INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 12, p. 78-83, 2014

RESEARCH PAPER OPEN ACCESS

Molecular detection of *Theileria ovis* and *T. lestoquardi* in vector ticks in Lorestan province, Iran

Hoghooghi-Rad Nasser[1], Hashemi Saeed [1*], AbdiGoudarzi Mohammad[2]

[1]*Department of Pathobiology, Faculty of Specialized Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Parasitology, Razi Vaccine and Serum Research Institute, Karaj,Iran*

Key words: Lorestan, *Rhipicephalus*, *Hyalomma*, *Theileria ovis, Theileria lestoquardi*.

http://dx.doi.org/10.12692/ijb/4.12.78-83 Article published on June 22, 2014

Abstract

Ixodidae ticks are carriers of pathogenic protozoa such as *Theileria* and *Babesia*. Recognition of these ticks is essential in each area considering treatment strategies and epidemiological studies Therefore, 219 ticks were collected from 150 sheep suffering from fever and anemia in different parts of Lorestan province during April-August 2012. Also, thin blood films were prepared from the peripheral blood of these animals. DNA of the tick salivary glands including 152 *Rhipicephalus sanguineuses*, 13 *R.bursas* and 54 *Hyalomma anatolicum anatolicums* was extracted. Then, PCR was performed using a pair of 520 bp specific primers of SSurRNA gene of *Theileria ovis* for the amplification of 785bp of *T.lestoquardi* merozoite surface antigens. PCR revealed that 37 out of 152 *R. sanguineus* (24/34%) were positive for *T.ovis* whereas none of *R.bursas* was positive. Also, 5 out of 54 *H.a anatolicums* (9.25%) were positive for *T. lestoquardi*. The microscopic examinations of blood smears showed that 19 out of 150 blood smears (12.66%) contained the piroplasmic forms of *Theileria* spcies. Regarding the vast distribution of *R.sanguineus* in the area, it seems that this tick may be the main vector of *T. ovis* in Lorestan.

* **Corresponding Author:** Hashemi Saeed ✉ Saeedhashemi2000@yahoo.com

Introduction

Theileriosis is a tick – borne disease of livestock caused by *Theileria* spp in the tropical and subtropical regions of the world . *Theileria lestoquardi*, *T. uilenbergi* and *T. luwenshuni* are the causative agents of malignant *Theileriosis* , and *Theileria ovis*, *T. separata,* and T. recondita are the causative factors of subclinical *Theileriosis* in small ruminants(Alani and Herbert., 1988; Ahmed *et al*.,2006). Hard ticks of Ixodidae family are vectors of *Theileria spp* that inject parasite's sporozoite to mammalian hosts during blood mealing. Recent studies indicate that in Iran, *T.ovis* and *T.lestoquardi* are transmitted to sheep and goats by the ticks of the genus *Hyalomma* and *Rhipicephalus* (Razmi *et al*.,2003; Telmadarraiy *et al*.,2011). Traditional methods for the detection of *Theileria spp*. in definitive hosts are based on parasite structure, host specificity and transmission ways and in the intermediate hosts, they may depend on the staining of salivary gland by various manners including Methylgreen-puronin, Giemsa and feulgen. These methods are not specific and in some cases where there are resemblance parasites such as *T. annulata* and *T.lestoquardi,* morphological similarity cannot be distinguished from the parasite species (Kirvar *et al*.,1998). Therefore, molecular techniques such as PCR and RFLP are used to identify the hemoparasites in the hosts nowadays because they are more sensitive and specific than the other common methods (Kirvar *et al*.,1998; Aktas *et al*.,2006). Although few studies have been done in this area in north eastern provinces of Iran, similar studies have been conducted on sheep Theileriosis. Maleki, (2000) studied 300 heads of sheep liver slaughterhouse in Khorramabad using Giemsa method and reported 10% incidence of *Theileriosis*. However, molecular studies on vector ticks of *Theileria* in West of Iran are insignificant and especially, the roles of climate in the degree of tick dispersion may be somewhat unclear so that they are more likely to be the main aims of this study.

