

Design of a Risk based Control System for *Toxoplasma gondii* in a pork supply chain

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Toxoplasma gondii is recognized as one of the major foodborne pathogens. The European Food Safety Authority (EFSA) concluded that *T. gondii* is one of the public health hazards in pigs to be covered within a modern meat inspection and advised to include serological testing of pigs and audits of pig farms to control *T. gondii*. A project is presented that develops a framework where this idea is practically implemented in a pork supply chain.

The aim of the program is to reduce the contribution from the pork chain to the human toxoplasmosis disease burden.

In the pilot at least one blood sample per batch at delivery at the slaughterhouse is tested with a Toxoplasma Elisa. With this screening 20 (1%) high risk herds were identified. These high risk farms and their controls, 2 per high risk farm, were followed up. More samples of risk farms and their controls were collected to get a better estimate of the within herd prevalence and to follow the development of serology over time. Additional MC-PCR was performed on hearts of pigs from these herds to confirm presence of infection, tissue cysts. Also farm assessments were done to verify the presence of risk factors at the identified farms.

In the first farm where hearts were tested by PCR the presence of toxoplasma tissue cysts was shown. The results give insight in how risk based approaches with food chain information (FCI) for animals sent to slaughter could work, by substantiating how risk herds can be practically identified and which interventions are feasible. The results are discussed in the perspective of human health risks.

Introduction

In humans, in the majority of *Toxoplasma* cases the course of infection is asymptomatic (Fricker-Hidalgo et al., 2009). When a woman is primarily infected with *Toxoplasma* during pregnancy, the parasite can cause congenital toxoplasmosis. This can lead to abortion, central nervous system abnormalities or subclinical infections at birth with the possibility to develop visual disorders later in life (Jones et al., 2003).

The integrated public health impact is globally considered to be very high (Torgerson et al., 2013). In the USA, *T. gondii* ranked third out of 14 foodborne pathogens (Batz et al., 2012) and in the Netherlands ranking *T. gondii* as the first among 14 enteral pathogens examined (Havelaar et al., 2010). Raw or undercooked meat is a major risk factor for infection with *T. gondii*, and 30-63% of infections in pregnant women could be attributed to meat consumption (Cook et al., 2000). Viable *T. gondii* have been isolated from tissues and meat of pigs infected with *T. gondii* (Dubey, 2009).

Prevalence of *T. gondii* infections in pigs is related to farm management (Kijlstra et al., 2004; Van der Giessen et al., 2007). The risk for *T. gondii* in pigs has been associated with outdoor access, the presence of cats, the occurrence of rodents, and the degree of cleaning and disinfection. A change of management aiming to reduce risk factors may contribute to the reduction of *T. gondii* infections in pigs.

All pigs must be inspected ante and *post mortem* during slaughtering. Food safety hazards that are currently identified to be of major importance for pigs (*Salmonella*, *Yersinia*, and *Toxoplasma*) cannot be controlled by visual inspection. Therefore, alternative methods using serological methods or management measures on the pig farm need to be implemented.

The European Food Safety Authority (EFSA) published an opinion on the modernization of meat inspection toward a risk-based system (EFSA, 2011). In this opinion, EFSA proposed harmonized epidemiological indicators (HEI) to control *T. gondii* infections in pigs. The measures include serological testing of pigs and audits of pig farms on risk factors for a *T. gondii* infection. In the EFSA proposal audits are done on farms with controlled housing and serological testing is done on farms with non-controlled housing.

Goal of the present project is to evaluate a serological surveillance system for both controlled and non-controlled housing systems.

Material and Methods

Monitoring of *Toxoplasma* was put on top of the surveillance implemented for *Mycobacterium avium* (MAA), which was already in place in the participating slaughter company (Hiller et al., 2013). In this system 1 sample per delivery is collected from herds with a low MAA risk, and 6 samples from herds with a higher risk. From organic farms routinely 6 samples are collected for serological testing on *toxoplasma*. Testing for *toxoplasma* started in 2012. Samples were tested with the PrioCHECK *Toxoplasma* Ab porcine ELISA (Thermo Fisher Scientific Prionics Lelystad B.V.) according to the manufacturer's instructions.

To identify herds at risk for *Toxoplasma*, cut offs need to be set to define high and low risk herds. Based on collected data the cut off will be re-evaluated. As a starting point high risk herds have a within herd prevalence of 15% or higher. Farms with a within herd prevalence below 5% are low risk farms.

From one high risk farm 50 hearts were collected in November 2014. Eight hearts, having the highest serological response, were tested with MC-PCR (Opsteegh, 2010)

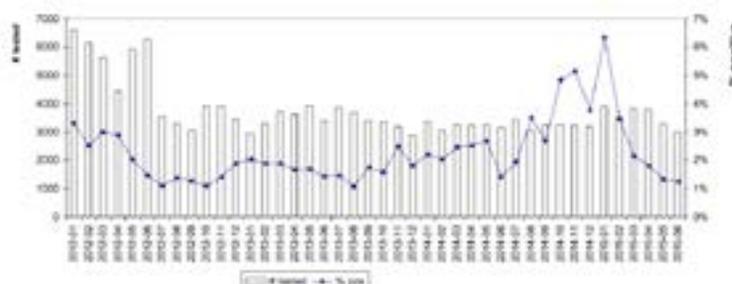
From 7 high risk herds at one delivery a large part of the delivery was sampled at bleeding in the slaughterhouse In Summer 2015. The samples were tested according standard procedures.

