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TOWARDS RISK-BASED MEAT INSPECTION – PREREQUISITES OF RISK-BASED MEAT INSPECTION OF PIGS IN FINLAND

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DOCTORAL DISSERTATION

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To my family

ABSTRACT

Salmonella spp., *Yersinia enterocolitica, Toxoplasma gondii* and *Trichinella* spp. are the most relevant biological hazards in the context of meat inspection of pigs in the European Union (EU). These zoonotic pathogens show no clinical symptoms or gross pathological lesions in pigs, and thus are not detectable with current meat inspection procedures, except *Trichinella* spp. by laboratory analysis. In this study, we analysed the serological prevalence of these pathogens in Finnish fattening pigs and evaluated serological monitoring as a control method.

In total, we studied 1353 meat juice samples and 1793 serum samples of fattening pigs using commercial ELISA kits. The seroprevalence of pathogenic *Yersinia* spp. was the highest of the studied pathogens, and *Yersinia* antibodies were detected in 57% and 66% of the meat juice and serum samples at the end of the fattening period, respectively. The seroprevalences of *Salmonella* spp. and *T. gondii* were low. *Salmonella* antibodies were detected in 3% of the meat juice samples and in 18% of the serum samples at the end of fattening. *T. gondii* antibodies were detected in 3% of meat juice samples and 1% of serum samples. *Trichinella* spp. antibodies were not detected. The seroprevalences at the end of the fattening or at slaughter were not associated with post-mortem findings of the current batch, which was expected. This indicates that we need new tools to control these public health hazards in pork.

Meat juice serology at slaughter was feasible and easy to perform. We observed huge differences between farms considering *Salmonella* spp., *Yersinia* spp. and *T. gondii* seroprevalences. This shows that farm-level serological data could be used as part of the food chain information (FCI) for risk-based decisions to improve food safety. Risk-based decisions include slaughtering arrangements, additional carcass processing, targeted sampling at the slaughterhouse and improved biosecurity measures at the farm. However, risk mitigation targets and procedures must be carefully adjusted for each pathogen. With targeted serological monitoring of *T. gondii* we could effectively target control measures and diminish the pathogen in pork. Serological monitoring of pathogenic *Yersinia* spp. could be the first step in the huge challenge of *Y. enterocolitica* in pigs, allowing for the possibility of slaughtering pigs from high-risk farms at the end of the day. Serological monitoring of *Salmonella* spp. would be beneficial, but would only have a limited positive impact on food safety, because the current situation is already excellent. Serosurveillance of *Trichinella* spp. would become meaningful, if current testing is to be diminished.

In addition to food safety issues, FCI could be useful for visual-only inspection, which should be the most common inspection method. We analysed authentic FCIs and meat inspection findings of 85 slaughter batches

of fattening pigs. In addition, we analysed on-farm health status indicators, assessed by a veterinarian, and the meat inspection findings of another 57 slaughter batches. The partial carcass condemnation rate of the current batch was best predicted by the partial carcass condemnation rate of the pigs from the same farm within one year. Constant coughing and tail biting at a farm were associated with partial carcass condemnations. On-farm health indicators (such as the healed tail biting rate at the end of fattening and constant coughing during fattening) together with previous meat inspection results could be used as part of the FCI to make decisions regarding the meat inspection procedure: visual-only or additional inspections. However, farmers must be properly advised to carefully report this information.

ACKNOWLEDGEMENTS

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I   Felin, E., Jukola, E., Raulo, S. and Fredriksson-Ahomaa, M. (2015). Meat juice serology and improved food chain information as control tools for pork-related public health hazards. *Zoonoses Public Health* 62, 456–464.

II   Felin, E., Jukola, E., Raulo, S., Heinonen, J. and Fredriksson-Ahomaa, M. (2016). Current food chain information provides insufficient information for modern meat inspection of pigs. *Prev. Vet. Med.* 127, 113–120.

III   Felin, E., Hälli, O., Heinonen, M., Jukola, E. and Fredriksson-Ahomaa, M. (2019) Assessment of the feasibility of serological monitoring and on-farm information about health status for the future meat inspection of fattening pigs. *Prev. Vet. Med.* 162, 76-82.

IV   Felin, E., Näreaho, A. and Fredriksson-Ahomaa, M. (2017). Comparison of commercial ELISA tests for the detection of *Toxoplasma* antibodies in the meat juice of naturally infected pigs. *Vet. Parasitol.* 238, 30–34.

The publications are referred to in the text by their Roman numerals.

These publications have been reprinted with the kind permission of their copyright holders: John Wiley and Sons(I), Elsevier B.V. (II–IV). Some previously unpublished material is additionally presented.

The author´s contributions to the publications included in this thesis:

I   Participated in designing the study. Had main responsibility for data handling, statistical analysis and interpretation of results, and writing of paper.

II   Had main responsibility for designing the study, data handling, statistical analysis and interpretation of results, and writing of paper.

III   Participated in designing the study. Had main responsibility for data handling, statistical analysis and interpretation of results, and writing of paper.

IV   Participated in designing the study, interpretation of the results and writing the paper.

ABBREVIATIONS

ANOVA	Analysis of variance
CI	Confidence interval
ECDC	European Centre for Disease Prevention and Control
EFSA	The European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
EVIRA	Finnish food safety authority
FCI	Food chain information
MAT	Modified agglutination test
NPV	Negative predictive value
OD	Optical density
OV	Official veterinarian
PP	Percentage of positivity
PPV	Positive predictive value
r	Pearson correlation coefficients
SE	Sensitivity
SP	Specificity
S/P	Sample/Positive
Yops	*Yersinia* outer proteins

1 INTRODUCTION

Meat inspection has been conducted in Europe for over a century. It has been the corner stone of food control preventing the transmission of meat-borne pathogens. During this century, the aims of meat inspection have broadened from public health into four major objectives: public health, animal health, animal welfare and organoleptic meat quality. However, the meat inspection protocol considering food safety issues has remained quite unchangeable over these years, despite the dramatic change in significance of various pathogens.

To incorporate a more risk-based approach, the European Union (EU) shifted the official *post-mortem* meat inspection of pigs to visual meat inspection in 2014 (European Commission, 2014). Routine palpations and incisions were omitted from inspection procedures, because the risk of microbiological cross-contamination was assessed to be higher than the risk of missing potential food safety hazards. However, this modernization of meat inspection did not enhance the detection of the current most relevant meat-borne zoonoses.

Salmonella spp., *Yersinia enterocolitica*, *Trichinella* spp. and *Toxoplasma gondii* are currently the most important biological hazards in the context of pig meat inspection (EFSA, 2011). These are zoonotic pathogens causing human infections frequently related to pork consumption (Fosse et al., 2008). However, these pathogens show no gross lesions in pigs and are not detectable within the current meat inspection of pigs. Meat inspection should therefore be developed to a more risk-based approach focusing on preventing the most relevant public health hazards occurring today.

The European Food Safety Authority (EFSA) stated that a comprehensive pork carcass safety assurance system 'from the farm to the fork' is needed to ensure the effective control of the main hazards (EFSA, 2011). Food chain information (FCI) should be the link between farms and slaughterhouses to provide information related to food safety. However, current information is limited, as monitoring programmes in Finland cover only *Salmonella* spp. and the routine analysis of *Trichinella* spp., which is expected to lessen (European Commission, 2015).

Besides the protection of human health, the aim of food control is also to protect consumer interests in relation to food (European Parliament and Council, 2002). Thus, during meat inspection, the meat is also condemned if it indicates any patho-physiological changes or anomalies in consistency or in organoleptic quality. Healthy pigs are the prerequisite for visual-only meat inspection. The official veterinarian (OV) should be able to recognize beforehand the slaughter batches of pigs expressing high frequencies of lesions, which need additional inspections and are unsuitable for visual-only

meat inspection. FCI is the intended tool for this purpose, in addition to food safety and animal health issues.

The aim of this doctoral thesis was to study the prerequisites of risk-based pig meat inspection in Finland. The scope of this thesis is food control and public health issues, concentrating on biological hazards. Specific aims were to investigate the seroprevalence of *Salmonella* spp., pathogenic *Yersinia* spp., *T. gondii* and *Trichinella* spp. in pigs, to evaluate the feasibility of serological monitoring in the context of risk-based meat inspection 'from the farm to the fork', to assess the usability of the current FCI as part of the risk-based and visual meat inspection, and to evaluate means for improving it.

2 REVIEW OF THE LITERATURE

2.1 RISK-BASED MEAT INSPECTION OF PIGS

'Risk' is a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard (European Parliament and Council, 2002). A high protection level of human health is one of the main objectives of food control (European Parliament and Council, 2002). In order to achieve this goal, food law and food control should be based on risk analysis consisting of risk assessment, risk management and risk communication (European Parliament and Council, 2002).

The systematic meat inspection procedure in Europe was developed in the 19th century based on a book by von Ostertag (1892). The primary objective was 'to protect man against the dangers which threaten him from eating meat' and the procedure was highly risk-based, considering risks relevant at that time (von Ostertag, 1904). However, the protocol has remained nearly unaltered until today, despite the risks having changed over the decades. Most of the significant zoonoses detected in traditional meat inspection have never been detected in Finnish pigs (eg. *Brucella suis*, *Taenia solium*), and Finland is currently officially free from *Mycobacterium bovis* (Finnish Food Safety Authority Evira, 2017a). After 2004, *Trichinella* spp. has been detected in Finland in only one pig in 2010 (Finnish Food Safety Authority Evira, 2017b).

The current meat inspection of pigs in the EU comprises of FCI, *ante-mortem* inspection, *post-mortem* inspection and inspections regarding animal welfare, animal by-products and laboratory testing (European Parliament and Council, 2004b). *Ante-* and *post-mortem* inspections are usually visual only. *Post-mortem* inspection can be enhanced by palpation and incisions when needed. In current meat inspection protocol, only conditions showing observable anomalies in animals or carcasses are detected. *Trichinella* spp. infection is the exception. It was formerly routinely checked for through laboratory analyses of each pig carcass at slaughter, but due to changes in legislation routine analysis is expected to become less frequent (European Commission, 2015).

EFSA (2011) conducted a qualitative risk assessment on the foodborne hazards in the context of pig meat inspection. Based on this assessment, *Salmonella* spp., *Y. enterocolitica*, *Trichinella* spp. and *T. gondii* were identified as the most relevant biological hazards in the context of pig meat inspection (EFSA, 2011). However, none of these pathogens show any gross lesions in pigs and are undetectable within the current pig meat inspection.

To meet the objectives of the European Food Law, meat inspection should be developed towards a more risk-based approach, focused on preventing the most relevant public health hazards occurring today. These hazards enter the

pork production chain mostly during the preharvest phase of production. Therefore, based on the risk analysis, the focus in risk-based meat inspection should be shifted from traditional meat inspection at the slaughterhouse to comprising the whole food chain from the farm until the carcass leaves the slaughterhouse (EFSA, 2011).

2.2 VISUAL-ONLY MEAT INSPECTION OF PIGS

The official *post-mortem* meat inspection of pigs shifted to visual-only meat inspection in the EU in 2014 (European Commission, 2014). This means that routine palpations and incisions are omitted from *post-mortem* inspection procedures. The change in legislation was performed based on the risk assessment made by EFSA (2011), where the risk of microbial cross-contamination was estimated to be higher than the risk of not detecting conditions targeted by these techniques. EFSA (2011) stated that incisions and palpations should only be conducted on pigs that are suspected of having conditions detectable with these techniques.

The OV decides whether any additional *post-mortem* inspection procedures are needed based on the FCI, *ante-mortem* inspection, *post-mortem* inspection or any other data regarding the animal that indicate a possible risk to public health, animal health or animal welfare (European Commission, 2014). Palpations and incisions can be conducted to fully inspect abnormal carcasses and offal, to achieve preliminary diagnoses and to decide on condemnations and possible laboratory analyses.

Studies conducted in the UK, Denmark and Australia (Mousing et al., 1997; Hamilton et al., 2002; A. Hill et al., 2013; Pacheco et al., 2013) show that visual meat inspection does not significantly reduce the detection of foodborne hazards in pig meat inspection.

The change made to visual meat inspection procedures through legislation was argued with the reduced risk of *Salmonella* and *Y. enterocolitica* cross-contamination (EFSA, 2011; European Commission, 2014). Discussion concerning this has surfaced, and a risk assessment in the UK concluded that no clear evidence points to visual-only meat inspection reducing the microbiological cross-contamination of carcasses (A. Hill et al., 2013). A. Hill et al. (2013) based their opinion mostly on the study by Hamilton et al. (2002), which compared the cross-contamination of carcasses with *Salmonella* spp. and *Yersinia* spp. However, Hamilton et al. (2002) also concluded that cross-contamination of carcasses may potentially occur when incising normal lymph nodes. This has also been shown by Pointon et al. (2000) and Nesbakken et al. (2003). Recently, in their quantitative microbiological risk assessment, Costa et al. (2017) concluded that an incision of the lymph nodes can be an important source of carcass contamination by *Salmonella* spp. In addition, a lower contamination level

with *Enterobacteriaceae* has been found after visual-only inspection than after traditional inspection (Tongue et al., 2013).

The visual-only meat inspection procedure is also expected to save time and resources, which could enable reallocation of resources to more risk-based procedures (Mousing et al., 1997). Visual-only meat inspection is assessed to lead to small decreases in the occupational exposure of meat inspectors to *Streptococcus suis* when a heart incision is omitted (A. Hill et al., 2013).

Most condemnations are not public health issues (EFSA, 2011). The meat is also to be declared unfit for human consumption if it indicates any patho-physiological changes or anomalies in consistency or in organoleptic quality (European Parliament and Council, 2004b). EFSA (2011) suggested that a meat quality assurance system by the food business operator could ensure the elimination of these abnormalities. However, this change in regulations has not been issued, and condemnations also due to merely aesthetic and meat quality issues are the responsibility of official meat inspection in the EU (European Parliament and Council, 2004b).

Moreover, the objectives of current meat inspection include animal health and animal welfare questions. Meat inspection is considered a key component in the surveillance of pig health and welfare (EFSA, 2011). Visual-only meat inspection has been shown to detect lesions related to these aspects comparably or even more sensitively than traditional meat inspection (Pacheco et al., 2013; Ghidini et al., 2018). However, animal health and welfare aspects are beyond the scope of this thesis, and thus are not further discussed.

The main deficiency in visual-only meat inspection, as in traditional meat inspection, is that only conditions associated with gross lesions are detected, while the most important pork-borne public health hazards are neglected (EFSA, 2011). Therefore, visual-only meat inspection is a specific change towards risk-based meat inspection, but insufficient on its own.

In Finland, visual-only meat inspection is implemented by Finnish Food Safety Authority Evira according to EU legislation (Finnish Food Safety Authority Evira, 2015). However, in practice visual-only meat inspection is only partly implemented in Finland and the EU, due to third country requirements concerning exports from slaughterhouses (Alban et al., 2018; personal communication, Marjatta Rahkio, Head of the Meat Inspection Unit, Finnish Food Safety Authority Evira). As this challenge is similar for all EU countries, full implementation would require EU trade policy.

Healthy pigs are the prerequisite for visual-only meat inspection. The benefits of visual-only meat inspection are lost if most of the pigs in a batch require additional inspections. Pig batches with high lesion frequencies should be slaughtered separately, as they need a slower line speed and adequate human resources at the trimming line. FCI is intended as the tool for providing information from the farm to the slaughterhouse, and could be

used to make decisions regarding the meat inspection procedure: visual-only or additional inspections.

2.3 FOOD CHAIN INFORMATION

According to EU regulations (European Parliament and Council, 2004a), adequate FCI must be presented to the slaughterhouse operator and to the OV no less than 24 h before the arrival of the animals to the slaughterhouse. The FCI must cover at least:

a) *the status of the holding of provenance or the regional animal health status;*
b) *the animals' health status;*
c) *veterinary medicinal products or other treatments administered to the animals within a relevant period and with a withdrawal period greater than zero, together with their dates of administration and withdrawal periods;*
d) *the occurrence of diseases that may affect the safety of meat;*
e) *the results, if they are relevant to the protection of public health, of any analysis carried out on samples taken from the animals or other samples taken to diagnose diseases that may affect the safety of meat, including samples taken in the framework of the monitoring and control of zoonoses and residues;*
f) *relevant reports about previous ante- and post-mortem inspections of animals from the same holding of provenance including, in particular, reports from the official veterinarian;*
g) *production data, when this might indicate the presence of disease; and*
h) *the name and address of the private veterinarian normally attending the holding of provenance.*

(European Parliament and Council, 2004a)

In addition, the national legislation specifies the relevant time periods during which the medications, diseases, analysis results, meat inspection reports and production data are to be declared (Ministry of Agriculture and Forestry, 2011).

In Finland, the FCI forms used by large slaughterhouses are usually electronic and generally contain the following information:

a) any relevant health status data regarding the farm or the animals in question (for example salmonellosis, trichinellosis, erysipelas, anthrax etc.)
b) any restrictions on the farm imposed by the authorities,
c) whether the farm is officially recognized to apply controlled housing conditions

d) any drug residues or unauthorized substances detected in animals or at the farm during the last year,

e) any pigs in the slaughter batch that have been treated with veterinary medicinal products that have a withdrawal period within 30 days prior to slaughter,

f) certain symptoms and signs detected in the slaughter batch (e.g. lame pigs, pigs with poor appetites, dirty pigs, abscesses/lumps, bitten tails, any changes in production parameters three months prior to slaughter)

g) anything else relevant considering slaughter or food safety,

h) contact information for the veterinary practitioner handling the farm.

The FCI does not routinely include reports about previous *ante-* and *post-mortem* inspections, as pigs originating from a certain farm are typically continuously slaughtered by the same slaughterhouse operator due to contracts, and thus historical information is available at the slaughterhouse. Finnish Food Safety Authority Evira also provides a template form in word-format (available in Finnish https://www.evira.fi/globalassets/tietoa-evirasta/lomakkeet-ja-ohjeet/elintarvikkeet/alkutuotanto/658928_alkutuotannon_toimijan_ilmoitus_liite_1_200617.doc, accessed in 2018-05-11), and it is used mostly in small-scale slaughterhouses.

According to EU regulations, the FCI should be two-way and feedback of relevant information to the farm should be provided (European Commission, 2005). This is specified in Finnish national legislation (Anon., 2006; Ministry of Agriculture and Forestry, 2012; 2014). In Finland, the OV provides a written meat inspection decision considering condemnations, and the slaughterhouse operator must deliver the information to the farm (Anon., 2006; Ministry of Agriculture and Forestry, 2014). In addition, the occurrence of pericarditis, pleuritis, pneumonia, ascariasis, arthritis, abscess and tail biting in fattening pigs at slaughter must be monitored by the slaughterhouse, and reported to the farm for each batch and for farm-specific half-year averages (Ministry of Agriculture and Forestry, 2012). In Finland, this feedback information is normally provided electronically to the farm, via the Sikava (Stakeholders health and welfare register for pig herds in Finland) interface.

