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Maxime Delsart, Jean-Michel Répérant, Chantal Benoit, Edouard Boudin, Jean-François Da-Costa, et al.. Bayesian estimation of the sensitivity and specificity of coprological and serological diagnostic tests for the detection of Ascaris suum infection on pig farms. International Journal for Parasitology, 2024, 10.1016/j.ijpara.2024.04.010. hal-04568350

# HAL Id: hal-04568350 https://enva.hal.science/hal-04568350

Submitted on 7 Jun2024

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International Journal for Parasitology xxx (xxxx) xxx



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# International Journal for Parasitology



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

# Bayesian estimation of the sensitivity and specificity of coprological and serological diagnostic tests for the detection of Ascaris suum infection on pig farms ☆

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#### ARTICLE INFO

Article history: Received 16 January 2024 Received in revised form 26 March 2024 Accepted 22 April 2024 Available online xxxx

Keywords: Parasitism Coprology Serology Diagnostic performance Pig

## ABSTRACT

Coprological and serological diagnostic tests were compared to define the status of a pig farm with regard to Ascaris suum. On each of the 100 farms in France visited for the study, 10 blood samples were taken from pigs at the end of fattening (at least 22 weeks old) and 20 to 30 faecal samples were taken, depending on the category of animals present on the farm (10 sows, 10 piglets aged 10 to 12 weeks and 10 pigs at the end of fattening, aged at least 22 weeks). A SERASCA® ELISA test (Laboratory of Parasitology, Ghent University) was performed on each blood sample (cut-off 0.5) and a coprological analysis on each faecal sample. A Bayesian approach was used to estimate the sensitivity and specificity of the coprological and serological tests. A farm was considered positive if at least one A. suum egg was observed in the faecal samples. With regard to the serological test, various hypotheses were tested in order to define the number of seropositive animals required to consider a farm positive for A. suum. The coprological test has very good specificity in the search for A. suum, whether 20 or 30 samples are taken per farm. However, even with an increase in the number of samples, the sensitivity of this diagnostic approach is very low (less than 30%). On the other hand, the serological diagnostic method, which consists of taking blood samples from 10 animals at the end of fattening, has good sensitivity and seems better suited to defining the status of a farm with regard to A. suum, provided that a farm is considered seropositive only if two out of 10 samples are positive.

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#### 1. Introduction

Swine ascariasis is an infection caused by Ascaris suum, the most widespread and common nematode (helminth) in pigs, affecting growing pigs more frequently than adult pigs (Brewer and Greve, 2019). Clinical signs are often limited. During larval migration through the lungs, asthmatic coughs may be observed, as well as reduced appetite. In rare cases, intestinal occlusions caused by the presence of numerous adult roundworms can lead to an animal's death. However, ascariasis is mainly associated with reduced

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productivity (growth and feed conversion rate) (Thamsborg et al., 2013). This is linked, in particular, to changes in the small intestine, including an increase in intestinal crypt depth and a decrease in villus height (Stephenson et al., 1980).

Larval migration is able to induce pulmonary lesions: petechiae, interstitial pneumonia, bronchiolitis and alveolar oedema. Larvae are also able to form lesions in the liver. Their migration causes eosinophilic infiltration of the migratory tract, as well as the formation of fibrous connective tissue, with the appearance of white spots known as "milk spots", which appear 7-10 days p.i. and disappear approximately 1 month later (Brewer and Greve, 2019).

In high income countries, most pigs are raised in closed buildings on slatted floors. However, changing societal demands are encouraging farmers to diversify their production methods by developing alternative systems. As a result, increasing numbers of pigs are being reared on litter and/or with outdoor access

Please cite this article as: M. Delsart, I.-M. Répérant, C. Benoit et al., Bayesian estimation of the sensitivity and specificity of coprological and serological diagnostic tests for the detection of Ascaris suum infection on pig farms, International Journal for Parasitology, https://doi.org/10.1016/j.ijpara.2024.04.010

<sup>\*</sup> Note: Supplementary data associated with this article.

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https://doi.org/10.1016/j.ijpara.2024.04.010

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(IFIP, 2022). However, these farms are particularly exposed to parasitism, and in particular to *A. suum* (Delsart et al., 2022).

Adult ascarides are easy to identify. They are very large, reaching 40 cm for females and 25 cm for males. However, they are not commonly seen. The diagnosis of infection often depends on the detection of milk spots on livers during autopsy or at the slaughterhouse. This diagnostic approach requires observation of a large number of dead animals to define the status of a farm with regard to A. suum. On live animals, infection can be confirmed using conventional egg identification methods such as standard flotation. Eggs are easily identifiable. They measure 50–70  $\times$  40–60  $\mu$ m and have a thick shell covered with asperities. Serological tests are available to detect antibodies to the parasite's haemoglobin (Vlaminck et al., 2012) or to L3 antigens (Vandekerckhove et al., 2017). Even though these two approaches do not really have the same objectives, one looking for eggs and current infection, the other for antibodies marking past infection, they are regularly used to define the status of a farm with regard to A. suum. However, data on the sensitivity and specificity of these approaches at herd level are scarce, as is information on the sampling required and the positivity criteria. This is why, in the absence of a reference test for diagnosing A. suum in live animals, a Bayesian approach was used in this study to estimate the sensitivities and specificities of serological and coprological diagnostic tests for A. suum at the farm level.

#### 2. Materials and methods

#### 2.1. Herd selection

The study was carried out from June 2020 to February 2022 on pig farms in France. These were alternative-system pig farms using bedding in housing and/or with access to the outdoors (outdoor courtyard, or free range), including farms operating under officially certified schemes identifying quality and origin, such as those for organic production or the French "Label Rouge" quality scheme. To be included, the farm also had to have been producing pigs under the alternative system for at least 18 months, so that the management elements could be stabilised and the biological markers truly reflect the effects of the farm's alternative management.

Twenty-six breeders' organisations or associations and veterinary offices were contacted to recruit farms. Fourteen of these organisations participated by contacting the farmers in their files who could be included in the study. Out of these files, 100 farms were contacted and agreed to take part in the study, of which 80 had both breeding stock and growing weaners (farrow-tofinishers), and 20 only growing weaners (fatteners).

Deworming was practised on at least one category of animal on 92 out of 100 farms. Only 12 out of 80 farrow-to-finisher farms did not deworm sows and 12 out of 100 farms did not deworm growing pigs (Table 1). The most commonly used molecules were benzimidazoles, accounting for 84% and 72% of dewormings of sows and growing pigs, respectively. Avermectin or levamisole were used to deworm sows in 12% and 4% of farms, respectively, and growing pigs in 4% and 25% of farms, respectively.

