Veterinary Parasitology 248 (2017) 33–38

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Serological detection of *Ascaris suum* at fattening pig farms is linked with performance and management indices

J.M. Martínez-Pérez[a,b], E. Vandekerckhove[c], J. Vlaminck[c], P. Geldhof[c], M. Martínez-Valladares[b,d,*]

[a] Department of Environmental Biology, Agricultural Chemistry and Biology Group, CMI Roullier, Faculty of Sciences, University of Navarra, 31008 Pamplona, Spain
[b] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad deLeón, Campus de Vegazana s/n, 24071 León, Spain
[c] Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[d] Instituto de Ganadería de Montaña (CSIC-Universidad de León), Finca Marzanas, Grulleros, 24346 León, Spain

ARTICLE INFO

Keywords:
Ascaris suum
Pig
Serodiagnosis
Management practices

ABSTRACT

The aim of the present study was to determine the association between the presence of *Ascaris suum* at fattening pig farms, using different serological methods and the percentage of affected livers at slaughter, with performance and management indices. In total, 21 fattening pig farms from the North of Spain were included in the study. Serum samples were collected from pigs at slaughter and analysed for the presence of anti-*Ascaris* antibodies. For this, two different ELISAs were used. The first was based on the antibody recognition of the *A. suum* haemoglobin (As-Hb) molecule whereas the second test used the total extract of *A. suum* lung stage L3. The serological results were subsequently correlated with the percentage of condemned livers at slaughter, management practices and technical performance parameters including average daily gain (ADG) and feed conversion ratio (FCR). According to the data from the slaughterhouse, 12 out of the 21 farms had livers condemned due to liver white spots. A total of 10 farms (48%) had an average optical density ratio (ODr) exceeding the test cutoff when the As-Hb ELISA was used. This number increased to 18 farms (81%) when using the As-Lung-L3 ELISA. The average ODr of the farms on both ELISAs correlated positively with the percentage of affected livers ($P < 0.01$). Only the average ODr values obtained with the As-Lung-L3 ELISA were positively correlated with the FCR ($P < 0.01$). No correlation was found between percentage of affected livers or serology and the ADG. In relation to management practices, farms with greater than or equal to 50% slatted flooring and that applied the 'all-in/all-out' flow system showed a lower percentage of liver condemnations ($P < 0.01$), lower average ODr results on the As-Lung-L3 ELISA ($P < 0.05$) and lower FCR ($P < 0.01$) compared with those with less than 50% slatted flooring. This study emphasizes that serology is a promising diagnostic tool for diagnosing ascariasis at fattening pig farms. It also supports earlier findings that the presence of *A. suum* can have a significant negative impact on farm productivity and that stable infrastructure or management practices can have a considerable impact on the control of this parasite.

1. Introduction

Ascariasis is a chronic illness in pigs caused by infection with the nematode parasite *Ascaris suum* and is present in traditionally managed indoor herds (Nansen and Roepstorff, 1999; Nissen et al., 2011) as well as industrialized pig farms, especially in old fatteners and sows (Roepstorff, 1997; Roepstorff et al., 1999, 2011). Ascariasis can also produce acute infections, where coughing is the first clinical sign to appear (Ducatti et al., 2017). High prevalence data have been reported in developed countries like Denmark (25–88%) and Canada (18–82%) (Nejsum et al., 2005; Haugegaard, 2010). In Spain animal-level prevalence on farms ranges from 28.7% (García-Vallejo, 1999) to 48.75% (Frontera-Carrión, 2000), while seroprevalence ranges between 17.6% and 27.3% in Iberian pigs reared under natural outdoor conditions (Sánchez-Murillo, 2003). More recently, lower prevalence values (18.6%) have been detected in wild boars in Spain (Vázquez-Rodríguez, 2015). In fact, ascariasis has a cosmopolitan distribution with an individual prevalence between 50% and 75% according to liver condemnations (Ortega-Mora, 1998). Since the presence of ascariasis on farms is associated with inadequate sanitation, it is essential to maintain clean housing facilities combined with prophylactic treatments to control possible infections (Vlaminck et al., 2014).

* Corresponding author at: Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain.
E-mail address: mmarva@unileon.es (M. Martínez-Valladares).

http://dx.doi.org/10.1016/j.vetpar.2017.10.009
Received 7 June 2017; Received in revised form 11 October 2017; Accepted 19 October 2017

During infection, larvae hatch in the intestine and migrate to the caecum and proximal colon where they penetrate the mucosa and arrive at the liver through the portal system (Holland, 2013). Larval migration in the host lung tissue induces pulmonary distress in pigs (Dold and Holland, 2011) and, due to this migration, pneumonia, pleuritis and/or allergic asthma can take place in infected animals (Vlaminck et al., 2014; Peng and Criscione, 2012). Furthermore, the presence of adult worms in the intestinal tract can lead to chronic malabsorption accompanied by villus atrophy in the intestine of pigs (Crompton, 2001; Pérez et al., 2001; Vázquez-Rodríguez, 2015). Consequently, ascariasis in pigs has a significant economic impact. The negative consequences of this infection have been correlated with decreased average daily growth (ADG), a higher feed conversion ratio (FCR) and increased liver condemnations (Roepstorff, 2003; Wieczorek et al., 2006; Knecht et al., 2012).

