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Seroprevalence of *Anaplasma* Infection in Sheep and Cattle in Kurdistan Province of Iran with an Overview of One Decades of Its Epidemiological Status in Iran

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aim: *Anaplasmosis* is an important economic livestock disease. Limited information on its epidemiology in Iran is still lacking thus the aim of the study was to determine the seroprevalence of the disease in sheep and cattle in Kurdistan province of Western Iran with an overview of one decades of its epidemiological status in Iran.
Study Design: This was a mixed cross sectional and longitudinal study carried out for a period of July to September 2013. Using competitive enzyme-linked immunosorbent assay (c-ELISA), for anti-*Anaplasma* antibodies.
Methodology: A total of 182 blood samples were collected from 105 cattle and 77 sheep for the detection of antibodies against *Anaplasma* species using cELISA method. For this purpose, cattle and sheep of different sex and age were examined.
Results: Examination of 182 blood samples revealed that 8 (7.62%) and 5 (6.49%) of cattle and

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sheep were infected with *Anaplasma* species, respectively. Also, the prevalence of *Anaplasma* infection in relation to age and sex was not statistically significant.
Conclusion: The study showed an increasing prevalence of the infection in cattle and sheep of Kurdistan area in western Iran which help to have appropriate prevention measures for *Anaplasmosis*.

Keywords: Antibodies; Anaplasma spp; Cattle; C-Elisa; Sheep.

1. INTRODUCTION

Anaplasmosis, a disease caused by various species of *Anaplasma*, poses important economic constraints to animal breeders. Besides the costs of the additional veterinary care, *Anaplasmosis* causes abortion in animals, reduction of milk production, body weight, and frequently leads to death [1]. *Anaplasma* is intracellular, gram negative bacteria and representatives of the order *Rickettsiales* classified into *Rickettsiaceae* and *Anaplasmataceae* families [2]. *Anaplasmosis* in cattle is common in South Africa, Australia, Russia, South America, and the United States, and *Anaplasmosis* of sheep and goats occurs in Africa, Mediterranean countries, Russia, and the United States [3]. *Anaplasmosis* in cattle is caused by *A. bovis* infecting monocytes [4]. *A. marginale* and *A. centrale were* which parasitize and replicate in red blood cells [5]. *A. bovis* is reported mostly from cattle, but also detected in small ruminants which could be a reservoir of this bacterium [6,7]. Bovine *anaplasmosis* results from infection with *A. marginale*. *A. centrale*, a less pathogenic but closely related organism, is used as a live vaccine for cattle in South Africa, South America and Australia [8]. *A. ovis*, the agent of ovine *Anaplasmosis*, may cause mild to severe disease in sheep, deer and goats but is not infectious for cattle [9]. Ovine *Anaplasmosis* is mainly caused by *A. ovis* and *A. marginale*. In the case of *A. ovis*, bacterial inclusions are found 35-40% of the time in the central or sub-marginal part of the host erythrocyte, and the remaining 60-65% in the marginal part [10]. Although *A. ovis* may infect domestic sheep and goats without clinical signs [11], it can predispose animals to other infections resulting in clinical disease and eventually death [12]. *Anaplasmosis* is transmitted by ticks, biting insects or inoculation of blood into susceptible animals can also transmit the disease [13,14]. Mechanical transmission occurs by contaminated mouthparts of biting flies but can only be achieved within a few minutes after the initial bite, although the pathogen can remain viable and infective in arthropods for several days after ingestion [15-17]. Other stress factors such as malnutrition and pregnancy also increase the susceptibility of animals to *Anaplasmosis* [11]. Key environmental factors, such as attitude, temperature, rainfall and humidity effect on influence the presence, development, activity and longevity of pathogens, vectors and zoonotic reservoirs of infection [18-20]. Due to the lack of documented information about *Anaplasma* species in cattle and sheep and having found clinical features and laboratory findings similar to *Anaplasmosis* in Kurdistan County during recent years, we conducted the present study to understand more about the *Anaplasma* infections in Western Iran. We also conducted an overview on the previously confirmed *Anaplasmosis* and demonstrated a one decade's prevalence in Iran. *Anaplasmosis* has been reported from some parts of Iran (Table 1); though few of cases were reported using molecular techniques, and none has yet been reported with c-ELISA methods since diagnosis of the infection is routinely performed using microscopic examination blood smears in Iran [21].

2. MATERIALS AND METHODS

2.1 Study Area

The Kurdistan Region is located within the western of Iran, and the weather conditions are similar to the Mediterranean area in which rainfall occurs in winter and moderate rain in autumn and spring and no rain fall in the summer season. With respect to the climate, the region is defined as having cold winters, hot summers, and neutral springs and autumns with a wide range of temperatures. The study was a mixed cross sectional and longitudinal study conducted in Kurdistan province in Western Iran for the period from July to September 2013.