#### Table 1

Distribution of farms according to the number of treatments against internal parasites (100 farms in France).

	Number of internal deworming treatments (annual for sows and per animal for growing pigs)			
	None	1	2	3 or more
Sows $(n = 80)$ Growing pigs $(n = 100)$	15% 12%	4% 33%	75% 36%	6% 19%

#### 2.2. Animals and sampling on farms

On each farm, and according to the categories of animals present, 10 samples of faeces from sows, 10 of faeces from piglets 10 to 12 weeks old and 10 of faeces from pigs at least 22 weeks old were taken. These samples were taken at the time of defecation or from the ground immediately after defecation. Animals were identified to limit the risk of sampling the same animal several times. The number of faecal samples collected differed according to whether the farm was a farrow-to-finisher or a fattener: in the first case, 30 coprological samples were collected per farm, while in the second, only 20 were collected due to the absence of breeding stock. These samples were individually placed in 180 ml polypropylene coprology jars.

A blood sample was taken from each of 10 pigs aged at least 22 weeks by a jugular vein puncture using evacuated tubes without an additive (Vacuette, Dutscher SAS, Brumath, France). The blood samples were not necessarily taken from the same pigs as the faecal samples. The samples were individually identified and sent to the laboratory for processing. The faeces were transported between the farm and the laboratory at a temperature of +4 °C to +6 °C. Serum was obtained by centrifugation for 10 min at 3500 g and stored at -20 °C until further analysis.

#### 2.3. Coprological tests

The faecal samples, refrigerated at 5 °C until examination, were analysed individually by four trained operators. After homogenisation with a spatula, 3 g of each sample were mixed for 5 min with 42 ml of saturated sodium chloride solution. Once the suspension had been sieved, the chamber of a McMaster cell was then filled with the supernatant. After 10 min, the contents of the McMaster cell chamber were observed at ×100 magnification. The number of occysts or eggs per gram of feces was obtained by multiplying the number of eggs counted in a McMaster chamber by 100. A sample was considered positive if at least one *A. suum* egg was detected.

# 2.4. Serological tests

An individual serological analysis for antibodies to *A. suum* was carried out on all blood samples taken from pigs aged at least 22 weeks. After centrifuging the samples, the sera were sent to Ghent University, Belgium. The total IgG antibodies for *A. suum* were detected using the SERASCA<sup>®</sup> ELISA (Laboratory of Parasitology, Ghent University, Belgium), as described by Vlaminck et al. (2012), using the purified haemoglobin antigen of *A. suum*. This test can detect antibodies from 6 - 8 weeks after infection. The analytical sensitivity and specificity of this test on experimentally-infected pigs are estimated at 99.5% and 100%, respectively, for a positivity threshold corresponding to an optical density ratio (ODr) greater than 0.5 (Vlaminck et al., 2012).

## 2.5. Positivity criteria at the farm level

For each diagnostic approach at farm level, different positivity criteria were tested.

#### 2.5.1. Coprological testing

Copro20: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 20 samples taken from growing pigs (10 piglets aged 10-12 weeks and 10 pigs aged at least 22 weeks -100 farms).

Copro30: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 30 samples

taken from both growing pigs (10 piglets aged 10–12 weeks and 10 pigs aged at least 22 weeks) and 10 sows (80 farms).

# 2.5.2. SERASCA<sup>®</sup> serological testing

SERASCA1: a farm was considered positive if at least one animal was seropositive out of 10 sampled at the end of fattening, when at least 22 weeks old (100 farms).

SERASCA2: a farm was considered positive if at least two animals were seropositive out of 10 sampled at the end of fattening, when at least 22 weeks old (100 farms).

# 2.6. Estimation of sensitivity and specificity of coprological and serological diagnostic tests

#### 2.6.1. Model description

The method described by Rose et al. (2010) was applied to estimate the characteristics of the two diagnostic approaches in the same population and without a gold standard. Sensitivity and specificity at herd level (i.e., respectively, the probability of obtaining at least one positive result in an infected herd and the probability of obtaining only negative results in an uninfected herd, hereafter called herd sensitivity (Se<sub>h</sub>) and herd specificity (Sp<sub>h</sub>)) vary according to the number of samples taken. This is why we differentiated between two populations, one with 100 farrow-tofinisher and fattener farms (with 20 coprological tests on growing pigs per farm) and another with 80 farrow-to-finisher farms (with 30 coprological tests on both growing pigs and sows per farm).

The model parameters are the two sensitivities, the two specificities and the true prevalence. Our data, y = (y11, y10, y01, y00), consisted of the cross-classified test results for the "*n*" farms tested in the population, where *n* was equal to 80 or 100, depending on the population and the coprological test; y11 was the number of farms positive for both tests, y10 the number of farms positive for the coprological test but negative for the serological test but positive for the serological test and y00, the number of farms negative for both diagnostic tests.

y ~multinomial (*n* (p11, p10, p01, p00)),

 $P11 = P (D1+, D2 + ) = \pi [Se1Se2] + (1 - \pi) [(1 - Sp1) (1 - Sp2)]$ 

 $P10 = P (D1+, D2-) = \pi [Se1 (1 - Se2)] + (1 - \pi) [(1 - Sp1) Sp2]$ 

 $P01 = P(D1-, D2 + ) = \pi[(1 - Se1)Se2] + (1 - \pi)[Sp1(1 - Sp2)]$ 

P00 = P (D1-, D2-) = 
$$\pi[(1 - \text{Se1})(1 - \text{Se2})] + (1 - \pi)[\text{Sp1Sp2}]$$

with *P* being the probability; D1 + and D1- the farms, respectively, positive and negative for the coprological test; D2 + and D2- the farms, respectively, positive and negative for the serological test;  $\pi$  the real prevalence of *A. suum*-positive farms; Se1 and Sp1 the sensitivity and specificity of the coprological test; Se2 and Sp2 the sensitivity and specificity of the serological test.

The model described above was used four times to estimate the sensitivity and specificity of the COPRO20 and COPRO30 coprological tests in conjunction with those of the SERASCA1 and SERASCA2 serological tests.

### *2.6.2. Prior distribution of the parameters*

Beta (a;b) or uniform (0;1) distributions were used as a priori probability distributions (=priors) for the parameters of interest (sensitivities, specificities, prevalence). External data from the literature were used to determine these beta distributions.

