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Tick diversity and detection of *Coxiella burnetii* in tick of small ruminants using nested Trans PCR in Southeast Iran

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Abstract. Ticks are obligatory bloodsucking arthropods, and probably the most harmful ectoparasites that may cause some tick born diseases. The main objective of this study was to determine the prevalence, diversity and seasonal distribution of ticks and using a nested Trans PCR to identify *Coxiella burnetii* in ticks collected from small ruminants in Sistan and Balouchestan province, southeast Iran. A total 1305 ticks were collected from 272 Sheep and 253 Goats during May 2014 to April 2015. Prevalence of ixodid tick infestation in small ruminants was 58.4%. Of all examined ticks, nine tick species were identified as follow: *Hyalomma anatolicum anatolicum* (30.3%), *Rhipicephalus sanguineus* (21%), *Hyalomma anatolicum excavatum* (19%), *Rhipicephalus turanicus* (9%), *Rhipicephalus bursa* (6.7%), *Hyalomma detritum* (4.7%), *Hyalomma dromedarii* (4.4%), *Hyalomma asiaticum asiaticum* (4.4%) and *Hyalomma marginatum* (0.5%). The nested Trans PCR examination of ixodid ticks revealed that *Hyalomma anatolicum anatolicum* and *Rhipicephalus sanguineus* were infected with *C. burnetii*. The results of the present study revealed that ixodid ticks infestation was widespread and shows their role as putative vectors and reservoirs for this pathogenic agent in southeast Iran. Hence; Q fever should be considered a significant public health threat in this region.

INTRODUCTION

Ticks are obligatory bloodsucking arthropods and probably one of the most harmful ectoparasites of mammals, birds, reptiles and amphibians. Approximately 10% of all known tick species, sheltering on variety of animals and feeding on their blood (Jongejan & Uilenberg 2004). Some human diseases of current interest in the world caused by tick-borne pathogens include Lyme disease, Rocky Mountain spotted fever, Q fever, Crimean-Congo hemorrhagic fever and tick-borne relapsing fever (Parola and Raoult 2001). *Coxiella burnetii* the etiological agent of Q fever is transmitted by ticks not only through their feces or saliva but also transstadially and transovarially (Capin *et al*., 2013). Q fever occurs worldwide except in New Zealand and infection in nature is identified in over 40 tick species (Nourollahi Fard & Khalili 2011). Cattle, sheep and goats are the most important recognized reservoirs of *C. burnetii*; they are usually asymptomatic however, abortion may occur in these animals (Capin *et al*., 2013). Recently, several investigators have reported the occurrence of *C. burnetii* in countries neighboring Iran, including Oman (Scrimgeour *et al*., 2003), Iraq (Leung-Shea

and Danaher 2006), Afghanistan (Bailey *et al*., 2011), United Arab Emirates (Lloyd *et al*., 2010), Turkey (Karabay *et al*., 2011). Currently Polymerase Chain Reaction (PCR) techniques are reported to be sensitive and specific for the detection of *C. burnetii* from ticks compared with serology methods (Capin *et al*., 2013). Although there have been a number of studies of the prevalence of ticks and Q fever in Iran over the past 50 years, there are still gaps in our information about the presence of *C. burnetii* in tick species. Therefore, this study was conducted to determine the tick diversity, seasonal variation activity of ixodid ticks and using nested Trans PCR for detection of *C. burnetii* on small ruminants' ticks in the region of Iran.

MATERIALS AND METHODS

Study design

Sistan and Balouchestan province lies on the southeast Iran. This province lies on the border with both Afghanistan and Pakistan. It has a hot and dry climate and moderately dry in the winter. During May 2014 to April 2015, a total 1305 ticks were collected from 272 Sheep and 253 Goats in four different age groups (less than 1 year, 1-2 years old, 2-3 years old and over 3 years old). The hard ticks were placed into labeled glass vials with 70% ethanol (Merck, Germany) and ticks were identified using standard keys (Walker *et al*., 2003).

DNA extraction

Firstly, for DNA extraction the collected ticks were grouped in to 95 pools with 12-18 ticks according to species. The genomic DNA extraction of *C. burnetii* was performed by using Genomic DNA Purification Kit (Fermentas, Germany).

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 this study.

