EFFECTS OF BABESIOSIS ON BLOOD PARAMETERS IN SHEEP AND GOATS

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ABSTRACT

Babesiosis is an infectious disease caused by tick-borne, intra-erythrocytic and generally host-dependent protozoan parasite of the genus Babesia. Babesia ovis, the main etiological agent of small ruminants. The infectious agent of Babesiosis is a small form of Babesia parasite (1-1.5 µm in diameter), which cause severe economic losses among sheep and goats in tropical and subtropical areas. This study conducted to determine the effects of Babesiosis on blood parameters (RBC, WBC and HB) of sheep and goats in Kunduz Province. A total 24 animals (12 Sheep and 12 Goats) selected; sheep and goats divided into two groups (infected with Babesia and clinically control). The infected group naturally infected with Babesia Spp. 5ml Blood collected in an EDTA tube from jugular vein. The result showed that RBC clearly decreased in sheep and goats ($P<0.05$) while WBC were increased, the level of HB were decreased in both species significantly ($P<0.05$). The study indicated that Babesia spp. affects the blood parameters of both species and decreased RBC/HB levels respectively.

Keywords: Babesia, Blood Parameters, Kunduz, Sheep & Goat

INTRODUCTION

Babesiosis is a protozoan disease, caused by various species of mites, an enteric protozoan parasite of red blood cells belonging to the genus Babesia ovis (Wormser, Dattwyler et al. 2006). A Babesia parasite 1-1.5 micrometer in diameter, transmitted by ticks, causes significant economic losses in sheep and goats in temperate and subtropical regions (Sevinc, Turgut et al. 2007). Babesia species is transmitted by ticks (Family: Ixodidae) which causes continuous fever in animals. The main symptoms of this disease are severe and with varying degrees of anorexia, lethargy, anemia, moderate diarrhea and the presence of hemoglobin in the urine (Rahbari, Nabian et al. 2008).

Babesia disease that shows chronic symptoms is characterized by fever, anorexia, increased respiratory rate, muscle tremors, malaise, fatigue, weight loss and, in the final stage, the presence of hemoglobin urea (Urquhart 1996). Anemia is common in all animals, but hemoglobin urea

may not be seen in animals infected with B. ovis (Popa 1998). Chronically infected sheep usually show no major symptoms except for the presence of parasites in the blood (Kozat, Yuksek et al. 2003). Babesia is an economically important disease that causes significant losses in small ruminant production rapidly in tropical and subtropical regions of the world (Muthuramalingam, Pothiappan et al. 2014). Babesia disease is epidemiologically the third most important sheep disease in Pakistan (Branscomb 1995).

Babesia is a parasitic disease of the blood in domestic and wild animals. Babesia mites are apicomplexan parasites that infect a wide range of vertebrate hosts. Some animals may slowly recover from treatment after a long period, but some develop shock or die from renal failure (Schetters, Kleuskens et al. 2009). Four forms of Babesia has been reported in sheep and goats, mainly one major form is B. motasi and three other minor forms are B. ovis, B. foliata and B. tyalori (Soulsby 1986). While Friedhoff said that, the three species that cause Babesia disease in domestic ruminants are B. ovis, B. motasi and B. crassa (Mehlhorn 1989). B. ovis is less pathogenic in sheep than B. motasia and B. crassa and causes relatively moderate hemolytic anemia (Aytuğ, Alaçam et al. 1990). According to previous reports, B. ovis is the most lethal organism that causes Babesia disease in sheep in most parts of Iran (Delpy 1936). These diseases caused by B. ovis, B. motasi and B. crassa (Hashemi-Fesharki 1997). Two species of Babesia commonly known to be pathogenic are B. ovis and B. motasi (Uilenberg 2006). B. ovis is a small (<2.5 μm) B. ovis, the most common species causing B. ovis disease in sheep in Iran (Rashid, Khan et al. 2010). B. motasi is not highly pathogenic and appears to be moderately virulent (Soulsby 1968). Babesia can have pear-shaped, round and ongated shapes. High incidences of B. ovis has been identified in sheep and goats in northeastern Iran (Razmi, Naghibi et al. 2003).

