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PREVALENCE OF COXIELLA BURNETII INFECTION IN MILITARY TRAINING AREAS IN THE CZECH REPUBLIC AND SLOVAKIA

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Abstract

L i t e r á k I.: *Prevalence of Coxiella burnetii Infection in Military Training Areas in the Czech Republic and Slovakia.* Acta vet. Brno 1995, 64:179-186.

Between 1987 and 1992, the presence of antibodies to the Q fever agent was investigated in seven military training areas (MTA) on the territory of the Czech republic and Slovakia. Using a complement fixation test (CFT), antibodies in cattle were confirmed (*Coxiella burnetii*, phase II, titer ≥ 8) in two MTA, and in small ground mammals in five MTA. In one MTA, the *C. burnetii* strain was isolated from cow milk. No antibodies were found in enlisted soldiers, horses, sheep, dogs or game. The presence of *C. burnetii* strains under the specific MTA conditions has been demonstrated and it is possible that people in those areas may be infected. The risk of infection, however, is the same as in a civilian environment. It seems that the virulence of *C. burnetii* strains circulating in MTA is relatively low.

Coxiella burnetii, antibodies, complement fixation test, military training areas, soldiers, cattle, sheep, small mammals, game

Military training areas (MTA) are large areas that have been used by the army forces for a long time. Some parts of those facilities have been extensively damaged by military activities, while others are well-preserved because they have been practically off-limits for the non-military, with farming and forest exploitation restricted to a minimum. The specific uses of MTA bring soldiers to a close contact with the environment inhabited by wild and, in some cases, domestic animals. This exposes the soldiers and animals to the risk of a contact with circulating zoonosis agents. These may occur in the environment, or may have been brought there recently by incoming soldiers or farm animals.

Q fever is a zoonosis, the causative agent of which, *Coxiella burnetii,* occurs in Central Europe in the nature, e.g. in ticks and reservoir wild mammals, as well as in places related to human activities, namely in herds of domestic animals, mainly cattle and sheep (Ř e h á č e k 1987). The aim of the present paper was to investigate a number of military training areas for the presence of the rickettsiae of *Coxiella burnetii,* indirectly by serological examination and by direct isolation, and to assess the risks of infection for people in this environment.

Materials and Methods

The study was carried out in seven military training areas:

1. Mimoň MTA in northern Bohemia. Area over 30 thousand ha, about half of which covered with forests. Cattle and horses production. Mimoň MTA is extensively poluted with oil products as a result of a long presence of the ex-Soviet Union troops.
2. Libavá MTA in northern Moravia. Area over 30 thousand hectares (ha), with almost 90% covered with forests. Cattle production.
3. Jince MTA in central Bohemia. Area about 25 thousand ha. Almost 90% of the area are forests. No animal production.

4. Boletice MTA in southern Bohemia. Area over 20 thousand ha, almost 60% of which are forests. Cattle and sheep production.

5. Vyškov MTA in southern Moravia. Area about 15 thousand ha, more than 13 thousand ha covered with forests. No animal production.

6. Hartmanice MTA in southern Bohemia. Area almost 20 thousand ha, forests about 17 thousand ha. Cattle production, some pigs and sheep.

7. Pliešovce MTA in central Slovakia. Large area partially covered with forests. Cattle and sheep production.

In MTA 1 to 6, which are now situated on the territory of the Czech Republic, the study was made from 1987 to 1989. In MTA 2 and 7, tests were made in 1991 and 1992. MTA 7 is now situated on the territory of the Slovak Republic.

Enlisted soldiers were examined in MTA 2 and 7.

Domestic and farm animals examined were cattle, sheep, horses and dogs. Cattle was examined in MTA 2, 4, 6 and 7, sheep and dogs in MTA 7, and horses in MTA 2.

Small mammals were caught using spring traps arranged in lines per 100 traps each. Seven such lines were usually placed at different locations in training areas for two nights in May, September and November. Antibodies to *C. burnetii* were examined in the so-called eluates, substances separated by elution from split hearts in 1 ml buffered physiological saline. The eluate was used like serum in a 1:10 dilution. Small mammals were examined in MTA 1-6.

In game from military training areas, blood was collected from individual animals immediately after they were killed and tests were made of their blood serum. Game animals were examined in training areas 1-6.

Serological examination

The complement fixation micromethod with antigen to phase II of the *C. burnetii* Nine Mile strain was used in all serologic examinations (Bodibion, MEVAK Nitra, Slovak Republic).