## International Journal for Parasitology xxx (xxxx) xxx

For serological tests, the individual sensitivity and specificity values proposed by Vlaminck et al. (2012) were used to determine an estimate of herd sensitivity and specificity with 10 samples per batch. In SERASCA1, the equations used to obtain herd sensitivity and specificity from individual sensitivity and specificity were Seh =  $1 - (1 - Se)^{10}$  and Sph =  $Sp^{10}$ , respectively. In SERASCA2, the equations used to obtain herd sensitivity and specificity from individual sensitivity and specificity were Seh =  $1 - (1 - Se)^{10} - (9 \times Se \times (1 - Se)^9)$  and Sph =  $Sp^{10} + (9 \times (1 - Sp) \times Sp^9)$ , respectively.

For coprological tests, there were far fewer data available, particularly with regard to sensitivity, which is difficult to estimate, but which nevertheless appears to be low at the individual level (Roepstorff and Nansen, 1998; Vlaminck et al., 2012; Vlaminck and Geldhof, 2013). To define the distribution mode, we used an individual sensitivity value of 5%. The equation Seh =  $1 - (1 - Se)^n$  was used to obtain the Se<sub>h</sub> (n = 30 for COPRO30 and n = 20 for COPRO20). However, the a priori distributions used were not very informative.

In contrast, the specificity of coprology was considered to be high, as the *A. suum* egg has a specific profile not easily confused with another egg.

For prevalence, the information in the literature varies, particularly because different types of test are used (coprological tests, serological tests, slaughterhouse liver examinations). The a priori distributions used were therefore uniform distributions (0;1).

The parameters of the beta distributions for each parameter (sensitivity and specificity of each diagnostic approach and prevalence) were defined by integrating the most probable value and the lowest value (95% confidence) of the parameter in the EpiR beta buster calculator at https://shiny.vet.unimelb.edu.au/epi/beta.buster/.

### 2.6.3. Model implementation

The models were run using the WinBUGS freeware program (Lunn et al., 2009). Parameter estimates were based on analytical summaries of 10,000 iterations of the Gibbs sampler with a burn-in phase of 1000 iterations (not included in the estimates). Three parallel chains were run with different starting values chosen at random from uniform distributions (0.3;1) for the sensitivities of the two diagnostic tests, (0.5;1) for the specificity of the serological test, (0.8;1) for the specificity of the coprological test and (0.1;1) for the prevalence.

### 2.6.4. Assessment of convergence

RStudio<sup>®</sup> software (RStudio<sup>®</sup>, Inc.) was used to evaluate the convergence of the resulting Markov chain Monte Carlo (MCMC) simulations. Successive trace plots were examined to detect slow mixing. The Heidelberger (Heidelberger and Welch, 1983) and Raftery and Lewis (Raftery and Lewis, 1992) tests were applied to validate the convergence of single chains. The Gelman-Rubin convergence diagnostic (Brooks and Gelman, 1998) was carried out to assess the convergence of the three parallel chains and autocorrelations.

# 2.6.5. Sensitivity analysis

In order to assess the influence of the a priori distributions on the estimated parameters of the model, different options were used with different levels of information in the priors. Table 2 describes the different options used according to the level of information.

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#### M. Delsart, J.-M. Répérant, C. Benoit et al.

International Journal for Parasitology xxx (xxxx) xxx

#### Table 2

Description of the different priors used in the model to estimate the sensitivity and specificity of coprological and serological diagnostic tests for Ascaris suum in pigs.

	Parameter	Information level	Assumption	Distribution
Option A	Seh COPRO20 test	Prior not very informative	> 0.3, mode = 0.65	Beta (4,124, 2.682)
	Sp <sub>h</sub> COPRO20 test	Moderately informative prior	> 0.8, mode = 1	Beta (13.425, 1)
	Seh COPRO30 test	Prior not very informative	> 0.3, mode = 0.78	Beta (3.14, 1.604)
	Sp <sub>h</sub> COPRO30 test	Moderately informative prior	> 0.8, mode = 1	Beta (13.425, 1)
	Seh SERASCA1 test	Informative prior	> 0.8, mode = 0.95	Beta (21.202, 2.063)
	Sph SERASCA1 test	Informative prior	> 0.7, mode = 0.81	Beta (40.756, 10.325)
	Seh SERASCA2 test	Moderately informative prior	> 0.7, mode = 0.9	Beta (15.034, 2.559)
	Sp <sub>h</sub> SERASCA2 test	Moderately informative prior	> 0.8, mode = 0.97	Beta (17.298, 1.504)
	Prevalence	Non-informative prior	Unknown	Uniform (0, 1)
Option B	Seh COPRO20 test	Prior not very informative	> 0.3, mode = 0.65	Beta (4.124, 2.682)
- <b>F</b>	Sp <sub>h</sub> COPRO20 test	Prior very informative	> 0.9, mode = 1	Beta (28.433, 1)
	Seh COPRO30 test	Prior not very informative	> 0.3, mode = 0.78	Beta (3.14, 1.604)
	Sp <sub>h</sub> COPRO30 test	Prior very informative	> 0.9, mode = 1	Beta (28.433, 1)
	Seh SERASCA1 test	Prior very informative	> 0.9, mode = 0.99	Beta (34.166, 1.335)
	Sph SERASCA1 test	Informative prior	> 0.8, mode = 0.95	Beta (21.202, 2.063)
	Seh SERASCA2 test	Prior very informative	> 0.9, mode = 0.99	Beta (34.166, 1.335)
	Sph SERASCA2 test	Prior very informative	> 0.9, mode = 0.97	Beta (53.581, 2.626)
	Prevalence	Non-informative prior	Unknown	Uniform (0, 1)
Option C	Seh COPRO20 test	Non-informative prior	Unknown	Uniform (0, 1)
	Sp <sub>h</sub> COPRO20 test	Informative prior	> 0.7, mode = 0.82	Beta (34.987, 8.461)
	Seh COPRO30 test	Non-informative prior	Unknown	Uniform (0, 1)
	Sp <sub>h</sub> COPRO30 test	Informative prior	> 0.6, mode = 0.74	Beta (26.458, 9.945)
	Seh SERASCA1 test	Non-informative prior	Unknown	Uniform (0, 1)
	Sph SERASCA1 test	Non-informative prior	Unknown	Uniform (0, 1)
	Seh SERASCA2 test	Non-informative prior	Unknown	Uniform (0, 1)
	Sph SERASCA2 test	Non-informative prior	Unknown	Uniform (0, 1)
	Prevalence	Non-informative prior	Unknown	Uniform (0, 1)

Option A, moderately informative option.

