Potential Risk Factors for the Presence of Anti-*Toxoplasma Gondii* Antibodies in Finishing Pigs on Conventional Farms in the Netherlands

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Research Article

Keywords: Toxoplasma gondii, pigs, risk factors, seroprevalence, cats

Posted Date: March 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1428804/v1

Abstract

Background

The parasite *Toxoplasma gondii (T. gondii)* is recognized worldwide as a pathogen causing a substantial human disease burden. Ingesting improperly cooked meat containing *T. gondii* is considered one of the major sources of human infection in Europe and North America. Consequently, control of *T. gondii* infections in pigs is warranted. The European Food Safety Authority (EFSA) advised to perform serological testing of pigs and farm audits for the presence of risk factors. Serological monitoring was implemented in several Dutch slaughterhouses. Blood samples from all deliveries of finishing pigs to the slaughterhouses were tested for the presence of anti-*T. gondii* antibodies. Using these test results, a case-control study was initiated to assess the association between the within-herd *T. gondii* seroprevalence and the presence of risk factors for *T. gondii* infections in 69 conventional finishing pig herds in the Netherlands.

Results

In a multivariable model twelve potential risk factors were significantly ($P \leq 0.05$) associated with the level of seropositive *T. gondii* blood serum samples.

Conclusions

Serological screening of finishing pigs in the slaughterhouse for *T. gondii* can be used to identify presence of *T. gondii* risk factors on Dutch conventional finishing pig farms. The use of serological screening seems therefore a valuable tool to guide and monitor the control of *T. gondii* in pork production.

Background

Toxoplasma gondii (*T. gondii*) is recognized worldwide as a pathogen causing a substantial human disease burden. It is estimated that up to one third of the world population has been exposed to the parasite [1]. In the Netherlands, toxoplasmosis ranks second on a list of prioritized emerging zoonosis [2] and also second in disease burden among 14 food-related pathogens [3].

T. gondii is an intracellular protozoan zoonotic parasite. Although sexual reproduction is only possible in felines, the definitive host, it can probably infect almost all warm-blooded animals including humans [4]. Human infection with *T. gondii* can occur by ingestion of sporulated oocysts present in soil or water or via contaminated fruit or vegetables or by ingestion of raw or undercooked meat from infected animals [4]. In humans, vertical transmission may occur from a mother, infected with *T. gondii*, to her unborn child. Transmission may also occur via blood transfusion or organ transplantation [5].

Ingesting raw or undercooked meat containing *T. gondii* is considered one of the major sources of human infection in Europe and North America [6–8]. In the Netherlands, it was estimated that pork contributed 12% to the total meat borne *T. gondii* infections [9]. Consequently, because of the high human disease burden of *T. gondii*, control of *T. gondii* infections in pigs or pork products is warranted.

As pigs are omnivorous animals they can be exposed to *T. gondii* by ingestion of sporulated oocysts in contaminated feed or water and by ingestion of bradyzoites through the consumption of infected rodents or birds. Few pigs become infected prenatally by trans-placental transmission [10]. Although the parasite can cause illness and mortality, especially in neonatal pigs, most pigs show hardly any clinical symptoms [10–12]. The level of *T. gondii* infections in pig herds depends on the farming system, with outdoor access leading to a higher reported seroprevalence compared to being held solely indoors [13–15]. Other reported risk factors for *T. gondii* infection include the presence of cats and occurrence of rodents and flies on the farm, the accessibility of cats, rodents and birds to pig feed, water and enrichment material, the feeding of goats whey and the degree of cleaning and disinfection on the farm[1, 10, 14, 16–23].

Detection of *T. gondii* in carcasses at slaughter by the currently practiced meat inspections is impossible due to the small size of tissue cysts and absence of pathological changes in carcasses [13]. To control *T. gondii* infections in pigs, the European Food Safety Authority (EFSA) advised to perform serological testing of pigs and farm audits for the presence of risk factors [13]. Indirect (serological) methods, based on the detection of antibodies against the parasite, have been developed [10, 24]. Among these methods, ELISA techniques have been used and validated for the diagnosis of *T. gondii* infection in pigs. These assays are easy to perform and enable testing of large serum sample numbers within a short time. In addition, several ELISA tests were standardized and commercialized [24–26].

