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## Q fever outbreaks in Poland during 2005–2011

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**Background:** Q fever is a health problem affecting humans and animals worldwide. In Poland, previous studies have pointed to 2 sources of outbreaks of the disease: the importation of infected animals and their products, and natural domestic foci. In the last decade, 5 outbreaks have occurred in cattle farms in south Poland in Malopolskie, Podkarpackie, Opolskie, and Silesian provinces. The aim of this study was to characterize the Q fever foci in Poland.





**Material/Methods:** A total of 279 individuals were included. Levels of serum IgM and IgG antibodies to phase I and II *C. burnetii* antigens were assayed by indirect immunofluorescence method. Bacterial DNA from all specimens were detected with PCR with primer pairs specific to the htpAB-associated repetitive element, and amplicons were sequenced.

**Results:** Infection was recognized in 67 individuals out of 279 tested in all foci. Twenty-five individuals presented clinical symptoms of acute Q fever. DNA of *C. burnetii* was found in 8 human blood samples obtained from 3 farm workers and 5 family members.

**Conclusions:** The described outbreaks demonstrate that the main source of human infections in Poland is infected cattle.

**Key words:** *Coxiella burnetii* • Q fever • outbreaks

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## Background

The *Coxiella burnetii* bacteria, the etiologic agent of Q fever, is an obligatory intracellular parasite. The disease occurs worldwide in various vertebrates and creates a health problem for humans. It has a wide range of acute and chronic manifestations. Most symptomatic patients develop a non-specific flu-like illness, and about 5% require hospitalization due to atypical pneumonia and/or hepatitis. About 60% of infected persons are asymptomatic. However, chronic Q fever can occur in about 1% of both symptomatic and asymptomatic groups of individuals, mainly as infective endocarditis [1]. Frequent exposure to *C. burnetii*-infected animals and their products is the main risk factor of Q fever affecting various professional groups, such as veterinarians, animal farmers, abattoir and tannery workers, and hunters [1,2].

Analysis of Q fever outbreaks in Poland in the past has shown 2 sources of the infection for humans. The first source was the importation of infected animals and their products, and the second source was farm animals, mainly cows. Animal infections were probably transmitted from natural domestic foci, originating from wild animals [1–3]. The first outbreak of Q fever in Poland was recorded in 1956. Infection was recognized and confirmed with serological methods in 63 farm workers. Epidemiological investigations revealed that the source of infection was a sheep flock imported from Romania. Tested wool samples from these animals were the source of the secondary outbreaks in a Zootechnical Institute laboratory in Cracow (20 cases) [4]. From 1956 to 2005, several outbreaks of Q fever were described. In the 1990s, 3 epidemics of Q fever were recognized. In 1992 an outbreak of Q fever was detected at a farm near Jawor in the Legnica district. *C. burnetii* infection was recognized serologically in 25 individuals (27 tested). It was found that cattle were the source of human infections. *C. burnetii* strain was isolated from bull semen. In the same year, Q fever was recognized among 18 workers of a tannery in Myslenice near Cracow. Antibodies to *C. burnetii* were detected in all workers (18 individuals) encountering imported hides. In 1993 several cases of Q fever were observed among people living in various regions of the country. Epidemiological investigations revealed that the infected patients were seasonal workers employed during the sheep shearing season in Spain. Specific serum antibodies reached a titer of 512 and strains of *C. burnetii* were isolated from the urine and semen of 2 patients [5].

In Poland, human cases of Q fever are notifiable. Information on animal cases is sent by the County District Veterinarian to the State Sanitary Inspectorate, and all individuals contacting infected animals are examined. Elimination of the source of infection is based on cooperation of both human and veterinary health services and is regulated by a Decree of the Ministry of Health.

