BRIEF COMMUNICATION AAEM

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PREVALENCE OF ANTIBODIES TO *COXIELLA BURNETII* AMONG FARMING POPULATION IN EASTERN POLAND

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Abstract: The presence of blood serum antibodies to *Coxiella burnetii* phase I antigen, indicative of chronic Q fever or convalescence, was found by indirect immunofluorescent test in 16 out of 90 examined farmers (17.8%) living in the Lublin region (eastern Poland) and in none of 30 examined urban blood donors living in the city of Lublin ($p < 0.05$). In both groups no antibodies to *C. burnetii* phase II antigen, indicative of acute Q fever, were detected. The frequency of antibodies among farmers was greater in females than in males (26.1% versus 9.1%, $p < 0.05$) and was significantly dependent on age ($\chi^2 = 146.42$, $p < 0.000001$) being greatest in the subgroup of farmers aged 41–55 (38.5%) while no positive reactions were found in young and elderly farmers (respectively, equal to or below 25 and above 56 years). The results suggest that the examined agricultural region could be in the past an epidemic area of Q fever, probably before 15–30 years.

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INTRODUCTION

Coxiella burnetii, the etiologic agent of Q fever, is a strictly intracellular, Gram-negative bacterium belonging to rickettsiae [9]. Q fever is a worldwide zoonosis occurring in ticks, birds and mammals. Humans are usually infected by contaminated aerosols from domestic animals, less often by tick bite [10, 11]. The disease may appear either in an acute form as a febrile flu-like illness, pneumonia, hepatitis and neurologic abnormalities ranging from headache to meningoencephalitis, or in a chronic form as endocarditis, hepatitis or a chronic fatigue syndrome [6, 9].

Human infections with *Coxiella burnetii* have been reported from many countries on all continents, including Poland [20, 21, 22]. To investigate an actual infection rate in eastern Poland, a study of seroprevalence to *Coxiella burnetii* was conducted among farming population in the Lublin region compared to urban blood donors.

MATERIALS AND METHODS

Examined population. Blood serum samples drawn from 90 farmers living in 11 villages located in the Lublin region (eastern Poland) were examined for the presence of antibodies to *Coxiella burnetii*. The population under study consisted of 44 males and 46 females, aged 43.8 ± 14.6 yrs (range 15–75 yrs). 76 out of 90 farmers (84.4%) kept farm animals, mostly pigs (64 farmers) and cows (41), fewer kept poultry, rabbits, horses, goats and sheep (respectively 8, 6, 3, 2, 1). 33 farmers reported past or present respiratory disorders (36.7%) which in 14 cases were work-related.

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Serum samples from 30 healthy urban blood donors living in the city of Lublin were examined as a reference group. The reference group consisted of 22 males and 8 females, aged 35.9 ± 9.8 yrs (range 21–55 yrs).

Examination of sera for the presence of antibodies to *Coxiella burnetii*. The presence of IgG antibodies to phase I and phase II *Coxiella burnetii* antigens was detected with the use of the indirect immunofluorescence test kit (MRL Diagnostics, Cypress, 90630 California, USA). The test was carried out in two stage "sandwich" procedure. In the first stage, examined sera were diluted and added to slide wells containing *C. burnetii* phase I and phase II antigen spots. Following incubation and washing, in the second stage, wells were overlaid with fluorescein-labeled goat antibody to human IgG that reacted with antigen-antibody complexes. After washing, drying and mounting, slides were viewed with a fluorescence microscope. The presence of bright apple-green fluorescent rickettsiae at the serum dilution 1:16 or higher was considered as a positive result of the test.

RESULTS

In 16 out of 90 examined farmers (17.8%) the presence of antibodies to *Coxiella burnetii* phase I antigen was found, indicative of chronic infection or convalescent phase of Q fever. Antibodies were not detected in 30 examined urban blood donors living in the city of Lublin and the difference between the groups of farmers and urban dwellers proved to be statistically significant (t-test, $p < 0.05$) (Tab. 1). In both groups no antibodies to *C. burnetii* phase II antigen, indicative of acute infection, were detected.

The frequency of antibodies among farmers was greater in females than in males (26.1% *versus* 9.1%) and the difference was statistically significant (t-test, $p < 0.05$) (Tab. 1). A highly significant relationship was found between the age of examined farmers and the presence of antibodies to *Coxiella burnetii* ($\chi^2 = 146.42$, $p < 0.000001$). The antibody response was found only in middle-aged farmers, being greatest in the subgroup of farmers aged 41–55 (38.5%) and about twofold lower (20.0%) in the subgroup aged 26–40 years (Fig. 1). No positive reactions were found in young and elderly farmers (respectively, equal to or below 25 and above 56 years).

Table 1. Seropositive reactions to *Coxiella burnetii* (phase I) in farming population of eastern Poland *versus* urban blood donors.

