Cross-sectional survey in pig breeding farms in Hesse, Germany: seroprevalence and risk factors of infections with *Toxoplasma gondii*, *Sarcocystis* spp. and *Neospora caninum* in sows

I.M. Damriyasaa, C. Bauer a,*, R. Edelhofer b, K. Failing c, P. Lind d, E. Petersen e, G. Scharfe f, A.M. Tenter g, R. Volmer h, H. Zahner a

aInstitute of Parasitology, Justus Liebig University Giessen, Rudolf-Buchheim-Strasse 2, 35392 Giessen, Germany
bInstitute of Parasitology and Zoology, Veterinary University Vienna, Veterinärplatz 1, 1210 Vienna, Austria
cUnit of Biomathematics and Data Processing, Faculty of Veterinary Medicine, Justus Liebig University Giessen, Frankfurter Strasse 95, 35392 Giessen, Germany
dDepartment of Immunology and Biochemistry, Danish Veterinary Institute, Bülowsvæj 27, 1790 Copenhagen V, Denmark
eLaboratory of Parasitology, Statens Serum Institute, Artillerivej 5, 2300 Copenhagen S, Denmark
fInstitute of Epidemiology, Seestrasse 55, 16868 Wusterhausen, Germany
gInstitute of Parasitology, School of Veterinary Medicine, Bünteweg 17, 30559 Hannover, Germany
hStaatliches Untersuchungsamt Hessen, Marburger Strasse 54, 35396 Giessen, Germany

Received 6 November 2003; received in revised form 13 July 2004; accepted 25 July 2004

Abstract

A cross-sectional survey was performed to estimate the prevalences of antibodies to *Toxoplasma gondii* (ELISA, IFAT), *Sarcocystis* spp. (ELISA, using *S. miescheriana* as antigen) and *Neospora caninum* (ELISA, immunoblotting) in sows from breeding farms in southern Hesse, Germany. A total of 2041 plasma samples of sows from 94 randomly selected farms was examined. Data on farm profiles, husbandry management and sows were collected by a questionnaire and exploratively analysed. For *T. gondii* the ELISA results agreed well with the results obtained by IFAT (kappa = 0.71). Antibodies to *T. gondii* were detected by ELISA in 19% of the sows. Sixty-nine percent of the...
farms had at least one seropositive sow, and a within-farm seroprevalence of ≥50% was observed in 14% of all farms. The prevalence of anti-\textit{T. gondii} antibodies was positively correlated with the age of sows. The within-herd seroprevalence was significantly higher in farms with reproductive disorders than in those without such problems. On the farm level, the farm type ‘piglet production’ (versus ‘pedigree breeding’ or ‘farrow-to-finish’) was the only risk factor associated with the presence of \textit{T. gondii}-seropositive sows. Antibodies to \textit{Sarcocystis} spp. were found in 29% of the sows. Seventy-two percent of the farms harboured at least one seropositive sow, and a within-farm seroprevalence of ≥50% was detected in 23% of all farms. The seroprevalence increased significantly with the age of sows. On the farm level, only the farm type ‘piglet production’ (versus ‘pedigree breeding’) and the replacement of sows by purchasing (versus raising on the own farm) were identified as risk factors for seropositivity. Antibodies to \textit{N. caninum} were detected in one sow using both the screening ELISA and the confirmatory immunoblotting technique. This may indicate the first natural \textit{N. caninum} infection in pigs. © 2004 Elsevier B.V. All rights reserved.

\textbf{Keywords:} Neospora caninum; Sarcocystis; Toxoplasma gondii; Pig protozoa; Epidemiology; Risk factors; Germany

1. Introduction

Infections with the tissue cyst-forming coccidia \textit{Toxoplasma gondii}, \textit{Sarcocystis miescheriana} and \textit{S. suihominis} may affect reproductivity of sows and productivity of pigs in general and thus play an important role in the pig industry. In addition, \textit{T. gondii} and \textit{S. suihominis} are of zoonotic importance (see Dubey, 1993), and the control of these parasitoses in pigs might become important for consumer’s protection. Infections with these parasites are common in pigs worldwide. In central Europe, however, most epidemiological data on \textit{T. gondii} infections in pigs were collected prior to the 1990s, and only few studies reported recent data on the prevalence of \textit{T. gondii} in different management systems (see Tenter et al., 2000). No reliable, representative data are available on the current status of \textit{Sarcocystis} spp. infections in domestic pigs in Europe. \textit{Neospora caninum}, another cyst-forming coccidia species, has a broad range of intermediate hosts. This protozoon is currently regarded as one of the most important causes of abortion in cattle (Dubey, 1999). Experimental infection of sows led to transplacental transmission of \textit{N. caninum} (Jensen et al., 1998) but nothing is known on its possible presence in the pig population of Germany.

