

Molecular-genetic typing of *Borrelia* circulating in Kyiv Oblast

Molekularno-genetyczne typowanie *Borrelia*,
które krążyły w obszarze Obwodu Kijowskiego

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ABSTRACT

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INTRODUCTION

Ixodes tick-borne borreliosis are the most widespread natural foci diseases on the European continent. The clinical manifestation of the illness is determined by the *Borrelia* species which infects humans. The purpose of the study is to define the *Borrelia* species that circulate in biotic objects on the territory of the Kyiv Oblast to optimize laboratory and clinical diagnostics of *Ixodes* tick-borne borreliosis.

MATERIALS AND METHODS

The *Ixodes* ticks were collected in different areas of the Kyiv Oblast. The tick suspensions to detect *B. burgdorferi sensu lato* were investigated using the real-time polymerase chain reaction (PCR-RT). The PCR agarose gel method for genotyping of the positive samples of *B. burgdorferi s.s.*, *B. afzelii*, *B. garinii* was employed.

RESULTS

Forty-three ($11.31 \pm 0.25\%$) positive pools of investigated ticks with *Borrelia burgdorferi sensu lato* complex have been determined. The average ratio of *Borrelia* infected ticks is $1.21 \pm 0.03\%$. The *Ixodes ricinus* has been treated as the main vector of *Borrelia* in this area. The *Borrelia* species have been identified in 27 tick pools. For the first time, the presence of *B. afzelii* ($90 \pm 12.14\%$) and *B. garinii* ($10 \pm 0.91\%$) have been found in the Kyiv Oblast.

CONCLUSION

Borrelia burgdorferi s. l. is endemic in the Kyiv Oblast where the main vector of agents is *Ixodes ricinus*. *B. afzelii* and *B. garinii* are the dominant *Borrelia* species in biotic objects.

KEY WORDS

Borrelia afzelii, *Borrelia garinii*, Kyiv area

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STRESZCZENIE

WSTĘP

Odkleszczowy typ boreliozy jest najbardziej rozpowszechniony na terytorium kontynentu europejskiego. Objawy kliniczne choroby uzależnione są od odmiany *Borrelia*, którą został zainfekowany człowiek. Celem pracy było zdefiniowanie odmian *Borrelia*, które krążą w obiektach biotycznych na terytorium Obwodu Kijowskiego dla optymalizacji diagnostyki laboratoryjnej i klinicznej odkleszczowego typu boreliozy.

MATERIAŁY I METODY

Kleszcze rodzaju *Ixodes* zebrano na różnych obszarach Obwodu Kijowskiego, sporządzono z nich zawiesinę. Zawiesina była badana w trakcie badania reakcji łańcuchowej polimerazy w czasie rzeczywistym (PCR-RT), pod względem obecności *B. burgdorferi sensu lato*. Przeprowadzono badanie PCR z wykorzystaniem żelu agarowego dla genotypowania pozytywnych próbek *B. burgdorferi s.s.*, *B. afzelli*, *B. garinii*.

WYNIKI

Wykryto 43 (11,31 ± 0,25%) pozytywnych próbek badanych kleszczy pod względem obecności *Borrelia burgdorferi sensu lato*. Średnia zainfekowanych kleszczy wyniosła (1,21 ± 0,03%). Wykazano, iż *Ixodes ricinus* jest głównym wektorem *Borrelia* na tym terytorium. Odmiany *Borrelia* zostały zidentyfikowane w 27 próbkach kleszczowych. Po raz pierwszy w Obwodzie Kijowskim stwierdzono obecność *B. afzelli* (90 ± 12,14%) oraz *B. garinii* (10 ± 0,91%).

WNIOSKI

Obwód Kijowski jest endemicznym w stosunku do *Borrelia burgdorferi s.l.*, gdzie głównym wektorem czynników chorobotwórczych jest *Ixodes ricinus*. Dominującymi odmianami *Borrelia* w obiektach biotycznych dla różnych regionów są *B. afzelli* oraz *B. garinii*.

SŁOWA KLUCZOWE

Borrelia afzelli, *Borrelia garinii*, Obwód Kijowski

INTRODUCTION

Lyme tick-borne borreliosis is the most common tick-borne infection in Europe and North America caused by *Borrelia burgdorferi sensu lato*. The polymorphism of clinical manifestations, staged course, the tendency to chronicity and recurrence with untimely treatment are the characteristic features of Lyme borreliosis. This multisystem disease leads to the damage of various tissues, organs and systems of the human body, including the skin, heart, nervous system, and in some cases eyes, kidneys and liver [1,2,3,4,5,6,7].

The pathogenic agents to humans are *B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelli*. The role of *B. lusitanae*, *B. Valaisiana* and *B. spielmani* in human pathology is being investigated [8].

In Europe, the *B. burgdorferi sensu lato* complex is represented by five separate genotypes: *B. afzelli*, *B. garinii*, *B. burgdorferi sensu stricto*, *B. lusitanae*, *B. valaisiana*. It is known that these taxonomic units are associated with different types of vertebrate hosts and have different clinical manifestations in humans.

Lyme tick-borne borreliosis is found throughout Europe, but is most common in eastern countries [8,9].

