

# An Epidemiological and Diagnostic Study of *Anaplasma ovis* Parasite in Native Goats in Anbar Province- Iraq

Suad Shallal Shahatha

Department of Biology, College of science, University of Anbar, Iraq

Received: 23<sup>rd</sup> Jan, 19; Revised: 25<sup>th</sup> Jan, 19, Accepted: 15<sup>th</sup> Feb, 19; Available Online: 25<sup>th</sup> Mar, 2019

## ABSTRACT

This study was conducted to investigate the epidemiology of *Anaplasma ovis* parasite in the native goat of some areas in Anbar province (Ramadi, Fallujah, Khalidiya, Hit and Baghdadi), by collecting 156 blood samples of both sexes and different ages ranging from one month to nine years for the period from March 2017 to February 2018, the parasite was diagnosed with microscopic examination using Giemsa stain. The results showed a total infection rate 34.6%, the infection rate in females was 38.8% higher than that of males 29.5% and significant differences ( $p \leq 0.05$ ). The highest rate of infection (40, 39.5%) was observed for the age group 4-5 years and 6-7 years respectively. The highest rate was 75% in April and lowest rate 18.1% in February. The study also included a number of hematological parameters, which showed a decrease in total erythrocyte count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV) in the infected goats.

**Keywords:** epidemiological, native goats, *Anaplasma ovis*, hematological parameters, Anbar.

## INTRODUCTION

The genus *Anaplasma* is an obligate parasite which live within cells and belong to a family Anaplasmataceae and class Rickettsiales<sup>1</sup>, which includes many species such as *A. ovis*, *A. bovis*, *A. marginale*, *A. centrale* that parasites on several types of animal's ruminants including sheep and goats<sup>2</sup>. Causing an illness called Anaplasmosis leading to serious health and economic problems<sup>3</sup>, the disease is widespread in tropical and subtropical regions<sup>4</sup> *Anaplasma ovis* affects approximately 10-90 % of the host's red blood cells leading to serious blood changes<sup>5</sup> Including decreased red blood cell count, decreased concentration of hemoglobin, and decreased blood cell volume<sup>6</sup> Symptoms appear at the acute stage which is characterized by severe fever, jaundice, anemia, underweight and wasting, idle, low production of milk and meat, abortion, and the loss of animals<sup>7</sup> the parasite is transmitted to the host either in a biological way by several species of ticks such as *Dermacentor*, *Rhipicephalus Ixodes*, *Hyalomma*<sup>8</sup> or mechanically by the bite of flies, especially horse flies<sup>9</sup> and also transmitted through contaminated tools while giving medicines to animals, and by collecting blood samples, or by placenta<sup>10</sup>. Several studies have been conducted to investigate the spread of *A. ovis* parasites in many countries of the world, including study of Altay *et al.*,<sup>11</sup> which recorded an infection rate of 71.3% in the eastern part of Turkey, and the study of Said *et al.*,<sup>12</sup> which recorded the infection rate of 65.3% in small ruminants in Tunisia, Zhang *et al.*,<sup>13</sup> reported an infection rate 18.2% during the examination of 710 goats in six regions of China, Zhou *et al.*,<sup>14</sup> pointed to infection rate 60 % in goats in the central regions of Turkey, as well, study of Ochirkhuu *et al.*,<sup>15</sup>

recorded a 57.5% infection rate in Mongolia, In addition to Patra *et al.*,<sup>16</sup> recorded a 32.2% infection rate during examination of 1053 blood samples from goats in India. In Iraq, Al-Amerey and Hasso<sup>17</sup> recorded a 32.1% infection rate in Baghdad province. Alsaad *et al.*,<sup>18</sup> mentioned a 24.7% of the total of 97 goats examined, Naqid and Zangana<sup>19</sup> found an 55.8% in the native goats in the Duhok region, and Renneker *et al.*,<sup>20</sup> reported a 66.6% in domestic goats in some areas of Iraq, moreover, Naqid<sup>21</sup> noted a 38.0% rate in Angor goats in Dahuk province, Northern Iraq. Due to the lack of studies in the country and its absence in Anbar province, the current study aimed to investigate the epidemiology of *A. ovis* parasite in the native goat in the province and the impact of infection on some blood standards for being important parasites that cause serious illness and health and economic damage may lead to the death of animals.