In order to fill these gaps, the present survey aimed to determine the seroprevalences of \textit{T. gondii}, \textit{Sarcocystis} spp. and \textit{N. caninum} in a regional pig population. The survey focussed on breeding sows because it was expected that these animals, due to their longer life span, might have the highest risk to acquire infections with these parasites.

2. Materials and methods

2.1. Selection of farms and sampling

A random, weighted sample of 117 farms, stratified by farm type and herd size, was selected from a total of 230 pig breeding farms associated with the Central Swine Health
Service in the southern part of the Federal State of Hesse, Germany. For this serological survey 94 of the 117 farms were sampled during February and August 1997. On the farm level, the sample size was chosen to ensure a 95% probability of detecting at least one positive animal for an assumed within-herd seroprevalence of 5% (Cannon and Roe, 1982). Blood samples from 2041 sows were collected in ethylenediaminetetra-acetate-coated (EDTA) tubes by puncture of the Vena jugularis. Plasma was isolated after centrifugation (800× g, 20 min) and stored at −20 ºC until used.

Informations on the farm profiles including the presence of cats and dogs, husbandry management and reproductive disorders, and on the parity number and reproduction status of examined sows were collected by personal interviews using a standardised questionnaire for assessing possible risk factors.

2.2. Determination of antibodies

2.2.1. Antibodies to T. gondii

Enzyme linked immunosorbent assay (ELISA): the Tx-12 antigen (batches Tx4797 and Tx0499; protein concentration: 11 mg/ml) used for detection of anti-\(T. gondii\) antibodies was a tachyzoite lysate antigen derived from the SSI/119 strain of \(T. gondii\). The ELISA was performed in principle according to Lind et al. (1997): 96-well polystyrene ELISA plates (Polysorb, Nunc, Wiesbaden, Germany) were coated for 15 h at 4 ºC with 100 µl of a 1:100 dilution of the Tx-12 antigen preparation in 0.01 M phosphate-buffered 0.5 M saline (PBS-0.5), pH7.2, and stored at 4 ºC for up to 1 week until used. All washing steps consisted of three washes for 5 min with PBS-0.5 containing 0.1% Tween 20 (PBS-0.5-Tween) using an Immunowash™ Wash 12 (Nunc). Plasma samples were diluted 1:100 in PBS-0.5-Tween and incubated for 1 h at room temperature (RT). After three washes conjugate (goat anti-swine IgG [\(\gamma\) chain-specific] horseradish peroxidase; Kierkegaard and Perry, Gaithersburg, MD, USA) was added in dilutions of 1:100 or 1:2000 (depending on the batch) in PBS-0.5-Tween containing 1% normal goat serum (Sigma, Deisenhofen, Germany) that was previously tested for absence of anti-\(T. gondii\) antibodies and incubated for 1 h at RT. After washes substrate solution (0.4 mg/ml \(o\)-phenylenediamine dihydrochloride [Sigma] and 0.012% hydrogen peroxide [Perhydrol, Merck, Darmstadt, Germany] in 0.05 M phosphate, 0.025 M citric acid, pH 5.0) was added. The reaction was stopped after 15 min at RT in the dark by an equal amount of 0.5 M sulfuric acid. Optical densities (OD) were measured at 490 nm. At the end of the measurements, a corrected OD-value (\(OD_{corr}\)) was calculated for each test sample taking into account the ODs obtained for the same positive and negative reference sera that were included on each ELISA plate as described by Lind et al. (1997). An optimised cut-off value of 0.196 for discrimination between positive and negative test results was calculated by two-graph receiver operating characteristic (TG-ROC) analysis (Greiner, 1996) from the \(OD_{corr}\) observed for positive sera that were collected from 10 pigs 13 weeks after experimental infection with 10,000 oocysts of \(T. gondii\) SSI/119 strain and for negative sera collected from 10 uninfected pigs (Lind et al., 1997).

Indirect immunofluorescence antibody test (IFAT): the test used whole tachyzoites of \(T. gondii\) from peritoneal exudates of mice infected intraperitoneally with the \(T. gondii\) SSI/119 strain. An anti-swine IgG fluorescein conjugated goat IgG fraction (Chemicon, Temecula, USA; 1:100 in PBS) served as conjugate. Sera of \(T. gondii\)-free and naturally
infected pigs were used as reference sera. The test was performed according to standard recommendations (Anon., 1977; Edelhofer, 1994). A titre of $\geq 1:16$ was considered positive.

