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RESEARCH ARTICLE

Prevalence of Theileriosis in sheep in Al-Kut province in Iraq

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Abstract

Ovine theileriosis is an important tick-borne disease of sheep in the tropical and subtropical regions of the world, which can cause severe economic loss in animal husbandry. A total of 87 blood samples was collected from sheep in Al-Kut province in the east of Iraq and examined to detect *Theileria* species. Regions of *Theileria*SSU rRNA genes were amplified to determination *T.ovis* and *T. annulata* and 30KDa gene to fixing *T.lestoquardi*. PCR analysis showed that there are 63%, 71.2% and 48.2% of examined blood samples were given a positive result for *T.ovis*, *T.annulata*and*T.lestoquardi*respectively in addition to 45% were recorded a mixed infection
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1- Introduction

Piroplasmsare atick-transmitted parasitic protozoa parasites divided into two genera *Theileria* and *Babesia.* They are the causative agents of theileriosis and babesiosis respectively (16,9). Theileriosis occurs over a wide geographic are aranging from Southern Europe and extending to Southern Russia, Middle East,Central Asia,China, India, Northern Africa and Sudan, Eritrea and Mauritania (19, 10, 18). Ticks belonging to family Ixodidae transmit a wide variety of pathogens to vertebrates ranging from viruses to helminthes; theileriosis is one of the widespread transmitted by ticks (14). Small ruminants are affected by *T.letoquardi, T.uilenbergi* and *T.luwenshni* which are very pathogenic;*T.ovis, T.seperata*, and *T.reconditte* are lesser (23).Among known *Theileria* parasites of small ruminants, *T.lestoquardi* is highly pathogenic and causes malignan to vinetheileriosis (24,13). The incubation period in infected animals varies from 9-25 days and the severity of the infection is dependent on the susceptibility of the animal, virulence of the parasite and the number of sporozoites that were transmitted to the animal during infection (20),the course of infection may consequently vary from per acute, acute or sub acute to chronic depending on the interaction between the host and the parasite. The present study aims to investigate the *Theileria* species in sheep in Al-Kut province in the east of Iraq by PCR amplification of SSU rRNA and 30KDa genes.

2. Materials and methods

2.1. Samples Collection

The study was conducted in rural areas of Al-Kut province in the east of Iraq. The climate in this province is characterized by being cool to moderate winters and hot, dry summer.

A total of 87 blood samples were collected from sheep selected randomly (77 samples from apparently healthy animals and 10 from suspected animals). Blood was drawn sterilely from the ear vein of these animals. The blood was put in sterile tubes containing EDTA and stored at -20C^0.

2.2 DNA Extraction and PCR Amplification

The genomic DNAs were extracted from the blood samples according to the manufacturer's instructionsof the DNA extraction kit (Genomic DNA mini kit (Blood, cultured cell), cat #GB100, GB300, Gene aid, USA).

Theileriaovis and *Theileriaannulata* were detected by amplification of SSUrRNA gene Whereas *Theilerialestoquardi* was detected by amplifying of 30 KDa gene with primer stets as describe in (Table 1).

Fivemicro litters (5µl) of genomic DNA was amplified in 20 µl reaction mixture, containing 1.5 µl of each F and R primers, 12 µl of DNAase water and lyophilized pellet of Accu power® PCR premix ((Top DNA P01) polymerase 14, each dNTP 250 µM, Tris-Hcl (PH 9.0) 10 mM, Kcl 30 mM and $mgcl_2$ 1.5 mM), Bio NEER, south Korea. (Pat No. 162770). Conventional PCR and Touchdown PCR methods were used using thermal cycler (Labent, USA). As follows:

The amplification conditions of *Theileriaovis* were at $94C^0$ for 5 minutes for initial denaturation followed by 35 cycles of denaturation at $94C^0$for 30 seconds, annealing at $60C^0$ for 60 seconds and extension at $72C^0$ for 60 seconds. The final extension was at $72C^0$ for 5 minutes. Touchdown PCR method was used to amplification of genomic DNAs in both *T.annulata* and *T. lestoquardi* .The amplification conditions of *T.annulata*were at $95C^0$ for 2 minutes for initial dentaturation followed by 15 cycles of denaturation at $95C^0$ for 30 seconds, annealing at $54.3C^0$ decrease at $0.5C^0$/ cycle for 30 seconds and extension at $72C^0$ for 40 seconds then 20 cycles of denaturation at $95C^0$ for 30 seconds, annealing at $47.3C^0$ for 30 seconds and extension at $72C^0$ for 40 seconds. The final extension was at $72C^0$ for 5 minutes.