Alani and Hebert described hematological and biochemical changes in sheep suffering from sarcoid and experimentally infected with B. motasi (Alani and Herbert 1988). Several studies have been previously conducted on the histopathology of lesions produced by B. ovis (Suteu, Vatic et al. 1975). To our knowledge, most studies previously conducted on hematological and biochemical changes in sheep naturally infected with B. ovis (Halacheva and Kyartov 1977, Yeruham, Hadani et al. 1998, Yeruham, Avidar et al. 2003). Recently study was conducted to study some hematological and serum biochemical parameters in sheep and goats naturally infected with B. ovis (Baby, David et al. 2001).

Babesia species cause severe economic losses in sheep and goats, especially in warm and semi-warm climates. From clinical symptoms perspectives, Babesia disease can be acute, subacute or chronic. Simply, Babesia disease is mostly divided into mild, moderate and severe disease depending on the severity of the bleeding (Lobetti, Dvir et al. 2002, Esmaeilnejad, Tavassoli et al. 2012). Analysis of spheroids with Babesia species showed signs of bleeding (Sayin, Dyncer et al. 1997). Red blood cell parasites, including Babesia and Plasmodium, thought to increase oxidative stress and lipid peroxidation due to the effect of blood analysis. Parasite infestation disrupts blood formation and thus causes anemia (Yur, Değer et al. 2010, KILINÇ, Göz et al. 2015).

Which is characterized by a decrease in the number of red blood cells or a sub-normal concentration of hemoglobin per unit volume of blood. Because of reduced red cell count, oxygen transfer decreases and tissue oxygen deficiency may occur. The climatic conditions of the country favor the growth and reproduction of mites, which are important external parasites in livestock and tropical Theileriosis (Sevinc,

Turgut et al. 2007, Inci, Ica et al. 2010). which caused reduction in livestock production (Shayan, Hooshmand et al. 2008). Therefore, it is necessary to investigate the effect of Babesia on the composition of the blood of animals, especially small ruminants. Since agriculture and livestock constitute the most significant part of income for rural residents livelihood. Livestock products contribute to the country economy and it is the only income source for living that incredibly contribute to our nation resilience. Given the significant humanitarian impact associated with livestock, noting the nutritional and livelihood importance of livestock, which is life saving and the fact that urban people heavy dependence on livestock.

In addition, agriculture and livestock are lagging behind in Afghanistan, and since long ago, the governor has been struggling with this backwardness, that livestock has taken a traditional form in Afghanistan as there are very few farms equipped with an advanced farming system. This study resulted to address the main challenge in reduction of livestock production, with taking preventive measures against Babesia can pave the way for improving yield productions and productivity, which will greatly contribute to the livestock sector in Afghanistan.

MATERIALS AND METHODS

Place of work

This study carried out in Kunduz Province under the direct guidance of Pre-Clinical department head, Faculty of Veterinary Sciences of Kunduz University.

Samples Size

In this study, a total of 24 sheep and goats studied. There were 12 sheep and 12 goats (divided into two groups), the control group and the infected group. Each main group was subsequently divided into 3 replicate groups; each replicate consisted of three sheep and three goats.

Blood Smear Examination

Blood was taken from the ear of the animal using antiseptics and a thin smear of the side of the slide was left to dry in the open air, after which it was fixed by methanol and after Giemza staining Oil immersion has been observed under a microscope with 100 power.

Hematological Examination

Using a sterile needle and syringe, 3 ml of blood was collected from the animal's jugular vein, placed in a tube containing anticoagulant material, and taken to the laboratory for hematological examination. Globally, the amount of red blood cells, hemoglobin and hematocrit is counted by Automated Cell Counts (ACT-8 Counter Miami-EUA) and manually i.e. by Neurochamber slide (Ozlem et al, 2015).

HB determination method

Hemomater device used to determine the amount of hemoglobin. In this method, first 20 microliters of HCL and 20 microliters of blood were taken and mixed in a Hemomater graded tube, and then left for five minutes and then its size is determined in the hierarchy.

WBC determination method

380 microliters of WBC solution were taken and then put in a 10 ml test tube. Twenty microliters of blood mixed with it and then a few drops of the solution put on the chamber slide and the cells counted under the microscope respectively.

