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Research paper

Evaluation of serology to measure exposure of piglets to *Ascaris suum* during the nursery phase

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ABSTRACT

The aim of this study was to evaluate whether serology can be used to measure exposure of piglets to *Ascaris suum* during the nursery phase. Experimental infection studies were performed in which 7 groups of 10 piglets of 4 weeks of age were orally infected with either 10, 20, 40, 60, 80, 100 and 500 *A. suum* eggs/day during 7 consecutive weeks, mimicking a nursery phase in an *A. suum* contaminated environment. Serum was collected on a weekly basis to monitor seroconversion on 2 ELISA tests based on the antibody recognition of either a haemoglobin protein purified from the pseudocoelomic fluid of adult *A. suum* or a water-soluble complete homogenate of the 3rd stage larvae isolated from the lungs (L3-lung). A dose-dependent seroconversion was measurable with the L3-lung ELISA starting from 4 weeks post-infection onwards, whereas this was not measurable with the haemoglobin-based test. After 7 weeks, equivalent to the end of the nursery phase, the L3-lung ELISA showed a 99% specificity and a 90% sensitivity to detect exposure of piglets to *A. suum,* with a minimum infection level of 20 *A. suum* eggs per day. To further evaluate the test under field conditions, a seroprevalence study was performed by sampling 10 piglets on 68 different nursery farms in Belgium. The results showed that for 38% of the farms analysed all piglets tested seronegative, whereas for the remaining 62% of the farms the percentage seropositive piglets ranged from 10 to 100%. This indicates contamination of the nursery facilities with *A. suum* eggs. In conclusion, the outcome of this study shows that serology can be used to measure exposure of nursery piglets to *A. suum*, thereby providing an additional tool in the control of this widespread parasite.

1. Introduction

Ascaris suum is a widespread parasitic nematode that infects pigs (Nansen and Roepstorff, 1999; Roepstorff et al., 1998). Despite the availability of effective anthelmintics, prevalences of *A. suum* on farm level remain high throughout the world (Thamsborg et al., 2010). *Ascaris* infections can occur in all age groups of pigs, but housing, production system (indoor vs outdoor) and management factors often determine which age group shows the highest intensity of infection (Boes et al., 2010). In most cases *Ascaris* infections are subclinical of nature without clear disease symptoms (Vlaminck et al., 2014). In the case of highly exposed animals the main clinical symptoms are respiratory problems (Urquhart et al., 1996) caused by the larvae migrating through the lungs (Boes et al., 2010).

Several studies have shown that exposure to *Ascaris* can result in significant losses for the pig producers. These losses can be directly caused by *Ascaris*, such as reduced growth and feed conversion (Hale et al., 1985), costs of anthelmintics, condemnation of affected livers (Perez et al., 2001) and lower carcass quality (Kanora, 2009). In addition, exposure to *Ascaris* can also indirectly affect the health status of the animals due to the immunomodulatory effect of this parasite, making the animals more susceptible to co-infections and/or interfere with vaccine efficacy (Steenhard et al., 2009).

Ascaris infections can be diagnosed post-mortem by the presence of worms in the small intestine or the presence of white spots on the livers (Vlaminck et al., 2012) or *in vivo* by detecting eggs in faecal samples (Roepstorff, 1998). Unfortunately, all these approaches have severe shortcomings. First, most pigs will expel the L4 larvae when they arrive back in the small intestine after the hepato-tracheal migration. Therefore, only a small number of pigs exposed to *Ascaris* will actually harbour egg-producing adults (Masure et al., 2013; Roepstorff et al., 1997). As a result of this strong over-dispersion, diagnosis based on the presence of worms or eggs in the faeces could severely underestimate true exposure and infection levels. Second, liver white spots are scar tissue, which heals and disappears over approximately 35 days (Copeman and Gaafar, 1972). Therefore, the number of liver white spots is only a reflection of recent larval migration and does not always reflect the level of long-term *A. suum* exposure (Nejsum et al., 2009b). Furthermore, the

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required visual assessment of the livers at the slaughterhouse also makes this parameter somewhat subjective.

As an alternative, the use of serology for diagnosis has recently been investigated. Vlaminck et al. (2012) reported on a serodiagnostic test that is based on the antibody recognition of a haemoglobin protein (Hb) produced by adult *A. suum*. Evaluation of the test on commercial farms showed that it had a better sensitivity for the detection of *A. suum* infections in comparison to faecal egg counts (Vlaminck et al., 2012) and that antibody levels measured at the end of the fattening period reflected infection intensity (Vlaminck et al., 2015). Based on the serological result, the deworming strategy applied in the fatteners can subsequently be adjusted if necessary.

Most commonly, in Belgian farms piglets remain suckling in the farrowing stable until 3–4 weeks of age, after which they are weaned and transferred to clean and warm (30 °C) nursery facilities for a duration of 7 weeks. Subsequently, they are transferred to the fattening units at approximately 10 weeks of age, weighing around 22 kg. At arrival, piglets are often routinely treated with anthelmintics to avoid the import of *A. suum* with these animals. As this is currently done without any form of diagnosis, serology could offer an interesting tool in this process. It can provide more information about the level of exposure at the onset of the fattening phase and the timing of a first anthelmintic treatment can be based on the results. The aim of the current study therefore was to investigate whether serology could also be used to measure exposure of piglets to *A. suum* during the nursery phase. For this, a 7 week nursery phase was mimicked during which piglets were trickle infected with different doses of *A. suum* eggs and their serum subsequently analysed on 2 ELISA tests based on the antibody recognition of either a haemoglobin protein purified from the pseudocoelomic fluid of adult *A. suum* worms or a water-soluble complete homogenate of the 3rd stage larvae that migrate through the lungs (L3-lung). In a second phase, the most promising serodiagnostic test was used in a seroprevalence study in Belgium to get more insights into the level of exposure to *A. suum* of piglets during the nursery phase.

