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Large-scale serological screening of slaughter pigs for *Toxoplasma gondii* infections in The Netherlands during five years (2012–2016): Trends in seroprevalence over years, seasons, regions and farming systems

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ABSTRACT

Toxoplasma gondii is the causative agent of the parasitic disease toxoplasmosis, which is an important foodborne zoonosis. Eating undercooked meat of infected animals, including pigs, has been considered the major transmission route of *T. gondii* to humans. Therefore, it is urgent to develop and implement intervention measures in the pork meat chain to reduce risks of acquiring a *T. gondii* infection. Proposed measures for control of *T. gondii* in pigs include serological testing of pigs and audits of pig farms on risk factors for *T. gondii* infection. So far, these ideas have not been tested in practice. In order to generate knowledge about the epidemiology and seroprevalence of *T. gondii*, as a basis for developing a surveillance system, we studied the long term seroprevalence over years, farming systems and regions, and seasonal patterns of *T. gondii* seroprevalence in Dutch slaughter pigs. During a five year study period from 2012 to 2016, serum samples were routinely collected in five Dutch pig slaughterhouses. The sera were tested in an ELISA for the presence of antibodies against *Toxoplasma*. In total 226,340 serum samples were collected and tested during the study period. The observed seroprevalence varied over years, with the highest overall seroprevalence in 2014 (2.8%) and the lowest in 2016 (1.4%). A higher seroprevalence was observed in pigs from organic farms compared to pigs from conventional farms. The overall risk of infection was on average 2.63 times significantly ($p < 0.001$) higher for organically raised pigs than for conventionally raised pigs. A seasonal pattern in seroprevalence was observed: the results showed a dominant annual periodicity with a seroprevalence peak in winter around week 1 and a minimum seroprevalence in summer around week 27.

To our knowledge, this is the first large scale study on the seroprevalence of *T. gondii* in slaughter pigs. In comparison to other European serological studies, the observed seroprevalence seems to be relatively low. However, care is needed when comparing the results with other studies because of differences in test setup, the number of samples and time period of sampling. The results can be used as a starting point for developing a surveillance system for *T. gondii*, and for implementation of intervention measures.

1. Introduction

Toxoplasma gondii is the causative agent of the parasitic disease toxoplasmosis. *T. gondii* is recognised as an important foodborne zoonosis. The human disease burden is regarded worldwide as very high (Torgerson and Mastroiacovo, 2013). In a global multicriteria based ranking, *T. gondii* ranked fourth out of 24 foodborne parasites (WHO, 2015); repeating of this ranking on a European level, *T. gondii* ranked together with *Trichinella spiralis* as second out of 23 foodborne parasites (Bouwknegt et al., 2018). In a study in the USA to explore the overall human health impact of domestically acquired foodborne illnesses (measured in DALY) *Toxoplasma* ranked second, just after non-typhoidal *Salmonella* (Scallan et al., 2015). All species of mammals can become infected with *T. gondii* (Dubey and Jones, 2008). Humans can become infected with *T. gondii* by intake of oocysts from cats via the environment or by ingestion of tissue cysts in raw or undercooked meat. In Europe, eating undercooked meat of infected animals, including pigs, has been considered the major transmission route of *T. gondii* to humans. Cook et al. (2000) estimated that *T. gondii* causes up to almost two-third of human infections. Viable *T. gondii* tissue cysts have been

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isolated from tissues and meat of pigs naturally and experimentally infected with *T. gondii* (Dubey et al., 1998; Dubey, 2009).

Given the high disease burden in humans, it is urgent to develop and implement intervention measures in the pork meat chain to reduce risks of acquiring a *T. gondii* infection. Research showed that prevalence of *T. gondii* infections in pigs is related to management on farms (Kijlstra et al., 2004). The number of pigs with antibodies against *T. gondii* in free-range farms was larger than on farms where pigs were kept indoors only (van der Giessen et al., 2007). The risk for *T. gondii* in pigs has also been associated with the presence of cats, occurrence of rodents and the degree of cleaning and disinfection (Villari et al., 2009; García-Bocanegra et al., 2010a; Hill et al., 2010; Veronesi et al., 2011). A change of management aimed at reducing risk factors above could thus contribute to the reduction of *T. gondii* infections in pigs.