## MATERIALS AND METHODS

### Collection and examination of samples

A total of 156 blood samples were collected from native goats and from both sexes and from different ages ranging from one month to nine years for the period from March 2017 to February 2018 and from several areas in Anbar province, included Ramadi, Fallujah, Khalidiya, Hit and Baghdadi. 6 ml of blood from the jugular vein was withdrawn after the area was sterilized with 70% ethyl alcohol, the blood samples were divided into two parts. The first section was used to detect the presence of the parasite and was diagnosis according to Jalali *et al.*,<sup>22</sup> by placing several drops of blood on a glass slide and

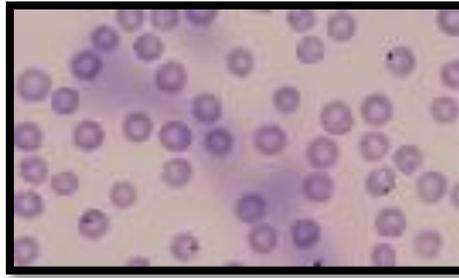


Figure 1: A smear of goat's blood dyed with a Giemsa stain showing *Anaplasma ovis* parasite inside the red blood cells (100x).



Figure 2: Photos of Goat infected with ticks.

Table 1: Number and percentage of *Anaplasma ovis* infection by sex.

Sex of the animal	Number of Examined animals	Number of infected animals	%
Males	71	21	29.5
Females	85	33	38.8**
Total	156	54	34.6
Significant variations			Less than 0.001

\*\*= significant

Table 2: Number and percentage of *Anaplasma ovis* infection by age.

Age of the animal (year)	Number of Examined animals	Number of infected animals	%
1 month – 1	15	1	6.6
2 – 3	16	4	25.0
4 - 5	40	16	40.0**
6 - 7	43	17	39.5
8 – 9	42	16	38.0
Total	156	54	34.6
Significant variations			Less than 0.001

\*\* = high significant

spreading it by another slide and left to dry in the air for several minutes. And then stabilized with 70% pure methyl alcohol for five minutes and dyed with Geimsa stain diluted with 10 % distilled water for 30 minutes, the examined was on oily lens (100x) by optical light

microscopy to watch the parasite. The second part of the blood samples were placed in test tubes containing anticoagulant and blood tests were carried out, which included total erythrocyte count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV)<sup>23</sup>.

*Statistical analysis*

The Chi-Square and the Fisher's test were used to determine the least significant difference L.S.D. at the probability level ( $p \leq 0.05$ ). And blood standards using a set of statistical software SAS<sup>24</sup>.

**RESULTS AND DISCUSSION**

The results showed that the native goats were infected with *Anaplasma ovis* parasite with a rate of 34.6%. The parasite was observed as black spherical bodies within the red blood cells in the blood samples examined (Figure 1). This is consistent with Al-Amerey and Hasso<sup>17</sup> where the rate of infection was 32.2% in goats in the surrounding areas of Baghdad and the study of Naqid<sup>21</sup> which reported a rate of infection 38.0% in Angor goats located in the province of Dohuk in northern Iraq and also agrees with the study of Nasir *et al.*,<sup>25</sup> where they recorded an infection rate of 39.29 % by examining 94 turkish a wassi sheep in the province of Baghdad, and higher than Zhang *et al.*<sup>13</sup> during the examination of 710 blood samples of goats in six regions in China which recorded an infection rate of 18.2%, moreover, study Alsaad *et al.*,<sup>18</sup> recorded 24.7% of the native goat in the Mosul area. The results of the present study were lower than Naqid and Zangana<sup>19</sup> where 55.8% were reported in the native goats of Dohuk, and Altaye *et al.*,<sup>11</sup> in the eastern part of Turkey recorded 71.3%, as well as, Said *et al.*,<sup>12</sup> with 65.3% in Tunisia. The high incidence of parasitic infection in native goats in Anbar province is due to the spread of ticks that transmit these parasites

Table 3: Number and percentage of *Anaplasma ovis* infection by month.

