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Serological responses to *Ascaris suum* adult worm antigens in Iberian finisher pigs

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Abstract

Adult *Ascaris suum* were dissected to obtain different worm components (body wall, body fluid, ovaries, uterus and oesophagus) which were used as antigens when testing 95 sera of naturally *A. suum*-infected Iberian pigs by enzyme-linked immunosorbent assay (ELISA) and Western blot (WB). Pigs with patent *Ascaris* infections had significantly lower ELISA optical density values than pigs without adult worms when using the body fluid and the body wall as antigens. A poor negative correlation was found between adult intestinal worm burden or eggs in faeces and specific antibody responses, measured by ELISA and WB using all antigens. By WB, the recognition of specific bands was variable, but three groups of bands with molecular weights of 97 kDa, 54–58 kDa and 42–44 kDa were generally recognized by sera from naturally infected pigs as well as from hyperimmunized pigs when using the five antigen extracts. The ELISA and WB techniques may be used for immunodiagnosis, using somatic adult worm antigens, to declare young pigs to be *Ascaris*-free but cannot be used for individual *Ascaris*-diagnosis in adult Iberian pigs.

Introduction

Ascaris suum is one of the most common intestinal nematode parasites in pigs, having a worldwide distribution (Urban, 1986). Patent infections are normally diagnosed by the presence of intestinal worms at slaughter or by eggs in faeces. However, these methods do not provide any information about the percentage of pigs with migrating larvae or pigs with past experience of infection. Furthermore, coprological analysis may give false-negative results due to the presence of immature worms or single sex infections (Jungersen *et al.*, 1997) or false-positive results due to coprophagia (Boes *et al.*, 1997).

Migrating *A. suum* larvae stimulate a well-documented immune response in pigs (Taffs, 1964; Roneus, 1966; Eriksen, 1981). The serological response has been studied using indirect agglutination (Taffs, 1964; Richharia *et al.*, 1975), indirect immunofluorescence (Stevenson & Jacobs, 1977; Rhodes *et al.*, 1977), indirect radioimmunoassay (Rhodes *et al.*, 1981) and more recently by enzyme-linked immunosorbent assay (ELISA) (Lind *et al.*, 1993; Bøgh *et al.*, 1994; Furuya *et al.*, 1995; Roepstorff, 1998). Most studies have reported a significant correlation between the level of serum antibody and the number of white spots in the liver, using larval antigens, egg antigen and even adult extract or body fluid antigens (Lind *et al.*, 1993; Bøgh *et al.*, 1994; Furuya *et al.*, 1995). However, a correlation between serum antibody levels and the number of adult worms has not been established. Therefore, there is a need to investigate the diagnostic value of using different adult worm antigens to demonstrate the presence of adult *A. suum* in the small intestine.

The Iberian pig is an old traditional breed raised on acorn pastures in the south-west of Spain. This particular production system results in meat products of high quality. Unfortunately, the prevalence of *A. suum* in this outdoor rearing system is considerable when measured by coprological methods (37%) (Pérez *et al.*, 1991). Therefore, the evaluation of other techniques for diagnosing this parasitic disease is very important.

The present study describes the serological response of naturally exposed Iberian finisher pigs to different adult *A. suum* extracts. The study furthermore evaluates the use

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of ELISA and immunoblotting techniques as diagnostic tools for *A. suum* infections in Iberian pigs, compared to standard coprological methods and post mortem findings.

Materials and methods

Antigens

Adult female *A. suum* were collected from the intestinal contents of naturally infected pigs at a local slaughterhouse, washed with tap water and dissected. The following body parts were preserved as antigens: body wall antigen (BWA), body fluid antigen (BFA), ovarian antigen (OVA), uterine antigen (UTA) and oesophageal antigen (OSA). Soluble extracts of the different tissues were prepared by ultrasonic vibration and then centrifuged (2500 g for 30 min), the supernatant filtered through a 0.28 μm filter, and stored at -80°C until further use in Western blotting and ELISA.