2.2 Materials

Blood samples were collected from 105 cattle and 77 sheep in Kurdistan province. Animal selection was random and Information about age and sex was recorded using with tool clearly

state either questionnaire or farmer response or by examination. Blood samples were them transferred to the laboratory of Protozoology and Production of Protozoal Vaccines, Razi State Serum and Vaccine Research Institute. Sera were extracted from 5ml venous blood samples, by centrifugation at 2000 g for 10 minutes and were stored at -20°C prior to testing. Data was arranged into groups for comparison i.e. age Sheep > 1 year. Also sheep <2 years and those > 2 years. Likewise the same was done for cattle. The data was compared in two groups based on sex, male and female.

2.3 Methods

A competitive enzyme-linked immunosorbent assay (c-ELISA) was performed using the *Anaplasma* Antibody Test Kit from VMRD Inc. (Pullmann, WA, USA) following the manufacturer's instructions. This assay detects serum antibodies to a major surface protein (MSP5) of *A. marginale*, *A. centrale* and *A. ovis* and *A. phagocytophilum*. Although approved only for bovines by the U.S. Department of Agriculture, it could detect seroconversion of experimentally infected sheep, since their antibodies compete successfully for free binding sites with monoclonal antibodies present in the detection system of the test kit [29]. Optical density (OD) values were determined using an automatic Multi-scan Plus microplate reader (model RS-232 C, Lab systems, Helsinki, Finland), and the percentage of inhibition was calculated as follows: I (%) = 100 – (sample OD x 100) / (mean OD of three negative controls). Samples with an inhibition ≥30% were regarded positive.

2.4 Statistical Analysis

Data was recorded as frequencies, expressed as percentages using SAS version 6.12 and Duncan's multiple range tests [30]. In group comparisons were carried out and a $P< 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSION

The results showed that the infection rate of *Anaplasma* spp. in sheep and cattle were 6.49% and 7.62% respectively (Table 2). There was no association between *Anaplasma* infection with age and sex in both sheep and cattle ($P > 0.05$).

This study was a preliminary study in Kurdistan province on seroprevalence of *Anaplasma* infection in sheep and cattle. The low seroprevalence observed would be due to the low tick vector population in Kurdistan province. *Anaplasma* spp. transmitted by at least 20 ticks' species, including *Argas persicus*, *Ornithodoros lahorensis*, *Boophilus annulatus*, *B. decoloratus*, *B. microplus*, *Dermacentor albipictus*, D. andersoni, D. *occidentalis*, D. *variabilis*, *Hyalomma excavatum*, *Ixodes ricinus*, *Rhipicephalus bursa*, *R. sanguineus* and *R. simus* [31]. Though, some of these tick vectors are widespread in Kurdistan province [32]. The prevalence of sheep *Anaplasmosis* was shown to be at 6.49% which is quite lower from that reported from surrounding countries in the region i.e. Turkey, Iraq, and Pakistan at 12.35%, 11.36% and 24.47% respectively [33-35]. *Anaplasmosis* is an important economical disease of the livestock industry in Iran and it has been shown that the infection can persist in cattle recovered from acute *Anaplasmosis* [33] thus acting as reservoirs for re-infection in herds hence complicating disease diagnosis and control further [36]. The prevalence of *Anaplasma* infection in cattle was shown to be at 7.62% which is well below 20% in comparison to recent findings in Iraq and Turkey were a prevalence of 2.5% and 55.35% has been reported respectively [37,38]. This would be due to the geographical differences thus affecting the epidemiological pattern of the dominant vectors in the region the differences in the infection rate with *Anaplasma* from area to area may be affected by many factors like climatic condition, seasonal variation of tick vector, susceptibility of breeds, distribution of vector, system of breeding, vaccination, and strategy of prophylactic and treatment methods [27].

3.1 Cattle

There are approximately 8 million cattle in Iran [39]. Cattle used for meat, milk and hides in Iran. *A. marginale* and *A. phagocytophilum* have long been recognized as bovine *anaplasmosis* agents. Recently, they have been detected in cattle of Iran by molecular approaches [24,40]. The carrier cattle can serve most probably as the reservoir of infection for vector ticks. Furthermore, the carrier status of cattle can function under severe nutritional or climatic stress for the clinical relapse. Control and management of livestock health could be understood as the two sides of a gold coin for a successful and healthy economy in stock farming. Here, the control of tick-borne diseases plays a prominent role. One of the most