Q fever is an underestimated infectious disease due to non-specific symptoms or course of the disease without any symptoms. However, asymptomatic infections do not exclude chronic Q fever in the form of endocarditis, valvular damage, hepatitis, and neurological abnormalities. To better understand Q fever, it is necessary to monitor the disease in humans contacting infected animals. The aim of this study was to present the results of research carried out in outbreaks of Q fever in Poland in the last decade.

## Material and Methods

### Description of outbreaks

Between 2005 and 2010, 5 outbreaks of Q fever in cattle farms located in the southern Poland were registered by the County District Veterinarian. *C. burnetii* infection was detected by serologic methods by veterinary services in all foci after cows' miscarriages. All tested persons were referred for testing by health professionals from the State Sanitary Inspectorates.

I. In May 2005 an outbreak of Q fever was recognized in dairy cattle farms in Malopolska province. The source of infection was not clear. At the beginning, an imported herd of cows was suspected, but the results of serological studies of the animals did not confirm this hypothesis.

Testing was done on 148 individuals, including 76 farm employees, 52 family members and incidental farm visitors, and 20 veterinarians. Four persons presented clinical symptoms of acute Q fever. In a herd of 170 cows, 137 (including 84 cows and 53 calves) were examined. Six placentas were examined by PCR.

II. The second outbreak of Q fever occurred in dairy cattle farms in Podkarpackie province in August 2008. In a herd consisting of 270 cows, 3 cows had miscarriages, and another 2 cows had miscarriages 3 weeks earlier.

Testing was performed on 27 farm workers, 69 family members or occasional visitors, and 8 veterinarians. Twenty persons presented symptoms of acute Q fever. A group of 27 individuals was tested 6 times at intervals of 2 weeks to 3 months. Nineteen specimens of bull semen were collected for examination.

III. In September 2009 a third outbreak appeared in Opolskie province. After abortion by 1 cow, serological tests were performed and Q fever was diagnosed among the animals. The herd consisted of 190 cattle. Eight persons directly engaged in handling the cattle were examined. One person presented atypical pneumonia.

**IV.** In November of 2010, infected cows were detected in Silesia province. A herd of dairy cattle consisting of 170 animals was affected. Nine people (7 farm workers and 2 veterinarians) were assayed. Moreover, 17 suspect animals were tested.

**V.** In December of 2010, a fifth outbreak was recognized, also in Silesia province.

Four individuals working at the farm and 6 veterinarians with direct contact with the infected herd were examined. Ten cows from the herd were also tested.

### Serological tests

Levels of serum IgM and IgG antibodies to phase I and II *C. burnetii* antigens were assayed by indirect immunofluorescence method with *Coxiella burnetii* I-II IFA IgG/IgM/IgA kit (Vircell, Spain) in all serum samples. Results were interpreted as positive in serum with titers  $\geq 40$  for IgM and IgA and  $\geq 256$  for IgG in a single sample or an at least 4-fold increase in antibody titers in 2 collected samples taken at intervals of at least 2 weeks.

In animals, levels of total antibodies (IgS) to phase I and phase II *C. burnetii* antigens were determined using the IFA commercial kit (Vircell, Spain) with some modification. Rabbit anti-cow immunoglobulins conjugated with FITC (Dakopatts, Denmark) were used in 1:40 dilution with phosphate-buffered saline. A titer of 128 or higher was considered as positive.

### PCR

Buffy coat from human and cattle EDTA blood samples, bull semen, and placentas were tested. Bacterial DNA from all specimens was extracted with the QIAamp Tissue kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was stored at  $-20^{\circ}\text{C}$  until testing. DNA samples were amplified with primer pairs specific to the *htpAB*-associated repetitive element [6]. The reaction mixtures of 50  $\mu\text{l}$  contained 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.1% gelatin, 200  $\mu\text{M}$  dNTPs, 50 pmol of each primer, and 1.5 U Taq DNA polymerase (Perkin-Elmer Cetus, USA). An aliquot of 5  $\mu\text{l}$  of DNA template was added to each reaction mixture. The cycling conditions were 5 min at  $95^{\circ}\text{C}$ , followed by 40 cycles of 1 min at  $95^{\circ}\text{C}$ , 1 min at  $57^{\circ}\text{C}$ , 1 min at  $72^{\circ}\text{C}$ , and 7 min at  $72^{\circ}\text{C}$ . PCRs were performed in a Mastercycler ep gradient apparatus (Eppendorf AG, Germany). Each run of PCR testing included DNA from *C. burnetii* Nine Mile strain and Henzerling strain as positive controls and water as a negative control. All amplicons were analyzed by electrophoresis in 1.5% agarose gel stained with ethidium bromide.

The QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany) was used to purify PCR products, according to the

manufacturer's protocol with some modifications. DNA was eluted with deionized water (instead of 10 mM Tris-Cl, pH=8.5 buffer).

All amplicons were sequenced with an ABI 377 DNA Analyzer (Applied Biosystems, USA) according to the manufacturer's recommendations. All sequences were edited using Autoassembler software (Applied Biosystem, USA) and identified using BLAST software and comparison with sequences available in GenBank.

### Results

Human blood samples were collected from 121 farm workers who had contact with infected animals, 122 family members and occasional visitors (students, inspectors, and seasonal workers), and 36 veterinarians taking care of infected cattle. Samples were taken and examined once in 279 persons, and at least twice in 141 persons, 1–4 weeks and 1–3 months after infection was recognized in animals (Table 1). The mean age of tested people was 43.7 years and the male to female ratio was 1.6. Among animals, 164 blood samples were collected from infected cattle herds from Malopolskie and from Silesian foci. Six placentas from aborted cows from a focus in Malopolskie and 19 semen samples from 9 bulls from Podkarpackie were tested.

**I.** At a cattle farm in Malopolskie province, 148 individuals were tested. At first testing, specific *C. burnetii* antibodies were found in 26% (39/148) of persons in titers ranging from 20 to 80 in phase I/II IgM and from 64 to 1024 in phase I/II IgG. Among farm workers, veterinarians, and incidental visitors, 35% (23/76), 55% (11/20), and 10% (5/52) of individuals were seropositive, respectively. Sixteen seropositive individuals were tested twice (1 month later). Antibodies were detected in 56% (9/16) of individuals. DNA of *C. burnetii* was found in 8 human blood samples obtained from 3 farm workers (2 of them had IgG phase II antibodies in titers of 32, and 1 in titer of 256) and 5 family members (4 seronegative and 1 with IgG phase II antibody titer of 32) (Table 2). The specificity of 4 positive results was confirmed by sequencing. A total of 89 nucleotide positions showed 98% nucleotide identity between sequences of *htpAB*-associated repetitive element of detected DNA and *C. burnetii* strains: RSA 331 (accession number CP000890.1) and RSA 493 (accession number AE016828.2). Because of the small amounts of DNA (weak band), 3 samples were not confirmed by sequencing.

We found phase II *C. burnetii* antibodies in 62% (85/137) of tested cattle, including 62 of 84 (74%) adult cows and 23 of 53 (43%) of their offspring. DNA of *C. burnetii* was not found in any cow's blood sample or placenta.

**II.** In the second outbreak (Podkarpackie province), specific *C. burnetii* antibodies were found in 18% (19/104) of all tested

**Table 1.** Number (percent) of seropositive individuals in all outbreaks in first and control (after one to four months) testing.

Individuals embraced by the outbreak	Serological testing in outbreaks (No. of seropositive/No. of tested)				No. of symptomatic cases <sup>#</sup>
	First testing <sup>**</sup>		Control testing <sup>***</sup>		
Milkmaids	3/5	(60%)	4/5	(80%)	3
Farm workers*	32/116	(28%)	11/49	(23%)	9
Veterinary staff	16/36	(44%)	5/12	(42%)	8
Incidental visitors	10/86	(12%)	10/42	(24%)	1
Raw milk consumers <sup>##</sup>	7/36	(19%)	7/36	(19%)	5
<b>Total</b>	<b>67/279</b>	<b>(24%)</b>	<b>38/141</b>	<b>(30%)</b>	<b>25</b>

\* Animal service with everyday contact with animals; \*\* testing 1–4 weeks after infection was recognized in animals; \*\*\* testing 1–3 months after infection was recognized in animals; # all presented acute Q fever symptoms such as: myalgia, arthralgia, headache, cough, asthenia, rigors, perspirations, stomach ache; ## family members.