Group	Gender	Number of persons (n)	Number of positive reactions (percent)
Farming population	Males	44	4 (9.1)
	Females	46	12 (26.1)[#]
	Total	90	16 (17.8)[*]
Urban blood donors (reference group)	Males	22	0
	Females	8	0
	Total	30	0

[#] significantly greater compared to males ($p < 0.05$);
[*] significantly greater compared to urban blood donors ($p < 0.05$).

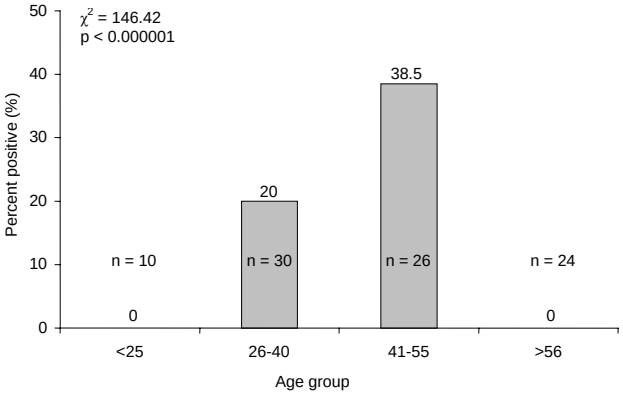

Figure 1. Seropositive reactions to *Coxiella burnetii* (phase I) in Polish farmers, depending on age.

No significant difference could be found between the farmers who kept farm animals and those who did not, as well as between those with and without respiratory disorders (t-test, $p > 0.05$).

DISCUSSION

The seroprevalence of *Coxiella burnetii* infection found among farming population in eastern Poland was higher compared to seroprevalence found among people exposed to farm animals in Turkey [3] and Ukraine [8]; - similar to that in India [23]; - lower compared to that in Zimbabwe [7], Sweden [2], Italy [19], and the Netherlands [4]; - and distinctly lower compared to that in the Q fever hyperendemic areas in Spain [12, 13, 14] and France [17]. The results suggest that the examined agricultural region could be an epidemic area of Q fever in the past, probably before 15–30 years. On one side, this presumption is based on the relatively high antibody response of middle-aged farmers to the *Coxiella burnetii* phase I antigen, indicative of chronic infection or convalescence [10], and on the other side - on the absence of antibodies to this antigen in the youngest and oldest farmers and the lack of antibody response to the *Coxiella burnetii* phase II antigen, indicative of acute infection [10], in the total farming population examined. This hypothesis is in accordance with the results of the long-term study by Klymchuk *et al.* [8] carried out in the Carpathian region of the Ukraine, not far from the area of the present study. The cited authors came to the conclusion that the outbreaks of Q fever in eastern Europe are intermitted with interepizootic periods.

A significant difference between the groups of farmers and urban dwellers in the present study confirms the view that people having contact with farm animals are under increased risk of infection with *Coxiella burnetii* and contracting Q fever [1, 3, 10, 15, 16, 18, 19], though no direct relationship could be found between tending of

animals and positive response within a farmers' group. The farmers examined in this study bred mostly pigs and cattle, and very rarely sheep and goats. Thus, the present study seems to support results indicating that cattle may play an important role in epidemiology of Q fever [5, 7, 18], in addition to sheep and goats which are considered the main reservoirs of *Coxiella burnetii* [11, 17, 19].

Cattle have also been identified as a source of earlier Q fever outbreaks in eastern Poland (Zamość district, 1983) and in western Poland (Leszno district, 1988; Legnica district, 1992) [20, 22]. Tylewska-Wierzbanowska *et al.* [20, 21] performed in the years 1988–1991, by the microagglutination and complement fixation tests, an extensive study on seroprevalence to *Coxiella burnetii* among people occupationally exposed to farm animals in different parts of Poland. The frequency of positive results found by these authors in western Poland was higher compared to the present work (up to 41.7%) whereas in eastern Poland it was much lower (0–0.4% in the Lublin region) [20]. The results of the present study, obtained with the use of ELISA method, seem to indicate that the entire territory of Poland should be considered as an endemic region of Q fever.

It was reported that the seroprevalence to *C. burnetii* among inhabitants of the Spanish regions of Cantabria [13], Leon [16], Salamanca [14], and Basque country [15] directly increased with age, being the highest in the oldest people, in contrast to the present study where it peaked in middle-aged farmers. Another difference concerned the dependence of seroprevalence on gender - the above cited authors found a greater serologic response in males [13, 15, 16], whereas in the present work there was a significantly greater seroprevalence in females, similar to the study by Abe *et al.* [1] in Japan. By contrast, no association between seroprevalence and age were found by Kelly *et al.* [7] in Zimbabwe and by Thomas *et al.* [18] in the United Kingdom.

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