Animals

Blood and faeces were collected from 95 Iberian pigs from 14 different farms in the Extremadura region, south-west Spain. All pigs were 14–15 months old and weighed 150–180 kg at slaughter. Faecal egg counts were performed using flotation and sedimentation techniques followed by a McMaster counting method (Hendrix, 1998). Intestinal worms were collected at slaughter by sieving the intestinal contents through 0.125 mm mesh sieve.

Hyperimmune sera against BWA, OVA, UTA, OSA and BFA antigens were obtained from ten 3.5-month-old *Ascaris*-free Iberian pigs (two pigs per antigen administered) from the Regional Selection Authoctonous Breed Centre (Extremadura region, Spain). Each pig was immunized with 10 μg kg^{-1} of antigen on days 0, 15 and 30. The sera were pooled and served as negative control serum (day 0) and positive control serum (day 45).

SDS–PAGE

Sodium dodecylsulphate–polyacrylamide 12% gel electrophoresis (SDS–PAGE) was carried out as described by Laemmli (1970) using a Protean® II cell (Bio Rad). Each antigenic extract was diluted with sample buffer and heated at 100°C for 5 min. The gels were stained with Coomassie blue staining and silver staining kit (Bio Rad) according to instructions of the manufacturer. Molecular weights (MW) were determined by comparison with standards markers (M-4038, Sigma).

Western blot

SDS–PAGE was performed as above except that antigen samples were applied as a single overlay on top of the stacking gel. Following completion of the SDS–PAGE run, the protein bands were transferred onto a 0.45 μm pore size nitrocellulose membrane using a semi-dry transfer cell apparatus (Bio Rad Mod. Transblot SD). The transfer was performed at 3 mA cm^{-2} for 1.5 h. The membrane was blocked for 1 h at 37°C with TRIS-NaCl buffer pH 8.2 (TBS) containing 5% non-fat dry milk. Immunorecognition was then performed by incubation for 1 h at 37°C with sera diluted at 1:8 in TBS with 0.05% Tween 20 (TBS-T) containing 1% non-fat dry milk. The membrane was then washed with TBS-T (4 × 10 min) and incubated for 1 h with affinity-isolated peroxidase-conjugated rabbit anti-pig immunoglobulins (Sigma, A-7042), at 1:1000 in TBS-T, containing 1% non-fat dry milk. In order to visualize bands, the membranes were washed with TBS-T (3 × 5 min) and incubated with the substrate (3 mg of 4-chloro-1-naphthol; 0.4 ml of methanol and 0.35 μl of H_2O_2 per ml of TBS) at 50°C. The reaction was stopped by exhaustive washing with distilled water.

ELISA

High binding plates were coated overnight at 4°C with 100 μl antigen extracts in phosphate-buffered saline (PBS, pH 7.2) at 5 μg ml^{-1}. Plates were blocked by adding 200 μl of 5% non-fat dry milk in PBS and incubated for 30 min at 37°C. After washing, 100 μl of serum samples with 1% non-fat dry milk diluted 1:400 in PBS-T were added in duplicate wells, and incubated at 37°C for 30 min. Once the plates were washed, 100 μl of an affinity-isolated peroxidase-conjugated rabbit anti-pig immunoglobulin G (Sigma, A-7042), diluted 1:10000 in PBS-T was added per well and incubated for 30 min at 37°C. After washing, 1.8 mg ml^{-1} of o-phenylene-diamine (Sigma, P-1063) and 0.0003% hydrogen peroxidase were incubated in phosphate-citrate buffer pH 5.0. The plates were left for a further 30 min in a dark environment, after which the absorbance was measured at 450 nm. All measurements were performed in duplicate. Positive control sera and negative control sera were applied to all plates and the optical density values (OD) were corrected for plate-to-plate variation.

Data analysis

Associations between the different parameters were estimated using Pearson's correlation coefficient. Differences between groups were analysed using analysis of variance (ANOVA) at a 95% confidence level. All calculations were performed using the SPSS 10.0 software package. Digital images of Western blot results were analysed using the TNImage 3.0.8a program for quantitative examination of antibody responses to three distinct precipitated bands (97 kDa, 42 kDa, 54–58 kDa). The scores were established as percentage of recognition in relation to the corresponding bands of the positive and negative control lanes on the same blot.

