SCREENING TICK-BORNE DISEASES IN SHEEP*

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***RESUME** : Sept groupes de moutons furent suivis en Iran. Des échantillons sanguins et des tiques furent récoltés pendant l'été 2003. Vingt neuf pour cent des moutons apparurent infectés par des bactéries (*Anaplasmataceae*) et 76% par des protozoaires (*Piroplasmidae*). Des variations entre les différentes régions d'Iran furent observées avec des infections bactériennes allant de 0% à 70% et des infections par des protozoaires de 33% à 100%. Le séquençage de l'ADN, après une PCR des ARN des ribosomes 16S et 18S, prouva que les moutons furent principalement infectés avec* Anaplasma ovis *et* Theileria lestoquardi.

***Mots-clés** : Petits ruminants, Iran, PCR, séquençage, tiques.*

***SUMMARY** : Seven groups of sheep were monitored in Iran. Blood and tick samples were collected during summer 2003. 29% and 76% of the sheep were infected with bacteria (*Anaplasmataceae*) and protozoa (*Piroplasmidae*), respectively. Geographic differences were observed for bacteria and protozoa infections ranging from 0 to 70% and from 33.3% to 100%, respectively. 16S and 18S-rRNA PCR reactions followed by DNA sequencing showed that* Anaplasma ovis *and* Theileria lestoquardi *were the major pathogens found in the sheep blood.*

***Keywords** : Small ruminants, Iran, PCR, sequencing, ticks.*

I - INTRODUCTION

Anaplasmosis, ehrlichiosis, theileriosis and babesiosis are serious tick-transmitted diseases in temperature, subtropical and tropical countries and lead to meats and milk production losses. Their main vectors and hosts are *Ixodidae* ticks and cattle, sheep, goats and wild ruminant, respectively.

For the detection and identification of mentioned pathogens the morphological and serological tests are replaced by methods of molecular biology, e.g. polymerase chain reaction (PCR), reverse line blot (RLB).

Fars province, with the highest level of sheep theileriosis in Iran was monitored for occurring of anaplasmosis and theileriosis in small ruminant based on the study of the 16S and 18S rRNA genes.

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* Texte de la conférence présentée au cours de la Journée AEEMA, 14 mai 2004

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II - MATERIALS AND METHODS

A total of 100 blood samples were monitored. Samples were collected in seven regions of the Fars province of Iran, from spring to summer 2003. The Fars province is located in the southern Iran.

DNA was extracted using the REDExtract-N-Amp Blood PCR Kit (Sigma) following the protocol of manufacture.

PCR was performed amplifying a fragment on the 16S and 18S rRNA targeted bacteria species and Piroplasmidae, respectively [Bekker *et al*., 2002; Gubbels *et al*., 1999].

Five microliter aliquots of the blood extracts were amplified in 50 µl PCR reaction mixture.

Touchdown PCR program of 10 min at 94°C, two cycles of 20 s at 94°C, 30 s at 67°C, 30 s at 72°C were used. During the subsequent two-cycle sets, the annealing temperature was lowered by 2°C until it reached 59°C. For the next 30 cycles, the annealing temperature was 57°C and PCR reaction was ended at 72°C for 5 min.

For each PCR reactions were used positive and negative controls.

Amplified PCR fragments were sequenced by Lark Technologies, Inc., Essex and sequences were confronted with GenBank Database.

III - RESULTS

Based on the results of PCR, 29.0% of blood samples were positive for bacteria, 93.1% of the bacteria-positive samples were also positive for Piroplasmidae. 76.0% of samples were positive for protozoa. The highest positivity for bacteria was in Sarvestan (70.00%). Positivity for protozoa was between 33.33% and 100.00%. (table I)

Based on sequencing, small ruminants were infected with *Anaplasma ovis* and *Theileria lestoquardi. A. ovis* was identified in Sarvestan and Kazeroon and *T. lestoquardi* in Sarvestan, Shiraz, Sepidan and Kavar.

Table I

Positivity of blood samples for bacteria and protozoa

Region	Sarvestan	Zargan	Shiraz	Sepidan	Kavar	Kazeroon	V-Strain	Total
Bacteria	21/30	0/10	0/9	1/15	1/8	6/25	0/3	**29/100 (29.0%)**
Protozoa	29/30	5/10	7/9	14/15	8/8	12/25	1/3	**76/100 (76.0%)**

IV - CONCLUSION

Future work will try to identify the tick vector *Hyalomma anatolicum anatolicum* and to develop species-specific probes for running a macro-based test. Therefore it will be possible to check multiple infections in small ruminants.

Unfortunately some published probes for *T. annulata* [Gubbels *et al*., 1999] and for *T. lestoquardi* [Schnittger *et al*., 2004] are cross-reacting and recognising each other.

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