2.2.2. Antibodies to S. miescheriana

ELISA: cystozoites of S. miescheriana were isolated by peptic digestion of the muscle samples obtained from pigs 3 months after experimental infection with 50,000 sporocysts of the S. miescheriana AOHP1 strain (Tenter et al., 1994). They were purified from contaminating host tissue by discontinuous density gradient centrifugation (Rommel et al., 1995), which rendered preparations containing $>99.98\%$ cystozoites, i.e. no host cell detected in Giemsa stained smears of 5000 cystozoites. Cystozoites were lyophilised and kept at $-20\,^\circ\mathrm{C}$ until used. For preparation of ELISA antigens, cystozoites were re-suspended in 0.01 M phosphate-buffered 0.15 M saline (PBS-0.15), pH 7.4, disrupted by three freeze-thaw cycles ($-80\,^\circ\mathrm{C}/+37\,^\circ\mathrm{C}$) and stored again at $-20\,^\circ\mathrm{C}$. The optimal working dilution of each test reagent was determined by checkerboard assays with serial dilutions of antigen, positive and negative reference sera, and conjugate. The final ELISA protocol was as follows: ELISA plates (Polysorb, Nunc) were coated for 15 h at 4 $^\circ\mathrm{C}$ with 100 $\mu$l per well of the S. miescheriana antigen preparation diluted in coating buffer (0.015 M carbonate 0.035 M bicarbonate, pH 9.6; working dilution: 10 $\mu$g protein/ml) and stored at 4 $^\circ\mathrm{C}$ for up to 1 week until used. Plates were washed three times for 5 min with PBS-0.5-Tween. All reagents were applied to the ELISA plates in volumes of 100 $\mu$l and incubations were carried out in the dark at 22 $^\circ\mathrm{C}$. Plasma samples were diluted 1:50 in PBS-0.5-Tween and incubated for 1 h. The conjugate (see Section 2.2.1) was diluted 1:500 in PBS-0.5-Tween containing 1% normal goat serum (Sigma), which had been previously tested for absence of anti-Sarcocystis antibodies. It was incubated for 1 h. The substrate, reaction time, stopping solution, measurement of OD and calculation of OD$_{corr}$ were the same as above (see Section 2.2.1). An optimised cut-off value of 0.354 for discrimination between positive and negative test results was calculated by TG-ROC analysis (Greiner, 1996) from the OD$_{corr}$ observed for S. miescheriana-positive sera that were collected from six experimentally infected pigs and negative sera collected from nine uninfected pigs (Daugschies et al., 1988).

2.2.3. Antibodies to N. caninum

Screening ELISA: $1 \times 10^8$ cell culture N. caninum tachyzoites (NC-1 strain) were sonicated (50% active cycle, output control level 2; VibraCell, Sonics & Material Inc., Danbury, CT, USA) in 0.1 M sodium bicarbonate, pH 8.3, 0.5% Triton X-100 90 s on ice. ELISA plates (Polysorb, Nunc) were coated (150 $\mu$l per well, 1 h, 37 $^\circ\mathrm{C}$) with antigen diluted 1:200 in 0.1 M sodium bicarbonate, pH 8.3, 0.015% Triton X-100 (final concentration). Blocking was performed with PBS-T substituted with 2% (v/v) gelatine (Serva, Heidelberg, Germany; 22156, liquid) (PBS-TG). Plasma samples were diluted 1:200 in PBS-TG and incubated 30 min at 37 $^\circ\mathrm{C}$. After three washes, incubation (37 $^\circ\mathrm{C}$, 30 min) with conjugate (peroxidase conjugated Affini Pure goat anti-swine IgG (H + L); Jackson Immunoresearch Laboratories, West Grove, PA, USA; 114-035-003) diluted 1:2000 in PBS-TG was performed. After three further washes substrate (100 $\mu$g/ml 3,3',5,5'-tetramethylbenzidine and 0.004% hydrogen peroxide in 0.2 M sodium acetate and
0.2 M citric acid at 37 °C) was added. The reaction was stopped with 50 μl 4 N sulfuric acid. OD was measured at 450 nm and expressed as ELISA-index according to Zahner et al. (1981). Based on the results obtained with the sera of one experimentally N. caninum-infected pig (Jensen et al., 1998) and of seven sows experimentally infected with T. gondii a preliminary cut-off value of 0.15 was selected.

