STUDIES ON THE OCCURRENCE OF COXIELLA BURNETII INFECTION IN TICKS IN SELECTED EASTERN AND CENTRAL REGIONS OF POLAND

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ABSTRACT

Arthropods may play a role in the transmission of CoxIELLA burnetti as they transmit pathogen both mechanically (as flies), and actively during the host blood suction. The aim of this study was to identify the C. burnetti occurring in ticks harvested from both domestic and livestock animals, as well as from wildlife in randomly selected regions of Poland. The total number 1126 ticks was collected in four regions of Poland: the Masovian, Lublin, Łódź and Podkarpackie Provinces. Among ticks collected from May 2011 to August 2012, the presence of IS1111 gene sequence was detected in 15 (1.33%) cases by real-time PCR test. Among the ticks harvested from domestic and livestock animals in 12 cases (3.1%), and in case of ticks found in open-space rodents in 3 cases (0.4%) we found the presence of marker sequences. The low percentage of C. burnetti infected ticks indicate a limited role of these arthropods in Q fever transmission.

Keywords: CoxIELLA burnetti, ticks, IS1111, real-time PCR

INTRODUCTION

In humans, the most frequent routes of infection are via inhalation (Dutkiewicz et al., 2011), direct contact of pathogen with damaged skin and mucous membrane, via the alimentary tract, and as a result of contact with C. burnetti infected ticks. In the latter case the infection can be transmitted during the tick blood suction as well as by contact with its excrements. In the Dermacentor andersoni faeces the bacteria survive even up to 580 days (Woldehiwet, 2004). The reservoir of C. burnetti comprises a wide range of species, including all vertebrates, as well as parasitic invertebrate animals. Reported data described cases of C. burnetti isolation from samples of animals such as sheep, goats, cattle, dogs, cats and many species of wild-living vertebrates, including bears, bison, deer, boars, rabbits, hares, shrews and marsupials. Arthropods may play a significant role in pathogen transmission, as they transmit C. burnetti both mechanically (e.g. flies), and actively during the host blood suction, the host may be also infected through the cutaneous or inhalation contact with the parasites faeces (Marrie, 1990; Anusz, 1995). The role of ticks in spreading the pathogen results from theirs manner of feeding and the ability of transstadial transmission. The ability of pathogen transmission (including transovarial) has been reported in about 40 ticks species (Mediannikov et al., 2010). Since ticks can reside on at least two to three different hosts (mainly mammals) during their life-cycle, they are considered as one of the factors determining the persistence of C. burnetti in the environment (Fard and Khalli, 2011). The infection of ticks by C. burnetti occurs during the blood sucking of on infected mammal or other vertebrate, whereby they become a vector of this microorganism (Norlander, 2000; Bossi et al., 2004; Hartzell et al., 2008; Oyston and Davies, 2011).

The aim of this study was to identify the C. burnetti occurring in ticks harvested from both domestic and livestock animals, as well as wildlife in randomly selected regions of Poland.

MATERIALS AND METHODS

Ticks collection

Ticks were collected in four regions of Poland: the Masovian Province (central region of Poland) - 27 Ixodes ricinus: 5 from wildlife (deer, boar), 2 from domestic animals (cats, dogs), 20 from cattle; 4 Dermacentor reticulatus from domestic animals, Lublin Province (eastern Poland) - 95 I. ricinus: 57 from wildlife, 18 from open-space rodents, 8 from cattle, 12 from domestic animals, 2 D. reticulatus from domestic animals, 16 Ixodes hexagonus from domestic animals, Łódź Province (central Poland) - 18 I. ricinus from domestic animals; 2 I. hexagonus from domestic animals, and Podkarpackie Province (south-eastern Poland, mountains and sub-mountains region) - 699 I. ricinus: 325 from wildlife, 75 from open-space rodents, 62 from domestic animals, 184 from cattle, 53 from goats; 5 I. persulcatus from wildlife; 257 D. reticulatus from wildlife; 1 Dermacentor marginatus from wildlife. The specimens were collected from May 2011 to August 2012. A total of 1126 ticks were collected, representing the following species: I. ricinus (74.5%), D. reticulatus (23.3%), I. persulcatus (0.45%), I. hexagonus (1.66%) and D. marginatus (0.09%).

Ticks were removed directly from animals, 743 specimens were taken from wildlife animals and another 383 ticks were harvested from domestic and livestock animals. Among the I. ricinus species 839 mature individuals were collected, including 707 females feeding, 120 males and 12 nymphs. From the D. reticulatus species 117 adult females and 146 males were caught, and also 5 females from the species I. persulcatus, 17 adult females and one nymph from the I. hexagonus species, and from the D. marginatus species - one adult female. Ticks were placed in sterile 2 ml tubes and stored at -20°C until further analysis.

