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Original Article

Snapshot Study of the Family Anaplasmataceae, *Anaplasma* spp., and *Ehrlichia* spp. Prevalence in Ticks of Sheep and Cattle in Jiroft City, Iran

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Abstract

Background: *Anaplasma* spp. and *Ehrlichia* spp. are amongst the most important tick-transmitted bacteria that can cause zoonotic disease in various hosts including ruminants and humans.

Methods: In this study, 16srRNA, *EE*, and *dsb* sequences were respectively used to screen Anaplasmataceae family, *Anaplasma* spp., and *Ehrlichia* spp. in tick samples (n= 100) collected from 100 domestic ruminants including 50 sheep and 50 cattle in Jiroft City, southeast of Iran, between June and August 2021.

Results: two genera were predominant among the ticks including *Hyalomma* spp. (64%; 43% from sheep and 21% from cattle) and *Rhipicephalus* spp. (36%; 22% from cattle and 14% from sheep); all ticks were adult and 73% of them were male. DNA of Anaplasmataceae was detected in 17% (17/100) of the ticks collected from cattle (18%; 9/50) and sheep (16%; 8/50). *Anaplasma* spp. was not found in the samples, but two ticks were positive for *Ehrlichia* spp.; all were positive for *Ehrlichia* spp. belonged to the cattle (4%; 2/50).

Conclusion: This study shows that Anaplasmataceae strains are circulating via ticks among domestic ruminants in the study area, emphasizing the need for effective tick control strategies by livestock farmers, health, and veterinary authorities. Surveillance, molecular characterization and further sequencing-based studies are crucial for informed control and prevention efforts.

Keywords: Ruminants; Ehrlichiosis; Anaplasmosis; Molecular detection; Tick-borne disease

Introduction

Ehrlichiosis and anaplasmosis are significant tick-borne zoonotic diseases caused by *Ehrlichia* spp. and *Anaplasma* spp., which are obligate intracellular bacteria from the family Anaplasmataceae; these are known as a non-contagious bacterial disease. The two pathogens are primarily transmitted by ticks such as *Ixodes* spp., *Hyalomma* spp., and *Rhipicephalus* spp. (1). Transmission occurs through transstadial routes but not transovarial routes; thus, each generation of ticks is infected by feeding on the reservoirs. Anaplasmosis and ehrlichiosis may be mechanically transmitted through the bite of flies or blood-contaminated equipment and there are reports of placental transmission from an infected mother to her offspring (2).

The symptoms of ehrlichiosis and anaplasmosis vary based on the bacterial species, the susceptibility of the infected vertebrate hosts, the prevalence of tick vectors, and co-infections (3). Clinical signs in humans are non-specific and include decreased appetite, fever, swollen lymph nodes, depression, weight loss, vomiting, and diarrhea (3). Animals that contract the infection may either recover independently, becoming asymptomatic reservoirs of infection, or develop a chronic illness with signs including loss of appetite, weight loss, pro-

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longed fever, weakness, and bleeding disorders in the animals leading to significant economic losses in developing countries due to its widespread occurrence (4).

Ehrlichiosis and anaplasmosis are endemic in tropical and subtropical regions, affecting various animals (5, 6); therefore, it is recommended to screen for Anaplasmataceae even in healthy hosts. Due to the low levels of bacteria in the blood, standard microscopy techniques struggle to detect infections in carriers. So, molecular assays including polymerase chain reaction (PCR) and sequencing can be considered as more precise techniques for identifying the infections. PCR can also detect pathogen DNA in the acute phase of infection when antibodies are not yet present (7).

Some important species of *Ehrlichia* spp. and *Anaplasma* spp. include *E. canis*, *E. ruminantium*, *E. chaffeensis*, *E. wingii*, *A. marginale*, *A. ovis*, *A. centrale*, *A. phagocytophilum*, and *A. bovis*. These strains affect domestic and wild ruminants, horses, dogs, and humans (farm workers, pet owners, hunters, and people living in the village). Best of our knowledge in Iran, there is little information on the prevalence of *Ehrlichia* spp. and *Anaplasma* spp. in sheep and cattle, in Iran. This research aimed to investigate the presence of *Ehrlichia* spp. and *Anaplasma* spp. in ticks collected from ruminants in Jiroft city, southeast Iran.