RBC determination method

3980 microliters of RBC solution has taken, 20 microliters of blood poured into a 10 ml test tube, mixed with it, and then after pouring a few drops on the chamber slide, the cells counted under the microscope.

Statistical analysis

The obtained results were analyzed by one-way ANOVA in SPSS software, the general confidence interval is 95% and the variable is ($P<0.05$).

RESULT

Part of this research, 24 sheep and goats chosen for this study and divided into two groups, control and infected. The animal was clinically sick and showed chronic symptoms such as fever, anorexia, anemia, moderate diarrhea and the presence of hemoglobin in the urine. The study revealed that Babesia parasite affected HB, RBC and WBC, in sheep and goats, there was a decrease in RBC and HB and an increase in WBC. The first graph shows, effects of Babesia parasite on the RBC of the sheep. There was a significant decrease ($P<0.05$) in the RBC size of the sheep in the infected group compared to the control group.

In the second graph, the effects of Babesia parasite on the HB of the sheep are shown, and there was a significant decrease ($P<0.05$) for HB of the sheep in the infected group compared to the control group. The third graph shows the effects of Babesia parasite on the WBC of the sheep. There was a significant increase ($P<0.05$) in the WBC of the sheep of the infected group compared to the control group. In the fourth graph, the effects of Babesia parasite on the RBC of goats have shown. There was a significant decrease ($P<0.05$) for RBC of infected goats compared to the control group. In the fifth graph, the effects of Babesia parasite on the HB of goats are shown, from which there was a significant decrease ($P<0.05$) for HB in the goats of the infected group compared to the control group.

The sixth graph shows the effects of Babesia parasite on the WBC of the goats. There was a significant increase ($P<0.05$) in the WBC of the infected goats compared to the control group.

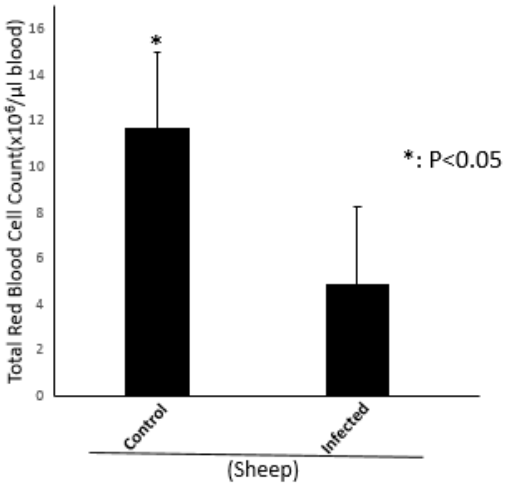

Figure 01: In the above graph, there was a significant reduction (P<0.05) in the size of the red blood cells of the infected sheep compared to the control.

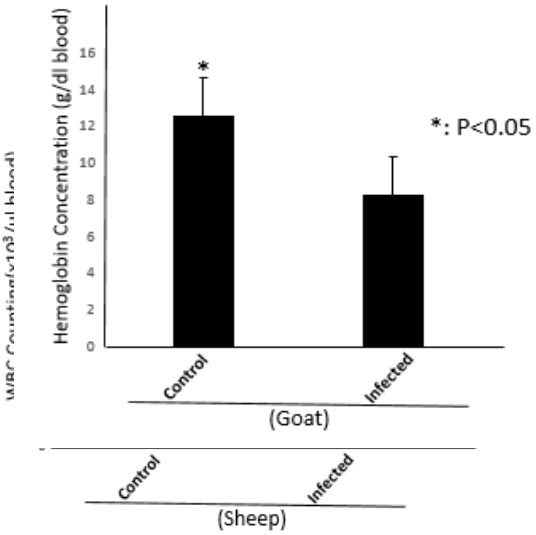

Figure 02: In the above graph, there was a significant decrease (P<0.05) for hemoglobin of sheep in the infected group compared to the control.