Diagnosis of *A. suum* infections in pigs is not straightforward since infections are usually sub-clinical and possible signs like respiratory distress and coughing are not specific enough to be automatically linked to ascariasis (Thamsborg et al., 2013). At present, the diagnosis of *A. suum* infection is carried out *in vivo* by the detection of eggs in faeces by means of a coprological method or by evidence collected post-mortem such as the presence of adult worms in the small intestine (Bernardo et al., 1990; Frontera et al., 2004) or white spot lesions on the liver (Pérez et al., 2001). However, due to the low sensitivity of the coprological methods, serodiagnostic tests could present an alternative for the *in vivo* detection of *A. suum* infection. A number of Enzyme-Linked Immunosorbent Assays (ELISAs) have been tested in pigs using protein extracts or excretory–secretory products of adult stage worms or larvae (Urban and Romanowski, 1985; Lind et al., 1993; Yoshihara et al., 1993; Bøgh et al., 1994; Roepstorff, 1998; Frontera et al., 2003). In 2012, Vlaminck et al. (2012) reported on a serological test based on the immune recognition of the *A. suum* haemoglobin (As-Hb) antigen (Vlaminck et al., 2011). Using sera from experimentally infected pigs, this ELISA had a diagnostic sensitivity and specificity of 99.5% and 100%, respectively. The same group also evaluated a different serological test based on the recognition of water soluble protein extract from the lung stage L3 s of *A. suum* (As-Lung-L3 ELISA) (Vandekerckhove et al., 2017).

The aim of the present study was to determine the association between the presence of *Ascaris suum,* using two different serological methods and the percentage of affected livers at slaughter, with performance and management indices at commercial fattening pig farms in Northern Spain.

2. Materials and methods

2.1. Selection of the farms

The present study was carried out at 21 randomly selected fattening pig farms from the North of Spain. On these farms animals were fattened from approximately 10 weeks of age for 12 weeks till slaughter weight was reached (90–100 kg). The mean number of animals per farm was 2088, divided into an average group size of 174 pigs, and on all farms animals were crossbred Belgian Landrace/Pietrain pigs.

2.2. Farm health and management survey

At each farm a survey was conducted in order to analyse different health and management practices that could have an effect on the presence and/or establishment of *A. suum* infections. The questions were related to: i) the kind of anthelmintic used for deworming, the frequency of application (0: once; 1: twice or more) and route of administration (only 19 farms); ii) management practices, such as type of flooring (0: less than 50% slatted flooring [< 50%]; 1: greater than or equal to 50% slatted flooring [≥50%]), type of feed (pellet forms or pulverized premix) and application of the 'all-in/all-out' (AI/AO) system (0: no; 1:yes); and iii) the presence of clinical signs such as coughs and their frequency (0: never, 1: infrequent, 2: frequent).

2.3. Collection of blood samples and analysis of serum samples

Between 10 and 15 serum samples were collected from the same homogeneous batch of fattening pigs on each farm, resulting in a total of 219 individual blood samples. These blood samples were randomly taken on the slaughter line using 5 ml Venoject® vacuum tubes, without anticoagulant. After collection, they were allowed to clot for an hour at 37 °C and refrigerated for another hour. Finally, samples were spun at 4000 × *g* for 5 min at 4 °C and serum was collected and frozen at −20 °C until used.

The sera were used to test for total IgG antibodies against the As-Hb and the As-Lung-L3 antigen on two separate ELISAs. The As-Hb- and As-Lung-L3 ELISAs were performed following the protocols described in Vlaminck et al. (2012) and Vandekerckhove et al. (2017) respectively. The results regarding reactivity to the antigens are shown as the average Optical Density ratio (ODr) [ODr sample = $(OD_{sample} - OD_{NC})/(OD_{PC} - OD_{NC})$] per farm. The cut-off score was previously established to be ODr = 0.500 for the As-Hb ELISA and ODr = 0.250 for the As-Lung-L3 ELISA. Positive samples had ODr values higher than the cut-off score previously established as 0.500 for the As-Hb ELISA and 0.250 for the As-Lung-L3 ELISA.

2.4. Technical performance parameters and post-mortem examination

In those batches from which the serum samples were collected, ADG was calculated as weight gain in kg/days and FCR as kg feed/kg weight gain by each farm veterinarian. The daily feed intake was calculated after weighing the food every day and correcting for feed residues collected on the following day.

The liver lesions were evaluated by official meat inspection personnel during necropsy in the slaughterhouse. The percentage of affected livers was calculated as the percentage of livers with at least one white spot lesion in each batch of pigs sampled.

2.5. Statistical analysis

The ODr data were expressed as arithmetic mean with standard deviation (SD). The data were analysed using the Statistical computer Package for Social Science (SPSS). The Kolmogorov-Smirnov test was carried out to determine if data were normally distributed. The statistical relationship between the different variables (percentage of affected livers, serology results and technical performance parameters) was calculated using the non-parametric Spearmańs rank correlation test. The non-parametric Mann-Whitney *U* test was used to evaluate significant differences ($P < 0.05$) between farms.

Moreover, multivariate linear regression analyses were conducted to assess the associations between the dependent variable (percentage of affected livers, ADG and FCR) and the independent variables (ODr using both ELISAs, frequency of anthelmintic administered, percentage of slatted flooring and application of the AI/AO system). A forward step-wise selection procedure was used to select the variables that were significantly ($P < 0.10$) associated with the different independent variables tested.

All data were analysed using SPSS 11.5 for Windows.

3. Results

3.1. Descriptive analysis of serological, production and slaughterhouse data

An overview of the percentage of affected livers, results for *Ascaris* serology, the cough frequency index and technical performance parameters (ADG and FCR) per farm is reported in Table 1. In 12 out of 21 farms affected at least one liver with white spot lesions was detected at