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ORIGINAL PAPER

Assessment of *Coxiella burnetii* presence after tick bite in north-eastern Poland

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Abstract

Purpose The aim of the study is to assess anti-*Coxiella burnetii* antibodies presence in inhabitants of north-eastern Poland, to assess the risk of Q fever after tick bite and to assess the percentage of co-infection with other pathogens.

Methods The serological study included 164 foresters and farmers with a history of tick bite. The molecular study included 540 patients, hospitalized because of various symptoms after tick bite. The control group consisted of 20 honorary blood donors. Anti-*Coxiella burnetii* antibodies titers were determined by *Coxiella burnetii* (Q fever) Phase 1 IgG ELISA (DRG International Inc. USA). PCR was performed to detect DNA of *C. burnetii*, *Borrelia burgdorferi* and *Anaplasma phagocytophilum.*

Results Anti-*C. burnetii* IgG was detected in six foresters (7.3%). All foresters with the anti-*C. burnetii* IgG presence were positive toward anti-*B. burgdorferi* IgG and anti-TBE (tick-borne encephalitis). Anti-*C. burnetii* IgG was detected in five farmers (6%). Four farmers with anti-*C. burnetii* IgG presence were positive toward anti-*B. burgdorferi* IgG and two with anti-TBE. Among them one was co-infected with *B. burgdorferi* and TBEV. Correlations between anti-*C. burnetii* IgG and anti-*B. burgdorferi* IgG presence and between anti-*C. burnetii* IgG presence and symptoms of Lyme disease were observed. *C. burnetii* DNA was not detected in any of the 540 (0%) patients.

Conclusions *C. burnetii* is rarely transmitted by ticks, but we proved that it is present in the environment, so it may be a danger to humans. The most common co-occurrence after tick bite concerns *C. burnetii* and *B. burgdorferi.*

Keywords *Coxiella burnetii* · *Borrelia burgdorferi* · *Anaplasma phagocytophilum* · Serology · PCR

Introduction

Ixodes ricinus ticks, which are common in Poland, transmit several different pathogens: *Borrelia burgdorferi* sensu lato (*Borrelia* species), tick-borne encephalitis virus (TBEV), *Anaplasma phagocytophilum* (*A. phagocytophilum)* and *Babesia* species (*Babesia* spp.). They may also transmit other less known pathogens, such as *Coxiella burnetii* (*C. burnetii*), *Rickettsiales or Candidatus Neoehrlichia mikurensis* [1].

Gram-negative *C. burnetii* is responsible for zoonosis called Q fever, which most often manifests as flu-like illness with fever, general malaise, severe headache, muscle pain, loss of appetite, dry cough and chills. Also other symptoms such as: vomiting, diarrhea, nausea, endocarditis and pneumonia may occur. The reservoir of bacteria is: cattle, sheep, goats, dogs and other domestic animals.

In vertebrates, infection affects reticulo-endothelial, vascular endothelial cells or erythrocytes. *C. burnetii* bacteria were found in the gut and hemolymph of ticks, suggesting that they are a natural vector [2]. Infection occurs most often by inhaling aerosols contaminated with particles of feces, urine or milk of animals. The bacterium is also rarely transmitted to humans by ticks. *C. burnetii* was detected in more than 40 tick species, mainly in those belonging to the genus *Ixodes, Rhipicephalus, Amblyomma* and *Dermacentor.*

Karol Borawski and Justyna Dunaj contributed equally to the manuscript.

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Moreover, ticks may play a role of a vector of Q fever. *C. burnetii* bacteria have the ability to penetrate their digestive tract and multiply in epithelial cells of the intestine and in the midgut. Ticks can transmit bacteria through saliva and feces, the latter contaminating the skin and fur of the animal. However, tick's participation in the epidemiological process in many cases is limited to the passive spread of the pathogen in the environment and is not a necessary link in the epidemical chain [3].