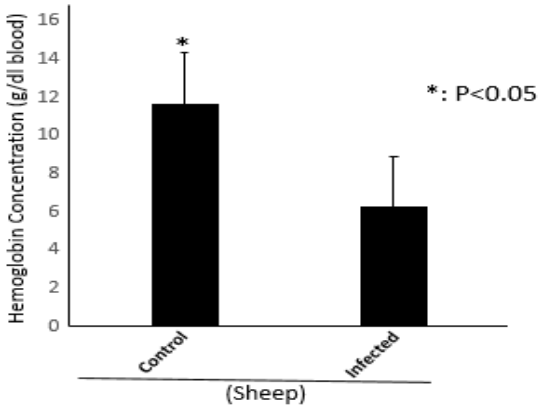

Figure 03: In the above graph, there was a significant increase (P<0.05) in the size of the white blood cells of the sheep in the infected group compared to the control.

Figure 04: In the above graph, there was a significant decrease (P<0.05) for hemoglobin of infected goats compared to the control.

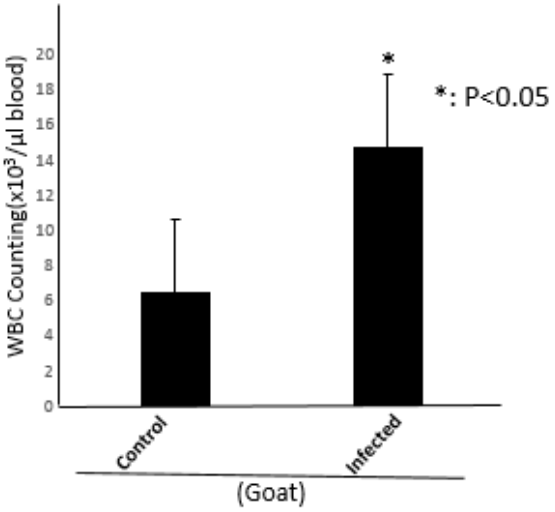

Figure 05: In the above graph, there was a significant decrease (P<0.05) in the size of the cells in the goats of the infected group compared to the control.

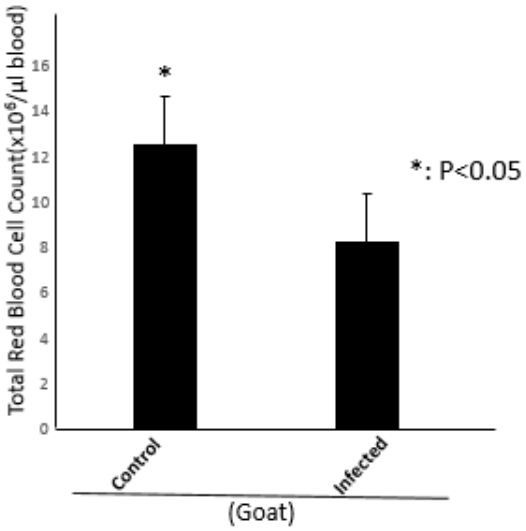

Figure 06: In the above graph, there was a significant increase (P<0.05) for white blood cells in the goats of the infected group compared to the control.

DISCUSSION

A study conducted by (KILINÇ, Göz et al. 2015) showed varying degrees of anemia in infected sheep with hematocrit ranging from 13.2 to 30.3, as well as RBC and hemoglobin levels in infected sheep. Infertility

came along with poverty. In this study, a total of 24 sheep and goats were studied, a significant decrease in RBC and HB of infected sheep was observed (P<0.05), this result is similar to the result of Ozlem.

The study conducted by (Sulaiman, Arslan et al. 2010) included 175 local goats, 27 goats on Babesia ovis, B. Motasi, B. Foliata and B. Taylori, these were infected and 25 year old normal goats selected as control. The percentage infected by Babesia is 15.42% and the percentage of Parasitemia varies from 3.5-10.4% with an average of 6.95%. , showed symptoms of nasal discharge, coughing, diarrhea and hemoglobin urea.

In addition, a statistically significant deficiency has seen in RBC, HB and Platelet count. A significant increase in red cell sedimentation rate and a significant increase in total white cell count, lymphocyte and neutrophil count observed (Sulaiman, Arslan et al. 2010). The present study conducted on two groups of sheep/goats, control and infected groups. A significant decrease (P<0.05) was observed in RBC/HB and an increase in the number of WBC was observed, so the study results are similar to the earlier study of (Sulaiman, Arslan et al. 2010).