The European Food Safety Authority (EFSA) suggested that *T. gondii* is one of the public health hazards to be covered by meat inspection of swine (EFSA, 2011). The traditional meat inspection is based on an individual visual inspection of animals at slaughter (Berends et al., 1993). This inspection was set up in times when contagious agents with visible deviations in carcasses were highly prevalent. An infection with *T. gondii* leads to little or no clinical abnormalities in pigs (Dubey, 2010) and *T. gondii* tissue cysts are too small to be seen in meat with the naked eye. Therefore, a *T. gondii* infection cannot be controlled at meat inspection in a visual way.

EFSA has proposed epidemiological indicators that make it possible to control *T. gondii* infections in pigs and safeguard it in the pork meat chain (EFSA, 2011). The instructions can be used by pig farms and slaughterhouses to prepare a package of measures, depending on the risk for a *T. gondii* infection. The measures advised by EFSA include serological testing of pigs on *T. gondii* infections and audits of pig farms on risk factors for *T. gondii* infection. However, the ideas of EFSA are abstract, not tested and not yet translated into working systems. Serological tests are developed and validated but not prepared for use in a system to control *T. gondii* infections (Steinparzer et al., 2015; Basso et al., 2013; Buholzer et al., 2010). Before developing a surveillance system based on serology for *Toxoplasma* in pigs, it is necessary to know the actual seroprevalence. Many studies on the seroprevalence of (finishing) pigs have been carried out in Europe in recent years. Different types of serological tests were used and different ages and categories of pigs (intensive, free range, organic) were sampled. Reported seroprevalence on animal level was between 0.4% (Deksne and Kirjušina, 2013; van der Giessen et al., 2007) and 10.0% (Meemken et al., 2014) for intensively kept pigs at finishing age, and went up to 27% for free range pigs (Hernandez et al., 2014) and 69.2% for organic pigs (Steinparzer et al., 2015). However, in most of the studies a limited number of samples was collected at one time point or within a limited time period. Until now, no large-scale long-term studies on serology in pigs have been reported.

In order to generate knowledge about the epidemiology and seroprevalence of *T. gondii*, as a basis for developing a surveillance system, we studied the long term seroprevalence over years, farming systems and regions, and seasonal patterns of *T. gondii* infections in Dutch slaughter pigs.

2. Materials and methods

2.1. Study population and design

Serum samples which were routinely collected in five slaughterhouses in the Netherlands for the serological monitoring of *Mycobacterium avium* infections in pigs (Hiller et al., 2013) were also tested for anti *T. gondii* antibodies. At every delivery (a group of pigs from the same farm, delivered on the same date to one slaughterhouse) of pigs, blood samples were collected haphazardly from pigs during bleeding. Criteria for the number of pigs sampled were based on the *M. avium* monitoring system (Hiller et al., 2013). Briefly, new suppliers were put on a regime of two samples per delivery. When 10 successively collected samples tested negative, then the regime was lowered to one sample per delivery. When a supplier had a test result between 20 and 50 (percentage positivity, PP), then six samples were collected from the next delivery. If these six tested negative, then the regime was lowered to one sample per delivery. If at least one sample tested positive, then six samples were taken per delivery until 18 consecutive samples tested negative. When at least one of the samples from a supplier tested positive with a PP $>$ 50, then the sampling regime was increased to six samples per delivery, until 18 consecutive samples tested negative. In practice, sometimes more than 6 samples were collected per delivery, especially in the first year when the sampling system was set up. For the analysis in this paper we used sera collected from January 2, 2012 until December 31, 2016. Pigs delivered to these slaughterhouses were raised on Dutch or Belgian pig farms and were slaughtered at approximately six months of age. The five slaughterhouses in which we sampled slaughtered approximately 50 percent of all pigs slaughtered in The Netherlands. Pigs originated from conventional and organic farms.