A questionnaire was set up to verify the absence and presence of risk factors. High risk and low risk farms were visited. The inventory was on biosecurity, including presence of cats, rodent control, and questions related to feed sources including whey. During farm visits the respective questions were visually checked.

Results

The summary of results in figure 1 shows that with the routine testing more than 3000 samples were analyzed per month. On average 2% of the samples was positive. The prevalences were a little higher in winter, in the last winter of 2014/2015 a significantly higher peak was seen, with 6% positive samples in January 2015.

Figure 1: results of *Toxoplasma* surveillance in slaughter pigs; numbers of collected samples and proportion of positive samples per year and month.



At January 1st, 20 and 60 farms could be defined as high risk farms based on respectively the last 60 and

last 20 samples. The 20 high risk farms represented 1,2% of all herds, and 11% of all positive samples. The 1504 low risk farms (84%) represented 48% of the positive samples.

Visits of the high risk farms are ongoing. The preliminary evaluation shows that at high risk farms rodent control is less well performed and many of these farms have outside bulk storage of some feed constituents, which may be accessible for rodents and/or cats.

In 7 out of 8 selected hearts with positive serology in their meat juice the presence of toxoplasma could be confirmed with MC-PCR.

The results of the sampling of one delivery of 7 farms is presented in table 1.

Table 1: *Toxoplasma* positive serological test of samples collected at one delivery during summer of high risk farms

| farm | matrix | Samples | Positive samples* | |
|------|---------------|---------|-------------------|--------------------------|
| 2 | Serum (blood) | 110 | 7 | (6,4% <2,6% ; 12,7% >) |
| 3 | Serum (blood) | 106 | 4 | (3,8% <1,0% ; 9,4% >) |
| 4 | Serum (blood) | 98 | 0 | (0,0% <0,0% ; 3,7% >) |
| 5 | Serum (blood) | 115 | 1 | (0,9% <0,0% ; 4,7% >) |
| 6 | Serum (blood) | 104 | 1 | (1,0% <0,0% ; 5,2% >) |
| 7 | Serum (blood) | 66 | 6 | (9,1% <3,4% ; 18,7% >) |
| 8 | Serum (blood) | 110 | 0 | (0,0% <0,0% ; 2,8% >) |

Number of positive samples, proportion and exact binomial (Clopper - Pearson) 95% confidence interval

Discussion

The strategy of the surveillance program is to identify high positive herds, and demand from farmers to improve control of *toxoplasma* in these herds. The aim is to decrease the overall prevalence of positive pigs, and therewith improve safety of pork. Where high risk farms are asked to improve control, intermediate risk farms are made aware of *toxoplasma* and the potential risk in their herds. The strategy is in principle to treat the meat of the high risk herds not differently by either controlled heating or freezing, but to prevent occurrence of *T. gondii* by verifying biosecurity control by means of serological screening and impose improvements at farm level.

The first result of the current surveillance program is unique insight in the prevalence of *Toxoplasma* and better understanding of the risks. Biosecurity in Dutch pig farming is in general at a high level, nevertheless a limited number of farms were identified that had weaknesses in their biosecurity, leading to introduction of *toxoplasma*. Accordingly, other pathogens may also be more easily introduced on these farms. The approach with screening and active follow-up may lead to an overall improvement of biosecurity to guarantee food safety and animal health.

Weaknesses that were seen in rodent control are storage of goods around the farms building, providing a hiding place for rodents, and irregular (re-)placement of rodenticides. It requires good motivation of the farmer to organize and manage this better.

Part of the ongoing project is to evaluate the sensitivity and specificity of the surveillance system. For that purpose the sample size is increased from 1 to 6 at every delivery for the identified risk herds and the control farms. The statistical validation of the system based on these data is ongoing. The results of the 7 high risk farms highlight research questions on sensitivity and specificity of the surveillance system. In previous periods the prevalence on these farms was higher (data not shown), hence these farms would not have been selected. With the prevalence in the tested period it is unlikely that these farms would have been categorized as high risk. Also the effect of one sample and irregular supply has to be studied more extensively.

The ongoing analysis will give more insight in how the surveillance system can be optimally designed, like

additional sampling at each delivery before measures are imposed on farms. On the other hand, there may be no harm when farms are given a false alarm and accordingly follow up on this signal by checking their biosecurity control. Estimates of the sensitivity and specificity of the system to detect high risk farms will become available in the next phases of the project.

Conclusion

With the implementation of the surveillance program a significant step has been set in implementing a modern way of meat inspection by using serological data as farm data (food chain information). The success of this scheme, being the reduction of *Toxoplasma* in the supply chain and especially on identified farms, and the effect of this scheme on the reduction of the number of human infections, will be assessed during the next stages of the project.

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