Results

Parasitological results

The numbers of pigs with or without *A. suum* adults in the small intestine and eggs in faeces are presented in table 1. Forty pigs (42.1%) harboured adult *A. suum* at slaughter, while 47 pigs (49.5%) excreted eggs in their faeces. Eighteen pigs (18.9%) had false-positive egg counts, while 11 pigs (11.6%) harboured intestinal worms without egg excretion. In 29 pigs (30.5%) both worms and eggs were found, whereas the remaining 37 pigs (38.9%) had neither adult worms nor eggs. Thus the

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Table 1. Numbers of Iberian pigs with/without adult *Ascaris suum* in the small intestine and/or eggs in the faeces.

	Worms present	Worms absent	Total no. of pigs
Eggs present	29	18	47
Eggs absent	11	37	48
Total no. of pigs	40	55	95

number of pigs showing evidence of parasite contact (worms and/or eggs) was 58 (61.1%). Worms and/or eggs were found in pigs from 13 of 14 farms examined.

SDS–PAGE

Ascaris suum antigens showed markedly different electrophoretic banding patterns (fig. 1), although all antigen extracts showed several bands of similar molecular weights between 14 and 190 kDa. The most complex antigens were BWA and UTA, while the body fluid antigen showed the lowest number of bands. UTA and OVA shared most of their fractions, while BFA showed remarkable differences in its electrophoretic pattern when compared with the other antigens.

The most intense protein bands were seen at 190, 90, 64, 54–56 and 42–45 kDa. Some faint polypeptide bands of 15 and 14 kDa were present in UTA and OVA.

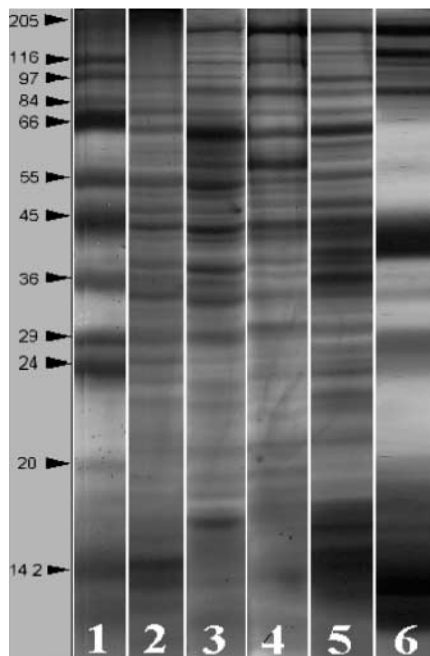

Fig. 1. SDS–PAGE 12% from different antigens of adult *Ascaris suum*. Silver stain. Lane 1, standard markers proteins; lane 2, oesophageal antigen; lane 3, uterine antigen; lane 4, ovarian antigen; lane 5, body wall antigen; lane 6, body fluid antigen. Relative molecular masses (kDa) of standard markers are shown to the left.

ELISA

Total OD levels showed a significant correlation ($P < 0.05$) when all different antigens were used. The highest correlation was observed between the OD of sera when UTA and OVA ($r = 0.93$) were compared, while the lowest correlation was between OVA and OSA ($r = 0.67$). Irrespective of antigen, no significant correlation was observed between worm burdens or egg counts and antibody levels.

No significant differences were found between the number of adult worms in the small intestine and the level of antibodies directed against the five adult antigens (fig. 2). However, in pigs with patent infections, the antibody response using BFA as antigen was significantly correlated ($r = 0.38$) with the number of adult *A. suum* (fig. 2). For each antigen, pigs without adult worms tended to have higher antibody levels than pigs harbouring adult worms, but these differences were only significant for BWA and BFA (table 2). No significant differences could be detected when comparing antibody levels of pigs with and without *A. suum* eggs in the faeces (table 2).

The ELISA test showed a high sensitivity but a poor specificity with regard to the presence of adult worms in the small intestine. Sensitivity was higher (100%) using BWA, OSA and OVA than BFA (92.3%) and UTA (89.74%). Because of the low specificity (0–2.43%), positive predictive values (PPV) were low (46.6–48.8%). When test sensitivity and specificity of the ELISA tests were measured with regard to the presence of worms and/or eggs, sensitivity improved slightly (91.1–100%) while PPV values increased to 71.3% (BWA, OSA and OVA), 70.1 (BFA) and 69.3% (UTA), respectively.

