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A survey of ixodid ticks feeding on cattle and molecular detection of *Coxiella burnetii* from ticks in Southeast Iran

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Abstract: Ticks are the main hematophagous arthropods and obligatory ectoparasites that are considered a vector of serious pathogens for animals and humans. The aims of the present survey were to assess the prevalence and diversity of ticks in cattle in Southeast Iran and to determine *Coxiella burnetii* in the ticks. From May 2014 to April 2015, 583 ticks were collected randomly from 257 cattle. Nested trans-PCR was performed to detect *C. burnetii*. The overall prevalence of hard tick infestation on cattle was 56.8%. In total, seven different species were identified: *Rhipicephalus sanguineus* sensu lato (32.6%), *Hyalomma anatolicum* (28.8%), *Hyalomma excavatum* (19%), *Hyalomma dromedarii* (11.9%), *Rhipicephalus bursa* (2.9%), *Hyalomma asiaticum* (2.7%), and *Hyalomma marginatum* (2.1%). Four out of 83 tick pools consisting of *H. anatolicum*, *H. excavatum*, and *R. sanguineus* sensu lato tested positive for *C. burnetii* in nested trans-PCR assay. This study is the first report of *C. burnetii* in ticks infesting cattle by nested trans-PCR assay and shows their role as putative vectors and reservoirs for this pathogenic agent in Iran.

Key words: Tick, *Coxiella burnetii*, cattle, nested trans-PCR, Iran

1. Introduction

Ticks (Arachnida; Acari; Parasitiformes) are economically the most important external parasite and are harmful for the host during blood feeding. Mammals, birds, reptiles, and amphibians are hosts for different stages of ticks. It has been estimated that about 80% of the world cattle population is infested with ticks (1). Ticks have a variety of direct and indirect effects on their hosts. Tick infestations can cause considerable irritation in animals and can lead to severe disorders, such as blood loss, general stress, damages to hide and skins, tick paralysis, and tick toxicosis (2,3). Several tick species are vectors of some microorganisms that cause theileriosis, babesiosis, anaplasmosis, Lyme disease, ehrlichiosis, Rocky Mountain spotted fever, tularemia, Q fever, Crimean–Congo hemorrhagic fever, and relapsing fever (4–8). *C. burnetii* is the etiological agent of Q fever and is transmitted by more than 40 tick species (9). This microorganism is an obligate intracellular parasite prevalent in large areas of world except New Zealand (4). Q fever cases have been reported from some countries neighboring Iran, such as Iraq in 2003 (10), Afghanistan in 2006 (11), and Turkey in 2010 (12). In nature, ticks can transmit *C. burnetii* not only horizontally, via feces or saliva, but also transstadially and transovarially (13). Q fever is a zoonotic disease that can manifest in both acute and chronic forms in humans (5). Cattle, sheep, and goats are considered the main reservoirs for *C. burnetii*, but some other mammals, birds, and arthropods, mainly ticks, have also been implicated in human disease/infection (5). *C. burnetii* has been detected in a variety of tick species, such as *Ixodes ricinus*, *Dermacentor reticulatus*, *D. marginatus*, *Haemaphysalis concinna*, and *H. inermis* in Slovakia (14); *R. sanguineus* in Switzerland (15); and *Hyalomma* spp. on the Greek island of Cephalonia (9). In Iran, two species of tick (*H. anatolicum* and *R. sanguineus*) are proven vectors for the agent (5). Therefore, when studying the tick infestation rate of livestock, investigating the tick fauna and tick-borne diseases seems to be necessary. Thus, this study was designed in Sistan and Baluchestan, southeastern Iran, to assess the diversity, seasonal variation, and frequency of ixodid ticks on cattle, using polymerase chain reaction (PCR) for the detection of *C. burnetii* in the collected ticks.

