Toxoplasma prevalence in Dutch slaughter pigs in the period 2012-2014

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Abstract

Toxoplasma gondii has frequently been named as one of the most important foodborne pathogens, in terms of its impact on human health. EFSA advised to include serological testing of pigs on *T. gondii* infections and audits of pig farms on risk factors for *T. gondii* infection (EFSA, 2011). In order to generate knowledge about the epidemiology and prevalence of *T. gondii* infections in pig herds we studied the long term seroprevalence on farms, persistence of infection and variation in results between and within farms. Sera which were routinely taken in Dutch pig slaughterhouses in the Netherlands for the serological monitoring of *Mycobacterium avium* infections in pigs (Hiller 2013) were also tested for anti *T. gondii* antibodies. Results of 120,666 sera, collected from January 2012 until August 2014, showed an average of 2% serological prevalence in pigs. Pigs from organic farms had a prevalence of 3,6%. Farm prevalence was much higher, ranging from approximately 30% for conventional farms to 90% for organic farms. Pigs delivered to the slaughterhouse during winter months had a higher prevalence than pigs delivered during summer months.It could be concluded that serological monitoring can be very useful in detecting farms infected with *T. gondii*. A test cut off of 20PP was the most appropriate.

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Introduction

To control *Toxoplasma gondii* infections, intervention measures can be taken in the animal reservoir. Research showed that prevalence of *T. gondii* infections in pig was related to management on farms (Kijlstra, 2004). The number of pigs with antibodies against *T. gondii* in free-range farms was larger than on farms where pigs are kept indoors only. 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. A change of management aimed at reducing these risk factors could thus contribute to the reduction of *T. gondii* infections in pigs. The European Food Safety Authority has proposed epidemiological indicators for controlling *T. gondii* infections in pigs and safeguarding 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. Moreover, serological tests were developed and validated but not prepared for use in a system to control *T. gondii* infections. Until now, no long-term serological studies have been carried out in slaughter pigs to look for antibodies against *T. gondii*. To generate knowledge about the epidemiology and prevalence of *T. gondii* infections in pig herds we studied the long term seroprevalence on farms, persistence of infection and variation in results between and within herds.

Materials and methods

Serum samples which were routinely collected in 5 slaughterhouses in the Netherlands for the serological monitoring of *Mycobacterium avium* infections in pigs (Hiller 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 randomly from pigs during bleeding. Per delivery, 1, 2 or 6 samples were collected (criteria for the number of pigs sampled were based on the *M. avium* monitoring system). For the

analysis in this paper we used sera collected from January 2, 2012 until August 13, 2014.

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 result is presented in PP (percentage positivity). PP is calculated as follows: PP = (ODsample-ODnegative control)/(ODpositive control-ODnegative control)*100.We used a cut off percentage of 20% (results ≥20% were considered positive), as advised in the test manual. Serological results were recorded in a data file, which contains, amongst others, the following information: UBN (unique farm number), slaughter date, PP (percentage positivity), test result (0 or 1, negative or positive), quality code (the quality program that a farm is in). The original 37 different quality codes were clustered in five so called "quality labels" (organic, intermediate, welfare, Belgian and other). 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 quality label and per year were counted. Mean PP's were calculated. A frequency distribution of the results in PP for all samples was made. The prevalence of positive farms, positive deliveries and positive samples was calculated per quality label. A positive farm was defined as a farm from which at least 1 sample had a positive test result in the study period. The Wald-Wolfowitz run test was used to test if test results were random or if a pattern could be found in the results of successive deliveries from one farm. In this test, the null hypothesis is that the series of zeros and ones is random from a distribution. If the "ones" are clustering, this test will result in a small probability and the null hypothesis will be rejected.

Results

During the total study period 3070 farms delivered pigs to the slaughterhouses. In total 120,666 samples from 96,711 deliveries of pigs were collected and tested during the study period. The average number of samples per delivery was 1.2, which ranged from 1.0 per delivery for the intermediate label to 5.0 per delivery for organic farms. Figure 1a and 1b show the frequency distribution of the serology results in PP for the whole testing period. Most samples had a result between 0 and 12 PP, but a very long "tail" with results up to PPs of almost 400 was present.

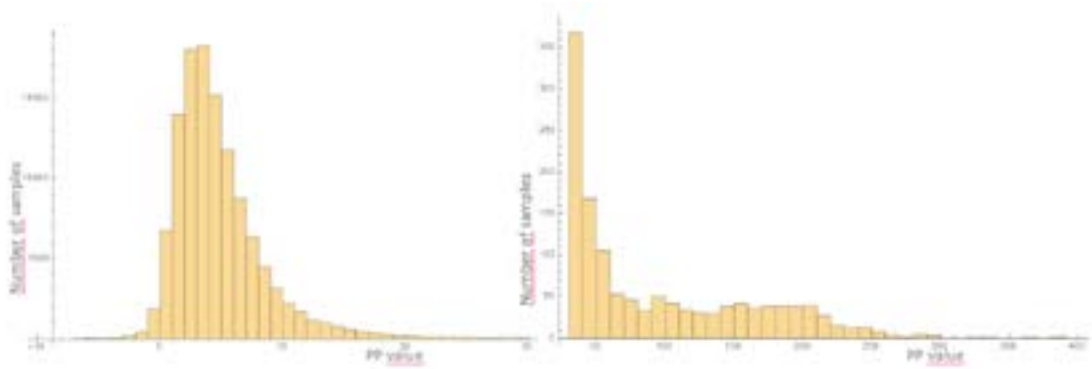

Figure 1a (left): Distribution of serology results for results with PP≤ 30 for the whole period
Figure 1b (right): Distribution of serology results for results with PP> 30 for the whole period

Table 1 shows the results per quality label in PP. It is obvious that the mean result for samples from organic farms was higher than for the other quality labels, although the range of the results within each label was wide. It can be seen that in all cases the median result was lower than the mean result, which follows from a low number of very high scoring results (see also figure 1b), that raise the mean. For all quality labels, the mean result was lowest in 2013 and highest in 2014. This trend can also be seen in figure 2, that shows the prevalence of positive samples per month, based on a test cut off of PP 20.