Western blot

The variability of antibody responses for all antigens was mainly quantitative and reflected in the band intensity. However, there were also differences in the

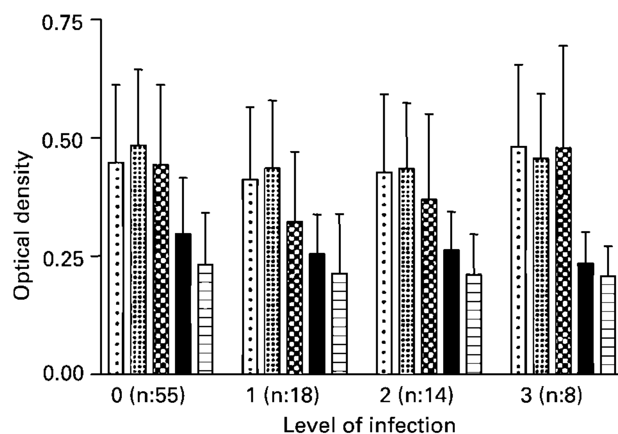

Fig. 2. Mean ELISA responses against five *Ascaris suum* adult antigens in 95 Iberian pigs in relation to the number of adult worms in the small intestine (**0**, not adults; **1**, 1–2 adults; **2**, 3–8 adults; **3**, >8 adults). ⊡, uterine antigen; ▦, ovarian antigen; ▩, body fluid antigen; ■, body wall antigen; ▤, oesophageal antigen.

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Table 2. Mean ELISA responses (optical density) ± standard deviation of 95 Iberian pigs to different *Ascaris suum* antigens in the presence or absence of adult worms in the small intestine and eggs in faeces.

	No. of samples	Antigens				
		OVA	BWA	BFA	OSA	UTA
Worms present	40	0.44 ± 0.13	0.25 ± 0.07*	0.37 ± 0.18*	0.21 ± 0.10	0.43 ± 0.16
Worms absent	55	0.48 ± 0.16	0.29 ± 0.12*	0.44 ± 0.17*	0.23 ± 0.10	0.45 ± 0.16
Eggs present	47	0.47 ± 0.16	0.27 ± 0.11	0.41 ± 0.19	0.22 ± 0.11	0.45 ± 0.18
Eggs absent	48	0.45 ± 0.14	0.28 ± 0.10	0.41 ± 0.15	0.23 ± 0.09	0.43 ± 0.14

* $P < 0.05$
BFA, body fluid antigen, BWA, body wall antigen, OSA, oesophageal antigen; OVA, ovarian antigen; UTA, uterine antigen.

presence or absence of recognition of some bands. Three protein groups of bands, with a MW of 97, 54–58 and 42 kDa were generally recognized by both hyperimmunized and most slaughtered pigs. In comparison to the positive serum in the same Western blot, the medium recognition percentage of the 97 kDa fraction varied from 8.3% using OSA to 34.3% using BFA as antigens in the 95 naturally infected pigs. The 42 kDa protein was recognized in 4.3% using OVA and 108.7% using the BWA. Finally, the recognition percentage of the 54–58 kDa fractions varied from 7.5% using OVA to 51.9% using the BFA as antigens.

As observed by ELISA, the reactivity of sera from slaughtered Iberian pigs with patent *Ascaris* infection was lower than in pigs without adult worms in the small intestine or eggs in faeces using all antigens (fig. 3), but we only found significant differences using the UTA as antigen. In fact, the Pearson's correlation between the recognition of the three bands with the presence of adult worms or the number of eggs in faeces was negative using all antigens.

Discussion

The comparison of antibody responses of pigs against different antigens, measured by ELISA and immunoblotting techniques, showed high correlations among all these antigens. Lind *et al.* (1993) showed a comparable correlation using BFA and antigens from larvae in experimentally infected piglets. These results show that the presence of specific fractions of antigens are not quantitatively important and, according to Lind *et al.* (1993) this also counts for larval antigens. Because antigens from adult worms are easily obtainable, the possibility of using these antigens for the detection of parasitic infection should be considered.