Materials and Methods

Sample collection

There were three main criteria for selecting ruminants for the study: (i) The large population of cattle and sheep in Jiroft made it an ideal location for the research. (ii) The animals selected appeared healthy, highlighting the importance of asymptomatic reservoirs in the distribution of pathogens. (iii) The presence of ticks on the ruminants was the main factor in selecting animals for sampling. A total of one hundred ticks were collected from domesticated ruminants including cattle (n= 50) and sheep (n= 50), from five herds in the Jiroft region (city and suburbs), between June and August 2021 (Fig. 1). Tick samples were stored in microtubes containing 70% ethanol and transferred to the veterinary microbiology and parasitology laboratory at Shahid Bahonar University of Kerman.

The sample size was determined by the below formula:

$$Sample\ size = \frac{Z^2_{1-\frac{\alpha}{2}}\, p(1-p)}{d^2} = \frac{3.84 \times 0.3 \times 0.7}{0.0081} = 99.55$$

95% confidence level (based on 0.05 error level or α); Z= 1.96; Z^2= 3.84

Expected proportion based on previous studies (6,8–10) (p)= 30% (0.3); 1-p= 0.7

Error determined by the researchers of this study (d)= 9% (0.09); d^2= 0.0081

DNA extraction from ticks

Tick samples were washed three times with a sterile 0.9% sodium chloride solution to remove ethanol. They were then placed on sterile paper to dry for 10 minutes. The ticks were crushed with a sterile scalpel and transferred to a sterile 1.5 ml microtube, where pre-lysis buffer (100 µl) and proteinase K (30 µl) were added. DNA was extracted using a commercial tissue DNA extraction kit (Sinapur, Iran) according to the manufacturer's instructions. The amount of DNA was measured with a spectrophotometer (NanoDrop, BioTek Epoch, US) at a wavelength of 260/280 nm. The extracted DNA samples were stored at -20 °C.

Molecular screening of Anaplasmataceae, *Ehrlichia* spp. and *Anaplasma* spp.

The DNA templates were evaluated for the presence of Anaplasmataceae, *Anaplasma* spp., and *Ehrlichia* spp. using the primers and procedures (Table 1) outlined by Zaid et al. (11), Han et al. (12), and Soares et al. (13) to identify the 16S rRNA (for Anaplasmataceae), 16S rRNA gene fragment in *Anaplasma* spp.) and dsb (disulfide bond formation protein in *Ehrlichia* spp.) genes, respectively.

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Polymerase chain reaction (PCR) was performed in a volume of 20 µL [2.5 µL of DNA extract, 0.5µL from each primer (for 0.5 µM final concentration), 10 µL of 2X ready-to-use Master Mix (Ampliqon, Odense, Denmark) and distilled RNase-DNase free water up to the reaction volume]. The cycling conditions were: 96 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s. A final step was performed at 72 °C for 10 min. In this study, extracted DNA from positive clinical samples for *Ehrlichia* spp. and *Anaplasma* spp., provided by Dr Mohammad Khalili (Shahid Bahonar University of Kerman), were used as positive controls. Also, distilled water was used as the negative control. PCR products were visualized by electrophoresis (100 V, 45 min on 1.3% agarose gel) and UV trans-illumination.

Results

In the present study, two tick genera were predominant among the collected ticks (n= 100) including *Hyalomma* spp. (64%; 43% from sheep and 21% from cattle) and *Rhipicephalus* spp. (36%; 22% from cattle and 14% from sheep); all ticks were adult and 73% of them were male. Among the 100 tick samples, 17 (17%; 95% CI: 10.2–25.8%) were positive for the family Anaplasmataceae (Fig. 2). These ticks were collected from cattle (9/50, 18%; 95% CI: 8.5–31.4%) and sheep (8/50, 16%; 95% CI: 7.1–29.11%). Two of the 17 samples (11.8%; 95% CI: 1.4–36.4%) were positive for *Ehrlichia* spp. DNA, but none were positive for *Anaplasma* spp. DNA (Fig. 2). The two *Ehrlichia*-positive ticks were collected from cattle (2/50; 4%, 95% CI: 0.4–13.7%).