A study conducted by (Muthuramalingam, Pothiappan et al. 2014) to determine the incidence of Babesia in Tellicherry goats in a private goat farm in Thalassery, China with a total number of 168 goats for the presence of Babesia. The study rev(Baby, David et al. 2001)ealed that 58 (34.5%) goats infected with B. ovis. The incidence of Babesia infection was non-significant between males and females and in different age groups of goats.

Infected goats show various annual symptoms such as in appetence, depression, increased breathing rate, weakness, coughing, nasal discharge, eye discharge, diarrhea, and increased body temperature between 39.5-41,2C and caused abortion. In blood parameters, RBC count, hemoglobin concentration, PCV volume, platelet count markedly decreased and lymphocytes and neutrophil count were increased. In this study, 12 goats were considered, nine were infected and three were control, and RBC and HB were significantly decreased in infected goats (P<0.05). Therefore, the results of the current study are similar to Muthuramalingan's study.

A study in 2012 by (Baby, David et al. 2001) showed varying amounts of parasites in the blood of infected sheep and goats. In addition, Razmi et al, 2003, Sevinc et al, 2007 and Aktas et al, 2007, showed the same results. A significant decrease in RBC and HB was observed in infected sheep and goats compared to healthy animals (P<0.05). The present study, conducted in sheep and goats, supports the study of Bijan and other researchers.

A study conducted by (Ijaz, Rehman et al. 2013) showed a decrease in HB, PCV, RBC, thrombocyte and WBC count in infected sheep compared to healthy sheep (P<0.05). In addition, there was a significant decrease in goat HB, PCV, RBC and thrombocyte count (P<0.05). However, there was no significant decrease in WBC count (P>0.05). This study is similar to (Rahbari, Nabian et al. 2008) who reported that blood volume was significantly different in most cases in animals infected by B. ovis. RBC, Hematocrit and Hemoglobin measurements clearly suggested that anemia is a consistent feature of infection.

Similar to the results of (Yeruham, Handani et al. 1992, Baby, David et al. 2001), they reported that a significant decrease in HB concentration and RBC count resulted in acute anemia caused by RBC destruction caused by Babesia. The present study showed that sheep and goats exposed a significant decrease in RBC and HB

compared to the healthy and an increase in the number of WBC, so this study is similar to the previous study conducted by M.

B. ovis plays an important role in causing anemia and renal dysfunction in infected animals. In this section, several extensive studies have conducted on hematological and biochemical findings of smallpox Babesia disease caused by B. ovis. The aim of this study was to evaluate the effects of Babesia disease on some hematological and biochemical parameters in infected small ruminants. A total of 280 sheep and 122 goats from 40 flocks randomly observed for the presence of B. ovis in blood. Out of these 402 samples, 67 animals (16.7%) were positive for B. ovis, which were 52 (18.5%) sheep and 15 (12.2%) goats respectively. Infected animals were divided into four subgroups according to the presence of parasites in the blood (<1%, 1%, 2% and 3%).

As a control group, 67 healthy animals selected in the same farm. A significant decrease was observed in the concentration of HB, PCV, RBCs, MCV and MCHC with increasing parasitemia ($P<0.05$). Also, a significant ($P<0.05$) increase in total white cell count, lymphocyte count, monocyte, neutrophil and eosinophil was shown (Baby, David et al. 2001, Azadeh, Reza et al. 2012). The present study also conducted in infected sheep and goats. There was a significant decrease in RBC and HB and an increase in WBC, so this study is similar to the previous study and confirm all above results accordingly.

CONCLUSION

Babesia parasite in sheep and goats is characterized by fever, loss of appetite, increased respiratory rate, muscle tremors, anemia, lethargy, weight loss, and the presence of hemoglobin urea in the last stage. Babesia species causes anemia due to the breakdown of spheroids. It found in large quantities in warm and semi-warm areas and transmitted by insects. This parasitic disease occurs worldwide, especially in warm and semi-warm regions. It is more common in climates. Babesia is an economically important disease that causes significant losses in small ruminant productions.