Immunoblotting: sera found to be positive in the screening ELISA were re-evaluated by immunoblotting (Schares et al., 1998). Plasma samples which recognized at least two of the five immunodominant antigens of N. caninum (17, 29, 30, 33, 37 kDa; Barta and Dubey, 1992; Schares et al., 1999) were regarded as seropositive.

2.3. Statistical analysis

Explorative data analyses were performed using general statistical software packages (BMDP: Dixon, 1993; BIAS: Ackermann, 2002). A farm was defined as positive for a parasite if at least one sow reacted with the respective antigen. For univariate description of data the median, percentiles, range and frequency were given. Associations between qualitative variables or between qualitative and quantitative variables were analysed for significance using the Chi-square test and the Kruskal–Wallis test followed by Dunn test, respectively. Correlations between quantitative variables were evaluated using Spearman’s rank correlation coefficient ($r_s$). On sow level, the effect of independent variables (age, reproductive status) on seropositivity was analysed for significance by the multiple logistic regression model. On farm level, raw odds ratios (OR) of management factors were calculated to assess their effects on seroprevalences. Differences with $P$ values < 0.05 were considered as statistically significant. Kappa statistic was calculated for assessing the agreement between the results of both the T. gondii ELISA and IFAT.

3. Results

3.1. Farm profiles and management

The mean herd size of the 94 farms was 42 (median: 26; range: 2–300). The mean numbers of litters and weaned piglets per sow and year were 2.1 (1.4–2.6) and 18.9 (12.3–23.3), respectively. Farm types were pedigree breeding (16%), piglet production (65%) and farrow-to-finishing (19%). Replacement sows were raised only on the own farm or only purchased from other herds in 28% and 39% of the properties, respectively; 33% of the farmers used both ways. Purchasing of replacements was more frequently ($P < 0.001$, Chi-square test) performed in piglet production farms than in pedigree breeding farms. Sows were kept on straw in gestation (78%) and farrowing pens (84%) in the majority of the farms. Twenty-one percent of the farmers cleaned the stable units only mechanically. Hand feeding of sows was carried out by 87% of the farmers. Cats and dogs were kept and had access to pig facilities in 62% and 66% of the farms, respectively. Pasturing of gilts and dry sows were performed in 9.5% of the farms (only pedigree breeding or farrow-to-finishing). The mean parity number of the examined sows was 3.2.
3.2. Antibodies to T. gondii

The results of the T. gondii ELISA substantially agreed with those of the T. gondii IFAT (kappa value: 0.71; 95% confidence interval: 0.66–0.74). The relative sensitivity and relative specificity of the ELISA was 80.6% and 93.7%, respectively, employing the IFAT as the gold standard.

The estimated overall prevalence of antibodies to T. gondii in sows was 16.5–18.5% depending on the assay. Using farms as the unit of analysis, more than 68% of the farms had at least one seropositive sow in the herd (Table 1). However, antibody levels were usually low. In case of the IFAT 73% of the reactive samples showed a titre of 1:16, i.e. just above the cut-off value. Similarly, the ODcorr in approximately 70% of the ELISA positive samples was found in the range of 0.2–0.3, i.e. slightly above the cut-off value. Otherwise, seropositivity clustered in particular farms, e.g. a within-farm seroprevalence of ≥50% was observed in 14% of all farms, i.e. 20% of the premises with at least one seropositive animal (Fig. 1). Sows responding to S. miescheriana antigen were found to be more frequently T. gondii-positive in the ELISA (21.0%) than Sarcocystis-negative animals (16.9%; P = 0.03, Chi-square test).

The within-farm prevalence of antibodies to T. gondii by ELISA was significantly higher in farms where reproductive disorders (repeat-breeders, abortion, neonatal mortality) had been recorded to occur during the past 5 years than in premises without such problems (Kruskal–Wallis test; Fig. 2). However, the within-farm seroprevalence was not significantly correlated with the number of weaned piglets in the farms (r_s = −0.19; P = 0.069).

The detection of antibodies to T. gondii by ELISA was positively correlated with the age of sows measured as parity numbers (r_s = 0.55; P < 0.05). For this variable an OR of 1.2 was determined in the multiple logistic regression model explaining seropositivity of sows (Table 2), i.e. as the parity number increased by 1, the odds to be seropositive increased by 20%. In contrast, the reproduction status of sows was not related to anti-T. gondii antibodies (Table 2). On the farm level, the only management factor significantly associated with seropositivity was the farm type (Table 3): piglet production farms had a 4–5 times higher chance to harbour seropositive sows than pedigree breeding or farrow-to-

### Table 1
Prevalence of antibodies to T. gondii, Sarcocystis spp., and N. caninum in sows from 94 randomly selected breeding farms in Hesse, Germany