In the Ukraine where the environmental conditions are favorable for the formation of natural foci of Lyme tick-borne borreliosis, this infection has been known since 1989. But a number of administrative areas, including the Kiev region, still remain poorly investigated concerning the spectrum of *Borrelia* genotypes circulation [10,11].

The Kiev area is geographically located in the north of Ukraine and divided into Polissia (in the north) and the steppe zone (in the south). The Dnieper divides the steppe zone into right and left banks.

MATERIALS AND METHODS

The suspension of *Ixodes* ticks which were collected in Polissia, as well as on the left and right banks of the steppe area was investigated.

The specific genetic sequences of *B. burgdorferi sensu lato* were determined by means of the polymerase

chain reaction (PCR) in real time using reagent kits “AmplifySens *Borrelia burgdorferi sensu lato-FL*” (FSEN CSRIE of Rosportebnadzor, Moscow, Russia) and “RealBest *Borrelia DNA burgdorferi sl (Set 2/RG)*” (“VektorBest”, Novosibirsk, Russia). The identification of *B. burgdorferi ss*, *B. afzelii*, *B. garinii* genotypes was performed by means of PCR with electrophoresis on agarose gel for the detection of lyophilized PCR; reagent kits “GenePak® DNA PCR test for the qualitative detection of *B. burgdorferi DNA*” (“Izogen”, Moscow, Russia); “GenePak® DNA PCR test for the qualitative detection of *B. afzelii DNA*” (“Izogen”, Moscow, Russia); “GenePak® DNA PCR test for the qualitative detection of *B. garinii DNA*” (“Izogen”, Moscow, Russia). The borrelia-tick ratio was calculated.

RESULTS

3551 specimens of *Ixodeaceae* ticks, combined into 380 pools, were examined to determine the presence of specific genetic sequences of microorganism *B. burgdorferi sensu lato complex*. Out of the total number of examined ticks, *Ixodes ricinus* make up 198 samples (1158 specimens) - $52,11 \pm 1,91\%$; *Dermacentor reticulatus* - 150 pools (1850 specimens) - $39,47 \pm 1,37\%$; *Dermacentor marginatus* - 32 pools (543 specimens) - $8,42 \pm 1,65\%$. Specific fragments of RNA or DNA of *B. burgdorferi sensu lato* in $11,31 \pm 0,25\%$ samples of all types of ticks were found. The average *Borrelia*-tick ratio for *Ixodeaceae* ticks is $1,21 \pm 0,03\%$.

The *Borrelia*-tick ratios by species of ticks are: for *Ixodes ricinus* - $3,27 \pm 0,04\%$; for *Dermacentor reticulatus* - $0,21 \pm 0,01\%$; for *Dermacentor marginatus* - $0,36 \pm 0,001\%$.

The highest *Borrelia*-tick ratios for all the geographical zones are observed among *Ixodes ricinus* ticks.

The borrelia genotyping for 35 samples of ticks (176 specimens) was conducted, including 31 samples (172 specimens) of *Ixodes ricinus* - $88,57\%$, and 4 samples (4 specimens) of *Dermacentor reticulatus* - $11,43\%$.

According to the research, specific DNA sequences of *B. afzelii* were found in 27 samples - $77,14 \pm 10,45\%$, and *B. garinii* - $8,57 \pm 0,67\%$. The mixed combination of *B. afzelii* and *B. garinii* was determined in $8,57 \pm 0,67\%$. One sample contained *B.*

afzelii and *B. burgdorferi s.s.* ($2,86 \pm 0,15\%$) simultaneously.

The results suggest that *B. afzelii* circulates in 15 districts of the Kyiv Oblast; *B. garinii* - in 4 districts; *B. burgdorferi s.s.* - in one district.

Thus, *B. afzelii* circulates in all the landscape-geographical zones of the Kyiv region, *B. garinii* are found within the area of Polissia and right bank of the Steppes; *B. burgdorferi s.s.* - on the right bank of the Steppes.

CONCLUSION

Borrelia burgdorferi s. l. is endemic in the Kyiv Oblast where the main vector of agents is *Ixodes ricinus* ticks. In different landscape regions, the dominant *Borrelia species* in biotic objects are *B. afzelii* and *B. garinii*.



Fig. 1. Result of study of ticks for DNA presence of *B. burgdorferi* (A), *B. garinii* (B), *B. afzelii* (C) by method of detection in agarose gels, where:

1A, 1B, 1C - positive controls;
17A, 17B, 17C - negative controls;
2A-16A - no detected *B. burgdorferi* DNA;
13B, 16B - detected *B. garinii* DNA;
2C-7C and 12C-16C - detected *B. afzelii* DNA.
Ryc. 1. Badanie kleszczy na obecność DNA *B. burgdorferi* (A), *B. garinii* (B), *B. afzelii* (C) w żelu agarozowym:
1A, 1B, 1C - kontrola pozytywna;
17A, 17B, 17C - kontrola negatywna;
2A-16A - negatywne wyniki badań kleszczy na obecność *B. burgdorferi*;
13B, 16B - pozytywne wyniki badań kleszczy na obecność *B. garinii*;
2C-7C and 12C-16C - pozytywne wyniki badań kleszczy na obecność *B. afzelii*.

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