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2. Materials and methods

2.1. Field study area

The present study was performed in Sistan and Baluchestan in southeastern Iran. It is located at 25°3′N–28°31′N and 58°48′E–63°19′E. This province is surrounded by Khorasan province and Afghanistan to the north, Pakistan and Afghanistan to the east, the Sea of Oman to the south, and Kerman and Hormozgan provinces to the west. The province has four distinct seasons: winter (January–March), spring (March–June), summer (July–September), and autumn (October–December). The average elevation of Sistan and Baluchestan is between 475 and 500 m above sea level and the average humidity is approximately 40%. The mean maximum and minimum temperature of the area is 40 °C and below 0 °C, respectively, and the average annual rainfall in the province is 120 mm.

2.2. Sample size

In this study, a total number of 257 cattle (132 female and 125 male) of different age groups (less than 1 year, 1–2 years old, 2–3 years old, and over 3 years old) were selected by stratified random sampling over the course of 1 year (May 2014 to April 2015). The examined cattle were raised under traditional husbandry practices (grazing on pastures during the day) without regular acaricide treatment. A total of 583 ticks were collected from cattle. Data for all specimens, including date, sex, age, and number of ticks, were recorded. All of the methods used in this study were confirmed by the Ethics Committee of Shahid Bahonar University of Kerman, respecting currently accepted animal welfare rules in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008.

2.3. Parasitological procedures

To reduce false positives, only unfed ticks were examined (ticks may test positive for *C. burnetii* due to blood from infected cattle). Ticks were removed from the host with rubbing alcohol pads surrounding the skin and blunt pointed forceps, avoiding damage to the mouthparts of the ticks and the skin of host. The collected specimens were transferred into holding tubes contain 70% ethanol (Merck, Darmstadt, Germany) and transferred to the Parasitology Research Laboratory of Shahid Bahonar University, Kerman, Iran. Following examinations under a stereomicroscope, ticks were identified by morphological characteristics using the key identification guide (16).

2.4. DNA extraction

Prior to DNA extraction, ticks were repeatedly washed with 70% ethanol and air-dried for 10 min on sterile paper. Later, ticks were divided into 83 pools of 5–8 ticks each, according to species, sex, and locality. Then the numbered tick groups were placed in aluminum foil and frozen in liquid nitrogen (–196 °C). Frozen ticks were triturated thoroughly in a mortar. The genomic DNA extraction of *C. burnetii* was performed using a Genomic DNA Purification Kit (QIAGEN, Hilden, Germany). The DNA extracts were stored at –20 °C until amplification.

2.5. Nested trans-PCR

The primers Trans 1, Trans 2, and 261 F-463 R targeting the IS1111 fragment, a transposon-like repetitive region in *C. burnetii,* were obtained from the literature (17). The amplification of the nested trans-PCR was performed in a reaction volume of 25 µL and based on the PCR protocol of Parisi et al. (17), then run in the MG thermal cycler (Bio-Rad, Hercules, CA, USA). The amplicons were analyzed on 1.2% agarose gel in 0.5X TBE buffer and visualized using ethidium bromide and a UV illuminator.

2.6. Standard strain of *C. burnetii*

Phenol-killed, purified, and lyophilized cells of the *C. burnetii* Nine Mile phase I strain (RSA 493) were used for the positive control. Negative control reactions contained distilled water instead of template DNA.

2.7. Statistical analysis

For data analysis, descriptive statistics for qualitative data with 95% confidence intervals (95% CI) were used and logistic regression was used to determine the effect of mentioned risk indicators (age, sex, and season) on the prevalence of infection. Data were analyzed using Stata, version 11.2 (College Station, TX, USA).

