Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in slaughtered pigs in the Czech Republic

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SUMMARY

In the Czech Republic, sera from 551 clinically healthy adult slaughtered pigs (females, 6–8 months old) were collected during the first half of June in 2010. Sera were tested for $Toxoplasma\ gondii$ -specific IgG antibodies by an enzyme-linked immunosorbent assay; samples with more than 50% S/P were considered as positive. The same samples were also analysed for $Neospora\ caninum$ antibodies using a commercial competitive-inhibition enzyme-linked immunosorbent assay; samples with more than 30% inhibition were considered as positive. Antibodies against T. gondii were found in 198 pigs (36%) in all districts with prevalences ranging from 18% to 75%. Antibodies against N. caninum were found in 16 pigs (3%); positive animals were found in 4 districts with prevalences ranging from 1% to 20%. Indication of mixed infections (concurrent presence of both N. caninum and T. gondii antibodies) was found in 8 (1·5%) pigs. The results of our study indicate that pigs in the Czech Republic have a relatively high seroprevalence for T. gondii, while they have only a low seroprevalence for N. caninum. Therefore, natural infection with T. gondii seems to be very common in Czech pigs. It is the first evidence of N. caninum antibodies in pigs in the Czech Republic. These results complete data about N. caninum infection in pigs in Europe.

Key words: toxoplasmosis, neosporosis, natural infection, serological survey.

INTRODUCTION

Toxoplasmosis is a common parasitic zoonosis that affects a wide range of warm-blooded animals and man. In pigs, clinical cases of the infection have been reported, but the real problem of toxoplasmosis in pigs lies in the fact that tissues of infected animals may contain *T. gondii* tissue cysts. Insufficiently cooked pork and poor personal hygiene principles during cooking may cause latent or even clinical infections in man.

Infections with this parasite are common in pigs worldwide. In central Europe, antibodies against *T. gondii* were found in pigs in Scotland (McColm *et al.* 1981), Italy (Genchi *et al.* 1991), Finland (Hirvela-Koski, 1992), Austria (Quehenberger *et al.* 1990; Edelhofer, 1994), Slovakia (Pleva *et al.* 1997), The Netherlands (Kijlstra *et al.* 2004), Germany (Damriyasa *et al.* 2004; Damriyasa and Bauer, 2005), Serbia (Klun *et al.* 2006), Poland (Gasior *et al.* 2010), Spain (García-Bocanegra *et al.* 2010) and the Czech Republic (Hejlíček and Literák, 1993; Vostalová *et al.* 2000).

Neospora caninum, another cyst-forming coccidian species, has a broad range of intermediate hosts.

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Neosporosis is a serious disease of cattle and dogs worldwide. Experimental infection of sows led to transplacental transmission of *N. caninum* (Jensen *et al.* 1998), but there is little information about *N. caninum* in pigs. Antibodies against *N. caninum* were detected in pigs from West Africa (Kamga-Waladjo *et al.* 2009), Brazil (de Azevedo *et al.* 2010) and Germany (Damriyasa *et al.* 2004); there are no other data from Europe.

This work was aimed to estimate N. caninum seroprevalence and to update T. gondii seroprevalence in pigs from the Czech Republic.

MATERIALS AND METHODS

In the Czech Republic, blood samples were collected from 551 clinically healthy adult pigs (*Sus scrofa domestica*) during the first half of June in 2010. The pigs came from 8 districts: Central Bohemian (n=350); Ústí nad Labem (71); Plzeň (65); South Bohemian (33); Karlovy Vary (11); Hradec Králové (8); Vysočina (8) and Liberec (n=5) (Fig. 1). Blood samples were collected in slaughterhouses. All pigs were females of slaughter age (6–8 months). Blood was centrifuged and serum was stored at $-20\,^{\circ}$ C.

Toxoplasma-specific IgG antibodies were measured by a commercial ELISA kit ID Screen Toxoplasmosis Indirect, Multi-species (ID Vet, France). The serum was considered as positive if

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Table 1.	Anti-Toxoplasma	gondii and anti-Neosp	ora caninum	antibody pro	evalences by	ELISA assay	y in pigs
from 8 d	istricts in the Czec	h Republic					

		$T.\ gondii$		N. caninum			
District	Number of samples	Positive (%)	S/P	Positive (%)	Inhibition	$T.\ gondii + N.\ caninum\ (\%)$	
Central Bohemia	350	114 (33%)	50-325	11 (3%)	32–44	7 (2%)	
Ústí nad Labem	71	33 (46%)	51-302	1 (1%)	33	1 (1.4%)	
Plzeň	65	17 (26%)	50-151	3 (5%)	35-60	_ ` ′	
South Bohemian	33	23 (70%)	52-289	_ ` ′	_	_	
Karlovy Vary	11	2 (18%)	60-69	_	_	_	
Hradec Králové	8	6 (75%)	53-337	_	_	_	
Vvsočina	8	2 (25%)	52-55	_	_	_	
Liberec	5	1 (20%)	58	1 (20%)	47	_	
Total	551	198 (36%)	50-337	16 (3%)	33-60	8 (1.5%)	



