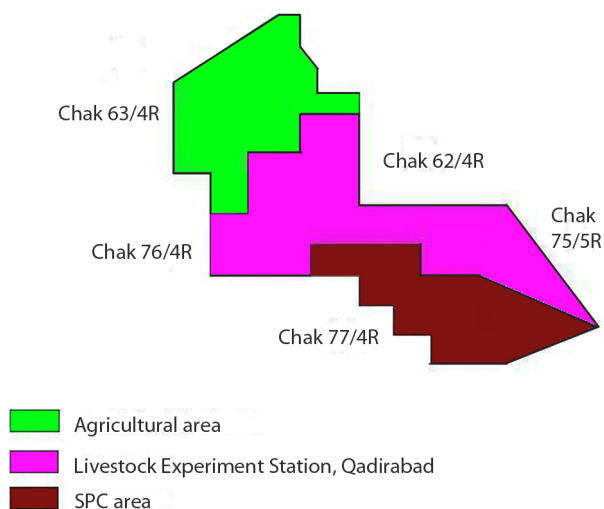
### Statistical Analysis

The data resulting from the present study were analyzed by Student's t-test. Associations were statistically significant when a *P*-value of less than 0.05 was obtained (21).

## RESULTS AND DISCUSSION

### Prevalence

During the study, a total of 310 Lohi sheep, including 250 adults and 60 lambs, were examined. The sheep were of different ages and both sexes. The overall prevalence of ovine babesiosis in and around the LES, Qadirabad, was 9.67% (Figure 2). Month-wise prevalence showed that three (3.6%), nine (8.18%) and 18 (15.38%) sheep were infected with *Babesia* in April, May and June,

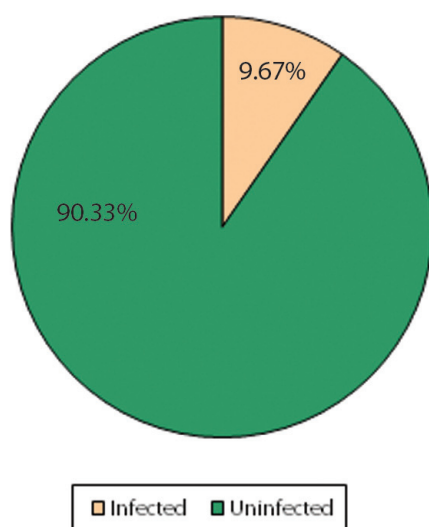


**Figure 1.** Map of the Livestock Experiment Station (LES), Qadirabad, showing the locations in the current study.

respectively. These results are in close agreement with those of Ferrer *et al.* (22), Yeruham *et al.* (23) and Aktas *et al.* (7), who reported 6.1%, 5.56% and 8.25% *Babesia ovis* infection, respectively. In the present study, the animals were apparently healthy but *Babesia* organisms were present in their circulation, corroborating the study of Tsomaya (24), who reported *Babesia ovis* infection in sheep for more than two years after recovery. However, the results of the present study are not in agreement with those of Mazyad and Khalaf (25), who reported very low prevalence, i.e. only 2.73% *Babesia ovis* among 475 sheep, which may be due to low existence of vector parasites that spread the disease. In addition, the results of the present study do not correlate to those of Papadopoulos *et al.* (26), Karatepe *et al.* (27) and Hosein *et al.* (28), who reported 52.1%, 23.63% and 50.92% ovine babesiosis, respectively. This difference may be attributed to the application of highly sensitive tests like indirect fluorescent antibody technique for diagnostic purpose.

### Chemotherapy

Diminazene®, administered to group A sheep at the dose rate of 3.5 mg/kg body weight, showed 80% efficacy at day 10 post-medication (Figure 3). These results agree with the study of Baby *et al.* (29), who treated concurrent babesiosis and anaplasmosis in goat with diminazene. Similar



**Figure 2.** Prevalence of babesiosis among Lohi sheep in and around the Livestock Experiment Station (LES), Qadirabad, Sahiwal (Pakistan).

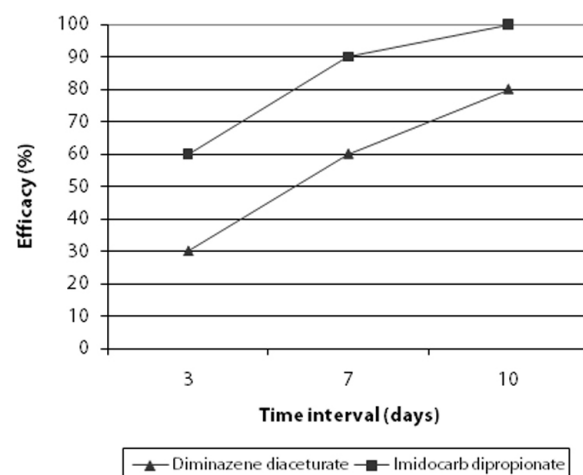
results were also found by Simitch *et al.* (30), Cordoves and Palano (31), Aliu and Odegaard (32), Mohamed and Yagoub (33) and Manget (34), who obtained a satisfactory effect of diminazene against babesiosis. On the other hand, the present results do not agree with those of Banerjee *et al.* (35) and Rao *et al.* (36), who recorded 100% recovery by diminazene. This may be due to the use of a high dose of diminazene against ovine babesiosis.

