

A study of *Neospora caninum* and *Toxoplasma gondii* antibody seroprevalence in healthy cattle in the Czech Republic

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Abstract

Introduction and objective. The aim of study was to test the sera of healthy dairy cows by ELISAs, the methods also used in other groups of animals in the Czech Republic, and thus to obtain actual data about *N. caninum* and *T. gondii* seroprevalence in cattle.

Material and methods. In the Czech Republic, sera from 546 clinically healthy dairy cows (*Bos primigenius f. taurus*) aged > 2 years from 49 farms in 7 districts were collected. Sera were tested for *Neospora caninum* antibodies by a commercial competitive-inhibition enzyme-linked immunosorbent assay; samples with more than 30% inhibition were considered as positive. The same samples were also analysed for *Toxoplasma*-specific IgG antibodies by an enzyme-linked immunosorbent assay; samples with more than 50% S/P were considered as positive.

Results. Antibodies against *N. caninum* were found only in 3 cows (0.5%) with inhibitions of 47, 78 and 85. Antibodies against *T. gondii* were found in 53 cows (9.7%) with S/P ranging from 51% to over 211%; positive animals were found in 4 of 7 districts, with prevalences ranging from 8% – 14%. Indication of mixed infections (concurrent presence of both *N. caninum* and *T. gondii* antibodies) was not proved.

Conclusion. The results of the study indicate that dairy cows in the Czech Republic have a relatively low seroprevalence for both *N. caninum* and *T. gondii*. Therefore, natural infection with *N. caninum* and *T. gondii* seems not to be very common in Czech cattle. These results show actual data about *N. caninum* and *T. gondii* infection in healthy dairy cattle from the Czech Republic.

Key words

Toxoplasmosis, Neosporosis, natural infection, serological survey, cows, Czech Republic

INTRODUCTION AND OBJECTIVES

Neospora caninum, a cyst-forming coccidian species, has a broad range of intermediate hosts; however, neosporosis is a serious disease of cattle and dogs worldwide. In cattle, neosporosis is a major cause of abortion, foetal malformations, pre-term deliveries, stillbirths and possible loss of milk yield, thus generating severe economic losses.

Serological prevalences of *N. caninum* in cattle worldwide have been summarized by Dubey and Schares [1]. In Europe, *N. caninum* antibodies were detected in Germany, Greece, Norway, Romania, Spain, Slovakia, Sweden, Turkey and the United Kingdom. There were considerable differences among countries, within countries, between regions and between beef and dairy cattle.

Toxoplasmosis is a common parasitic zoonosis that affects a wide range of warm-blooded animals and humans. In cattle, clinical cases of the infection have not been reported, but the real problem of toxoplasmosis in cattle lies in the fact that the tissues of infected animals may contain *T. gondii* tissue cysts. Insufficiently cooked meat and poor personal hygiene principles during cooking may cause latent or even clinical

infections in humans. Antibodies against *T. gondii* were found in cattle from several countries in Europe: France, Italy, the Netherlands, Portugal, Spain, Switzerland and Turkey.

During the 10 year period 2000–2010, several studies focusing on the detection of both *N. caninum* and *T. gondii* antibodies in different groups of animals – sheep, horses, pigs, goats, wild boars, hares, wild ruminants and zoo animals – were conducted in the Czech Republic. In the case of cattle, there is only limited data. There are only two studies detecting *N. caninum* antibodies in cattle [2, 3]; and two studies detecting *T. gondii* antibodies by the Sabin Feldman Test (SFT), a method that is no longer used [4, 5].

For this reason, the presented study aimed to test sera of healthy dairy cows from the Czech Republic by the same methods used in other groups of animals, and thereby obtain actual data about *N. caninum* and *T. gondii* seroprevalence in cattle with the possibility to compare the data with other groups of animals in the Czech Republic, and with seroprevalence in other countries in Europe.

MATERIALS AND METHOD

Blood samples were collected from 546 clinically healthy adult dairy cows (*Bos primigenius f. taurus*), aged > 2 years, without any cases of abortions recorded. The cows came from 49 farms

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in 7 districts of the western part of the Czech Republic: Central Bohemia (n=185); Plzeň (n=147); Ústí nad Labem (n=125); Liberec (n=43); Hradec Králové (n=30); Prague (n=11) and Karlovy Vary (n=5) (Fig. 1). The farms produce cows for milk production. The cows are stabled in cow-sheds; there are also yards with shelters in some cases; however, pastures during whole year are not provided. Blood samples were collected by veterinarians from the caudal vein of cows during 2009. Blood was centrifuged and serum stored at -20°C .