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Assay</th>
<th>Percentage of seropositive sows (95% CI)</th>
<th>Percentage of farms with seropositive sows (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. gondii</td>
<td>ELISA(^b)</td>
<td>18.5 (16.8–20.2)</td>
<td>69.1 (59.2–79.0)</td>
</tr>
<tr>
<td></td>
<td>IFAT(^c)</td>
<td>16.5 (14.8–18.2)</td>
<td>68.1 (58.1–78.1)</td>
</tr>
<tr>
<td>Sarcocystis spp.</td>
<td>ELISA(^b)</td>
<td>28.6 (26.6–30.6)</td>
<td>72.3 (62.7–81.9)</td>
</tr>
<tr>
<td>N. caninum</td>
<td>ELISA(^b)</td>
<td>3.3 (2.5–4.1)</td>
<td>27.7 (18.1–37.3)</td>
</tr>
<tr>
<td></td>
<td>Immunoblotting(^d)</td>
<td>0.04</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(^a)</td>
<td>Confidence interval.</td>
</tr>
<tr>
<td>(^b)</td>
<td>N = 2041 sows tested in ELISA.</td>
</tr>
<tr>
<td>(^c)</td>
<td>N = 1967 sows tested in IFAT.</td>
</tr>
<tr>
<td>(^d)</td>
<td>Serial testing of 67 sows with N. caninum-positive reaction in ELISA.</td>
</tr>
</tbody>
</table>
Fig. 1. Within-farm seroprevalence of antibodies to T. gondii (T) and Sarcocystis spp. (S) in sows from 94 randomly selected farms in Hesse, Germany using ELISA. The median, 10th, 25th, 75th and 90th percentiles and extreme values are shown.

Table 2
Effects of age and reproduction status of sows on the presence of antibodies to T. gondii and Sarcocystis spp. using ELISA on farms in Hesse, Germany (multiple logistic regression model)

<table>
<thead>
<tr>
<th>Factor</th>
<th>T. gondii</th>
<th></th>
<th>Sarcocystis spp.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (β)</td>
<td>Odds ratio (95% CI)</td>
<td>Coefficient (β)</td>
<td>Odds ratio (95% CI)</td>
</tr>
<tr>
<td>Intercept</td>
<td>−2.14</td>
<td></td>
<td>−2.24</td>
<td></td>
</tr>
<tr>
<td>Age (parity number)</td>
<td>0.17</td>
<td>1.2***</td>
<td>0.06</td>
<td>1.06*</td>
</tr>
<tr>
<td>In early pregnancy vs. nonpregnant</td>
<td>0.34</td>
<td>1.4b (0.99–2.0)</td>
<td>1.51</td>
<td>4.5*** (3.1–6.6)</td>
</tr>
<tr>
<td>In late pregnancy vs. nonpregnant</td>
<td>−0.11</td>
<td>0.9b (0.6–1.5)</td>
<td>1.39</td>
<td>4.0*** (2.6–6.3)</td>
</tr>
<tr>
<td>Suckling vs. nonpregnant</td>
<td>0.26</td>
<td>1.3b (0.8–2.0)</td>
<td>1.36</td>
<td>3.9*** (2.5–6.0)</td>
</tr>
</tbody>
</table>

*a* Confidence interval.

b Not significant.

*P < 0.05.

***P < 0.001.
finishing farms (Table 4). Correspondingly, the within-farm seroprevalence in piglet production farms was on average significantly higher than in the others (data not shown). In contrast, the origin of replacement sows (Table 4), herd size, floor type, cleaning methods, access of cats to stable units and pasturing of sows were not identified as risk factors for *T. gondii* seropositivity (Table 3).

### 3.3. Antibodies to *Sarcocystis* spp.

The estimated prevalence of antibodies to *Sarcocystis* spp. in sows was 28.6%, and 72.3% of the farms harboured at least one seropositive sow (Table 1). A within-farm seroprevalence of $\geq 50\%$ was detected in 23% of all farms (Fig. 1), i.e. in 32% of those with seropositive animals.

Both the age and reproduction status of sows were independently related to the presence of antibodies to *Sarcocystis* spp. in individual sows (Table 2): as the parity number increased by 1, the odds to react seropositively increased by 6%, and the chance to react with *S. miescheriana* antigen was about four times higher in pregnant and suckling sows than in nonpregnant ones.

