Anti-*Ascaris suum* IgG antibodies in fattening pigs with different respiratory conditions

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ABSTRACT:

During their migration through the pig's body, *Ascaris suum* larvae cause significant damage to the lungs. Little is known about the actual impact of this tissue damage on the occurrence and severity of respiratory problems in industrial pig fattening farms. In this study, we evaluated the link between the serological response to two different *A. suum* antigen preparations and respiratory or meat inspection outcomes. Two different serological tests

were used that measure antibodies against either the *A. suum* haemoglobin molecule or complete homogenate of the 3rd stage larva that migrate through the lungs. Firstly, serum samples were analysed that were collected from 19 herds in which the cause of acute clinical respiratory symptoms was either *Actinobacillus pleuropneumoniae*, *A. suum*, or a miscellaneous cause. This was done to test whether serological results could confirm pathological findings. Secondly, serum samples from 60 herds of finishing pigs with a history of high or low frequency of pleuritis at meat inspection (MI), but without acute respiratory symptoms at the time of sampling, were also submitted for serological evaluation using both tests. Regression models were used to search for potential associations between the proportion of pigs testing seropositive with MI results, in particular pathological changes related to the lungs. The results of both ELISAs were strongly associated ($P < 0.001$) with pigs belonging to a herd where the respiratory problems could be attributed to *A. suum* by histology, indicating that both tests can be used to diagnose clinical respiratory outbreaks due to *A. suum*. In the herds without acute clinical respiratory symptoms, a positive association was found between the proportion of pigs testing seropositive and the percentage of livers rejected due to milk spots and with whole carcass condemnations. No association was found between *Ascaris* serology and lung pathology (pneumonia and pleuritis) registered at MI, however, challenging the likely involvement of *Ascaris* in the development of these lesions.

Keywords*: helminth, swine, pleuritis, pneumonia, respiratory, farm, meat inspection*

Highlights

- ELISA tests could associate acute respiratory outbreaks wirh *Ascaris suum*.
- Anti-*Ascaris* antibodies were not associated with pneumonia nor pleuritis.
- Milk spots and carcass condemnations were associated with anti-*Ascaris* antibodies.

1. Introduction

The roundworm *Ascaris suum* is a parasite of importance to the pig industry, as the adverse effects of the infection mainly affect growing pigs (Nansen and Roepstroff, 1999; Lassen et al., 2017). When swallowed, infective eggs hatch in the gut, and larvae start their migration through their host before returning to the intestine, where they can develop into adult worms. Migration of the larvae through the liver occurs 0-6 days post infection (p.i.) (Roepstorff et al., 1997) causing mechanical injury and triggering an inflammatory reaction (Roepstorff, 2003). When the larvae continue their migration, and reach the lungs around 7 days p.i., they can cause small petechial haemorrhages (Underhahl and Kelly, 1957; Rivera and Gaafar, 1976; Yoshihara et al., 1983). The severity and recognisability of clinical manifestations may also be driven by the number of larvae reaching the lungs simultaneously. Pigs can show respiratory symptoms such as coughing, increased breathing rate, and dyspnoea from emphysema and oedema in the lungs (Spindler, 1947; Taffs, 1968; Eriksen, 1981; Yoshihara et al., 1983; Curtis et al. 1987). The presence of *Ascaris* in the lungs attract eosinophils and can result in eosinophilic pneumonia. Though reports exist on the association between respiratory symptoms and *Ascaris* in pigs (Miskimins et al., 1994; Haimi-Hakala et al., 2017), observational studies rarely describe it. Explanations for this could be that *Ascaris* infections triggering respiratory symptoms are uncommon, they may be mild and easily overlooked, or they may be difficult to observe as an abnormality under endemic conditions (Roepstroff, 2003).