The purpose of FCI is to provide information related to food safety from the farm to the slaughterhouse. This information was intended to be used as an integral part of the inspection procedures (European Commission, 2005). However, because no regular monitoring programmes currently exist for the most relevant zoonoses during the pre-harvest phase in most European countries (including Finland), information considering food safety is limited in the FCI. In practice, information is available only if there is a known outbreak on a farm (e.g. *Salmonella* spp.) or previous findings have been made during meat inspection (*Trichinella* spp.). No information concerning

Y. enterocolitica and *T. gondii* is available from farms to slaughterhouses, neither in Finland nor in most other EU countries.

As part of the comprehensive pork carcass safety system, EFSA (2011) recommended that herd status considering *Salmonella* spp., *Y. enterocolitica*, *T. gondii* and *Trichinella* spp. should be systematically monitored via sampling. Consequently, this information, included in FCI, would enable risk differentiation of pig batches in relation to hazards (EFSA, 2011). However, EFSA further recommended research on the use of FCI for risk categorization and on hazard testing, and stressed the need for refinements reflecting differences between regions (EFSA, 2011).

The FCI was also intended to assist slaughterhouse operators in organizing slaughter operations (European Commission, 2005). The FCI could facilitate visual meat inspection, if the slaughter batches of pigs with high lesion frequencies could be recognized beforehand with reliable FCI reporting. Identifying which batches of slaughter pigs are suitable for visual meat inspection and which are more likely to need additional inspections is necessary beforehand, to ensure an efficient visual meat inspection process. Thus, suspicious batches can be slaughtered separately with slower line speed and adequate human resources at the trimming line and inspection. Suspect batches may be recognized during *ante-mortem* inspection, but changing the slaughter order at this stage of the process is usually laborious and impractical.

2.4 *SALMONELLA* SPP. IN PORK PRODUCTION

Salmonellae are gram-negative bacteria belonging to the genus *Enterobacteriacae*. All of the most relevant salmonellae belong to species *S. enterica* subsp. *enterica*, which consists of more than 1500 serovars (Grimont and Weill, 2007). *Salmonella* spp. are widespread and infect a wide range of hosts and can survive prolonged periods in the environment (Waldner et al., 2012).

Salmonella infections in humans may induce gastroenteritis and fever, and occasionally complications occur such as bacteremia and reactive arthritis (Cohen et al., 1987). Pigs/pork are the second most common infection source to humans, after egg layers/eggs (Mughini-Gras et al., 2014).

Salmonellosis is the second most common zoonosis in Europe, directly after campylobacteriosis (EFSA and ECDC, 2018). In total, 91 662 confirmed human cases were reported in the EU in 2017 (EFSA and ECDC, 2018). EFSA has estimated that approximately 10–20% of human *Salmonella* infections in the EU may be attributable to the pig reservoir (EFSA, 2010). In Finland, 1535 human cases were reported in 2017 and most (76%) of these were travel-related (EFSA and ECDC, 2018). Of the domestic human cases, 14% are estimated to be due to domestic pork (Finnish Food Safety Authority Evira, 2018).

Pigs carrying *Salmonella* spp. are typically asymptomatic, but they may shed the bacteria in their faeces and carry it in their tonsils and lymph nodes for up to 28 weeks (Wood et al., 1989). Pigs can acquire the infection from other pigs (piglets, replacement animals) at the farms, from the feed or the environment (e.g. pens, birds, rodents, bedding, visitors, staff) or during transport and lairage (Berends et al., 1996). Carrier pigs introduce *Salmonella* to the slaughterhouse, where it may contaminate the carcass and the slaughterhouse facilities during the slaughter process and cross-contaminate other carcasses (Morgan et al., 1987). Control measures during the pre-harvest production phase reduce the pathogens in fattening pigs and reduce the risk of transmitting them into the food chain. *Salmonella* spp. can be inactivated by heating the meat thoroughly to 70 °C (Murphy et al., 2004).

Salmonella spp. prevalence in pigs is very low in Finland, Sweden and Norway compared to other European countries (EFSA and ECDC, 2018). The Finnish *Salmonella* Control Programme for pigs includes monitoring at slaughterhouses, where the quantity of *Salmonella* culture-positive lymph node samples at slaughter has been <0.1% and no *Salmonella* spp. have been found in carcass swabs or pork during the 2010s (Anon., 2017; https://www.ruokavirasto.fi/globalassets/teemat/zoonoosikeskus/zoonoosit /bakteerien-aiheuttamat-taudit/salmovalvontaohj_siat2016paivheinakuu2017.pdf, visited January 13, 2019). The Finnish *Salmonella* Control Programme includes an eradication strategy and positive finding results in epidemiological investigations and official restrictions (Ministry of Agriculture and Forestry, 2013; Ministry of Agriculture and Forestry, 2014). Similar *Salmonella* control programmes with eradication strategies exist in Sweden and Norway (Heier et al., 2017). Denmark and Germany run *Salmonella* control programmes with reduction strategies based on meat juice serology (Alban et al., 2012; QS Qualität und Sicherheit GmbH, 2018).

2.5 PATHOGENIC *YERSINIA ENTEROCOLITICA* IN PORK PRODUCTION

Yersiniae are gram-negative bacteria belonging to the genus *Enterobacteriacae*. There are three human pathogenic *Yersinia* species: *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. *Y. enterocolitica* is ubiquitous in pork production, while *Y. pseudotuberculosis* is isolated more seldom (Laukkanen-Ninios et al., 2014). *Y. pestis* is not currently found in Europe (Raoult et al., 2013).

Y. enterocolitica and *Y. pseudotuberculosis* cause enteral yersiniosis in humans (Drummond et al., 2012). Typical symptoms are gastrointestinal, but long-term sequelae, such as reactive arthritis and erythema nodosum, also occur (Drummond et al., 2012).

Yersiniosis is the third most commonly reported zoonosis in Europe, directly after campylobacteriosis and salmonellosis (EFSA and ECDC, 2018). In total, 6823 confirmed human cases were reported in the EU in 2017 (EFSA and ECDC, 2018). In Finland, 423 human cases were reported in 2017, and Finland has the highest country-specific notification rate for yersiniosis in Europe (EFSA and ECDC, 2018). In 2017, 39% of the human cases reported in Finland were travel-associated (EFSA and ECDC, 2018), and *Y. enterocolitica* was the causative agent in over 95%. Pigs are a major reservoir of human pathogenic *Y. enterocolitica* strains (Fredriksson-Ahomaa et al., 2001; Laukkanen-Ninios et al., 2014).

Y. enterocolitica and *Y. pseudotuberculosis* have been isolated from 52% and 4% of slaughter pigs´ tonsils in Finland, respectively (Niskanen et al., 2002; Korte et al., 2004). Pigs are usually asymptomatic carriers of *Y. enterocolitica*, and they typically shed the bacteria in their faeces for a few weeks and carry them in their tonsils for several months after infection (Nielsen et al., 1996). Contamination sources at farms remain unclear (Laukkanen-Ninios et al., 2014). Virtanen et al. (2012) showed that piglets transport *Y. enterocolitica* to the fattening unit, where it spreads effectively during fattening. In addition to pig-to-pig contacts and the purchase of new pigs, pigs can acquire the infection from floors or other structures of a contaminated pen (Fukushima et al., 1983; Laukkanen-Ninios et al., 2014; Virtanen et al., 2014). *Y. enterocolitica* of bioserotype 4/O:3 is most frequently isolated in pigs, but it is only infrequently isolated from the outdoor farm environment or pests (Laukkanen-Ninios et al., 2014). However, wild animals and outdoor environments can introduce other pathogenic bioserotypes to pigs (Laukkanen-Ninios et al., 2014).

The pigs introduce *Y. enterocolitica* to the slaughterhouse, where it frequently contaminates carcasses, pluck sets, and the slaughterhouse equipment and facilities during the slaughter process (Fredriksson-Ahomaa et al., 2000; Laukkanen et al., 2009). Lowering the occurrence of the pathogen in fattening pigs would reduce the risk of transmitting it into the food chain. However, no surveillance or control programmes are in place for *Y. enterocolitica* in pigs. Controlling *Y. enterocolitica* requires the possibility of buying piglets from *Y. enterocolitica*-negative farms (Skjerve et al., 1998; Virtanen et al., 2012; Vilar et al., 2013; Laukkanen-Ninios et al., 2014), and good farming practices as such are not effective in preventing contamination (Laukkanen-Ninios et al., 2014). Currently, strict slaughter hygiene and bagging of the rectum are the most important measures for preventing the contamination of pork (Laukkanen-Ninios et al., 2014). In addition, removing the unsplit head from the carcass with the tonsils and tongue intact would be beneficial (Christensen and Luthje, 1994). *Y. enterocolitica* can be inactivated by heating the meat thoroughly to 70 ºC, lower temperatures (60 ºC) are effective if the temperature is upheld for several minutes (D_{60} value 1.7 min) (Shenoy and Murano, 1996).

2.6 *TOXOPLASMA GONDII* IN PORK PRODUCTION

T. gondii is an intracellular, protozoan parasite. *T. gondii,* the only species in the genus, worldwide infects virtually all warm-blooded animals (including humans) as intermediate hosts, while felines (family *Felidae*) serve as definite hosts (Tenter et al., 2000).

A *T. gondii* infection can be acquired through multiple ways: congenitally, by ingesting tissues cysts or oocysts, or through the horizontal transmission of tachyzoites. Cats shed a large number of oocysts in their faeces after the primary infection, and thus contaminate the environment (soil, water, plants, feed) (Frenkel et al., 1970; Tenter et al., 2000). Sporulated, infectious oocysts survive for a prolonged time in the environment and are resistant to diverse conditions (Tenter et al., 2000). When the intermediate host ingests the sporulated oocyst, it results in tissue cysts forming within the muscles, central nervous system and eyes, and in the visceral organs to a lesser extent (Tenter et al., 2000). When another host eats the tissues (e.g. meat), the infection is transmitted when tissue cysts are ingested. In definite hosts, the infection results in sexual reproduction of the parasite and shedding of oocysts in the faeces, while in intermediate hosts this results in asexual reproduction and the forming of tissue cysts (Tenter et al., 2000). This means that humans, as intermediate hosts, can acquire the infection by eating meat containing infective tissue cysts, or by ingesting sporulated oocysts from an environment contaminated with cat faeces (e.g. garden, vegetables, litter box, water).

Toxoplasmosis in women during pregnancy may lead to miscarriage or congenital toxoplasmosis in the offspring, leading to neurological impairment, learning difficulties, ocular disorders with impaired vision or even neonatal death (Koppe et al., 1986; Romand et al., 2004; EFSA, 2007; Berrébi et al., 2010). Immunocompromised persons may also develop severe consequences from the infection (Mele et al., 2002; EFSA, 2007). In healthy humans, toxoplasmosis is generally considered asymptomatic or a mild disease; however, it has been linked to several behavioural changes, psychological disorders and ocular diseases (Bosch-Driessen et al., 2002; Holland, 2003; Flegr, 2007; Yolken et al., 2009; Flegr, 2013).

Toxoplasmosis is the most reported parasitic zoonosis in humans in the EU, despite being under-detected (EFSA, 2007). Only congenital toxoplasmosis is reported to the European Centre for Disease Prevention and Control (ECDC). In total, 40 congenital toxoplasmosis cases were reported in the EU and none in Finland in 2017 (EFSA and ECDC, 2018). In total, approximately 20-40 toxoplasmosis cases are annually reported in Finland (National Infectious Diseases Register). In the study by Koskiniemi et al. (1992), 20% of the pregnant women were seropositive for *T. gondii* in Finland, while globally up to one third of the human population is estimated to be infected (Tenter et al., 2000). In the Netherlands, *T. gondii* infections (congenital and postnatal) are estimated to cause the highest disease burden

amongst all food pathogens (Havelaar et al., 2012). Consumption of raw or undercooked meat has been consistently identified as a risk factor for toxoplasmosis, but the type of meat varies between countries (Cook et al., 2000; EFSA, 2007). In Europe, pork has generally been considered a major source, whilst studies in Norway and France show lamb a stronger risk factor (Kapperud et al., 1996; Baril et al., 1999; Tenter et al., 2000; EFSA, 2007)

Pigs serve as an intermediate host to *T. gondii* and can thus contract the infection postnatally by ingesting sporulated oocysts or tissue cysts, or more rarely congenitally. Cat faeces or rodents are the most probable infection sources to indoor pigs (Kijlstra et al., 2008; Dubey, 2009). Seroprevalences in fattening pigs in other European countries have varied between 0% to 45%, and the highest seroprevalences are detected in pigs with access to the outdoors (Kijlstra et al., 2004; Klun et al., 2006; van der Giessen et al., 2007; Dubey, 2009; Villari et al., 2009; Deksne and Kirjusina, 2013; Wallander et al., 2016; Limon et al., 2017). Seroprevalences of intensively farmed pigs have been low in Europe (van der Giessen et al., 2007; Deksne and Kirjusina, 2013). A previous study on Finnish pigs was conducted over 30 years ago, and the seroprevalence was 2.5% (Hirvelä-Koski, 1992). Pigs are typically asymptomatic carriers and severe toxoplasmosis in pigs is considered rare (Dubey, 2009).

Pigs infected with *T. gondii* during fattening harbour tissue cysts in their muscles and the cysts remain infective until the age of slaughter (Dubey et al., 1984). Tissue cysts remain infective in refrigerated carcasses (Dubey et al., 1990). Consequently, the only way to lower the occurrence in fresh pork is to lower the occurrence of the pathogen in fattening pigs. However, no surveillance or control programmes exist for *T. gondii*, except voluntary monitoring conducted by private meat companies in the Netherlands and Germany (Oorburg et al., 2017). Tissue cysts can be killed by heating the meat thoroughly to 67 °C (Dubey et al., 1990). However, it is generally recommended to cook pork to 70 °C. Most tissue cysts are killed at freezing below -12 °C; however, some cysts may be resistant to freezing (Kotula et al., 1991; Tenter et al., 2000).

2.7 *TRICHINELLA SPP.* IN PORK PRODUCTION

Trichinella spp. are nematode parasites that globally infect virtually all warm-blooded animals (including humans) and also reptiles (Pozio and Zarlenga, 2005; Pozio, 2007; Korhonen et al., 2016). *Trichinella* spp. are divided into encapsulated and non-encapsulated clades. The encapsulated clade is comprised of *T. spiralis, T. nativa, T. britovi, T. murrelli, T. nelsoni, T. patagoniensis* and three additional taxonomically undefined genotypes Trichinella T6, T8 and T9 (Pozio and Zarlenga, 2005; Korhonen et al., 2016). The non-encapsulated clade comprises three species: *T. pseudospiralis, T.*

papuae, T. zimbabwensis (Pozio and Zarlenga, 2005; Korhonen et al., 2016).

Trichinella spp. infection is acquired by ingesting raw or undercooked meat containing *Trichinella* spp. larvae (Gottstein et al., 2009). These larvae mature into adult worms and reproduce in the intestines, and newborn larvae disseminate throughout the host and settle down in the striated muscles (Gottstein et al., 2009).

The main symptoms in human trichinellosis are gastrointestinal symptoms during the first intestinal phase, followed by fever, myalgia, myocarditis, encephalitis and facial oedemas during the muscular phase (Gottstein et al., 2009).

In 2017, 224 human trichinellosis cases were reported in the EU and none in Finland (EFSA and ECDC, 2018). The last human trichinellosis case in Finland was in the 1970s, and it was acquired from bear meat (The Zoonosis Centre team, 2012). Traditionally, trichinellosis has been associated with pork, but nowadays human infections in many countries commonly result from other undercooked meats (Dupouy-Camet, 2000; Gottstein et al., 2009; Rostami et al., 2017).

In the EU, *Trichinella* spp. infections were not detected in over 71 million fattening pigs kept under controlled housing conditions and tested in 2017 (EFSA and ECDC, 2018). In total, 224 infected fattening pigs were found among the pigs kept under non-controlled housing conditions (over 121 million pigs tested) (EFSA and ECDC, 2018). Most of the positive pigs were found in Romania (EFSA and ECDC, 2018).

Since 2004, over 25 million pigs have been tested in Finland using the digestion method, and only one positive pig was detected in 2010 (Finnish Food Safety Authority Evira, https://www.evira.fi/elaimet/zoonoosikeskus/zoonoosit/loisten-aiheuttamat-taudit/trikinelloosi/, accessed in 8 July, 2018). However, the parasite is abundant in wildlife in Finland (Airas et al., 2010; The Zoonosis Centre team, 2012). The predominant *Trichinella* species in Finnish wildlife is *T. nativa*, which has low infectivity in pigs, but *T. spiralis and T. britovi* are also commonly found in wild animals in Finland (Kapel and Gamble, 2000; Airas et al., 2010; Oksanen et al., 2018). Recently, the prevalence of *T. spiralis* among wildlife in Finland has reduced significantly in the absence of spill-over from the domestic cycle, while the prevalence of *T. britovi* remained stable (Oksanen et al., 2018).

Pigs may contract the infection from contaminated feed (e.g. offal, scraps) or from eating infected rodents (through predation or accidentally mixed in feed) (Oivanen et al., 2002; Franssen et al., 2017). Pigs infected with *Trichinella* spp. during fattening harbour the larvae in their muscles and the larvae remain infective until the age of slaughter. Tissue larvae remain infective in refrigerated carcasses (Malakauskas and Kapel, 2003). All pigs (and other animals considered a risk) are tested for *Trichinella* spp. during meat inspection, except pigs raised in officially recognized controlled housing

conditions (European Commission, 2015). Controlled housing conditions include requirements related to feed safety, rodent control, building requirements and management practices to prevent the risk of infection (European Commission, 2015). Pigs from controlled housing in the EU represent an extremely low risk for humans and no human trichinellosis cases in the EU are associated with pork products originating from controlled housing systems (Franssen et al., 2018). However, pigs from non-controlled housing system pose a higher risk, and without *Trichinella* testing during meat inspection, the estimated number of human trichinellosis cases from pigs from non-controlled housing systems would annually be over 59 000 in the EU (Franssen et al., 2018).

Tissue larvae can be killed by heating the meat thoroughly to 60 °C (Kotula et al., 1983; Gamble et al., 2000), however, pork meant for consumer preparation is generally recommended to cook until 70–71 °C (Gamble et al., 2000). *T. spiralis* and *T. pseudospiralis* are killed by freezing the meat at -18 °C for one week. However certain species, such as *T. nativa,* are resistant to normal freezing temperatures (Malakauskas and Kapel, 2003). Processors and consumers are recommended to freeze pork meat at risk for *T. spiralis* to -15 °C for three to four weeks, depending on the size of the cuts (Gamble et al., 2000).