Table 1: Test result in PP per delivery per year per quality label.

Label	Year	Median	Mean	95% confidence interval
Organic	2012	5.24	7.96	0.90 - 36.89
	2013	4.90	7.08	1.32 - 33.60
	2014	6.43	8.39	2.60 - 32.40
Intermediate	2012	3.79	5.66	0.03 - 16.30
	2013	3.30	4.64	-0.45 - 15.16
	2014	5.00	6.65	0.50 - 19.50
Welfare	2012	3.97	5.48	0.34 - 16.64
	2013	3.25	4.66	-0.39 - 15.60
	2014	5.00	6.62	0.40 - 18.10
Belgian	2012	4.08	6.27	0.47 - 20.52
	2013	3.50	4.94	-0.20 - 15.60
	2014	5.40	6.63	1.00 - 19.00
Other	2012	3.96	5.92	0.25 - 16.84
	2013	3.48	5.74	-0.31 - 18.82
	2014	4.90	6.97	0.00 - 18.00

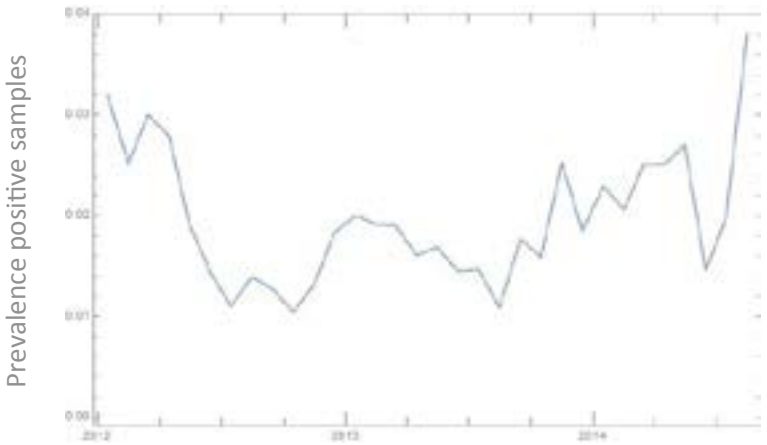

Figure 2: Prevalence of positive samples per month, based on a test cut off of PP 20; period January 2012 until August 13, 2014.

Prevalences of positive farms ranged from 27% in Belgian farms to 90% in organic farms. Overall sample prevalence was 2,0%, ranging from 1,7% in samples from welfare and intermediate farms to 3,6% in samples from organic farms. We found that most organic farms were positive, but the within farm prevalence was not very high. In the Belgian farms we also found a higher prevalence in samples, but this coincides with a low farm prevalence. Here, a few farms with a high within-farm prevalence must have caused the higher prevalence in samples. Figure 2 shows there was a seasonal variation in the prevalence of positive samples per month. The prevalence seemed to decline in autumn. The Wald-Wolfowitz runs test for randomness of results of successive deliveries from one farm resulted in a p value of 0.016, which meant the null hypothesis was rejected and there was some kind of persistence in test results of deliveries from one farm. Farms could be divided into three categories: 1. (Always) negative farms; these farms had a probability of 2% to be found positive in one of the following months. 2. Farms

that were positive only in winter months; in summer months these farms carried the same risk as any of the negative farms from category 1. 3. Farms that were positive during summer and winter months; these farms had a year round higher probability (6% per delivery) to be found positive in the next months.

Discussion and Conclusions

Serological prevalence in all samples was low (2%), whereas prevalence of positive farms (at least one positive sample from that farm) was much higher, ranging from 27% of the Belgian farms to 90% of the organic farms. The high prevalence of positive organic farms does not mean that the within farm prevalence in organic farms was also high. When selecting farms with high within farm prevalence for a case-control study, only one organic farm was selected. Most farms with a high within farm prevalence were from the welfare label.

For this project, a positive farm was defined as a farm with at least one positive sample in the whole study period. This means that farms, from which more than one sample are collected per delivery, like organic farms, or with many deliveries, theoretically have a higher probability to become a positive farm. Therefore, the farm prevalence was perhaps higher for some quality labels with more frequent deliveries per farm. This effect does not extend to the sample-prevalence. The mean sample PP was highest in 2014 and lowest in 2012. It is not known if this is caused by natural variation or represents a trend. Possible explanations are to be found in the test or control samples, different weather conditions per year, different farms that have delivered, changes in the rodent densities, etc.

A very interesting result was finding that positive farms showed different seasonal patterns in persistence of positivity. Part of the positive farms only delivered positive pigs in winter, and were serologically negative during summer months, whereas other farms delivered positive pigs the whole year around. In the next stage of this project, we will analyse this aspect in combination with a case-control study to see if there are risk factors for being a “summer positive” or a “winter positive” farm, for example quality label, farm size, delivery pattern, etc.

Furthermore, we hope to get more insight into why more farms/pigs are serologically positive during winter months. Is this caused by a recent infection? Or were these pigs already serologically positive during the last months of their lives, and did they become infected in the end of summer or autumn?

Conclusion

It could be concluded that serological screening of pigs in the slaughterhouse is a suitable method to divide pigs farms in high risk and low risk farms. The results of this work will be used as an input for a case control study and for developing surveillance scenarios.

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