Chronic non-pyemic lesions in the lungs caused by migrating *Ascaris* larvae may not commonly contain pus nor show signs of necrosis (Liljegren et al., 2003), but the damage to the alveolar and bronchial air space may have both direct and opportunistic side effects. The side effects that are observed at slaughter include secondary lung infections that have been associated with damage to the mucosal barrier by *A. suum* (Bernardo et al., 1990; Martinsson

et al., 1991; Nilsson et al., 1991). Migrating larvae may introduce, or pave the way for, opportunistic viral and bacterial infections, which have been observed in experimental infections in pigs (Underdahl and Kelly, 1957; Adedji et al., 1989; Christensson et al., 1991; Vlaminck et al., 2015).

Ascaris infections are relevant to both pig welfare and the industry. Slower weight gain and discarded livers due to milk spots are well known effects (Nansen and Roepstroff, 1999; Roepstorff, 2003; Lassen et al., 2017). The potential effects and side-effects on the lungs are less well studied, and may result in unspecific pathological findings such as pleuritis and pneumonia in meat inspection (MI) after slaughter. The role of *A. suum* causing respiratory problems in pig production systems merits more attention than has been received thus far. The presence of anti-*Ascaris* antibodies and the timing of the antibody reaction may be a sensitive way of identifying not only the animals or groups of animals exposed to migrating larvae (Vlaminck et al., 2012), but also the associations with effects observable at slaughter (Eriksen et al., 1992; Nejsum et al., 2009; Vlaminck et al. 2015).

Firstly, using two different serological tests, we examined the serum of fattening pigs to investigate whether specific antibodies against *A. suum* could confirm previous findings in herds with acute respiratory problems (ACUTE herds) caused either by *Actinobacillus pleuropneumoniae*-infection (APP), *A. suum*, or miscellaneous causes. Secondly, we examined whether *Ascaris* serology in herds experiencing no respiratory symptoms (NON-ACUTE herds), with a history of high or low pleuritis in MI, was associated with MI findings.

2. Methods

2.1. Ethics statement

The experiment was approved by Southern Finland Regional State Administrative Agency (ESAVI/5547/04.10.07/2013). Farmers were asked to participate in the experiment voluntarily and they had the possibility to withdraw from the study at any time.

2.2. Experimental setup

2.2.1. *ACUTE herds*

The first group consisted of 19 case herds from farms with finishing or farrow-to-finish pigs investigated for pathogens involved in acute respiratory outbreaks in southern and southwestern Finland. The results were reported elsewhere in detail (Haimi-Hakala et al., 2017), and were used in this study to investigate the connection with *A. suum* antibodies in serum. In short, these herds had finishing pigs with acute respiratory problems (cough, fever, and reduced appetite) diagnosed between May 2011 and January 2014. The herds were visited for the first time during the respiratory outbreak, which was around 3 weeks (mean 26.2 days ± 14.1 SD) after the pigs had arrived to the fattening room, and within three days of farmers informing the research group about the respiratory symptoms in their finishing pigs. In the herd that had more than one room for finishers, pigs were selected in the room with the most severe respiratory problems for sampling. The second herd visit and sampling occurred about one month (33.1 ± 5.1 days) later.

During the first visit to each herd, three pigs with the most evident respiratory problems were euthanized and necropsied. Histopathological results were used to classify the herds into three groups, describing the most important causes for the acute respiratory problems: APP, *A. suum*-infection, or a miscellaneous reason (other than APP or *A. suum*) described in detail by Haimi-Hakala and colleagues (2017).

From each herd blood samples were collected during both visits from the same, individually ear-tagged pigs (n = 20) into serum tubes. The samples were kept at 4°C for up to 24 h before being centrifuged at 3000 rpm for 10 min. Sera were collected and stored at -18°C until analysis.