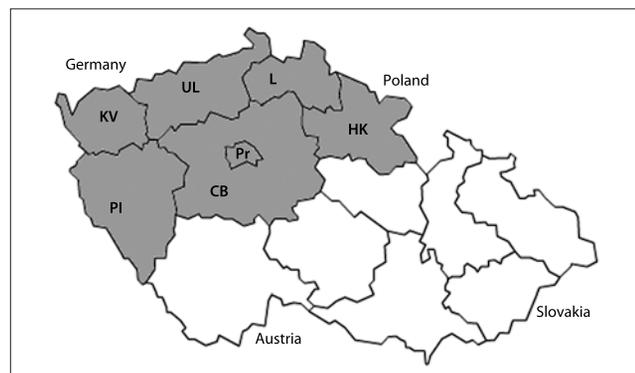


Figure 1. Map of the Czech Republic showing sampled areas. Sampled areas shaded grey: Central Bohemia (CB), Prague (Pr), Plzeň (Pl), Karlovy Vary (KV), Ústí nad Labem (UL), Liberec (L), Hradec Králové (HK)

Neospora caninum antibodies were measured using a commercial competitive-inhibition enzyme-linked immunosorbent assay (cELISA) validated for ruminants (VMRD, Pullman, USA), according to the manufacturer's instructions. Optical densities (OD) were measured spectrophotometrically at 620 nm. The test results were expressed as percentage of inhibition (%I) according to the following formula: $\% I = 100 - (\text{OD sample} \times 100 / \text{mean OD negative control})$. Tested samples which produced $\geq 30\%$ inhibitions were considered to be positive.

The same samples were also analysed for *Toxoplasma*-specific IgG antibodies by a commercial ELISA (ELISA *Toxoplasma gondii* serum screening (Institut Pourquier, Montpellier, France), according to the manufacturer's instructions. Optical densities were measured spectrophotometrically at 450 nm. The consequential colouration depends on the quantity of specific antibodies present in the specimen to be tested. For each sample, the ratio of optical densities of examined serum to mean OD of positive control was calculated as S/P (%), according to the

formula: $S/P (\%) = (\text{OD sample} / \text{OD positive control}) \times 100$. Samples with the S/P (%) $\geq 50\%$ were classified as positive.

Data analysis was performed by Chi-Square test for independence using STATISTICA Cz 10 [6]. The null hypothesis that *T. gondii* seroprevalence does not depend on origin (district) of cows was also tested. The differences were considered statistically significant when $p < 0.05$. Odds ratio (OR) and 95% confidence intervals (CI) for the odds ratio were computed to quantify the association between selected districts and serological *T. gondii* status.

RESULTS

Antibodies against *N. caninum* were detected in 3 (0.5%) of 546 cows with inhibitions of 47, 78 and 85. Positive animals came from 3 different farms in 2 districts. Statistical analysis (Chi-Square test) could not be performed, because of the very low frequency of *N. caninum* prevalence.

Antibodies against *T. gondii* were detected in 53 (9.7%) cows, with S/P ranging from 50–100, 100–150, 150–200 and ≥ 200 in 34, 10, 7 and 2 cows, respectively. Positive animals were found in 4 of 7 districts, with prevalence ranging from 8% – 14% (Tab. 1). Indication of mixed infections (concurrent presence of both *N. caninum* and *T. gondii* antibodies) was not proved. We found statistically different *T. gondii* prevalence in cows from the district of Ústí nad Labem with respect to all other examined districts ($p = 0.046$; OR 1.841; 95% CI 1.003, 3.380); however *T. gondii* seroprevalence does not depend on the origin (district) of cows ($p = 0.200$). The statistical results must be interpreted carefully because of the small numbers of cows in the districts of Hradec Králové, Prague and Karlovy Vary.

DISCUSSION

In Europe, antibodies against *N. caninum* have been detected in 2.8–60% cattle. There are considerable differences among countries, within countries, and between regions, with the highest in Turkey – 60% [7] and the lowest in Sweden – 2.8% [8]. The differences in prevalence could be influenced by many factors, such as the method and cut-off used, the number of cows examined, their health status, age, gender and breed. There are some studies documenting different *N. caninum* seroprevalence in cattle breeds, whereas on the

Table 1. *Toxoplasma gondii* and *Neospora caninum* antibody seroprevalence determined by ELISA in dairy cows from western Czech Republic