Isolation assay of *Coxiella burnetii*

Cows in MTA 7 were tested by injecting 1 ml of milk from selected cows intraperitoneally to serologically negative laboratory mice of a Swiss strain. Two of four mice were sacrificed three weeks after the milk had been injected, and impression preparations of their spleens were made and stained for a coxiella assay according to Giménez. Spleens were then frozen to -18 °C. The remaining two mice were sacrificed 6 weeks after the milk was injected, and their blood was collected at that time for serological examination. Impression preparations from spleens of these mice were also stained. Spleens of the first two mice were thawed, mixed with the spleens of the other two mice and a suspension was made of them and buffered physiological saline. This was injected intraperitoneally (second passage) to two laboratory mice (1 ml each). The mice were sacrificed one week later, impression preparations from their spleens were stained, and the passage repeated. Repeated passages were made of samples which had caused seroconversion in mice after injection.

Results

Military Personnel

Examinations were made in the Libavá and Pliešovce training areas, with a total of 72 and 30 soldiers respectively. No antibodies to *C. burnetii* were detected in any of the tests (Tab. 1 and 2).

Table 1

Results of serologic examinations for antibodies to *C. burnetii* in Libavá MTA in 1991 and 1992

	No. of tests	No. of positive tests	CFT Titres
People	72	0	–
Cattle			
Czech Spotted			
Cows	136	7(5.1 %)	2x8.3x16.2x32
Calves	13	3	8,16,64
Hereford	35	0	–
Horses	14	0	–

Table 2
Results of serologic examinations for antibodies to *C. burnetii* in Pliešovce MTA in 1991 and 1992

	No. of tests	No. of positive tests	CFT Titres
People	30	0	–
Cattle (cows) 1991 on-vaccinated	127	27 (21.3%)	7x8,6x16 6x32,5x64 3x128
1992 After Bodibion vaccination	129	31 (24.0%)	15x8,10x16 3x32,2x64 1x128
Sheep	53	0	–
Dogs	11	0	–

Cattle

In the Boletice and Hartmanice training areas, a total of 202 and 336 head of cattle respectively were examined, and no antibodies were found.

In the Libavá MTA, a 5.1% prevalence of antibodies was ascertained in a production herd of the Czech Spotted Cattle. No antibodies were found in a Hereford herd, quarantined there after arrival from Canada (Tab. 1).

In 1991, when cattle was not vaccinated against Q fever, antibodies were found in 21.3% of the Pliešovce MTA cattle, a local breed reared in traditional cowsheds. When, independently of our investigation, the high seroprevalence of antibodies to Q fever in the Pliešovce area was ascertained by the State Veterinary Institute in Zvolen, local veterinary authorities decided that all the cattle should be vaccinated against Q fever in early 1992 (Bodibion vaccine, i.e. *C. burnetii* in phase I). In 1992, antibodies were found in 24 % of the cows tested (Tab. 2). In the tests, CFT with a phase II antigen was used (the CFT used does not detect the postvaccination antibodies to phase I antigen). In the Pliešovce MTA, no statistically significant difference was found between the 1991 and 1992 incidence of antibodies (χ^2 test, $\chi^2 = 0.36$, 1 df, $P > 0.5$). A statistically highly significant difference was, on the other hand, found in seroprevalence of antibodies to Q fever between the Libavá and the Pliešovce MTA cattle (χ^2 test, $\chi^2 = 20.7$, 1 df, $P < 0.001$).

In isolation tests of milk from 24 cows, the *C. burnetii* strain was detected in 4 cows by a seroconversion in laboratory mice which had been injected with the milk tested. The *C. burnetii* strain was directly detected in the spleen of experimental mice in two cases (Tab. 3).

Sheep, Horses and Dogs

A total of 53 sheep and 11 dogs from the Pliešovce MTA and 14 horses from the Libavá MTA were tested. In all these cases, results were negative (Tabs 1 and 2).

Small Mammals

A total of 1.486 small mammals were tested. In 12 cases (0.8 %), antibodies to *C. burnetii* were detected (Tab. 4). In small mammals, antibodies were found in five of the six military training areas surveyed. The seroprevalence ranged from 0.3 % to 1.9 %. Antibodies were detected in 11 specimens of the *Apodemus* sp. and 1 specimen of *Microtus arvalis* (Tab. 5). Small mammals with antibodies to *C. burnetii* were usually trapped in places used by man or domestic animals.

Game animals

No antibodies to *C. burnetii* were detected (Tab. 6) in any of the 169 animals hunted in MTA 1 to 6 (Tab. 6).