Regarding to the amplification conditions of *T. lestoquardi* were the same as the conditions in *T. annulata* except the annealing temperature of the first 15 cycles was at $61.7C^0$ decrease $0.5C^0$/ cycle and at $54.7C^0$ in second 20 cycles, and the final extension at $72C^0$ for 80 seconds. Next, 5µl of PCR products were electrophoresis in 1.5% a garose gel containing 0.5 µg/µl of ethidium bromide.

3. Results

To detect the *Theileria* species in sheep, the amplification of two regions of*Theileria*SSUrRNA and 30 KDa genes by conventional PCR and touchdown PCR were performed.

Out of 87 examined blood samples, 55 (63.2%), 62 (71.2%) and 42 (48.2%)samples were shown a positive result for *T. ovis*, *T. annulata*and *T. lestoquardi*, respectively. In addition to that 40 (45.9%) samples were recorded a mixed infection of two or all of these species (Table 2, Figures 1,2 and 3).

Table 1: *T. ovis, T. annulata* and *T. lestoqaurdi* used primers

T. ovis:520bp SSU rRNA gene (8)

F; 5-'TCG AGA CCT TCG GGT-3'

R; 5'-TCC GGA CAT TGT AAA ACA AA-3'

T. annulata: 370bp SSU rRNA gene (26)

F; 5'-AGT TTC TGA CCT ATC AG-3'

R; 5'-TGC ACA GAC CCC AGA GG-3'

T. lestoquardi: 785bp 30 KDa gene (26)

F; 5'-GTG CCG CAA GTG AGT CA-3'

R; 5'-GGA CTG ATG AGA CGA AGA G-3'

Table 2: Showing PCR positive cases of theileriosis according to species of parasite.

No. of examined blood samples	No. of positive samples & percentage (%)							
	T. ovis	**%**	***T. annulata***	**%**	***T.lestoquardi***	**%**	**mixed**	**%**
87	55	63.2	62	71.2	42	48.2	40	45.9

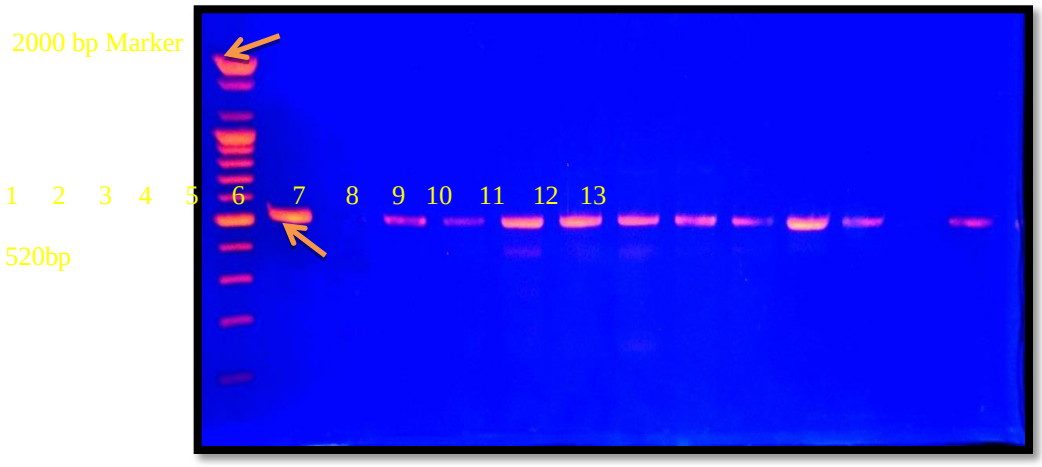

Figure 1:Detection of *Theileriaovis* in sheep by conventional PCR. Using amplified of SSU rRNA gene. Lanes 1,3,4,5,6,7,8,9,10,11 and 13 represented positive blood samples (520bp). Lanes 2 and 12 appeared a negative blood samples.