Serological monitoring for *T. gondii* was implemented in several Dutch slaughterhouses [27]. Samples from all batches of finishing pigs were tested for the presence of anti-*T. gondii* antibodies. Based on these results, a case-control study was initiated to assess the association between the within-herd *T. gondii* seroprevalence and the presence of risk factors for *T. gondii* infections in finishing pig herds in the Netherlands.

Results

Descriptive results

In the end, 69 farms agreed to participate in this study. Approximately 5% of the initially contacted farmers declined cooperation. Reasons for this were: farm biosecurity, lack of time, lack of motivation or a (temporary) cessation of raising pigs. Fourteen of the participating farms were farrow-to-finish operations. Of the 69 farms, 25 farms (36%) had no positive blood samples in the year before the audit and from the total of 5134 serum samples analysed for these farms, 259 (5%) were considered positive (Table 1).

Table 1
Frequency distribution (n) of 69 Dutch finishing pig farms, their tested sera, and the positive tested sera related to the percentage of within-farm **T. gondii** positive seroprevalence.

Seroprevalence at finishing pig farm (%)	N (%) farms		N sera tested	N sera positive
0	25	(36)	800	0
0–5	19	(28)	2836	51
5–10	8	(12)	555	33
10–20	11	(16)	744	106
> 20	6	(9)	199	69
Total	69	(100)	5134	259

Farm and management characteristics related to the within-farm T. gondii seroprevalence

In total, 25 of the 30 examined potential risk factors reached a $P \leq 0.15$ in the univariable logistic regression (Table 2). One risk factor ('feeding compost, soil or peat') had too few observations in a category to have the model run adequately and was excluded from the multivariable analysis. The risk factor 'use of straw' was excluded from the multivariable analysis due to missing values. The variables 'wet/liquid feed', 'roughage' and 'corncob mix' were excluded from the multivariable analysis due to correlations with the variables 'compound feed heated' (i.e. $r > 0.7$), 'bedding pigs accessible for rodents' (i.e. $r > 0.7$) and 'pig feed accessible for cats' (i.e. $r > 0.5$) respectively. The variables 'pig feed accessible for rodents' and 'pig feed accessible for cats' were strongly correlated (i.e. $r > 0.7$). Given cats are the definitive host, the variable 'pig feed accessible for cats' was retained. Correlations between other variables were ≤ 0.5.

Table 2
Univariate analysis of potential risk factors for *T. gondii* infection in pigs from 69 Dutch finishing pig farms.

Risk factor/variable	Variable values	Frequency (n)	% positive blood samples	*P*-value
General farm characteristics				
Type of farm	Closed / Open	14 / 55	3.6 / 5.5	0.008
Presence of dogs	Absent / Present	22 / 47	6.9 / 4.5	0.002
Presence of poultry	Absent / Present	59 / 10	4.0 / 10.4	< 0.001
Presence of ruminants *	Absent / Present	41 / 28	4.2 / 6.8	< 0.001
Biosecurity				
Well-defined clean/dirty zones	No / Yes	52 / 17	5.7 / 3.2	< 0.001
Boots only used inside stables	No / Yes	43 / 26	5.8 / 3.3	< 0.001
Shower and farm clothing	Absent / Present	61 / 8	6.0 / 2.0	< 0.001
Supply of pigs				
Purchase of breeding gilts [a]	No / Yes	58 / 11	5.2 / 4.1	0.195
Cleaning every round of pigs	No / Yes	21 / 47	7.8 / 4.2	< 0.001
Cats				
Presence of cats				< 0.001
- Absent		19	3.1	

* Cattle, sheep and/or goats

[a] Risk factors not included in the multivariable analysis due to $P > 0.15$ in univariable analysis.

[b] Risk factors not included in the multivariable analysis due to low frequency counts.

[c] Risk factors not included in the multivariable analysis due to collinearity issues.

[d] Risk factors not included in the multivariable analysis due to missing values.