2.2. Outcome measurements

In the laboratory at the Animal Health Service, sera were tested for the presence of antibodies against *Toxoplasma* by the PrioCHECK *Toxoplasma* Ab porcine ELISA (Thermo Fisher Scientific Prionics Lelystad B.V.) according to the manufacturer's instructions. Test results were presented in percentage positivity (PP). PP was calculated as follows: PP = (OD sample-OD negative control)/(OD positive control-OD negative control)*100. We used a cut off PP value of 20% (results $\geq$20% were considered positive), as advised in the test manual by the manufacturer.

2.3. Study variables

Data for this study were delivered by the slaughterhouse company to which the five slaughterhouses in this study belong. Variables available for this study on pig-level were: sampling date, province of the farm the pig originated from, PP and type of farm (conventional or organic). Conventional farms fulfil all requirements of IKB (integral chain management system). These requirements cover for instance rodent control, preventing pets from entering the pig housing, feed of GMP (Good Manufacturing Practice) source, and veterinary care. These pigs have no outdoor access. Organic farms were farms that fulfil the requirements of IKB and SKAL (the Dutch organisation that checks if European requirements for organic production are fulfilled). The pigs were housed with compulsory outdoor access throughout their lives. Outdoor access could be a natural pasture or a paddock like system with concrete flooring. During the study period, no pigs from free range farms were delivered to the participating slaughterhouses, because almost all Dutch free range farmers had changed to organic farming due to the higher price paid for organically produced meat.

2.4. Data analysis

As part of initial data inspection, summaries of the total number of farms that delivered pigs, number of deliveries of pigs that were slaughtered and total number of samples collected during the total study period per year per farming system were generated. Carrying out surveillance at slaughter houses provides comparable results to simple random sampling when data are used to quantify the prevalence of an infection at the animal level (Schärrer et al., 2015). Therefore we used the data to (i) estimate the yearly seroprevalence of *T. gondii* infections in pigs and (ii) to evaluate seasonality in that seroprevalence. A potential source of bias was the difference in sample size per delivery (see section 2.1). This was adjusted for, as described by Gelman (2007).

(i) The yearly seroprevalence was estimated by fitting a logistic

regression model. In this model the serological result classifying the pig as positive or negative was the response variable. The explanatory variables were the year of surveillance, the farming system (organic, conventional), a categorical variable to adjust for the effect of sample size (categories: 1, (1,4], (4,10], > 10 samples), the province in the Netherlands (with Belgium as 13th region) in which the farm was located and the potential interactions between geographical location, the year and farming system. Significant interactions were only observed between year and farming system and between geographical location and farming system. Therefore these interactions were kept in the model. As a sensitivity analysis we also fitted a weighted model where sample size was used as a weight rather than an adjusting variable.

(ii) To test for seasonal variations in seroprevalence, we used sine (sin) and cosine (cos) functions in a logistic regression model. This type of models are known as harmonic regression models. These models explicitly include time as a covariate and characterise seasonal patterns in terms of amplitude (ratio of the peak seroprevalence to the trough (minimum) seroprevalence) and phase shift (Stolwijk et al., 1999). We evaluated whether seasonal patterns had a yearly cycle (one peak and trough per year (52 weeks period)), semi-yearly cycles (26 weeks period) or a combination of both. The final model had a combination of both:

$$logit(p) = \alpha + \beta_1 sin\left(\frac{2\pi t}{52}\right) + \beta_2 cos\left(\frac{2\pi t}{52}\right) + \beta_3 sin\left(\frac{2\pi t}{26}\right) + \beta_4 cos\left(\frac{2\pi t}{26}\right) + \beta_5 sample + \beta_6 farm + \beta_d f(t, d)$$

Where p is the seroprevalence, α is the model intercept, β_1 to β_4 are the parameters describing the seasonal cycles. These parameters were used to identify the periods of peak and minimum seroprevalence as well as the amplitude following formulas described elsewhere (Stolwijk et al., 1999). The parameter β_5 and β_6 are the parameters used to adjust for the number of samples tested per delivery (*"sample")* and farming system (*"farm"*) in the estimation of the seroprevalence, respectively. Finally, t is the week when the pig was sampled (study period consisted of 265 weeks) and $\beta_d f(t, d)$ are the set of the parameters (β_d) describing the trends in the seroprevalence during the study period. This trend was modelled using a function of natural cubic splines with 5 degrees of freedom (Faraway, 2006). Following the model fit, the weekly seroprevalence p was estimated by taking the inverse of the $logit(p)$. We examined whether or not the seasonality analyses should be stratified for farming system.