On the farm level, both the farm type and origin of female replacements were significantly associated with the presence of *Sarcocystis*-seropositive sows (Table 3). The chance to detect seropositive sows was higher in piglet production farms than in pedigree breeding farms and also higher in farms that purchased female replacements than in those that replaced culled sows by the own progeny (Table 4). Correspondingly, significantly higher rates of seropositive sows were found, on average, in piglet production farms and in premises that purchased replacements than in the respective counterpart (data not shown).
Other farm parameters including presence of dogs were not significantly associated with the occurrence of antibodies to *Sarcocystis* spp. in sows (Table 3).

### 3.4. Antibodies to N. caninum

Antibodies to *N. caninum* were detected by the screening ELISA in 3.3% of the sows from 27.7% of the farms (Table 1). In half of the positive premises only one animal reacted with *N. caninum* antigen; in one farm a seroprevalence of 60% was found. Samples of *Sarcocystis*-seropositive sows reacted more frequently with *N. caninum* antigen (5.7%) than those of *Sarcocystis*-seronegative animals (1.9%; \( P < 0.0001 \), Chi-square test). In contrast, cross-reaction between *N. caninum* and *T. gondii* (ELISA) was not observed.
4. Discussion

This cross-sectional survey is the first in central Europe to give representative data on the prevalence of antibodies to *T. gondii*, *Sarcocystis* spp. and *N. caninum* in the breeding pig population of a region.

4.1. *T. gondii*

Antibodies to *T. gondii* were comparatively estimated using IFAT and ELISA. The results of both assays agreed substantially as already reported by others (Hirvelä-Koski, 1990; Arko-Mensah et al., 2000). *T. gondii*-seropositive sows were observed in about 69% of the farms but in many of them only a small proportion of animals reacted suggesting only sporadic infections. However, in 14% of the premises ≥50% of the sows were seropositive indicating that *T. gondii* clusters on some farms. Similar results were reported in market weight pigs from farms in Switzerland, Canada and the USA (Weigel et al., 1995a; Gajadhar et al., 1998; Wyss et al., 2000).

One observation of the present survey was that antibody levels in most cases were very low. This may be due to a decrease of *T. gondii*-specific antibodies in sows over time after infection. There is only scanty information on the time course of antibody levels to *T. gondii* in naturally infected pigs (Berends et al., 1991; Lind et al., 1995), but it has been shown using ELISA that IgG antibodies appear from 10–21 days after experimental infection and may persist on a similar level for more than 2 years (Dubey et al., 1997; Lind et al., 1997). Another explanation for low levels of antibodies to *T. gondii* may be a potential antigenic cross-reactivity between *T. gondii* and other coccidian parasites. Cross-reaction between *T. gondii* antigen used and *Eimeria* spp. or *Isospora suis* antigens has not been studied. However, the prevalence of antibodies to *T. gondii* detected by ELISA was significantly higher in *Sarcocystis*-seropositive sows than in *Sarcocystis*-negative animals.
in the present survey. Using the same ELISA protocol for sera of pigs that had been experimentally infected with *S. miescheriana*, three of nine animals showed low levels of *T. gondii*-reactive antibodies between 6 and 15 weeks after infection (Lind et al., 1997). Both explanations would mean that *T. gondii* infections in sows are less common than suggested by the mere figures of seropositivity.

Isolation of viable *T. gondii* appears to be less successful from slaughter pigs with low levels of antibodies (Dubey et al., 1995a) and failed in some studies (Gajadhar et al., 1998). However, it should be noted that the currently available viability tests for *T. gondii* vary greatly in their sensitivities (Dubey et al., 1995a) and also that viable *T. gondii* stages were occasionally isolated from meat of seronegative pigs (Hejlícek and Literák, 1993; Dubey et al., 2002). Therefore, seropositivity of pigs does not accurately reflect the infection risk for humans, but it is likely that a seropositive pig harbours infective tissue cysts of *T. gondii* and hence the consumption of under-cooked meat or meat products derived from this animal is a potential risk factor for *T. gondii* transmission to humans. Also, *T. gondii* seropositivity is an indicator for the hygienic status and the infection risk for pigs on the respective farm.