2.2.2. *NON-ACUTE herds*

The second group consisted of herds without acute clinical respiratory symptoms. Information on the frequency of pleuritis at MI in the slaughterhouse was used to ensure the samples originated from herds that potentially had both high and low levels of pleuritis. Three large slaughterhouses aided in finding the herds based on their MI data for one year (July 2010 to June 2011). High pleuritis (HP) or low pleuritis (LP) herds were defined based on the proportion of pigs diagnosed to have pleuritis higher than the mean plus-one standard deviation (SD) or lower than mean minus SD of the mean in the slaughterhouse listing, respectively. Other inclusion criteria were a farm size of over 1000 pigs slaughtered per year and location in south-/southwest Finland.

Of the 92 herds contacted, 32 (35%) opted out or were unable to participate. Altogether 60 herds participated in the study: 33 HP herds and 27 LP herds. The first visit was made at the beginning of the finishing period (week 1, mean 8 days ± 10.5 SD), and the second visit was around 10 weeks (week 10, mean 74 days ± 14.8 SD) after entering the unit, and right before slaughter. In 37 herds, the same pigs were sampled twice both during week 1 and week 10, and in 27 herds they were sampled only during week 10. If the herd had several rooms, only one room per herd was included in the study. Blood samples were collected from 20 pigs with no clinical respiratory symptoms in each herd and handled the same way as described for samples collected from ACUTE herds.

Herd owners provided information about the dates when pigs were sent to slaughter and authorized the researchers to obtain the MI findings of all pigs slaughtered in the herds during the same time period when the sampled study pigs were slaughtered. The following variables were used to examine the association with *Ascaris* infections: number of pigs <61 kg of slaughter weight (N); slaughter weight (kg); proportion of pigs with milk spots, pleuritis, pneumonia, abscesses, partial carcass condemnations, or whole carcass condemnations (% of pigs slaughtered from the herd).

2.3. Blood sample analysis

Serum samples were analysed at the laboratory of Parasitology at Ghent University (Belgium) using two ELISAs.

The As-Hb ELISA detects the presence of antibodies against *A. suum* haemoglobin, as previously described by Vlaminck et al. (2012). The adjusted optical density (ODR) was calculated: ODR = (OD sample - average OD negative controls) / (average OD positive controls (PC) - average OD negative controls (NC)). Samples were considered *Ascaris*-positive if ODR $\geq$ 0.500.

The As-L3-Lung ELISA detects the presence of antibodies recognising *A. suum* lung phase L3 larvae, as previously described by Vandekerckhove et al. (2017). The adjusted optical density (ODR) was calculated: ODR = (OD_{sample} - OD_{NC}) / (OD_{PC} - OD_{NC}). Samples were considered As-L3-Lung ELISA-positive if ODR > 0.250.

2.4. Statistics

Mixed logistic models were used for studying associations between pigs testing positive for antibodies against *A. suum*, and the cause of acute respiratory problems in ACUTE herds. Pigs

testing positive/negative for antibodies against *A. suum* at different sampling times were included as an outcome variable, and cause of outbreak as an explanatory variable (3-level categorical variable: *A. suum*, APP infection or miscellaneous causes) in all 4 models. Farm was included as a random variable.

Herd-level linear regression, tobit regression, and logistic regression models were used for studying the association between herd level MI results, and the proportion of pigs testing positive for antibodies against *A. suum* in the herd at different sampling times (week 1 and week 10). Linear regression models were used when outcome variables at herd level were: the average of slaughter weight, proportion of pigs with abscesses after logarithmic transformation, or proportion of pigs with partial carcass condemnation after square root transformation. Tobit regression models were used if outcome variables were (all after square root transformation): herd level proportion of pigs with liver milk spots, proportion of pigs with pneumonia, proportion of pigs with whole carcass condemnation, or proportion of pigs with pleuritis. The tobit regression model was chosen because there was a high proportion of herds with a result of 0%. This would violate the regression model's assumptions of normal distribution of the outcome variable. In the tobit regression, all cases falling below a specified threshold value (all 0% results in this case) are censored, although these cases remain in the analysis (Long, 1997). A logistic regression model was used when the outcome variable was MI recordings of pigs <61 kg weight at slaughter (yes/no). The proportion (%) of pigs testing positive for antibodies *A. suum* at different sampling times (week 1 or week 10) were included as continuous explanatory variables in the described herd level models. The herds history of pleuritis found at MI (HP and LP), and herd size categories according to the quartiles of herds number of pigs slaughtered (<500, 500–874, 875–1434, and >1434 pigs), were included in all these models as categorical explanatory variables.