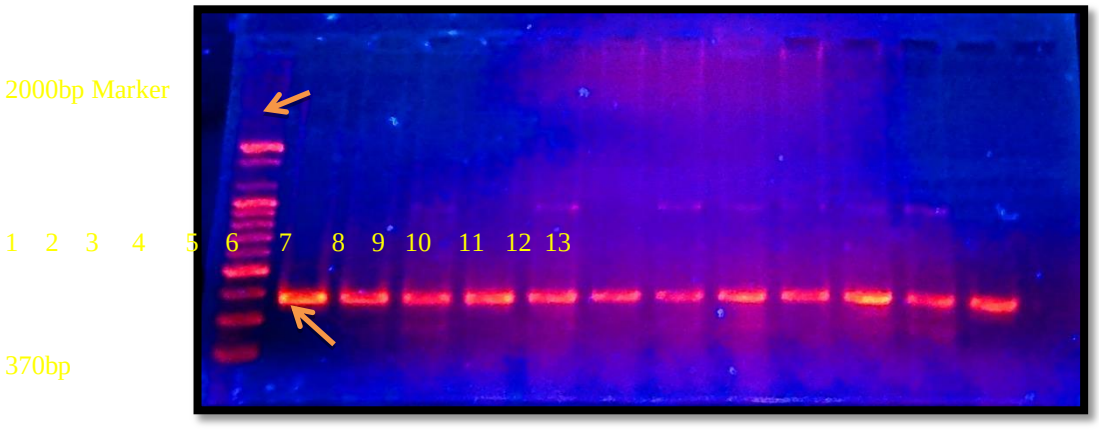

Figure 2:Detection of *Theileriaannulata* in sheep by touchdownPCR. Using amplified of SSU rRNA gene. Lanes 1- 12 representatives as positive blood samples (370bp). Lane 13 was negative blood sample.

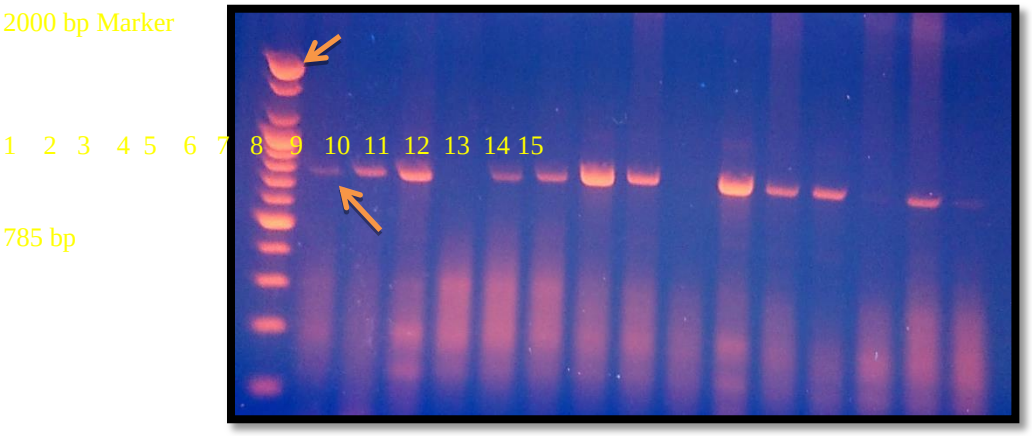

Figure 3:Detection of *Theilerialestoquardi* by touchdown PCR. By use amplified of 30 KDa gene. Lanes 1,2,3,5,6,7,8, 10,11,12,14 and 15 representatives positive blood samples (785bp). Lane 4,9 and 13 were negative blood samples.

The results reflected that in study region the sheep can infect with specific species (*T. ovis, and T. lestoquardi*) and nonspecific species (*T. annulata*).