Option B, very informative option.

Option C, not very informative option.

# 3. Results

# 3.1. Ascaris suum coprological tests

Ascaris suum eggs were detected in 80 samples (i.e. 2.9% of samples) from 18 of the 100 farms selected (positive for COPRO20). Of the 80 farrow-to-finish farms, 16 had at least one positive sample out of the 30 taken (COPRO30-positive farms). Pigs aged at least 22 weeks were most frequently found to have *A. suum* eggs (Table 3), with almost one pig in three having *A. suum* eggs on infected farms. Sows were never the only infected animals on the farms. The average number of *A. suum* eggs per infected sample is highest in sows (3443 +/- 929), but can be very high in some farms in pigs aged at least 22 weeks (2418 +/- 2705) (Fig. 1). It is lower for piglets aged 10–12 weeks (640 +/- 384).

## 3.2. Ascaris suum serological tests

Out of all the samples, 44.5% had an ODr greater than 0.5. Eighty-nine per cent of farms had at least one positive sample (SERASCA1-positive farms), and 80% had at least two positive samples (SERASCA2-positive farms). The average rate of seropositive animals per farm was 44.5% (S.D. = 30,5; median = 40; min = 0; max = 100). The distribution of serological values by farm is shown in Supplementary Fig. S1.

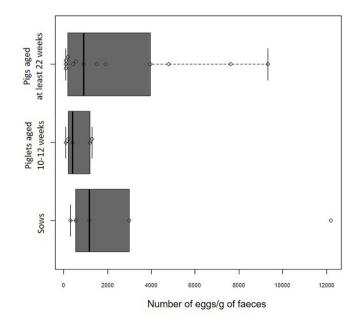
#### Table 3

Proportion of infected animals on farms infected with *Ascaris suum* (80 farms with sows, 100 farms with growing pigs, in France).

	Number of <i>A. suum</i> -positive farms	Average % of <i>A. suum</i> -positive animals	S.D.
Sows	16	9.4	18.8
Growers (10-12 weeks old)	18	5.0	9.9
Finishers ( $\geq$ 22 weeks old)	18	31.1	32.7

3.3. Association between coprological and serological diagnostic test results

No farm was positive for coprological tests but negative for SER-ASCA1 (Table 4), whatever the number of faecal samples taken on the farm (20 samples for COPRO20 and 30 for COPRO30). Only one SERASCA2-negative farm had *A. suum* eggs in faecal samples (in this case, from a single sample taken from pigs at the end of fattening). There was a significant link between the mean ODr per farm



**Fig. 1.** Distribution of the average count of *Ascaris suum* eggs in infected samples per pig farms in France and per production phase (n = 18 farms).

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### M. Delsart, J.-M. Répérant, C. Benoit et al.

#### Table 4

Contingency table of the number of studied farms in France considered positive according to the diagnostic method for the detection of infections by Ascaris suum.

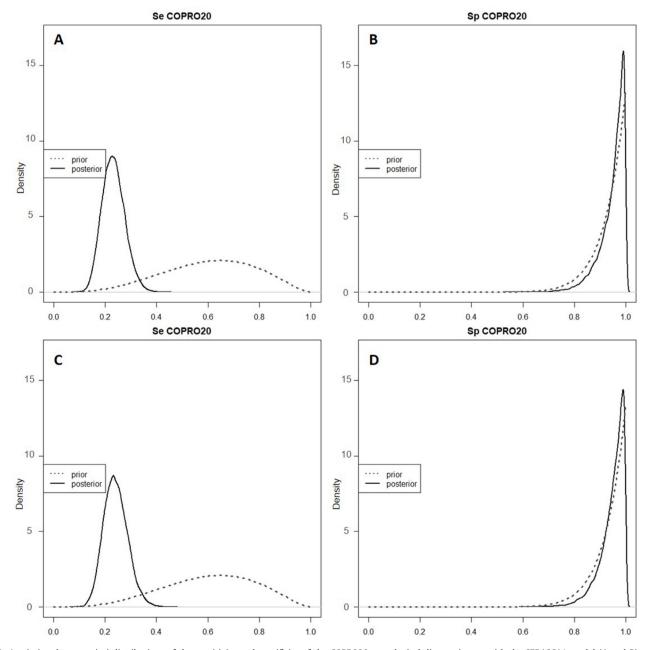
		COPRO20			COPRO30		
		Negative	Positive	Total	Negative	Positive	Total
SERASCA1	Negative	11	0	11	10	0	10
	Positive	71	18	89	54	16	70
	Total	82	18	100	64	16	80
SERASCA2	Negative	19	1	20	17	1	18
	Positive	63	17	80	47	15	62
	Total	82	18	100	64	16	80

COPRO20: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 20 samples taken from growing pigs (10 piglets aged 10–12 weeks and 10 pigs aged at least 22 weeks).

COPRO30: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 30 samples taken from growing pigs (10 piglets aged 10– 12 weeks and 10 pigs aged at least 22 weeks) and 10 sows.

SERASCA1: a farm was considered positive if at least one animal was seropositive out of 10 pigs aged at least 22 weeks.

SERASCA2: a farm was considered positive if at least two animals were seropositive out of 10 fattening pigs.



**Fig. 2.** A priori and a posteriori distributions of the sensitivity and specificity of the COPRO20 coprological diagnostic test with the SERASCA1 model (A and B) and the SERASCA2 model (C and D) for the detection of *Ascaris suum* infection (Option A: moderately informative option; see Section 2.6.5). Se, sensitivity; Sp, specificity. COPRO20: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 20 samples taken from growing pigs (10 piglets aged 10–12 weeks and 10 pigs aged at least 22 weeks). SERASCA1: a farm was considered positive if at least one animal was seropositive out of 10 pigs aged at least 22 weeks. SERASCA2: a farm was considered positive if at least two animals were seropositive out of 10 pigs aged at least 22 weeks.