The high sensitivity of the ELISA test contrasts with the lack of specificity when compared to the presence of adult worms in the small intestine. Pigs without adult *A. suum* may have a positive reaction by ELISA and Western blotting because they have been previously sensitized by worms that have been removed by anthelmintic treatment or by migrating larval stages in the liver and lungs that were able to stimulate a strong humoral response. Besides, there is much evidence that naturally or experimentally infected pigs often do not develop an intestinal population of adult worms (Buchwalder *et al.*, 1984; Urban *et al.*, 1988; Eriksen *et al.*, 1992a,b). The correlation between uptake of infective eggs and number of intestinal worms is poor (Urban *et al.*, 1988, 1989; Eriksen *et al.*, 1992b; Stankiewicz *et al.*, 1992; Roepstorff & Murrell, 1997; Roepstorff *et al.*, 1997). Eriksen *et al.* (1992b) obtained a prevalence of 25% patent infections following repeated inoculations. Paradoxically, in these same animals, Lind *et al.* (1993) found the highest immune response in pigs without adult worms by using antigens from adult worm body fluid, hatching fluid of embryonated eggs and excretory/secretory antigens from

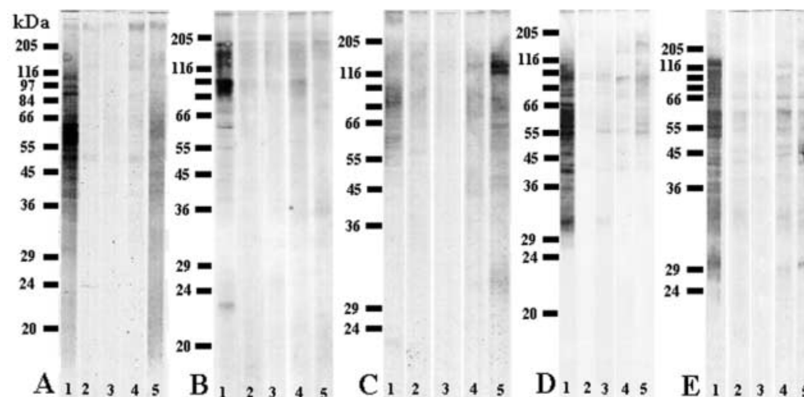

Fig. 3. Western blotting of *Ascaris suum* antigen preparations (A, body wall antigen; B, oesophageal antigen; C, ovarian antigen; D, body fluid antigen; E, uterine antigen) with sera from Iberian pigs (lane 1, hyperimmunized pigs; lane 2, slaughtered pigs with adults and eggs; lane 3, slaughtered pigs with adults and without eggs; lane 4, slaughtered pigs without adults and with eggs; lane 5, slaughtered pigs without adults and without eggs). Relative molecular masses (kDa) are shown to the left of each antigen.

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infective larvae (L2/L3). Furthermore, there is another important aspect to consider. The coprological results are not specific regarding the numbers of adult worms in the small intestine due to a high percentage of false positive and false negative results. Thus, Bernardo *et al.* (1990), Eriksen *et al.* (1992a) and Boes *et al.* (1997) indicated that some *A. suum* egg-excreting pigs in a herd are responsible for the remaining pigs to excrete low numbers of *A. suum* eggs. In this situation, it is extremely difficult to affirm that a positive reaction against a given *A. suum* antigen is not due to a continuous exposure to *Ascaris* eggs. However, Eriksen *et al.* (1992a) found an age-dependent resistance in helminth-free SPF pigs, in which the OD increased with age. If this phenomenon is commonly occurring, it is evident that the ELISA test could not be used for adult pigs, at least not with non-purified antigens, although it could certainly be used for piglets.