Risk factor/variable	Variable values	Frequency (n)	% positive blood samples	*P*-value
- Present, no kittens spotted, cats not in stable		29	3.3	
- Present, kittens spotted, cats not in stable		11	11.8	
- Present, no kittens spotted, stable accessible		3	7.1	
- Present, kittens spotted, stable accessible		6	13.5	
Pig feed accessible for cats	No / Yes	46 / 23	2.8 / 8.1	< 0.001
Bedding pigs accessible for cats [a]	No / Yes	65 / 4	5.1 / 4.8	0.882
Feed supply				
Compound feed heated	No / Yes	37 / 32	5.6 / 3.8	0.004
Compost, soil, peat [b]	No / Yes	68 / 1	4.9 / 10.4	0.032
Whey (goat and/or cow)	No / Yes	51 / 18	4.4 / 5.9	0.021
Whey (cow) [a]	No / Yes	59 / 10	5.2 / 4.3	0.246
Whey (goat)	No / Yes	65 / 4	4.1 / 23.5	< 0.001
Wet/Liquid feed [c]	No / Yes	35 / 34	4.4 / 5.4	0.137
Roughage [c, d]	No / Yes	61 / 6	5.8 / 1.3	< 0.001
Corncob mix [c, d]	No / Yes	52 / 15	4.4 / 6.9	< 0.001
Use of straw [d]	No / Yes	54 / 13	5.6 / 4.0	0.059
Garden/kitchen waste	No / Yes	66 / 3	4.9 / 14.4	< 0.001
Water supply				

[*] Cattle, sheep and/or goats

[a] Risk factors not included in the multivariable analysis due to $P > 0.15$ in univariable analysis.

[b] Risk factors not included in the multivariable analysis due to low frequency counts.

[c] Risk factors not included in the multivariable analysis due to collinearity issues.

[d] Risk factors not included in the multivariable analysis due to missing values.

Risk factor/variable	Variable values	Frequency (n)	% positive blood samples	*P*-value
Pig drinking water	Mains / Well	32 / 37	6.2 / 4.6	0.017
Pest control and prevention				
Shielding of flies	No / Yes	49 / 20	4.4 / 6.2	0.006
Shielding of birds	No / Yes	8 / 61	8.2 / 4.8	0.014
Professional rodent control [a]	No / Yes	41 / 28	5.5 / 4.6	0.152
Mode of rodent control				0.051
- No or only with traps		4	4.6	
- Poison		47	4.6	
- - Poison and traps		18	6.8	
Stable accessible for rodents [a]	No / Yes	31 / 38	5.2 / 5.0	0.736
Pig feed accessible for rodents [c]	No / Yes	36 / 33	2.7 / 6.4	< 0.001
Bedding pigs accessible for rodents	No / Yes	64 / 5	5.3 / 1.6	0.004

[*] Cattle, sheep and/or goats

[a] Risk factors not included in the multivariable analysis due to $P > 0.15$ in univariable analysis.

[b] Risk factors not included in the multivariable analysis due to low frequency counts.

[c] Risk factors not included in the multivariable analysis due to collinearity issues.

[d] Risk factors not included in the multivariable analysis due to missing values.

Analysis of the multivariable model resulted in 12 variables significantly ($P \leq 0.05$) associated with the presence of positive blood serum samples for *T. gondii* on 69 farms (Table 3).

Table 3
Multivariable analysis of potential risk factors for *T. gondii* infection in finishing pigs from 69 Dutch finishing pig farms using backward elimination and inclusion criterion of $P \leq 0.05$.