The incubation time of Q fever is 9–40 days. Q fever is considered one of the most contagious diseases in the world, because only one bacterium can be sufficient to cause infection in susceptible patients [4].

Q fever has gained renewed attention after the large outbreak in the Netherlands in 2007–2009, indicating its importance as an emerging public health threat [5].

The data concerning *C. burnetii* epidemiology in Poland is scarce. There are only individual cases of patients with Q fever described in Poland. The last epidemics occurred in 1983 in the Lublin region, then in 2005–2011 25 individuals presented clinical symptoms of acute Q fever and DNA of *C. burnetii* was found in 8 human blood samples obtained from 3 farm workers and 5 family members [6]. According to the annual report of the National Institute of Public Health–National Institute of Hygiene, only a single case of Q fever has been recognized since 2010.

The main aim of the study is to assess the prevalence of anti-*C. burnetii* antibodies in the inhabitants of the Podlaskie Voivodship (Fig. 1), which is considered as endemic area of tick-borne diseases. Another goal is to assess the risk of Q fever development after tick bite. Moreover, we assessed the percentage of co-infection with *C. burnetii* and other pathogens.

Fig. 1 The location of Podlaskie Voivodship

Materials and methods

Material and patient group (Fig. 2)

The study was divided into two separate steps:

– Serological, which assessed the prevalence of anti-*C. burnetii* antibodies in people endangered by tick bites, but without symptoms;
– Molecular, which aimed at detecting *C. burnetii* DNA in patients with symptoms suggestive of Q fever.

The serological study included 184 people divided into three groups:

- Group Ia—82 foresters from the Podlaskie Voivodship remaining in cooperation with the Department of Infectious Diseases and Neuroinfections: 4 women and 78 men;
- Group IIa—82 patients—farmers living in the Podlaskie Voivodship, hospitalized in the Department of Infectious Diseases and Neuroinfections in 2015–2018 due to various symptoms after tick bite: 36 women and 46 men;
- Group III—control group—20 honorary blood donors from the Regional Centre for Transfusion Medicine, Bialystok, Poland, who have never been bitten by ticks.

The molecular study included 560 people:

- Group Ib—540 patients, hospitalized in the Department of Infectious Diseases and Neuroinfections in 2015–2018

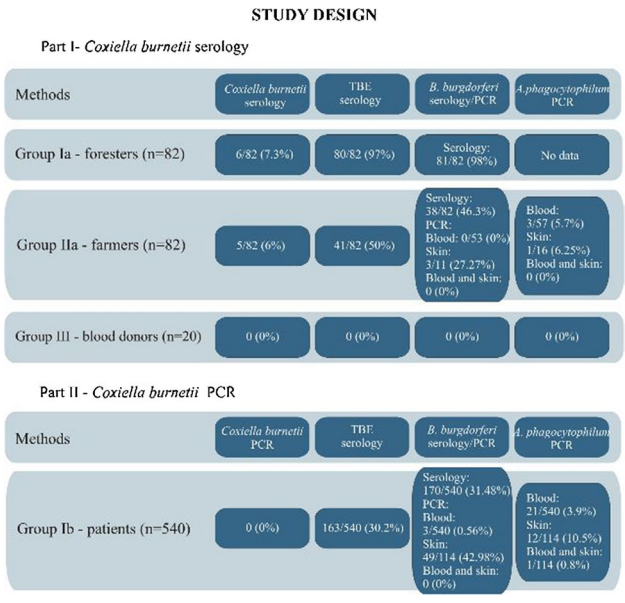

Fig. 2 The categories of patients, the tests performed and the obtained results

because of various symptoms after tick bite. For molecular study, the control group was the same as for serological tests (20 blood donors—Group III).

