

## ***Toxoplasma gondii* and *Neospora caninum* antibodies in goats in the Czech Republic**

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**ABSTRACT:** *Toxoplasma gondii* is zoonotic protozoan parasite that causes infections in many vertebrate species. The present study determined the seroprevalence of *T. gondii* and *N. caninum* in goats from the Czech Republic. Serum samples were collected from 251 healthy adult goats in the Czech Republic during the years 2006 to 2009. Sera samples were tested for serum antibodies to *Toxoplasma gondii* by an enzyme-linked immunosorbent assay with cut off equal to or higher than 50% S/P. The same samples were tested for serum antibodies to *Neospora caninum* by a competitive-inhibition enzyme-linked immunosorbent assay with cut off equal to or higher than 30% inhibition; positive sera were confirmed by an indirect fluorescent antibody test with cut-off titre equal to or higher than 40. Sera positive in both tests were marked as positive. In total, 166 (66%) and 15 (6%) goat sera reacted positively for *T. gondii* and *N. caninum* antibodies, respectively. All sera positive for *N. caninum* antibodies were simultaneously positive for *T. gondii* antibodies. This is the first detection of *N. caninum* antibodies in goats in the Czech Republic. Our findings indicate that goats in the Czech Republic are frequently exposed to *T. gondii*, but less frequently to *N. caninum*.

**Keywords:** neosporosis; toxoplasmosis; *Capra aegagrus*; serological survey; ELISA

*Toxoplasma gondii* and *Neospora caninum* are two closely related protozoan parasites with carnivores as definitive hosts. Toxoplasmosis in goats can lead to abortion, foetal death and weak kids (Dubey 1990). Reproductive disorders (abortions and births of weak kids) accompanied by increased seroprevalence were recorded also in the Czech Republic (Slosarkova et al. 1999). In Europe, *Toxoplasma gondii* antibodies were found in the range of 5–91% in goats in Austria, Bulgaria, Croatia, Germany, Greece, France, Italy, Poland, Netherlands, Norway, Slovakia, Spain and Turkey (Dubey 2010). In the Czech Republic, a seroprevalence of 21% to 82% has been found in goats in previous studies (Hejlíček and Literák 1994; Slosarkova et al. 1999; Misurova et al. 2009).

Neosporosis is a serious disease of cattle and dogs worldwide, although clinical neosporosis was reported also in sheep and goats (Dubey 2003). In Brazil, congenital neosporosis was described in

a goat kid with difficulty rising, ataxia, opisthotonos and non-suppurative encephalitis (Corbellini et al. 2001). In Italy, *N. caninum* was found by PCR in 2 of 23 (8.7%) aborted fetuses (Masala et al. 2007). Furthermore, one goat foetus aborted. Antibodies against *Neospora caninum* were reported in goats from Sri Lanka (Naguleswaran et al. 2004), Argentina (Moore et al. 2007) and Brazil (Faria et al. 2007). In Europe there is only one report from Poland (Czopowicz et al. 2011).

This work aimed to estimate the prevalence of *T. gondii* and simultaneously *N. caninum* antibodies in goats from the Czech Republic.

### **MATERIAL AND METHODS**

Serum samples of 251 healthy adult ( $\geq 12$  months of age) goats were collected in the period 2006 to

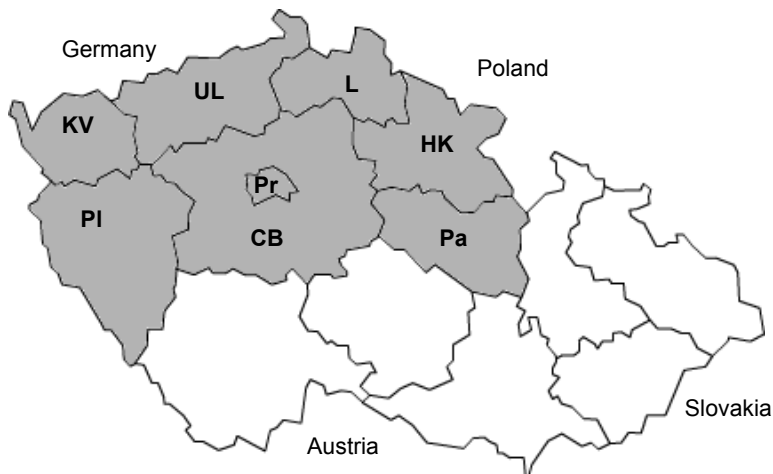


Figure 1. Map of the Czech Republic showing the sampled areas

Sampled areas are shaded: Central Bohemia (CB), Prague (Pr), Plzeň (Pl), Karlovy Vary (KV), Ústí nad Labem (UL), Liberec (L), Hradec Králové (HK), Pardubice (Pa)