2.8 SEROLOGICAL MONITORING

The presence of antibodies against a specific pathogen can be detected using an enzyme-linked immunosorbent assay (ELISA). The analysis is performed using a microtiter plate coated with specific antigen from the pathogen of interest. The diluted sample (serum, plasma, meat juice) is pipeted to the microtiter test plate and, during sample incubation, the specific antibodies bind to the antigen fixed on the plate. Unbound material is removed by rinsing. A conjugate is pipeted to the microtiter test plate, and the conjugate detects the antibodies bound to the antigen. Unbound conjugate is removed by rinsing. Substrate is added to initiate a colorimetric reaction. The conjugate catalyzes a colour development. The optical density (OD) is measured in a spectrophotometer. The OD values correlate with the concentration of specific antibodies in the sample. Usually IgG-conjugate is used, and it detects IgG antibodies. The conjugate can be a multi-species conjugate (horseradish peroxidase), which can be used with many species, or a pig-specific peroxidase-labelled anti-pig antibody conjugate. ELISA methods can be semi-automated. ELISA tests are cost-effective, fast and convenient diagnostic tools for large-scale screening purposes. (Nielsen et al., 1995; Nielsen et al., 1996, Manufacturers´ instructions)

Antibody levels are lower in meat juice compared to serum, and to compensate this, a lower dilution factor is used for meat juice samples

(Nielsen et al., 1998; Meemken and Blaha, 2011; Forbes et al., 2012; Wallander, Frossling, Vagsholm, Burrells et al., 2015).

The presence of antibodies to a specific pathogen indicates that the animal has been exposed to the pathogen at some stage of life, although the seropositive animal may no longer carry or shed viable pathogen cells (Nielsen et al., 1995; Nielsen et al., 1996). Antibodies typically remain until the age of slaughter (Gamble et al., 1983; Nielsen et al., 1995; Nesbakken et al., 2006; Basso et al., 2017), but an immune response cannot be measured immediately after infection. At least 12 days are required for *Yersinia* and seven days for *Salmonella* antibodies to rise after infection (Nielsen et al., 1995; Nielsen et al., 1996). Sometimes the animal´s immune system is unable to produce a measurable immune reaction, although they are carrying the pathogen. Hence serological methods are unsuitable for individual carcass testing for purposes of assuring food safety.

These physiological phenomena described in the previous paragraph may also explain the results from several studies comparing serological methods and bacteriological methods and for the lack in correlation between serological and microbiological results in the detection of pig salmonellosis at the individual level (Nollet et al., 2005; Farzan et al., 2007; Mainar-Jaime et al., 2008; Vico et al., 2010; Methner et al., 2011).

Serological methods have been considered useful tools for population-level and herd-level surveillance programmes in several studies (Nesbakken et al., 2003; Gamble et al., 2004; Gamble et al., 2005; Nowak et al., 2007; Alban et al., 2012; Basso et al., 2013; Meemken et al., 2014). Recent studies by Casanova-Higes et al. (2017) and Mainar-Jaime et al. (2018) found a significant relationship between within-farm *Salmonella* serology and *Salmonella* shedding at slaughter. Nesbakken et al. (2003) showed an association between *Yersinia* serology and occurrence in tonsils. Serological testing of *T. gondii* has been conducted in practice in the Netherlands with good experiences (Oorburg et al., 2017). Serological monitoring systems for *Salmonella* antibodies in meat juice have been proven effective for identifying high-risk herds for *Salmonella* in Denmark, Germany and Ireland (Safefood, 2010; Merle et al., 2011; Alban et al., 2012; QS Qualität und Sicherheit GmbH, 2018). Serological testing of *Salmonella* in blood samples has been used in Belgium and the Netherlands (Hanssen, 2011; Méroc et al., 2012). However, Belgium stopped its serological monitoring programme for *Salmonella* in 2015 (Mainar-Jaime et al., 2018). Also, the UK has tested meat juice samples for *Salmonella*, but discontinued the programme in 2012 (https://www.pigprogress.net/Health-Diseases/Health/2012/6/UK-New-direction-for-Zoonoses-National-Control-Programme-ZNCP-PP008961W/, accessed on 4th August 2018). In the UK, this decision was made to make way for on-farm *Salmonella* risk assessment. To be effective, a surveillance programme needs to be accompanied with proper interventions. National surveillance was continued in Belgium, but on-farm risk-categorization was

discontinued (http://www.arsia.be/?page_id=3801&lang=de, accessed on 4th August 2018).

EFSA (2011) stated that incoming pig batches should be risk-ranked based on the herds' status of *Salmonella* spp., *Y. enterocolitica*, *T. gondii* and *Trichinella* spp., and suggested that this ranking could be based on historical serological testing of meat juice.

3 AIMS OF THE STUDY

The main objective of this work was to study the prerequisites of risk-based meat inspection of pigs in Finland in the context of food control and public health issues, concentrating on biological hazards 'from the farm to the fork'. The objective was to investigate current hazard testing, the usability of FCI as part of risk-based and visual meat inspection and to reveal the possible needs for refinements reflecting differences between Finland and the EU.

Specific aims of the studies were:

1. To assess the seroprevalence of *Salmonella* spp., pathogenic *Yersinia* spp., *T. gondii* and *Trichinella* spp. in fattening pigs in Finland and to evaluate the feasibility of serological monitoring at the slaughterhouse and the usability of the results as part of the FCI (I)

2. To assess the usability of the current FCI and to evaluate the possibility of risk-ranking incoming slaughter batches according to previous meat inspection data and current FCI statements for the needs of visual and risk-based meat inspection (II)

3. To investigate the serological *Salmonella* spp., pathogenic *Yersinia* sp. and *T. gondii* status in pigs during the fattening period, to investigate factors predicting condemnations at slaughter and to evaluate the contribution of this data to risk-based meat inspection (III)

4. To compare commercially available ELISA kits for detecting *T. gondii* antibodies in the meat juice of naturally infected fattening pigs (IV).

4 MATERIALS AND METHODS

4.1 SAMPLING

4.1.1 SAMPLING OF THE PIG CARCASSES AT THE SLAUGHTERHOUSES FOR SEROLOGICAL ANALYSIS (I, II, IV)

Sample collection, study I

Meat samples of ca. 10 g of muscle from the diaphragm of fattening pigs were collected by the OVs and official auxiliaries randomly at the slaughter line in two large slaughterhouses in Finland. Meat samples were placed in plastic bags, and frozen to below -18 °C. Samples were transported to a laboratory, where they were thawed and mechanically squeezed to obtain meat juice (Figure 1). Meat juice was stored at -70 °C until testing and thawed before analysis, then frozen again for possible reanalysis.

Figure 1 Meat was mechanically squeezed to obtain meat juice. Photo by Maria Stark, 2013.

In total, 1353 meat samples from fattening pigs originating from 259 conventional farms were collected between November 2012 and April 2013 (Table 1). Farms were allocated according to farm types: 36% were large fattening farms (≥1000 pig places), 33% were small fattening farms (<1000 pig places) and 31% were farrow-to-finish farms. On average, five (range 3–15) pigs were sampled per farm. The sampling represents the current situation in Finland, as these two slaughterhouses receive animals throughout Finland and slaughter approximately 75% of the fattening pigs in Finland.

Sample size is adequate for evaluating seroprevalences of *Salmonella*, *Yersinia* and *Toxoplasma*, as the seroprevalences were pre-estimated to be 1%, 60% and 3%, respectively, for sample-size estimation (Naing et al., 2006). Regarding *Trichinella*, the aim was to indicate that the seroprevalence was below 1%, as it was assumed to be nearly 0%.

Samples used for study II

Study II included samples (n=431) from one of the slaughterhouses from study I (Table 1). This slaughterhouse slaughters approximately 30% of the fattening pigs in Finland.

Samples used for study IV

To compare the ELISA tests for the detection of *Toxoplasma* antibodies in study IV, 90 samples were selected from study I (Table 1). All the 43 *T. gondii*-seropositive samples from study I were included. In addition, 27 samples just below positive with a percentage of positive (PP) -ratio between 0.10 and 0.14 (10–14%) were selected. Finally, 20 random samples (SPSS Random selection) with a PP below 0.10 (10%) were included.

4.1.2 COLLECTING BLOOD SAMPLES FROM PIGS AT THE FARMS FOR SEROLOGICAL ANALYSIS

In total, 1793 blood samples were collected from 1140 pigs in 57 fattening farms in southern Finland during 2012–2014 (Table 1). Farms were allocated according to farm types: 39% were large fattening farms (≥1000 pig places), 21% were small fattening farms (<1000 pig places) and 40% were farrow-to-finish farms.

The farms were selected for the needs of another study investigating chronic pleurisy in fattening farms, and participation was based on the willingness of individual farmers. Only farms producing at least 1000 fattening pigs per year were included in the study. If a farm had several compartments, only one compartment per farm was included in our study.

An average of 20 pigs were randomly selected from various parts of the compartment. Pigs were captured with a snout snare, ear-tagged and blood samples were collected from the jugular vein. Samples were stored at 4 °C for up to 24 hours and then centrifuged in the laboratory at 3000 rpm for 10 minutes. Sera was separated and stored frozen at -18 °C until analysis.

Individual pigs from 34 farms were sampled twice. The first sampling (Sampling A) occurred during the first fattening week (median six days, range 0–11 days after arrival to the fattening unit). The second sampling (Sampling B) of the same pigs occurred at the end of the fattening period (median 11 weeks, range 7–15 weeks after arrival at the fattening unit). In addition, individual pigs from 23 farms were sampled only at the end of the fattening period (only Sampling B, median 10 weeks, range 6–14 weeks after arrival at the fattening unit). In total, 653 individual pigs were sampled twice (Samples A and B) and 487 pigs once (463 pigs only sample B and 24 pigs only sample A).

For sample size estimation, the seroprevalences of *Yersinia* spp., *T. gondii* and *Salmonella* spp. were assumed to be 57%, 3% and 3%, respectively, at the end of fattening, based on results from study I. Consequently, the sample size (1116 fattening pigs at the end of fattening and 653 fattening pigs sampled twice) was adequate to estimate the seroprevalences of *Salmonella* spp. and *T. gondii* with 1–1.5% precision and the seroprevalence of *Yersinia* spp. with 3–4% precision with 95% confidence (Naing et al., 2006). Within each farm, the sample size of 20 fattening pigs enabled us to estimate the within-farm seroprevalence of *Salmonella* spp. and *T. gondii* with 95% confidence and 8% precision and *Yersinia* spp. with 95% confidence and 22% precision (Naing et al., 2006).

The experiments were approved by the National Animal Experiment Board in Finland.

Table 1 *Samples collected during studies I to IV.*

Samples	Study	Sampling Time	Sampling place	No. of animals	No. of farms	Farm type
Meat juice	I	2012–2013	Slaughterhouses A and B	492	93	Large FF[a]
				436	86	Small FF[b]
				425	80	FFF[c]
	II	2012–2013	Slaughterhouse A	431	80	
	IV	2012–2013	Slaughterhouses A and B	90	58	
Blood	III	2012–2014	Farms 1–57	441	22	Large FF[a]
				240	12	Small FF[b, d]
				459	23	FFF[c, d]
Meat inspection data	II	2012–2013	Slaughterhouse A	8954	80	
	III	2012–2014	Slaughterhouses A–C	25552	57	

[a]Large FF = large fattening farm, ≥1000 pig places
[b]Small FF = small fattening farm, <1000 pig places
[c]FFF = farrow-to-finish farm
[d] Only farms slaughtering at least 1000 pigs/year were included

4.2 SEROLOGICAL ANALYSIS

4.2.1 SEROLOGICAL ANALYSIS OF MEAT JUICE SAMPLES (I, II)

The meat juice samples were analysed using commercial ELISA tests suitable for pig meat juice samples (Table 2). From each sample for each analysis, 10 µl of meat juice was diluted to 1:10, and analysed and interpreted following the manufacturers' instructions. The reactions were read using a spectrophotometer (Multiskan Ascent V1.24, Thermo Electron Corporation, Waltham, MA, USA) at 450 nm.

The *Salmonella* antibodies were analysed using the SALMOTYPE Pig Screen test (Labor Diagnostik GmbH, Leipzig, Germany) with a cut-off optical density (OD) value of 20%. Antibodies to O-antigens 1, 4, 5, 6, 7 and 12 were detected. According to the manufacturer, the sensitivity and specificity of the test are 98.5% and 99.8%, respectively.

The *Yersinia* antibodies were analysed using the PIGTYPE® YOPSCREEN test (Labor Diagnostik) with a cut-off OD value of 30%. The antigens used in

the test are *Yersinia* outer proteins (Yops), which are expressed only by pathogenic Yersinia strains carrying the virulence plasmid. According to the manufacturer, the sensitivity and specificity of the test are near to 100%.

The *T. gondii* antibodies were analysed using the Prio-CHECK® Toxoplasma Ab Porcine test (Prionics AG, Schlieren-Zurich, Switzerland) with a cut-off PP value of 0.15 (15%). (PP = a percentage of positivity = OD Sample/OD positive control *100 %). Sensitivity and specificity of the test have been demonstrated to be 98.9% and 92.7%, respectively (Basso et al., 2013).

The *Trichinella* antibodies were analysed using the PIGTYPE® Trichinella Ab Test (Labor Diagnostik) with a cut-off OD value of 30%. The sensitivity of the test was 98.9% and the specificity was 95.4%, and nearly 100% specificity is achievable by confirming positive results with the Western blot analysis(Frey et al., 2009; Knoop et al., 2011).

4.2.2 SEROLOGICAL ANALYSIS OF BLOOD SAMPLES (III)

The sera were analysed using commercial ELISA tests (Table 2) to test for antibodies to salmonellae (Pigtype® Salmonella Ab, Qiagen, Leipzig, Germany), *Yersinia* spp. (Pigtype® Yersinia Ab, Qiagen, Leipzig, Germany) and *T. gondii* (Pigtype® Toxoplasma Ab, Qiagen, Leipzig, Germany). The tests were performed, and the results were calculated and interpreted following the manufacturer's instructions. Samples with a sample/positive (S/P)-ratio ≥30% were considered positive. OD% values for *Salmonella* spp. were calculated according to the following equation: $OD\% = \frac{S/P \times 100\%}{2}$. We considered samples with an OD ≥15% positive for *Salmonella* spp. According to the manufacturer, the sensitivity and specificity of the kits used are nearly 100%.

4.2.3 SEROLOGICAL ANALYSIS OF MEAT JUICE SAMPLES FOR *TOXOPLASMA* ANTIBODIES (IV)

Samples were analysed parallel with four commercial ELISA tests (tests I–IV) and a commercial modified agglutination test (MAT). Samples were analysed in 2016.

All the tested ELISA kits (Table 2) are intended for meat juice analysis. The analyses were performed according to the manufacturers´ instructions. Negative and positive controls supplied by the manufacturer were included on each ELISA plate. Duplicate meat juice samples were tested. OD was measured at 450 nm after 15 min in a spectrophotometer (Multiskan Ascent V1.24, ThermoElectron Corporation, Waltham, MA, USA). The S/P-ratio was calculated. $\text{S/P-ratio} = \frac{Sample\ OD - mean\ OD\ of\ the\ negative\ control}{Mean\ OD\ of\ the\ positive\ control - mean\ OD\ of\ the\ negative\ control}$.

Test I was Priocheck® Toxoplasma Ab Porcine (Prionics). Antigens in the test were whole tachyzoites, which are pig-specific using a peroxidase-labelled Anti-pig IgG antibody. The cut-off value recommended by the manufacturer was S/P-ratio 0.20.

Test II was Pigtype® Toxoplasma Ab (Qiagen, Leipliz, Germany). Antigens in the test were whole tachyzoites. The test is a multi-species test, using a multi-species horseradish peroxidase to detect IgG antibodies. The cut-off value recommended by the manufacturer was S/P-ratio 0.30.

Test III was ID Screen® Toxoplasmosis Indirect Multi-species (IDvet, Grabels, France). Antigens in the test were P30 proteins of the tachyzoites' surface. A multi-species horseradish peroxidase is used in the test to detect IgG antibodies. The cut-off value recommended by the manufacturer was S/P-ratio 0.50 and values between 0.40–0.50 were considered weakly positive according to the manufacturer.

Test IV was the *Toxoplasma gondii* Antibody Test Kit (SafePath Laboratories, Carlsbad, CA, USA). Antigens in the test were whole tachyzoites. A pig-specific peroxidase-labelled Anti-pig IgG antibody was used in the test. The cut-off value recommended by the manufacturer was OD ≥0.30.

Test V was a commercial MAT, ToxoScreen DA® test kit (Biomerieux, France). The test was performed according to the manufacturer´s instructions. All samples were analysed in two dilutions: 1:40 representing the cut-off value, and 1:4000 to avoid false-negative results. The higher dilution is used to prevent false-negative results, which may result if high quantities of IgG antibodies inhibit antigen cross-linking. The borderline positive results were interpreted as positive to compensate the lower antibody levels in meat juice samples compared to serum samples. The test is originally validated to serum samples.

Table 2 *Elisa kits used for the detection of Salmonella, Yersinia, Toxoplasma and Trichinella antibodies in studies I to IV.*

Elisa kit	*Salmonella*	*Yersinia*	*Toxoplasma*	*Trichinella*
Pigtype® Salmonella Ab, Qiagen	I, II, III			
Pigtype® Yersinia Ab, Qiagen		I, II, III		
Pigtype® Toxoplasma Ab, Qiagen			III, IV	
Prio-CHECK®Toxoplasma Ab, Prionics			I, II, IV	
ID Screen®Toxoplasmosis, IDvet			IV	
Toxoplasma antibody test, SafePath			IV	
Pigtype® Trichinella Ab, Qiagen				I, II, III

4.3   DATA COLLECTION

4.3.1   FARM-LEVEL DATA, FOOD CHAIN INFORMATION AND MEAT INSPECTION RESULTS (I & II)

Study I

The slaughterhouses provided information considering farm types for all the 259 conventional farms included in study I. Farms were allocated into large fattening farms (≥1000 pig places), small fattening farms (<1000 pig places) and farrow-to-finish farms (Table 1). In addition, the exact number of pig places at the farms was obtained from 177/179 fattening farms studied.

Study II

For study II, meat inspection results and FCI covering 85 slaughter batches from 80 farms were obtained from one of the slaughterhouses (Table 1). The median size of these 85 slaughter batches was 87 pigs/batch (range 20−271) and they were comprised of a total 8954 fattening pigs.

The meat inspection data covered the meat inspection results of the current batch and the data covering all the slaughter batches sent from the same farm during the previous year. In total, this meat inspection data covered more than 280 000 pigs, which is over 13% of all fattening pigs slaughtered in Finland annually in 2012 and 2013 (http://statdb.luke.fi/PXWeb/pxweb/en/LUKE/). At the time of slaughter, meat inspection was performed in accordance with the traditional procedure, including palpations and incisions.

FCI was provided by the respective pig producers using the FCI form used by Finnish large slaughterhouses described in chapter 2.3. The mortality rate for each slaughter batch during the last three months was obtained from Sikava (Stakeholders' health and welfare register for pigs in Finland).

All collected data are described in detail with their respective results in Table 16.

4.3.2   PIG HEALTH AND MEAT INSPECTION RESULTS (III)

During the farm visits (4.1.2), the veterinarian collected data concerning the health of the pigs (Table 3). Health status was evaluated by observing ca. 100 pigs (Visit A: median 110 pigs, range 19−181 pigs; Visit B: median 105 pigs, range 19−180 pigs) for signs of lameness and fresh/healed tail-biting lesions. In addition, all pigs in each compartment were forced to stand up and the number of coughing episodes during the following five minutes was

registered. A coughing index was calculated by dividing the number of coughing episodes by the number of pigs in the compartment.