An overall comparison of the seroprevalence observed in the present survey with data from other regions or countries is difficult, because the methods used in epidemiological studies are not standardised and a broad range of serological tests with varying sensitivity and specificity for *T. gondii* were used (see Tenter et al., 2000). On the farm level, about 69% of the Hessian farms harboured at least one seropositive sow, which is similar to respective figures for pig breeding farms in some regions of the USA (47–64%; Weigel et al., 1995a; Patton et al., 1996) but quite lower than in breeding farms in Switzerland (93%; Wyss et al., 2000). In recent studies in northern and eastern Germany seropositivity of sows varied from 8 to 30% (Seineke, 1996; Fehlhaber et al., 2003); however, the numbers of animals and farms examined in those studies were too small, and their selection was not at random to be representative. Average seroprevalences of 12% and 17%, which are similar to the present results, were recorded for 807 sows from 30 Danish farms (Lind et al., 1995) and 110 slaughter sows from Sweden (Lundén et al., 2002), respectively. In contrast, 31% of 1009 slaughter sows from the Netherlands (Van Knapen et al., 1995) and 32% of 1720 sows from 82 farms in Switzerland (Wyss et al., 2000) were seropositive by ELISA, while an average seroprevalence of only 3–4% was recorded in sows from Austria involving the same IFAT method as in the present study (Quehenberger et al., 1990; Edelhofer, 1994). Other recent surveys showed antibodies to *T. gondii* in 9% of slaughter sows in different Canadian provinces (Smith, 1991) and in 12–36% of breeding sows in different states of the USA (Assadi-Rad et al., 1995; Dubey et al., 1995b; Weigel et al., 1995a; Patton et al., 1996, 1999). These differences may be explained by varying risks and sources of infection in the different regions, different serological tests and also different sampling procedures. For example, the sows in our survey were the present breeding stock and probably younger (mean parity number 3.2) than the slaughter sows examined in the Dutch study (Van Knapen et al., 1995). In fact, a positive correlation between age of pigs and seroprevalence was evident in the present survey and previous reports (Smith et al., 1992; Lind et al., 1995; Weigel et al., 1995a; Arko-Mensah et al., 2000). In conclusion and in spite of the above-mentioned uncertainties these data clearly demonstrate considerably higher prevalences of anti-*T. gondii* antibodies in sow herds when compared with those in
fattening pigs where regional seropositivities decreased during the past decades to \( \leq 6\% \), e.g. in several European countries (Edelhofer, 1994; Lind et al., 1995; Van Knapen et al., 1995; Wyss et al., 2000; Lundén et al., 2002) and some states of the USA (Weigel et al., 1995a; Patton et al., 1996).

In the present survey the only management factor with a significant relation to the presence of *T. gondii*-seropositive sows on a farm was the farm type ‘piglet production’. However, there is no really plausible explanation for the higher chance of this farm type to have seropositive sows as compared to pedigree breeding or farrow-to-finishing premises. Pasturing of sows, performed in pedigree breeding and farrow-to-finishing farms, was not significantly associated with seroprevalence in Hessian sow herds. This corresponds with the results of Weigel et al. (1995b) who also found no significant difference between seroprevalences of pigs kept in confined or non-confined facilities, but disagrees with those of other studies in the USA, which suggest that access to outdoor facilities is a risk factor in transmission of *T. gondii* to pigs due to the possibility of ingestion of either oocysts shed by cats or tissue cyst-infected rodents (Smith et al., 1992; Assadi-Rad et al., 1995; Patton et al., 1999). In fact, the presence of cats on the farm was proved, under American conditions, to be an important risk factor for seropositivity in sows (Assadi-Rad et al., 1995; Weigel et al., 1995b; Patton et al., 1999; Lehmann et al., 2003). However, in our survey, the seroprevalence of sows was also not significantly associated with the access of cats to pig units on the farms. Presence of rodents, which are known to be reservoir hosts of *T. gondii*, may increase the infection risk in pigs as observed in the USA (Assadi-Rad et al., 1995; Dubey et al., 1995b; Weigel et al., 1995b). In contrast, bioassay testing of rodents trapped on farms in northwest Germany suggested that rodents are not a major factor in transmission (Von den Driesch, 1987), and the risk of transmission posed by rodents may vary with the infection rate in the rodent population of a particular farm (Weigel et al., 1995b; Mateus-Pinilla et al., 1999).

The association between recorded reproductive disorders and prevalence of antibodies to *T. gondii* in respective farms observed in the present study corresponds with several previous reports (see Dubey, 1986; Seineke, 1996). This might be of clinical and economic importance for the farmers and requires further investigation.