The frequency of negative samples for Anaplasmataceae was significantly higher than the positives (P< 0.05). Among the Anaplasmataceae-positive samples, the prevalence of negatives for *Ehrlichia* spp. and *Anaplasma* spp. was significantly higher than the positives for *Ehrlichia* spp. (P<0.05). The prevalence of the family Anaplasmataceae and *Ehrlichia* spp. in sheep was not significantly different from the prevalence in cattle (P> 0.05). There was no significant association between tick species and the studied bacterial agent.

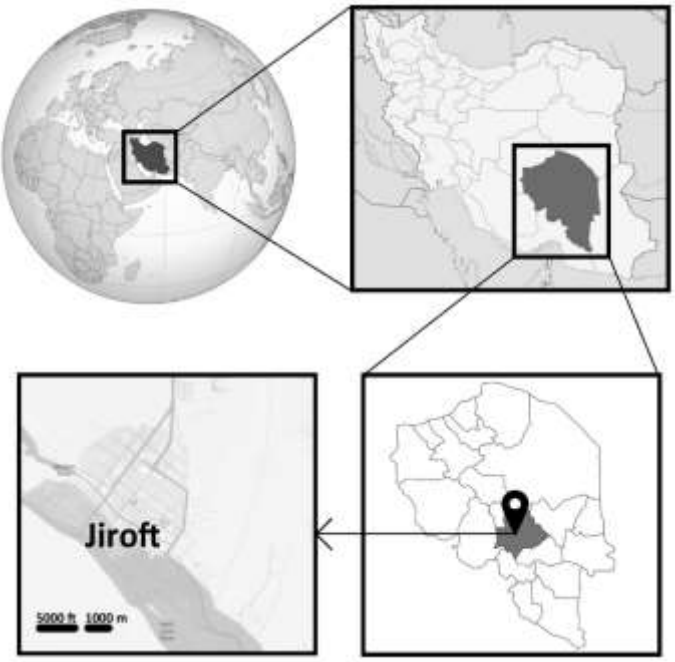

Fig. 1. Location of tick sampling between June and August 2021 in Jiroft City

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Table 1. Sequence of the specific primers used for detection of Anaplasmataceae, *Ehrlichia* spp., and *Anaplasma* spp. in ticks collected in Jiroft City, June- August 2021

Gene name	Target	Primer name (Ref.)	Primer sequence (5′–3′)	PCR product size (bp)
16s rRNA	Anaplasmataceae	EHR16SR	F- GGTACCYACAGAAGAAGTCC	345
		EHR16SD (11)	R- TAGCACTCATCGTTTACAGC	
16s rRNA	*Anaplasma* spp.	EE1	F-TCCTGGCTCAGAACGAACGCTGGCGGC	1400
		EE2 (12)	R-AGTCACTGACCCAACCTTAAATGGCTG	
dsb (disulfide oxi-doreductase)	*Ehrlichia* spp.	dsb-330	F-GATGATGTTTGAAGATATSAAACAAAT	401
		dsb-720 (13)	R-CTATTTTACTTCTTAAAGTTGATAWATC	
		dsb-380	F-ATTTTTAGRGATTTTCCAATACTTGG	349
		dsb-720 (13)	R-CTATTTTACTTCTTAAAGTTGATAWATC	