Materials and methods

The study area

This study looked at samples of blood smear and tick, were collected from sheep flocks of five regions in Lorestan, including Khorammabad(Zaqeh) , Bourujerd (Araban), Dourud (Meidanak and Aziz-abad), Aligodarz (Absharsefid) and Poldokhtar(Chammort). (fig. 1)

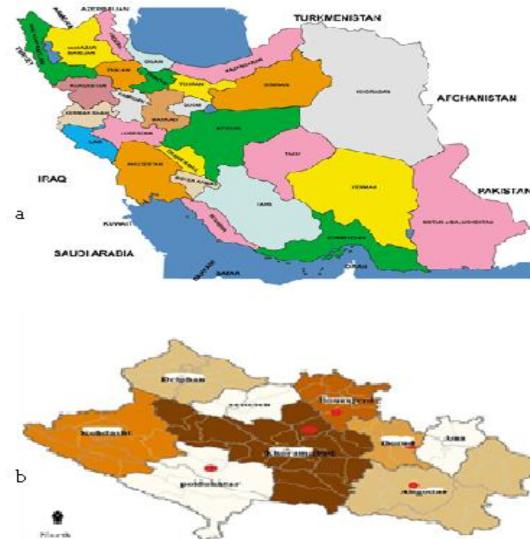

Fig. 1. Lorestan's situation in Iran (a) with close-up (b). The study areas were showed with red points.

Climate conditions of the area under study

Lorestan has various climates and mean temperature, relative humidity and annual precipitation of northern and southern areas vary. Therefore, mean temperature, annual precipitation and relative humidity rates have been shown in Table (1) for the last ten years in Lorestan.

Blood smear and tick sampling

Totally, 219 samples of hard ticks from the ears, groin and surrounding breast and 150 blood smears from the ear vein of sheep which have a history of fever and anemia were collected. Blood smears were fixed on glass slides with methanol. Afterwards, they were stained by 5% Giemsa (acidity of 7.2) for 45 min and then, analyzed by light microscopy regarding piroplasmic forms. Genus and species of tick samples have been detected by the means of standard guide key (Hoogstraal and Wassef.,1985).). From ventral surface, each tick was fixed inside a Petri dish by the use of paraffin, and its scutum was removed by Scissors and Micro sterile scalpel. Then, grapes-like salivary glands were removed and kept in1.5 mL of

Eppendorf at freezing temperature of -20 °C for DNA extraction.

DNA Extraction

In this study, the salivary glands of 152 *Rhipicephalus sanguineus*, 13 *Rhipicephalus bursa* and 54 *Hyalomma anatolicum anatolicum* ticks in separate bonds were used for DNA extraction. This proce using DNA extraction Kit (Cinagene, Iran) Was based on the manufacturer's protocol. 5µl of extracted DNA was analyzed on a 1% agarose gel at 85 V for 45 min and then visualized under UV light after staining with ethidiumbromide. DNA samples to the next steps in, -20 °C were being held.

PCR method

In the present study, a pair of specific primers (TSsr) with the sequences of 170F; 5'-TCGAGACCTTCGGGT-3' and 670R; 5'-TCCGGACATTGTAAAACAAA-3' was used for the amplification of a gene fragment with the size of 520 bp belonging to small subunit ribosomal RNA (ssu rRNA) gene of *T. ovis* along with a pair of specific primers (30 kDa) with the sequences of F; 5'-GTGCCGCAAGTGAGTCA-3' and R; 5'-GGACTGATGAGAAGACGATGAG-3' used to amplify 785 fragments of *T. lestocuardi* merozoite surface gene. Positive control of *T. ovis* and *T. lestocuardi* was done through cell culture and infected sheep blood, respectively.

PCR was done on positive control resulting in a suitable response. PCR reaction had been designed for a volume of 25 µL including 12.5 µl of PCR master mix containing 0.5 µL dNTPs, 0.5 µL Taq DNA polymerase and 1µL $Mgcl_2$, 2µL 10× PCR buffer, 2 µL of each primer, 2 µL template DNA and the final volume with the distilled water.