Meat inspection data (Table 3) were obtained from the veterinary meat inspection reports covering the slaughtering periods of the animals in the studied batches. Data covered all fattening pigs (n=25 552) slaughtered concurrently with the studied pigs from each farm.

Table 3 *Farm-level data concerning on-farm health and meat inspection findings in fattening pigs from pig farms in Finland. Published in Felin et al., 2018 (III), reprinted with permission of the copyright holder.*

Subject	Collected data	No. of farms	Description of data		
			Mean	Median	Range
On-farm pig health	Coughing index at the beginning of the fattening period [a]	34	0.6%	0.5%	0–2.6%
	Coughing index at the end of the fattening period [a]	57	0.6%	0%	0–5.5%
	% of pigs with fresh tail-biting injuries at the beginning of the fattening period	34	2.8%	0%	0–36.1%
	% of pigs with fresh tail-biting injuries at the end of the fattening period	57	0.7%	0%	0–11.2%
	% of pigs with healed tail-biting injuries at the beginning of the fattening period	34	8.0%	2.7%	0–38.0%
	% of pigs with healed tail-biting injuries at the end of the fattening period	57	16.1%	11.3%	0–94.7%
	% of lame pigs at the beginning of the fattening period	34	0.9%	0%	0–6.0%
	% of lame pigs at the end of the fattening period	57	1.7%	1.7%	0–8.0%
Within-farm meat inspection data at slaughter	Partial carcass condemnation %	57	7.0%	6.8%	1.0–16.0%
	Arthritis %	57	3.3%	3.1%	0–10.0%
	Abscess %	57	4.5%	3.8%	1.0–13.0%

[a]Coughing index: Pigs in one compartment were forced to stand up, the coughing episodes were recorded for 5 minutes and the number was divided with the number of pigs in the compartment.

4.4 STATISTICAL ANALYSIS

Several statistical analyses were used in the thesis (Table 4). P-values <0.05 were considered to indicate statistical significance. The analyses were mainly conducted with SPSS® Statistics (IBM Corporation, New York, USA) (Table 4).

Table 4 *Statistical analyses used in studies I to IV.*

Statistical analysis	Programme	Studies			
		I	II	III	IV
Confidence intervals	Open-Epi	x			
Cross tabulations	SPSS® Statistics version 21	x			
Pearson chi-square test	SPSS® Statistics version 21	x			
Pearson correlation	SPSS® Statistics versions 21, 22, 23	x	x	x	x
Variance (ANOVA)	SPSS® Statistics versions 21, 22, 23	x		x	
Independent sample t-test	SPSS® Statistics version 22		x		
Linear regression	SPSS® Statistics version 22		x		
Regression model (GenLinMixed)	SPSS® Statistics version 23			x	
Spearman correlation	SPSS® Statistics version 23			x	
Wilcoxon signed rank test	SPSS® Statistics version 23			x	
McNemar test	SPSS® Statistics version 23			x	
Kappa	SPSS® Statistics version 23				x
ROC	SPSS® Statistics version 23				x

4.4.1 STATISTICAL ANALYSIS OF SEROLOGICAL AND FARM-LEVEL DATA (I)

An animal was considered seropositive if its meat juice sample was positive. A farm was considered seropositive when at least one of the sampled animals tested positive.

The Open-Epi programme and the Wilson method were used to calculate the 95% confidence intervals (CIs) for the seroprevalences (Dean et al., 2013). Cross-tabulations and the Pearson chi-square test were used to analyse relationships between seropositivity to various pathogens studied both at the animal and farm levels. Bivariate Pearson (two-tailed) was used to calculate correlations between variables. The seropositivity of pigs originating from various farm types was compared using one-way analysis of variance (ANOVA) and Tukey´s honestly significant difference.

4.4.2 STATISTICAL ANALYSIS OF MEAT INSPECTION RESULTS, FCI AND SEROLOGICAL DATA (II)

Correlations between variables were analysed by calculating Pearson correlation coefficients (r).

Independent sample t-tests were used to compare the differences between mean values of the groups. For the t-tests, variables were transferred using the arcus sine of the square root transformation to achieve homogenous variances and approximately normal distributions.

Stepwise linear regression analysis was used to predict meat inspection results to indicate which slaughter batches could have been suitable for visual meat inspection. Partial condemnation rate, organ condemnation rate and the sum of the partial and total condemnation rates were the response variables used in the analysis. These indicators were assumed to retrospectively indicate, whether a slaughter batch could have been suitable for visual meat inspection. This was because batches with high frequencies of lesions require additional inspection procedures. Total condemnations were so rare that the variable was not considered a relevant response variable as such. The response variables were transformed using the arcus sine of the square root transformation to achieve homogenous variances and approximately normal distributions. Regression models were also estimated for untransformed responses using the regressors found in the stepwise procedures.

The predictors tested in the linear regression analysis were all variables in previous meat inspection reports, i.e. the mortality rate and the information on the batch declared in the FCI. The previous year´s organ condemnation rate and the previous year´s rate of tail biting, both of which had 54 missing values, were exceptions to this. One extreme value for the previous year´s partial carcass condemnation rate (24%) was discarded from the analysis, as it would have resulted in illogical models. FCI data regarding the farm were not included in the analysis, because none of the farms declared salmonellosis, trichinellosis or any restrictions imposed by the authorities, and only three farms declared erysipelas.

4.4.3 STATISTICAL ANALYSIS IN STUDY III

An animal was considered seropositive for a pathogen if its serum sample was positive. Seroprevalence at a farm was calculated by dividing the number of seropositive pigs from the farms by the number of samples taken from the farm. 'Within-farm seroprevalence' means the number of positive samples from a farm per the number of samples taken from the farm, presented as a percentage.

Logistic regression analysis for associations to serological status of the pigs

Statistical analyses were conducted to evaluate the comorbidity and the effect of the within-farm seroprevalence at the beginning of the fattening. Each dependent variable was separately analysed. The dependent variables were

1. *Yersinia* seropositivity of the pig (seropositive/seronegative) at the beginning of fattening,
2. *Yersinia* seropositivity of the pig (seropositive/seronegative) at the end of fattening,
3. Seroconversion of the pig to *Yersinia* seropositive during fattening (yes/no),
4. *Salmonella* seropositivity of the pig (seropositive/seronegative) at the beginning of fattening,
5. *Salmonella* seropositivity of the pig (seropositive/seronegative) at the end of fattening,
6. Seroconversion of the pig to *Salmonella* seropositive during fattening (yes/no).

Independent variables were a pig's simultaneous seropositivity to the pathogen (*Yersinia/Salmonella,* binomial variable) other than the dependent variable. The within-farm seroprevalence (%) of the studied pathogen at the beginning of fattening (scale variable) was included as an independent variable for dependents 2, 3, 5 and 6.

First, a univariable generalized linear mixed model, with farm as a random effect using the logistic link function, was performed to screen the association between each independent and dependent variable. Independent variables with P≤0.1 in the univariable analysis were introduced to the multivariable generalized linear mixed model with farm as a random effect using the logistic link function. These risk factors were checked for collinearity by calculating Spearman correlation coefficients. Correlations were moderate at maximum (all <0.35).

Risk factors for *Toxoplasma* seropositivity were not analysed because of the low number of seropositive pigs.

Logistic regression analysis for risk factors for condemnations at slaughter

The associations between condemnations at slaughter and on-farm health data were analysed to examine whether on-farm health data predicted condemnations. The dependent variables were

1. Partial carcass condemnation rate,
2. Arthritis rate,
3. Abscess rate.

Dependent variables were introduced to the model as 'events/trials', e.g. 'n of condemned pigs/n of pigs'. Independent variables were scale farm-level

variables, which are described in Table 3 (Health of pigs). Independent variables also included the within-farm seroprevalences of *Salmonella* and *Yersinia* at the beginning and end of fattening.

First, a univariable generalized linear mixed model, with farm as a random effect using the logistic link function, was performed to screen the association between each independent and dependent variable. Independent variables with P≤0.1 in the univariable analysis were introduced to the multivariable generalized linear mixed model with farm as a random effect using the logistic link function. These risk factors were checked for collinearity by calculating Spearman correlation coefficients. Correlations were moderate at maximum (all <0.35). Each dependent variable was separately analysed with the factors in effect at the beginning of fattening (n=34 farms) and with the factors in effect at the end of fattening (n=57 farms).

Other statistical analysis

Differences between within-farm seroprevalences at the beginning and end of fattening were tested with a Wilcoxon signed-rank test. The McNemar test was used to compare the seroprevalence of all pigs sampled twice at the beginning and the end of fattening.

The number of seropositive pigs originating from various farm types was compared using ANOVA and the Games Howell post hoc test.

4.4.4 STATISTICAL ANALYSIS COMPARING COMMERCIAL ELISA TESTS FOR THE DETECTION OF *TOXOPLASMA* ANTIBODIES

MAT was used as a reference test in the calculations. Sensitivity (SE), specificity (SP), accuracy, positive predictive value (PPV) and negative predictive value (NPV) were calculated. SE was calculated as the probability (percentage) that seropositive pigs determined by the MAT result will have a positive result using the test under evaluation (TDR diagnostics evaluation expert panel et al., 2010). SP was calculated as the probability (percentage) that seronegative pigs determined by the MAT result will have a negative result using the test under evaluation (TDR diagnostics evaluation expert panel et al., 2010). Accuracy was calculated as the percentage of correct results obtained by the test under evaluation compared with the MAT results (TDR diagnostics evaluation expert panel et al., 2010). NPV was calculated as the probability that a negative result accurately indicates the absence of infection (TDR diagnostics evaluation expert panel et al., 2010). PPV was calculated as the probability that a positive result accurately indicates the presence of infection (TDR diagnostics evaluation expert panel et al., 2010). PPV and NPV also depend on infection prevalence in the population studied (TDR diagnostics evaluation expert panel et al., 2010). The agreement level

between the ELISA tests and MAT was tested by calculating. Agreement was considered good or very good with Kappa values between 0.6 and 0.8 or over 0.8, respectively. We additionally estimated the Pearson correlation between the ELISA tests and intra-class correlation between the two repeated analyses with the same test. ROC curves were also drawn.

5 RESULTS

5.1 THE SEROPREVALENCE OF *SALMONELLA* SPP., PATHOGENIC *YERSINIA* SPP., *T. GONDII* AND *TRICHINELLA* SPP. IN FATTENING PIGS IN FINLAND (I, III, UNPUBLISHED)

5.1.1 SEROPREVALENCE OF *SALMONELLA* SPP. IN FATTENING PIGS IN FINLAND

We analysed *Salmonella* antibodies in two separate studies (I, III). *Salmonella* antibodies were detected in 3.1% of the meat juice samples collected at slaughter (study I) and in 17.6% of the blood samples collected from the farms (study III) at the end of the fattening period (Table 5). Most of the farms (91.2%) where blood samples were taken (an average 20 pigs/farm sampled) were seropositive at the end of fattening and 13.5% of the farms where meat juice samples were taken at slaughter (an average of five pigs/farm sampled) were seropositive.

Table 5 *Salmonella seroprevalence estimates in fattening pigs and fattening pig farms in Finland.*

Sample	Seropositives at the end of the fattening period[a]					
	All pigs			All farms[b]		
	N	%	95% CI	N	%	95% CI
Meat juice	42/1353	3.1	2.3–4.2	35/259	13.5	9.9–18.2
Serum	196/1116	17.6	15.3–19.8	52/57	91.2	83.7–98.8

[a] Blood samples were collected at the farm at the end of the fattening period and meat juice samples were collected at slaughter
[b] Seropositive farm = at least one positive sample

No differences were found between farm types at the end of fattening (I and III). We observed significant differences between individual farms: within-farm *Salmonella* seroprevalences at the end of fattening ranged from 0 to 60% (I) and from 0 to 55% (III).

The OD values of *Salmonella* were low (Figure 2; Figure 3). When the cut-off value OD40% was used according to the German *Salmonella* monitoring programme (QS Qualität und Sicherheit GmbH, 2018), *Salmonella* seroprevalence at the end of the fattening period was 0.1% in study I (Figure 2) and 1.9% in study II (Figure 3).
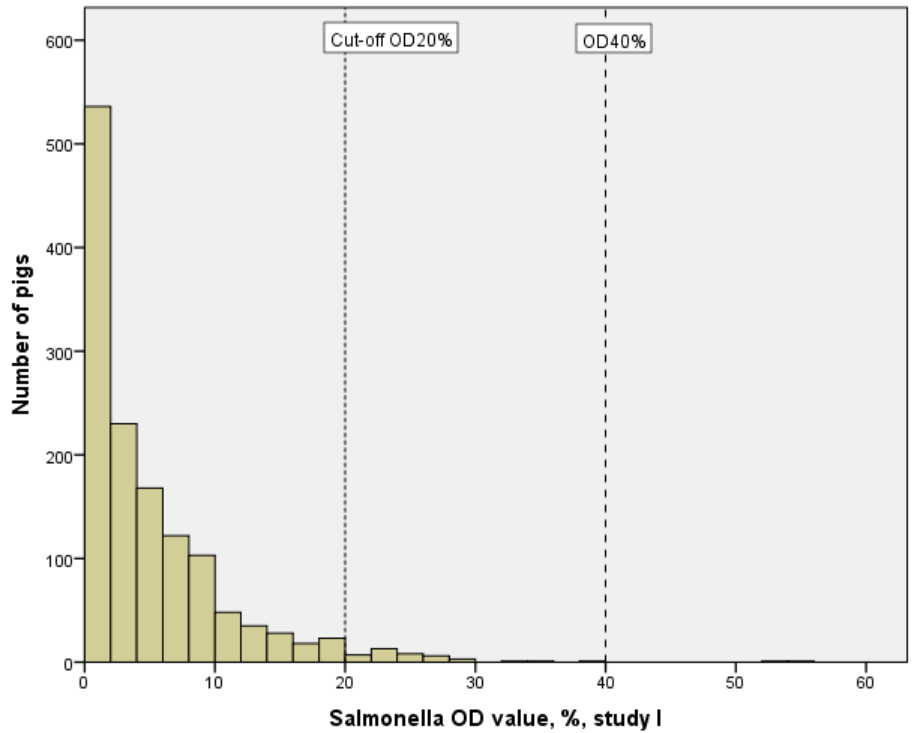
Figure 2 Salmonella OD values in meat juice samples of 1353 fattening pigs at slaughter in Finland. Published in Felin et al., 2015 (I), reprinted with permission of the copyright holder.
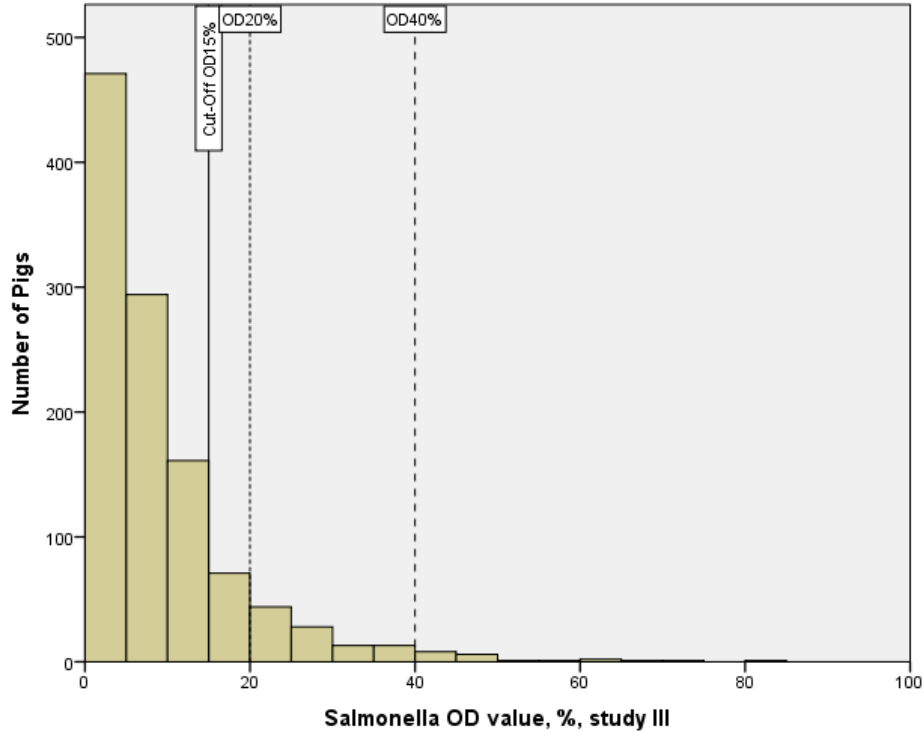
Figure 3 Salmonella OD values of 1116 blood samples taken at the end of the fattening period in Finland. Published in Felin et al., 2018 (III), reprinted with permission of the copyright holder.

In study III, we also analysed changes in *Salmonella* seropositivity of individual pigs during the fattening period (Table 6). *Salmonella* seroprevalence rose significantly (P<0.001) during the fattening period: from 8.1% to 17.2% seropositive pigs. At the beginning of fattening, the seroprevalence was highest among pigs from farrow-to-finish farms (10.4%), but the difference was not statistically significant (P=0.145, One-way ANOVA, compared to large fattening farms 6.0%).

Table 6 *Salmonella-seropositive blood samples from fattening pigs in Finland.*

Pigs sampled twice				Farms sampled twice			
Sampling A[a]		Sampling B[b]		Sampling A[a]		Sampling B[b]	
n	%	N	%	N	%	N	%
53/653	8.1	112/653	17.2	23/34	67.6	30/34	88.2

[a]Sampling A = at the beginning of the fattening period
[b]Sampling B= at the end of the fattening period

In total, 13 farms were included in both studies I and III. When considering these farms only, the mean *Salmonella* seroprevalence of a batch of fattening pigs at slaughter was 3.1% in study I (meat juice samples) and 13.2% at the end of fattening in study III (blood samples). The difference was statistically significant (paired sample t-test, P=0.03). We found no significant correlations between the seroprevalence of the batches in studies I and III. However, the results of different batches from the same farm are quite similar when considering the sample size: five samples/batch in study I, 20 samples/batch in study III (Table 7) (unpublished).

Table 7 *Salmonella seroprevalences of different fattening pig batches originating from 13 pig farms in Finland.*

Farm	Sampling date in study I	Seroprevalence of the batch in study I, % [a]	Sampling date in study III	Seroprevalence of the batch in study III, % [b]
1	09-11-2012	<20	15-04-2014	5
2	09-11-2012	<20	15-08-2012	5
3	09-11-2012	<20	25-11-2012	5
4	03-01-2013	<20	12-03-2013	15
5	07-01-2013	<20	12-03-2014	<5
6	07-01-2013	<20	11-04-2014	5
7	08-01-2013	<20	30-08-2012	30[c]
8	09-01-2013	<20	03-07-2014	15
9	11-01-2013	<20	05-04-2013	17
10	13-02-2013	20	11-07-2013	20
11	21-02-2013	20	11-10-2012	<5[c]
12	25-02-2013	<20	30-01-2013	40[c]
13	25-02-2013	<20	27-01-2014	15

[a]Five meat juice samples/batch studied at slaughter, a value <20 means that no seropositive samples were found
[b]20 blood samples/batch studied at the end of the fattening period, a value <5 means that no seropositive samples were found
[c]Difference shown between batches from the same farm

Farms were allocated into risk categories according to *Salmonella* serological results using the Danish and German schemes (Alban et al., 2012; QS Qualität und Sicherheit GmbH, 2018). All farms were in German category 1 (Table 8). Most (98%) farms were in Danish category 1 and only 2% of farms were in Danish category 2 (Table 9). In addition, we allocated farms according to our modified categorization (Table 10) (unpublished).