Fig. 1. Map of the Czech Republic showing the sampled areas. Districts: CB, Central Bohemian (350); Ú, Ústí nad Labem (71); PL, Plzeň (65); SB, South Bohemian (33); KV, Karlovy Vary (11); HK, Hradec Králové (8); V, Vysočina (8); LI, Liberec (5).

more than 50% S/P was found. The same samples were also analysed for *N. caninum* antibodies using a commercial competitive-inhibition enzyme-linked immunosorbent assay (cELISA) (VMRD, Pullman, USA). The serum was positive if more than 30% inhibition was found.

RESULTS

The antibodies against T. gondii were detected in the 198 (36%) of 551 pigs with S/P % ranging from 50% to over 337%. Positive animals were found in all districts with prevalence ranging from 18% to 75% (Table 1). Antibodies against N. caninum were detected in 16 (3%) pigs with inhibition of $30\cdot1-40$, $40\cdot1-50$ and $60\cdot1-70$ in 9, 5 and 2 pigs, respectively. Positive animals were found in 4 districts with prevalence ranging from 1% to 20% (Table 1). Indication of mixed infections (concurrent presence of both N. caninum and T. gondii antibodies) was found in 8 ($1\cdot5$ %) pigs. This is the first evidence of N. caninum antibodies in pigs in the Czech Republic

and the first evidence of *T. gondii* antibodies detected by ELISA in the Czech Republic.

DISCUSSION

In Europe, antibodies against T. gondii were found in 0.4-64% pigs depending on the number of pigs examined, the method and cut-off used. The highest prevalence, 64%, was found in pigs in Italy (Genchi et al. 1991). In other countries, the prevalence in pigs was as follows: 29% in Serbia (Klun et al. 2006), 19% in in Spain (García-Bocanegra et al. 2010) and Poland (Gasior et al. 2010), 18.5%-9% in Germany (Damriyasa et al. 2004; Damriyasa and Bauer, 2005), 9% in Scotland (McColm et al. 1981), 4% in Slovakia (Pleva et al. 1997), 2.5% in Finland (Hirvela-Koski, 1992) and 2% in the Netherlands (Kijlstra et al. 2004). In Austria, a trend towards lower seropositivity has been observed during the years 1982-1992 with prevalences of 13.7% and 0.9%, respectively (Quehenberger et al. 1990; Edelhofer, 1994). In the Czech Republic, a similar trend was observed in previous studies. The prevalence ranged from 41.9% to 10.7% during the years 1948–1970, as tested by the Sabin-Feldman test (SFR), complement fixation test (CFT) and microprecipitation on agar gel (MPA) (summarized by Kouba et al. 1974) and decreased to 5.9%, 0.3% and 0.1% in the years 1979–1990, as tested by SFR, CFT and MPA, respectively (Hejlíček and Literák, 1993) and to 0.5% and 0.4% as tested by CFT and SFR, respectively (Vostalová et al. 2000). In the present study, we found a higher prevalence compared to the last study. The difference could be explained by the origin of the pigs. Whilst in the last study the pigs came from one large farm, the pigs included in the present study came from different farms of different districts. We found prevalences ranging from 18% to 70% in different districts; the differences were observed also on farms. Based on previous studies, the higher prevalence is found among pigs from small back-yard operations, while the prevalence among pigs from traditional large farms and modern large-scale farms is usually lower. Another explanation for higher seropositivity could be that ELISA is more sensitive compared to other serological methods. Dubey et al. (1997) infected pigs and compared different serological methods and found the modified agglutination test (MAT) and ELISA to be the most sensitive tests compared to the dye test (DT), latex agglutination test (LAT) and indirect haemagglutination test (IHA).

Antibodies against *N. caninum* and *T. gondii* were detected in 3% and 36% of pigs, respectively. Our results are in agreement with a similar study from Brazil. Antibodies against *N. caninum* and *T. gondii* were detected by an indirect fluorescence test in 3·1% and 36·2% of 130 pigs, respectively (de Azevedo *et al.* 2010). In Germany, Damriyasa *et al.* (2004) used ELISA to test 2041 pigs and found 3·3% and 18·5% pigs to have *N. caninum* and *T. gondii* antibodies, respectively. Our serological results bring new information about *N. caninum* prevalence in pigs in Europe and thus complete our knowledge about this parasite.

In conclusion, the results of the present study indicate that the risk of natural infection with T. gondii seems to be relatively high for pigs in the Czech Republic. However, there are local differences in prevalences. These data also demonstrate that natural infections with N. caninum can occur in pigs from the Czech Republic, but only sporadically.

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REFERENCES

Damriyasa, I.M. and Bauer, C. (2005). Seroprevalence of *Toxoplasma gondii* infection in sows in Münsterland, Germany. *Deutsche Tierärztliche Wochenschrift* **112**. 201–240.

