Research paper

Effect of strategic deworming on *Ascaris suum* exposure and technical performance parameters in fattening pigs

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ABSTRACT

The aim of this study was to investigate the effect of a strategic deworming program on *Ascaris suum* infection levels and technical performance parameters in fattening pigs. Eighteen fattening stables were selected and divided into two groups. Group 1 consisted of 9 stables in which the fattening pigs tested seropositive for *Ascaris*, indicative for the presence of *Ascaris* eggs in the stable, whereas group 2 consisted of 9 stables in which the fattening pigs tested seronegative for *Ascaris*, indicating of a low or absent environmental contamination with *Ascaris* eggs. The production in each stable was monitored for a period of 7 consecutive fattening rounds. The first of these 7 fattening rounds (i.e. round 0), during which no intervention took place in the deworming strategy applied in the stable, served as a historical control. A deworming program using 200 mg/ml fenbendazole oral suspension in drinking water for 2 days every 6 weeks was implemented for a period of 6 consecutive fattening rounds. For each fattening round and for each stable, technical performance parameters including average daily growth, feed conversion ratio, days in fattening and the percentage of affected livers were obtained from the producers. Blood was collected from 10 randomly selected animals per stable at the end of each fattening round and evaluated for the presence of anti-*Ascaris* antibodies using 2 different serological tests, namely the AsHb- and the L3-Lung ELISA. The serological results obtained indicated a lower exposure of the animals to *Ascaris* after the implementation of a strategic deworming program. A significant decline in anti-*Ascaris* antibody levels was detectable in the stables that originally tested positive for *Ascaris* and was already visible after one treatment round. The outcomes of hierarchical linear mixed models indicated that the level of L3-Lung antibody reactivity was a significant predictor of decreased ADG, increased FCR and prolonged DIF for the *Ascaris*-positive herds, indicating an effect of *Ascaris* infections on productivity.

1. Introduction

Ascaris suum is a widespread parasitic nematode that causes infection in pigs worldwide (Dold and Holland, 2011; Nansen and Roepstorff, 1999; Roepstorff et al., 1998). A single *A. suum* female worm may produce close to two million eggs per day and these eggs can remain viable in the environment for several years. After oral uptake, the L3-stage larvae will hatch from the egg in the gastrointestinal tract, penetrate the caecum wall and migrate via the blood stream to the liver and subsequently the lungs. Here, the larvae are coughed up, swallowed and subsequently arrive back in the small intestine. During this migration, damage is caused in the respective organs (Roepstorff et al., 1997). In the liver, the characteristic lesions caused by the inflammatory response to the larvae are called hepatic white spots and are a major reason for liver rejection at slaughter. The acute phase of a severe infection is characterized by frequent coughing (Boes et al., 2010) due to pneumonia. The damage caused in the lungs can pave the way for opportunistic bacterial and viral infections (Adedeji et al., 1989; Curtis et al., 1987; Tjornehoj et al., 1992). Finally, it has also been shown that *A. suum* impairs the effects of a *Mycoplasma hyopneumoniae* vaccine resulting in increased pulmonary lesions (Steenhard et al., 2009).

Several studies state that infections with *A. suum* result in significant economic losses such as decreased average daily growth (Bernardo et al., 1990; Lassen et al., 2017; Pedersen et al., 2002; Stewart et al., 1972; Urban et al., 1989; Vlaminck et al., 2015) in combination with an increased feed conversion ratio (Hale et al., 1985; Kipper et al., 2011; Stewart and Hale, 1988; van Krimpen et al., 2010; Zimmerman et al., 1973) and lower meat quality (Jankowska-Makosa and Knecht, 2015; Knecht et al., 2012, 2011). However, pigs harbouring patent infections,

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even with large numbers of adult worms, often appear to be clinically healthy (Boes et al., 2010). The consequence of this is that pig farmers and veterinarians underestimate both the infection levels as well as the potential impact on production.