important diseases in cattle farms is the infection with *Anaplasma* organisms, which cause annually high economic losses in Iran. Furthermore, reviews of tick-borne diseases have been increasingly recognized worldwide as highlighting this animal health problem [40]. A recent experimental study showed that cattle can be co-infected with *A. phagocytophilum* and *A. marginale* [41]. There have been other reports of evidence of simultaneous infection with two or more species from the *Anaplasma* genus in ticks, deer and cattle in different areas of the world [8,42-46]. In Isfahan, of the 150 cattle, 4 (2.66%) was positive for *A. bovis* by nested-PCR [26]. The prevalence rate of *Anaplasma* infection in cattle by PBS method is 19.37; 50 and 3% in Mashhad suburb, Isfahan and Kerman, respectively [23,26,27]. In Kerman and Ghaemshahr, rate of infection in cattle by PCR assay is 77 and 22.22%, respectively [7,27]. In South Africa, A total of 87% of the cattle were seropositive for *Anaplasma* by enzyme-linked immune-sorbent assay [47]. In Iran, PCR analysis of *A. marginale* 16S ribosomal RNA (rRNA) gene on bovine blood samples showed 58 out of the total 150 blood samples to be positive for *Anaplasma* spp. [26]. In Sicily analyzed the prevalence of *A. marginale* by PCR and sequence analysis of MSP4 amp-icons and reported 50% positivity among the tested bovine samples [8]. Recently, PCR amplification of the segment spanning the V1 region of the 16S rRNA gene of *Anaplasma* species, followed by reverse line blot (RLB) hybridization assay identified *Anaplasma* infections in 9.0% (35/389) of the bovine samples from Turkey [48]. Also the highest rates of positive prevalence *A. marginale* (9.09%) were diagnosed in cattle while lower value (3.36%) in sheep [37].

3.2 Goats and Sheep

There are approximately 88 million goats and sheep in Iran [39]. *A. ovis* and *A. marginale* infect goats [10]. *A. marginale* (the type species for cattle) also causes latent *Anaplasmosis* in sheep and goats [49]. Experimental inoculation of goats with *A. ovis* induces an acute disease characterized by depression, anorexia, fever, and progressive anemia [11]. Reported that goat also can be a susceptible host for *A.* ovis [49]. The prevalence of *Anaplasma* infection was studied in goats in the Mashhad area of Iran from 1999 to 2002, 80.3% and 47.53% of sheep and goats were infected with *Anaplasma*, respectively [23]. In a study in the northeast of Iran using PCR-RFLP of the MSP4 gene, 63.7% (123/193) of examined goats were *Anaplasma* positive, all of which were *A. ovis* [25]. They recommended this method as a useful tool for the detection of *A. ovis* in goats. A molecular surveillance of tick-borne diseases of sheep in the south of Iran showed 29.0% *Anaplasma* positive blood samples [22]. Also, demonstrated that 87.4% and 43.08% of sheep infected by *Anaplasma* species in Ahvaz and Mazandaran (Ghaemshahr) by using PCR method [7,28]. Evaluation prevalence of sheep blood parasites in 2013 in Ahvaz, Iran and was declared that 33.6% of animals were *Anaplasma* positive by PBS method [28]. In Mazandaran (Ghaemshahr) province reported that 25% of goats were infected with *Anaplasma* by using PCR [7].

3.3 Vector

A tick survey was carried out in four different geographical areas of Iran, where the majority of the domestic ruminants in Iran exist (Fig. 1) [32]. Tick studies were initiated by Delpy [50-52]. Later, Abbasian-Lintzen and Mazlum compiled a list of adult ticks collected from domestic animals [53-57]. The influence of temperature and moisture on the survival and diversity of ticks is well known, and it is also well understood that different species have different requirements for survival and reproduction. Hence, climatic condition of a country should be considered in the study of ticks and tick-borne disease. According to data published by the Iranian Ministry of Agriculture, the major differences in climatic condition result in four different zones in Iran. These zones are the Caspian region in the north, mountainous areas in the northwest to southeast, the desert boundary area in the central region and the Persian Gulf region in the south. There has been little study on tick fauna in recent years in Iran, and the present study therefore aimed at determining the distribution of ticks infesting ruminants [32].

The tick infestation has thus been shown to occur in areas of high livestock density, and this may indicate that special attention should be directed to certain areas concerning certain ticks. A long time has passed since the previous studies on tick fauna in Iran, and the intensity of livestock has been changing in different places (Table 3). Together with climatic changes of recent years, these factors can influence the diversity of ticks found in Iran [32].

Table 1. Results of *Anaplasma* infection in Iran

Province	Year	Animal host	Methods	% infection	References
Fars	2004	Goats	PCR*	29	[22]
Khorasan razavi (Mashhad)	2006	Cattle, sheep, goats	PBS**	19.37,80.3,38.92	[23]
Isfahan	2009	Cattle	PBS,PCR	50,77	[24]
Golestan and Khorasan razavi	2009	Goats	PBS,PCR	22.3,63.7	[25]
Isfahan	2010	Cattle	PCR	2.66	[26]
Kerman	2011	Cattle	PBS	3	[27]
Ahvaz	2013	Sheep	PBS,PCR	33.6,87.4	[28]
Mazandaran (Ghaemshahr)	2014	Cattle, sheep, goats	PCR	22.22,43.08,25	[7]

**PCR: polymerase chain reaction; **PBS: Peripheral blood smear*

Table 2. Number of infected animals by *Anaplasma*

Animal	Animals tested	Seropositive animals (%)	The first age group-seropositive animals (%)	The second age group-seropositive animals (%)	Male seropositive animals (%)	Female seropositive animals (%)
Cattle	105	8 (7.62)	4 (7.69)	4 (7.55)	3 (5.77)	5 (9.43)
sheep	77	5 (6.49)	3 (7.69)	2 (5.26)	2 (5.13)	3 (7.89)

Table 3. Prevalence of ixodid ticks in Iran [32]

