

## MOLECULAR BIOLOGICAL DETECTION OF EMERGING TICK-BORNE ZOOBOTIC PATHOGENS IN IXODID TICK SPECIES

Narantsatsral S., Myagmarsuren P., Davaasuren P., Enkhtaivan B., Battsetseg B. and Battur B.

Institute of Veterinary Medicine

e-mail: [naran69@gmail.com](mailto:naran69@gmail.com)

### ABSTRACT

*Tick infestation activity has increased in Mongolia in recent years. Rickettsiosis, ehrlichiosis, Lyme disease and Q fever are all tick borne zoonoses. We aimed to survey dispersal of these zoonotic disease agents in vector tick population by a molecular diagnostic method. In total, 1462 ticks were harbored from various geographical regions of Mongolia. Genomic DNA was extracted from 400 pasture ticks (*Dermacentor nuttalli*, *Dermacentor daghestanicus*, *Hyalomma dromedarii*, *Hyalomma as. asiaticum*) and 100 forest ticks (*Ixodes persulcatus*). PCR was performed to detect zoonotic pathogens and the prevalence of *Rickettsia* spp., was 12.5 % and 22.9% in pasture and forest tick DNA samples respectively. *Ehrlichia* spp., were detected in 28.5 % and 75 % in pasture and forest ticks respectively. Interestingly, Lyme disease agent *Borrelia* spp. has not detected in 200 pasture tick DNA samples, but found in one out of 96 forest tick DNA samples. Contrary to this, *Coxiella burnetii*, the causative agent of Q fever found only in one out of 240 pasture ticks and was not detected in 96 forest tick DNA samples. Results have proven that molecular diagnostic PCR method is the fastest reliable tool to detect zoonotic pathogens in vector ticks.*

**KEY WORDS:** Rickettsiosis, ehrlichiosis, Lyme disease, Q fever

### INTRODUCTION

Ticks are second only to mosquitoes as vectors of bacterial, viral, and protozoan agents and tick borne zoonotic diseases are emerging or reemerging worldwide [1]. Primary vectors for Rickettsiosis, Ehrlichiosis, Lyme disease and Q fever are arthropod ticks.

Q fever, Lyme disease, zoonotic Ehrlichiosis and Rickettsiosis are the most detected

zoonotic pathogens transmitted through tick engorgement in Mongolia, which makes the area as an endemic by these diseases [2, 3, 4, 5, 6]. Preventing tick infestation completely in free ranging livestock farming circumstances is barely impossible in today's condition in many countries around the world.

### MATERIALS AND METHODS

In the frame of this survey, we have collected ticks from various geographical regions of Mongolia. All ticks were screened for tick species identification by using Zolotariev A. H.

method [7]. Genomic DNA of ticks was extracted by commonly used method of phenol/ chloroform extraction and ethanol precipitation.

We have performed polymerase chain reaction (PCR) to detect vector-borne zoonotic pathogens. PCR was performed in total of 50 µl of volume which contains 1 U Taq polymerase (iNtRon Bio, Korea), 10 pM of each primer, 1.5 mM of MgCl<sub>2</sub> and 2.5 mM of each dNTPs and pathogen DNA was amplified in an automatic DNA thermocycler (Eppendorf, Germany). For the detection of *Rickettsia* spp., primers RCK/23-5-F and RCK/23-5-R targeting to amplify 345 bp product from intergenic spacer 23S-5S of *Rickettsia* were used in the PCR [8]. In order to amplify *Ehrlichia* spp. genus specific gene, ECC and ECB primers targeting 16S rRNA of *Ehrlichia* spp. were used to amplify 477 bp of

PCR product [9]. The species-specific LD1 and LD2 primer set, which was designed to amplify all species associated with Lyme disease, generates 357 bp of amplification fragment from 16S ribosomal DNA of *Borrelia* genus [10]. For the detection of *Coxiella burnetii*, PCR primers CB1 and CB2 were designated based on DNA sequence of the gene encoding the superoxide dismutase enzyme of *C. burnetii* and it generates 257 bp of the PCR product [11].