In recent years, several studies have investigated the use of serology to more accurately measure the level of exposure of pigs to *A. suum* in comparison to other diagnostic techniques such as liver white spots and faecal egg counts (Vlaminck et al., 2014). First, Vlaminck et al. (2012) reported on a serological test that is based on the antibody recognition of a haemoglobin protein (AsHb) mainly produced by the late larval and adult stages of *A. suum*. More recently, Vandekerckhove et al. (2017) described an ELISA test that is based on the recognition of antigens present in a water-soluble protein homogenate of L3 larvae that migrate through the lungs. Interestingly, applying these tests on commercial fattening farms in both Belgium and Spain showed significant correlations between *Ascaris* antibody levels and technical performance parameters, such as daily weight gain and feed conversion ratio (Vlaminck et al., 2015; Martínez-Pérez et al., 2017), suggesting that serology could potentially also be used to estimate economic impact of *Ascaris* infection.

Based on these observations, the aim of the current study was to monitor the effect of strategic anthelmintic treatment in fattening pigs on both *A. suum* antibody levels and technical performance parameters over time. For this, two groups of 9 fattening stables with different levels of contamination with *A. suum* eggs were monitored for 7 consecutive fattening rounds. In each stable a deworming program was applied using fenbendazole every 6 weeks in order to interrupt the development of adult worms in the fattening pigs. For every stable and every fattening round, technical performance parameters such as average daily growth, feed conversion ratio, days in fattening and percentage of condemned livers as well as anti-*Ascaris* antibody levels were monitored in the fattening pigs.

2. Material and methods

2.1. Selection of the fattening stables

A total of 41 stables from commercial fattening herds that were part of the integration of the cooperative Covavee cvba (Belgium) were visited and screened for potential inclusion in the study. Of these 41 stables, eventually 2 groups of 9 stables were selected for inclusion based on the following parameters: conventional indoor stables with fully- or semi-slatted floors, the presence of climate control, the absence of bedding material, piglets sourced from outside the herd, no specific cleaning procedure between fattening rounds apart from rinsing with water, the availability of production data for each fattening round and, finally, the possibility to treat animals via drinking water. Group 1 consisted of 9 stables in which the average antibody response of 10 randomly chosen fattening pigs to AsHb was positive as measured by ELISA (Average ODR > 0,5), indicating for the presence of *Ascaris* eggs in the stable. Group 2 consisted of 9 stables in which 10 randomly chosen fattening pigs tested seronegative for *Ascaris* (Average ODR < 0,5), indicating a low or absent environmental contamination level (Vlaminck et al., 2012). Some characteristics of the selected stables, such as the type of floor and capacity, are summarized in supplementary Table 1 together with the AsHb ELISA results obtained in round 0. All pigs were typically fattened from approximately 10 weeks old with an average weight of approximately 22 kg until slaughter weight was reached (approximately 100 kg) at an age of approximately 28 weeks old. An all-in/all-out management system was employed in all stables.

2.2. Deworming program

The stables were monitored for a period of 7 consecutive fattening rounds. The first of these 7 fattening rounds (i.e. round 0), during which

Table 1
Correlation coefficients between the results for the serology (AsHb and L3-Lung) and Liver white spots (LWS) for the stables of both groups, as calculated by Spearman correlation analysis (*P < 0.05).

		L3-lung	LWS
Group 1	**AsHb**	0.694*	0.386*
	L3-Lung		0.560*
Group 2	**AsHb**	0.574*	0.156
	L3-Lung		−0,093

no intervention took place in the deworming strategy applied on the farms, served as a historical control. From the second fattening round onwards (i.e. round 1) a deworming program using 200 mg/ml fenbendazole oral suspension for use in drinking water (Panacur Aquasol) for 2 days was implemented at week 0 (onset), 6 and 12 of the fattening period.

2.3. Collection of blood

Blood was collected approximately 14 weeks after onset of the fattening round from 10 animals randomly selected from different pens in the stable in order to get a sample set representative for the whole herd. Samples were collected in 5 ml serum tubes that were subsequently centrifuged at 4000*g* during 10 min at 4 °C. Serum was collected and stored at −20 °C until used.

### 2.4. Slaughter line data and technical performance indicators

After approximately 18 weeks, when the animals weighed around 100 kg, animals were transported to commercial slaughterhouses in Flanders. For each fattening round and for each stable, technical performance parameters including average daily growth (ADG) (total weight of all animals weighed at the time of slaughter minus total weight of all animals at onset of the fattening round divided by the number of animals and the number of days in fattening, expressed as gram/day), feed conversion ratio (FCR) (Amount of feed used during the whole fattening period divided by the average daily growth, expressed in kg) and days in fattening (DIF) (number of days in the fattening stable) were obtained from the producer. Official meat inspection personnel visually assessed all the livers from the slaughtered animals for the presence of white spot lesions (LWS) during routine post-mortem meat inspection at the slaughterhouse.