Species	Region
Ixodes ricinus	Caspian Sea region
Boophilus annulatus	Mazenderan, Gilan, Khoozestan, West Azerbaijan
Dermacentor marginatus	Khorassan, West and East Azerbaijan, Khoozestan, Mazenderan, Kerman and Markazi province
Dermacentor daghestanicus	Khorassan, Mazenderan, Kerman and Isfahan
Rhipicephalus bursa	All over Iran especially in Khorassan, Khoozestan, Fars, Sistan and Baluchistan, Gilan, Mazenderan, Kerman and Markazi province
Rhipicephalus sanguineus	All over Iran especially in Caspian Sea region, Northwest Iran and Boushehr
Rhipicephalus turanicus	North and Northwest Iran and Khorassan
Haemaphysalis concinna	Gilan, Mazenderan, Khorassan and Northwest Iran
Haemaphysalis cholodkovskyi	Northwest Iran, Caspian Sea region, Kerman, Boushehr, Khorassan
Haemaphysalis cinnabarina punctata	Mazenderan, Golestan, South and North Azerbaijan, Khorassan
Haemaphysalis inermis	Mazenderan, Golestan
Haemaphysalis parva	South and North Azerbaijan, Kurdistan, Khorassan
Hyalomma aegyptium	Tehran, Kerman, Kurdistan, Kermanshah
Hyalomma schulzei	Tehran, Fars, Kerman, Khorassan, Sistan-Baluchistan
Hyalomma dromedarii	Sistan–Baluchistan, Khorassan, Boushehr, Quom
Hyalomma anatolicum anatolicum, H.anatolicum excavatum	All over Iran
Hyalomma asiaticum asiaticum	All over Iran especially Southern and Southwest provinces
Hyalomma detritum	Khorassan, West and East Azerbaijan, Khoozestan, Boushehr, Mazenderan, Gilan, Fars
Hyalomma rufipes, H. rufipes glabrum, H. rufipes turanicum	All over Iran
Hyalomma marginatum marginatum, H. plumbeum plumbeum, H. impressum, H. savignii	Caspian Sea region, Khoozestan and Markazi province

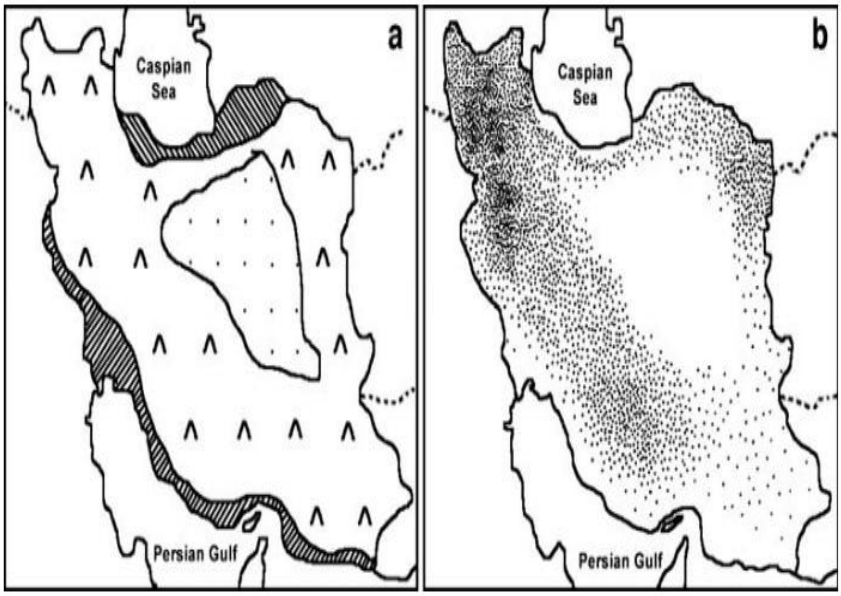

Fig. 1 **a** The four different geographical zones of Iran. Caspian, mountain plateau, Persian Gulf lowlands, desert. **b** The distribution area of domestic ruminants in Iran