This research carried out in sheep and goats in Kunduz province. A total of 24 sheep and goats, both control and infected, were considered, and the results showed that Babesia species had a significant effect on the blood parameters of sheep and goats. When compared with the control group, there was a significant ($P<0.05$) decrease in RBC and HB in the diseased sheep and goats, and significant ($P<0.05$) increase in WBC. The study suggest that Babesia species can adversely affect the livestock productions and productivity.

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REFERENCES

1. Alani, A. and I. Herbert (1988). "The pathogenesis of Babesia motasi (Wales) infection in sheep." Veterinary Parasitology 27(3-4): 209-220.
2. Aytuğ, C. N., E. Alaçam, Ü. Özkoç, B. Yalçın, H. Gökçen and H. Türker (1990). "Koyun-keçi hastalıkları ve yetiştiriciliği." Tüm Vet Hayv Hiz Yay(2).

3. Azadeh, S., F. S. Reza, A. Sara, V. Mohsen, M.-D. Bijan and Z. R. Zali (2012). "Four years incidence rate of colorectal cancer in Iran: a survey of national cancer registry data-implications for screening." Asian Pacific Journal of Cancer Prevention 13(6): 2695-2698.
4. Baby, P., P. David, P. Ravindran and R. Ravindran (2001). "A subacute case of concurrent babesiosis and anaplasmosis in a she-goat." Veterinary Journal (India).
5. Branscomb, A. W. (1995). "Anonymity, autonomy, and accountability: Challenges to the first amendment in cyberspaces." The Yale Law Journal 104(7): 1639-1679.
6. Delpy, L.-P. (1936). Agents pathogènes observés en Iran dans le sang des animaux domestiques, Masson.
7. Esmaeilnejad, B., M. Tavassoli and S. Asri-Rezaei (2012). Investigation of hematological and biochemical parameters in small ruminants naturally infected with Babesia ovis. Veterinary research forum, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
8. Halacheva, M. and N. Kyartov (1977). "Histopatholigical changes in spleneclomized sheep infected with Babesia ovis." Vet Sci 14: 50-56.
9. Hashemi-Fesharki, R. (1997). "Tick-borne diseases of sheep and goats and their related vectors in Iran." Parassitologia 39(2): 115-117.
10. Ijaz, M., A. Rehman, M. Ali, M. Umair, S. Khalid, K. Mehmood and A. Hanif (2013). "Clinico-epidemiology and therapeutical trials on babesiosis in sheep and goats in Lahore, Pakistan." The Journal of Animal and Plant Sciences 23(2): 666-669.
11. Inci, A., A. Ica, A. Yildirim and Ö. Düzlü (2010). "Identification of Babesia and Theileria species in small ruminants in Central Anatolia (Turkey) via reverse line blotting." Turkish Journal of Veterinary & Animal Sciences 34(2): 205-210.
12. KILINÇ, Ö. O., Y. Göz, N. Yüksek, Y. BAŞBUĞAN, A. B. Yilmaz and A. D. ATAŞ (2015). "Determination of serum cardiac biomarkers and plasma D-dimer levelsin anemic sheep with babesiosis." Turkish Journal of Veterinary & Animal Sciences 39(5): 606-610.
13. Kozat, S., N. Yuksek, N. Altug, Z. Agaoglu and F. Ercin (2003). "Studies on the effect of iron (Fe) preparations in addition to Babesiosis treatment on the haematological and some mineral levels in sheep naturally infected with Babesia ovis." Fak Derg 14(2): 18-21.
14. Lobetti, R., E. Dvir and J. Pearson (2002). "Cardiac troponins in canine babesiosis." Journal of Veterinary Internal Medicine 16(1): 63-68.
15. Mehlhorn, H. (1989). Babesiosis of Domestic Animals and Man, M. Ristic (Ed.), CRC Press Inc., Boca Raton, Florida (1988),£ 74, 50, Urban & Fischer.
16. Muthuramalingam, T., P. Pothiappan, P. T. Gnanaraj, S. M. Sundaram, T. Pugazhenthi and S. Parthiban (2014). "Report on an outbreak of babesiosis in tellicherry goats." Indian J Vet Anim Sci Res 43: 58-60.
17. Popa, E. (1998). "Ixodid ticks vectors of Babesiosis in animals in Romania." Revista-Romana-de-Medicina-Veterinara 8(2): 61-67.
18. Rahbari, S., S. Nabian, Z. Khaki, N. Alidadi and H. J. ASHRAFI (2008). "Clinical, haematologic and pathologic aspects of experimental ovine babesiosis in Iran."
19. Rashid, A., J. Khan, M. Khan, K. Rasheed, A. Maqbool and J. Iqbal (2010). "Prevalence and chemotherapy of babesiosis among Lohi sheep in the Livestock Experiment Station, Qadirabad, Pakistan, and environs." Journal of Venomous Animals and Toxins including Tropical Diseases 16: 587-591.