3. Results

This research revealed that 56.8% (95% CI: 50.5–63.0) of cattle were infested by seven species of tick. A total of 583 ticks (279 female and 304 male) were collected from the examined cattle (Table 1). Tick infestation was significantly higher in females at 62.8% (83/132) than in males at 50.4% (63/125) ($P < 0.04$). In the examined cattle, the highest rate of hard tick infestation, 82.5% (66/80), was observed in the >3 years old age group ($P < 0.00$). The highest seasonal frequency occurred in the spring 86% (80/93), followed by summer 72.2% (39/54), while in the autumn and winter, rates dropped to 40% (20/50) and 11.6% (7/60), respectively (Table 2). Nested trans-PCR revealed that four out of 83 tick pools, which consisted of seven *H. anatolicum* in two pools and six *H. excavatum* and five *R. sanguineus* s. l. in the other two pools, respectively, were infected with *C. burnetii* (Figure).

4. Discussion

As the results show, more than half of the cattle were infested with ticks. There are publications on the prevalence of tick infestation in cattle in different parts of Iran and other countries, including 32.49%, 75.8%, and

Table 1. The prevalence of tick species in examined cattle in Sistan and Baluchestan, Iran.

Tick species	No. of male ticks	No. of female ticks	Total	(%)
Rhipicephalus sanguineus sensu lato	102	88	190	32.6
Hyalomma anatolicum	87	81	168	28.8
Hyalomma excavatum	58	53	111	19
Hyalomma dromedarii	37	32	69	11.9
Rhipicephalus bursa	10	7	17	2.9
Hyalomma asiaticum	5	11	16	2.7
Hyalomma marginatum	5	7	12	2.1
Total	304	279	583	100.0

Table 2. Prevalence of ixodid tick infestation according to the season, age, and sex of examined cattle in Sistan and Baluchestan, Iran.

Season	Number of animals	Number of infested animals	Prevalence (n/N) (%)	Age (years) (%)				Sex (%)	
				<1 N = 54	1–2 N = 58	2–3 N = 65	>3 N = 80	M N = 125	F N = 135
Spring	93	80	86	9 (16.6)	14 (24.1)	14 (21.5)	43 (53.7)	35 (28)	45 (33.3)
Summer	54	39	72.2	3 (5.5)	9 (15.5)	10 (15.3)	17 (21.2)	18 (14.4)	21 (15.5)
Autumn	50	20	40	5 (9.2)	3 (5.1)	6 (9.2)	6 (7.5)	8 (6.4)	12 (8.8)
Winter	60	7	11.6	4 (7.4)	2 (3.4)	1 (1.5)	-	2 (1.6)	5 (3.7)
Total	257	146	56.8	21 (38.8)	28 (48.2)	31 (47.6)	66 (82.5)	63 (50.4)	83 (62.8)

F, Female; M, male; n, animals infested with ticks; N, total animals examined.

24.63% in the Sari, Golestan, and Kermanshah regions of Iran, respectively, and 86.1%, 72.9%, and 29.6% in Ethiopia, Pakistan, and Turkey, respectively (3–22). In this investigation, the frequency of tick infestation was higher than that of two recent studies carried out in Turkey, where 34% and 36.90% of cattle were infested with at least one tick species (23,24). The variation in the prevalence of tick infestation might be due to geographical distribution, climate condition, and management systems (20). Furthermore, the methods and some other factors used in the field study could also affect the results. In Iran, 16 species from the family Ixodidae have been reported in different parts of the country (25). In the current study, *R. sanguineus* s. l. was the major tick that infested cattle, but the results from other research on ixodid ticks revealed that the genus *Hyalomma* was predominant in Iran, which was not consistent with our results (26).

The most probable reasons for this difference may be various factors that influence both tick and host populations, geographical location, climate condition, and temperature (3).