**Table 2.** *C. burnetii* DNA detection in human blood samples in Malopolskie outbreak.

Individuals embraced by the outbreak	Titers of phase II <i>C. burnetii</i> antibodies*		PCR
	IgM	IgG	
Family member	Negative	32	Positive
Family member	Negative	Negative	Positive
Family member	Negative	Negative	Positive
Family member	Negative	Negative	Positive
Family member	Negative	Negative	Positive
Farm workers	Negative	32	Positive
Farm workers	Negative	32	Positive
Farm workers	Negative	256	Positive

\* Antibodies detected with IFA in sera collected at the same time with EDTA blood samples. All sera were negative with phase I *C. burnetii* antigens.

persons at first testing, 24% (25/103) 2 months later, and in 19% (20/104) 4 months after the outbreak was detected. Antibodies were found in 4 of 5 (80%) milkmaids, 15% (4/22) of other farm workers contacting the animals, 63% (5/8) of veterinarians, 6% (2/33) of incidental visitors and family members, and 19% (7/36) of raw milk consumers. As the result of treatment, the number of seropositive persons decreased in the groups of incidental visitors and family members to 6% (2/33) and in raw milk consumers it decreased to 14% (5/36). Serum samples of 27 seropositive individuals (with or without symptoms) were tested 6 times (every 2 months) to monitor the level of specific antibodies.

*C. burnetii* DNA was not found in any human blood samples or in bull semen.

III. In Opolskie province, 2 of 8 farm workers showed significant levels of specific serum antibodies. The following titers of IgG phase I and phase II were observed in asymptomatic patients: 4096 and 512, and IgA phase I and phase II – 384 and 756, respectively. Level of IgG phase II in serum of the second farm worker, with atypical pneumonia diagnosed 4 weeks earlier, was 2048. IgM antibodies were not present. A 2- to 4-fold decrease of the antibody levels was observed after antibiotic therapy 2 months after the first testing. *C. burnetii* DNA was not found in any tested sample.

IV. In a Silesian province outbreak in November of 2010, antibodies to *C. burnetii* were detected in 3 out of 7 farm workers. Phase II IgG antibodies at titer 64 were detected. Among the tested cattle, 25% (4/16) were positive with IFA. *C. burnetii* DNA was not detected in any tested sample from humans or cows.

**Table 3.** Epidemiologic data from survey in group of children with specific antibodies to *C. burnetii*.

Age	Risk factor	IFA test results		Profession of parents	
		IgM-phase II	IgG-phase II	Father	Mother
9	Raw milk consumer	Negative	128	Farm worker	Milkmaid
10	Raw milk consumer, visitor of the farm	48	512	Farm worker	Milkmaid
10	Visitor of the farm	20	64	Nd.	Veterinary assistant
15	Raw milk consumer, visitor of the farm	Negative	64	Veterinary assistant	Nd.
13	Raw milk consumer, visitor of the farm	Negative	64	Veterinary assistant	Farm worker
16	Raw milk consumer	Negative	64	Veterinarian	Nd.
15	Raw milk consumer	Negative	64	Veterinarian	Nd.
13	Visitor of the farm	Negative	64	Veterinarian	Veterinarian

Nd. – not done.

V. In December, in a farm in Silesian province, 2 farm workers of 3 tested and 3 veterinarians of 6 tested were seropositive. *C. burnetii* antibodies were detected in 22% (2/9) of tested cattle with IFA test. In studied human and cow blood samples, *C. burnetii* DNA was not found.