In the same way, antigenic recognition by Western blotting was higher in pigs without patent infections. It could thus be speculated that a high level of antibody response against preadult *A. suum* stages is associated with a protective response being able to prevent reinfection and the presence of adults in the intestine. Another possibility is that adult worms in the small intestine result in immunodepression in the host. This can explain the low response of pigs observed by the ELISA test and Western blotting, but there are no conclusive results that support this assumption. Nevertheless, Soares & Mota (1992) isolated two components of 530 and 29 kDa in somatic extracts from adult worms, which produced immunosupression and induction, respectively, in guinea pigs. Stankiewicz & Froe (1995), Barta *et al.* (1986), Komatsu *et al.* (1979) and Crandall *et al.* (1978) also detected some immunosuppressing effects of *A. suum*.

Bøgh *et al.* (1994), Furuya *et al.* (1995) and Eriksen *et al.* (1992a) only indicated that there is a significant positive correlation between the number of white spots and the reactivity of sera measured by ELISA using several antigens. However, there was no significant correlation between ELISA OD and adult worms. These results are in agreement with the present study, where more than 61% of the pigs harboured *A. suum* in the small intestine or eggs in faeces, and more than 92% of the farms were contaminated with *A. suum* adults or eggs, but the ELISA values were not correlated with adult worms nor eggs counts.

There is evidence that the contact of pigs with larval stages that do not develop to adult worms, can induce a positive antibody response against adult worms antigens. Therefore, the use of ELISA tests or Western blotting techniques for the immunodiagnosis of ascariasis using adult worm antigens may be useful in piglets and fattening pigs to guarantee the absence of *A. suum*.

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References

Barta, O., Stewart, T.B., Shaffer, L.M., Huang, L.J. & Simmons, L.A. (1986) *Ascaris suum* infection in pigs sensitises lymphocytes but suppresses their responsiveness to phytomitogens. *Veterinary Parasitology* **21**, 25–36.

Bernardo, T.M., Dohoo, I.R. & Ogilvie, T. (1990) A critical assessment of abattoir surveillance as a screening test for swine ascariasis. *Canadian Journal of Veterinary Research* **54**, 274–277.

Boes, J., Nansen, P. & Stephenson, L. (1997) False-positive *Ascaris suum* egg counts in pigs. *International Journal for Parasitology* **27**, 833–838.

Bøgh, H.O., Eriksen, L., Lawson, L.G. & Lind, P. (1994) Evaluation of an enzyme-linked immunosorbent assay and a histamine release test system for the detection of pigs naturally infected with *Ascaris suum*. *Preventive Veterinary Medicine* **21**, 201–214.

Buchwalder, R., Hiepe, T.H., Ribbeck, R. & Rummel, C.H. (1984) Investigations into the course of experimental *Ascaris suum* invasions in pigs under field conditions. *Helminthology* **21**, 221–229.

Crandall, R.B., Crandall, C.A. & Jones, J.F. (1978) Analysis of immunosuppression during early acute infection of mice with *Ascaris suum*. *Clinical and Experimental Immunology* **33**, 30–37.

Eriksen, L. (1981) Host parasite relations in *Ascaris suum* infection in pigs and mice. Thesis, Royal Veterinary and Agricultural University, Copenhagen.

Eriksen, L., Lind, P., Nansen, P., Roepstorff, A. & Urban, J. (1992a) Resistance to *Ascaris suum* in parasite naive and naturally exposed growers, finishers and sows. *Veterinary Parasitology* **41**, 137–149.

Eriksen, L., Nansen, P., Roepstorff, A., Lind, P. & Nilsson, O. (1992b) Response to repeated inoculations with *Ascaris suum* eggs in pigs during the fattening period. I. Studies on worm population kinetics. *Parasitology Research* **78**, 241–246.

Furuya, T., Yano, Y., Yoshiharu, S., Saeki, H. & Ishii, T. (1995) Diagnosis of hepatic milk spots in swine using enzyme-linked immunosorbent assay with adult female *Ascaris suum* antigen. *Journal of Veterinary Medicine* **48**, 191–195.

Hendrix, C.M. (1998) *Diagnostic veterinary parasitology.* Mosby, Inc., St Louis, USA.

Jungersen, G., Eriksen, E., Nansen, P. & Fagerholm, H.P. (1997) Sex-manipulated *Ascaris suum* infections in pigs: implications for reproduction. *Parasitology* **115**, 439–442.