The present investigation demonstrated that tick infestation was significantly higher in female cattle than in male cattle ($P < 0.04$). In Egypt and northwest Ethiopia, Asmaa et al. (27) and Werede and Afera (22) reported higher tick infestation rates in female cattle than in male cattle. The difference in the infestation rate may be due to the fact that higher levels of prolactin, progesterone hormones, and stresses such as pregnancy and lactation play some role in predisposing female cattle to tick infestations. The results of this study showed that the highest number of hard ticks was recorded from cattle older than 3 years. Sohrabi et al. (20) recorded the maximum number of ticks in cattle older than 3 years in Kermanshah province, Iran. Their findings were similar to our results. There is variation in the rate of tick infestation between age groups in different records, and this can be justified due to differences in nutrition, hormonal level of the host,

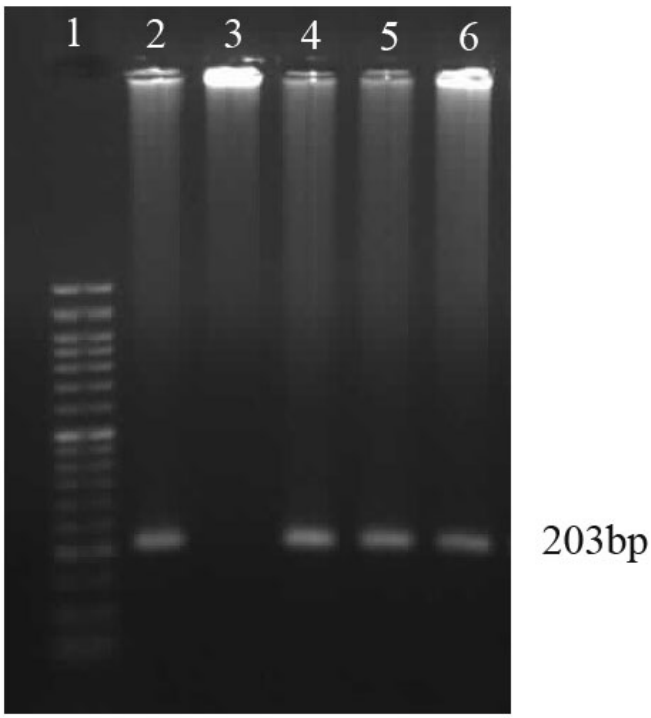

Figure. Detection of *C. burnetii* DNA in ticks found on cattle. The amplified 203-bp product was subjected to electrophoresis in 1.5% agarose gel and stained with ethidium bromide. Lane 1: 50-bp ladder. Lane 2: Reference strain RSA 493 *C. burnetii.* Lane 3: Nontemplate control (NTC). Lanes 4–6: Positive samples.

and management. In the current investigation, hard ticks were more prevalent during spring than other seasons, while the fewest were observed in winter. The results of our study are similar to the findings by Sofizadeh et al. (21). In contrast, Yakhchali and Hosseine (28) reported higher tick prevalences in winter and lower prevalences in summer seasons. It is an established fact that climate condition and temperature affect tick prevalence (3).

Coxiella burnetii should be considered a public health problem. In the enzootic cycle, ticks and vertebrates are important components. Molecular tools were more sensitive and specific than serological techniques in detecting *C. burnetii* infection in ticks (29). The current study confirmed the presence of *C. burnetii* in *H. anatolicum*, *H. excavatum*, and *R. sanguineus* s. l. ticks via nested trans-PCR. The results of our study are similar to studies conducted by Nourollahi Fard and Khalili (5) in Iran and Capin et al. (29) in Turkey. In those studies, using trans-PCR and PCR-RFLP, respectively, *C. burnetii* positivity was reported in *H. anatolicum*, *R. sanguineus*, *R. turanicus*, *R. bursa*, and *H. excavatum*. This study is the first study on the presence of *C. burnetii* in ticks infesting cattle in Iran.

The results of this study give an overview of data on the species composition and distribution of ticks infesting cattle, as well as the presence of *C. burnetii* in these ticks in southeastern Iran. Consequently, further studies should be considered to evaluate the effect of ticks on public health and the role of ticks in the epidemiology of Q fever, and to prepare educational programs for the prevention and control of ticks.

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