198 Assumptions of all herd-level linear regression and tobit models were confirmed using
199 normality and scatter plots of model residuals. Stata 14.0 (StataCorp, TX) software was used
200 for statistical analyses and a *P*-value ≤ 0.05 was considered to be significant.
201
202 **3. Results**
203 *3.1.* Ascaris suum-*Hb and -L3-Lung ELISA* results in *ACUTE herds*
204 The causes of acute respiratory problems on the farms studied were APP (13/19, 68.4%), *A.*
205 *suum* (3/19, 15.8%), or miscellaneous causes (3/19, 15.8%) (Haimi-Hakala et al., 2017).
206 Using the As-Hb ELISA in herds with respiratory outbreaks caused by APP, *A. suum,* and
207 miscellaneous causes, 19.8% (55/278), 86.4% (51/59), or 8.3% (5/60) of the pigs were
208 positive at the first sampling, and 25.2% (69/274), 96.4% (53/55), or 15.8% (9/57) one month
209 later, respectively.
210 The serological results for each of the 19 farms for both serological tests are presented in
211 Figure 1. In herds where no respiratory outbreaks were caused by *A. suum,* the mean As-Hb
212 ODR was 0.35 ± 0.23 SD (median 0.31, min-max -0.08–1.70) and 0.40 ± 0.23 SD (median
213 0.36, min-max -0.25–1.84) at first sampling and one month later, respectively. In contrast, the
214 mean As-Hb ODR in herds with acute respiratory symptoms caused by *A. suum* infection was
215 1.26 ± 0.70 SD (median 1.26, min-max 0.11–2.69) at the first sampling and 1.78 ± 0.54 SD
216 (median 1.81, min-max 0.42–2.85) at second sampling, one month later.
217 Using the As-L3-Lung ELISA in herds with respiratory outbreaks caused by APP, *A. suum,*
218 and miscellaneous causes, 1.6% (4/258), 84.8% (50/59), or 1.7% (1/60) were positive at the
219 first sampling, and 2.0% (5/253), 98.2% (54/55), or 3.5% (2/57) one month later, respectively.
220 In herds where respiratory outbreaks were not caused by *A. suum*, mean As-L3-Lung ODR
221 were 0.02 ± 0.12 SD (median 0.01, min-max -0.20–1.38) at first sampling and 0.00 ± 0.10 SD
222 (median -0.01, min-max -0.19–0.45) one month later. The mean As-L3-Lung ODR in herds

with respiratory outbreaks attributable to *A. suum* infection were 1.02 ± 0.66 SD (median 0.01, min-max -0.20–1.38) at first sampling, and 2.02 ± 0.77 SD (median 1.72, min-max 0.03–3.21) one month later.

Pigs that were positive on both ELISAs at the first sampling ($P < 0.001$, Supplementary materials: Table S1, $P < 0.001$ and Table S2, $P < 0.001$, respectively) and one month later ($P < 0.001$, Supplementary materials: Table S3, $P < 0.001$ and Table S4, $P < 0.001$, respectively), had higher odds of belonging to a herd in which *A. suum* was detected in the lungs by histology.

3.2. Ascaris suum *antibodies and MI in NON-ACUTE herds*

Descriptive data about the MI findings in *NON-ACUTE* herds is presented in Table 1. The proportion of pigs testing positive using the As-Hb and As-L3-Lung ELISA is presented in Table 2. Mean As-Hb ODR values were 0.13 ± 0.21 SD (median 0.08, min-max -0.11–1.47) at week 1 and 0.35 ± 0.30 SD (median 0.26, min-max -0.18–2.39) at week 10. Mean As-L3-Lung ODR values were 0.04 ± 0.28 SD (median -0.03, min-max -0.20–2.07) at week 1 and 0.21 ± 0.43 SD (median 0.06, min-max -0.28–2.73) at week 10.