All analysis were done using the statistical software R. The package "Splines" was used to introduce cubic splines to model the trends in the seroprevalence of *T. gondii*.

3. Results

3.1. Sampling

During the total study period 3114 farms delivered pigs to the slaughterhouses. In total 226,340 serum samples from 173,851 deliveries of pigs were collected and tested during the study period. The average number of pigs per delivery was approximately 110 (not calculated; personal communication from the slaughterhouse company), with a large variation between deliveries. Table 1 shows the numbers of farms, deliveries and serum samples per farming system. The average number of serum samples taken per delivery of pigs from organic farms ranged from 3.8 in 2012 to 5.8 in 2016. For the conventional farms, mostly one serum sample was taken per delivery, with slightly higher number of samples taken in 2012 (Table 1).

3.2. T. gondii seroprevalence in pigs

In Table 2, crude and adjusted seroprevalence estimates and corresponding 95% confidence intervals (CI) are presented. A logistic regression model was used to calculate adjusted seroprevalence values. The highest overall seroprevalence was observed in 2014 [p = 0.028 (95%CI:0.019 – 0.042)] and the lowest in 2016 [p = 0.014 (95%CI:0.010 – 0.021)] (Table 2), with higher seroprevalence of infection observed in pigs from organic farms than in pigs from conventional farms (Table 2). The overall risk (adjusted) of infection for pigs from organic farms was on average 2.63 (95%CI: 1.6–4.18) times significantly higher than for pigs from conventional farms (Table 3).

The seroprevalence of infection stratified by geographical location (province of origin) is presented in Fig. 1. No interactions between province and calendar year were observed, which showed that yearly changes in seroprevalence applied to the whole country.

3.3. Seasonality of T. gondii infections in pigs

Significant similar seasonal patterns ($P < 0.05$) were observed in both organic and conventional pigs. Therefore, data of both systems could be pooled. Seasonal patterns were best explained by a combination of yearly and semi-yearly cycles. The identified periods (weeks) of peak and minimum seroprevalence were similar (overlapping confidence intervals) among conventional and organic pigs (data not shown), therefore we pooled the data from these labels to assess seasonality and included farming system in the model to adjust the final estimates. The analysis showed a dominant annual periodicity with a seroprevalence peak in winter around week 1 (95%CI: 52 – 2) and a minimum seroprevalence peak in summer around week 27 (95%CI 25–28) (Fig. 2). The mean ratio of the peak to minimum seroprevalence (amplitude), which can be considered as average relative risks of infection during the peak period, was 1.7 (95%CI: 1.6–1.9). The mean duration of the high seroprevalence period was around 19 weeks.