Komatsu, T., Nishimura, T., Sano, R. & Shinka, S. (1979) *Ascaris suum* suppression of reaginic and haemaglutinating antibody responses in the mouse by crude

https://doi.org/10.1079/JOH2002163 Published online by Cambridge University Press

extract and maintenance fluid. *Experimental Parasitology* **47**, 158–168.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **277**, 680–685.

Lind, P., Eriksen, L., Nansen, P., Nilsson, O. & Roepstorff, A. (1993) Response to repeated inoculations with *Ascaris suum* eggs in pigs during the fattening period. II. Specific IgA, IgG, and IgM antibodies determined by enzyme-linked immunosorbent assay. *Parasitology Research* **79**, 240–244.

Pérez, J.E., Serrano, F.J., Reina, D., Breña, M. & Navarrete, I. (1991) Efecto de la climatología sobre la parasitofauna del cerdo ibérico de montanera en el sur de Extremadura (España). p. 297 in *I Congreso Internacional de las Asociaciones Suboccidental Europeas de Parasitología*. Valencia, J. Aguilar Press.

Rhodes, M.B., Mccullough, R.A., Mebus, C.A., Klucas, C.A., Ferguson, D.L. & Twiehaus, M.J. (1977) *Ascaris suum*: hatching of embryonated eggs in swine. *Experimental Parasitology* **42**, 356–362.

Rhodes, M.B., Staudinger, L.A. & Hart, R.A. (1981) Indirect solid-phase microradioimmunoassay for detection of *Ascaris suum* antibodies in swine sera. *American Journal of Veterinary Research* **42**, 868–870.

Richharia, V.S., Jeska, E.L. & Grover, J.H. (1975) Delayed hypersensitivity response of swine to *Ascaris suum* infection. *Parasitology* **61**, 113–115.

Roepstorff, A. (1998) Natural *Ascaris suum* infections in swine diagnosed by coprological and serological (ELISA) methods. *Parasitology Research* **84**, 537–543.

Roepstorff, A. & Murrell, K.D. (1997) Transmission dynamics of helminth parasites of pigs on continuous pasture: *Ascaris suum* and *Trichuris suis*. *International Journal for Parasitology* **27**, 563–572.

Roepstorff, A., Eriksen, L., Slotved, H.C. & Nansen, P. (1997) Experimental *Ascaris suum* infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs. *Parasitology* **115**, 443–452.

Roneus, O. (1966) Studies on the aetiology and pathogenesis of white spots in the liver of pigs. *Acta Veterinaria Scandinavica* **7**, 97–104.

Soares, M. & Mota, I. (1992) Effect of purified components of *Ascaris suum* extracts on IgE antibody response in guinea pigs. *Brazilian Journal of Medical and Biological Research* **25**, 1137–1140.

Stankiewicz, M. & Froe, D.L. (1995) Unabbreviated *Ascaris suum* immunising infections of piglets lead to immunosupression and increased numbers of intestinal parasites. *Acta Parasitologica* **40**, 152–155.

Stankiewicz, M., Jonas, W. & Froe, D.L. (1992) Patent infections of *Ascaris suum* in pigs: effect of previous exposure to multiple, high doses of eggs and various treatment regimes. *International Journal for Parasitology* **22**, 597–601.

Stevenson, P. & Jacobs, D.E. (1977) *Toxocara* infection in pigs. The use of indirect fluorescent antibody test and in vitro larval precipitate test for detecting specific antibodies. *Journal of Helminthology* **51**, 149–154.

Taffs, L.F. (1964) Immunological studies on experimental infection of pigs with *Ascaris suum* Goeze, 1782: III. The antibody response and acquired immunity. *Journal of Helminthology* **38**, 129–150.

Urban, J.F. (1986) The epidemiology and control of swine parasites: immunity and vaccines. *Veterinary Clinics of North America – Food Animal Practice* **2**, 765–778.

Urban, J.F., Alizadeh, H. & Romanowski, R.D. (1988) *Ascaris suum*: development of intestinal immunity to infective second-stage larvae in swine. *Experimental Parasitology* **66**, 66–77.

Urban, J.F., Gamble, H.R. & Katona, I.M. (1989) Intestinal immune responses of mammals to nematode parasites. *American Zoology* **29**, 469–478.

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