2.5. Analysis of the serum samples

The AsHb and L3-lung ELISA tests were performed as described by Vlaminck et al. (2012) and Vandekerckhove et al. (2017) respectively. To compensate for variation, a negative and positive control sample was included on each plate. The negative control (NC) was a pooled serum sample from 10 piglets without previous exposure to *A. suum*. The positive control (PC) for the AsHb test was a pooled serum sample from pigs after 18 weeks of daily infection with 100 *A. suum* eggs. The positive control (PC) for the L3-lung test was a pooled serum sample from piglets after 7 weeks of daily infection with 100 *A. suum* eggs. Reactivity to the antigen is shown in ODR (Optical Density Ratio) (ODR sample = (OD sample – OD NC)/ (OD PC – OD NC)). Serological results are expressed as the arithmetic mean of the ODR values of all 10 samples per stable.

2.6. Statistical analysis

Spaghetti plots were prepared for each parameter to visualize the trends of the variables over time and paired, non-parametric statistical analysis was performed using the Friedman test combined with a posthoc Dunn's multiple comparison test to evaluate significant changes
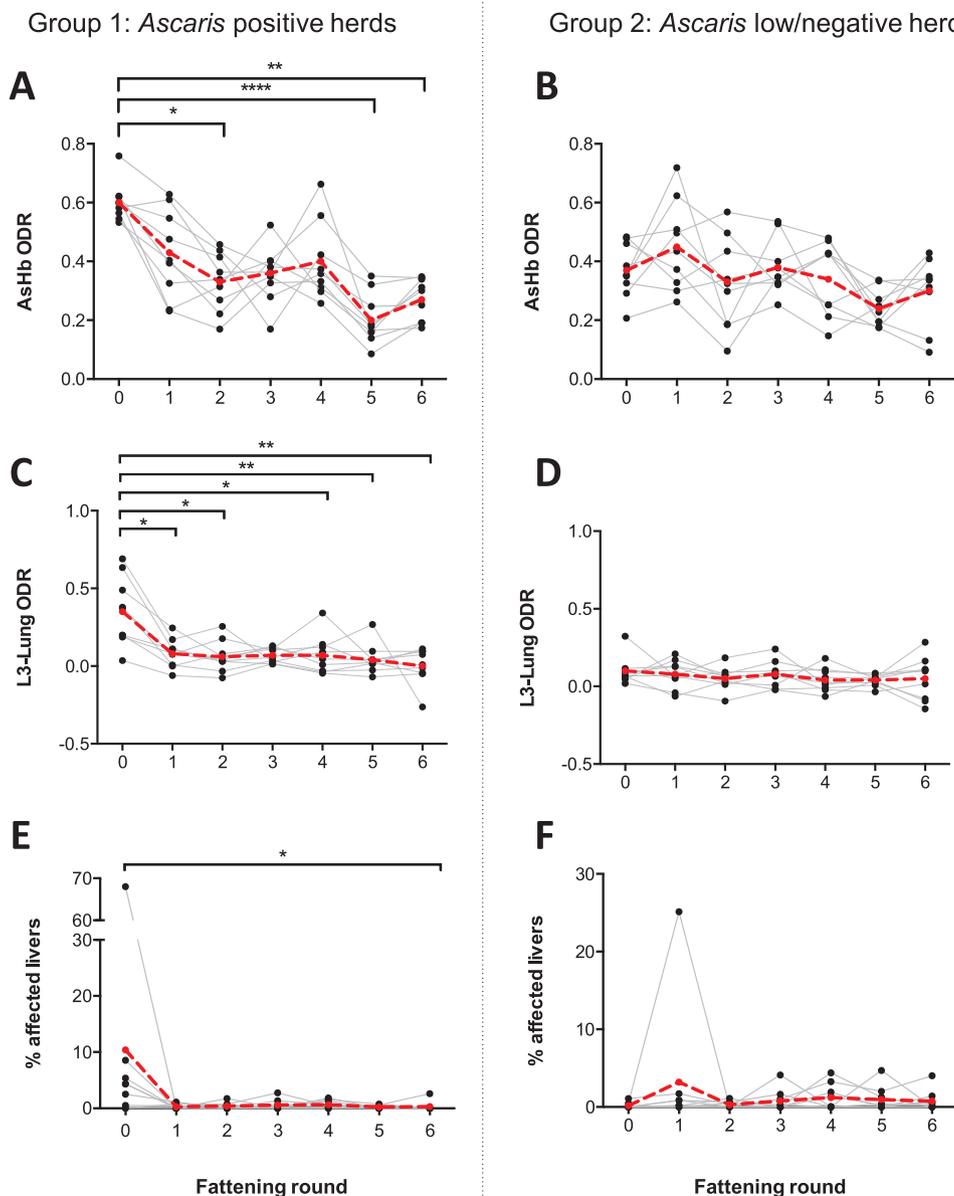
Fig. 1. Evolution of the Anti-*Ascaris* antibody levels as measured by the AsHb- (panels A and B), the L3-lung ELISA (panels C and D) and percentage of livers with white spots (panels E and F) for *Ascaris* positive stables (panels A, C, E) and stables negative/low for *Ascaris* (panels B, D, F). Serological results are shown as the average serological result of 10 animals. The red dotted lines represent the average results of the 9 stables over time. (Statistical analysis: * P < 0.05, ** P < 0.01, *** P < 0.001). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