Table 8 *Serological results from Finnish fattening pig farms allocated according to the German QS Salmonella control programme[a] using a cut-off value of OD40%.*

Risk category	Results from study I (259 farms)	Results from study III (57 farms)	Corrective actions in German QS
Category 1, Low, within-farm seroprevalence ≤20%	100% of farms	100% of farms	None
Category 2, Medium, within-farm seroprevalence >20-40%	0% of farms	0% of farms	Check and document the hygiene status
Category 3, High, within-farm seroprevalence >40%	0% of farms	0% of farms	Bacteriological sampling, epidemiological investigation, corrective actions at farm

[a] QS Qualität und Sicherheit GmbH 2018

Table 9 *Serological results from Finnish fattening pig farms allocated according to the Danish Salmonella control programme[a] using a cut-off value of OD20%.*

Risk category	Results from study I (259 farms)	Results from study III (57 farms)	Corrective actions in Danish programme
Category 1, Low, within-farm seroprevalence <40%[b]	98.1% of farms	98.2% of farms	None
Category 2, Medium, within-farm seroprevalence 40-65%[b]	1.9% of farms	1.8% of farms	Penalty fee
Category 3, High, within-farm seroprevalence >65%[b]	0% of farms	0% of farms	Penalty fee, slaughtered separately

[a]Alban et al., 2012
[b]Weighted average based on data from the previous three months

Table 10 *Serological results from Finnish fattening pig farms allocated according to modified categories using a cut-off value of OD20%.*

Risk category	Results from study I (259 farms)	Results from study III (57 farms)
Category 1, Low, within-farm seroprevalence <20%	88.4% of farms	75.4% of farms
Category 2, Medium, within-farm seroprevalence 20-40%	9.7% of farms	22.8% of farms
Category 3, High, within-farm seroprevalence >40%	1.9% of farms	1.8% of farms

5.1.2 SEROPREVALENCE OF PATHOGENIC *YERSINIA* SPP. IN FATTENING PIGS IN FINLAND

Antibodies for pathogenic *Yersinia* spp. were analysed in two separate studies (I, III). *Yersinia* antibodies were detected in 56.6% of the meat juice samples collected at slaughter (I) and in 66.1% of the blood samples collected at farms (III) at the end of the fattening period (Table 11). In both studies, most farms were seropositive at the end of fattening (Table 11).

Table 11 *Pathogenic Yersinia seroprevalence estimates in fattening pigs and fattening pig farms in Finland.*

Sample	Seropositives at the end of the fattening period[a]					
	All pigs			All farms[b]		
	N	%	95% CI	N	%	95% CI
Meat juice	766/1353	56.6	54.0–59.2	220/259	84.9	80.1–88.8
Serum	738/1116	66.1	63.3–68.9	50/57	87.7	78.9–96.5

[a] Blood samples were collected at the farm at the end of the fattening period and meat juice samples were collected at slaughter
[b] Seropositive farm = at least one positive sample

Pigs at large fattening farms were more often seropositive than pigs at farrow-to-finish farms in study III (69.7% vs. 62.2%, P<0.05). The differences between farm types were not statistically significant in study I: 60.6%, 55.0% and 53.6% of meat juice samples from large fattening farms, small fattening farms and farrow-to-finish farms were positive. We found significant differences between individual farms: on-farm *Yersinia* seroprevalences at the end of fattening ranged from 0 to 100% in both studies. All pigs tested seropositive for *Yersinia* at the end of fattening at 60 farms (23%) in study I and at 14 farms (25%) in study III.

In study III, we also analysed changes in *Yersinia* seropositivity of individual pigs during the fattening period. *Yersinia* seroprevalence rose significantly (P<0.001) during this period: from 30.3% to 72.3% (Table 12). Interestingly, seroprevalence at the beginning of fattening was highest in farrow-to-finish farms compared to the other two farm types.

Table 12 *Yersinia-seropositive blood samples from fattening pigs in Finland.*

Pigs sampled twice				Farms sampled twice			
Sampling A[a]		Sampling B[b]		Sampling A[a]		Sampling B[b]	
n	%	N	%	n	%	N	%
198/653	30.3	472/653	72.3	20/34	58.8	32/34	94.1

[a]Sampling A = at the beginning of the fattening period
[b]Sampling B= at the end of the fattening period

In total, 13 farms were included in both studies I and study III. When only considering these farms, mean *Yersinia* seroprevalences of a batch of fattening pigs at slaughter were similar in both studies, 50.8% in study I and 56.5% in study III. We observed no significant correlation between the seroprevalence of the batches in studies I and III (Table 13). Two farms (farms no. 5 and 8) were *Yersinia* seronegative in both studies (unpublished).

Table 13 *Yersinia seroprevalences of various fattening pig batches originating from 13 pig farms in Finland.*

Farm	Sampling date in study I	Seroprevalence of the batch in study I, %[a]	Sampling date in study III	Seroprevalence of the batch in study III, %[b]
1	09-11-2012	80	15-04-2014	100
2	09-11-2012	100	15-08-2012	84
3	09-11-2012	40	25-11-2012	32
4	03-01-2013	20	12-03-2013	100
5	07-01-2013	<20	12-03-2014	<5
6	07-01-2013	60	11-04-2014	75
7	08-01-2013	60	30-08-2012	100
8	09-01-2013	<20	03-07-2014	<5
9	11-01-2013	100	05-04-2013	89
10	13-02-2013	100	11-07-2013	50
11	21-02-2013	100	11-10-2012	10
12	25-02-2013	<20	30-01-2013	90
13	25-02-2013	<20	27-01-2014	5

[a]Five meat juice samples/batch studied at slaughter, a value <20 means that no seropositive samples were found
[b]20 blood samples/batch studied at the end of the fattening period, a value <5 means that no seropositive samples were found

5.1.3 SEROPREVALENCE OF *T. GONDII* IN FATTENING PIGS IN FINLAND

Antibodies for *T. gondii* were analysed in two separate studies (I, III). In study I, 3.2% of fattening pigs originating from 9.3% of farms were seropositive for *T. gondii* at slaughter. Only 0.7% of fattening pigs originating from 10.5% of farms were *T. gondii* seropositive at the end of the fattening period in study III (Table 14).

Table 14 *Toxoplasma gondii seroprevalence estimates in fattening pigs and fattening pig farms in Finland.*

Sample	Seropositives at the end of the fattening period[a]					
	All pigs			All farms[b]		
	N	%	95% CI	N	%	95% CI
Meat juice	43/1353	3.2	2.4–4.3	24/259	9.3	6.3–13.4
Serum	8/1116	0.7	0.2–1.2	6/57	10.5	2.3–18.7

[a] Blood samples were collected at the farm at the end of the fattening period and meat juice samples were collected at slaughter
[b] Seropositive farm = at least one positive sample

We found statistically significant differences between farm types in study I. Fattening pigs originating from small fattening farms showed significantly higher seroprevalences (6.0%, p<0.01) than fattening pigs from large

fattening farms (1.8%) and from farrow-to-finish farms (1.9%). Significant differences were also found between individual farms in study I: on-farm *T. gondii* seroprevalences at the end of fattening ranged from 0 to 100%. High seroprevalences (>20%) were only found in small fattening farms with <500 pig places at the farm. A strong negative correlation was observed between the pigs' seropositivity for *T. gondii* and the number of pig places on the farm of origin (P<0.001, n=918 pigs, study I).

In study III, we only observed minor differences between farms, most of the *T. gondii*-seropositive pigs were scattered and the highest within-farm seroprevalence was 10%. *T. gondii* seroprevalence remained at 0.6% during the entire fattening period at farms that were sampled twice in study III, though certain changes were observed in the seropositivity of individual pigs during the fattening period.

5.1.4 SEROPREVALENCE OF *TRICHINELLA* SPP. IN FATTENING PIGS IN FINLAND

Antibodies for *Trichinella* spp. were analysed in study I. *Trichinella* antibodies were not detected. All the samples (n=1353) were clearly negative.

5.1.5 ASSOCIATIONS BETWEEN PATHOGENS

We found strong positive associations at the animal level between the pigs´ seropositivity for *Salmonella* and *Yersinia* in both studies I and III.

In study I, 83% of the *Salmonella*-seropositive pigs were also *Yersinia* seropositive (χ^2, P<0,001). At the farm level, no significant association was observed between farm seropositivity for *Salmonella* and *Yersinia* (χ^2, p=0.25). However, the within-farm seroprevalences of *Salmonella* and *Yersinia* did correlate positively (r=0.15, P<0.05). The within-farm seroprevalence of *Yersinia* was higher in *Salmonella*-seropositive farms than in *Salmonella*-seronegative farms (71% and 54%, respectively, P<0.05).

Animal-level (but not farm-level) associations between *Salmonella* and *Toxoplasma* seropositivity were found in study I (χ^2, P<0.05), but not in study III, where *Toxoplasma*-seropositive pigs were rare.

In study III, the simultaneous *Salmonella* seropositivity of a pig was a risk factor for *Yersinia* seropositivity of the same pig, and vice versa, and we found multiple associations between *Salmonella* and *Yersinia* seropositivity of the pigs in the multivariable model (Table 15).

'*Yersinia* seroprevalence (%) at the farm at the beginning of fattening' was a protecting factor for *Yersinia* seroconversion of the pig during fattening (OR 0.98, P=0.02).

Interestingly, *Salmonella* seroprevalence at the farm at the beginning of fattening was not associated with a pig's seropositivity at the end of fattening (p=0.49). Meanwhile, whenever *Yersinia* seroprevalence at the farm at the beginning of fattening increased by a one per cent unit, the pressure of a pig

being *Yersinia* seropositive at the end of fattening increased by 3% (OR 1.03, p=0.005, Table 15).

Table 15 *Variables significantly (P<0.05) associated with Yersinia and Salmonella seropositivity in fattening pigs in Finland using a generalized linear mixed model with farm as a random effect.*

Dependent	Factor	Odds ratio	95% CI	p-value	n
Yersinia seropositivity of a pig (seropositive/seronegative) at the beginning of fattening	Simultaneous *Salmonella* seropositivity of the pig	6.37	2.59–15.69	<0.001	677
Yersinia seropositivity of a pig (seropositive/seronegative) at the end of fattening	Simultaneous *Salmonella* seropositivity of the pig	6.47	2.33–17.94	<0.001	656
	Yersinia seroprevalence (%) at the farm at the beginning of fattening	1.03	1.01–1.05	0.005	656
*S*eroconversion of a pig to *Yersinia* seropositive during fattening (yes/no)	Seroconversion of the pig to *Salmonella* seropositive during fattening	5.83	3.01–11.28	<0.001	653
	The *Yersinia* seroprevalence (%) at the farm at the beginning of fattening	0.98	0.97–1.00	0.02	653
Salmonella seropositivity of a pig (seropositive/seronegative) at the beginning of fattening	Simultaneous *Yersinia* seropositivity of the pig	5.10	2.94–8.85	<0.001	677
Salmonella seropositivity of a pig (seropositive/seronegative) at the end of fattening	Simultaneous *Yersinia* seropositivity of the pig	4.05	2.44–6.74	<0.001	1116
Seroconversion of a pig to *Salmonella* seropositive during fattening (yes/no)	Seroconversion of the pig to *Yersinia* seropositive during fattening	6.45	3.55–11.73	<0.001	653

5.2 THE FEASIBILITY OF THE SEROLOGICAL MONITORING AND THE USABILITY OF THE RESULTS AS PART OF THE FCI (I, III)

Commercial ELISA kits were easy to use. Collecting meat juice samples at slaughter was practical and effortless, and handling and sending the meat juice samples was simple. A sufficient quantity of meat juice was obtained from a 10-g piece of diaphragm muscle. Collecting the blood samples at the farm was resource consuming and more difficult to implement in practice, as it required on-farm visits and competence in taking blood samples from live animals.

Results from the blood samples taken at the farms are available for each batch before slaughter and can be included in FCI, while the meat juice sample results for a batch taken at slaughter are available only after slaughter, and the information can be included in the FCI of forthcoming batches.

We observed significant differences between individual farms concerning *Salmonella* and *Yersinia* within-farm seroprevalences in both studies (see 5.1.1 and 5.1.2). *Toxoplasma*-seropositive pigs were sporadic in study III, while farms with exceptionally high *Toxoplasma* seroprevalence were found in study I. Consequently, differences were seen between farms and could be taken into account if this information were part of the FCI.

Trichinella antibodies were not detected, so no differences occurred between farms, and including this information in the FCI would mainly be confirmation of the favourable situation (I).

5.3 THE USABILITY OF THE FCI FOR THE NEEDS OF VISUAL MEAT INSPECTION AND RISK-BASED MEAT INSPECTION (I, II, III)

5.3.1 DECLARATIONS IN CURRENT FCI, MEAT INSPECTION RESULTS, SEROLOGICAL RESULTS AND MORTALITY RATES (II)

None of the farms declared salmonellosis, trichinellosis or any restrictions imposed by the authorities in their FCIs, and erysipelas was noted by only three batches (Table 16). In total, 74% (63/85) of the FCI documents included at least one declaration regarding the current batch.

The meat inspection results corresponded well with the national meat inspection statistics for the same year (Table 16). Pleuritis rates were higher due to even the smallest lesions being reported in this slaughterhouse, though those may not lead to condemnations.

The samples used in study II were obtained from study I, and the serological results from study II corresponded well with the overall

serological results from study I (Table 16). The mortality rate of the batches during fattening had a mean of 1.7% (range 0–8.4%)

Table 16 *Data collected from 85 randomly selected slaughter batches of finishing pigs from a Finnish slaughterhouse. Published in Felin et al., 2016 (II), reprinted with permission of the copyright holder.*

A. Food chain information regarding the farm	% (number) of batches (n=85)	% of pigs in current batches (n=8954)	Of concern to
Salmonellosis present	0	0	P
Trichinellosis present	0	0	P
Erysipelas present	3.5 (3)	n/a	P
Official restrictions	0	0	A / P
Drug residues detected in animals	0	0	P

B. Food chain information regarding the batch			Of concern to
Includes pigs medicated within the last three months	52.9 (45)	n/a	A / P
Includes pigs with hernia	35.3 (30)	0.7	W / (P, faecal contamination)
Includes pigs with abscesses/lumps	21.2 (18)	0.4	W
Includes lame pigs	15.3 (13)	0.3	W
Includes pigs with bitten tails	44.7 (38)	1.4	W
Constant coughing three months prior to slaughter: somewhat / a lot	4.7 (4) / 0.0	n/a	A

C. Meat inspection results	Study data Mean % (variation interval) of batches (n=85)	Mean % (variation interval) of farm results[a] (n=80)	Official statistics[b] % of all fattening pigs slaughtered in Finland 2013	Of concern to
Total condemnation	0.2 (0.0–2.9)	0.3 (0.0–3.4)	0.3	A / P
Partial condemnation	5.0 (0.0–17.6)	5.6 (1.5–23.9)	6.4	A / W
Condemned organs	1.6 (0.0–19.5)	1.7 (0.2–11.7)[e]	n/a	A / W / P
Pneumonia	3.8 (0.0–29.2)	2.0 (0.0–11.7)	2.2	A
Pleuritis	35.0 (1.7–90.0)	17.8 (0.0–58.8)	15.9	A
Pericarditis	3.6 (0.0–32.9)	n/a	2.3	A
Condemned livers	9.1 (0.0–54.4)	6.0 (0.0–52.5)	6.3	A
Arthritis	1.7 (0.0–17.6)	2.4 (0.6–18.0)	3.0	W
Bitten tails	1.5 (0.0–8.3)	0.5 (0.0–1.7)[e]	1.0	W
Abscesses	4.1 (0.0–12.9)	4.5 (0.0–11.3)	3.2	A / W
Findings related to *Mycobacterium avium* complex	0.5 (0.0–6.8)	0.4 (0.0–4.7)	0.3	P

Table 16 (*Continued*)

D. Serological analysis results	Positive batches [c], % (n=85)	Positive pigs, % (n=431)	Of concern to
Salmonella spp.	14.1	3.5	P
Pathogenic *Yersinia* spp.	81.2	56.1	P
Trichinella spp.	0.0	0.0	P
Toxoplasma gondii	8.2	2.6	P
E. Other data	Mean of batches, % (variation interval)		Of concern to
Mortality rate of the batch during the fattening period	1.7 (0.0–8.4) [d]	n/a	A / W

Note: A = animal health; W = animal welfare; P = public health; adapted from EFSA, 2011.
n/a= not available
[a]farm result = result of all pigs slaughtered from the farm the previous year
[b]meat inspection statistics 2013 from Food Safety Authority Evira
[c]3–10 pigs per batch tested
[d]data from Sikava - Stakeholders health and welfare register for pig herds in Finland, available for 82/85 batches
[e]previous years meat inspection data considering organ condemnations and tail biting was available for 31/80 farms

5.3.2 ASSOCIATION BETWEEN CURRENT FCI REGARDING THE BATCH, MORTALITY DURING FATTENING AND MEAT INSPECTION RESULTS (II)

Slaughter batches were divided into two groups according to the FCI reports: a group with "nothing to declare" in their FCIs (n=22) and a group with something to declare in their FCIs (n=63). Batches with something to declare had lower condemnation rates for livers than batches with nothing to declare (6.6% and 16.0%, p<0.01). The organ condemnation rate was also lower for batches with something to declare, but the difference was not statistically significant (1.1% and 2.9%, p=0.13).

Only four batches declared constant coughing during the three months prior to slaughter, which lowers the generality of our results. However, these four batches had higher mean arthritis prevalence (6.4% and 1.5 %, p<0.01), and partial (8.8% and 4.8%, p=0.05) and total (0.8% and 0.2%, p=0.07) condemnation rates than the other batches. The difference between the partial and total condemnation rates was not statistically significant. In contrast, pneumonia and pleuritis rates observed at meat inspection did not differ in batches for which coughing was declared in the FCI.

Batches that, according to FCI, included pigs medicated within the last three months prior to slaughter had lower mean condemnation rates for livers (6.3% and 12.2%, p<0.01).

Batches declaring lameness had a statistically significantly higher mean prevalence of pleuritis (45.3% and 33.1%, p=0.04) at meat inspection. No other statistically significant differences were found.

The FCI declaration rates, i.e. the percentages of pigs in a batch declared to have a symptom/sign for one of the following: hernias, abscesses/lumps, and lameness, correlated with higher pleuritis rates at meat inspection (r=0.24, 0.26, 0.31 and p=0.03, p=0.02, p<0.01 respectively).