4. Discussion

More than 226,000 serum samples from slaughter pigs obtained in the period 2012–2016 were tested for the presence of *T. gondii* antibodies. To our knowledge, such a large scale study never has been carried out concerning *T. gondii* infections in pigs. The overall adjusted seroprevalence of pigs varied between years from 0.014 (1.4%) to 0.028 (2.8%). This is comparable to the results of Van der Giessen et al. (2007), who tested 845 pig serum samples from The Netherlands at slaughter age, and found a seroprevalence of 0.4% in intensively kept pigs, 5.6% in free range pigs and 2.7% in organic pigs. Other studies within Europe came up with largely differing seroprevalences (see also Table S1 in supplementary material). High seroprevalences were found in Spain by Hernandez et al. (2014), in the Czech Republic by Bártová and Sedlák (2011) and in Serbia by Klun et al. (2006), with seroprevalences of 58.2%, 36% and 29% respectively. Somewhat lower seroprevalences were reported in Switzerland by Berger-Schoch et al. (2011b), in Spain (García-Bocanegra et al., 2010a), Portugal (Lopes et al., 2013), Italy (Veronesi et al., 2011) and Germany (Meemken et al., 2014) with seroprevalences ranging from 9.8 to 16.1%. Relatively low seroprevalences were reported for Ireland by Halová et al. (2013), with a seroprevalence of 4.7%, and for Latvia by Deksne and Kirjušina (2013), with a result of 0.4% for intensively kept pigs and 6.2% for free range pigs. However, the large differences in seroprevalences in the above mentioned studies must be interpreted with care. Many different serological tests were used, with different cut off levels. In some studies meat juice was tested instead of serum. Also, in most studies the number of tested pigs was limited, and sampling was sometimes done in a limited period of the year. Furthermore, the types of originating farms of the pigs differed among studies. Therefore, *Toxoplasma* seroprevalences resulting from different studies and countries, cannot be directly compared.

In our study we found that pigs from organic farms had a higher probability to have antibodies against *T. gondii* than pigs from

Table 1
Number of pig farms, deliveries and samples tested for *Toxoplasma* antibodies per farming system per year and for the 5 year period in total.

Farming system	Year	Total number of farms	Total number of deliveries	Total number of samples	Average number of deliveries per farm (min-max)	Average number of samples per delivery (min-max)
All	2012	2734	41006	55681	15 (1-157)	1.8 (1-90)
	2013	2295	35550	41151	15.5 (1-170)	1.1 (1-6)
	2014	1820	32105	38752	17.64 (1-113)	1.2 (1-6)
	2015	1899	32196	44462	17.0 (1-98)	1.3 (1-8)
	2016	1877	32994	46294	17.6 (1-86)	1.4 (1-8)
	Total	3114	173851	226340		
Conventional	2012	2677	39927	51482	14.9 (1-157)	1.8 (1-90)
	2013	2240	34464	35413	15.4 (1-170)	1.0 (1-2)
	2014	1763	30947	32197	17.6 (1-113)	1.0 (1-3)
	2015	1859	31409	40046	16.9 (1-98)	1.2 (1-8)
	2016	1837	32264	42061	17.6 (1-86)	1.3 (1-8)
	Total	3049	169011	201199		
Organic	2012	57	1079	4199	18.9 (1-42)	3.8 (1-6)
	2013	55	1086	5738	19.6 (2-50)	5.2 (1-6)
	2014	57	1158	6555	20.3 (1-44)	5.7 (1-6)
	2015	40	787	4416	19.7 (2-43)	5.6 (1-6)
	2016	40	730	4233	18.3 (1-46)	5.8 (1-6)
	Total	65	4840	25141		

Table 2
Seroprevalence of *Toxoplasma* antibodies in Dutch slaughter pigs at animal level, per farming system (conventional / organic) and monitoring year. Seroprevalence values are given as proportion.

Farming system	Year	Crude seroprevalence (proportion of positives)	Adjusted seroprevalence (95% confidence intervals)
All	2012	0.021 (0.019 – 0.022)	0.020 (0.013 – 0.029)
	2013	0.017 (0.016 – 0.018)	0.016 (0.011 – 0.024)
	2014	0.030 (0.028 – 0.031)	0.028 (0.019 – 0.042)
	2015	0.021 (0.020 – 0.023)	0.021 (0.014 – 0.031)
	2016	0.015 (0.013 – 0.016)	0.014 (0.010 – 0.021)
Conventional	2012	0.018 (0.017 – 0.020)	0.017 (0.011 – 0.027)
	2013	0.015 (0.014 – 0.017)	0.015 (0.010 – 0.023)
	2014	0.027 (0.025 – 0.029)	0.026 (0.017 – 0.041)
	2015	0.021 (0.019 – 0.022)	0.020 (0.013 – 0.031)
	2016	0.014 (0.013 – 0.015)	0.014 (0.009 – 0.021)
Organic	2012	0.048 (0.042 – 0.055)	0.053 (0.041 – 0.069)
	2013	0.029 (0.025 – 0.034)	0.032 (0.025 – 0.042)
	2014	0.043 (0.038 – 0.048)	0.048 (0.037 – 0.061)
	2015	0.024 (0.020 – 0.029)	0.029 (0.022 – 0.039)
	2016	0.017 (0.013 – 0.022)	0.020 (0.015 – 0.028)

Table 3
Crude and adjusted odds of infection with *T. gondii* in slaughter pigs from organic farms. Adjusted estimates are adjusted for sample size, calendar year and geographical location.