20. Razmi, G., A. Naghibi, M. Aslani, K. Dastjerdi and H. Hossieni (2003). "An epidemiological study on Babesia infection in small ruminants in Mashhad suburb, Khorasan province, Iran." Small Ruminant Research 50(1-2): 39-44.
21. Sayin, F., S. Dyncer, Z. Karaer, A. Cakmak, B. Yukary, H. Eren, S. Deger and S. Nalbantoglu (1997). "Status of the tick-borne diseases in sheep and goats in Turkey." Parassitologia 39(2): 153-156.
22. Schetters, T. P., J. Kleuskens, J. Van De Crommert, P. De Leeuw, A.-L. Finizio and A. Gorenflot (2009). "Systemic inflammatory responses in dogs experimentally infected with Babesia canis; a haematological study." Veterinary parasitology 162(1-2): 7-15.
23. Sevinc, F., K. Turgut, M. Sevinc, O. D. Ekici, A. Coskun, Y. Koc, M. Erol and A. Ica (2007). "Therapeutic and prophylactic efficacy of imidocarb dipropionate on experimental Babesia ovis infection of lambs." Veterinary parasitology 149(1-2): 65-71.
24. Shayan, P., E. Hooshmand, S. Nabian and S. Rahbari (2008). "Biometrical and genetical characterization of large Babesia ovis in Iran." Parasitology research 103: 217-221.
25. Soulsby, E. (1986). "Helminths, Arthopods, and Protozoa of Domesticated Animal Seventh Editon." London: Bailliere Tindall.
26. Soulsby, E. J. L. (1968). "Helminths, arthropods and protozoa of domesticated animals." Helminths, arthropods and protozoa of domesticated animals.
27. Sulaiman, E., S. Arslan, Q. Al-Obaidi and E. Daham (2010). "Clinical, haematological and biochemical studies of babesiosis in native goats in Mosul." Iraqi Journal of Veterinary Sciences 24(1): 31-35.
28. Suteu, E., N. Vatic and A. Cosma (1975). "New data and observation on babesiosis in sheep in Transylvania." Bull Inst Agr 29: 107-109.
29. Uilenberg, G. (2006). "Babesia–a historical perspective." Vet Parasitol 138: 3-10.
30. Urquhart, G. M. (1996). "Veterinary parasitology." (No Title).
31. Wormser, G. P., R. J. Dattwyler, E. D. Shapiro, J. J. Halperin, A. C. Steere, M. S. Klempner, P. J. Krause, J. S. Bakken, F. Strle and G. Stanek (2006). "The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America." Clinical Infectious Diseases 43(9): 1089-1134.
32. Yeruham, I., Y. Avidar, I. Aroch and A. Hadani (2003). "Intra-uterine Infection with Babesia bovis in a 2-day-old Calf." Journal of Veterinary Medicine, Series B 50(2): 60-62.
33. Yeruham, I., A. Hadani and F. Galker (1998). "Some epizootiological and clinical aspects of ovine babesiosis caused by Babesia ovis—A review." Veterinary Parasitology 74(2-4): 153-163.
34. Yeruham, I., A. Handani, F. Galker, S. Rosen and J. Schlien (1992). "A field study of haemoparasites in two flocks of sheep in Israel." Israel Journal of Veterinary Medicine 47(3): 107-111.
35. Yur, F., Y. Değer and S. Dede (2010). "Na+/K+ ATPase activity in sheep with natural babesiosis." Acta Veterinaria Brno 79(2): 233-236.