Risk factor	No. of Farms	Blood samples			OR	95% CI	*P*-value
Variables		No. positive	No. tested	% positive			
Type of farm							
Closed	14	42	1180	3.6	1.00		0.0488
Open	55	217	3954	5.5	0.63	0.40–1.00	
Dogs							
Absent	22	83	1211	6.9	1.00		0.0161
Present	47	176	3923	4.5	0.60	0.40–0.91	
Ruminants (cattle, sheep and/or goat)							
Absent	41	145	3463	4.2	1.00		0.0071
Present	28	114	1671	6.8	1.67	1.15–2.42	
Boots only used inside stables							
No	43	207	3578	5.8	1.00		0.0068
Yes	26	52	1556	3.3	1.91	1.20–3.04	
Shower and farm clothing							
No	61	235	3947	6.0	1.00		0.0106
Yes	8	24	1187	2.0	0.37	0.17–0.79	
Mode of rodent control							
No or only with traps	4	12	262	4.6	1.00		0.0056
Poison	47	175	3808	4.6	3.37	1.23–9.23	
Poison and traps	18	72	1064	6.8	5.57	1.90–16.3	

Risk factor	No. of Farms	Blood samples			OR	95% CI	*P*-value
Bedding pigs accessible for rodents							
No	64	253	4767	5.3	1.00		0.0002
Yes	5	6	367	1.6	0.17	0.07–0.44	
Presence of cats							
Absent	19	47	1530	3.1	1.00		< 0.0001
Present, no kittens, no stable access	29	82	2498	3.3	1.90	1.11–3.27	
Present, kittens, cats not in stable	11	82	697	11.8	11.80	6.23–22.5	
Present, no kittens, stable accessible	3	7	99	7.1	2.87	0.67–12.3	
Present, kittens, stable accessible	6	41	304	13.5	4.20	2.04–8.55	
Drinking water for pigs							
Tapwater	32	96	1555	6.2	1.00		0.0095
Well	37	163	3579	4.6	0.60	0.40–0.88	
Feed heated							
No	37	195	3459	5.6	1.00		0.0129
Yes	32	64	1675	3.8	0.42	0.21–0.83	
Whey (goat)							
No	65	201	4887	4.1	1.00		< 0.0001
Yes	4	58	247	23.5	11.30	7.12–18.0	
Shielding of birds							
No	8	26	316	8.2	1.00		0.0035
Yes	61	233	4818	4.8	0.18	0.06–0.57	

Discussion

In this study the association was assessed between *T. gondii* seroprevalence and potential risk factors for *T. gondii* infections in finishing pig herds in the Netherlands. Twelve out of 30 variables were identified as potential risk factors. Most of these 12 potential risk factors are already well known for *T. gondii* and in general related to the presence of cats, presence of other animals, the accessibility of cats, rodents and birds to the stables and feeds and rodent control [18]. To determine the association, the seroprevalence in the selected herds was calculated on the basis of a serological surveillance system for which from every delivery of finishing pigs to the slaughterhouse one or six serum samples were taken and tested for anti *T. gondii* antibodies [27]. Because of the significant association with known farm risk factors, it can be concluded that this serological surveillance system can be used to identify finishing pig farms where the typical *T. gondii* risk factors are present. This finding emphasizes that determination of the within-herd *T. gondii* seroprevalence is valuable to guide and monitor the control of *T. gondii* in pig herds. Recently, we performed an intervention study on five pig farms in which the within-herd *T. gondii* seroprevalence was successfully used to evaluate the effectiveness of the interventions on T. gondii risk factors [29]. These results confirm that determination of within-herd *T. gondii* seroprevalence is a useful part of a surveillance system based on serology for detection of *T. gondii* infections in pigs.

As in other studies, in our study the presence of cats at the barnyard or in the pig stables was associated with a significantly increased seroprevalence of *T. gondii* in pigs. Pigs can get infected by uptake of soil, feed and water contaminated with oocysts shed by cats in the environment, or by ingestion of cysts in the tissues of infected intermediate hosts (e.g. rodents, birds, meat and cannibalism) [30].

Our results also showed that not just the presence of cats on pig farms is a significant risk factor but that this significance increased when kittens were present. Kittens pose the highest risk of spreading oocysts in the environment, because most cats are infected with *T. gondii* as juveniles [34] or even as suckling kittens [35]. Cats only spread *T. gondii* in their feces for 1–3 weeks following the first episode of infection and they become immune to re-shedding of oocysts [36]. Neutering adult cats to prevent kittens to be born was found to be a successful intervention to achieve a significant reduction in *T. gondii* seroprevalence in a pig herd [29]. On farms, cats are often used to control rats and mice surrounding the pig stable which is also important in preventing a *T. gondii* infection and in improving biosecurity. Thus, many pig farmers might not want to remove all cats from the farm. Instead, the advice can be given not to keep kittens on the farm and that neutering of cats is a suitable approach to achieve this.