3.4 Diagnosis

Diagnosis of *Anaplasmosis* in ruminants mainly based in the identification of the *Rickettsia* in stained blood smears. However, below 0.1% *Rickettsia* in chronic carriers are not detected by this method [58]. This method is suitable in detection of *Anaplasmosis* in acute phase, but it is not applicable in identifying pre-symptomatic and carrier animals [21]. However, it is difficult to differentiate the organism from other similar structures like Howell-Jolly bodies, or staining artifacts, especially in carrier animals with low level of *rickettsia* [10,59]. This makes microscopic assessment unreliable for the detection of persistent infections [23]. Hence, alternative diagnostic techniques, such as serological tests [59-61] and nucleic acid based assays [26,62,63]. In these instances, the infection is generally diagnosed by serologic demonstration of antibodies with confirmation by molecular detection methods. Several serological tests have been employed extensively for epidemiological studies: complement fixation (CF) test, capillary agglutination assay (CAA), card agglutination test (CAT), indirect fluorescent antibody (IFA) test, as well as various enzyme linked immunosorbent assays (ELISA) such as a c-ELISA, indirect ELISA and dot ELISA. The two serological tests currently preferred for identifying infected animals are the c-ELISA and the CAT [64]. Serological assays, based on Major Surface Protein 5(MSP-5) of *A. marginale* have been successfully used, for the detection of antibodies against *Anaplasma* [65]. In contrast, the development and persistence of antibodies following *Anaplasma* infection provide a means to detect infected animals at all stages of infection [66]. It has proven very sensitive and specific for the detection of *Anaplasma* infected animals [60,65]. However, the test cannot differentiate between *A. marginale* and some of the other *Anaplasma* species, because they all express the MSP5 antigen [66,67]. Molecular methods, as more sensitive and specific diagnostic tools, have been increasingly used to detect and differentiate *Anaplasma* in carrier animals and tick vectors [21,25,26]. Nucleic-acid-based tests [polymerase chain reaction (PCR)] have also been developed that are capable of detecting the presence of low-level infection in carrier cattle [64].

3.5 Vaccination

Vaccination has been an economical and relatively effective way to control bovine *Anaplasmosis* worldwide. Both killed and live vaccines have relied on erythrocyte-derived antigen sources to induce protective immunity or to prevent clinical disease. However, neither one prevents cattle from becoming persistently infected with *A. marginale* or becoming reservoirs of infections [68] Killed (inactivated) vaccines developed in the USA in the 1960s were marketed until 1999, when they were withdrawn from the marketplace owing to company restructuring [68]. The vaccine was effective in preventing clinical *Anaplasmosis* in the south

central United States where geographical strains were cross-protective. Live vaccines involve inoculating cattle with erythrocytes infected with less pathogenic (attenuated) strains of *A. marginale* or *A. centrale*. The immune response is similar to natural infection with vaccinated animals developing mild and in apparent infections and becoming persistently infected with the vaccine strain. *A. centrale* is used as a vaccine in Africa, Australia, Israel and Latin American countries. However, it does not provide effective cross-protection in widely separated geographical areas, as was demonstrated in Paraguay [69]. The ideal vaccine for bovine *Anaplasmosis* would be one that prevents infection as well as induces protective immunity. Additionally, the possibility of blocking the biological transmission of *A. marginale* is an important goal of vaccines for bovine *Anaplasmosis* [9].

3.6 Control

The differences in the infection rate with *Anaplasma* spp. From area to area may be affected by many factors like climatic condition, seasonal variation of tick vectors and of hematophagous flies, susceptibility of breeds, and distribution of vector, system of breeding, vaccination, and strategy of prophylactic and treatment methods [27]. *Anaplasmosis* is endemic or potentially endemic to 42 countries. Although *Anaplasmosis* is not endemic to Iran, imported, expatriate or other presentations of the disease have been associated with this country [70]. Control measures for bovine *Anaplasmosis* may vary with geographical location, but they have not varied markedly during the past 50 years [71]. Control and prevention measures include (i) maintenance of *Anaplasma*-free herds through import and movement control, testing, and elimination of carrier cattle; (ii) vector control; (iii) prevention of iatrogenic transmission; (iv) administration of antibiotics; and (v) preimmunization with live vaccines and immunization with killed vaccines [9].

4. CONCLUSION AND RECOMMENDATIONS

It was found that 7.14% of domestic animals in Kurdistan province in west of Iran using of c-ELISA were infected by *Anaplasma* which is slightly lower than that reported from surrounding countries. Further epidemiological studies would be conducted to assess the major risk factors and the economic burden of the infection in the province.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Author hereby declares that all experiments have been examined and approved by the ethics committee of Razi Vaccine and Serum Research Institute, Alborz, Iran.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Stuen S, Nevland S, Moum T. Fatal cases of tick-borne fever (TBF) in sheep caused by several 16S rRNA gene variants of *Anaplasma phagocytophilum*. Annals of the New York Academy of Sciences. 2003;990:433-4.
2. Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, et al. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: Unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int J Syst Evol Microbiol. 2001;51:2145-65.
3. Rafyi A, Maghami G. Under study contribution of some blood parasites of sheep and chevreen Iran and neighboring countries. Bulletin of the Office International des Epizooties.1691;65:1769-83.