The Month	Number of infected animals	Number of Examined animals	%
March 2017	12	4	33.3
April	20	15	75.0**
May	15	11	73.3
June	15	8	53.3
July	13	5	38.4
August	12	5	41.6
September	13	3	23.0
October	10	1	10
November	10	0	0
December	9	0	0
January 2018	16	0	0
February	11	2	18.1*
Total	156	54	34.6
Significant variations			Less than 0.001

\* = significant      \*\* = high significant

(Figure 2). due to the availability of favorable climatic conditions of high temperature and humidity suitable for

Table 4. Rates of blood standards for native goat infected with *Anaplasma ovis* parasites

Blood standards	uninfected goats (control) Average $\pm$ standard error	The infected goat Average $\pm$ standard error
Total number of red blood cells Cm <sup>3</sup> x10 <sup>6</sup> / microliter	7.321 $\pm$ 0.12	4.677 $\pm$ 1.055
Concentration of hemoglobin g / 100 ml	11.978 $\pm$ 1.559	6.511 $\pm$ 0.176
Blood cell volume %	29.894 $\pm$ 5.821	21.149 $\pm$ 2.441

the growth and reproduction of tick, which is the intermediate host of the parasites, as well as the spread of blood-sucking flies that transmit parasite mechanics to animals in livestock breeding fields. The differences in the results of our current study with the previous studies may be due to the different methods used in the diagnosis, the size of the samples examined, and the different study areas.

There is significant difference between the rates among females (38.8%) and males (29.5%) as Table (1) presents. This is consistent with the study of Naqid<sup>21</sup> in Duhok province, the reason for the high incidence of infection among females because most breeders keep females longer than males for breeding purposes and the early sale of males this increases the chances of female exposure to parasitic infection more than males.

Over (4 years) had the highest rate of infection, which was (40, 39.5) % for ages 4 -5 years and 6-7 years, respectively,

while the lowest rate of infection was 6.6% in ages 1 month – 1year (Table 2). This is consistent with the study of Naqid<sup>21</sup> which found that the highest rate of infection 66.6% at the age of more than 3 years in the Angor goats in the province of Dohuk, due to the large age groups are more susceptible to ticks through the passage of animals in many seasons where the prevalence of tick, which is the transfer of blood parasites among animals and the high incidence of infection, the younger animals also have increased immunity against the parasite for obtaining antibodies with colostrum during breastfeeding than their mothers. This makes them resistant to parasite infection and low infection rate. Kuttler<sup>26</sup> reported that all ages are infected with parasites, but older animals are more vulnerable than small animals.

Table (3) shows that the highest rate of infection of *Anaplasma ovis* was (75, 73.3%) in April and May respectively and the lowest of 18.1% in February. This is in line with the study of Patra *et al.*,<sup>16</sup> in India and the study of Abed and Alsaad<sup>27</sup> in Basrah, this is due to the availability of favorable climatic and humidity conditions during the spring and summer months, which help to reproduce the vector host to this parasite of various species. Dharanesha<sup>28</sup> pointed out that the high infection rate of the blood parasites in the months of spring and summer are due to the high temperature and humidity that stimulate the vector host to transport the parasite to the ruminants. The results showed a significant effect of *A. ovis* on the blood parameters of the infected goat. The infection resulted in a decrease in the total number of red blood cells, concentration of hemoglobin, volume of blood cells and significant differences in these values in control animals (Table 4). This is consistent with Petrucelli and Bermúdez<sup>29</sup>, Shah *et al.*,<sup>30</sup> a significant reduction in blood standards in infected animals causes anemia because of parasite reproduction within red blood cells, his causes red blood cells to break down, causing hemolytic anemia<sup>31</sup>. Esmailnejad *et al.*,<sup>32</sup> indicated that the mechanism of cellular immune action and the phagocytosis of the red blood cells infected with parasite and their disposal through the thrombocytopenic cells in the endothelial system leads to a significant decrease in the total number of red blood cells, Parasitic infection also leads to weak blood cells leading to easy crashin