Our questionnaire included several questions about feed-related variables, because uptake by pigs of sporulated oocysts of *T. gondii* in animal feed represents an important route by which pigs can be infected. Open or less confined feed storage or feeding area represent an increased risk for exposure of livestock to the parasite [30]. However, most of the feed-related variables could not be analyzed in our multivariable analysis due to collinearity with other variables or due to missing values. The only feed-related variable which we could analyze was the use of heated feed for feeding the pigs, and this was found to be significantly reduce *T. gondii* seroprevalence. High temperatures during the production of pig

feed can inactivate the parasite. More research is needed to analyze the impact of other feed-related variables.

We found that feeding of goat whey is associated with a high seroprevalence. Although there were only four farms where whey was fed, the difference in seroprevalence with an OR of 11.30 between these four farms and the other 63 farms was considerable. This is in line with other studies that showed that feeding of pigs with raw milk goat whey is an important risk factor for infection with *T. gondii* [20, 31].

As in other studies, rodent control was identified as a risk factor for *T. gondii* infections in pigs [14, 17]. Besides that, in this study we found that rodent control using a combination of poison and traps has a higher OR than the use of poison and traps separately. It could be that simultaneous application of the two approaches for rodent control is more effective than each single approach. As in other studies, we identified shielding of birds as a preventive factor for *T. gondii* infections in pig herds [30]. Birds can acquire *T. gondii* infection through ingestion of oocysts from the ground or through ingestion of tissue cysts present in infected prey. Like rodents, birds are incidentally caught and eaten by pigs.

In our study, presence of other farm animals (cattle, sheep and/or goats) on the farm was found to be a risk factor for *T. gondii* infection in finishing pigs, while in other studies it does not seem to be a risk factor [19, 32]. However, in line with our findings, a recent review [30] suggested that the presence of multiple animal species on a farm could serve as an indicator of low farming intensity and that this low intensity was often related to a higher risk of *T. gondii* seropositivity.

In our study presence of dogs on the farm was found to be a preventive factor for *T. gondii* infection in pigs (OR = 0.5). Hill *et al*. (2010) also found that the presence of dogs was significantly associated with a reduced number of *T. gondii* seropositive samples on surveyed farms and the explanation is that dogs could be used for rodent control [16]. In contrast, other studies identified presence of dogs as a significant risk factor for *T. gondii* infection in pigs [14, 33] or did not find a significant effect [23].

The use of boots only in the stables was identified as potential risk factor, although the crude percentage of positive samples was lower for this category compared to the reference. A similar apparent mismatch was observed for the variable ‘type of farms’. Additional modelling (forward multivariable selection, bi- and trivariable logistic regression; data not included), showed that confounding and effect modification were unlikely to explain this observation. We hypothesize that these observations result from the effect explained by other variables in the multivariable model. The remaining effect attributed to the two mentioned risk factors is thus with an sign opposite to what one would initially expect. We hypothesize that these observations result from the combined effect explained by all other variables in the multivariable model.

A further difference was found for the water source as a risk factor for *T. gondii* infection in pigs. In our study, use of well water was found as a preventive factor for *T. gondii* infection compared to tap water. A recent review [30] concluded that it is hard to quantify the risk for a *T. gondii* infection of pigs through water, because in some studies well water was associated with an increased risk, while it seemed to have

a protective statistical effect in others. A potential reason for these differences could be that in some studies cats had access to the water at any stage before it reached the pigs and contaminated it with oocysts, whereas in other studies they did not. Water can be supplied to the pigs from a variety of sources and on different ways, which may depend on different production systems and regional differences. It should be noted that changing from water source might not be a possible intervention for all pig farmers, because the production system might prescribe a certain source or regional circumstances prevent implementation of certain sources.