F: forward; R: reverse

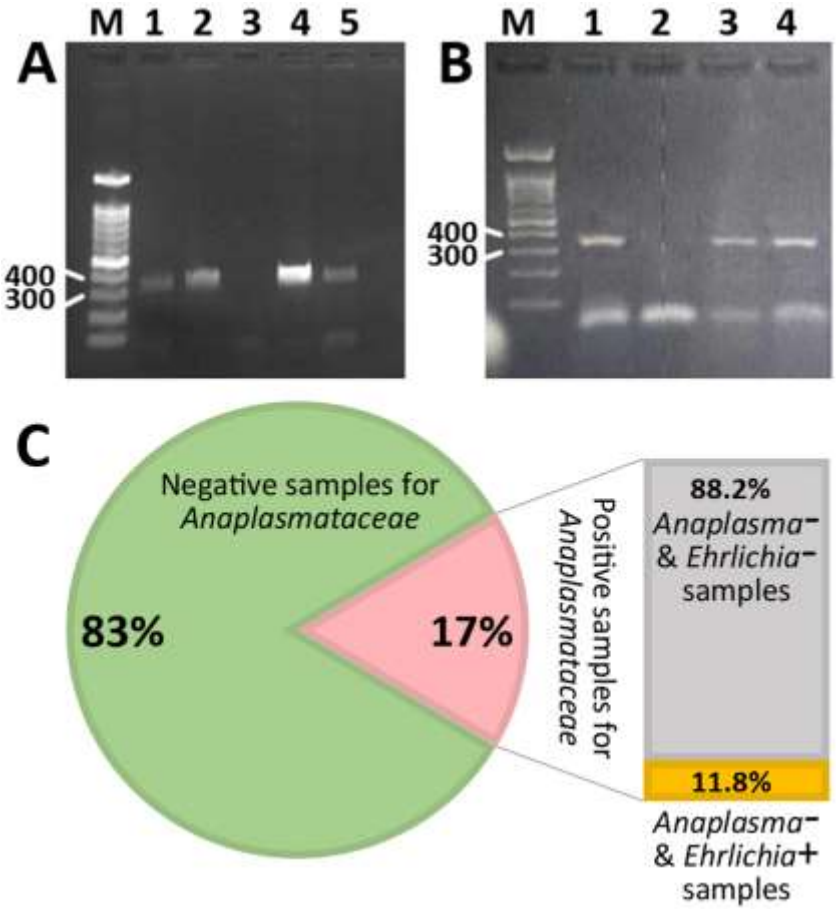

Fig. 2. Detection of Anaplasmataceae, *Ehrlichia* spp., and *Anaplasma* spp. in ticks collected in Jiroft City between June and August 2021. **A:** agarose gel electrophoresis image of the 16s RNA PCR products (345 bp), M, Marker [100 bp ladder (Cinna Clone; Iran)]; 1, 2, and 5, positive samples; 3, negative control (distilled water); 4, positive control (extracted DNA of positive clinical samples for Anaplasmataceae provided by Shahid Bahonar University of Kerman). **B:** dsb gene PCR products (349 or 401 bp), M, Marker (100 bp ladder); 1, positive control (extracted DNA of positive clinical samples for *Ehrlichia* spp. provided by Shahid Bahonar University of Kerman); 2, negative control (distilled water); 3 and 4, positive samples. **C:** Prevalence rate of the family Anaplasmataceae, *Anaplasma* spp., and *Ehrlichia* spp. in the ticks studied

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Discussion

In the present study, *Hyalomma* spp. accounted for 64% of the ticks (43% from sheep and 21% from cattle), while *Rhipicephalus* spp. constituted 36% (22% from cattle and 14% from sheep); there was no significant association between tick species and the studied bacterial agents. These results differ significantly from the findings in Thailand (14), where *Rhipicephalus* spp. dominated the tick population (93.18%) in cattle, with *Haemaphysalis* spp. making up the remaining 6.82%. Research in Iraq (15) reported the predominance of both *Rhipicephalus* spp. and *Hyalomma* spp. in ticks from sheep. In France (16) *Rhipicephalus* spp. and *Hyalomma* spp. were detected from cattle with the frequencies 84.5% and 15.5%, respectively. Furthermore, in Iran (9) a different distribution was found for *Rhipicephalus* spp. (41%), *Hyalomma* spp. (29%), and *Dermacentor* spp. (30%) on various ruminants. These differences suggest that environmental factors, host availability, and regional ecology play crucial roles in shaping tick species distribution across various geographic regions.

In this research, the frequency of Anaplasmataceae among the ticks collected from cattle and sheep was on average 17%. Of these, 52.9% (9/17 ticks) were from cattle and 47.1% (8/17 ticks) from sheep. None of the ticks were positive for *Anaplasma* spp., but two were positive for *Ehrlichia* spp.; all positive ticks for *Ehrlichia* belonged to cattle. Ranjbar et al. (17) found that approximately one-fourth of ovine ticks in Kerman province were positive for *Anaplasma* spp. which is higher than the present study. Absence of *Anaplasma* spp. in the samples but the presence of *Ehrlichia* spp. underscores the complexity and diversity of tick-borne pathogens in different ecological settings (18).