International Journal for Parasitology xxx (xxxx) xxx

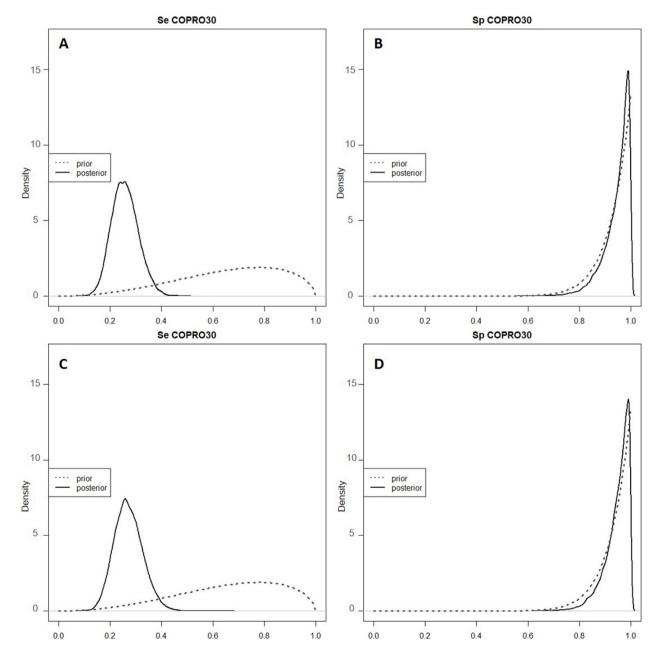
obtained with the SERASCA<sup>®</sup> test and the status assessed by coprological analysis (Student test, P < 0.01). The mean ODr was 0.78 in the coprology-positive farms and 0.48 in the others. However, there was no correlation between the average ODr per farm and the average quantification of parasites in faecal samples.

# 3.4. Bayesian estimation of the sensitivity and specificity of coprological and serological diagnostic tests for Ascaris suum

The MCMC convergence diagnostics obtained for each model (whatever the choice of a priori distributions = priors) indicated good convergence and a sufficient number of iterations, except for the study between COPRO20 and SERASCA1, where the number of iterations of the Gibbs sampler had to be increased to 20,000. The successive plots of the chains indicate good mixing.

The characteristics of the a posteriori distributions for the sensitivity and specificity of coprological and serological diagnostic tests for *A. suum* and of the prevalence according to the informative level of the a priori distributions (priors) are presented in Supplementary Tables S1 and S2.

Estimates of the sensitivities of coprological tests are globally low (Fig. 2), whatever the number of samples taken per farm, the serological test with which they are compared and the level of information provided by the priors. These estimates vary little for COPRO20, from 23.2% [15.3%; 32.3%]<sub>95%</sub> to 24.8% [16.3%; 34.4]<sub>95%</sub> depending on the model and whether the priors are moderately informative (Option A) or very informative (Option B). It should



**Fig. 3.** A priori and a posteriori distributions of the sensitivity and specificity of the COPRO30 coprological diagnostic test with the SERASCA1 model (A and B) and the SERASCA2 model (C and D) for the detection of *Ascaris suum* infection (Option A: moderately informative option; see Section 2.6.5). Se, sensitivity; Sp, specificity. COPRO30: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 30 samples taken from growing pigs (10 piglets aged 10–12 weeks and 10 pigs aged at least 22 weeks) and 10 sows. SERASCA1: a farm was considered positive if at least one animal was seropositive out of 10 pigs aged at least 22 weeks. SERASCA2: a farm was considered positive if at least two animals were seropositive out of 10 pigs aged at least 22 weeks.

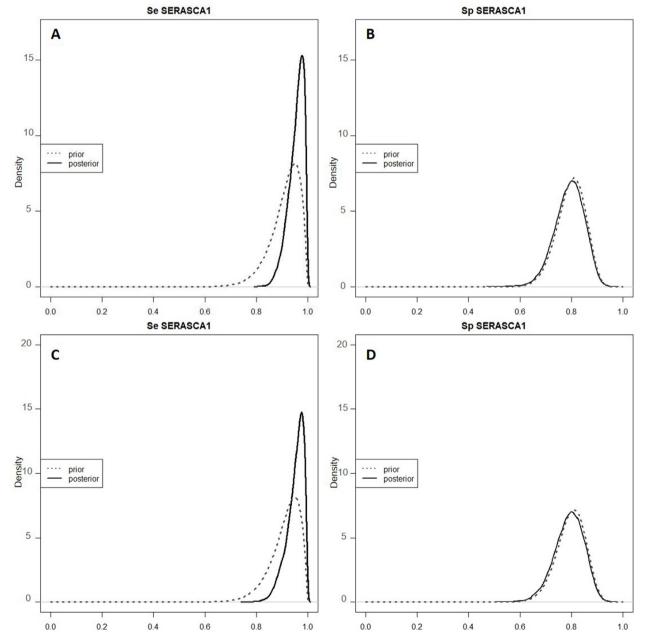
be noted that for option C (priors with little or no information), the estimated sensitivity is higher (approximately 30%) but with very wide credible intervals (approximately 85%). The results are similar for COPRO30 (Fig. 3), with improved sensitivity due to the greater number of samples taken. Sensitivity varied between 25.9% [16.6%; 36.4%]<sub>95%</sub> and 27.7% [17.7%; 38.8%]<sub>95%</sub> depending on the model and the level of information in the priors. Here again, option C offers a higher estimate of sensitivity, with a very wide credible interval.

As with sensitivity, estimates of the specificity of coprological tests vary little according to the model and the level of information provided by the priors. For COPRO20 (Fig. 2), they range from 95.0% [82.2%; 99.9%]<sub>95%</sub> to 97.2% [90.2; 99.9%]<sub>95%</sub> depending on the level

International Journal for Parasitology xxx (xxxx) xxx

of information provided by the priors. The maximum value (99.9%) is systematically within the credible intervals. For COPRO30 (Fig. 3), the estimates are nearly the same, ranging from 94.6%  $[82.2\%; 99.8\%]_{95\%}$  to 97.1%  $[89.9\%; 99.9\%]_{95\%}$ . For option C, the estimates are lower (around 82.5% for COPRO20 and 76.3% for COPRO30), with wider credible intervals.