Imizol®, administered to group B animals at the dose rate of 2 mg/kg body weight, showed 60% efficacy at day 3, 90% at day 7 and 100% at day 10 post-medication (Figure 3). These results are in agreement with those of Ramin (37) and McHardy *et al.* (18), who recorded 97.28% and 100% imidocarb efficacy, respectively, while treating sheep with babesiosis.

### Hematology

For group A, at day zero, mean hemoglobin concentrations and hematocrit values were  $7.12 \pm 0.37$  g/dL blood and  $20.3 \pm 0.78\%$ , respectively. After treatment with diminazene, at days 3, 7 and 10 post-medication, mean hemoglobin concentrations were  $7.66 \pm 0.68$ ,  $8.38 \pm 0.74$  and  $8.88 \pm 0.76$  g/dL blood, respectively, while mean hematocrit values were  $23.0 \pm 0.63$ ,  $25.5 \pm 1.1$  and  $27.9 \pm 1.5\%$ , respectively.

For group B, at day zero, mean values of hemoglobin and hematocrit were  $7.0 \pm 0.45$  g/dL blood and  $19.8 \pm 0.97\%$ , respectively (Figures 4 and 5). After treatment with imidocarb dipropionate, there was a marked improvement in the health of animals. At days 3, 7 and 10 post-medication,



**Figure 3.** Comparative efficacy (%) of drugs at days 3, 7 and 10 post-medication for groups A and B.

mean hemoglobin concentrations were  $8.48 \pm 0.57$ ,  $9.64 \pm 0.89$  and  $11.1 \pm 0.94$  g/dL, respectively, while mean hematocrit values were  $25.2 \pm 1.32$ ,  $28.6 \pm 1.0$  and  $32.8 \pm 1.1\%$ , respectively.

For the infected control group, hemoglobin and hematocrit values were much more decreased at days 3, 7 and 10 compared to day zero, while group D (healthy control) showed normal values at days zero, 3, 7 and 10 (Figures 4 and 5). The mean values of hemoglobin and hematocrit for all groups were statistically significant ( $P < 0.05$ ). The results of present study correlated to those of Banerjee *et al.* (35), who reported 6.4 g/dL hemoglobin for naturally infected goat with *Babesia motasi*. The present results also agree with those of Voyvoda *et al.* (38), who reported hemoglobin and hematocrit values of  $7.00 \pm 0.3$  g/dL and  $19.07 \pm 0.78\%$ , respectively, for naturally infected white karaman sheep with *Babesia ovis*. In addition, the present findings are similar to those of Baby *et al.* (29), Gautan and Chaudhary

(39), Reid *et al.* (40) and Yeruham *et al.* (10, 41), who reported a decrease in hemoglobin and hematocrit values during babesiosis. Red blood cell counts reduced due to severe anemia, as well as due to destruction by *Babesia*.

For group D, hematocrit value was normal (Figure 5). This study is in agreement with that of Bell-Sakyi *et al.* (42), who reported normal mean packed cell volume for sheep without hemoparasite infection.

In conclusion, the results obtained in this study clarifies that *Babesia* is an important prevalent pathogen among sheep in and around the LES, Qadirabad. Hematological examination revealed a significant decrease in hemoglobin and hematocrit values for sheep infected with babesiosis. Moreover, imidocarb dipropionate showed better results whereas diminazene diaceturate was the second in efficacy against *Babesiosis*.

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## CONFLICTS OF INTEREST

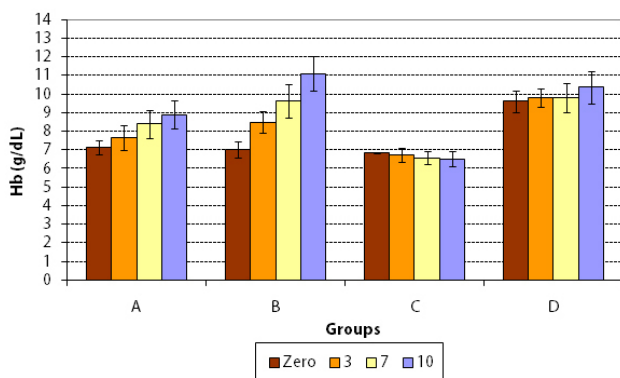
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## CORRESPONDENCE TO

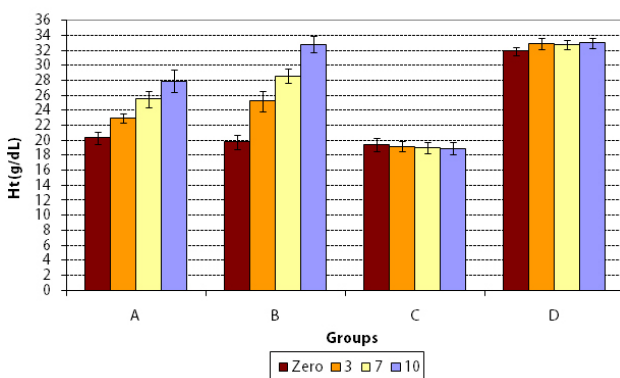
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**Figure 4.** Hemoglobin concentrations for Lohi sheep of different groups at days 0, 3, 7 and 10 post-medication.



**Figure 5.** Hematocrit values for Lohi sheep of different groups at days 0, 3, 7 and 10 post-medication.

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