2009. The goats originated from 15 farms located in the eight regions of Karlovy Vary ( $n = 101$ ), Ústí nad Labem ( $n = 93$ ), Central Bohemia ( $n = 30$ ), Liberec ( $n = 9$ ), Prague ( $n = 9$ ), Hradec Králové ( $n = 4$ ), Plzeň ( $n = 3$ ) and Pardubice ( $n = 2$ ) (Figure 1). Blood was taken from goats by veterinarians by jugular punctures and sent to the State Veterinary Institute for routine virological and bacteriological serological examination. This blood was centrifuged, serum stored at  $-20^{\circ}\text{C}$  and later assayed for *T. gondii* and *N. caninum* antibodies.

Antibodies against *T. gondii* were detected by a commercial enzyme-linked immunosorbent assay (ELISA, Institut Pourquier, Montpellier, France) with cut off equal to or higher than 50% S/P (sam-

ple-to-positive percentage). This ELISA is used to detect *T. gondii* antibodies in cats, pigs and ruminants. Antibodies against *N. caninum* were detected by a commercial competitive-inhibition enzyme-linked immunosorbent assay (cELISA, VMRD, Pullman, USA) with cut off equal to or higher than 30% inhibition. This commercial cELISA test is used to detect *N. caninum* antibodies in cattle and goat sera. Samples positive in cELISA were confirmed by an indirect fluorescence test (IFAT) using commercially available NC-1 tachyzoite slides and an anti-goat IgG conjugate (VMRD, Pullman, USA). A titre equal to or higher than 40 was considered positive. Samples positive in both cELISA and IFAT were regarded as positive.

Table 1. Serological results (*N. caninum* and *T. gondii* antibodies) in goats positive for *N. caninum* antibodies

Goat number	<i>N. caninum</i>		<i>T. gondii</i>	
	IFAT (titre)	cELISA (% of inhibition)	ELISA (% S/P)	evaluation
1	640	92.02	207.07	high positive
2	320	55.03	164.97	positive
3	320	55.87	207.07	high positive
4	80	42.07	178.66	positive
5	40	49.08	173.72	positive
6	40	85.24	102.66	positive
7	40	66.24	142.00	positive
8	40	85.34	119.38	positive
9	40	86.26	249.91	high positive
10	40	91.37	198.32	positive
11	40	75.50	190.77	positive
12	40	50.52	147.74	positive
13	40	71.65	168.04	positive
14	40	41.23	192.82	positive
15	40	69.96	200.37	high positive

## RESULTS

In total, 166 (66%) and 15 (6%) goats sera reacted positively for *T. gondii* and *N. caninum* antibodies, respectively. All goat sera positive to *N. caninum* antibodies were also positive to *T. gondii* antibodies. In different regions, the prevalence ranged from 43% to 83% for *T. gondii* and from 11% to 44% for *N. caninum* (Table 1).

*T. gondii* antibodies in ELISA were detected in 166 (66%) goats with % S/P ranging from 55.98 to 290.84 in positive samples (Figure 2). Of those, the sera of 123 goats were highly positive (% S/P equal to or higher than 200).

*N. caninum* antibodies in cELISA were detected in 64 (25%) goats, with 30.08–92.02% inhibition in positive samples (Figure 3). Sera, positive in cELISA, were examined in IFAT; of those only 15 (23%) sera were positive with titres of 40, 80, 320 and 640 in 11, one, two and one goats. Detailed results of the serological examination in 15 goats positive for *N. caninum* antibodies are summarized in Table 1.

## DISCUSSION

Anti-*T. gondii* antibodies were found in 166 (66%) goats. Similarly, antibodies against *T. gondii* were found in 60–69% of goats from Austria (Edelhofer and Aspöck 1996), Italy (Sindoni et al. 1989), Poland (Michalski and Platt-Samoraj 2004) and Bulgaria (Prelezov et al. 2008). In previous studies in the Czech Republic, antibodies against *T. gondii* were found in 21% to 66% goats (Hejlíček and

Literák 1994; Slosarková et al. 1999). Our result is comparable with these earlier reports, even though a different serological method was used.