The estimates of the sensitivity of the SERASCA1 serological test are very close to each other (Supplementary Tables S1 and S2), whatever the model and the level of information provided by the priors (with the exception of option C, which gives lower values). These estimates ranged from 95.0% [87.2%; 99.4%]<sub>95%</sub> to 97.9% [92.6%; 99.9%]<sub>95%</sub>, almost systematically including the maximum value (Fig. 4). However, these estimates are slightly more variable



**Fig. 4.** A priori and a posteriori distributions of the sensitivity and specificity of the SERASCA1 serological diagnostic test with the COPRO20 model (A and B) and the COPRO30 model (C and D) for the detection of *Ascaris suum* infection (Option A: moderately informative option; see Section 2.6.5). Se, sensitivity; Sp, specificity. COPRO20: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 20 samples taken from growing pigs (10 piglets aged 10–12 weeks and 10 pigs aged at least 22 weeks). COPRO30: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one *A. suum* egg was detected in at least one *A. suum* egg was detected in at least one of the 30 samples taken from growing pigs (10 piglets aged 10–12 weeks). COPRO30: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 30 samples taken from growing pigs (10 piglets aged 10–12 weeks) and 10 pigs aged at least 22 weeks) and 10 sows. SERASCA1: a farm was considered positive if at least one animal was seropositive out of 10 pigs aged at least 22 weeks.

in the case of the SERASCA2 test's sensitivity, with values close to 100% (97.1% [91.2%; 99.8%]<sub>95%</sub> in the COPRO20 model, option B, and only 91.0% [78.8%; 98.2%]<sub>95%</sub> in the COPRO30 model, option A (Fig. 5)).

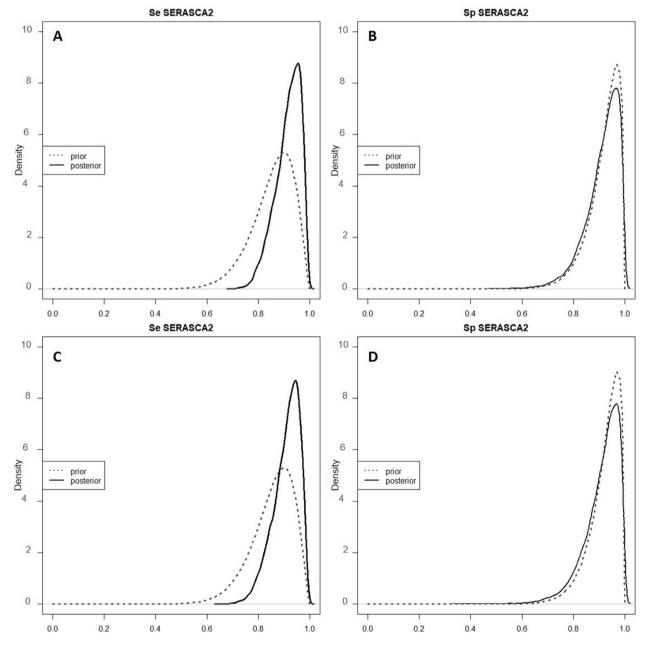
The estimates of the specificities of the serological diagnostic tests vary significantly, mainly depending on the level of information provided by the priors, and sometimes depending on the model (Supplementary Tables S1 and S2). The modal value of the specificity distributions for options A and B differ by approximately 4% to 10%, whatever the model (COPRO20 and COPRO30) and serological test (SERASCA1 or SERASCA2). These differences are much greater for option C, with credible intervals of over 90%. For SERASCA1 (excluding option C), specificity estimates range from 79.2% [67.0%; 89.1%]<sub>95%</sub> (option A, COPRO30 model) (Fig. 4) to

International Journal for Parasitology xxx (xxxx) xxx

90.6% [75.9%; 98.7%]<sub>95%</sub> (option B, COPRO30 model). These estimates are systematically higher for SERASCA2, with credible intervals approaching the maximum value (Fig. 5).

By compiling the results of the different models and options A and B, the results presented in Table 5 are obtained. Specificity is high for coprological analysis (slightly better than for SERASCA2), but sensitivity is much lower than serological tests, whatever the number of faecal samples taken per farm.

The estimated prevalence varies between models. However, if we exclude option C, which proposes credible intervals close to 100%, the prevalence of *A. suum* in our sample would be between 77.9% [67.3%; 88.1%]<sub>95%</sub> (COPRO30/SERASCA2 model, option B) and 89.0% [78.1%; 98.7%]<sub>95%</sub> (COPRO20/SERASCA1 model, option A).



**Fig. 5.** A priori and a posteriori distributions of the sensitivity and specificity of the SERASCA2 serological diagnostic test with the COPRO20 model (A and B) and the COPRO30 model (C and D) for the detection of *Ascaris suum* infection (Option A: moderately informative option; see Section 2.6.5). Se, sensitivity; Sp, specificity. COPRO20: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 20 samples taken from growing pigs (10 piglets aged 10–12 weeks and 10 pigs aged at least 22 weeks). COPRO30: a farm was considered infected (positive) if at least 22 weeks) and 10 sows. SERASCA2: a farm was considered positive if at least two animals were seropositive out of 10 pigs aged at least 22 weeks.

#### Table 5

Mean sensitivity and specificity estimates, and 95% credible intervals for serological and coprological diagnostic tests for *Ascaris suum* on studied farms in France (options A and B).

	Sensitivity	Specificity
COPRO20	23.7% [15.6%, 33.0%] <sub>95%</sub>	96.0% [85.0%, 99.9%] <sub>95%</sub>
COPRO30	26.7% [17.0%, 37.8%] <sub>95%</sub>	95.9% [85.0%, 99.9%] <sub>95%</sub>
SERASCA1	96.6% [89.3%, 99.8%] <sub>95%</sub>	84.8% [68.8%, 98.1%] <sub>95%</sub>
SERASCA2	94.0% [81.4%, 99.6%] <sub>95%</sub>	93.2% [78.4%, 99.2%] <sub>95%</sub>

COPRO20: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 20 samples taken from growing pigs (10 piglets aged 10–12 weeks and 10 pigs aged at least 22 weeks).

COPRO30: a farm was considered infected (positive) if at least one *A. suum* egg was detected in at least one of the 30 samples taken from growing pigs (10 piglets aged 10–12 weeks and 10 pigs aged at least 22 weeks) and 10 sows.

SERASCA1: a farm was considered positive if at least one animal was seropositive out of 10 pigs aged at least 22.

SERASCA2: a farm was considered positive if at least two animals were seropositive out of 10 pigs aged at least 22 weeks.

Option A, moderately informative option; Option B, very informative option.

#### 4. Discussion

This study compared two imperfect techniques commonly used in the diagnosis of *A. suum* in live pigs with the final aim of providing advice to farmers. Other diagnostic methods exist, in particular the examination of livers at the slaughterhouse. However, in this study we wanted to focus on diagnostic approaches applicable to live animals, with samples taken during a farm visit. In addition, the presence of milk spots on livers at the slaughterhouse indicates recent migration of *A. suum* larvae (Brewer and Greve, 2019). However, our objective was to define the status of a farm. This is why we took faecal samples from different categories of animals and blood samples from pigs at the end of their growth period. The presence of antibodies at this stage indicates contact with the parasite in its larval or adult state during the animal's life (Vlaminck et al., 2012).