Nested Trans-PCR

For nested Trans PCR assay, two pair primers Trans 1 – Trans 2 and 261 F – 463 R were designed based on a repetitive, transposon-like element (Trans-PCR) as previously described (Berri *et al*., 2000; Parisi *et al*., 2006). The amplification of the nested Trans PCR performed in reaction volume of 25 µl and based on PCR protocol of Berri *et al*. (2000) and Parisi *et al*. (2006) then, run in the MG thermal cycler (Biorad, USA). The amplicons were analyzed on 1.2% agarose gel in 0.5 times TBE buffer and visualized using ethidium bromide and UV-eluminator.

Statistical analysis

For analysis of data, descriptive statistics for qualitative data with 95% confidence interval (95% CI) was used and Logistic regression was used to determine the effect of mentioned risk indicators on the prevalence of infection. P value less than 0.05 was considered as statistical significant. Data were analyzed using Stata, version 11.2.

RESULTS

During the study a total number of 1305 ticks were collected and identified at the species level. The results of this investigation revealed that 58.4% (307/525) of small ruminants were infested with ticks. Of the 272 sheep and 253 goats examined, 168 (61.7%) and 139 (54.9%) were infested with one or more genera of ticks, respectively. Of all examined ticks, nine tick species were identified as follow: *Hyalomma anatolicum anatolicum* (30.3%), *Rhipicephalus sanguineus* (21%), *Hyalomma anatolicum excavatum* (19%), *Rhipicephalus turanicus* (9%), *Rhipicephalus bursa* (6.7%), *Hyalomma detritum* (4.7%), *Hyalomma dromedarii* (4.4%), *Hyalomma asiaticum asiaticum* (4.4%) and *Hyalomma marginatum* (0.5%). Prevalence and statistically significance level of tick infestation in different seasons, sex and age groups in sheep and goats presented in Table 1 and 2. The nested Trans-PCR examination of 1305 ixodid ticks revealed that seven out of 95 pools included *Hyalomma anatolicum anatolicum* and *Rhipicephalus sanguineus* were infected with *C. burnetii*. (Fig. 1).

Table 1. Prevalence of ixodid tick infestation according to the seasonal, age groups and gender of examined sheep in Sistan and Balouchestan, Iran

Examined animals	Season	Number of animal	Number of infested animals	Prevalence (*n/N*) (%)	Age (years) (%)				Gender (%)	
					<1 N= 61	1–2 N=69	2-3 N=70	>3 N=72	M N=129	F N=143
Sheep (272)	Spring	99	86	86.8*	18 (29.5)	35 (50.7)	18 (25.7)	15 (20.8)	35 (27.1)	51 (35.6)
	Summer	61	44	72.1	9 (14.7)	15 (21.7)	9 (12.8)	11 (15.2)	18 (13.9)	26 (18.1)
	Autumn	61	33	54.1	3 (4.9)	11 (15.9)	8 (11.4)	11 (15.2)	15 (11.6)	18 (12.5)
	Winter	51	5	9.8	3 (4.9)	–	2 (2.8)	–	3 (2.3)	2 (1.3)
Total		272	168	61.7	33 (54)	61 (88.4)*	37 (52.8)	37 (51.3)	71 (55)	97 (67.8)*

Notes: M, male; F, female; n, animals infested with ticks; N, total animals examined. (* P<0.05).

Table 2. Prevalence of ixodid tick infestation according to the seasonal, age groups and gender of examined goats in Sistan and Balouchestan, Iran

Examined animals	Season	Number of animal	Number of infested animals	Prevalence (*n/N*) (%)	Age (years) (%)				Gender (%)	
					<1 N= 60	1–2 N=71	2-3 N=61	>3 N=61	M N=118	F N=135
Goats (253)	Spring	89	75	84.2*	15 (25)	40 (56.3)	11 (18)	9 (14.7)	34 (28.8)	41 (30.3)
	Summer	51	34	66.6	9 (15)	11 (15.4)	10 (16.3)	4 (6.5)	12 (10.1)	22 (16.2)
	Autumn	57	26	45.6	5 (8.3)	8 (11.2)	9 (14.7)	4 (6.5)	10 (8.4)	16 (11.8)
	Winter	56	4	7.1	1 (1.6)	–	–	3 (4.9)	3 (2.5)	1 (0.7)
Total		253	139	54.9	30 (50)	59 (83.1)*	30 (49.1)	20 (32.7)	59 (50)	80 (59.2)

Notes: M, male; F, female; n, animals infested with ticks; N, total animals examined. (* P<0.05).