All amplified PCR products were loaded in 1.5 % agarose gel for gel-electrophoresis and stained with ethidium bromide, then visualized through ultra violet trans-illuminator.

## RESULTS

In total, 1462 of Ixodid ticks were harbored from 10 aimags (provinces) of Mongolia consisting from *D. nuttalli*, *D. daghestanicus* (*Dermacentor niveus*), *H. dromedarii*, *H. as. Asiaticum* and *I. persulatus* species. *D. nuttalli*, *D. daghestanicus*, *H. dromedarii*, *H. as. asiaticum* species were considered as

pasture ticks and *I. persulatus* ticks were adjudged as forest ticks.

### Rickettsiosis

Prevalence of the pathogen in tick population were 13.65% in overall assessment and in pasture and forest ticks and these results differed moderately as 12.5% and 22.9% respectively.

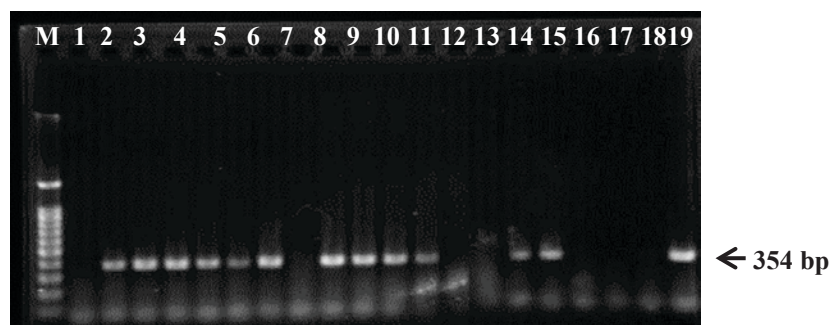


Fig. 1. Agarose gel electrophoresis of the PCR product. M- 100 bp ladder; 2- 7, 9-12, 15, 16 and 20<sup>th</sup> samples are positive for *Rickettsia* spp.; 1, 8, 13, 14, 17-19<sup>th</sup> samples are

Table 1

Pathogens	Prevalence in pasture ticks		Prevalence in forest ticks		Overall prevalence (± SD)
	Tested	Positive (%)	Tested	Positive (%)	
<i>Rickettsia</i> spp.	384	48 (12.5 %)	48	11 (22.9 %)	432 / 13.65 % ± 1.6
<i>Ehrlichia</i>	200	57 (28.5 %)	32	24 (75 %)	232 / 34.9 % ± 3.1
<i>Borrelia</i> genus	200	0 (0 %)	96	1 (1.04 %)	296 / 0.33 % ± 0.5
<i>C. burnetii</i>	240	1 (0.41 %)	96	0 (0 %)	336 / 0.29 % ± 0.2

SD= standard deviation was calculated as,  $\sqrt{\frac{pX(100-p)}{n}}$  where  $p$  is the percentage of positive samples and  $n$  is the sample size.

**Ehrlichiosis**

In total, 232 pasture and forest tick genomic DNA were screened for genus *Ehrlichia* spp. specific gene and the prevalence was 34.9%. Distribution of *Ehrlichia* genus pathogens in pasture and forest ticks was substantially high as much as 28.5% in pasture ticks and 75% in forest ticks. Results are shown in figure 2.

**Lyme disease**

Causative agents from *Borrelia* genus were not detected in 200 pasture tick DNA, but found only in 1 sample positive result out of 96 forest

tick DNA and it is shown in figure 3. Based on this data, the prevalence of Lyme disease agent in forest ticks is 1.04%, which is relatively low.

**Q fever**

Contrary to Lyme disease prevalence result, *C.burnetii* was not detected in forest ticks, but one out of 240 pasture ticks DNA samples found to be positive . Results are shown in figure 4. The prevalence of Q fever agent in pasture ticks is 0.41%.