#### Group of children (all outbreaks)

Among all the individuals included in the study, 31 were children ranging from 1 to 16 years old. Eight of them (26%) had specific IgG phase II antibodies in titers of 64 to 512, including 6 children of the veterinary staff and 2 children of farm workers (Table 3).

## Discussion

From 2005 to 2010, five Q fever outbreaks were recognized in southern Poland. In total, 67 humans were seropositive. Twenty-five individuals presented symptoms of acute Q fever: myalgia (71%), arthralgia (58%), headache (45%), cough (38%), asthenia (38%), and rarely observed: rigors, perspirations, stomach ache (information from the State Sanitary Inspectorates) (Table 1).

The outbreaks described in Poland previously, as well as recently, show that for many years cows have been the main source of Q fever in humans. In southern Europe, the main sources of infection are goats and sheep. The main route of *C. burnetii* transmission from animals to veterinarian and farm workers was the inhalation of contaminated aerosols. Consumption of raw milk (7 seropositive from 36 individuals, including 5 symptomatic cases) was also the route of infection and risk factor of the disease in outbreaks reported in Poland.

From 1973 to 1991, a serological survey of Q fever among cattle and sheep was performed in Wielkopolskie province (western

Poland). From 1973 to 1985, over 28 000 blood samples derived from cattle and sheep were tested and all were seronegative. The first infected cattle were found in 1986 (5.5% seropositives) and in the next years the percentage increased to 12.3% to 17.5%. In 1986–1991, among 19 900 cattle tested, specific antibodies were detected in 2670 (13.2%) animals. At the same time, among 3500 sheep tested, only 2.3% were seropositive. In the same farms where infected cattle were living, sheep were free of infection [7,8].

According to Central Statistical Office data on farming, in 2009 there were 5.7 million cattle and 286 000 sheep in Poland (Statistical Yearbook of Agriculture 2010) [9]. Analysis of data shows that in 4 provinces in which Q fever occurred, 588 000 thousand cows (10% of total number) and 124 000 sheep (43%) were registered. In Malopolskie province, 196 000 cows and 95 000 sheep were raised (sheep to cow ratio, 1: 2). Despite the large number of sheep in this area, infections among sheep were not detected. A similar situation was observed earlier in Wielkopolskie province [7,8].

Six *C. burnetii* strains isolated in outbreaks between 1956 and 1992 in Poland were characterized genetically. Genotyping with MST method of 2 strains isolated from cattle placentas in Wielkopolskie province revealed that both belonged to sequence type 18 (ST18) group [10]. The highly discriminatory MLVA system showed differences between genotype panels of those strains and ST18 strains isolated from humans, goats, and sheep in neighboring countries [11,12]. These differences were detected in 5 among 10 tested *loci*. This may suggest that strains occurring in Poland have a special affinity for cows. Furthermore, in 2007 in Poland an epidemiological study was carried out to determine the prevalence of *C. burnetii* antibodies in 48 herds of goats located in different parts of the country. The survey did not reveal *C. burnetii* antibodies in animals, meaning that goat-to-human transmission of *C. burnetii* seems to be very rare in Poland [13].

Surveillance data from 2 neighboring countries, the Czech Republic and the Slovak Republic, show that *C. burnetii* has occurred mainly in cattle farms. Since the 1980s, the number of human infections has been decreasing, probably due to vaccination of cattle in those countries [14,15], but in the 1990s the resurgence in number of Q fever cases was associated with goats. In Germany from 1947 to 1999, 40 outbreaks of Q fever in humans were documented. Sheep were the source of Q fever in humans in at least 24 of the detected outbreaks and cattle were recognized as the source of infection in 6 outbreaks [16]. In Ukraine, due to a significant reduction in the number of livestock herds, from 1996 to 2005, only 38 cases of Q fever were recorded. Currently, outbreaks occurring in Ukraine are related to the circulation of *C. burnetii* in ticks and wild animals [17,18].