4.Discussion

This study was the first report on the identification of *Theileria* infection using PCR technique in Al-Kut province in the east of Iraq; in addition to that the infection with *T. annulata* was firstly recorded in sheep in Iraq (17, 3,6,7,1,27,4,5).

Three species were investigated and detected in this paper, *T. Ovis*, *T. annulata*,and *T. lestoquardi* with rates of infection 63.2%, 71.6% and 48.2% respectively, also mixed infection recorded in 45.9% of examined cases. The results of the current study have identified with many other studies (2,12,25).This suggests that the all above *Theileria* species are prevalent in Al-KutProvince, and the detection of them refer to that there are risky circumstance especially when knowing that the infections were occurring among apparently healthy sheep (14, 11, 21).

The presence of detecting*Theileria* species in the study area as an endemic pathogen may be closely related to the suitable environmental conditions there, that which play an important role in the activation and proliferation of the vector (ticks) (22,15).

References

1. **Abdo, J. (2011).** Development of two diagnostic tools (ELISA and ICT) for detection of antibodies against ovineand bovine Theileriosis. M.Sc. thesis, College of Ludwig Maximilians, University of Munich Germany.

2. **Aktas, M., Altay, K., and Dumanli,N. (2005).** Survey *Theileria* parasites of sheep in eastern Turkey using polymerase chain reaction .Small Ruminant Research.; 60,289–293.

3. **Al-Amery, M. A. Y., and Hasso, S. A. (2002)**. Laboratory diagnosis of novel species of *Theileriahirci*, *Eimeria caprovina* and *Eimeriapallida* in goats in Iraq. Small Ruminant Res.; 44: 163-166.

4. **Al–Emarah, Gh Y. A., Khudor, M. H., and Daham, H. R. (2012).** Clinical, haematological and biochemical study to cattlenaturally infected with *Theileriaannulata* in north of Basrahprovince .AL-Qadisiya Journal of Vet.Med.Sci.; Vol. /11 No. /1.

5. **AL-Janabi, M.H.K.(2012)**.Clinical, hematological and biochemical tests for diagnosis of infected locally breed goats with Theileriosis in three governments. MSC thesis. College of Education. University of Baghdad. Iraq.

6. **ALKhaledi, M. J. (2008).** Epidemiological study of (Theileriosis, Babesiosis, and Anaplasmosis) in cattle of ALQadisiya province.MSC thesis. College of Vet. Med. University of Baghdad. Iraq.

7. **Al-Saad, K. M.; Al- Obaidi, Q. T. and Esmaeel, S. A. (2009).** Hematological and biochemical study on the effect some common blood parasites in native goats in Mosul area. Iraqi. J. Vet. Sci., 23(1): 101-106.

8. **Altay, K., Dumanlia, N., Holmanb, P. J., and Aktasa, M. (2005).** Detection of *Theileriaovis* in naturally infected sheep by nested-PCR. Vet. Parasitol. ; 127: 99-104.

9. **Altay, K. M., Aktas, and Dumanli, N. (2007**). *Theileria* infection in small ruminants in the East and Southeast Anatolia. Turkey Parasitol. Derg.; 31: 268-271.

10. **d'Oliviera, C., Weide, I.M.V.D., Miguel, I., Habela ,H., Jacquiet, P., and Jongejan, F. (1995) .**Detection of Theileria*annulata*in blood samples ofcarrier cattle by PCR. J. Clin. Microbiol., 33: 2665–2669.

11. **Darghouth, M.A. (2004)**. Piroplasmids of livestock in Tunisia. Arch. Inst. Pasteur Tunis. ; 81: 21-25.

12. **Duran, A., Younus, M., Ksmal, N., Mehmood, N., and Shakoori, A.R. (2011).** Prevalence of ovine *Theileria* species District Lahore ,Pakistan .Pakistan J. Zool.,vol. ; 43(1),pp.57-60.