The FCI declaration rate for tail biting correlated with higher pneumonia rates (r=0.25, p=0.02) at meat inspection and the FCI declaration rate for lameness correlated with more observations of tail biting (r=0.26, p=0.02) at meat inspection. However, two exceptional batches occurred in the data: one batch with 29% pneumonia found at meat inspection and another batch with 7% lameness declared in FCI. When these batches were discarded from the analysis, these correlations disappeared.

The percentage of bitten tails declared in the FCIs correlated with observations of tail biting at meat inspection, but this correlation was not statistically significant (r=0.20, p=0.06, Figure 4). However, when the zero values were discarded, the correlation was clear (r=0.56, p<0.01) (Figure 4).
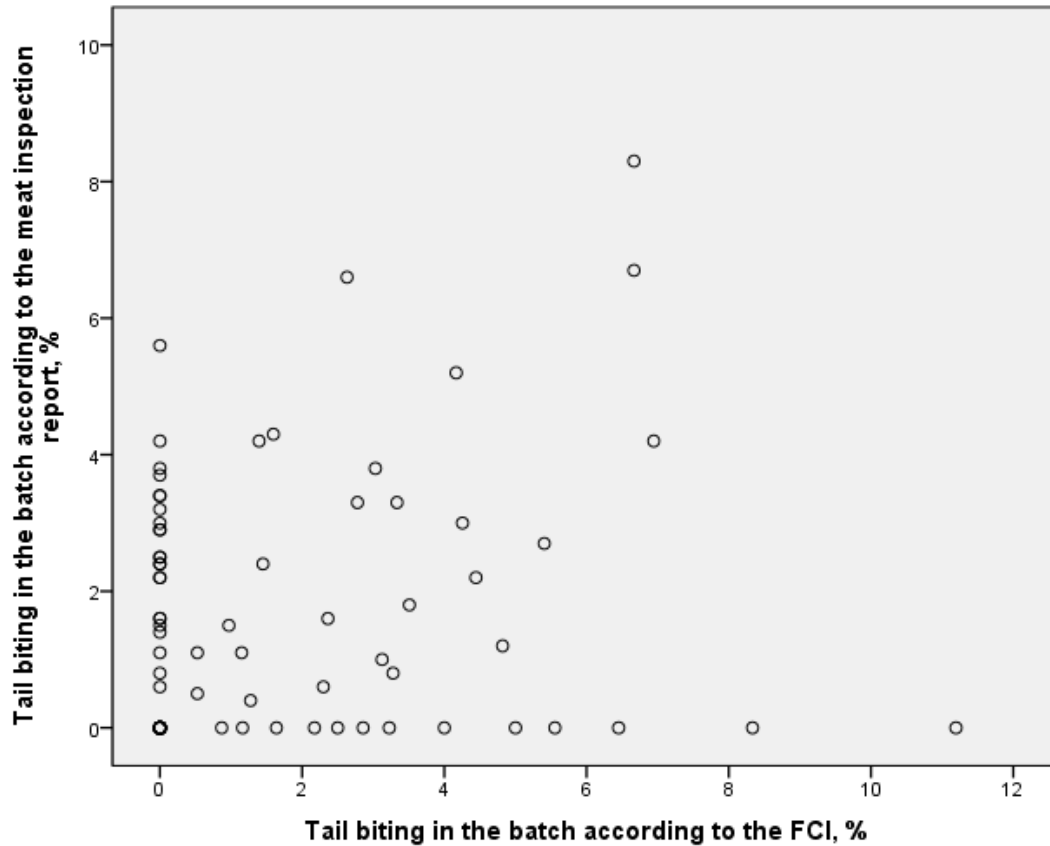
Figure 4 The correlation between food chain information and meat inspection reports of tail biting in finishing pigs from 85 slaughter batches.

We found clear positive correlations between FCI declaration rates for hernias, abscesses/lumps, abnormalities in gait and bitten tails (r=0.49-0.71, p<0.001).

We suspected that certain FCIs with "nothing to declare" were unreliable. Therefore, all the analyses were repeated so that only batches with something declared in the FCI (n=63) were included. No new associations were found.

The mortality rate during fattening did not correlate with any of the meat inspection results.

5.3.3 ASSOCIATION BETWEEN MEAT INSPECTION RESULTS OF CURRENT AND PREVIOUS BATCHES FROM THE SAME FARM (II)

Several meat inspection results of all the pigs sent for slaughter during the previous year and the meat inspection findings from the currently slaughtered batch correlated at the farm level (Table 17). We found no correlations solely for the total condemnation, pneumonia and arthritis rates of the currently slaughtered batch.

Table 17 *Statistically significant correlations (p<0.05) between meat inspection results for the currently slaughtered batch (n=85) and between meat inspection results for pigs sent from the same farm for slaughter the previous year. Published in Felin et al., 2016 (II), reprinted with permission of the copyright holder.*

Currently slaughtered batch	Slaughtered pigs from the farm during previous year	Pearson correlation coefficient	p-value	Number of batches concerned
partial condemnations %	partial condemnations %	0.32	<0.01	85
	abscesses %	0.37	<0.01	85
	tail biting %	0.42	0.02	31
organ condemnations %	organ condemnations %	0.42	0.02	31
	pleuritis %	-0.25	0.02	85
	pneumonia %	-0.23	0.03	85
abscesses %	abscesses %	0.41	<0.01	85
	tail biting %	0.51	<0.01	31
	partial condemnations%	0.31	<0.01	85
tail biting %	tail biting %	0.45	0.01	31
	abscesses %	0.35	<0.01	85
pleuritis %	pleuritis %	0.28	<0.01	85
pericarditis %	organ condemnations %	0.91	<0.01	31
	liver condemnations %	0.27	0.01	85
liver condemnation %	liver condemnations %	0.56	<0.01	85
	organ condemnations %	0.63	<0.01	31
Mycobacterium avium – complex %	*Mycobacterium avium –* complex, %	0.30	<0.01	85

5.3.4 RISK FACTORS FOR CONDEMNATIONS AT SLAUGHTER (II, III)

Linear regression analysis in study II

The partial condemnation rate of the previous year was revealed as the most important variable for predicting condemnations at slaughter and thus also whether the current batch would have been suitable for visual-only meat inspection.

The regression analyses revealed that the partial condemnation rate of the previous year (p<0.001, Figure 5) and a declared cough in the current FCI (p=0.02) were the best predictors for the partial condemnation rate of the batch. Total condemnations were so rare that the predictors were the same for the sum of the total and partial condemnation rates. When the untransformed partial condemnation rate was predicted by the two best predictors, the adjusted R² was 0.31, the regression coefficient of the partial condemnation rate of the last year was 0.93 and the coefficient of the

declared cough in the FCI was 4.5. This means that for each additional per cent in partial condemnation rate of the last year, the partial condemnation rate of the current batch increased by an average of 0.93 per cent units and that the predicted partial condemnation rate increased by 4.5 per cent units if coughing was reported in the slaughter batch.

The best predictor for the organ condemnation rate of the current batch was the farm's organ condemnation rate of the previous year (n=31, p=0.03). When the untransformed organ condemnation rate was predicted by the best predictor, the adjusted R^2 was 0.15, and the regression coefficient of the organ condemnation rate of the last year was 0.19.
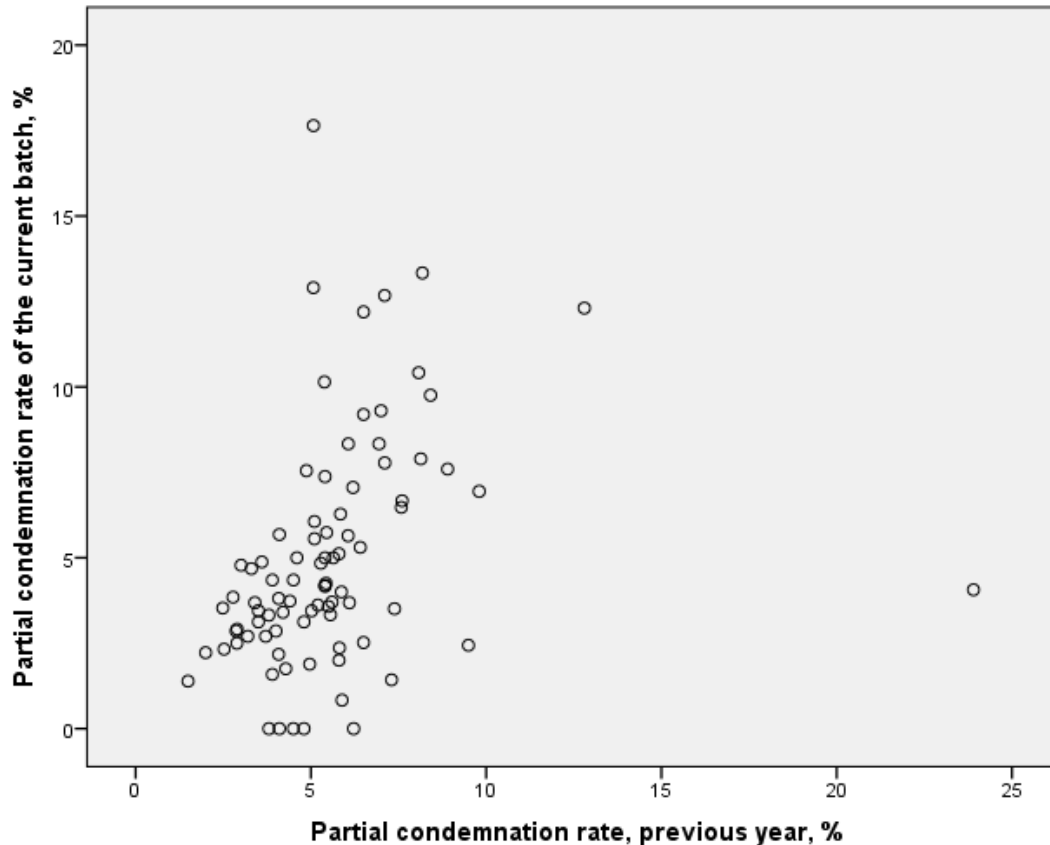
Figure 5 The partial condemnation rate of 85 slaughter batches of fattening pigs and the batches from the same farm during the previous year. Published in Felin et al., 2016 (II), reprinted with permission of the copyright holder.

Logistic regression analysis in study III

The healed tail-biting rate at the end of the fattening period and the fresh tail-biting rate at the beginning of fattening were significant risk factors for a partial carcass condemnation rate at slaughter (Table 18). No significant risk factors were observed for abscess rate, and only one for arthritis rate, namely the fresh tail-biting rate at the beginning of the fattening period (Table 18). The odds ratios detected were small, e.g. 1.009 for healed tail biting. However, this means that the odds of a pig carcass being partially condemned were 0.9% higher when the percentage of pigs affected with

healed tail biting at a farm increased by one per cent unit (Figure 6). For example, if a farm had a problem and the healed tail-biting index was 50, the probability of a pig carcass being partially condemned was 8.8% (OR 0.097; p(probability)=OR/(1+OR); logit=b_0+ b_1*x_1; OR=exp(logit)). *Salmonella* and *Yersinia* seroprevalences at the farm did not associate with any of the meat inspection findings.

Table 18 *Farm-level factors significantly (P<0.05) associated with farm-level condemnation rates in fattening pigs in Finland using a generalized linear mixed model with farm as a random effect. Published in Felin et al., 2018 (III), reprinted with permission of the copyright holder.*

Dependent	Factor	Odds ratio	95% CI	P-value	N of farms
Partial condemnation rate	Fresh tail biting A[a]	1.028	1.000–1.057	0.047	34
	Healed tail biting B[a]	1.009	1.002–1.016	0.008	57
Arthritis rate	Fresh tail biting A[a]	1.035	1.010–1.061	0.008	34

[a] A= at the beginning of fattening, B= at the end of fattening
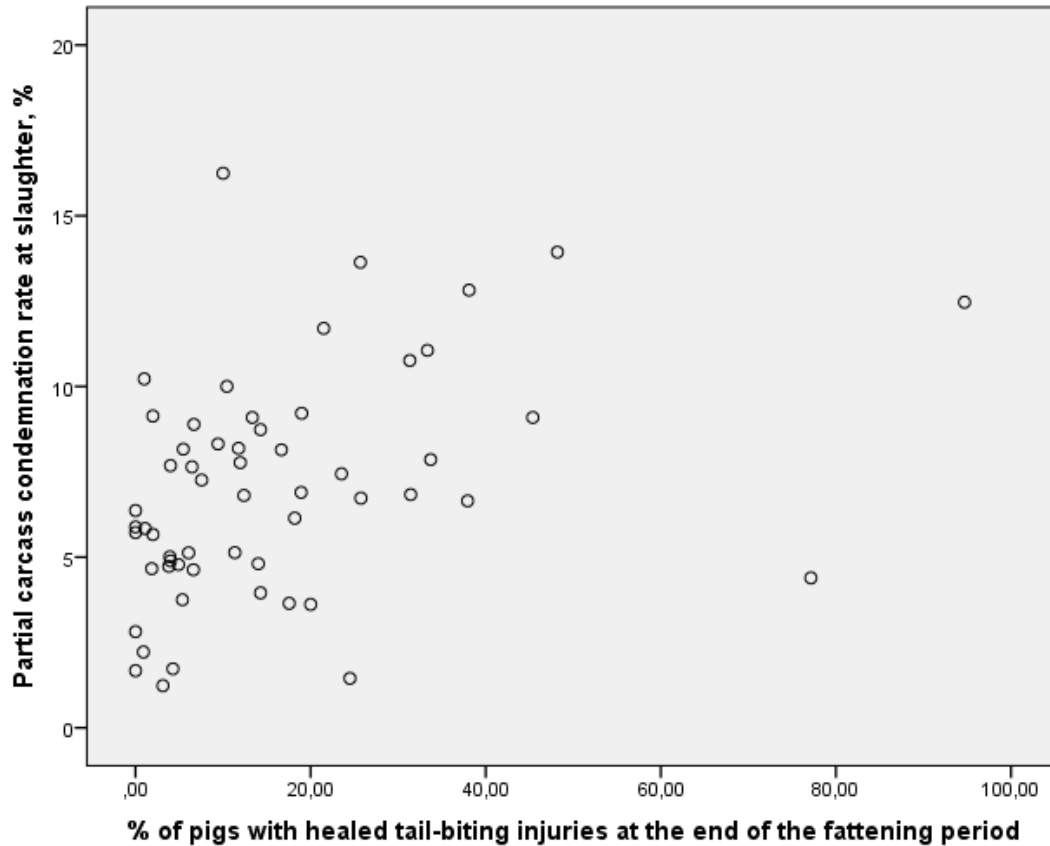
Figure 6 The association between the healed tail-biting injuries observed by a veterinarian at the farm and partial carcass condemnation rate during the meat inspection of fattening pigs from 57 farms in Finland. Published in Felin et al., 2018 (III), reprinted with permission of the copyright holder.

Scoring system to predict slaughter batches unsuitable for visual-only meat inspection (II)

A scoring system was developed to allocate slaughter batches into two categories in advance, namely: "suitable for visual-only meat inspection" with scores of 0 to one and "needs additional inspection procedures" with scores of two to four (Table 19). Scoring was done using the statistically significant risk factors for condemnations: the previous year´s partial condemnation rate (%) and the FCI regarding the current slaughter batch declaring the occurrence of constant coughing during the three months prior to slaughter. Threshold limits for scoring were calculated using the 10 and 90 deciles from the previous year´s condemnation rates: 0 points for below the 10 decile, one point for 10 to 90 decile, and two points above the 90 decile. The declaration for a constant coughing added another two points. Note that only four batches (4.7%) were declared to have had constant coughing within the three months prior to slaughter, which compromises the results (Table 19).

Table 19 *Scoring of 85 batches of slaughter pigs according to food chain information provided. Published in Felin et al., 2016 (II), reprinted with permission of the copyright holder.*

Scoring points	0	1	2	3	4
Previous year's partial condemnation rate	< 3%	3–8%	>8%	3–8%	>8 %
Declaration for constant cough	no	no	no	yes	yes
% of batches	9.4	75.3	10.6	4.7	0.0

Eight batches (9.4%) were given a score of zero points, which indicated that they would have been suitable for visual-only meat inspection (Table 19). Analysis of the meat inspection reports for these batches showed them to be suitable for visual-only meat inspection (partial condemnation rate 1.4–3.8%, organ condemnation rate 0.0–3.5 %).

A total of 64 (75.3%) batches were scored with one point (Table 19). An analysis of the meat inspection reports of these batches showed a majority to be suitable for visual meat inspection. However, five batches could not be considered suitable for visual meat inspection only because of a high rate of partial condemnations (9–13%), and these could not have been detected beforehand with the suggested scoring system. The FCI of these five batches only revealed that two batches had pigs that had been treated with veterinary medicinal products within the three months prior to slaughter. Their historical meat inspection data and mortality rates during fattening did not markedly differ from the mean. The available prior information was insufficient to discriminate these batches beforehand.

Thirteen batches (15.3%) were considered to need additional inspection procedures, as they had scores of two points or more (nine batches with last year's partial condemnation rate over 8% and four batches with FCI on constant coughing) (Table 19). However, seven of these batches had current partial condemnation rates of less than 9% and therefore could have been suitable for visual-only meat inspection. Nevertheless, the high condemnation rates for the previous year or coughing reported in the FCI for the current slaughter batches indicated that carrying out additional inspection procedures would have been appropriate.

We suggested that batches with two points or more (last year's partial condemnation rate over 8% or FCI declarations for constant coughing in the current slaughter batches) should be indications for additional meat inspection procedures. With this classification, the worst eighth of the batches were classified as unsuitable for visual-only meat inspection. Current batches with high partial condemnation rates (>9%) were classified as not being suitable for visual meat inspection only.

SP for identifying batches suitable for visual meat inspection was 91% (CI95: 82–95 %) (Table 20). The SE of the scoring system to identify the

batches unsuitable for visual meat inspection was 55% (95 % confidence interval: 28–79 %). The accuracy of identifying the batches unsuitable for visual meat inspection was 86% (CI95: 77–92 %).

Table 20 *Sensitivity and specificity of the scoring system for finding slaughter batches of pigs unsuitable for visual-only meat* inspection. *Published in Felin et al., 2016 (II), reprinted with permission of the copyright holder.*

	Previous year´s partial condemnation rate >8% or reported cough	Previous year´s partial condemnation rate≤ 8% no reported cough	
Batches unsuitable for visual-only meat inspection	6	5	11
Batches suitable for visual-only meat inspection	7	67	74
Total	13	72	85

Sensitivity 55% (95% confidence interval 28–79%) and specificity 91% (CI95: 82–95%)

5.3.5 ASSOCIATIONS BETWEEN SEROLOGY AND MEAT INSPECTION RESULTS (II, III)

No statistically significant correlations were found between any of the tested seroprevalences and current meat inspection findings at the slaughter batch level (II, III).

In study II, we found a positive correlation between the current seroprevalence of *Salmonella* and the historical prevalence of pneumonia (r=0.28, p<0.01) and pleuritis (r=0.24, p=0.02) in the previous year's meat inspection reports.