Ticks identification and treatment, Genetic material isolation

Identification of tick species was carried out according to the identification key (Siuda, 1993) using a dissecting microscope at magnification range 3.5 - 90 × in the reflected artificial light, and with regard to the larva in transmitted light. For tick treatment, the modified method described by Mediannikov et al. (2010) was implemented. In order to eliminate the possible microbial contamination including Bacillus cereus group, ticks were treated with 0.5% solution of sodium hypochlorite for 10 minutes. In the next stage, the ticks were rinsed with distilled water, dried and re-suspended in 70% ethanol for 10 minutes. Finally, the samples were rinsed with distilled water and PBS. Remains of the fluids were then removed and dry ticks were re-suspended in 0.6 ml of MEM (Minimum Essential Medium, Sigma-Aldrich, USA) without antibiotic and homogenized by Stuart®SHM-1 apparatus. The homogenate was filled up to 2 ml
with MEM. Chitin remains were precipitated and liquid layer (1 ml) was centrifuged at 25 000 × g, the pellet was re-suspended in MEM (5 ml) and then twice filtered through 0.45 µm membrane. The filtrate was used for infection for the BGM cell line (African monkey green kidney, HPA Culture Collection, UK) (Mediannikov et al., 2010). Bacterial DNA was isolated from 1 ml of the suspension obtained after homogenization of the ticks, using Genomic AX Tissue Mini Spin Kit (A&A Biotechnology, Gdynia, Poland) according to manufacturer's recommendations.

**Real-time PCR**

Real-time PCR method was used for detecting the presence of insertion sequence IS1111 (transposase gene, GenBank: M80806) (Klee et al., 2006) using the following primers and probe sequence: IS1111F: 5'-GCTTAAAGTTGGCCTGCGTG-3', IS1111R: 5'-CCCCGAATCTCATTGACG-3', IS1111P: 5'- FAM – AGCGAACCATTGTACGACGTT-TAMRA-TATGG-Gho-3'.

The real-time PCR reaction was performed in a capillary system in the LightCycler 2.0 (Roche, Poland) in a final reaction volume of 20 µl. The reaction mixture consisted of the LightCycler TagMan Master Kit (Roche, Germany) - 10 × conc., primers - 0.5 µM, probe - 0.15 µM and 5 µl of template DNA. During the real-time PCR reaction, the samples were denatured at 95°C for 15 s, followed by 40 cycles of amplification: 15 s at 95°C, 30 s at 60°C and 30 s at 40°C. As a positive control DNA extracted from C. burnetii strain Nine Mile phase I was used, and as a negative control a reaction mixture without template DNA was used. Real-time PCR was performed for 40 cycles, and the test samples for which Ct (Cycle threshold) was lower than 36 were considered as positive. Additionally, the biological material isolated from ticks and cultured in BGM cell line (African monkey green kidney, HPA Culture Collection, UK) (Mediannikov et al., 2010). Bacterial DNA was isolated from 1 ml of the suspension obtained after homogenization of the ticks, using Genomic AX Tissue Mini Spin Kit (A&A Biotechnology, Gdynia, Poland) according to manufacturer's recommendations.

**Table 1** Summary of positive test results for the presence of C. burnetii infections in ticks

<table>
<thead>
<tr>
<th>No.</th>
<th>Province</th>
<th>Place/District</th>
<th>Animal species/ source</th>
<th>Tick species</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lublin</td>
<td>Puławy</td>
<td>Dog</td>
<td>I. ricinus</td>
<td>A, P, F</td>
</tr>
<tr>
<td>2.</td>
<td>Lublin</td>
<td>Ryki</td>
<td>Dog</td>
<td>D. reticulatus</td>
<td>A, G, F</td>
</tr>
<tr>
<td>3.</td>
<td>Lublin</td>
<td>Puławy</td>
<td>Cat</td>
<td>I. ricinus</td>
<td>A, P, F</td>
</tr>
<tr>
<td>5.</td>
<td>Łódź</td>
<td>Łódź</td>
<td>Dog</td>
<td>I. ricinus</td>
<td>A, P, F</td>
</tr>
<tr>
<td>6.</td>
<td>Łódź</td>
<td>Łódź</td>
<td>Cat</td>
<td>I. ricinus</td>
<td>A, P, F</td>
</tr>
<tr>
<td>7.</td>
<td>Łódź</td>
<td>Łódź</td>
<td>Dog</td>
<td>I. hexagonum</td>
<td>A, P, F</td>
</tr>
<tr>
<td>8.</td>
<td>Łódź</td>
<td>Łódź</td>
<td>Open-space rodent1</td>
<td>I. ricinus</td>
<td>A, P, F</td>
</tr>
<tr>
<td>9.</td>
<td>Łódź</td>
<td>Łódź</td>
<td>Open-space rodent1</td>
<td>I. ricinus</td>
<td>A, P, F</td>
</tr>
<tr>
<td>10.</td>
<td>Łódź</td>
<td>Łódź</td>
<td>Open-space rodent1</td>
<td>I. ricinus</td>
<td>A, P, F</td>
</tr>
<tr>
<td>11.</td>
<td>Podkarpackie</td>
<td>Sanok</td>
<td>Open-space rodent1</td>
<td>I. ricinus</td>
<td>A, P, F</td>
</tr>
<tr>
<td>12.</td>
<td>Podkarpackie</td>
<td>Sanok</td>
<td>Open-space rodent1</td>
<td>I. ricinus</td>
<td>A, G, F</td>
</tr>
</tbody>
</table>