PCR reactions were observed by the help of thermal cycler (Bio Rad, USA). The cycling program regulated for *T.ovis* includes three steps; in the first, second and third steps, temperature and time are estimated as 96˚c and 3 min, 94 ˚c and 30s along with 60˚c and 30s and 72˚c and 2min for almost 40 cycles and 72˚c and 10 min, respectively. Also, cycling program regulated for *T. lestocuardi* includes temperature calculated as 94˚c for 3 min; in the second step, 94 ˚c for 1min and 60˚c for 1min as well as 72˚c for 1min for almost 40 cycles; and in the last step, 72˚c for 5 min. PCR product was electrophoresed on 1% agarose gel and then, was stained by ethidium bromide and visualized under UV light.

Sequencing

Two PCR products of *T. ovis* and *T. lestocuardi* for sequencing were sent to Takapouzist Company. Gene sequencing results of the present study were compared to genes obtained from the gene bank by the blast.

Results

Microscopic examination

The light microscopic observation of about 150 blood smears which were stained with Giemsa(5%), proved the existence of oval and ring forms of *Theileria spp*. In 19(12.66%) cases of the samples. The highest and lowest prevalence respectively was seen in Aligodarz and Borujerd ($P<0.05$).(table 2).

Table 1. From 2003 to 2012 the cities of Lorestan Weather(The Weather Lorestan 2013).

Decennial mean	Khorammabad	Poldokhtar	Borujerd	Dourud	Aligodarz
Temperature(c°)	17.2	22.8	13.48	16	12.7
Rainfall(mm)	426	354	415	605	609
Relative humidity (%)	54	49	58	55	58

PCR Observations

In the PCR test , using specific primers, existence of 520bp fragment of *T.ovis* genum were indicated in 37 out of 152 *R. sanguineus* ticks(24.34%) including 21/68(30.88%) of female ticks and 16/84(19.04%) male ticks but this parasite, were not showed in other

ticks(fig.2).

In infected *Rhipicephalus* ticks , the highest infection rate was seen in Aligodarz 16(32.65%) and the lowest infection rate was indicated in Borujerd 3(18.75%).(table 3).

DNA was extracted from 54 salivary *Hyalomma a. anatolicum* were examined using primers 30KDa and existence of 785bp fragment of *T. lestocuardi* genum were indicated in 5 out of 54 of theses ticks (9.25%) including 3 (5.55%) of female ticks and 2(3.70%) male ticks (fig 3).These infected ticks were collected from Aligodarz.

Table 2. Microscopic examination of blood smears: Bs : Blood smear.

regions	Bs examined NO	Bs infected to *Theileria spp* NO	Mean (%)
Aligodarz	46	7	15.21
Dourud	34	4	11.76
Borujerd	23	2	8.69
Khorammabad	26	3	11.53
Poldokhtar	21	3	14.28
Total	150	19	12.66

Table 1. Prevalence of 520 bp gene fragment of *T.ovis* in *R. sanguinus* ticks collected of Lorestan sheep.

Sampling area	Ticks		Total
	female	male	
Aligodarz	10/19	6/30	16/49(32.65%)
Dourud	3/12	4/20	7/32(21.87%)
Borujerd	2/10	1/6	3/16(18.75%)
Khorammabad	4/15	3/21	7/36(19.44%)
Poledokhtar	2/12(16.66%)	2/7(28.57%)	4/19(21.05%)
Total	21/68(30.88%)	16/84(19.04%)	37/152(24.34%)

The present study genes of T. *lestocuardi* and T. *ovis* were sequenced and were submitted to GenBank (accession numbers: KC599235 and KC599236**).**

Discussion

Ixodidae ticks play important roles in transporting such pathogens as *Theileria* and *babesia* to domestic and wild ruminants in different parts of world. Identification of these vector ticks and their prevalence and distribution is critical for understanding the epidemiology of *Theileriosis* (Namavari *et al.*,2007 ; Maleki,2002). Molecular methods such as PCR are more sensitive and specific as compared to traditional ones for detecting definitive and intermediate hosts which are infected with parasites. Due to climatic differences in different parts of Lorestan, prevalence and distribution of hard ticks are very different and significant studies related to the carrying of ticks in this province have not been done. In this paper using specific primers, it has been determined that *R. sanguinus* and *H. a. anatolicum* ticks have respectively transferred *T. ovis* and *T. lestocuardi* to sheep. Similar studies have been carried out in Iran and other countries. Razmi *et al.*, (2003) investigated salivary glands of R. *sanguinus* and *Hyalomma. a. anatolicum* by staining fulgen and tick infestation rate with *T. ovis* and *T. lestoquardi* reported as 4 and 15%, respectively. Abdi Goodarzi, (2013) showed that *H. a. anatolicum* ticks collected from Fars province and *H. detritum* from Aligoodarz may be the carriers of *T. lestoquardi*. The studies have shown that the most prevalence of T. *lestoquardi* exists in eastern and central regions of