Odd.Ratio	Mean	LCL[a]	UCL[b]	P value
Crude	1.78	1.65	1.92	< 0.001
Adjusted	2.63	1.60	4.18	< 0.001

[a] LCL: lower 95% confidence limit.
[b] UCL: upper 95% confidence limit.

conventional farms (Table 3). The main difference between organically kept pigs and conventionally kept pigs is that pigs from organic farms have outdoor access, and therefore have more possibilities to pick up oocysts from the environment and to come into contact with infected rodents.

Studying the seroprevalence of *Toxoplasma* in pigs for five consecutive years made it possible to look for seasonal patterns, and to compare seroprevalences between years and different types of farms. We discovered that the seroprevalence in pigs showed an annual periodicity with a peak in winter around week 1 and a minimum in summer around week 27. These findings are in line with Schulzig and Fehlhaber (2005) who found significantly more pigs to be infected during the autumn/winter than in the spring/summer period. Apparently, there is a seasonality in *Toxoplasma* infections in farmed pigs. The explanation for this seasonal pattern is not easy. The seroprevalence of *Toxoplasma* in pigs in this study was determined in slaughter pigs of approximately six months old. Dubey et al. (1997) showed that pigs can have *Toxoplasma* antibodies from two to three weeks after infection to at least a year after infection, so a positive serum sample at slaughter age does not tell us at which timepoint in life the pig had become infected. However, our results do indicate, although not when, that infections follow a seasonal pattern and further research needs to be done to identify this period of increased probability of infections in pigs. Performing, for example, longitudinal studies following a cohort of pigs in different farms, for six months before the identified seroprevalence peak, or multiple cross sectional surveys where pigs from different age strata are sampled (say every two months) could help identify the age and likely period of infections. Schares et al. (2016) found that cats shed oocysts predominantly in summer and autumn (June-November). It is possible that these oocysts infect mice and rats which have entrance to the pig houses and feed, or the cats have access to the pig feed and contaminate this with oocysts. The peak in winter in pigs might therefore be a consequence of this summer/autumn peak in cats. It might also be an effect of mice that live outside in summer, and that come into the pig houses in winter. Schares et al (2016) also showed an association between temperature and oocyst shedding of cats two months after a temperature peak. They also noticed an association between precipitation amount and oocysts shedding, with a three month time lag. In our study we measured antibodies in finished pigs (five - six months old) which could had been infected any time within the previous 0.5 to 6 months. Hence assessing associations between antibody prevalence and weather variables recorded for the time of sampling would lack biological relevance. Seasonality was also seen in human toxoplasmosis cases, and although not all authors report the same peak periods, winter seems to be the main season for acute toxoplasmosis in humans (Bobić et al., 2016; Contopoulos-Ioannidis et al., 2015; Morin et al., 2012; Logar et al., 2005). This peak of human cases in winter could partially be caused by the winter peak in pigs (eating raw pork products), but it is also possible that pigs and humans get infected by the same source, most likely oocysts shedded by cats during autumn. When comparing seroprevalence between years, the overall seroprevalence was highest in 2014 and lowest in 2016 (Table 2, Fig. 2). We cannot explain this easily. According to the manufacturer of the