Table 3

Results of isolation surveys of cow milk from two farms in Pliešovce MTA to assay the strain of *C. burnetii*

(K.V. - Kamenný Vrch Farm, V.V. - Vidov Vrch Farm)

Cow No.	Titres of antibodies in cows (a)	Antibody prevalence in mice (b)	*C. burnetii* incidence in mice (c)
1 K.V.	-	-	- 1st passage
2 K.V.	-	-	- 1st passage
3 K.V.	-	-	- 1st passage
4 K.V.	-	-	- 1st passage
5 K.V.	8	-	- 1st passage
6 K.V.	8	-	- 1st passage
7 K.V.	8	-	- 1st passage
8 V.V.	8	-	- 1st passage
9 K.V.	16	-	- 1st passage
10 V.V.	16	-	- 1st passage
11 K.V.	16	-	- 1st passage
12 V.V.	16	-	- 1st passage
13 V.V.	16	-	- 1st passage
14 V.V.	16	+	- 8th passage
15 K.V.	32	-	- 1st passage
16 V.V.	32	-	- 1st passage
17 K.V.	32	+	+ 4th passage
18 V.V.	32	+	+ 6th passage[d]
19 V.V.	64	-	- 1st passage
20 K.V.	64	+	- 8th passage
21 K.V.	128	-	- 1st passage
22 V.V.	128	-	- 1st passage
23 V.V.	128	-	- 1st passage
24 V.V.	128	-	- 1st passage

a - CFT of antibodies in cows at the e of milk collection (October 1991)

b - CFT of antibodies in mice 6 weeks after i.p. injection with infected milk

c - impression preparations of the spleen stained according to Giménez, speen suspension passaged i.p. every 6 days

[d] - strain identification confirmed by the WHO reference laboratory (Virological Institute of the SAS, Bratislava, Slovak Republic)

Table 4
Antibodies to *C. burnetii* in small mammals in individual MTA between 1987 and 1989

Species	Prevalence of antibodies Positive/Total tests MTA 1	2	3	4	5	6
Common Vole *Microtus arvalis*	0/20	1/38	0/17	0/134	0/70	1/54
Field Vole *M. agrestis*		0/2	0/9	0/1		0/3
Bank Vole *Clethrionomys glareolus*	0/52	0/3	0/23	0/12	0/13	0/17
Field Mouse *Apodemus* sp.	4/87	0/92	2/126	1/99	0/155	1/114
Striped Field Mouse *A. agrarius*	0/8	2/96		0/2		
House Mouse *Mus musculus*	0/2				0/1	
Norway Rat *Rattus norvegicus*	0/3					
Common Dornmouse *Muscardinus avellanarius*			0/1			
Common Shrew *Sorex araneus*	0/39	0/30	0/39	0/64	0/16	0/28
Pygmy-Shrew *Sorex minutus*	0/2		0/8	0/2		
Lesser White-Toothed Shrew *Crocidura suaveolens*				0/2		
Water-Shrew *Neomys fodiens*				0/1		
Total	4/213	3/261	2/223	1/315	0/258	2/216
%	1.9	1.2	0.9	0.3	0.0	0.7

Table 5
List of small mammals with antibodies to *C. burnetii*

MTA	Date	Species	Titre	Location of traps
1	Sep. 1987	*Apodemus* sp.	10	Log cabin
1	– // –	– // –	10	Farm
1	– // –	– // –	10	– // –
1	Nov. 1988	– // –	10	Pasture
2	Sep. 1987	*Microtus arvalis*	10	Log cabin
2	Sep. 1988	*Apodemus agrarius*	10	Rubbish heap
2	Nov. 1989	– // –	320	Log cabin
3	Sep. 1987	*Apodemus* sp.	10	Rubbish heap
3	– // –	– // –	10	– // –
4	– // –	– // –	10	Forest edge
6	Nov. 1987	– // –	40	Log cabin
6	– // –	– // –	20	Chemical warfare training area

Table 6
Results of game survey for antibodies to *C. burnetii* (results of all tests were negative)

Species	MTA 1	2	3	4	5	6
Red Deer *Cervus elaphus*	1	40	7	7	13	32
Roe Deer *Capreolus capreolus*		7		11	3	4
Fallow Deer *Dama dama*	2					
Hog *Sus scrofa*	1	7	3	8	14	3
European Hare *Lepus europaeus*		1				
Common Red Fox *Vulpes vulpes*		3				
Pine-Marten *Martes martes*						1
Total	4	58	10	26	30	40

Discussion

The prevalence of antibodies to Q fever in the population of the Czech Republic is at present only about 1 % (Řeháček et al. 1985; Literák 1994a). It was not at all surprising that no antibodies were found in the 72 people from the Libavá MTA or the 32 people from the Pliešovce MTA, particularly because these people (draftees doing their national service) did not get into any close contact with potentially infected cattle.