Iran (HeidarpourBami *et al*.,2010 ; Namavari *et al*.,2007). Haddadzadeh *et al.,* (2004) has reported that optimum temperature for T. *lestoquardi* and *Hyalomma* ticks is 20-25 °c in Iran . Weather conditions in Eastern Lorestan (Aligodarz) have such features so that T. *lestoquardi* was seen in this area; it has been confirmed by this paper.

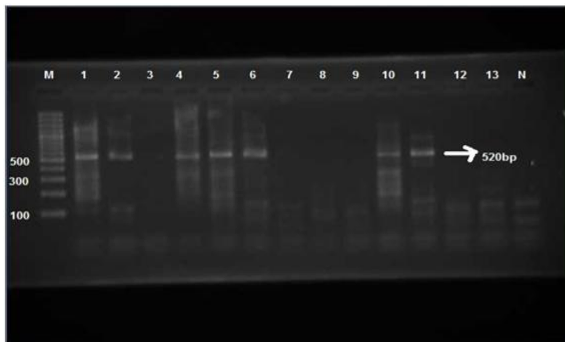

Fig. 2. Determine of *Theileria ovis* in infected *Rhipicephalus* ticks. Lane M, 100 bp DNA ladder; lane 1, purified piroplasm DNA obtained from *T. ovis*-infected sheep blood .(positive control); lanes 2,4,5.6 infected *R.sanguinus*(female ticks); lanes 10,11infected *R.sanguinus* (male ticks**)** ; lane N , negative control(no DNA); other lanes, non infected *Rhipicephalus* ticks.

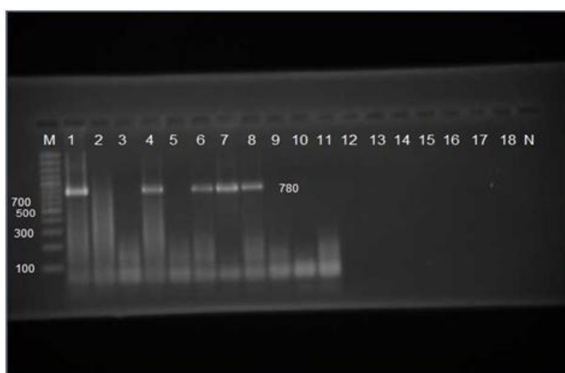

Fig. 3. Determine of *T. lestocuardi* in infected *Hyalomma a. anatolicum* ticks . Lane M, 100 bp DNA ladder; lane 1, positive control ; lane 4, infected male tick; lanes 6,7,8 infected female ticks ; lane N , negative control(no DNA); other lanes, non infected ticks **.**

Telmadarraiy *et al.*, (2012) has reported that *R. sanguinus* is the major carriers of *T.ovis* in North East Iran sheep (Mazandaran), but the infection rate was 55%, which indicates the difference between northern climate and Lorestan's . The prevalence of subclinical Theileriosis in Eastern Turkey calculated as 54.03% has been reported. In another study in eastern Turkey, it became clear that *R.bursa* is more likely to be the main vector of *T. ovis*.(Aktas *et al*.,2006; Altay *et al*.,2005). In Pakistan, Durrani *et al*.,(2012)) showed that 65/8% of *R. sanguinus* ticks may be the carriers of *T.ovis* and 6/66% of *Hyalomma a. anatolicum* ticks are considered as the carriers of *T. lestoquardi*). However, in most of these studies, *R.sanguinus* as the main vector of *T. ovis* and *Hyalomma a. anatolicum* as the main vector of *T. lestoquardi* have been observed in different parts of Iran and the Middle East that may be consistent with our results.