Blood samples for molecular and immunoserological diagnostics were collected from patients. Clinical analysis of all patients was performed based on medical documentation and a personal questionnaire form prepared specifically for this study. We recorded the presence or absence of the presenting symptomatology including fever, headache, dizziness, musculoskeletal pain, back pain, neck pain, malaise, photophobia, nausea/vomit, cranial nerve paresis and duration of fever determined by review of clinical notes made by physicians following diagnosis.

The study was approved by the Bioethical Commission of Medical University of Bialystok (R-I-002/329/2018). All patients signed a written informed consent form for the study.

Methods: serological analyses

Anti-*Coxiella burnetii* antibodies titers were determined by ELISA: *C. burnetii* (Q fever) Phase 1 IgG ELISA (DRG International Inc. USA).

TBE was confirmed by detection of specific antibodies with enzyme-linked immunosorbent assay (ELISA) using the kit of Virion/Serion (Wurzburg, Germany) according to the manufacturer's instructions.

NB (neuroborreliosis) was confirmed by detection of specific anti-*B. burgdorferi* antibodies in serum and CSF with enzyme-linked immunosorbent assay (ELISA) using the kit of *Borrelia* IgM, IgG (DRG, Germany) confirmed by Western blot (DRG, Germany) according to the manufacturer's instructions and with intrathecal synthesis of anti-*B. burgdorferi* antibodies (EcoLine; Virotech).

Methods: DNA extraction

DNA was extracted with the Qiagen DNAeasy Blood and Tissue Mini kit. Whole blood was gently mixed (200 µl) and skin biopsies were enzymatically digested at 56 °C before extraction. Purified DNA isolates were frozen at − 20 °C.

Methods: molecular techniques (PCR)

Molecular analysis for *Coxiella burnetii*

The Hum PCR Coxiella burnetii detection kit (Bioingentech Ltd., Chile) for in vitro diagnostics was used for *C. burnetii* molecular detection.

One-tube type of conventional qualitative PCR was performed. To each PCR tube with 2.7 µl of HumPCR *C. burnetii* premixture, 6 µl of Free Water and 2 µl of sample DNA, negative or positive control, was added. Internal control samples was prepared with 2.7 µl of Internal Control Mixture, 6 µl of Free Water and 2 µl of sample DNA. The total volume of the PCR mixture with template DNA was 10.7 µl. To all PCR tubes on the top of the mixture, 8 µl of Mineral Oil was added.

PCR was performed on the SensoQuest LabCycler (SensoQuest, Germany) in compatibility to Bioingentech's instruction: initial denaturation at 94 °C for 2 min, amplification for 30 cycles (denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 30 s) and final extension at 72 °C for 5 min.

Amplification products were separated on 1.5% agarose gel (Sigma-Aldrich, Germany) containing Midori Green (5 µg/1 ml; Nippon Genetics, Japan) in electrophoresis at 100 V for 45 min. Amplicons were visualized by means of UV illumination in Gel Logic System 100 (Kodak Imaging System, Inc., USA). Positive samples were those with amplification products with the length of 340 bp fragments of *C. burnetii* gene. Additionally, 140 bp-long fragments of internal standard were detected in all samples.

Molecular analysis for *B. burgdorferi* and *A. phagocytophilum*

Molecular detection of *Borrelia* species was performed by using *The Borrelia burgdorferi PCR kit* (GeneProof, Czech Republic) which amplifies a specific DNA sequence of a 276 bp fragment of the flagellin encoding gene by a nested one-tube PCR on the SensoQuest LabCycler (SensoQuest, Germany) [7]. To further confirm the results, PCR amplification was performed with a real-time PCR assay targeting the *16S rRNA* gene as previously described [8].

For *A. phagocytophilum* in vitro detection, a gene fragment encoding a part of the small ribosomal *16S rRNA* subunit (546 bp) was amplified (Blirt-DNA Gdańsk, Poland). Analyses were conducted by nested PCR on a SensoQuest LabCycler (SensoQuest, Germany) [7].