In recent years, a generalized prevalence of antibodies to *C. burnetii* in cattle herds in the Czech Republic and Slovakia has been observed. Antibodies to *C. burnetii* have also been found in small mammals in several areas (Řeháček et al. 1979, 1985, 1987ab; Řeháček 1987; Lisák et al. 1989; Vošta et al. 1988, 1989; Cempírková et al. 1993; Literák 1994b, 1995; Literák and Calvo Rodríguez 1994). Similar situation also exists in neighbouring central European countries (Cygan et al. 1983; Schweighardt et al. 1984; Rady et al. 1985; Krauss et al. 1987; Řeháček et al. 1993). For that reason, we really cannot consider the finding of antibodies to *C. burnetii* in cattle and small mammals in Czech and Slovak military training areas as something unusual. The prevalence, however, is a proof that the agent of the zoonosis circulates both in cattle herds, i.e. close to human settlements, and in the nature. The importance of these findings depends mainly on the virulence of autochthonous strains of *C. burnetii*. In the herds in the training facilities investigated, no clinical symptoms of Q fever were found. In cattle herds with seroprevalence of antibodies to *C. burnetii* on civilian farms, we observe, for the most part, latent infections without any clinical manifestations. No relationship was confirmed, for example, between abortions in the cattle in southern Moravia and seroprevalence of antibodies to *C. burnetii* (Literák and Rodríguez 1994). An indispensable prerequisite for an epidemiological and epizootiological evaluation of antibody findings is an isolation of *C. burnetii* strains and testing their virulence in experimental infections. It seems that strains of *C. burnetii* currently circulating in Moravia are less virulent for laboratory animals than the standard *C. burnetii* strain, i.e. the Nine Mile strain (V a l k o v á et al. 1994).

The fact that prevalence of antibodies in the Libavá MTA cattle was significantly lower than in the Pliešovce MTA can be explained by objective differences between Q fever in cattle in the Czech Republic and Slovakia (Ř e h á č e k 1987). In the Czech Republic, prevalence is lower than in Slovakia, and so is probably virulence of autochthonous circulating strains of *C. burnetii*. It is assumed that the reason is the incidence of *Dermacentor marginatus*, the tick that is an important vector and reservoir of coxiellas. *Dermacentor marginatus* occurs mainly in southern areas of central Slovakia, including also the Zvolen district where the Pliešovce MTA is situated. In northern Moravia, where the Libavá MTA is, *Dermacentor marginatus* does not occur.

In cattle herds with a level of seroprevalence of antibodies to *C. burnetii*, coxiellas are excreted in milk. In the Pliešovce MTA, coxiellas were found in 4 out of 24 milk samples. However, normal excretion of *C. burnetii* by latently infected cows was reported by, e.g., R e u s c h (1982) or S c h a a l and S c h a f e r (1989). This fact, however, is not assumed to have much importance for human health (D u r a n d and L i m o u z i n 1983).

The prevalence of antibodies to *C. burnetii* in small mammals deserves particular attention. After all, infected specimens were found in five out of six military training areas investigated at places which, like, e.g., log cabins, rubbish heaps, farm, shooting range, were related man's activities. In unaffected areas of the countryside, however, antibodies to *C. burnetii* were absent not only from small mammals but also from game. Very similar results in small mammals at municipal waste dumps in southern Bohemia were reported by V l č e k (1991). The sources of infection, which might play a role in human infections, as well as the origin and virulence of individual strains of *C. burnetii*, are some of the questions to which answers must be sought.

Výskyt infekcí způsobovaných riketsií *Coxiella burnetii* ve vojenských výcvikových prostorech v České republice a na Slovensku

V letech 1987-1992 byly v sedmi vojenských výcvikových prostorech (VVP) na území dnešní České republiky a Slovenska sledovány protilátky proti původci Q horečky. Protilátky byly prokázány komplementfixační reakcí (antigen *Coxiella burnetii* ve fázi II, titr ≥ 8) u skotu ve dvou VVP a u drobných zemních savců v pěti VVP. Kmen *C. burnetii* byl izolován z mléka krav v jednom VVP. Protilátky nebyly prokázány u vojáků základní služby, koní, ovcí, psů a lovné zvěře. Ve specifických podmínkách VVP kmeny *C. burnetii* cirkulují a existuje tedy možnost infekce lidí v těchto oblastech. Tato možnost infekce se však neliší od možnosti infekce v civilním sektoru. Virulence kmenů *C. burnetii* cirkulujících ve VVP je zřejmě poměrně nízká.

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