4. Uilenberg G. Other ehrlichiosis of ruminants. In: Woldehiwet Z. In: Ristic M, editor. Rickettsial and chlamydial diseases of domestic animals. Pergamon Press, Oxford. 1993;293-332.
5. Kuttler KL. Clinical and hematologic comparison of *Anaplasma marginale* and *Anaplasma centrale* infections in cattle. American Journal of Veterinary Research. 1966;27:941-6.
6. Goethert HK, Telford III SR. Enzootic transmission of *Anaplasma bovis* in Nantucket cottontail rabbits. Journal of Clinical Microbiology. 2003;41:3744-7.
7. Hosseini-Vasoukolaei N, Oshaghi MA, Shayan P, Vatandoost H, Babamahmoudi F, Yaghoobi-Ershadi MR, et al. *Anaplasma* infection in ticks livestock and human in Ghaemshahr Mazandaran Province Iran. Journal of Arthropod-Borne Diseases. 2014;8:204-11.
8. de la Fuente J, Lew A, Lutz H, Meli M, Hofmann-Lehmann R, Shkap V, et al. Genetic diversity of *Anaplasma* species major surface proteins and implications for *Anaplasmosis* serodiagnosis and vaccine development. Animal Health Research Review. 2005;6:75-89.
9. Aubry P, Geale DW. A review of bovine *anaplasmosis*. Transboundary and Emerging Diseases. 2011;58:1-30.
10. Shompole S, Waghela SD, Rurangirwa FR, McGuire TC. Cloned DNA probes identify *Anaplasma ovis* in goats and reveal a high prevalence of infection. Journal of Clinical Microbiology. 1989;27:2730-5.
11. Splitter EJ, Anthony HD, Twiehaus MJ. *Anaplasma ovis* in the United States: Experimental studies with sheep and goats. American Journal of Veterinary Research. 1956;17:487-91.
12. Kocan KM, de la Fuente J, Blouin EF, Garcia-Garcia JC. *Anaplasma marginale* (*Rickettsiales*: *Anaplasmataceae*): Recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology. 2004;129:285-300.
13. Yasini SP, Khaki Z, Rahbari S, Kazemi B, SalarAmoli J, Gharabaghi A, et al. Hematologic and Clinical Aspects of Experimental Ovine *Anaplasmosis* caused by *Anaplasma ovis* in Iran. Iranian Journal of Parasitology. 2012;7:91-8.
14. De Echaide ST, Knowles DP, Mcguire TC, Palmer GH, Suarez CE, Mcelwain TF. Detection of cattle naturally infected with *Anaplasma marginale* in a region of endemicity by nested PCR and a competitive enzyme linked immunosorbent assay using recombinant major surface protein 5. Journal of Clinical Microbiology. 1998;36:777-82.
15. Ewing SA. Transmission of *Anaplasma marginale* by arthropods. Proceedings of the 7th National *Anaplasmosis* Conference. 1981;395-423.
16. Hornok S, Foldvari G, Elek V, Naranjo V, Farkas R, de la Fuente J. Molecular identification of *Anaplasma marginale and Rickettsial endosymbionts* in blood-sucking flies (*Diptera*: *Tabanidae*, *Muscidae*) and hard ticks (*Acari*: *Ixodidae*). Veterinary Parasitology. 2008;154:354-9.
17. European Food Safety Authority. Scientific opinion on geographic distribution of tick-borne infections and their vectors in Europe and the other regions of the Mediterranean basin1. European Food Safety Authority Journal. 2010;8:1723.
18. Chaput EK, Meek JI, Heimer R. Spatial analysis of human granulocytic ehrlichiosis near Lyme Connecticut. Emerging Infectious Diseases. 2002;8:943-8.
19. Daniel M, Kola JR, Zeman P. GIS tools for tick and tickborne disease occurrence. Parasitology. 2004;129(Suppl):329-52.
20. Sedaghat MM, Shemshad K, Kamali K, Charrahy Z, Biglarian A, Rafinejad J. The eco-epidemiology of tick-borne hemo parasitic diseases using geographic information systems. Zahedan Journal of Research in Medical Sciences. 2013;15:17-22.
21. Carelli G, Decaro N, Lorusso A, Elia G, Lorusso E, Mari V, et al. Detection and quantification of *Anaplasma marginale* DNA in blood samples of cattle by real-time PCR. Veterinary Microbiology. 2007;124: 107-14.
22. Spitalska E, Namavari MM, Hosseini MH, Shad-del F, Amrabad OR, Sparagano OAE. Molecular surveillance of tick-borne diseases in Iranian small ruminants. Small Ruminant Research. 2005;57:245-8.
23. Razmi GR, Dastjerdi K, Hosseini H, Naghibi A, Barati F, Aslani MR. An epidemiological study on *Anaplasma* infection in cattle, sheep, and goats in Mashhad suburb Khorasan province Iran. Annals of the New York Academy of Sciences. 2006;1078:479-81.
24. Noaman V, Shayan P, Amininia N. Molecular diagnostic of *Anaplasma*