Conclusion

Since Aligoodarz region as compared to southern and Eastern Lorestan is of moderate temperature, precipitation and relative humidity, the risk of melignant *Theileriosis* is greater while *R. sanguinus* has been seen in the most parts of the studied province with different ratios. Thus, our results indicate that this tick can be discussed as the main vector of *T. ovis* in Lorestan.

Acknowledgements

The authors are thankful to Dr.Karimi, the head of Department of Parasitology, Razi Vaccine and Serum Research Institute, Karaj, Iran.

References

Abdigoudarzi M. 2013. Detection of Naturally Infected Vector Ticks (Acari: Ixodidae) by Different Species of *Babesia* and *Theileria* Agents from Three Different Enzootic Parts of Iran. Journal of Arthropod -Borne Disease **7(2),** 164-172.

Ahmed JS, Luo J, Schnittger L , Seitzer U , Jongejan F, Yin H. 2006. Phylogenetic position of small ruminant infecting piroplasms.Annals of the New York Academy of Sciences **1081,** 498-504

Alani AJ, Herbert IV. 1988. pathogenesis of

infection with *theileria recondita* isolated from *Haemaphysalis punctate* from NorthWales. Veterinary Parasitology **4,** 293-301.

Altay K, Dumanlia N, Holman PJ, Aktas M. 2005. Detection of Theileria ovis in naturally infected sheep by nested PCR. Veterinary Parasitology **127,** 99–104.

Aktas M, Altay K, Dumanli N. 2006. PCR-based detection of *Theileria ovis* in *Rhipicephalus bursa* adult ticks. Veterinary Parasitology **140,** 259-263

Durrani AZ, Younus M, Kamal N, Mehmood N, Shakoori AR. 2011. Prevalence of ovine *Theileria* species in District Lahore, Pakistan. Pakistan Journal of Zoology **43(1),** 57-60.

Haddadzadeh HR, Rahbari S, Khazraiinia P, Nabian S. 2004. New concepts on limiting factors of ovine and caprine malignant *theileriosis* (OCMT) in Iran. Iranian Journal of Veterinary Research **5,** 43-46.

HeidarpourBami M, Khazraiinia P, Haddadzadeh H, Kazemi B. 2010. Identification of *Theileria* species in sheep in the eastern half of Iran using nested PCR-RFLP and microscopic techniques.Iranian Journal of Veterinary Research Iranian **11(3),** 262-266.

Hoogstraal H, Wassef YH. 1985 . *Hyalmoma anaolicum* and Indian *Pakistani cattle tick* parasitizing bovine in Oman. Veterinary Parasitology **71(1),** 129-130.

Kirvar E, Ilhan T, Katzer F, Wilkie G, Hooshmand-Rad P, Brown CGD. 1998. Detection of *Theileria lestoquardi (hirci)* in ticks, sheep, goats using polymerase chain reaction. Annals of the New York Academy of Sciences **894,** 52-62.

Maleki SH. 2002. Case study of *Theileria* contamination in liver of disease sheep perished and slaughtered in the slaughter house of khorramabad. Journal of Veterinary Research **57(1),** 97-99.

Namavari MM, Seghatoleslam A, Hosseini MH, Amrabadi O, Shirazi-Nejad A, Tahamtan Y. 2007. Use of the polymerase chain reaction for identification and quantification of protozoa in *Hyalomma anatolicum anatolicum* ticks. 15 th International Congress of Mediterranean Federation of Health and production of Ruminants ,Kusadasi Turkey. 210-215.

Razmi GR, Hosseini M, Aslani MR. 2003. Identification of tick vectors of *ovine theileriosis* in an endemic region of Iran . Veterinary Parasitol **116,** 1-6.

Telmadarraiy Z, Oshaghi MA, Hosseini Vasoukolaei N, Yaghoobiershadi MR, Babamahmoudi F, Mohtarami F. 2011. First molecular detection of Theileria ovis in Rhipicephalus sanguineus tick in Iran. Asian Pacific Journal of Tropical Medicine **4,** 29-32.