Conclusion

Twelve potential risk factors for *T. gondii* infection of finishing pigs were identified using serological screening of Dutch intensive pig farms. The use of serological screening seems therefore a valuable tool to guide and monitor the control of *T. gondii* in pork production.

Materials And Methods

Farm selection and study

Multiple Dutch slaughterhouses from one company in the Netherlands run a serological monitoring programme for *T. gondii*. From every delivery of finishing pigs, a minimum of one and a maximum of six serum samples were taken [27]. The serum samples were used for antibody testing by a PrioCHECK™ Toxoplasma Antibody ELISA. A cut-off of 20% positivity was considered to classify samples as positive, as described by the manufacturer. The performance of this ELISA test was determined in two different studies [24, 26]. The within-herd seroprevalence was estimated per farm using the test results from all serum samples from the preceding 12 months. A list was prepared with farms ranked on the basis of their within-herd seroprevalence. Subsequently, conventional finishing pig farms that delivered pigs at least three times per year were approached for voluntary participation when they were on the top (‘cases’) or the bottom (‘controls’) of this list.

Questionnaire

Participating farmers were audited using a questionnaire that was based on an earlier developed questionnaire [18]. Our questionnaire used the Hazard Analysis Critical Control Points (HACCP) framework to identify the most important control measures to prevent, reduce or control the introduction and spread of *T. gondii* on a pig farm. The questionnaire contained questions about farm and management characteristics potentially related to *T. gondii* infection in the pigs, general farm biosecurity measures, outdoor access, rodent control, presence of cats, feed and water supply. The questionnaire is available in the supplementary material and contained questions about 30 potential risk factors (Table 1). On each farm, a project researcher completed the questionnaire by interviewing the farmer during a farm visit. Furthermore, the interior of the stables as well as the outside environment of the stables were subjected to a visual inspection to verify elicited answers.

Statistical analysis

The effect of possible risk factors (RF_j, $j = 1$ to J, where subscript j indicate the risk factor) on the within-herd seroprevalence of anti-*T. gondii* antibodies was assessed using logistic regression [28]. The presence of antibodies in a blood serum sample was considered a binomial process, with the number of blood serum samples taken from farm i, n_i, being the number of trials. The probability of a test-positive sample for farm i, p_i, was defined as a linear function (including intercept) of the J possible risk factors, RF_j after a logit transformation through:

$$\text{logit}(p_i) = \beta_0 + \sum_{j=1}^{J} \beta_j \times RF_{ij} + \varepsilon_{ij} \qquad (2)$$

The statistical analysis was done in SAS version 9.4 (SAS Institute, Cary, NC, USA). Univariable analysis was used to preselect variables for multivariable analysis, where RF_j showing a probability < 0.15 were selected. Correlation between selected variables was assessed via the Pearson's correlation coefficient. If that coefficient was >|0.5|, then the correlated RF with the most likely biological explanation was included. The multivariable model was trimmed through a backward procedure as described by Hosmer and Lemeshow [28] and was considered completed when remaining variables all had a P-value < 0.05. The fit of the multivariable model was assessed with Hosmer and Lemeshow goodness-of-fit test.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Funding

This research was part of the project "*Toxoplasma* infections in pigs: a system for risk based monitoring in the pork production chain" within the public-private partnership "One Health for Food" in The

Netherlands. The research was co-funded by the Vion Food Group and the Dutch Ministry of Economic Affairs.

Acknowledgments

The authors sincerely thank all farmers for their cooperation. Also special thanks to Maud Klein Koerkamp, Janneke Heijltjes en Lourens Heres as participating auditors for the farm visits.

Author Contributions

Conceptualization, D.M.E., M.B. and H.J.W.; methodology, D.M.E. and M.B.; formal analysis, M.B.; investigation, D.M.E; writing—original draft preparation, D.M.E, M.B. and H.J.W.; writing—review and editing, J.W.B.vd.G., M.S., D.O., H.A.P.U., C.P.A.v.W. and M.A.P.M.v.A.; visualization, D.M.E., M.B. and H.J.W.; supervision, H.J.W.; project administration, H.J.W.; funding acquisition, M.B. and H.J.W. All authors read and approved the final manuscript.

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