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.

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
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 are 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 then 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 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 Anaplama spp. in sheep and cattle were 6.49% and 7.62% respectively (Table 2). There was no association between Anaplama 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 Anaplama infection in sheep and cattle. The low seroprevalence observed would be due to the low tick vector population in Kurdistan province. Anaplama 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 Anaplamosis 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]. Anaplamosis 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 Anaplamosis [33] thus acting as reservoirs for re-infection in herds hence complicating disease diagnosis and control further [36]. The prevalence of Anaplama 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 Anaplama 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-sorben 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

<table>
<thead>
<tr>
<th>Province</th>
<th>Year</th>
<th>Animal host</th>
<th>Methods</th>
<th>% infection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fars</td>
<td>2004</td>
<td>Goats</td>
<td>PCR*</td>
<td>29</td>
<td>[22]</td>
</tr>
<tr>
<td>Khorasan razavi (Mashhad)</td>
<td>2006</td>
<td>Cattle, sheep, goats</td>
<td>PBS**</td>
<td>19.37,80.3,38.92</td>
<td>[23]</td>
</tr>
<tr>
<td>Isfahan</td>
<td>2009</td>
<td>Cattle</td>
<td>PBS,PCR</td>
<td>50.77</td>
<td>[24]</td>
</tr>
<tr>
<td>Golestan and Khorasan razavi</td>
<td>2009</td>
<td>Goats</td>
<td>PBS,PCR</td>
<td>22.3,63.7</td>
<td>[25]</td>
</tr>
<tr>
<td>Isfahan</td>
<td>2010</td>
<td>Cattle</td>
<td>PCR</td>
<td>2.66</td>
<td>[26]</td>
</tr>
<tr>
<td>Kerman</td>
<td>2011</td>
<td>Cattle</td>
<td>PBS</td>
<td>3</td>
<td>[27]</td>
</tr>
<tr>
<td>Ahvaz</td>
<td>2013</td>
<td>Sheep</td>
<td>PBS,PCR</td>
<td>33.6,87.4</td>
<td>[28]</td>
</tr>
<tr>
<td>Mazandaran (Ghaemshahr)</td>
<td>2014</td>
<td>Cattle, sheep, goats</td>
<td>PCR</td>
<td>22.22,43.08,25</td>
<td>[7]</td>
</tr>
</tbody>
</table>

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

Table 2. Number of infected animals by *Anaplasma*

<table>
<thead>
<tr>
<th>Animal</th>
<th>Animals tested</th>
<th>Seropositive animals (%)</th>
<th>The first age group - seropositive animals (%)</th>
<th>The second age group - seropositive animals (%)</th>
<th>Male seropositive animals (%)</th>
<th>Female seropositive animals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>105</td>
<td>8 (7.62)</td>
<td>4 (7.69)</td>
<td>4 (7.55)</td>
<td>3 (5.77)</td>
<td>5 (9.43)</td>
</tr>
<tr>
<td>sheep</td>
<td>77</td>
<td>5 (6.49)</td>
<td>3 (7.69)</td>
<td>2 (5.26)</td>
<td>2 (5.13)</td>
<td>3 (7.89)</td>
</tr>
</tbody>
</table>

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

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

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