District	No. of samples	<i>N. caninum</i> (cELISA)		<i>T. gondii</i> (ELISA)				
		Positive (%)	% I	Positive (%)	S/P (%)	<i>p</i> -value	OR	95%CI
Central Bohemia	185	2 (1.1%)	78 and 85	19 (10%)	51 – 211	0.774	1.091	0.604; 1.971
Plzeň	147	1 (0.7%)	47	12 (8%)	51 – over	0.444	0.770	0.392; 1.509
Ústí nad Labem	125	0 (0%)	–	18 (14%)	56 – 182	0.046	1.841	1.003; 3.380
Liberec	43	0 (0%)	–	4 (9%)	60 – 125	0.916	0.944	0.324; 2.753
Hradec Králové	30	0 (0%)	–	0 (0%)	–	0.064	0	–
Praha	11	0 (0%)	–	0 (0%)	–	0.270	0	–
Karlovy Vary	5	0 (0%)	–	0 (0%)	–	0.460	0	–
Total	546	3 (0.5%)	47 – 85	53 (9.7%)	51 – 211			

%I (percentage of inhibition) – results of cELISA expressed as %I according to the formula: $\% I = 100 - (\text{OD sample} \times 100 / \text{mean OD negative control})$; OD = optical density. Samples with $\geq 30\%$ inhibitions were considered to be positive. S/P(%) – calculated according to the formula: $S/P(\%) = (\text{OD sample} / \text{OD positive control}) \times 100$. Samples with the S/P(%) $\geq 50\%$ were classified as positive. OR – odds ratio (in the case of OR with zero, 95% interval could not be calculated), 95% CI – confidence interval for the odds ratio. Chi-Square test could not be calculated for neosporosis, because of very low frequency of *N. caninum* prevalence.

contrary [9] found similar *N. caninum* seroprevalence 25.6% and 22.5% in 20,206 beef and 37,090 dairy cattle, respectively. In Slovakia, *N. caninum* antibodies were found in 20.1% cows post-abortion, while only 2.3% in cows without any reproductive problems [10]. In the Czech Republic, 3.9% *N. caninum* seroprevalence was found in cows post-abortion [3]. Bulk milk samples collected from 495 dairy herds were analysed for the presence of specific antibodies by a commercial *N. caninum* iscom ELISA, and only 5 (1%) herds were considered positive [2]. In the present study, similarly very low *N. caninum* seroprevalence (0.5%) was found in dairy cows without any cases of abortion. The risk of natural infection with *N. caninum* seems to be relatively low for cows in the Czech Republic.

In Europe, antibodies against *T. gondii* were found in 2–76% of cows by using different serological methods, e.g. SFT, Modified Agglutination Test, Direct Agglutination Test and ELISA. From previous studies in the Czech Republic, 2–24% seroprevalence was in cattle by SFT [4,5]. In the current study, *T. gondii* antibodies were found in 9.7% of cows by ELISA. A similar seroprevalence of 11% was found in cows from Switzerland using the same method [11], while a higher seroprevalence of 22% was found in cows from the Netherlands, also by ELISA [12].

In cattle, a usually higher *N. caninum* seroprevalence was found compared to *T. gondii* infection, e.g. 24.1% and 7.3% seroprevalence in cows from Spain [13], in Turkey, however, a higher *T. gondii* seroprevalence was found compared to *N. caninum* infection: 6.8% and 13.6% for cows with abortions, and 15.8% and 31.3% for cows without abortions, respectively [14]. Similarly, the presented study also found higher *T. gondii* seroprevalence compared to *N. caninum* (9.7% vs. 0.5%).

Simultaneous detection of both *N. caninum* and *T. gondii* antibodies were found in 4.8% of cows in Brazil [15] and in 1.1% of cows in Switzerland [11]. In the presented study, only three cows were found to be positive for *N. caninum*, but negative for *T. gondii* antibodies.

The results of this study indicate that the risk of natural infection with *N. caninum* and *T. gondii* seems to be relatively low for dairy cattle in the Czech Republic. The cows positive for *N. caninum* antibodies in this study came from small farms in different districts. There was no case of abortion or other reproduction problems in these cows, which is why transplacental transfer of infection can be ruled out, and the possible source of infection could be pastures contaminated with *N. caninum* oocyst.

CONCLUSION

In the Czech Republic, several seroprevalence studies have been conducted in recent years among domestic and wildlife animals without clinical symptoms. In the group of domestic animals, *N. caninum* and *T. gondii* were found in 24% and 23% of horses, 12% and 59% of sheep, 6% and 66% of goats and in 3% and 36% of pigs, respectively [16, 17, 18, 19]. *N. caninum* and *T. gondii* antibodies were also found in 6% and 32% of wildlife ruminants, respectively [20].

Compared to different groups of domestic animals and wildlife ruminants, the herds of healthy dairy cattle in the Czech Republic are not so much exposed to *T. gondii* infection and represent low risk for human consumption. However, further studies with regard to risk factors are needed.

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