## REFERENCE

1. Silaghi, C., Santos, A. S., Gomes, J., Christova, I., Matei, I. A., Walder, G., ... & Oteo, J. A. Guidelines for the direct detection of *Anaplasma* spp. in diagnosis and epidemiological studies. Vector-Borne and Zoonotic Diseases, 2017;17(1), 12-22.
2. Han, R., Yang, J., Liu, Z., Gao, S., Niu, Q., Hassan, M. A., ... & Yin, H. Characterization of *Anaplasma ovis* strains using the major surface protein 1a repeat sequences. Parasites & vectors, 2017; 10 (1), 447.
3. Battilani, M., De Arcangeli, S., Balboni, A., & Dondi, F. Genetic diversity and molecular epidemiology of *Anaplasma*. Infection, Genetics and Evolution, 2017;49, 195-211.

4. Sharma, A., Orr, L., & Shore, E. T. ARDS Due to *Anaplasma* and *Borrelia* Co-Infection; Can Corticosteroids Help? In C51. critical care case reports: you give me fever-infection and sepsis 2018; pp. A5201-A5201. American Thoracic Society.
5. Jayalakshmi, K., Prasath, N. B., Kavitha, S., Krishnakumar, S., Veeraselvam, M., & Yogeshpriya, S. Concurrent Infection of Sheep Pox, Orff, Theileriosis and Anaplasmosis in a Sheep, 2017.
6. Ullah, N., Durrani, A. Z., Avais, M., Ahmad, N., Ullah, S., Ullah, S., & Khan, N. U. A first report on prevalence of caprine theileriosis and its association with host biomarkers in Southern Khyber Pakhtunkhwa, Pakistan. *Small Ruminant Research*, 2018;159, 56-61.
7. Dahmani, M., Marié, J. L., Scandola, P., Brah, S., Davoust, B., & Mediannikov, O. *Anaplasma ovis* infects sheep in Niger. *Small Ruminant Research*, 2017;151, 32-35.
8. Sidouin, D. M. K. Molecular Identification and Characterization of *Anaplasma* Haemoparasites Isolated from Cattle and Sheep in Homabay County, Kenya (Doctoral Dissertation, University of Nairobi);2017.
9. Stiller, D., & Marchette, N. The Anaplasmataceae, Bartonellaceae, and Rochalimaea Quintana. In *Ecological Relationships and Evolution of Rickettsiae*, 2017; pp. 97-126. CRC Press.
10. Noaman, V. A Review of Anaplasmosis and The Prevalence of *Anaplasma marginale* in Cattle in Iran and The World; 2017.
11. Altay, K., Dumanli, N., Aktas, M., & Ozubek, S. Survey of *Anaplasma* infections in small ruminants from east part of Turkey. *Kafkas Univ. Vet. Fak. Derg.*, 2014; 20 (1), 1-4.
12. Said, M. B., Belkahia, H., Alberti, A., Zobba, R., Bousrih, M., Yahiaoui, M., & Messadi, L. Molecular survey of *Anaplasma* species in small ruminants reveals the presence of novel strains closely related to *A. phagocytophilum* in Tunisia. *Vector-Borne and Zoonotic Diseases*, 2015; 15(10), 580-590.
13. Zhang, Y., Lv, Y., Zhang, F., Zhang, W., Wang, J., Cui, Y., ... & Ning, C. Molecular and phylogenetic analysis of *Anaplasma* spp. in sheep and goats from six provinces of China. *Journal of veterinary science*, 2016; 17(4), 523-529.
14. Zhou, M., Cao, S., Sevinc, F., Sevinc, M., Ceylan, O., Ekici, S., & Iguchi, A. Molecular detection and genetic characterization of Babesia, *Theileria* and *Anaplasma* amongst apparently healthy sheep and goats in the central region of Turkey. *Ticks and tick-borne diseases*, 2017; 8(2), 246-252.
15. Ochirkhuu, N., Konnai, S., Odbileg, R., Murata, S., & Ohashi, K. Molecular epidemiological survey and genetic characterization of *Anaplasma* species in Mongolian livestock. *Vector-Borne and Zoonotic Diseases*, 2017; 17(8), 539-549.