2. Material and methods

2.1. Ethics statement

All animal experiments were conducted in accordance with the E.U. Animal Welfare Directives and VICH Guidelines for Good Clinical Practice, and ethical approval to conduct the studies were obtained from the Ethical Committee of the Faculty of Veterinary Medicine at Ghent University (Identification number EC2015/55 and EC2013/143) who approved the document.

2.2. Parasite material

Adult female *A. suum* worms were collected from the intestines of naturally infected pigs at the local abattoir. The eggs were obtained by dissection of the worm uteri and suspended in a 2% potassium dichromate solution ($K_2Cr_2O_7$) as an oxidizing agent, to a volume of 50 ml and placed in a culture flask. The eggs were incubated restricted from light at 27 °C approximately 30 days until fully embryonated and then used to infect the piglets.

Infective L3 lung larvae were obtained by removing the lungs from 10 week old pigs experimentally infected with 250 000 eggs 7 days post infection. The collected lung tissue was cut using scissors into pieces of approximately 0,5–1 cm^3 and poured upon a Baermann filter filled with phosphate buffered saline (PBS) at 37 °C and kept overnight, where the larvae were allowed to migrate out of the tissue (Urban et al., 1981). After collection at the bottom of the funnel, the parasites were washed 3 times with PBS, grounded in liquid nitrogen and the pellet resuspended in PBS with a protein inhibitor mix (Vlaminck et al., 2016). After centrifugation, the supernatant was collected and the protein concentration determined by the Bicinchoninic acid assay (BCA).

2.3. Animal experiments

All piglets used in the infection experiments came from the same conventional farrowing-to-finish farm and were randomly selected from different litters. All animals were 4 weeks of age at the onset of the trial, weighing approximately 7.8 kg in average. In a first study, 3 groups (n = 10) of 4-week old pigs were individually infected on a daily basis during 7 consecutive weeks by oral application of the egg solution using a 2 ml syringe. The piglets received an infection dose of approximately 10, 100 or 500 eggs/day. One group of 10 naïve piglets served as a negative control group. Blood was collected every week, using 5 ml serum tubes. The first collection took place before any infection occurred, at the age of 4 weeks. At the end of the study, at an age of approximately 11 weeks, all animals were euthanized by electrocution. Intestinal content of each piglet was collected and sieved over a 220 µm sieve in order to determine the number of *Ascaris* worms present. A second identical study was subsequently performed with 4 groups (n = 10) of 4-week old pigs that were individually orally infected using a probe, with approximately 20, 40, 60 or 80 eggs/day for 7 consecutive weeks. In addition to the number of worms present in the intestines, the number of liver white spots were also quantified in the second study, based on macroscopic assessment.

Finally, an additional 100 individual serum samples were collected from *A. suum* naïve piglets at an age of 10 weeks. Three week old piglets born from worm-free sows, as determined by ELISA, were housed in a new stable that had not been used before. In addition, the animals received three treatments with flubendazole in the drinking water during the 7 weeks of housing to further avoid the development of adult worms and the subsequent contamination of the stable with eggs. All piglets had access to feed and water *ad libitum* throughout the experimental period.

2.4. Analysis of the serum samples

Serum samples were individually analysed on two different ELISA tests which were based on the haemoglobin purified from the pseudocoelomic fluid from adult *A. suum* worms and the water-soluble complete homogenate from L3 larvae migrating through the lungs (L3-Lung ELISA). The haemoglobin ELISA was performed essentially as previously described (Vlaminck et al., 2012). For the ELISA test based on the L3-lung larvae, plates were coated overnight with 5 µg/ml L3-lung protein homogenate at 4 °C. The wells were washed three times with wash buffer (PBS with 0.05% Tween 20) followed by the addition of blocking solution (150 µl/well of 5% milk powder in PBS) for 2 h at 4 °C. Sera were added at a dilution of 1/200 in wash buffer. After 1 h at room temperature, the conjugate was added (HPRO-conjugated goat anti-pig IgG) at a dilution of 1/12,500 in wash buffer + 5% milk powder. The plates were incubated for 1 h at room temperature. O-phenylenediamine 0.1% in citrate buffer (pH 5.0) served as a substrate and optical density (OD) was measured at 492 nm. In order to compensate for variation between different plates, a negative and positive control sample was included on each plate. The negative control (NC) was a pooled serum sample taken from 10 naive piglets without previous exposure to *A. suum*. The positive control (PC) was a pooled serum sample from experimentally infected piglets with 100 eggs/day. Reactivity to the antigen is shown in ODR (Optical Density ratio) (ODR sample = (OD sample − OD NC)/(OD PC − OD NC)).

2.5. Maternal transfer of anti-Ascaris antibodies

To determine whether anti-*Ascaris* antibodies detected in weaned piglets were maternally derived, serum was collected from 6 sows and 5 of their 1 week old piglets in the farrowing stable and subsequently analysed on the L3-lung ELISA. In addition, serum was collected from 20 piglets 3 days after birth and used to measure both total immunoglobulin levels using the Ig immunocrit method (Vallet et al.,