marginale in carrier cattle. Iranian Journal of Parasitology. 2009;4:31-8.
25. Ahmadi-Hamedani M, Khaki Z, Rahbari S, Kazemi B, Bandehpour M. Molecular identification of *Anaplasmosis* in goats using a new PCR-RFLP method. Iranian Journal Veterinary Research. 2009;10:367-72.
26. Noaman V, Shayan P. Molecular detection of *Anaplasma bovis* in cattle from central part of Iran. Veterinary Research Forum. 2010;1:117-22.
27. Saleh Zadeh S, Fathi S, Mirzaei Dehaghi M, Norouzi Asl E, Asgary Nezhad H. Survey of *Theileria* and *Anaplasma marginale* in cattle in Kerman area, southeast of Iran. Scientia Parasitologica. 2011;12:61-6.
28. Jalali SM, Khaki Z, Kazemi B, Bandehpour M, Rahbari S, Razi Jalali M, et al. Molecular detection and identification of *Anaplasma* species in sheep from Ahvaz, Iran. Iranian Journal Veterinary Research. 2013;14:50-6.
29. Dreher UM, de la Fuente J, Hofmann-Lehmann R, Meli ML, Pusterla N, Kocan KM, et al. Serologic cross-reactivity between *Anaplasma marginale* and *Anaplasma phagocytophilum*. Clinical and Diagnostic Laboratory Immunology. 2005;12:1177-83.
30. SAS. Statistical Analysis, 6.12 SAS user's guide. Statistics SAS institute Inc. 6.12 SAS User's Guide ed. Cary North Carolina, USA; 1987.
31. Marchette N, Stiller D. The *Anaplasmataceae Bartonellaceae* and *Rochalimaea quintana*. Florida USA: CRC Press Boca Raton; 1982.
32. Rahbari S, Nabian S, Shayan P. Primary report on distribution of tick fauna in Iran. Parasitology Research. 2007;101(Suppl 2):175-7.
33. Gokce HI, Genc O, Akca A, Vatansever Z, Unver A, Erdogan HM. Molecular and serological evidence of *Anaplasma phagocytophilum* infection of farm animals in the black sea region of Turkey. Acta Veterinaria Hungarica. 2008;56:281-92.
34. Latif B, Al-Izzi SA, Majeed MA, Sultan AS. Prevalence of piroplasmosis among sheep in the central part of Iraq. The Iraqi Journal of Veterinary Medicine. 1987;11:132-8.
35. Kashif M, Ahmad MS. Geographical seroprevalence of *Anaplasma marginale* infection (*Anaplasmosis*) by ELISA in *Ovis aries*, in district Peshawar Pakistan. The Journal of Zoology Studies. 2014;1:15-8.
36. Eriks IS, Palmer GH, McGuire TC. Detection and quantitation of *Anaplasma marginale* in carrier cattle by using a nucleic acid probe. Journal of Clinical Microbiology. 1989;27:279-84.
37. Ameen KAH, Abdullah BA, Abdul-Razaq RA. Seroprevalence of *Babesia bigemina* and *Anaplasma marginale* in domestic animals in Erbil, Iraq. Iraqi Journal of Veterinary Sciences. 2012;26:109-14.
38. Birdane FM, Sevinc F, Derinbay O. *Anaplasma Marginale* infection in dairy cattle: Clinical disease with high seroplevalence. Bulletin of the Veterinary Institute in Pulawy. 2006;50;467-70.
39. Ministry of Agriculture. A Statistics of Agriculture. 2012; 2.
40. Noaman V, Shayan P. Molecular detection of *Anaplasma phagocytophilum* in carrier cattle of Iran-first documented report. Iranian Journal of Microbiology. 2009;1:37-42.
41. Hoar BR, Nieto NC, Rhodes DM, Foley JE. Evaluation of sequential co- infection with *Anaplasma phagocytophilum* and *Anaplasma marginale* in cattle. American Journal of Veterinary Research. 2008;69:1171-8.
42. Wen B, Jian R, Zhang Y, Chen R. Simultaneous detection of *Anaplasma marginale* and a new *Ehrlichia* species closely related to *Ehrlichia chaffeensis* by sequence analyses of 16S ribosomal DNA in boophilus microplus ticks from Tibet. Journal of Clinical Microbiology. 2002;40:3286-90.
43. Hofmann-Lehmann R, Meli ML, Dreher UM, Gonczi E, Deplazes P, Braun U, et al. Concurrent infections with vector-borne pathogens associated with fatal hemolytic anemia in a cattle herd in Switzerland. Journal of Clinical Microbiology. 2004; 42:3775-80.
44. Chahan B, Jian Z, Xuan X, Sato Y, Kabeya H, Tuchiya K, et al. Serological evidence of infection of *Anaplasma* and *Ehrlichia* in domestic animals in Xinjiang Uygur Autonomous Region area, China. Veterinary Parasitology. 2005;134:273-8.
45. Kawahara M, Rikihisa K, Lin Q, Isogai E, Tahara K, Itagaki A, et al. Novel genetic variants of *Anaplasma phagocytophilum*, *Anaplasma bovis*, *Anaplasma centrale*, and a novel *Ehrlichia* sp. in wild deer and ticks on two major islands in Japan. Applied and