In total, 15 out of 251 goats (6%) were positive for *N. caninum* antibodies. This is the first detection of *N. caninum* antibodies in goats in the Czech Republic. Antibodies against *N. caninum* were found in 6.4%, 6.6% and 0.7% of goats from Brazil (Faria et al. 2007), Argentina (Moore et al. 2007) and Sri Lanka (Naguleswaran et al. 2004), respectively. In Poland, 9% of 1060 female goat sera were positive for *N. caninum* antibodies by cELISA (Czopowicz et al. 2011). There are no other studies with which to compare our results. The cELISA method is more sensitive, but IFAT is more specific, and for this reason we used IFAT as a reference method. We did not find a correlation between the size of titres in IFAT and values obtained in cELISA.

All goats positive for *N. caninum* were simultaneously positive for *T. gondii* antibodies. Samples with high *N. caninum* titres in IFAT (equal to or higher than 320) also had higher *T. gondii* values in ELISA (two of them were marked as highly positive with titres equal to or higher than 200). In contrast however, we found the highest *T. gondii* value by ELISA (249.91) in a goat serum that was *N. caninum*-negative. We cannot exclude cross-reactivity in samples positive for both parasites, but the serologic methods used in our study are highly specific and sensitive, and thus we believe it is probably suitable for diagnosis of simultaneous *N. caninum* and *T. gondii* infection.

Compared to other countries in Europe, the total number of goats in the Czech Republic is very low.

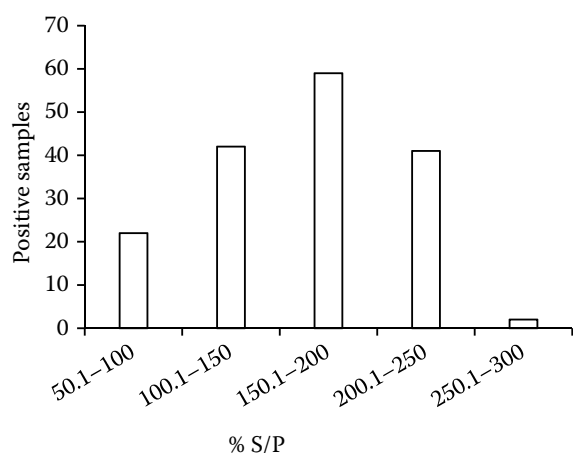


Figure 2. The ratio S/P (%) of *Toxoplasma gondii* antibodies detected by ELISA in domestic goats in the Czech Republic

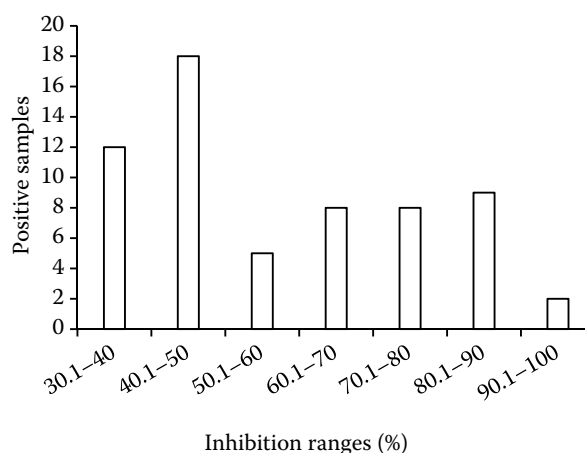


Figure 3. The inhibition ranges (%) of *Neospora caninum* antibodies detected by cELISA in domestic goats in the Czech Republic

Currently, there are about 14 thousand goats registered in the whole of the Czech Republic; but 90% of breeders have only one to three goats, and just 25 breeders have more than 15 goats. Goat farms are not equally distributed in all regions; the bigger farms are located in regions with extensive agriculture where the goats are used to graze the grass in meadows. In small farms, goats are usually stabled together with other groups of animals and in such environments can be found cats and dogs that can contaminate water, pasture and stables with *T. gondii* and *N. caninum* oocysts. Toxoplasmosis and neosporosis in goats result in economic losses due to intrauterine transmission of parasites to the foetus leading to the risk of abortion or foetus malformation. The meat of goats may harbour tissue cysts of *T. gondii* and may represent a vehicle of toxoplasmosis for humans.

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