Methods: statistical analysis

The statistical analysis was performed using the Statistica 10.0 program. Kruskal–Wallis and Spearman rank correlation testes were used. P values <0.05 were considered statistically significant.

Results

Results of serological tests

Among 184 people included in the study, 82 were foresters: 4 women and 78 men, and 82 patients were farmers hospitalized in the Department of Infectious Diseases and

Neuroinfections in 2015–2018 due to various symptoms after tick bite: 36 women and 46 men.

Group Ia

Anti-*B. burgdorferi* IgG antibodies were identified in 81 (98%) of the 82 foresters included in this study (ELISA confirmed with Western blot). IgM anti-*B. burgdorferi*-specific antibodies were present in serum of ten (12.2%) patients. In 80 (97%) patients, anti-TBE antibodies were detected after the vaccination (Fig. 2).

Anti-*C. burnetii* IgG were detected in six foresters (7.3%). All foresters with the anti-*C. burnetii* IgG presence were positive toward anti-*B. burgdorferi* IgG and anti-TBE.

Group IIa

Forty-one patients were hospitalized because of TBE, while 38 because of Lyme disease: 10 with EM and 10 with NB and 18 with musculoskeletal symptoms (among them 8 were co-infected with *B. burgdorferi* and tick-borne encephalitis virus). In three patients, anaplasmosis was diagnosed.

Anti-*C. burnetii* IgG were detected in five farmers (6%). Four farmers with anti-*C. burnetii* IgG presence were positive toward anti-*B. burgdorferi* IgG and two with anti-TBE (Fig. 2). Among them, one was co-infected with *B. burgdorferi* and tick-borne encephalitis virus. No co-infection with *A. phagocytophilum* was observed.

Group III

In the control group, no anti-*C. burnetii* IgG, anti-*B. burgdorferi* IgG, anti-TBE IgG and DNA of *A. phagocytophilum* were detected.

No statistical significance was detected between the frequency of anti-*C. burnetii* IgG between both groups; however, there were differences between group Ia and CG, IIa and CG ($p < 0.05$).

Analysis of correlations

The most common symptoms reported by patients from group IIa were headache—57.3% (47/82) and fever (over 38 °C)–51.2% (42/82). Less common manifestations were: nausea—25.6% (21/82) and vertigo—18.2% (21/183). Symptoms which appeared rarely were vomit—12.1% (10/82), facial nerve paresis—7.3% (6/82) and muscle pain—3.6% (3/82). Fever lasted from 1 to 20 days (mean: 4.04 ± 4.03; median 3 days). No correlation between symptoms reported by patients and anti-*C. burnetii* antibodies was detected.

IgM anti-*B. burgdorferi*-specific antibodies were present in the serum of 25 (30%) patients and IgG antibodies–in 34 (41%) patients. Seventeen (21%) patients were positive in both classes.

There was correlation between anti-*C. burnetii* IgG presence and anti-*B. burgdorferi* IgG presence ($r = 0.974$; $p < 0.05$) and between anti-*C. burnetii* IgG presence and symptoms of Lyme disease ($r = 0.231$; $p < 0.05$).

No correlation between anti-*C. burnetii* IgG presence and anti-TBE antibodies presence was seen.

Results of molecular analyses

In none of the 540 (0%) patients, the DNA of *C. burnetii* was detected using conventional PCR. The results of molecular tests for *B. burgdorferi* and *A. phagocytophilum* infection are presented in Fig. 2.

Discussion

In our study, we concentrated on tick bite as a risk factor of *C. burnetii* infection. This is not the main route of Q fever spread and therefore our results should be interpreted with care.

