MONO- AND MIXED-INFECTIONS OF TICK-BORNE PATHOGENS IN VARIOUS ECOLOGICAL FOCI IN MOLDOVA

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Prevalenţa diferiţilor agenţi zoonotici a fost studiată la căpuşele *Ixodes ricinus*, *Dermacentor marginatus*, *Dermacentor reticulatus* şi *Haemaphysalis inermis*. Căpuşele au fost colectate în diferite biocenoze din Republica Moldova. ADN-ul *Borrelia burgdorferi sensu lato* a fost detectat la o căpuşă *Ixodes ricinus* şi o căpuşă *Dermacentor marginatus*. Două căpuşe *Ixodes ricinus* şi şase căpuşe *Dermacentor marginatus* au

fost pozitive pentru Rickettsia sp. (R. helvetica şi R. slovaca). *Babesia microti* a fost detectată în 20 de căpuşe *Ixodes ricinus*, 12 căpuşe *Dermacentor marginatus* şi patru căpuşe *Haemaphysalis punctata*. ADN *Babesia odocolei* a fost detectat într-o singură căpuşă *Dermacentor marginatus*. O infecţie dublă cu *Borrelia burgdorferi sensu lato* şi *Babesta microti* a fost detectată în şase căpuşe *Ixodes ricinus*. La o căpuşă *Ixodes ricinus* şi o căpuşă *Dermacentor marginatus* a fost identificată o infecţie dublă cu *Rickettsia* sp. şi *Babesia microti*. O infecţie triplă cu *Borrelia burgdorferi* s. l., *Rickettsia* sp. şi *Coxiella burnetii* a fost detectată la o singură căpuşă *Ixodes ricinus*.

Cuvinte cheie: agenţi patogeni generaţi de căpuşe, *Borrelia burgdorferi*, *Coxiella burnetii*, *Ixodes*, *Dermacentor, Haemaphysalis*, PCR

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Introduction

Ticks are important as vectors for the transmission of zoonotic pathogens like *Borrelia (B.) burgdorferi* sensu lato (s. l.), *Coxiella (C.) burnetii, Babesia (Bab.) odocolei, Bab. microti*. and *Rickettsia (R.)* sp. Spirochetes of the *B. burgdorferi* s. l. complex causes Lyme disease, one of the most important tick-transmitted diseases in Europe. Infection occurs through tick bite. The disease is characterized by erythema migrants, a disseminated infection (skin, nervous system, heart, joints), fever, headache, arthritis, and neuropathy. *C. burnetii* is the aetiological agent of Q fever [1]. In contrast to borreliae *C. burnetii* is shed in tick feces and transmission to humans occurs via inhalation of infected dust. Babesiosis is a zoonosis caused by intraerythrocytic protozoa of the Phylum Apicomplexa [2]. Investigations of the last years indicated that many of the tick-borne diseases are able to be transmitted as mixed infections [3]. There are only few reports about the distribution of tick transmitted diseases in the Republic of Moldova. Koci et al. (2007) [4] investigated *I. ricinus* ticks collected in Chisinau City by PCR. *B. burgdorferi* s. l. was detected in 25.2% of tested ticks. 2.5% of the ticks showed a co-infection with *Anaplasma phagocytophilum*. Investigations in ticks collected from wild birds showed a prevalence of 14% in *I. ricinus* and 5.5% in *I. lividus* ticks. Borreliae were most prevalent in *I. ricinus* ticks collected from blackbirds (17%) [5]. First investigations on the prevalence of mixed infections with *C. burnetii* and *B. burgdorferi* s. l. in ticks collected in different biocenoses in the Republic of Moldova were performed by Movila et al. (2006) [6]. Unfortunately, the ecology and distribution of tick-borne diseases is not explored enough on the territory of Republic of Moldova. Therefore ticks of the species *D. marginatus*, *D. reticulatus*, *I. ricinus*, and *H. inermis* were sampled in different biocenoses and then the prevalence of various tick-borne pathogens were compared.