Acute Q fever patients are rarely reported in Poland. Chronic Q fever cases (endocarditis) in previous Q fever outbreaks have been reported sporadically [19,20]. It is not clear whether this is the result of the low pathogenicity of circulating strains or low detection and notification of the disease.

The results of the above-mentioned study with MST analysis of *C. burnetii* strains isolated in Poland between the 1960s and the 1980s, showed that all of them represent ST18 type (although MLVA typing showed their genotypic heterogeneity). The strain isolated in 1956 from sheep belongs to the unique VNTR and MST patterns, which since then have never been recognized among strains isolated in Poland. This may suggest that this imported strain was effectively eliminated due to sanitary intervention and elimination of infected animals. As a result of these actions, this particular strain did not subsequently spread in Poland, even though it is known that *C. burnetii* strains with ST16 profile occur in Europe (Romania, Germany and France) as well as on other continents. It is known that ST18, ST16, ST1, and ST4 correlate with acute form of the disease and they have never been isolated from chronic Q fever cases. In countries bordering Poland, there is a wide variety of isolated strains; for example in Germany there are at least 6 sequence types, and in Slovak Republic at least 3. So far, however, their pathogenicity has not been recognized [11,12,21].

The small number of human Q fever cases recognized in Poland may indicate that the disease is not fully diagnosed and is underestimated. Between 2005 and 2010, less than 100 acute cases were reported. At the same time, our studies of patients with serious cardiologic disorders referred to heart transplantation have shown infections of their valves and myocardium with *C. burnetii* (data prepared for publication). In France during 2005 to 2009, over 47 000 serum samples were tested. Among them, 1326 (3%) and 1083 (2.3%) samples were

positive and met the criteria of acute and chronic Q fever, respectively [22].

Between 1949 and 2005, chronic Q fever was reported the most often in France (264 cases), in the United Kingdom and Ireland (227 cases), in Spain (62 cases), and in Switzerland (21 cases). In 22 European countries, there were 583 cases in 2006 and 637 cases in 2007, and one-third of them were reported in more than 4 countries (European Centre for Disease Prevention and Control Annual Epidemiological Report on Communicable Diseases in Europe 2010. Stockholm: ECDC; 2010). MST genotyping of human isolates from France and Spain revealed the presence of strains belonging to ST8 (which is correlated with the chronic form of the disease) in addition to other sequence types of *C. burnetii* [21].

Human cases and epidemics occurred in rural communities and in urban areas located close to animal farms. In outbreaks in Malopolskie and Podkarpackie provinces, a group of incidental visitors consisted of those living in houses located closely to the farms. This situation was observed in a large outbreak (331 cases) that occurred in 2005 on a sheep farm bordering a residential area in Germany [23]. Since 2007, the Netherlands is faced with the largest outbreak of Q fever ever reported. In the last 4 years, over 4000 cases have been reported. One of the reasons is the recent expansion of high-intensity goat farming in highly populated areas [24].

Symptoms of acute Q fever often are non-specific. Proper recognition is based on serological methods. PCR should be performed together with serologic tests in the first weeks of the disease, especially in seronegative patients [6]. In our study, DNA of *C. burnetii* was detected in blood of 8 humans: 4 persons had no specific antibodies and the others had *C. burnetii* phase II antibodies, which are characteristic for the acute form of Q fever. Such early detection of infection enables immediate application of effective treatment.

## Conclusions

In southern and western European countries, sheep and goats are the sources of infection in humans, whereas in Poland cattle are the main source of Q fever. The sudden appearance of infections in southern Poland indicates that Q fever outbreaks can occur in other parts of the country and can become a nation-wide problem. This calls for research in other regions to assess the current epidemiological situation. The present studies should induce better cooperation between public health and veterinary services to prevent an emergence of new outbreaks in other regions of the country.

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