Environmental Microbiology. 2006;72:1102-9.
46. Jilintai N, Seino D, Hayakawa M, Suzuki H, Hata S, Kondo K, et al. Molecular survey for *Anaplasma bovis* and *Anaplasma phagocytophilum* infection in cattle in a pastureland where sika deer appear in Hokkaido Japan. Japanese Journal of infectious diseases. 2009;62:73-5.
47. Mtshali MS, De Waal DT, Mbati PA. A sero-epidemiological survey of blood parasites in cattle in the north-eastern Free State, South Africa. Onderstepoort Journal of Veterinary Research. 2004;71:67-75.
48. Aktas M, Altay K, Dumanli N, Kalkan A. Molecular detection and identification of *Ehrlichia* and *Anaplasma* species in ixodid ticks. Parasitology Research. 2009;104: 1243-8.
49. Bazargani TT, Rahbari S, Nadalian M, Ghodsian I. A report of some cases of *Anaplasmosis* in cattle and goats. Journal Faculty of Veterinary Medicine University of Tehran. 1985;40:95-105.
50. Delpy L. Note on *ixodidae* hyalomma (Koch). Annels Parasitology. 1936;14:206-45.
51. Delpy L. The especes iranienes gender *Haemaphysalis* Koch. Annalles de Parasitologie Humaineet Comparee. 1938;16:1-10.
52. Delpy RLP. Agentsen Iran dans le sang des animaux domestiques. Bulletin of the Exotic Pathology. 1963;29:157-61.
53. Abbasian-Lintzen R. A preliminary list of ticks (*Acarina*: *Ixodidae*) occurring in Iran and their distributional data. Acarologia. 1960;2:43-61.
54. Abbasian-Lintzen R. Records of tick (*Acari*na: *Ixodidae*) occurring in Iran and their distributional data. Acarologia. 1961;3:546-59.
55. Mazlum Z. Tick species of Iran its distribution, host and seasonal activity. Journal of Faculty of Veterinary Medicine. University of Tehran. 1972;72:1-28 (In Persian, with English Abstract).
56. Mazlum Z. Hyalomma asiaticum asiaticum. (Schulze and Schlottke) 1929: Its distribution, hosts, seasonal activity life cycle and role in transmission of bovine theileriosis in Iran. Acarologia. 1968;10:437-42.
57. Mazlum Z. Ticks of domestic animals in Iran: Geographic distribution host relation and seasonal activity. Journal Faculty of Veterinary Medicine University of Tehran. 1971;27:1-32.
58. Potgieter FT, Stoltsz WH. Bovine *Anaplasmosis*. Livestock I Do, editor. Southern Africa Cape Town: Oxford University Press; 2004.
59. Ndung'u LW, Aguirre C, Rurangirwa FR, McElwain TF, McGuire TC, Knowles DP, et al. Detection of *Anaplasma ovis* infection in goats by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. Journal of Clinical Microbiology. 1995; 33:675-9.
60. Knowles DP, Torioni de Echaide S, Palmer GH, McGuire TC, Stiller D, McElwain TF. Antibody against an *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. Journal of Clinical Microbiology. 1996; 34:2225-30.
61. Scoles GA, Goff WL, Lysy TJ, Lewis GS, Knowles DP. Validation of an *Anaplasma marginale* cELISA for use in the diagnosis of *A. ovis* infections in domestic sheep and *Anaplasma* spp. in wild ungulates. Veterinary Microbiology. 2008;130:184-90.
62. Molad T, Mazuz ML, Fleiderovitz L, Fish L, Savitsky I, Krigel Y, et al. Molecular and serological detection of *A. centrale* and *A. marginale* infected cattle grazing within an endemic area. Veterinary Microbiology. 2006;113:55-62.
63. De La Fuente J, Passos LMF, Van den Bussche RA, Ribeiro MFB, Facury-Filho EJ, Kocan KM. Genetic diversity and molecular phylogeny of Anaplasma strains from Minas Gerais. Brazilian Journal of Veterinary Parasitology. 2004;121:307-16.
64. OIE. Bovine *Anaplasmosis*. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 6 ed. Paris. World Organization for Animal Health. 2008;599-610.
65. Strik NI, Alleman AR, Barbet AF, Sorenson HL, Wamsley HL, Gaschen FP, et al. Characterization of *A. phagocytophilum* major surface proteins 5 and the extent of its cross-reactivity with *A. marginale*. Clinical and Vaccine Immunology. 2007;14:262-8.
66. Visser ES, McGuire TC, Palmer GH, Davis WC, Shkap V, Pipano E, et al. The *Anaplasma marginale* msp 5 gene encodes a 19-kilodalton protein conserved in all recognized *Anaplasma* species. Infection and Immunity.1992;60:5139-44.

67. McGuire TC, Davis WC, Brassfield AL, McElwain TF, Palmer GH. Identification of *Anaplasma marginale* long-term carrier cattle by detection of serum antibody to isolated MSP-3. Journal of Clinical Microbiology. 1991;29:788-93.
68. Kocan KM, de la Fuente J, Guglielmone AA, Melendez RD. Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. Clinical Microbiology Review. 2003;16:698-712.
69. Brizuela CM, Ortellado CA, Sanabria E, Torres O, Ortigosa D. The safety and efficacy of Australian tick-borne disease vaccine strains in cattle in Paraguay. Veterinary Parasitology. 1998;76:27-41.
70. Berger S. Infectious disease of Iran. Los Angeles, California, USA: GIDEON informatics Inc; 2014. E-Book Series. Available:http://www.Gideononline.com/ebooks/
71. Kocan KM, Blouin EF, Barbet AF. *Anaplasmosis* control. Past present and future. Annals of the New York Academy of Science. 2000;916:501-9.

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