16. Patra, G., Ghosh, S., Mohanta, D., Kumar Borthakur, S., Behera, P., Chakraborty, S., & Mahata, S. Prevalence of haemoprotozoa in goat population of West Bengal, India. *Biological Rhythm Research*, 2018; 1-10.
17. Al-Amerey MA, Hasso SA. Epizootiological Survey of some blood and fecal parasitic protozoa of goats around Baghdad City. *Basrah J Vet Res.* 2002;1(2):41-48.
18. Alsaad KM, Al-obaidi QT, Esmael SA. Hematological and biochemical study on the effect of some common blood parasites in native goats in Mosul area. *Iraq. J Vet Sci.* 2009; 23:101-106.
19. Naqid IA, Zangana IZ. Hematological and serological (cELISA) studies of caprine anaplasmosis in Duhok governorate of Kurdistan region of Iraq. *J Duhok Univ.* 2011;13(1):153-161.
20. Renneker, S., Abdo, J., Salih, D. E. A., Karagenc, T., Bilgiç, H., Torina, A., & Seitzer, U. Can *Anaplasma ovis* in small ruminants be neglected any longer? *Transboundary and emerging diseases*, 2013; 60, 105-112.
21. Naqid, I. A. Prevalence of *Anaplasma ovis* infection in Angora goats of Duhok province, Kurdistan region-Iraq. *Iraqi Journal of Veterinary Sciences*, 2017; 31(2), 73-79.
22. Jalali S, Khaki Z, Kazemi B, Bandehpour M, Rahbari S, Razi Jalali M, Yasini S. Molecular detection and identification of *Anaplasma* species in sheep from Ahvaz, Iran. *Iranian J Vet Res.* 2003;14(1):50-56. 15
23. Meyer DJ, Harvey JW. *Veterinary laboratory medicine*. 3rd ed. WB. Saunders Co London. 2004;17- 24, 63-65, 163.
24. SAS. *Statistical Analysis system, users Guide*. Statistical version 9.1th ed. SAS. Inst. Inc. Cary., N.C. the USA 2012.
25. Nasir, M. A., Al-Anbery, Taha, N. N. A. A. 2017. The effect of kind blood parasite infection on some productive traits in Turkish a wassi sheep. *The Iraqi Journal of Agricultural Sciences –1672-1676*: (6) 48 (in Arabic).
26. Kuttler, K. L. *World-wide impact of babesiosis. In Babesiosis of domestic animals and man 2018*; (pp. 1-22). CRC Press.
27. Abed, F. A., & Alsaad, K. M. Clinical, Hematological and Diagnostic Studies of Hem Mycoplasma Infection (*Mycoplasma Ovis*) In Sheep of Basrah Governorate. *Basrah Journal of Veterinary Research.*, 2017; 16(2), 284-304.
28. Dharanesh, N. K., Giridhar, P., Byregowda, S. M., Venkatesh, M. D., & Ananda, K. J. Seasonal prevalence of blood parasitic diseases in crossbred cattle of Mysore and its surrounding districts of Karnataka. *Journal of Parasitic Diseases*, 2017; 41(3), 773-777.
29. Petrucelli, J. V., & Bermúdez, S. Clinical and Serological Evidence of Canine Anaplasmosis and Ehrlichiosis in Urban and Rural Panama. *Ann. Clin. Cytol. Pathol.*, 2017; 3(1), 1050.
30. Shah, S. S. A., Khan, M. I., & Rahman, H. U. Epidemiological and hematological investigations of tick-borne diseases in small ruminants in Peshawar and Khyber agency. *Pakistan. J. adv. Parasitol.* 2017; 4(2).

31. Goda ASA, Osman WA, Mona AM, Abou-Elnaga, TR. Seroprevalence of *Anaplasma ovis* antibodies in small ruminants by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. Suez Canal Vet Med J. 2009; 1:287–297. 17
32. Esmailnejad, B., Tavassoli, M., Samiei, A., Hajipour, N., Imani-Baran, A., & Farhang-Pajuh, F. Evaluation of oxidative stress and antioxidant status, serum trace mineral levels and cholinesterase's activity in cattle infected with *Anaplasma marginale*. Microbial pathogenesis; 2018.