Other studies in Iran revealed varying prevalence rates for these bacteria; Mohammadian et al. (19) reported a higher prevalence of *Anaplasma* spp. in cattle compared to sheep. In a study conducted by Choubdar et al. (5), *Anaplasma* spp. (89.9%) and *Ehrlichia* spp. (9.1%) were found in *Hyalomma* spp. on the Iran-Pakistan border. Yousefi et al. (8) detected *A. ovis* in over one-fifth of sheep and goats in Iran, while Jafar Bekloo et al. (9) identified the DNA of *Anaplasma* and/or *Ehrlichia* spp. in approximately 5% of ticks isolated from ruminants. Noaman and Bastani (10) reported a low frequency of *Anaplasma* spp. in sheep and cattle. Hosseini-Vasoukolaei et al. (6) detected *A. ovis* from 8% of tick samples, 2.5% of human blood, and 5.1% of sheep blood samples.

Some studies in Asian countries show similar prevalence rates to the present findings; Galay et al. (20) found the frequency of *Anaplasmataceae* and *Ehrlichia* spp. to be approximately 16% and 2%, respectively which aligns with the results of this study. In Iraq, the prevalence of *Anaplasma* spp. is similar to present findings (15). Thinnabut et al. (14) conducted a study in northeastern Thailand, detecting both *Anaplasma* spp. (21.7%) and *Ehrlichia* spp. (7.2%) in beef cattle ticks; they reported a significant genetic diversity of tick populations, which could contribute to the differences in prevalence rates observed across different studies. Yan et al. (21) detected *Anaplasma* spp. (23%) closely related to *A. phagocytophilum* in small ruminants from China, showing significant regional variations in pathogen prevalence and species diversity. Their results support the region-specific distribution pattern of pathogens, highlighting the need for localized studies to understand the epidemiology of tick-borne diseases.

However, studies in African and South American countries generally show higher prevalence rates (22–26), while European studies report lower prevalence rates (16, 27). Abdel-Shafy et al. (7) in Egypt identified *Anaplasmataceae* in 25% of cattle and 26.3% of sheep, (Table 2). Matos et al. (28) in Mozam-

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bique, found *A. marginale* and *E. ruminantium* in beef cattle with varying prevalence rates (9–77%) and reported a higher prevalence of *Anaplasma* spp. (76.5%) compared to the present study. Similarly, Silva et al. (29) in Brazil, detected *Anaplasma* spp. in sheep (2.9%) and goats (17.4%) indicating a widespread presence of these pathogens in various types of livestock across different continents., which could be attributed to differences in environmental factors, tick species, and host immunity.

Differences in prevalence rates across studies may be due to variations in tick control strategies, climatic conditions affecting tick growth, farm management programs, and animal husbandry practices**.** Anaplasmosis is endemic in many regions of Iran, with infection rates increasing in spring and summer (30). Additionally, different detection protocols can affect results; for instance, higher prevalence rates are often found in blood samples compared to tick samples possibly due to the difficulty in DNA extraction from ticks. The family Anaplasmataceae includes other genera such as *Neorickettsia* and *Wolbachia* which may explain why some Anaplasmataceae-positive samples were negative for *Anaplasma* and *Ehrlichia* spp.

Conclusion

The family Anaplasmataceae is potentially circulating among ruminants via ticks in the Jiroft region. Therefore, livestock farmers, health, and veterinary authorities must adopt appropriate strategies for tick control. The findings contribute to evidence on the distribution of Anaplasmataceae in different regions. The observed variations in prevalence rates and species diversity underscore the importance of continuous surveillance and molecular characterization of these pathogens to inform effective control and prevention strategies. So, further molecular and sequencing-based studies are recommended in Iran to investigate the Anaplasmataceae pathogens in various hosts.

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Ethical considerations

Ethical approval does not apply to this study.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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