over time. Correlations between the different diagnostic variables (AsHb ELISA, L3-lung ELISA and LWS) were investigated using the non-parametric Spearman's rank correlation test. Probability (P) values < 0.05 were considered to indicate significant changes or correlations. These calculations were performed in Prism Version 5.0b.

Subsequently we aimed to estimate the effect of *A. suum* infection intensity on production parameters by producing models. Hierarchical linear mixed models were applied to the data because the serial measurements are nested within the stable levels over time. Different models were prepared for each production parameter or dependent variable (ADG, FCR or DIF). For each dependent variable, three different models were created, each time including a single different diagnostic test or explanatory variable (AsHb and L3-lung ELISA or LWS) since diagnostic methods are highly correlated. The data on the percentage of livers showing white spots per stable was highly zero-inflated which required transformation of the parameter into an ordinal parameter with value 0 for when LWS were absent and value 1 if LWS

were present (> 0%).

Differences in trajectories of economic parameters between stables are expected. Adding a random intercept is thus a conceptual necessity for repeated measures analysis. This allows the baseline score of each production parameter of each stable to be taken into account. The factor 'stable' was thus incorporated as a random effect. The basic model we started from was: Y ~ X + (~ 1|Farm), where Y is any of the response or dependent variables (ADG, FCR, DIF) and where X is any of the explanatory or independent variables (AsHb, L3-lung, LWS). The change in economic parameters and explanatory variables is associated with the sampling round and this relationship is not always completely linear. It can be expected that changes in these parameters are more pronounced during the first treatment rounds and that they reach equilibrium over time as environmental contamination with parasite eggs is reducing. Therefore, it was also tested whether the inclusion of a time and/or $time^2$ (quadratic effect) fixed parameter increased the model fit. All models were fit in R using the hglm package for