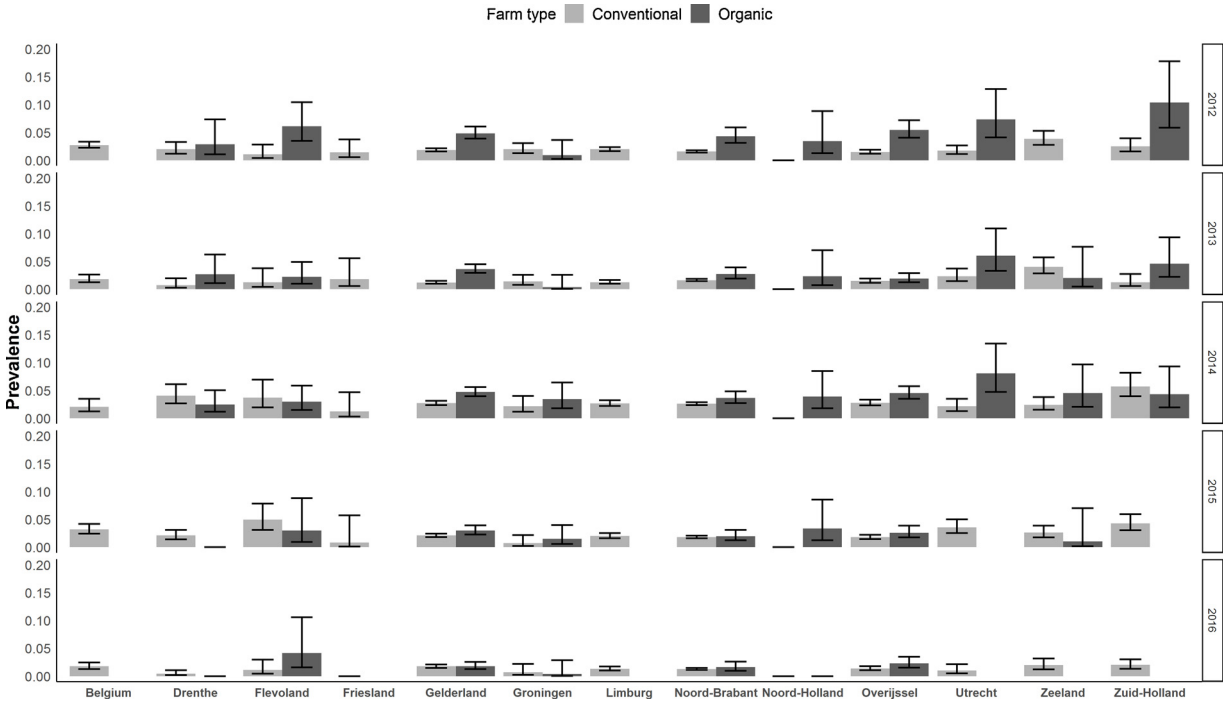

Fig. 1. *T. gondii* seroprevalence in Dutch slaughter pigs, per farming system, per province[a] and per monitoring year. Seroprevalence values are given as proportion[b].
[a]The country Belgium is reported here in the same way as the provinces of The Netherlands.
[b]If no values could be calculated, because of no samples in this period/province, the graph is blank. If seroprevalence was 0, the graph shows a black horizontal line at 0.

ELISA test, in this period nothing changed in the manufacturing of the test, and the same control samples were used. In Fig. 2, a real peak in seroprevalence can be seen in the autumn of 2014. This peak is the main explanation for the higher seroprevalences in 2014. A possible explanation might be the fact that there was a high infestation of mice in the winter of 2014/2015 in The Netherlands, especially in the northern provinces (Friesland) (Wymenga et al., 2016). To check if the bigger mice population could have caused the higher seroprevalence in pigs, we compared the seroprevalences per province. However, the analysis showed that the highest seroprevalences (for all five years) were found in Zuid-Holland and Zeeland (Fig. 1), which are located in the south-western part of The Netherlands. In each province the highest seroprevalence was measured in 2014, but the rise in seroprevalence in 2014 was not higher in the northern provinces, from which we conclude that the mice infestation does not seem to be a logical explanation for the seroprevalence peak in 2014. It might be interesting to mention here that Kik et al. (2015) reported about a sudden increase in dead red squirrels in The Netherlands in the autumn of 2014, which seemed to be caused by a *Toxoplasma* outbreak. This outbreak in squirrels perhaps was a result of the same cause as the seroprevalence peak in pigs. After