The presence of liver milk spots was associated with the proportion of pigs testing seropositive in the herd, both when tested during week 1 (Supplementary materials: Table S5, $P = 0.030$) and week 10 (Table 3, $P = 0.026$), using the As-L3-Lung ELISA. The final regression models could also detect a negative association between the proportion of pigs with abscesses at MI and the proportion of pigs testing seropositive in the herd during week 10 (Supplementary materials: Table S6, $P = 0.028$) using the As-L3-Lung ELISA. A positive association was found between whole carcass condemnation at MI and the proportion of pigs

testing seropositive in the herd during week 10, when tested using the As-Hb (Table 4, P = 0.008) and As-L3-Lung ELISA (Supplementary materials: Table S7, P = 0.046).

4. Discussion

4.1. Association between Ascaris antibodies and causes of respiratory outbreaks

We examined if the As-Hb ELISA (Vlaminck et al., 2012; Vlaminck et al., 2015) and the As-L3-Lung ELISA (Vandekerckhove et al., 2017) could confirm previous findings of a respiratory outbreak caused by *A. suum* (Haimi-Hakala et al., 2017). At both sampling times, 3 weeks after the pigs had arrived to the fattening room and 1 month later, and for both ELISAs, an association was found between the level of antibodies against *Ascaris* and belonging to a herd with a respiratory outbreak where *Ascaris* larvae were found in the lungs at necropsy. This adds further support to the conclusion that *A. suum* can be an important cause of herd-level respiratory problems (Haimi-Hakala et al. 2017). The study by Haimi-Hakala and colleagues concluded that serology was not optimal in the diagnosis of the respiratory outbreaks caused by APP and swine influenza. Our results suggest that serological methods for detecting *A. suum* antibodies in pigs is a useful tool to diagnose such outbreaks. The connection between the serological response and the conditions of *A. suum* infection that lead to respiratory symptoms are poorly studied. However, continuous low-dose infections with 100 infective *Ascaris* eggs five times weekly resulted in a measureable IgG response using the As-Hb ELISA already 4 weeks post infection, with a peak around 8 weeks post infection, and remained positive under stimulation from a continuous infection (Vlaminck et al., 2012). Slightly higher experimental trickle infections of fattening pigs with 500 eggs twice weekly, kept under similar management conditions as in this study, resulted in a large increase in blood eosinophils (Eriksen et al., 1992). The rise in eosinophils is a result of the larvae migrating through the lungs (Miskimins et al., 1994), as a result from relatively low

infection doses, and *Ascaris* is known to potentially cause verminous pneumonia in individual pigs (Corwin and Steward, 1999).

4.2. Association between Ascaris antibodies and pathological changes in MI

The most common approach to study the connection between presence of *A. suum* and the effects observed at slaughter have been based on the presence of eggs in faeces prior to slaughter or harvesting the parasites post mortem (Carstensen et al., 2002; Knecht et al., 2011; Kalai et al., 2012). Eggs in the faeces of pigs are indicative of the final adult stage of *A. suum* larvae and is likely an underestimation of the effects that migrating *Ascaris* larvae may have on the health of pigs. In this study, blood samples from 60 fattening herds were analysed with both ELISAs to see if associations could be found between findings at MI with the proportion of pigs carrying antibodies against *A. suum*. The selected herds lacked known acute respiratory symptoms (NON-ACUTE herds) at the time of sampling. Herds were selected based on historical data regarding pleuritis at slaughter. We selected both HP and LP herds in the expectation of improving chances of finding associations between seropositive pigs and *Ascaris*-related pathological changes, particularly to lungs and pleura, which would be observable at MI. The history of pleuritis observed at slaughter was offered as a variable to all the models. However, no difference could be observed between HP and LP herds at the level of pleuritis or pneumonia scores of this batch of slaughtered pigs. Similar to the results of our study, Vlaminck et al. (2015) found no association between serology and the percentage of affected lungs at MI using the As-Hb ELISA. However, they found positive correlations with findings of *M. hyopneumonia (M. hyo),* Porcine Circovirus Type 2 (PCV2), and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) (Vlaminck et al., 2015). In this study, herds were very likely to be free from *M. hyo* and PRRSV and the influence of respiratory pathogens in the pigs may have been different. The presence of *A. suum* larvae has