Fig. 2. Agarose gel electrophoresis of the PCR product. M- 200 bp ladder; 3, 17, 18 and 24<sup>th</sup> samples are positive for *Ehrlichia* spp.; 1, 2, 4- 16 and 19- 23<sup>rd</sup> samples are negative.



Fig. 3. Agarose gel electrophoresis of the PCR product. M- 100 bp ladder; P- positive control, 3<sup>rd</sup> sample is positive for *Borrelia* spp.; 1, 2, 4 to 14<sup>th</sup> samples are negative.

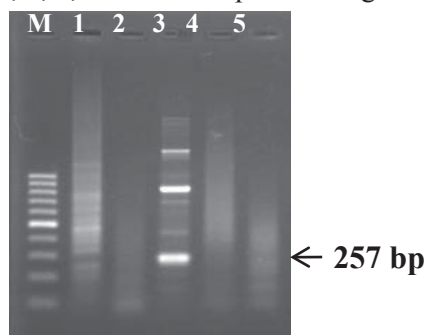


Fig. 4. Agarose gel electrophoresis of the PCR product. M- 100 bp ladder; 3<sup>rd</sup> sample is positive for *C.burnetii*; 1, 2, 4 and 5<sup>th</sup> samples are negative

## DISCUSSION

*D.nuttalli*, *I.persulcatus* species of ticks were vectoring the *Rickettsia* spp. and prevalence rate in *I.persulcatus* ticks (22.9%) was almost two times higher compared to *D.nuttalli* ticks (12.5%). The prevalence result is relatively lower compared to other studies such as Speck et al., 2012 and Glushenkova et. al., 2010 which they included only vector ticks into their study [2, 12] and in our survey, different species of ticks were included from various different geographical regions of Mongolia, which explains the lower prevalence rate. Surprisingly is that in the *Ehrlichia* spp. detection result, all *D.nuttalli*, *D.daghestanicus*, *H.dromedarii*, *H.as.asiaticum* species of Ixodid ticks were vectoring the disease agent unlike other three disease agents. These species of ticks never have been documented as a vector for

*Ehrlichia* spp. the overall prevalence of *Ehrlichia* spp. in Ixodid ticks was 34.9%, which itself manifests first time evidence of molecular detection method application in Mongolia.

By dissipating the result, *Ehrlichia* spp., were detected in 28.5 % and 75 % in pasture and forest ticks respectively. Compared the results of prevalence in pasture and forest ticks also has similar pattern to the *Rickettsia* spp. and it's detection in forest ticks was twice as much more than those of the prevalence in pasture ticks. Lyme disease agents - *Borrelia* spp. have not detected in 200 pasture tick DNA samples, but found in 1 out of 96 forest tick DNA samples. We could conclude that, pasture ticks widely distributed in Mongolia are not a vector for this organism and the prevalence in forest tick population is also low.

Table 2

### Vector tick of zoonotic pathogens

Pathogens	Pasture ticks, carrying zoonotic pathogens				
	Pasture tick species				Forest tick
	<i>D. nuttalli</i>	<i>D. daghestanicus</i>	<i>H. dromedarii</i>	<i>H. as. asiaticum</i>	<i>I. persulcatus</i>
<i>Rickettsia</i> spp.,	+				+
<i>Ehrlichia</i> spp.,	+	+	+	+	+
<i>Coxiella burnetti</i>			+		
<i>Borrelia</i> spp.,					+

Risk of been infected by Lyme disease agent through tick bite in Mongolia is significantly rare based on this results and easily preventable as if we refrain to engage in any work into forested area in April and May.

*C. burnetii*, the causative agent of Q fever found only in 1 out of 240 pasture ticks and

was not detected in 96 forest tick DNA samples. Conclusion is that *I. persulcatus* ticks are not a reservoir species for this agent and the prevalence rate in pasture ticks is low as 0.41%.

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