Damriyasa, I. M., Bauer, C., Edelhofer, R., Failing, K., Lind, P., Petersen, E., Schares, G., Tenter, A. M., Volmer, R. and Zahner, H. (2004). Cross-sectional survey in pig breeding farms in Hesse, Germany: seroprevalence and risk factors of infections with *Toxoplasma gondii*, Sacrocystis spp. and Nespora caninum in sows. Veterinary Parasitology 126, 271–286.

de Ayevedo, S. S., Pena, H. F. D., Alves, C. J., Guimaraes, A. A. D., Oliveira, R. M., Maksimov, P., Schares, G. and Gennari, S. M. (2010). Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora canimum* antibodies in swine from Northeastern Brazil. *Revista Brasileira de Parasitologia Veterinaria* 19, 80–84.

Dubey, J. P., Andrews, C. D., Thulliez, P., Lind, P. and Kwok, O. C. H. (1997). Long-term humoral antibody responses by various serologic tests in pigs orally inoculated with oocysts of four strains of *Toxoplasma gondii. Veterinary Parasitology* **68**, 41–50.

Edelhofer, R. (1994). Prevalence of antibodies against *Toxoplasma gondii* in pigs in Austria – an evaluation of data from 1982 and 1992. *Parasitology Research* **80**, 642–644.

García-Bocanegra, I., Dubey, J.P., Simon-Grifé, M., Cabezón, O., Casal, J., Allepuz, A., Napp, S. and Almería, S. (2010). Seroprevalence and risk factors associated with *Toxoplasma gondii* infection in pig farms from Catalonia, north-eastern Spain. *Research in Veterinary Science* 89, 85–87.

Gasior, L., Kur, J., Hiszczynska-Sawicka, E., Drapala, D., Dominiak-Górski, B. and Pejsak, Z. (2010). Application of recombinant antigens in serodiagnosis of swine toxoplasmosis and prevalence of *Toxoplasma gondii* infection among pigs in Poland. *Polish Journal of Veterinary Science* 13, 457–464.

Genchi, G., Polidori, G. A., Zaghini, L. and Lanfranchi, P. (1991). A survey of *Toxoplasma* infection in pig intensive breeding. *Archivio Veterinario Italiano* 42, 105–111.

Hejlíček, K. and Literák, I. (1993). Prevalence of toxoplasmosis in pigs in the region of South Bohemia. *Acta Veterinaria Brno* **62**, 159–166

Hirvela-Koski, V. (1992). The prevalence of *Toxoplasma* antibodies in swine sera in Finland. *Acta Veterinaria Scandinavica* **33**, 21–25.

Jensen, L., Jensen, T. K., Lind, P., Henriksen, S. A., Uggla, A. and Bille-Hansen, V. (1998). Experimental porcine neosporosis. *Acta Pathologica*, *Microbiologica et Immunologica* 106, 475–482.

Kamga-Waladjo, A.R., Chatagnon, G., Bakou, S.N., Boly, H., Diop, P.E.H. and Tainturier, D. (2009). Neospora caninum antibodies and its consequences for reproductive characteristics in wandering sows from Senegal, West Africa. Asian Journal of Animal and Veterinary Advances 4, 263–266.

Kijlstra, A., Eissen, O. A., Cornelissen, J., Munniksma, K., Eijck, I. and Kortbeek, T. (2004). *Toxoplasma gondii* infection in animal-friendly pig production systems. *Investigative Opthalmology and Visual Science* **45**, 3165–3169.

Klun, I., Djurkovic-Djakovic, O., Katic-Radivojevic, S. and Nikolic, A. (2006). Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: Seroprevalence and risk factors. *Veterinary Parasitology* **135**, 121–131.

Kouba, K., Jíra, J. and Hübner, J. (1974). *Toxoplazmóza*. Avicenum, Praha, Czech Republic.

McColm, A.A., Hutchison, W.M. and Siim, J.C. (1981). The prevalence of *Toxoplasma gondii* in meat animals and cats in central Scotland. *Annals of Tropical Medicine and Parasitology* 75, 157–164

Pleva, J., Sokol, J., Cabadaj, R. and Saladiová, D. (1997). Epizootologický a epidemiologický význam toxoplazmózy. *Slovenský Veterinárský Časopis* 22, 127–129.

Quehenberger, P., Schuller, W. and Awad-Masalmeh, M. (1990). Occurence of complement-fixing antibodies against *Chlamydia psittaci* and *Toxoplasma gondii* in pigs in the individual counties of Austria. *Deutsche Tierärztliche Wochenschrift* 77, 285–286.

Vostalová, E., Literák, I., Pavlásek, I. and Sedlák, K. (2000). Prevalence of *Toxoplasma gondii* in finishing pigs in a large-scale farm in the Czech Republic. *Acta Veterinaria Brno* **69**, 209–212.