297 also been demonstrated to affect the immune response towards vaccines aimed at preventing
298 respiratory infections and their related pathology. Pigs vaccinated with killed *M. hyo*, infected
299 with *A. suum*, and challenged with living *M. hyo*, displayed more severe lung pathology
300 compared to vaccinated pigs that were *A. suum*-free (Steenhard et al., 2009).
301
302 Herds with higher numbers of seropositive pigs using the As-L3-Lung ELISA had a higher
303 proportion of livers rejected at MI due to milk spots. This is consistent with what has been
304 observed in other studies and is a consequence of *Ascaris* larvae migrating through the liver
305 (Roepstorff et al., 1997; Vlaminck et al., 2015; Martínez-Pérez et al., 2017; Lassen et al.,
306 2017). The same association could not be demonstrated, however when the As-Hb ELISA
307 was employed. This can be explained by the fact that the As-L3-Lung ELISA is better at
308 detecting exposure to the larval stages of the parasite than the As-Hb ELISA, and likely
309 reflects that the haemoglobin molecule is mostly produced and excreted by the later larval
310 stages and adult worms in particular (Vlaminck et al., 2012).
311
312 Linear regression analysis further predicted that pigs that tested seropositive at week 10 using
313 the As-L3-Lung ELISA and originated from a HP herd had a lower chance of having
314 abscesses at MI. The reason for this unexpected result is not clear, and it may be expected the
315 damage to the mucosal barrier in the lungs by migrating *Ascaris* larvae burrowing through the
316 tissue may ease access to bacteria and viruses (Underdahl and Kelly, 1957; Christensson et
317 al., 1991; Roepstorff, 2003) and therefore one could expect a higher chance of abscesses. The
318 synergistic effects of migrating *Ascaris* larvae and *Escherichia coli* has been associated with
319 the occurrence of abscesses in pigs (Adedeji et al., 1989), and ascariasis has been linked to
320 increased susceptibility to *Pasteurella multocida*, *Escherichia coli* and *Salmonella* spp. in the
321 lungs (Curtis et al., 1987; Tjornehoj et al., 1992; Smith et al., 2011).

The As-Hb ELISA demonstrated a stronger association with the proportion of pigs with whole carcass condemnation from a herd, compared with the As-L3-Lung ELISA, during week 10. The reason for the association is challenging to speculate on as data on the underlying reason for rejecting the whole carcass of a pig are many and was not collected.

Our results reflect that lung pathology may be a multifactorial effect in which *A. suum* plays a role both mechanically and immunologically. How *A. suum* infections affect the development of other infectious diseases is not well studied and merits more attention.

5. Conclusion

Both ELISA tests, As-L3-Lung and As-Hb, were considered good diagnostic tools in differentiating herds experiencing clinical respiratory outbreaks due to *Ascaris suum*. However, there was no association between the presence of antibodies against *A. suum* at the herd level and lung pathology (pneumonia and pleuritis) registered in MI in herds without acute respiratory signs. The results presented support the fact that ascariasis should be considered in the differential diagnosis of pigs showing acute signs of respiratory symptoms. More attention should be directed towards studying the role of *A. suum* as a factor that can lead to pathological changes observed at MI.

6. Conflict of interest

The authors do not have any financial or personal conflicts of interest that could bias the study.