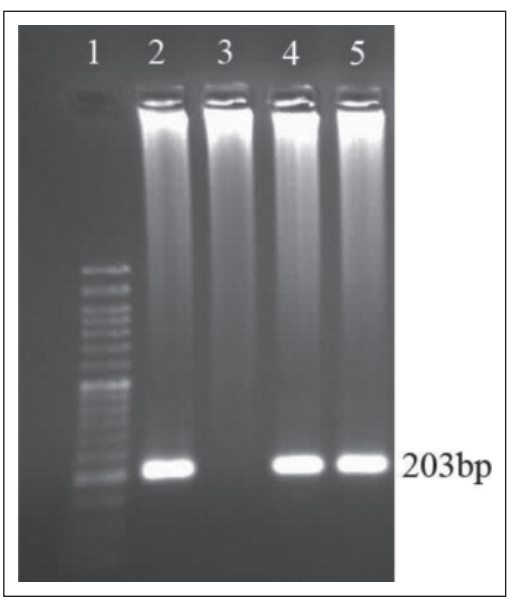

Figure 1. Detection of *C. burnetii* DNA in ticks of Sheep and goats. The amplified 203-bp product was subjected to electrophoresis in 1.5% agarose gel and stained with ethidium bromide. Lane 1: 50-bp ladder, Lane 2: Reference strain RSA 493 *C. burnetii*, Lane 3: Non Template Control (NTC), Lane 4 and 5.

DISCUSSION

In our study, prevalence of ixodid ticks infestation was 58.5%. These results were compatible with previous studies in Iran and other countries. Yakhchali *et al.* (2012) that reported 37.2% prevalence of tick infestation in domestic ruminants in Sanandaj suburb, Iran. Asmaa *et al.* (2014) and Werede and Afera (2014) reported 30.1% and 86.1% prevalence of infestation with tick in Egypt and Ethiopia, respectively. Ticks species identified in current study are similar with ticks species reported by other researchers (Sajid *et al.*, 2009; Nourollahi Fard *et al.*, 2012; Yakhchali *et al.*, 2012; Taddese and Mustefa 2013). The most frequent and predominant tick species seen sheep and goats were *Hyalomma anatolicum anatolicum*. This finding is in disagreement with the observation made by Nabian *et al.* (2007). The difference in prevalence and diversity of tick could be due to the acaricides application, geographical location, climate

condition and temperature (Perret *et al*., 2000; Sajid *et al*., 2009).

In current study tick infestation in female animals was higher than male. The results on goats concurred with the results of Yakhchali *et al*. (2012). Although, the exact cause of higher prevalence of tick infestation in female ruminants cannot be explained but may be due to higher levels of prolactin, progesterone hormones and stresses of production such as pregnancy and lactation that may make the individual more susceptible to any infection (Kabir *et al*., 2011). The results of this study showed that, 1-2 years-old female sheep and goats had highest infestation rate with hard ticks. Our results agre with Sohrabi *et al*. (2013) who reported the maximum infestation in the 1-2 years-old female sheep and goats in Kermanshah province, Iran. It is very difficult to explain exactly the frequent occurrence of tick infestation in younger and older animals but, combination of factors, including some form of innate protection, nutrition, hormonal level of the host and management could be influence on tick infestation (Kabir *et al*., 2011). The highest prevalence of ticks in spring was more than other seasons and the least was observed in winter, which it was in accordance with the previously reported studies (Sofizadeh *et al*., 2014; Ghashghaei *et al*., 2015). Therefore, geographical condition, temperature and altitude very effective on prevalence of ticks in different seasons (Sajid *et al*., 2009).

In this work, molecular findings indicated that *Hyalomma anatolicum anatolicum* and *Rhipicephalus sanguineus* ticks were positive for *C. burnetii* infection. In other studies, *C. burnetii* was reported in *Hyalomma anatolicum anatolicum*, *Rhipicephalus sanguineus*, *Rhipicephalus turanicus*, *Rhipicephalus bursa* and *Hyalomma excavatum* (Nourollahi Fard and Khalili 2011; Capin *et al*., 2013).

In conclusion the finding of the present study gives an update of the prevalence and seasonal diversity of ticks and presence of *C. burnetii* in ticks in the southeast of Iran. Although the results of the present study represent relatively low *C. burnetii* infections in ticks, still there is a potential risk for human infection. The authorities and the public should be alerted to the necessity of controlling the tick's population and its role in human and domestic animals diseases; further studies are needed.

Ethical approval

This article does not contain any studies with human or animals participants performed by any of the authors.

Ethic and conflict of interest statement

The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript. This study had an ethics approval certificate from research council of Shahid Bahonar University with grant number vt1394.

Statement of animal rights

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.

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