Group 1: *Ascaris* positive herds   Group 2: *Ascaris* low/negative herds
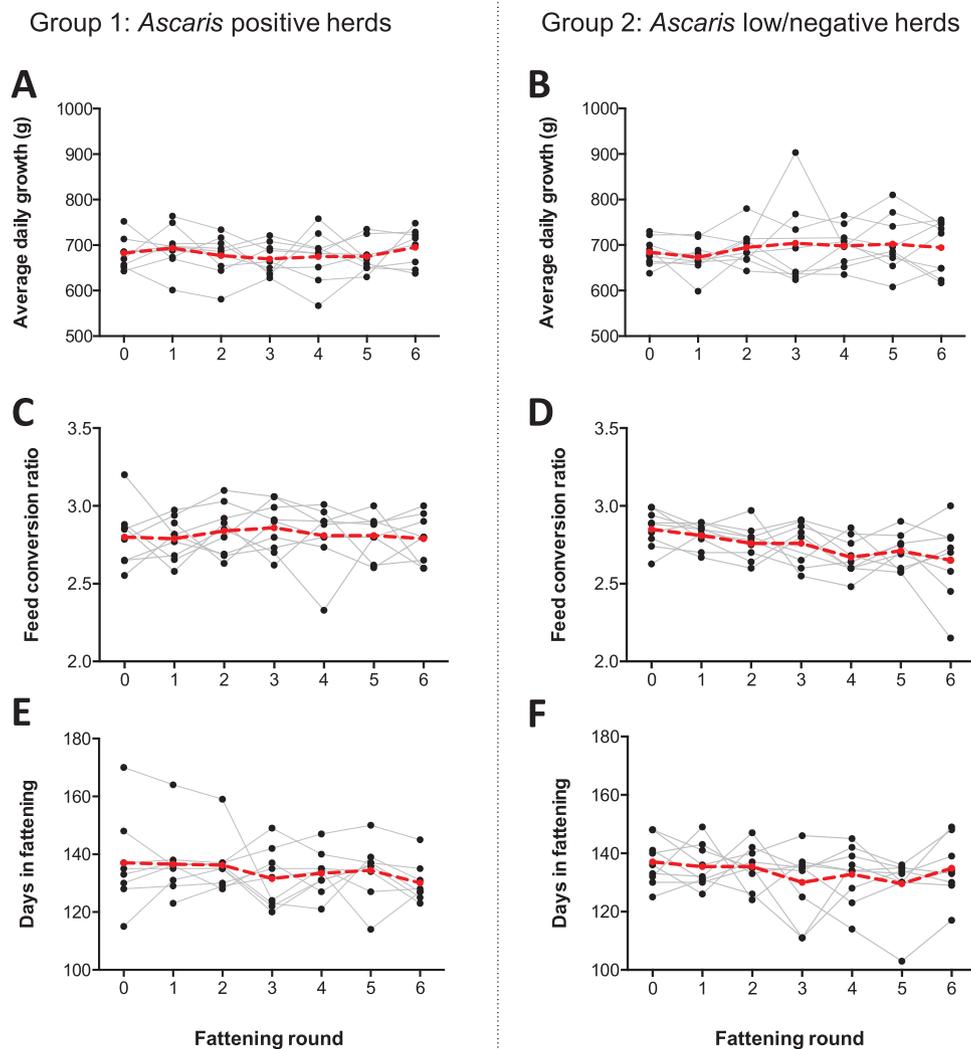
Fig. 2. The results obtained for daily growth (panels A and B), feed conversion ratio (panels C and D) and the number of days in fattening (panels E and F) for *Ascaris* positive stables (panels A, C, E) and stables negative/low for *Ascaris* (panels B, D, F). The red dotted lines represent the average results of the 9 stables over time. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

hierarchical general linear models (Ronnegard et al., 2010). The package fits generalized linear models with random effects, where the random effect may come from a conjugate exponential-family distribution (normal, gamma, beta or inverse-gamma). Model fit was evaluated using the conditional Akaike Information Criterion (cAIC) as the calibrating parameter. The best model was selected as the model with the lowest cAIC value.

3. Results

3.1. Effect of repeated deworming on Ascaris infection intensity

The *Ascaris* infection levels in all stables were monitored over 7 consecutive rounds using the AsHb- and the L3-Lung ELISA and the LWS. The results of the ELISA analyses are represented as the average ODR of 10 animals analysed individually per herd per round. The LWS are reported as percentages for the whole herd. The results obtained for the *Ascaris*-positive stables (group 1) are shown in Fig. 1 panels A, C and E, respectively. A significant decrease in anti-*Ascaris* antibody levels over time was observed with both serological tests. Similarly, the percentage of affected livers decreased significantly after only one round of deworming. In contrast, no significant decrease in anti-*Ascaris* antibody levels and percentage of affected livers could be detected for

the *Ascaris*-negative/low stables (group 2) (Fig. 1 panels B, D and F). Finally, a significant correlation was found between the results for the 2 serological tests and LWS for the positive stables (Table 1). For the stables of group 2, a significant correlation was only found between the results of the 2 serological tests (Table 1).