The results of our study indicate the possibility of *C. burnetii* infection after tick bite in Poland. Although we have not detected *C. burnetii* DNA in the samples, the presence of antibodies against this pathogen in 11 patients confirms the circulation of *C. burnetii* in the environment. The risk of symptomatic infection seems to be minimal; however, it has to be taken into consideration in the differential diagnosis of fever after tick bite, as the symptoms of Q fever are nonspecific. It is worth underlining that the study was performed in an area where annual incidence of tick-borne diseases such as TBE or LB is 16–28 times higher than in the whole country throughout the years. Moreover, the population included in the study comprised foresters and farmers, so people were occupationally exposed to frequent tick bites. So even in the endemic area for tick-borne diseases and in the group of patients with frequent tick bites, the risk of Q fever is low. It reflects well the data from the National Institute of Health, where registered incidence on Q fever is low—one case in 2014 and five cases in 2009 [9].

Studies performed in specific regions of Poland indicate that *C. burnetii* prevalence varies in different studies and throughout the country.

Szymańska-Czerwińska et al., tested 2082 serum samples taken from 936 goats, 933 cattle, 89 sheep and 124 horses, including various horse breeds, and revealed that Polish horses were seronegative, while in the populations of cattle and small ruminants, seropositive animals were present. The percentage of seropositive cattle, goats and sheep was 4.18%, 6.3%, and 13.48%, respectively [10].

The prevalence of *C. burnetii in* ticks ranged from 0.45 to 3.45% in north-western Poland [11] to 15.9% in south-eastern Poland [12], although the samples, isolated from ticks collected from places where local outbreaks occurred, accounted

for 33.3%, which may suggest that ticks can be an important vector for *C. burnetii* [13].

The results of studies performed on humans were equivocal. Wójcik-Fatla et al. detected the presence of *Coxiella burnetii* antibodies in 16 out of 373 (4.3%) veterinarians [14]. The study included people from 12 districts of Poland. On the other hand, Szymańska-Czerwińska et al. examined 151 farmers from six regions of Poland and acquired significantly different results. The samples tested with indirect fluorescent antibody (IFA) were positive in 31.12%, with ELISA 39.07% and with complement fixation test (CFT) 15.23%. Of the three test types, IFA results were considered the most sensitive. Real-time PCR confirmed the presence of DNA specific for *C. burnetii* in ten patients [15]. Moreover, the authors analyzed patients with direct contact with animals and our study concentrated on patients after a tick bite.

In central Poland *C. burnetii* IgG antibodies have been found in sera of 4.4% of the farmers and in 12% of waste collectors [16].

In south-eastern Poland (Lublin area), the prevalence of anti-*C. burnetii* antibodies among hunters was higher—16.2% of 104 sera [17]. Another study conducted on a group of farmers inhabiting this region yielded similar results (17.8% of sera positive) [18].

Our study showed the correlation between anti-*C. burnetii* IgG presence and anti-*B. burgdorferi* IgG presence, which might suggest that these pathogens are transmitted by the same tick and lead to co-infection.

In persons with occupational risk of tick bites—forestry workers and farmers—anti-*B. burgdorferi* antibodies are more frequently detected and they are more often co-infected with various tick-borne pathogens than persons from the control group, as shown in previous studies [19]. Clinical analysis of symptomatic cases showed no infection caused by *C. burnetii* among 540 analyzed patients. It seems that although north-eastern Poland is an endemic region for tick-borne diseases, the prevalence of *C. burnetii* in humans is rather low and the possibility of Q fever development after tick bite is even lower.

Conclusions

1. Seroprevalence *of C. burnetii* antibodies in north–eastern Poland is low, but this bacterium is present in the environment and may cause disease in humans.
2. The most common co-occurrence after a tick bite concerns *C. burnetii* and *B. burgdorferi.*
3. Q fever development after a tick bite in north-eastern Poland is very unlikely.

Authors contribution KB, AMM and JD—planned and conducted the study; wrote manuscript. AMM—organized the study; PCz, JZ and SG—collected samples from patients; RŚ—performed serological analyses; AŻ—supplied blood from donors.

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Compliance with ethical standards

Conflict of interests None.

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