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Author contributions

Study design (MH, BL, TO, PG, CO, OH), sampling (CO, MH, OH), analysis (BL, TO, PG, JV), writing the manuscript (BL), final approval of the manuscript (BL, CO, TO, MH, OH, PG, JV).

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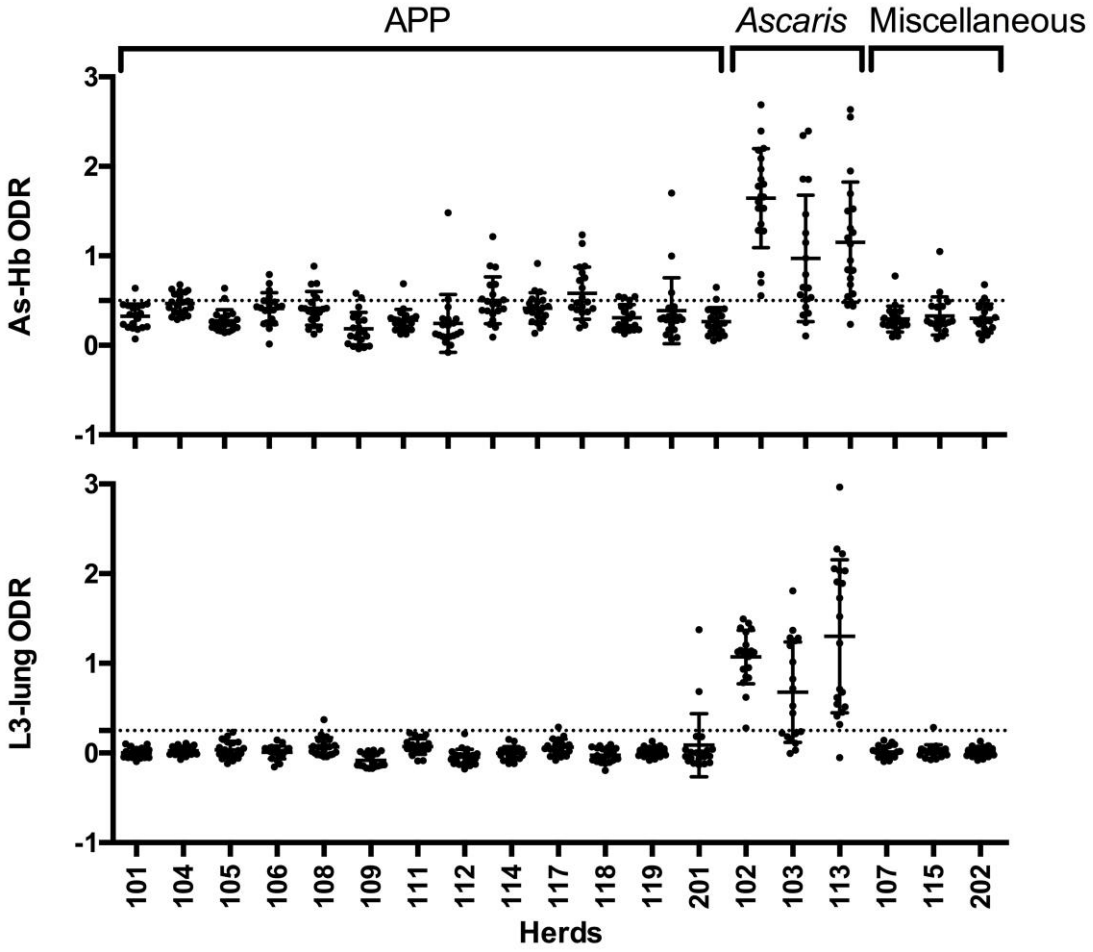

Fig. 1. *Ascaris suum*-Hb and -L3-Lung ELISA optical density ratio (ODR) from 19 herds sampled at the time of the respiratory outbreaks. Dotted lines indicate OD cut-off of a positive result for the assay. The herds are grouped to indicate the herds with pathological results suspecting *Actinobacillus pleuropneumoniae*-infection (APP), *A. suum*, or miscellaneous reason as the cause of the outbreak (Haimi-Hakala et al., 2017).