5.4 COMPARISON OF COMMERCIALLY AVAILABLE ELISA KITS FOR DETECTING *TOXOPLASMA* ANTIBODIES IN THE MEAT JUICE OF NATURALLY INFECTED FATTENING PIGS (IV)

The MAT detected 31% and the ELISA tests detected 1–41% of the 90 selected meat juice samples as *Toxoplasma* antibody-positive with the cut-offs recommended by the manufacturers (Table 21). The highest number of positive samples (47.8% and 41.1%) was obtained by test I, which had the lowest cut-off levels 0.15 (old) and 0.20 (new), respectively. Test III had the highest cut-off level (0.50), and a clearly lower number of positive samples was obtained compared to tests I and II. The number of positive samples was extremely low using test IV, which also gave very low OD values varying between 0.04 and 0.45. However, the OD values were high for the positive

controls (1.48–1.73) for test IV. The samples were tested a second time with another lot of test IV, but the OD values were not higher than in the first run.

Table 21 *Prevalence of Toxoplasma-positive samples using different commercial ELISA tests with various cut-off values for 90 pig meat juice samples. Published in Felin et al., 2017 (IV), reprinted with permission of the copyright holder.*

Test	S/P ratio[a]				Cut-off value	No. of positives	Prevalence (%)
	Min	Max	Mean	Median			
I	0.02	1.51	0.33	0.14	0.15[b]	43	47.8
					0.20[c]	37	41.1
					0.30	30	33.3
					0.40	26	28.9
					0.50	23	25.6
II	0.00	2.27	0.33	0.04	0.20	27	30.0
					0.30[c]	25	27.8
					0.40	22	24.4
					0.50	20	22.2
III	0.04	1.77	0.36	0.14	0.20	40	44.4
					0.30	28	31.1
					0.40[d]	24	26.7
					0.50[c]	22	24.4
IV	0.04[e]	0.45	0.06	0.04	0.07[f]	12	13.3
					0.20	3	3.3
					0.30[c]	1	1.1
					0.40	1	1.1
					0.50	0	0

[a] S/P ratio = (sample optical density (OD) – mean OD of the negative control) / (mean OD of the positive control – mean OD of the negative control)
[b] Old cut-off value used by Felin et al. (2015)
[c] Cut-off value recommended by the manufacturer
[d] S/P values between 40 and 50 are considered weakly positive
[e] OD value
[f] According to Hill et al. (2006)

The SE, SP, accuracy and Kappa value of the ELISA tests were determined using MAT as a reference test (Table 22). The SEs of tests I (cut-off=0.20), II (cut-off=0.30) and III (cut-off=0.50) were 96.4, 89.3 and 78.6, respectively, and the SPs were 83.9, 100.0 and 100.0, respectively. SE was unacceptably low (3.6) for test IV (cut-off=0.30).

Table 22 *The sensitivity (SE), specificity (SP), accuracy, positive predictive values (PPV), negative predictive values (NPV) and Kappa (K) values of four commercial ELISA tests using a commercial modified agglutination test as the reference test. Published in Felin et al., 2017 (IV), reprinted with permission of the copyright holder.*

ELISA test	Cut-off Value	SE (%)	SP (%)	Accuracy (%)	PPV High P^d (%)	NPV High P^d (%)	PPV Low P^e (%)	NPV Low P^e (%)	K value
I	0.15[a]	96.4	74.2	81.1	62.8	97.9	7.1	99.9	0.62
	0.20[b]	96.4	83.9	87.8	73.0	98.1	10.9	99.9	0.74
	0.30	92.9	93.5	93.3	86.7	96.7	22.6	99.8	0.85
	0.40	85.7	96.8	93.3	92.3	93.8	35.3	99.7	0.84
	0.50	78.6	98.4	92.2	95.7	91.0	50.1	99.6	0.81
II	0.20	92.9	98.4	96.7	96.3	96.8	54.2	99.9	0.92
	0.30[b]	89.3	100.0	96.7	100.0	95.4	100.0	99.8	0.92
	0.40	78.6	100.0	93.3	100.0	91.2	100.0	99.6	0.84
	0.50	71.4	100.0	91.1	100.0	88.6	100.0	99.4	0.78
III	0.20	96.4	79.0	84.4	67.5	98.0	8.6	99.9	0.68
	0.30	92.9	96.8	95.6	92.9	96.8	37.2	99.9	0.90
	0.40[c]	85.7	100.0	95.6	100.0	93.9	100.0	99.7	0.89
	0.50[b]	78.6	100.0	93.3	100.0	91.2	100.0	99.6	0.83
IV	0.07	42.9	100.0	82.2	100.0	79.5	100.0	98.8	0.51
	0.20	10.7	100.0	72.2	100.0	71.3	100.0	98.2	0.14
	0.30[b]	3.6	100.0	70.0	100.0	69.7	100.0	98.1	0.05
	0.40	3.6	100.0	70.0	100.0	69.7	100.0	98.1	0.05
	0.50	0.0	100.0	68.9	-	68.9	-	98.0	0.00

For equations see: TDR diagnostics evaluation expert panel, 2010
[a] Old cut-off value recommended by the manufacturer
[b] Cut-off value recommended by the manufacturer
[c] S/P ratios between 40 and 50 are considered weakly positive
[d] PPV and NPV values calculated for a high prevalence population 31% seropositive rate
[e] PPV and NPV values calculated for a low prevalence population 2% seropositive rate

Accuracy was high for tests II and III: 96.7% and 93.3%, respectively. Tests II and III showed very good agreement (K=0.92 and 0.84, respectively) with the reference test (MAT) (Table 22). Tests I, II and III all had the highest accuracy and the best agreement with the MAT when a cut-off of 0.30 was used for the tests (Figure 7). A very strong positive correlation (Pearson correlation>0.89) was observed between the S/P values of the three ELISA tests. The highest positive correlation (0.96) was between tests II and III. Majority of the samples were tested twice with the same test, and the repeatability was shown to be good with all three tests: intra-class correlation was 0.92 (n=90), 0.81 (n=63) and 0.89 (n=61) for tests I, II and III, respectively.
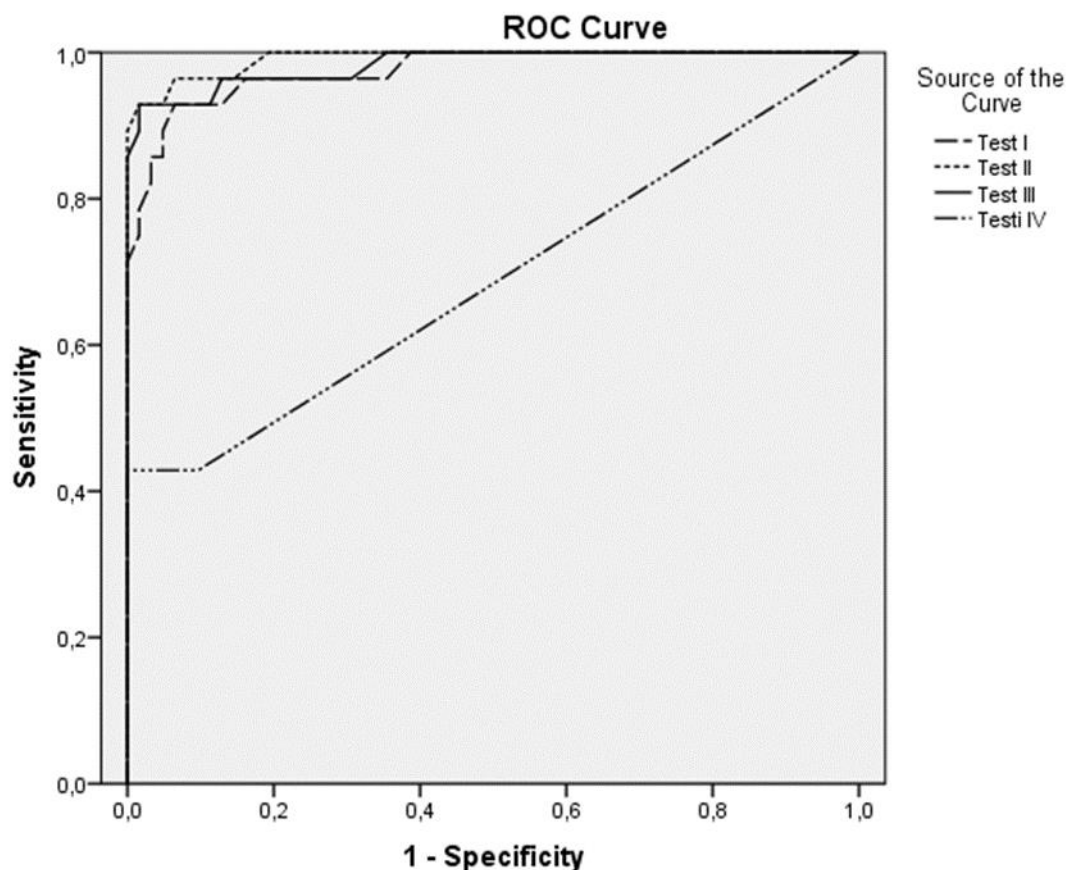
Figure 7 The ROC Curve of four commercial ELISA tests' S/P ratios using a commercial modified agglutination test as the reference test. Published in Felin et al., 2017 (IV), reprinted with permission of the copyright holder.

The 90 samples were selected from the study I and included all 43 positive samples with S/P ratios ≥0.15. The positive samples were from slaughter pigs originating from large fattening farms (nine pigs), small fattening farms (26 pigs) and from farrow-to-finish farms (eight pigs). The number of positive samples decreased from 43 to 37 when using the new cut-off of 0.20 instead of 0.15 used in test I. This mostly affected the large fattening farms: the number of positive pigs decreased from nine to four. This did not affect the number of positive pigs from farrow-to-finish farms, because most of the positive pigs originating from these farms had high (0.30–0.50) or very high (>0.50) S/P values. The new cut-off of 0.2 for test I lowered the apparent seroprevalence in large fattening farms observed in our study I, when five previously positive samples were re-rated as negative. These five samples were also negative when tested with other tests used in our present study. However, three out of these five samples had slightly raised S/P values also with tests II and III.

6 DISCUSSION

6.1 CONTROLLING PORK-RELATED PUBLIC HEALTH HAZARDS DURING MEAT INSPECTION

6.1.1 OCCURENCE OF THE MAIN HAZARDS IN FATTENING PIGS IN FINLAND

Salmonella spp., *Y. enterocolitica*, *T. gondii* and *Trichinella* spp. are the main pork-related biological hazards within the EU (EFSA, 2011). The seroprevalences of *Salmonella* spp. and *T. gondii* were low in Finland. However, we still appear to have some small farms with high *T. gondii* occurrence in pigs. *Trichinella* antibodies were not detected. The seroprevalence of pathogenic *Yersinia* spp. was the highest of the studied pathogens.

Salmonella spp.

The seroprevalences of *Salmonella* spp. were lower than results previously reported from other European countries (Lo Fo Wong et al., 2003; Hautekiet et al., 2008; Smith et al., 2011; Alban et al., 2012; Wacheck et al., 2012; Meemken et al., 2014). This reflects the favourable *Salmonella* situation of pig farms in Finland, Sweden and Norway (EFSA and ECDC, 2018) and is consistent with results from the Finnish National *Salmonella* Control Programme.

Salmonella seroprevalence in study III rose significantly during fattening. However, *Salmonella* seroprevalence at the farms at the beginning of fattening was not associated with pig seropositivity at the end of fattening. This may indicate that *Salmonella* infections were most often acquired during fattening. During a recent risk analysis, the Finnish Food Safety Authority Evira assessed that 34% of *Salmonella* infections in fattening pigs in Finland are attributable to feed (Finnish Food Safety Authority Evira, 2018).

The seroprevalence of *Salmonella* spp. was higher in study III (17% at the end of fattening) than in study I (3% at slaughter). Samples were taken approximately during the same time period (2012 and 2013 study I, 2012–2014 study III). The ELISA kits used were practically the same, although product name and manufacturer changed between studies. Cut-off values recommended by the new manufacturer were slightly different, but because the calculation formula of the OD value was changed simultaneously, the final results were influenced only minimally. The sample matrix was the largest difference influencing the results between studies: meat juice samples

in study I and serum samples in study III. Serum samples are shown to result in higher OD values than meat juice samples, despite the different dilution factor (Vico and Mainar-Jaime, 2011) and this is likely to also explain our results. Considering the sample matrix and cut-off value is important whenever comparing serological results between studies or countries.

However, *Salmonella* seroprevalence, especially in study III, was higher than expected considering the results from the Finnish *Salmonella* Control Programme, where the prevalence of *Salmonella* culture-positive lymph node samples at slaughter has been <0.1% and no *Salmonella* has been found in carcass swabs or pork during the 2010s (Anonymous, 2015; https://www.evira.fi/globalassets/elaimet/zoonoosikeskus/zoonoosit/taudit /salmonella/salmovalvontaohj_siat2016paivheinakuu2017.pdf, visited July 8, 2018). However, OD values of positive samples were considerably low. Low-dose infections during fattening with feed- or environment-associated *Salmonella* serotypes may explain the considerably high seroprevalence associated with low OD values in positive pigs, while the findings in lymphatic tissues at slaughter are rare (van Winsen et al., 2001; Österberg and Wallgren, 2008). In addition, the SP of the test in natural infections may be far less than 100% (Vico et al., 2010), especially when using low cut-off values, in which case the estimated true prevalence decreases close to 0% (Reiczigel et al., 2010). However, in our studies, 0.1–1.9% of the pigs were seropositive with a cut-off of OD40%. The estimated true seroprevalence does not differ greatly from this apparent seroprevalence, as the test is expected to be highly specific but less sensitive, with a cut-off of OD40%.

Recent studies by Casanova-Higes et al. (2017) and Mainar-Jaime et al. (2018) found a significant relationship between on-farm *Salmonella* serology and *Salmonella* shedding at slaughter. However, if we wish to assess the risk of shedding at slaughter, reflecting the food safety aspect, a cut-off of OD40% would be more suitable (Casanova-Higes et al., 2017; Mainar-Jaime et al., 2018). Our cut-off corresponds to OD15%, which likely sensitively reflects the *Salmonella* exposure during fattening, but not the shedding at slaughter (Methner et al., 2011).

Our results indicate some level of exposure to *Salmonella* spp. in pigs during fattening, which we cannot find in the lymph nodes in the current Finnish *Salmonella* Control Programme. This is not a food safety issue as such, because the vast majority of these pigs most probably do not shed the pathogen at slaughter (only 0.1–1.9% had OD%>40).

Pathogenic *Yersinia* spp.

The high seroprevalence of pathogenic *Yersinia* spp. (57–66%) was expected, based on a previous study in Finland, in which the isolation rate of pathogenic *Y. enterocolitica* in slaughter pig tonsils was 52% (Korte et al., 2004). The occurrence of pathogenic *Yersinia* spp. in fattening pigs appears

to be at the same level in Finland as in the other EU countries (von Altrock et al., 2011; Van Damme et al., 2014; Meemken et al., 2014).

Yersinia-seropositive pigs were detected more often at large fattening farms than at farrow-to-finish farms. This is in accordance with a previous study by Skjerve at al. (1998) and may result from the limited traffic of live animals to farrow-to-finish farms, which seldom buy piglets from other farms. Piglets transport *Y. enterocolitica* to the fattening unit, where it spreads effectively during fattening (Virtanen et al., 2012). This may also explain our results in study III: *Yersinia* seroprevalence rose significantly during the fattening period and higher within-farm *Yersinia* seroprevalence at the beginning of fattening associated with more seropositive pigs at the end.

However, all tested pigs were *Yersinia* seronegative at the end of fattening at seven (12%) farms in study III and 39 (15%) farms in study I. Two of these *Yersinia*-negative farms were included in both studies (I and III) and consequently these two farms tested negative during each of the three samplings over a 1.5-year period. Despite the high seroprevalence of pathogenic *Yersinia* spp. in the pork production chain, certain farms have recurrently low *Yersinia* prevalence.

T. gondii

The seroprevalence of *T. gondii* was low (3.2%) in study I and very low (0.7%) in study III. Differences between results are presumably due to the different ELISA test kits we utilized and to our farm selection. The test used in study I was more sensitive in our comparison than the test used in study III.

Study III included farms that slaughter at least 1000 pigs per year. The seropositive pigs were sporadic and the overall seroprevalence remained low (<1%) during fattening, despite certain changes in individual pig seropositivity. Considering the test does not have 100% specificity, the estimated true prevalence in study III was close to 0% (Reiczigel et al., 2010). These results are in accordance with previous results from Latvia and the Netherlands, where the seroprevalences in intensively farmed pigs were 0.4% (van der Giessen et al., 2007; Deksne and Kirjusina, 2013).

Study I also included smaller fattening farms, and the total seroprevalence was 3.2%, which is similar to a previous Finnish study conducted over 30 years ago (1984) and a Swedish study conducted in 1999 (Hirvelä-Koski, 1992; Lunden et al., 2002). Most (60%) of the seropositive pigs in study I originated from small fattening farms and, on three small-scale fattening farms, all samples tested were *Toxoplasma* seropositive. Other studies have also shown that pigs from smaller herds have a higher risk for *Toxoplasma* seropositivity (Villari et al., 2009; Limon et al., 2017).

The seroprevalences of fattening pigs in other European countries have varied between 0% to 45%, and the highest seroprevalences are detected in

pigs with outdoor access (Kijlstra et al., 2004; Klun et al., 2006; van der Giessen et al., 2007; Dubey, 2009; Villari et al., 2009; Deksne and Kirjusina, 2013; Wallander et al., 2016; Limon et al., 2017). All pigs sampled in study III were raised indoors. Information on access to the outdoors was not available for all the pigs in study I, but fattening pigs in Finland are raised nearly exclusively indoors (Finnish Food Safety Authority Evira, 2011).

Results show that the prevalence of *T. gondii* in fattening pigs in Finland is generally negligible, however; certain small farms had very high prevalences, and biosecurity measures require improvements in these locations.

Trichinella spp.

Antibodies were not found for *Trichinella* spp.. This was expected, based on results from the digestion analyses performed at meat inspection. Since 2004, over 25 million pigs have been tested using the digestion method, and only one positive pig was detected in 2010 (Finnish Food Safety Authority Evira, https://www.evira.fi/elaimet/zoonoosikeskus/zoonoosit/loisten-aiheuttamat-taudit/trikinelloosi/, accessed in 8 July, 2018).

Comorbidity with *Salmonella* spp. and *Yersinia* spp.

We observed a clear positive association between an individual pig`s seropositivity for *Salmonella* spp. and *Yersinia* spp.. This is in contrast with findings from other countries (von Altrock et al., 2011; Nathues et al., 2013; Powell et al., 2016). However, Nathues et al. (2013) and Powell et al. (2016) used a bacterial culturing method, which detects only present infections, while serological methods reflect the past exposure of an animal to a pathogen, which may partly explain the different results. In addition, none of the other studies analysed the association of seropositivity at the individual pig level. Vico et al. (2010) speculated the possible cross-reactivity of the *Salmonella* ELISA with e.g. *Yersinia enterocolitica* antibodies because of the use of polyclonal peroxidase conjugate, but the test we used has the anti-IgG conjugate. The antigens used in the tests are specific, and no cross-reactivity is suspected between *Yersinia* spp. and *Salmonella* spp. (Nielsen et al., 1995). The difference between the results in Finland and other countries may be due to the low prevalence of *Salmonella* spp. infections in Finnish pigs, which would affect the epidemiology. This finding indicates that these two pathogens have certain similar infection routes (e.g. rodents and wild birds) in fattening pigs in Finland.