### 4.2. Sarcocystis spp.

Knowledge on the prevalence and risk factors of *Sarcocystis* infections in pigs is scanty, and representative data from recent years are missing worldwide. Tissue cysts of *Sarcocystis* spp. were found in 36% of slaughter sows in southern Germany (Boch et al., 1978), and *Sarcocystis* spp. cystozoites were isolated from the diaphragm muscle of 8% of 2338 pigs slaughtered in northwest Germany with higher prevalence in sows (18%) than in fattening pigs (4%) (Furmanski, 1987). Sarcocysts were found in 32% of 348 slaughter sows in Austria (Hinaidy and Supperer, 1979), 18% of 893 slaughter sows in Iowa, USA (Dubey and Powell, 1994) and 16% of 104 sows in Japan (Omata et al., 1993). Antibodies to *Sarcocystis* spp. were detected in 15–35% of 133 slaughter pigs from southern Germany depending on the assay used (Neumayer, 1982), and in Spain 39% of 100 fattening pigs reacted seropositively (Pereira and Bermejo, 1988). In the present survey 72% of the Hessian breeding farms harboured seropositive sows, and the overall seroprevalence in
sows was about 29%. However, *Sarcocystis* seropositivity does not necessarily correspond with the presence of cystozoites in muscles: for example, 69% of 118 slaughter sows were seropositive in an IFAT but cystozoites were isolated from only 23% of these animals (Furmanski, 1987). From this reason and because older investigations were based often on rather limited numbers of animals or had sampling bias, it is not possible to compare the data of the present study directly with those from previous studies in Germany or other European countries. Nevertheless, data available from the past decades give no indication for a decreasing prevalence of *Sarcocystis* infection in pigs in Germany.

Concerning the possible zoonotic importance of the seroprevalence data presented here it should be emphasized that serology currently does not allow to differentiate between *Sarcocystis* spp. According to Furmanski (1987) and Hinaidy and Supperer (1979) the human species *S. suihominis* is less common in pigs than the dog-derived species *S. miescheriana*, at least in Germany and Austria. But irrespective of this aspect, *Sarcocystis* infections may represent an economic problem for the pig industry affecting growth rate and productivity and may sporadically cause acute disease (Boch et al., 1980; Daugschies et al., 1988; Dubey, 1993).

In the present survey, a positive age-dependency of the *Sarcocystis* seroprevalence in sows was recorded, similarly as for *T. gondii*. Additionally, pregnant and suckling sows had independent of their age a significantly higher chance to be *Sarcocystis*-seropositive than dry sows. This observation remains to be plausibly explained. On the farm level, the farm type ‘piglet production’ (versus ‘pedigree breeding’) and purchasing of replacement sows from other farms (versus raising on the own farm) were found to be risk factors. The risk of introducing an infection will be higher, of course, if replacements are purchased from elsewhere, as documented for piglet production farms. Other management practices including access of dogs, as definitive hosts of *S. miescheriana*, to pig facilities were not identified as risk factors for *Sarcocystis* seropositivity.

### 4.3. *N. caninum*

Concerning *N. caninum* an occurrence of antibodies in the sows would have not been totally surprising since at least experimental infections of pigs with tachyzoites resulted in seroconversion (the positive reference serum used in the present survey was derived from one of these animals) and vertical infection of offsprings (Jensen et al., 1998). In fact, 67 of the sow sera tested here reacted in the screening ELISA. However, only one case was confirmed by immunoblotting indicating that the other samples were false-positive in the ELISA. This discrepancy reflects the current uncertainty in estimating antibodies to *N. caninum* in pigs. Thus, Wyss et al. (2000) observed 3% and 1% seropositive sows and fattening pigs, respectively, in Switzerland when using an ELISA but could not confirm any case by polymerase chain reaction (PCR). In case of sera from aborted sows in southern Britain even 8.8% reacted when tested by ELISA but the reaction could be verified neither by IFAT nor by a specific inhibition ELISA (Helmick et al., 2002). In the present study, *Sarcocystis*-positive sera reacted significantly more frequently with *N. caninum* antigen than *Sarcocystis*-negative samples, which suggests that ELISA results are affected by cross-reactions with *Sarcocystis* spp. as have been reported for cattle (Dubey et al., 1996). Nevertheless, considering the recognition of *N. caninum* specific antigens by the one
particular plasma sample in our study, it could indicate the first natural infection in pigs reported so far. We would have liked to proceed the donor animal to further investigation, e.g. PCR analyses, however, the sow was no longer available.

5. Conclusions

In conclusion, the results of the present serological survey indicate that the risk of infection with both *T. gondii* and *Sarcocystis* spp. is still high in breeding sows from the southern part of the Federal State of Hesse, Germany. These data also demonstrate that natural infections with *N. caninum* may occur in pigs only very sporadically and thus play a negligible role in the epidemiology of this protozoon.

Acknowledgements

We thank Andrea Bärwald (Wusterhausen) and Angelika Duttmann (Hannover) for technical assistance and Anja R. Heckeroth for carrying out the TG-ROC analyses. I.M. Damriyasa was supported by a research grant of the German Academic Exchange Service (DAAD).

References