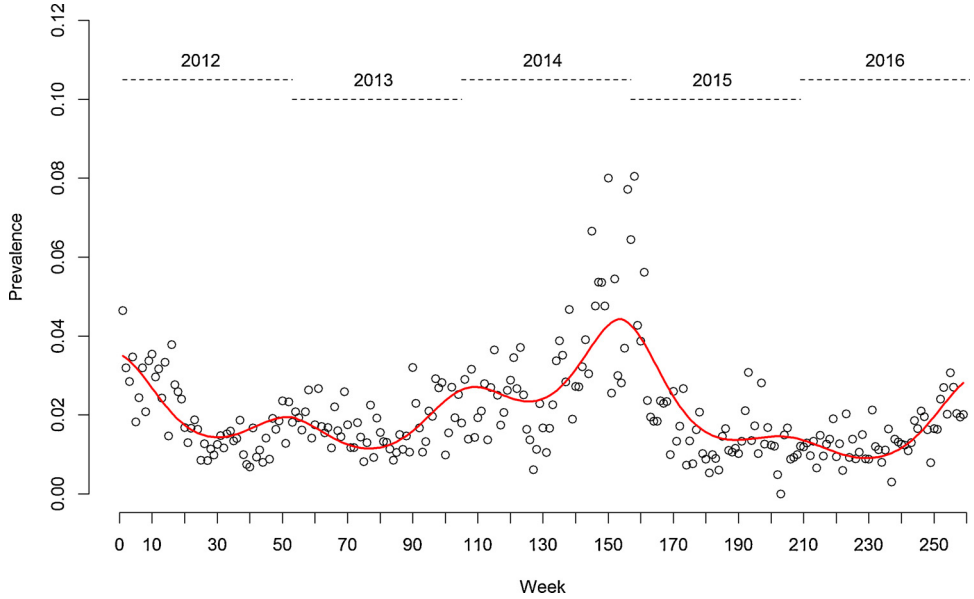

Fig. 2. Trends in *T. gondii* seroprevalence in Dutch slaughter pigs over years and seasons. Week 1 starts at January 1st 2012. The total study period was 5 years = 260 weeks.

the seroprevalence peak in the autumn of 2014 the seroprevalence decreased during the first months of 2015 (Fig. 2), like it did each year, to the "normal" seasonal pattern.

In this study we quantified farming system (organic, conventional) and seasonal risk factors (peak and duration) for seropositive detection of toxoplasmosis in pigs. These risk factors can be used to optimise the current surveillance programme and monitoring methods to assess efficacy of intervention, by for example targeting sampling to be done during the weeks of identified high risk and adjusting sample size as a function of the farming system.

5. Conclusion

From this largescale serological study it could be concluded that the *Toxoplasma* seroprevalence in Dutch slaughter pigs in the years 2012–2016 varied between 0.014 (1.4%) and 0.028 (2.8%). This seems to be relatively low, compared to other European serological studies, although we should be careful with comparing between different studies. We discovered that the seroprevalence varied between years, and that a seasonal pattern was present with a peak in winter. These results can be used as a starting point for developing a more efficient surveillance system for *T. gondii*, and for implementation of intervention measures.

Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication (Large-scale serological screening of slaughter pigs for *Toxoplasma gondii* infections in The Netherlands during five years (2012–2016): trends in seroprevalence over years, seasons, regions and farming systems) and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author.

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CRediT authorship contribution statement

Manon Swanenburg: Conceptualization, Methodology, Writing - original draft, Investigation, Writing - review & editing. **Jose L. Gonzales:** Methodology, Validation, Formal analysis, Data curation, Writing - original draft, Visualization. **Martijn Bouwknegt:** Conceptualization, Writing - review & editing. **Gert Jan Boender:** Formal analysis, Investigation, Data curation. **Derk Oorburg:** Conceptualization, Resources, Funding acquisition. **Lourens Heres:** Conceptualization, Investigation, Resources. **Henk J. Wisselink:** Conceptualization, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

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