6.1.2 SEROLOGICAL MONITORING AND IMPROVED FOOD CHAIN INFORMATION AS RISK-BASED CONTROL TOOLS

The seroprevalences at the end of fattening and at slaughter were not associated with the *post-mortem* findings of the current batch, which was expected. This means that current post-mortem inspection cannot find these pathogens and we thus need new tools to control these public health hazards in pork.

Practical aspects of conducting serological monitoring

EFSA (2011) concluded that improved FCI should be used to risk-differentiate slaughter batches in relation to the most relevant biological hazards (*Salmonella* spp., *Y. enterocolitica*, *T. gondii*, *Trichinella* spp.). This could be done by sampling pigs at farms or at slaughterhouses and analysing the within-farm seroprevalences of these pathogens and including these data to the FCI. In our study, this was tested in practice by collecting blood and meat juice samples from fattening pigs at farms and at slaughterhouses, respectively.

Meat juice samples were more feasible than blood samples as a sample matrix. Collecting the meat samples at slaughter was easy and handling the samples was simple. In addition, we experienced no problems with haemolysis, as with the serum samples. However, meat juice is not a homogenous serological matrix and diaphragmatic meat juice samples give lower OD values than blood despite the different dilution factor (Vico and Mainar-Jaime, 2011; Wallander, Frossling, Vagsholm, Burrells et al., 2015). Considering the sample matrix when adjusting the cut-off values is therefore important.

Sampling at the slaughterhouses was far more feasible than sampling at the farms. Mainar-Jaime et al. (2018) have suggested that sampling should be performed on-farm for each batch to determine beforehand the risk of *Salmonella* spp. shedding at slaughter, because of the variation between batches originating from the same farm. However, on-farm sampling would be very difficult to conduct in practice. Thus, meat juice sampling at slaughter would be the method of choice in large control programmes and it is already in use for *Salmonella* control in Germany and Denmark (Alban et al., 2012; QS Qualität und Sicherheit GmbH, 2018). Sampling at slaughter indicates the biosecurity level of the farm rather than the risk of a certain batch, and this approach would also fit in the Finnish context.

Serological monitoring of *Salmonella* spp.

Results from the Finnish *Salmonella* Control Programme have been successful (Maijala et al., 2005; Finnish Food Safety Authority Evira, 2018). However, individual farms show differences in *Salmonella* seroprevalences

and a certain level of *Salmonella* exposure, which we cannot find within the current control programme. The current Finnish *Salmonella* Control Programme operates as an eradication strategy (Finnish Food Safety Authority Evira, 2018). Because the cost of *Salmonella* eradication is very high on pig farms, the eradication decision cannot be based only on highly sensitive serological monitoring. However, serological monitoring would provide us with large-scale farm-level data. With serological testing, we could monitor farm-level *Salmonella* risks and react promptly by improving preventive measures at the farms.

In Finland, serological *Salmonella* sampling would have only a limited positive impact on food safety, because the current situation is already excellent. However, we would be able to follow farm-level trends, detect changes readily, target microbiological sampling at slaughterhouses and locate infected farms more quickly, which may ease eradication, or possibly even improve biosecurity measures before expensive eradications are needed.

When allocating farms to risk categories, the targets of the programme and corrective actions must be considered. The German and Danish serological sampling programmes are part of their reduction strategies, while Finland is applying an eradication policy. Consequently, the German and Danish categorizations are not directly applicable in the Finnish context. In Finland, we could use a modified allocation of farms using the same cut-off OD20% as Denmark, but modified corrective actions and modified limits of within-farm seroprevalences (5.1.1). For example, if the within-farm seroprevalence were 20–40% (Category 2), the farmer would be recommended to self-check the biosecurity measures using a specific checklist. If meat juice samples were used, approximately 10% of the farms would fall within this category in the current Finnish situation. A within-farm seroprevalence exceeding 40% (Category 3) would indicate an elevated food safety risk, which would result in bacteriological sampling and a biosecurity check at the farm in question. Approximately 2% of farms would fall into this Category 3 in the current Finnish situation. In the Finnish context, subsequent procedures for eradicating the pathogen from a farm follow whenever *Salmonella* spp. is isolated from animals at the farm. This modified categorization system is only an example, and it would need to be adjusted and optimized after additional data collection. This programme could also have a positive effect considering *Yersinia* spp., as the two appear to coexist in the Finnish epidemiological situation.

Serological monitoring of *Yersinia* spp.

We observed huge differences between farms considering within-farm *Yersinia* seroprevalence. By serological monitoring at slaughter, farms with low *Yersinia* prevalence could be found and supported to maintain the situation. However, this would also require the possibility of buying piglets

from *Yersinia*-negative farms (Skjerve et al., 1998; Virtanen et al., 2012; Vilar et al., 2013; Laukkanen-Ninios et al., 2014). Currently, *Y. enterocolitica* prevalence is high in fattening pigs (especially in the tonsils) at slaughter and good slaughter hygiene is therefore a major control measure to reducing carcass contamination. However, serosurveillance could be the first step in overcoming the huge challenge of *Y. enterocolitica* in pigs. Surveillance would increase our understanding of the occurrence and trends of *Y. enterocolitica* at farms, even if it would not incorporate farm-level control measures at first. Serological results in FCI would provide slaughterhouses with the possibility of risk-ranking farms according to the *Yersinia* spp. shedding risk or risk of carrying it in their tonsils. Pigs from high-risk farms could then be slaughtered at the end of the day, thus improving food safety.

Serological monitoring of *T. gondii*

The true prevalence was negligible in indoor fattening farms slaughtering at least 1000 pigs per year and no extensive serological monitoring for *T. gondii* is thus required at slaughterhouses. However, despite the seroprevalence of *T. gondii* being low in general, we found certain farms with 100% seroprevalence. Therefore, serological monitoring could be beneficial if targeted risk-based small fattening farms and outdoor farms. Serological monitoring would enable detecting these farms where improvement in biosecurity measures are needed. These farms should be visited and advised to improve their biosecurity measures. Carcasses from high-risk farms could be frozen or heated until the situation is improved.

Serological monitoring of *Trichinella* spp.

We found no seropositive pigs, so we could not risk-rank farms according to their *Trichinella* risk using serological monitoring. Current regulation (European Commission, 2015) requires 10% of the pigs raised in a controlled housing system to be tested yearly using digestive methods. However, if the risk is negligible, as it is in Finland (prerequisites defined by the European Commission, 2015), a Member State can apply for a derogation and subsequently exempt pigs raised in a controlled housing system from the *Trichinella* testing. If *Trichinella* testing is to be lessened, as the legislation (European Commission, 2015) enables, then serology could be a feasible way for surveying the situation and the effectivity of the controlled housing conditions (EFSA, 2011). Serology is more sensitive to detecting light infections than the current digestive method (Gamble et al., 1983). Surveillance is important, as the parasite is abundant in wildlife in Finland, although *T. spiralis* prevalence has been significantly reduced in recent years (Airas et al., 2010; The Zoonosis Centre team, 2012; Oksanen et al., 2018).

Serological monitoring as part of the risk-based meat inspection of pigs

As part of the comprehensive pork carcass safety assurance system, serological monitoring could be used to allocate pig farms into risk categories, for which targeted control measures could be applied. By continuously collecting farm-level serological data we could create serological farm profiles, which could be used as part of the FCI reflecting the food-safety risk of certain batches. Risk-based decisions include additional carcass processing, separate slaughter, targeted sampling at the slaughterhouse and improved biosecurity measures at the farm. However, risk mitigation targets and procedures must be carefully adjusted for each pathogen, as described earlier in this chapter.

A cost-benefit analysis was beyond the framework of this study. It is obvious that the suggested serological monitoring would raise the costs of official controls, if it were conducted additionally to current controls. However, serological analyses are relatively cheap, and all these pathogens can be analysed from the same meat juice sample. The cost of serological sampling can be estimated at 5€/sample. If we were to sample all 1000 farms raising fattening pigs in Finland with 60 samples/farm/year, the total annual cost would be 300 000 € for one pathogen. The additional cost of analysing more pathogens from the same sample would be less, because the sampling and preparation of the sample has already been done. Considering *Salmonella* spp., with this investment we would acquire a huge data (60 000 samples annually) compared to the current random 3000 lymph node samples from fattening pigs. The analysis of current lymph node samples can be estimated to cost 75 000 € annually (3000 samples x 25 €/sample). The serological *Salmonella* results could be used to target lymph node sampling or even possibly replace it.

Serological monitoring for *Salmonella* is already in use in Germany and Denmark, and at least one large meat company in the Netherlands voluntarily surveys *T. gondii* serology. We should shift towards this system, if we wish official controls to be risk-based and effective at improving food safety. Probably, considering the challenges of the industry, the change to multi-serology would require the simultaneous change of the entire meat inspection process and subsequent reallocation of the resources, which would also require changes to current EU legislation (see more in section 6.2).

6.1.3 DIFFERENCES BETWEEN COMMERCIAL ELISA TESTS FOR THE DETECTION OF *T. GONDII* ANTIBODIES

We compared four commercial ELISA tests for the detection of *T. gondii* antibodies in pigs at slaughter with each other and with a MAT as a reference. Comparison is challenging, as no reference method with 100% SE and SP is available. The MAT was used as a reference test, in the same

manner as in other studies (Forbes et al., 2012; Steinparzer et al., 2015; Wallander, Frossling, Vagsholm, Uggla et al., 2015). It has been shown to work even for meat juice samples with low antibody levels (Forbes et al., 2012). However, it must be noted that the MAT is not a "gold reference test", which influences the result. To overcome this, we also compared the ELISA tests with each other.

The cut-off values provided by the manufacturers varied, and this appeared to most significantly influence the results with tests I–III. We found a very strong correlation between the S/P values of these three ELISA tests, and observed the highest accuracy and best agreements with the MAT when the cut-off value 0.30 was used for each of the tests.

One of the tests (test IV) had poor performance. The antibody levels in the diaphragm muscle tissue of naturally infected pigs appear to be too low for proper detection by this test. Forbes et al. (2012) have previously shown that this test can be used to test meat juice, but the study was performed on experimentally infected pigs. Gamble et al. (2005) and Hill et al. (2006) also reported that the sensitivity of this test was clearly lower for meat juice samples than for serum samples of naturally infected pigs.

Results show that three of the ELISA tests performed comparably when cut-off values were adjusted, while one of the tests could not detect antibodies properly. To ensure proper sensitivity, the validation protocols should include meat juice samples from experimentally infected animals with different infection doses and samples from naturally infected pigs.

6.2 FOOD CHAIN INFORMATION IN VISUAL-ONLY MEAT INSPECTION

FCI should be the link between the farm and the slaughterhouse in providing information related to food safety, animal health and animal welfare. The main purpose of the FCI is that the pig producer declares that no restrictions exist for normal slaughter considering these issues.

In addition to food safety and animal health issues, meat is also declared unfit for human consumption if it indicates patho-physiological changes or anomalies in consistency or in organoleptic quality (European Parliament and Council, 2004). As the official EU *post-mortem* inspection of pigs has shifted to visual-only meat inspection, palpation and incision procedures should be carried out only for abnormal or suspect carcasses and offal. Pig batches with high frequencies of lesions are unsuitable for visual-only meat inspection and should be slaughtered separately, as they need a slower line speed and adequate human resources at the trimming line. FCI could be a tool for food business operators and OVs to recognize beforehand the slaughter batches with high frequencies of lesions, which need additional inspections and are not suitable for visual-only meat inspection.

Our study showed that FCI could be used to assess the likelihood of carcass condemnations in meat inspection. The partial carcass condemnation rate of the current batch was best predicted by the partial carcass condemnation rate of the pigs from the same farm within one year. However, historical meat inspection data cannot predict unexpected variation between batches (Harbers et al., 1991). We found that properly reported health indicators in FCI could be used to partly overcome this problem. Tail biting and coughing were shown to be the best on-farm health indicators predicting carcass condemnations. A scoring system could easily be established ranking batches using the historical (one-year average) partial carcass condemnation rate, and tail biting and coughing as additional indicators. Farmers should additionally be advised to separately report in the FCI if they have any doubts that the current batch could result in more condemnations than normally.

In study II, we analysed the authentic FCIs provided by farmers to the slaughterhouses and compared them to the meat inspection findings. Reports were analysed anonymously and farmers did not know that their FCIs were to be included in the study. Our results suggest that FCIs were not always accurate. This is in accordance with a questionnaire study by Luukkanen et al. (2015), where Finnish OVs reported serious problems in receiving accurate FCIs. We found that batches with "nothing to declare" had poorer meat inspection results than batches that did declare. Moreover, the FCI reports and meat inspection records on tail biting did not correlate, and a high level of false reporting appears to have occurred. In study II, reports of constant coughing during the last three months prior to slaughter was the only factor in the FCI that predicted higher partial carcass condemnation and arthritis rates. However, only four batches were declared to have had constant coughing, so the result may not be generalizable.

In study III, we wished to examine associations between on-farm health data and condemnations at slaughter using accurate assessments by veterinarians, to overcome the problem of inaccurate reporting in study II. The main shortage in study III was that on-farm health status was assessed for only approximately 100 pigs and the meat inspection findings represented the entire batch. Despite this, we found that the fresh tail-biting rate at the beginning and the healed tail-biting rate at the end of fattening observed by a veterinarian predicted partial carcass condemnations at slaughter. Tail-biting victims have previously been shown to express an increased incidence of abscesses and arthritis at slaughter, which leads to carcass condemnations (Valros et al., 2004; Marques et al., 2012). These results together show that a correctly reported tail-biting rate in FCI could be used in addition to the information on previous meat inspection results at slaughterhouses to predict the condemnations of incoming batches. The coughing index in study III was not associated with partial carcass condemnations. However, it was defined differently than coughing in study II. Declarations of constant coughing during the last three months should

result in additional inspections, as it is nonconforming and appears to predict a higher frequency of meat inspection findings.

In a non-peer-reviewed questionnaire, farmers reported inaccuracies in FCI due to the time-lag between sending the FCI and the delivery of pigs to slaughter, because they often submit the FCI concurrently as they announce their intention to send pigs for slaughter (Nieminen, 2015). In addition, farmers found it difficult to assess the number of animals in the batch with specific lesions (Nieminen, 2015). However, Finnish farmers appeared to regard the FCI with high motivation. The majority of respondents believed that the FCI currently in use improves food safety and prevents animal diseases (Nieminen, 2015).

These results together show that proper guidelines for FCI reporting are needed so that farmers can provide more useful and accurate information. However, farmers appear to be willing to perform this duty, as they consider FCI important.

However, despite scoring of the historical meat inspection data and the health indicators (constant coughing and increased tail-biting rate), some unexpected variation between batches will still occur (e.g. a sudden "bad" batch from a "good" farm). This was also shown in our study. These batches probably included considerable numbers of remnant pigs collected from several compartments of the farm. Such pigs are typically not the best individuals. However, when the partial carcass condemnation rate is over 10%, the farmer should be expected to notice something unusual in the batch during fattening. Improving the reliability of FCI reporting, including information on irregular slaughter batches and informing farmers why this information is important are the only solutions for detecting these remnant and outlier batches beforehand.

To conclude, well-chosen on-farm health indicators (such as healed tail-biting rate at the end of fattening and constant coughing during fattening) together with previous meat inspection results could be used as part of the FCI to make decisions regarding the meat inspection procedure: visual-only or additional inspections. Slaughterhouses could adjust their thresholds and use this information as an automatized scoring system. Farmers would also report abnormal batches for other reasons. They must be properly advised to carefully report this information.

Such allocation of slaughter batches is not a food safety issue, but important for visual-only meat inspection. It would be beneficial regardless of who is conducting the task of condemning meat due to quality issues. Currently this is the task of official control. EFSA (2011) suggested a shift to visual-only meat inspection and that a meat quality assurance system conducted by slaughterhouse operators could ensure the elimination of abnormalities due to aesthetic/meat quality grounds. Only the first-mentioned change was included in the legislation. In our study, we did not assess the distribution of the meat inspection task. In a recent study by Luukkanen et al. (2015), the majority of red meat slaughterhouse

representatives and OVs considered that *post-mortem* inspections should be performed by official auxiliaries employed by an authority to ensure consumer confidence. However, 75% of red meat slaughterhouse representatives considered redistributing meat inspection tasks essential for increasing cost-efficiency, although they were not willing to redistribute whole-carcass condemnations (Luukkanen et al., 2015). Considering food safety, applying a risk-based approach would be crucial in meat inspection (6.1.2). However, if reallocation of current resources is required to finance this change, further research is needed to also ensure the meat quality, animal health and animal welfare aspects included in current meat inspection.

7 CONCLUSIONS

The seroprevalence of pathogenic *Yersinia* spp. was the highest of the studied pathogens. The seroprevalences of *Salmonella* spp. and *T. gondii* were low. *Trichinella* antibodies were not detected.

Differences between farms were huge when considering *Salmonella* spp., *Yersinia* spp. and *T. gondii* seroprevalences. As part of a comprehensive pork carcass safety assurance system, serological monitoring of these pathogens could be used to allocate pig farms into risk categories for which targeted control measures could be applied. Risk mitigation targets and procedures must be carefully adjusted for each pathogen.

Serological *Salmonella* monitoring would enable us to follow farm-level trends and detect changes readily and sensitively. However, this would have only a limited positive impact on food safety, because the current situation is already excellent.

Considering *Yersinia* spp., serological results in FCI would provide the slaughterhouse with the opportunity of risk-ranking farms according to their pigs' risk of shedding *Yersinia* spp. or carrying it in their tonsils, and to slaughter pigs from high-risk farms at the end of the day.

As the seroprevalence of *T. gondii* was very low, no extensive serological monitoring is needed at slaughterhouses. However, monitoring could be targeted to small fattening farms and outdoor farms. Serological results in FCI provide the possibility for slaughterhouses to risk-rank farms according to their *T. gondii* risk, and to direct carcasses from high-risk farms to freezing or heating.

Serological monitoring of *Trichinella* spp. is not necessary in the current situation, as virtually all pigs are tested at slaughter using the digestive methods and the seroprevalence is 0%. However, as routine *Trichinella* spp. testing is to be diminished, serosurveillance could be used to verify the effectivity of controlled housing conditions.

The partial carcass condemnation rate for a batch was best predicted by the partial carcass condemnation rate of the pigs from the same farm within one year. In addition, constant coughing and tail biting at a farm were associated with partial carcass condemnations.

On-farm health status indicators (such as tail biting and coughing) together with previous meat inspection results could be used as FCI to allocate

batches beforehand and to make decisions regarding the meat inspection procedure: visual-only or additional inspections.

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