3.2. Effect of Ascaris infections on technical performance parameters

The ADG, FCR and DIF for all the stables of both groups are shown in Fig. 2. In both the *Ascaris* positive and negative group, production data was missing for 1 time point for 3 stables as a result of random absence of information rather than attrition from the study. Missing values were omitted from the datasets before statistical evaluation. No significant changes were observed over time for any of the parameters monitored.

To investigate the relative effect of *Ascaris* infections on performance parameters, hierarchical linear mixed models were prepared for each of the three performance parameters (ADG, FCR and DIF) in combination with each of the three explanatory variables (AsHb ELISA, L3-Lung ELISA and LWS). The outcomes of the fixed effects estimates of the best modelled interactions for the positive stables are presented in Table 2. The results indicated that the level of L3-Lung antibody reactivity was a significant predictor of decreased ADG, increased FCR

Table 2

Summary of fixed effects estimates for each modelled interaction between response variables (ADG, DIF and FCR) and fixed explanatory variable or diagnostic test (L3 Lung ELISA, AsHb ELISA, LWS) for the *Ascaris*-positive stables (group 1).

Explanatory Variables	Response Variables	Estimate	SE	t-value	P-value
		ADG			
L3Lung	Intercept	761.3	23.8	32.0	< 0.001
	L3lung	**−128.4**	**34.3**	**−3.7**	**< 0.001**
	Time	−35.8	10.6	−3.4	< 0.01
	Time^2	3.8	1.3	3.1	< 0.01
AsHb	Intercept	746.3	35.7	20.9	< 0.001
	AsHb	−53.0	41.8	−1.3	NS
	Time	−24.7	12.1	−2.1	< 0.05
	Time^2	2.7	1.4	2.0	0.057
LWS	Intercept	715.6	22.1	32.4	< 0.001
	LWS	−0.5	0.57	−0.9	NS
	Time	−20.2	11.3	−1.8	0.079
	Time^2	2.4	1.4	1.8	0.086
		DIF			
L3Lung	Intercept	132.1	2.1	62.6	< 0.001
	L3lung	**20.9**	**7.6**	**2.8**	**< 0.01**
	Time	/	/	/	/
	Time^2	/	/	/	/
AsHb	Intercept	128.6	3.6	35.6	< 0.001
	AsHb	14.7	7.8	1.9	0.064
	Time	/	/	/	/
	Time^2	/	/	/	/
LWS	Intercept	131.8	2.5	52.6	< 0.001
	LWS	5.2	2.3	2.2	< 0.05
	Time	/	/	/	/
	Time^2	/	/	/	/
		FCR			
L3Lung	Intercept	2.61	0.10	27.38	< 0.001
	L3lung	**0.38**	**0.14**	**2.68**	**< 0.05**
	Time	0.09	0.04	2.12	< 0.05
	Time^2	−0.01	0.01	−1.90	NS
AsHb	Intercept	2.82	0.06	50.08	< 0.001
	AsHb	0.01	0.12	0.07	NS
	Time	/	/	/	/
	Time^2	/	/	/	/
LWS	Intercept	2.80	0.04	74.03	< 0.001
	LWS	0.04	0.04	1.15	NS
	Time	/	/	/	/
	Time^2	/	/	/	/

and prolonged DIF for the *Ascaris*-positive stables. This was not observed for the AsHb antibody reactivity or the percentage affected livers. An identical analysis performed on the data of the *Ascaris* negative stables also did not reveal any significant results (Supplementary Table 2).

4. Discussion

The results obtained in this study clearly indicated an improvement in terms of exposure of the animals to *A. suum* after the implementation of a strategic deworming program, as measured by 2 different serological tests and the percentage of affected livers. A significant decline in anti-*Ascaris* antibody levels was detected in the stables that originally tested positive for *Ascaris*. The 6-weekly treatment program is based on the prepatent period of *Ascaris*, which is approximately 42 days. By applying this program one avoids the development of adult worms and, as a consequence, the secretion of new *Ascaris* eggs in the stables. The decrease in antibodies against the haemoglobin antigen, which is mainly produced by the intestinal stages of *Ascaris*, could be explained by a reduced exposure to the adult worms. On the other hand, the decline in antibody reactivity towards the L3-lung extract suggests that the animals were less exposed to the migratory larvae, which can also explain the significant decrease in liver white spots. The amount of eggs

present in the stable determines the level of exposure of the animals. These eggs are either excreted during previous fattening rounds or the fattening round itself. It typically takes a couple of weeks for *Ascaris* eggs to fully embryonate and become infectious, at least under laboratory conditions. The fact that the decreased exposure, as measured by serology and liver white spot counts, was already visible after one round of strategic deworming, without the implementation of additional cleaning procedures between the rounds, suggests that the observed reduction in seroconversion and damage to the livers was a result of a reduced auto-infection, i.e. eggs excreted by the animals themselves.