1. – Striped field mouse (Apodemus agrarius), 2 – common vole (Microtus arvalis), A – Adult individual, P – The individual was feeding, G – The individual was not feeding, F – Female

C. burnetii culture

Culturing of the BGM cell line was carried out in the presence of MEM culture medium containing: 2 mM glutamine (Sigma-Aldrich, USA), 1% Non-essential amino acids (NEAA, Sigma-Aldrich, USA), 10% FBS (Fetal bovine serum, Sigma-Aldrich, USA), 100 µg/ml streptomycin and 100 µl penicillin (Sigma-Aldrich, USA) at 37°C in the presence of 5% CO₂.

RESULTS AND DISCUSSION

From 1126 ticks harvested in the selected regions of Lublin, Podkarpackie, Masovian, and Łódź Provinces, the presence of C. burnetii-specific IS1111 gene sequence was found in 15 (1.33%) cases (Tab. 1). Among the positive samples, five were acquired in Lublin Province (4 – I. ricinus, 1 – D. reticulatus), seven in Masovian and Łódź Provinces (6 – I. ricinus, 1 – I. hexagonum) and three in Podkarpackie Province (I. ricinus). In case of seven tick samples, the Ct fluorescence had the value of 31, for the next two it was above 35 cycles, and for others the signal was detected in the second round of real-time PCR. Among ticks harvested from domestic and livestock animals in 12 cases (3.1%), and in case of ticks found in open-space rodents in 3 cases (0.4%) we found the presence of marker sequences.

Khalli, 2011; Špitalská et al., 2003; Toledo et al., 2009; Hildebrandt et al., 2011; Sprong et al., 2012). In our studies only two isolates were recovered from all 15 BGM cell line cultures. Due to intracellular type of C. burnetii infection, the method of bacteria recovery is crucial – significant losses of material may be sustained during the isolation procedure. The digitonin, which has high affinity to PV racellular structure is the place of C. burnetii proliferation. On the other hand, bacterial confections (such as Borrelia spp.), noted mainly among samples obtained from ticks, disturbed some cell cultures and impaired C. burnetii recovery from the cells. Sonication method, which is an alternative option for C. burnetii isolation, was not used due to accompanying risk related to unintentional generation of biological aerosol (Cockrell et al., 2008). We used multi-copy IS1111 gene (7 to 110 per one cell of C. burnetii), which is a very convenient and sensitive genetic marker as a tool for C. burnetii screening in ticks (Klee et al., 2006; Bielawska-Dróżdż et al., 2013). Pathogens may be transmitted by number of ticks species, the most epidemiologically important species in Poland (Tylęska-Wierzbanska et al., 1996; Bielawska-Dróżdż et al., 2013). Not
only it is the most prevalent species, but also it has a significant range of hosts. This assumption was confirmed in the current study, showing that the most of infected ticks were *I. ricinus*. The meadow tick (*D. reticulatus*) may be taken into account as the second most predominant tick in the eastern Poland (Tylewska-Wierzbanowska and Chmielewski, 2010).

Currently, the role of ticks as vectors and reservoir of Q fever is disputable. Some researchers (Mediannikov et al., 2010; Tylewska-Wierzbanowska and Chmielewski, 2010; Sprong et al., 2012) claim that ticks, as vectors of *C. burnetii* do not play a significant role in disease transmission among animals and humans. Contrarily, Toledo et al. (2009) observed high number of infected individuals among *H.lusitanicum* and *D. marginatus* population, which support the hypothesis about their role in Q fever transmission. Also other authors claim that ornate sheep tick (*D. marginatus*) is the most significant vector of *C. burnetii* in the middle Europe (Hildebrandt et al., 2011). Results of the current study demonstrated a low proportion of infected ticks (1.33%), which may confirm the first thesis. Nonetheless, ticks may be an important factor in *C. burnetii* transmission among wild animals, such as rodents, lagomorphs or wild birds.

(Fard and Khalili, 2011; Astobiza et al., 2011).

**CONCLUSION**

In our research, a low percentage of *C. burnetii* infected ticks (1.33%) indicated by the probe specific real-time PCR for multi-copy insertion sequence (IS1111), reveals limited role of these arthropods in Q fever transmission in Poland.

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REFERENCES