Material and methods

The tick specimens were sampled from recreation parks of Chisinau and Tiraspol.

The tick surveys were conducted according to the standard entomological methods using flagging technique. Tick habitats such as open pastures, forests and public parks were surveyed. The ticks and also in *D. marginatus*. SFG rickettsial DNA was detected in 6.3% tick specimens. Specimens were identified to species according to standard taxonomic keys [7, 8].

DNA was extracted with a commercial test kit (QIA-quick PCR purification kit, Qiagen GmbH) according to the manufacturer's instructions. The DNA samples were then subjected to specific PCRs to detect fragments of specific genes as described earlier [9, 10, 11, 12]. PCR products were separated by 1.5% agarose gel electrophoresis and visualised by ethidium bromide staining under UV light.

Results and discussion

Altogether, DNA was extracted from 142 ticks and examined for pathogens. A total of 39.4% ticks were found to be infected. Out of these 35.4% were infected by one pathogen, 5.6% showed a double infection, and only one tick (0.7%) was infected by three pathogens (Table 1, 2). The predominant group with a prevalence of 31% were the ticks infected with *Bab. microti*. This pathogen was frequently identified in *I. ricinus.*

Table 1. Diversity of tick-borne pathogens at tick-borne foci

Tick foci	PATHOGENS							Total infected ticks
	Single infection				Dual infection		Triple infection	
	B. burg. **s.l.**	*Rick.* **sp.**	**Bab. micr.**	**Bab. odocl.**	*B. burg* **s.l.** + *Bab. micr.*	*Rick***. sp.** + *Bab. micr.*	*B. burg.* **s.l.** + *C.b.* + *Rick.* **sp.**	
Chisinau city	1	1	11	-	3	1	1	18/45
Chisinau recreation parks	-	6	13	1	-	-	-	20/60
Tiraspol recreation park	1	1	12		3	1	-	18/37
TOTAL:	2	8	36	1	6	2	1	56/142

In our investigation *I. ricinus* ticks showed a low prevalence for borreliae (6.3 %). These results are in contrast to the results of Movila et al. (2007) [5] who detected *B. burgdorferi* s. l. DNA in 14% of individual *I. ricinus* ticks. Also the high prevalence (25.2%) which was reported by Koci et al. (2007) [4] does not fit to our results. Corresponding to the results of Movila (2006) [6] also in this study *I. ricinus* seems to be the most important species for the transmission of borreliae. Since this time the Q fever agent has been detected in many tick species [13], the second question of this study was the meaning of ticks for the transmission of Q fever. The results of our study showed a low evidence (one positive sample) for a role of ticks in the transmission of Q fever. This may be caused by the sampling places (forest and urban biocenoses). Even Movila et al. (2006) [6] found *C. burnetii* in 30 different areas in the central and the southern region of Moldova high prevalences were only detected in agrarian biocenoses while the results from ticks which were collected in forests were negative. A high number of ticks (30.8%) showed an infection with *Bab. microti*. This agent was found at all sampling sites and in three of the four tested species.

Altogether, these data indicate a high prevalence of pathogenic agents in Moldavian ticks which may have a meaning for forest workers, recreation areas and also urban biocenosis where the contact between ticks and humans can be expected to be high.

Table 2. Diversity of tick-borne pathogens detected

Tick foci	PATHOGENS							Total infected ticks
	Single infection				Dual infection		Triple infection	
	B. burg. s.l.	Rick. sp.	Bab. micr.	Bab. odocl.	B. burg. s.l. + Bab. micr.	Rick. sp. + Bab. micr.	B. burg. s.l. + C.b. + Rick. sp.	
Ixodes ricinus	1	2	20	-	6	1	1	31/68
Dermacentor reticulatus	-	-	-	-	-	-	-	0/11
Dermacentor marginatus	1	6	12	1	-	1	-	21/54
Haemophysalis punctata	-	-	4	-	-	-	-	4/9
TOTAL:	2	8	36	1	6	2	1	56/142

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