The McMaster quantitative method used in this study was simple, quick and inexpensive. The people in charge of the analysis, who were few in number, had all been trained beforehand and were used to carrying it out regularly. This helped to limit errors in sample preparation and identification of *A. suum* eggs. According to Pereckienė et al. (2007), the addition of a centrifugation step before adding the supernatant to the cell could have improved the sensitivity of the technique.

In our study, we chose to define the A. suum status of a farm on the basis of the presence or absence of parasite eggs in the faeces or the number of seropositive animals. Other studies use the mean OD obtained on a farm to define its serological status (Vandekerckhove, E., Vlaminck, J., Vercruysse, J., Geldhof, P., 2014. Study on the relationship between seroprevalence of Ascaris suum in fatteners, farm management factors and production parameters. Presented at the 6th European Symposium of Porcine Health Management, Sorrento, Italy; Martínez-Pérez et al., 2017). It may appear risky to average the ODrs with an ELISA test, as the relationship between the ODr and the antibody level is not linear, particularly at extreme values (Ramirez, 2022. https://www.3trois3.com/articles/le-testelisa-comme-outil-de-diagnostic-1-2-principes-de-base\_15907 (accessed 8.18.23)). In our sample, there was indeed a significant link between the mean ODr per farm obtained with the SERASCA® test and the status assessed by coprological analysis. However, we did not find any sensitivity or specificity data for this diagnostic approach. The importance of providing information for the model in a Bayesian approach is one of the reasons that led us not to estimate the sensitivity and specificity for serological tests using the average ODr to define the status of a farm, even though studies have shown that this test can be used quantitatively to objectivise

#### International Journal for Parasitology xxx (xxxx) xxx

the consequences of *A. suum* in a farm, particularly on animal growth (Vandekerckhove et al., 2014, cited above).

Other tests based on the parasite's haemoglobin are commercially available. A study could be carried out with these tests to assess whether the estimates are close to those obtained with the SERASCA<sup>®</sup> test. This is a highly probable hypothesis as their design is very similar (Biox diagnostic, 2020, MONOSCREEN<sup>TM</sup> AbE-LISA – Ascaris suum.).

The proportion of farms considered to be positive for A. suum differed greatly, depending on whether a coprological or serological test was used. Thus, coprological testing for eggs appears to underestimate the proportion of pigs exposed to A. suum. The SER-ASCA® test detects antibodies to a purified A. suum haemoglobin protein. It can therefore be used to determine whether a pig has been infected by larval or adult forms of the parasite, whereas coprological analysis looks for parasite eggs in faeces (Vlaminck et al., 2014). Boes et al. (1997) found that no A. suum eggs could be observed by coprological analysis despite the presence of adult worms in the intestine. This can be observed both when the adult worms infecting the pig are of the same sex and when it is infected by immature larvae (Roepstorff and Nansen, 1998). In addition, once these larvae have completed their hepato-tracheal migration, most are expelled from the small intestine between 17 and 21 days p.i. (Nejsum et al., 2009), and do not reach adulthood to lay eggs. Finally, in the event of parasitic infection, there is an immune reaction, which may result in a reduction in egg production (Roepstorff and Nansen, 1998). Serology does have a few limitations, however, such as the seroconversion time, which is at least 6 weeks with the SERASCA<sup>®</sup> test (Vlaminck et al., 2012). Furthermore, the presence of antibodies at the end of fattening does not mean that the pig is still infected at that stage, but that it has been in contact with A. suum (larval or adult stage) during its life. SERASCA<sup>®</sup> test ODr levels begin to fall 8-10 weeks after infection but remain above the positivity threshold at least 14 weeks after infection (Vlaminck et al., 2012). Thus, serology provides a cumulative and historical picture, while coprology provides a one-off measurement of infection by adult and sexed forms.

As no reference test was available, the real status of the farms with regard to A. suum was unknown. In the absence of this information, it was difficult to estimate the characteristics of the tests used (sensitivity and specificity). We chose to use the Bayesian methodology, which has proven its usefulness in estimating the characteristics of diagnostic tests without a reference test in pigs (Fablet et al., 2010; Rose et al., 2010; Delsart et al., 2019). The basic idea behind the Bayesian approach is to free ourselves from the need to know the characteristics of the tests by first constructing a prior probability distribution for all the unknown parameters (a priori distributions). The data, using the likelihood function (cross-classifications between test results), is then combined with the a priori distributions to obtain posterior distributions using Bayes' theorem. This allows simultaneous inferences to be made about all the parameters, even though their number is greater than the degrees of freedom. The posterior distributions contain updated estimates of the values of the model parameters after taking into account the information provided by the data (Joseph et al., 1995). With a Bayesian rather than a frequentist approach, the uncertainty about all the parameters is modelled by a probability distribution that reflects the uncertainty of the unknown quantities (Joseph et al., 1995).

The choice of a priori distributions (=priors) is therefore a key point in a Bayesian approach and affects the results, depending on the informative level of these priors. In this study, the priors were combined using different options with different levels of information to estimate and compare their effect on the parameters. Option C (low information option) provided almost no information to the models, apart from information on the specificity of coprology. As a result, most of the estimates produced using this option are inaccurate, with very wide credible intervals.

To our knowledge, the sensitivity of coprology had not previously been quantified. According to the literature, it is variable and depends both on the number of parasite eggs excreted (lower detection limit of 100 eggs per g of faeces (Pereckiene et al., 2007)) and on the type of parasite: sensitivity decreases with the length of the parasite's prepatent period (Roepstorff and Nansen, 1998). Additionally, sensitivity is influenced by sampling and the probability of selecting animals that excrete parasite eggs. Only a small proportion of pigs exposed to A. suum will develop and carry adult worms in their intestines. This is why we chose to provide little information in our model. We calculated the distribution modes with an individual sensitivity of 5%, and we opted for very spread-out distributions, for both options A and B. The estimates of the sensitivities of the coprological tests obtained with option C are close to the values used for the priors of options A and B. However, we provided almost no information to the models in option C, and none concerning the sensitivity of the coprological test. In the absence of any information, the models in option C therefore seem to support our choices.