Despite the improvement in seroconversion over time in the seropositive herds of group 1, there were no significant changes in the technical performance parameters. Although several studies have shown an effect of *Ascaris* on ADF and FCR (Bernardo et al., 1990; Hale et al., 1985; Kipper et al., 2011; Lassen et al., 2017; Pedersen et al., 2002; Stewart and Hale, 1988; Stewart et al., 1972; Urban et al., 1989; van Krimpen et al., 2010; Zimmerman et al., 1973) other studies were not able to correlate *Ascaris* infections with productivity, even in controlled experiments (Urban et al., 1989; Boes et al., 2010). Importantly, however, the *Ascaris*-positive stables in the current study were selected based on serology, which has shown to be more sensitive to detect exposure to *Ascaris* in comparison to faecal egg counts (Vlaminck et al., 2012). Other studies that looked at the effect of *Ascaris* on production mostly used faecal egg counts as a parameter to select *Ascaris*-positive herds. Given the large number of commercial herds and animals included in the current study as well as the low sensitivity of both faecal egg counts and worm counts to detect *Ascaris* infections (Vlaminck et al., 2014), it was practically impossible to include these parameters in the current study. As a consequence, perhaps the infection intensities in the stables included in the current study were too low to observe an improvement in the technical performance parameters. It would therefore be interesting to repeat this type of study in stables with an initial higher infection level. Other factors that influence pig production, such as type of feed and housing (Hale et al., 1985; Zimmerman et al., 1973) and bacterial and viral infections (Bernardo et al., 1990) may potentially also mask the effects of an *Ascaris* infection. In the current study, we monitored the evolution of the infection levels and technical performance parameters over time within each farm rather than comparing between farms. Although this approach allowed us to keep certain parameters constant over time, such as management practices, housing and feed, a drawback is that the study relies on the data collected during one historical control round. For this reason, factors such as the potential seasonal impact on the parasite transmission can not be accounted for.

Vlaminck et al. (2015) and Martinez-Pérez et al. (2017) previously observed significant correlations between anti-*Ascaris* antibody levels and technical performance parameters in fatteners. In the current study, hierarchical linear mixed models indicated that the level of L3-Lung antibody reactivity was a significant predictor of decreased ADG, increased FCR and prolonged DIF for the animals housed in the *Ascaris*-positive stables. The results basically indicated that a theoretical increase of 1 ODR on the L3-Lung test was indicative for a drop in ADG with approximately 128 g, an increase of the numbers of days in fattening with 21 days and an increase of FCR with 0.38. A similar trend was also observed for the AsHb ELISA test and the percentage of livers showing white spots, although these did not reach significance. However, the differences in ODR on the L3-lung test before and after the implementation of the strategic deworming on the *Ascaris* positive herds ranged between 0.027 and 0.678, indicating that the expected impact of the deworming on the technical performance parameters is likely to be lower.

In conclusion, the outcome of this study showed that a strategic treatment program with fenbendazole oral suspension was effective to reduce the exposure of fatteners to *A. suum* and that this effect was measurable by serology. Furthermore, the results also showed that anti-

Ascaris antibody levels were a significant predictor of decreased technical performance of the animals. This association has previously also been found by Vlaminck et al. (2015) and Martinez-Perez et al. (2017). However, although a high anti-*Ascaris* ODR result indicates a potential loss in productivity due to *Ascaris*, the implementation of strategic deworming does not necessarily mean that the productivity will significantly improve. Many factors influence productivity and if some of these are sub-optimal, the positive effect of reducing *Ascaris* infections levels might still be masked. Despite this, serology still proves to be a useful tool to assess *Ascaris* infection levels in fatteners.

Declaration of interests

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2019.03.006.

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