13. **Durrani, S., Khan, Z., Khattak, R. M., Andleeb, M., Kiran, S., Anwar, H., Riaz, M., Sajid, M., Sheikh, R. S., Ali, M., and Iqbal, F. (2012).** A comparison of the presence of *Theileriaovis* by PCR amplification oftheir SSU rRNA gene in small ruminants from two provinces of Pakistan.Asian Pacific Journal of Tropical Disease 43-47.

14. **Georges, K., Loria, G. R., Riili,S., Greco, A., Caracappa, S., Jongejan, F. , and Sparagano, O. (2001).**Detection of haemoparasites in cattle by reverse line blot hybridization with a note on the distribution of ticks in Sicily. Vet. Parasitol. ; 99:273–286.

15. **Gosh, S.G.C., Bansal, S.C., Gupta, D., Ray, M.Q., Khan, H.,Irshad, M., Shahiduzzaman,U., Seitzer, and Ahmed, J.S.(2007)**.Status of tick distribution in Bangladesh, Indiaand Pakistan. Parasitol. Res.; 101:207-216.

16. **Heidarpour, B. M., Haddadzadeh, H.R. ,Kazemi, M., Khazraiinia, P., Bandehpour,M., and Aktas, M. (2009).** Molecular identificationof ovine *Theileria* species by a newPCR–RFLP Method. Vet Parasitol .;161:171–177.

17. **Karawan, A.J. (2007).** Study of Theileriosis in cattle and effects of (Butalex®) Buparvaquone.MSC thesis. College of Education. University of Bahgdad. Iraq.

18. **Leemans, I., Hooshmand-Rad, P., and Uggla, A. (1997).** The indirect fluorescent antibody test based on schizont antigen for study of the sheep parasite *Theilerialestoquardi*. Vet. Parasitol.; 69:9–18.

19. **Leemans, I., Brown, D., Hooshmand-Rad, P., Kirvar, E., and Uggla, A. (1999b)**. Infectivity and cross-immunity studies of *Theilerialestoquardi*and *Theileriaannulata* in sheep and cattle: I. In vivo responses. Vet. Parasitol. ; 82(3), 179-192.

20. **Minjauw, B., and Mcleod, A. (2000).** Epidemiological economics of ticks tick borne diseases their effects on the livelihoods of the poor in east and southern Africa and India. Consulting Report to Animal Health Programed (AHP). Nairobi, Kenya. 94.

21. **Nagore, D., Garcia –Sanmartin, J., Garcia, Perez, ALJuste, R.A., and Hurtado, A.** (2004).Identification, genetic diversity and prevalence of *Theileria* and *Babesia* species in a sheep population from Northern Spain. Int. J. Parasitol. ; 34:1059–1067.

22. **Rashidi, A., and Razmi, G. (2013).** Molecular detection of *Theileria* spp. in sheep and vector ticks in the North Khorasan province, Iran. Trop. Anim. Health Prod.; 45:299-303.

23. **Salih, D. A., El Hussein, A. M., Hayat, M., and Taha, K. M. (2003).** Survey of *Theilerialestoquardi* antibodies among Sudanese sheep. Vet. Parasitol. ; 111: 361-367.

24. **Shayan, P., andRahbari, S. (2005).** Simultaneous differentiation between *Theileria*spp. and *Babesia* spp. on stained blood smear using PCRParasitol. Res.; 97: 281-286.

25. **Taha, K.M. (2009).** Experimental transmission of *Theilerialestoquardi* and *Theileriaannulata* (Apicomplexa: *Theileridae*) and their cross transmission between cattle and sheep. Ph.D. Thesis. Sudan Academy of Science. ; p. 89.

26. **Taha, K.M., Salih, D.A., Ali, A.M., Omer, R.A. and Elhussein, A.M.(2013).**Naturally occurring infections of cattle with *Theilerialestoquardi*and sheep with *Theileriaannulata* in the Sudan. Vet. Parasitol.; 191:143-145.

Zangana, I. K. and Naqid, I. A. (2011). Prevalence of piroplasmosis (Theileriosis and Babesiosis) among goats in Duhok Governorate. AL-Anbar J. Vet. Sci.;Vol: 4 No.2.