On the other hand, we provided a great deal of information about the specificity of coprology. It is very difficult to confuse an *A. suum* egg with another helminth egg. The literature has nonetheless reported false positives (Boes, 1997). This occurs when a faecal sample contains eggs even though the animal is not infected, which may be the case during coprophagy or geophagy (ingestion of infected soil). As coprophagy or geophagy occurs within a farm's boundaries, this had no impact in our study on the definition of the farm's infection status, and we considered the specificity of coprology to be almost perfect for the diagnosis of *A. suum* in our a priori distributions.

For the serological tests, we used the sensitivity and specificity data proposed by Vlaminck et al. (2012). With 10 samples per farm, herd sensitivity is almost 100%. This is the modal value used in option B (very informative option). In option A (moderately informative option), we gave the model less information, with a substantially more spread-out distribution of the prior. We took into consideration the low number of samples (10 blood samples). However, we know that with 10 samples per farm, the limit prevalence rate, i.e. the prevalence threshold for detection, is high (26%  $(\alpha = 0.05))$  for a test with perfect sensitivity. However, it should be remembered that the rate of A. suum-seropositive animals per farm was 44.5% in our sample, well above the prevalence limit. Ten samples per farm therefore do not appear to be a limiting factor for the herd sensitivity of our serological diagnostic test. We therefore took this into account in the information provided to the model, by limiting the spread of the prior's distribution. For specificity, we voluntarily downscaled the data from Vlaminck et al. (2012) from experimental inoculations with Trichuris suis and chose an individual specificity of 99.5% for option B priors and 98% for option A priors to define the mode of our distributions.

For prevalence, the information in the literature is divergent and depends very much on the type of analysis. This is why we chose not to include any information in our models (uniform distribution). It should be remembered that the prevalence of farms infected by *A. suum* in our sample is estimated, according to our Bayesian approach, at approximately 80%. Our sample is exclusively made up of alternative farms. However, *A. suum* is more frequently detected on this type of farm than on conventional farms, where pigs are reared on slatted floors (Eijck and Borgsteede, 2005), probably due to the resistance of the parasite's eggs, which can remain viable for up to 10 years in the environment (Roepstorff and Nansen, 1994). It is therefore difficult to extrapolate these prevalence results to the whole pig farming population.

### International Journal for Parasitology xxx (xxxx) xxx

In the end, between the models and options, we obtained 12 estimates for prevalence and six for each of the sensitivities and specificities of each serological or coprological diagnostic test (COPRO20, COPRO30, SERASCA1 and SERASCA2). With the exception of the estimates obtained with option C, for which we provided little information, the sensitivity and specificity estimates for each of these diagnostic approaches are very close to each other, which fosters confidence in the results obtained.

As expected, coprology showed excellent specificity. The absence of seronegative farms with the SERASCA1 diagnostic test in the COPRO20- or COPRO30-positive farms led us to expect that the data would validate the information provided by the priors. Despite the increase in the number of samples, the specificity hardly deteriorated. The credible intervals are also relatively narrow, making the modal value obtained all the more plausible, with the maximum value systematically included.

Concerning the sensitivity of coprology, the credible intervals are much wider. Above all, the estimates are very low, below 30%, and the increase in the number of samples taken does very little to improve this parameter. This tends to indicate that coprology cannot be considered as the reference diagnostic test for *A. suum* in pig farming. This was probably to be expected based on the literature. However, an estimate of the herd sensitivity of this diagnostic approach can now be proposed.

While we were aware of the strengths and weaknesses of coprology, one of the aims of this work was to estimate the performance of serology at the farm level, using standard practices (analyses of 10 pigs at the end of the growth period). The sensitivity of the SERASCA1 test (farm positive if there is at least one seropositive animal out of 10 on the farm) is excellent, as we expected from the performances described by Vlaminck et al. (2012), remaining above 95% in all the models and the maximum value being almost always within the credible interval. The results suggest that the specificity of SERASCA1, on the other hand, is not so good, despite the individual specificity of the SERASCA® test being estimated at 100% by Vlaminck et al. (2012). However, this estimate was only obtained from ODs observed in animals experimentally inoculated exclusively with T. suis. Cross-reactivity with other parasites cannot therefore be ruled out. Furthermore, specificity at herd level is systematically lower than individual specificity. The specificity of the serological diagnostic approach is much improved if we consider that a farm is positive for *A. suum* when there are at least two positive samples (SERASCA2). According to our models, SERASCA2 is therefore much more specific, while maintaining a sensitivity close to that of SERASCA1.

In conclusion, this study confirms the very good specificity of coprological analysis in the search for *A. suum*, whether 20 or 30 samples are taken per farm. However, the sensitivity of this diagnostic approach remains very low (less than 30%), even when adding samples taken from breeding animals to those taken from growing animals. On the other hand, the serological test, which entails taking blood samples from 10 animals at the end of fattening, has good diagnostic sensitivity and seems to be more suitable for defining the status of a farm with regard to *A. suum*, provided that a farm is only considered seropositive if two out of 10 samples are positive.

#### **CRediT authorship contribution statement**

**Maxime Delsart:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Jean-Michel Répérant:** Formal analysis, Investigation, Methodology, Validation, Writing – review & editing. **Chantal Benoit:** Investigation. **Edouard Boudin:** 

Investigation. Jean-François Da-Costa: Investigation, Writing – review & editing. Virginie Dorenlor: Investigation. Florent Eono: Investigation. Eric Eveno: Investigation. Stéphane Kerphérique: Investigation. Gilles Poulain: Investigation. Marie Souquière: Investigation. Martine Thomas-Hénaff: Investigation. Françoise Pol: Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. Barbara Dufour: Conceptualization, Supervision, Validation, Writing – review & editing. Nicolas Rose: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. Christelle Fablet: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

### Acknowledgements

The authors would like to thank all who took part in this study (farmers, technicians, veterinarians, farmers' organizations). This study was carried out as part of the PIGAL (acronym for pig alternative) research programme led by ANSES in Ploufragan-Plouzané-Niort (France). This work was supported by the following financial partners: Avril Nutrition Animale SAS (France), Cooperl (France), Elanco France SAS, FranceAgriMer (France), Herta (France), Inaporc (France), Sofral le Gouessant (France) and Zoetis France, and with the help of the Institut Carnot AGRIFOOD Transition (France). Ethical review and approval were waived for this study in accordance with the opinion of the ANSES animal welfare structure.

# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2024.04.010.

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