453 **Table 1.** Descriptive data of meat inspection findings in slaughterhouses from pig herds
454 without acute respiratory symptoms and *Ascaris suum* antibody results in pigs, at the
455 beginning (week 1) and at the end (week 10) of the finishing period.

Variable	n of herds	Overall (median (min-max) unless otherwise indicated)
Number of pigs slaughtered (median, min–max)	52	347 (58–1594)
Average slaughter weight (mean ± SD)	29	87.5 ± 7.5
Proportion (%) of pigs <61 kg weight at slaughter	27	0.0 (0.0–1.0)
Proportion (%) of pigs with liver milk spots	50	1.2 (0.0–25.1)
Proportion (%) of pigs with pleuritis	52	4.1 (0.0–60.9)
Proportion (%) of pigs with pneumonia	52	1.1 (0.0–5.7)
Proportion (%) of pigs with abscesses	52	3.9 (0.9–12.7)
Proportion (%) of pigs with whole carcass condemnation	52	0.0 (0.0–1.0)
Proportion (%) of pigs with partial carcass condemnation %, median (min-max)	52	6.8 (1.2–16.2)
Proportion (%) of pigs testing positive for As-Hb antibodies at week 1	29	0.0 (0.0–50.0)
Proportion (%) of pigs testing positive for As-Hb antibodies at week 10	52	0.1 (0.0–68.4)
Proportion (%) of pigs testing positive for As-L3 antibodies at week 1	29	0.0 (0.0–60.0)

Proportion (%) of pigs testing positive for As-L3 antibodies at week 10	52	0.1 (0.0–100.0)

Table 2. Proportion of pigs in herds without acute respiratory symptoms testing positive in herds and at animal level at the beginning (week 1) and at the end (week 10) of the finishing period using As-Hb and As-L3 ELISA.

	n	n positive	As-Hb ELISA % (95% CI)	n	n positive	As-L3-Lung ELISA % (95% CI)
Herd level						
Week 1	29	14*	48.3 (30.7–66.2)	29	13*	44.8 (27.7–63.0)
Week 10	52	42*	80.8 (68.4–89.8)	52	37*	71.2 (57.8–82.2)
Animal level						
Week 1	575	30	5.2 (3.6–7.3)	576	45	7.8 (5.8–10.2)
Week 10	1016	210	20.7 (18.2–23.2)	1014	209	20.6 (18.2–23.2)

* At least one pig testing positive in the herd

Table 3. Tobit regression model results where outcome variable is proportion of pigs (%) in farms (n = 50) with liver milk spots at meat inspection after square root transformation. The model has 15 left-censored observations at milk spots proportion 0%.

Variable	n of herds	Coef.	SE	*P*-value
Proportion (%) of *As*-L3-lung ELISA positive pigs per herd at week 10 of finishing period	50	0.030	0.013	0.026
Finishing unit size: *				0.369
<500	10	0		
500-874	14	0.109	0.939	0.908
875-1434	13	1.293	0.817	0.120
>1434	13	0.367	0.817	0.655
Constant		0.054	0.661	0.935

* Variable retained in model because it was a confounder.

482 **Table 4.** Tobit regression model results where outcome variable is proportion of pigs (%) in
483 farms (n = 52) with whole carcass condemnation at meat inspection after square root
484 transformation. The model has 26 left-censored observations at whole carcass contamination
485 proportion 0.

Variable	n of herds	Coef.	SE	*P*-value
Proportion (%) of Hb-antibody-positive pigs in the herd during week 10 of finishing period	52	0.015	0.005	0.008
Finishing unit size:				<0.001
<500	11	0		
500–874	14	0.072	0.327	0.827
875–1434	13	0.987	0.297	0.002
>1434	14	0.804	0.297	0.009
Constant		-0.737	0.292	0.015

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