



DESCRIPTIVE EPIDEMIOLOGY OF THE MAIN INTERNAL PARASITES ON ALTERNATIVE PIG FARMS IN FRANCE

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KEY WORDS ABSTRACT

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Alternative pig farms, which do not raise animals in closed buildings with slatted and/or concrete floors, have critical points that need particular attention. Internal parasitism is one, as the farming conditions in such structures are more favorable to the development and survival of parasites. The objectives of this study, carried out on 70 alternative farms in continental France, were to (i) estimate the frequency and level of infestation by the main internal parasites on these farms, and (ii) define their typology according to the level of parasitism. For this purpose, fecal samples were taken for coprological analysis from 10 sows, 10 pigs aged 10–12 wk, and 10 pigs at the end of the fattening period. Blood samples were also taken for serological analysis (targeting *Ascaris suum* and *Toxoplasma gondii*) from 10 sows and 10 pigs at the end of the fattening period. Of the 70 farms, only 5 had no helminth egg or coccidian oocyst. *Coccidia* oocysts were observed in 79% of the farms, while eggs of *Oesophagostomum* spp./*Hyostromylyus rubidus*, *Ascaris suum*, and *Trichuris suis* were found in 47%, 16%, and 36% of the farms, respectively. On each infested farm, an average of 56.8% of sows, 23.8% of grower pigs, and 38.9% of finisher pigs were parasitized. At least 1 *Ascaris suum*-seropositive finisher pig was found on 91% of the farms, and at least 1 *Toxoplasma gondii*-seropositive finisher pig or sow on 60% of the farms. Data on housing, animal management, and health management (particularly parasite control) were collected to characterize the typology of farms according to their level of parasitism. The variables defining these farm typologies differed according to the parasites. Access to the outdoors for breeding stock was a characteristic of the farms most heavily infested with helminths or *T. gondii*. Conversely, the farms with the lowest frequency of coccidia oocyst infestation were characterized by free-range farrowing facilities and also by the presence of slatted floors, mostly plastic in our study, rather than straw bedding in the farrowing rooms. The level of biosecurity concerning the storage of straw for pig bedding was another discriminating factor for parasitism level of helminths and *T. gondii*. Farms with the highest levels of helminth parasitism were more likely not to practice an all-in-all-out postweaning system and to deworm their grower/finisher pigs less frequently than farms with the lowest levels of helminth parasitism.

Internal parasites are common pests of swine worldwide (Brewer and Greve, 2019). Among the most frequently isolated internal parasites in the pig production sector in temperate countries are protozoa such as coccidia or *Toxoplasma gondii*, or helminths such as *Ascaris suum*, *Trichuris suis*, and the strongylate nematode species *Oesophagostomum* sp. and *Hyostromylyus rubidus*, the eggs of which are morphologically indistinguishable. In addition to the food safety significance of *Toxoplasma gondii* for consumers, infection with these parasites is an acknowledged

contributor to production losses due to poor growth and feed conversion performance (Ózsvári, 2018). It can also be a risk factor for other digestive tract diseases such as *Lawsonia intracellularis* infection (Pearce, 1999) or intestinal *Salmonella* carriage (Steenhard et al., 2002). Finally, it can interfere with vaccination uptake, as shown previously for *A. suum* and *Mycoplasma hyopneumoniae* vaccination (Steenhard et al., 2009).

The presence of internal parasites and the intensity of infestations are strongly influenced by the production system



Table I. Criteria for inclusion.

Geographical location	Continental France
Type of farm	Farrow-to-finishers Breeders Fatteners
Livestock size	Using alternative housing for at least 18 mo If presence of a farrowing unit ≥ 20 sows If presence of a fattening unit ≥ 100 grower/ finisher pigs
Type of housing	
Sows	Gestating sows in semi-open buildings* on bedding + Farrowing units in closed buildings Pregnant sows with outdoor courtyards or free-range + Farrowing units in closed buildings Pregnant sows with litter or outdoor courtyards or free-range + outdoor farrowing units
Fattening unit	Post-weaning and/or fattening in semi-open buildings* with bedding Post-weaning and/or fattening with outdoor access and concrete floor (outdoor courtyard) Post-weaning and/or fattening with outdoor access and natural floor

* Non-hermetic building, open on at least 1 side (at least with a windbreaking system).

(Nansen and Roepstorff, 1999). Although a decrease in the number of parasite species and infestation pressure has been observed with slatted floors (Nansen and Roepstorff, 1999), internal parasitism remains a major preoccupation in systems with bedding and/or outdoor access (Yaeger et al., 2009; Rousing, 2011; Delsart and Houlbert, 2012; Früh et al., 2018; Delsart et al., 2020). These rearing conditions are more favorable to the development and survival of oocysts or eggs of parasites in the environment (Salajpal et al., 2013). Soil type is an important parameter that affects parasitism depending on whether manure is removed from the enclosure or not, by limiting the contact time with potentially infected excreta.

The term “alternative farms” can have several meanings. In the remainder of this paper, we define as “alternative” any farming system that does not raise all the pigs in closed buildings on slatted and/or concrete floors, i.e., that differs from the predominant contemporary structures (Guy and Edwards, 2006). Alternative production systems vary widely. There are (i) free-range systems, which allow pigs to have access to the outdoors and to be in contact with the ground and plants (Honeyman et al., 2001), and (ii) bedded systems (straw, sawdust, hay, etc.). Popular with consumers (Sato et al., 2017), alternative systems generally attach great importance to animal welfare and product quality (Bonneau et al., 2011). Their numbers are currently growing (Honeyman, 2005; Willer and Lernoud, 2017). There are few numerical data on alternative livestock production, especially in France, where rearing on litter is estimated to represent about 5% of pig farms (Boulestreau-Boulay et al., 2012) and organic production 1.7% of the sow population (FNAB, 2020).

In many studies, the diversity of parasites isolated from alternative pig farms is often limited, with 3 helminths (*A. suum*, *Oesophagostomum* spp., and *T. suis*) and coccidia most frequently identified (Carstensen et al., 2002; Baumgartner et al., 2003; Eijck and Borgsteede, 2005), although other species such as *H. rubidus*, *Metastrongylus* spp., *Strongyloides ransomi* or *Stephanurus dendatus* have also been observed (Roepstorff and Nansen, 1994; Carstensen et al., 2002; Prunier, 2010). Together with coccidia, *A. suum* is the most frequently isolated endoparasite according to Eijck and Borgsteede (2005) (63% and 60% of the alternative farms studied, respectively), followed by *T. suis* (37% of the farms) and *Oesophagostomum* spp. (25%). Most of the descriptive studies on the subject of parasitism in alternative farms offer only a partial view by focusing on only 1 production phase of the pigs, including only a few farms, or by ignoring their diversity. These studies generally only include coprological analyses, without using new tools such as serological tests that can detect antibodies against *A. suum* or *T. gondii*, whose seroprevalence has been estimated at 7.9% in pigs from alternative farms in Sweden (Wallander et al., 2016).

The objectives of the present study were to (i) estimate the frequency and level of infestation by the most common internal parasites (*Strongyles*, *A. suum*, *T. suis*, coccidia genus *Eimeria* and *Cystoisospora*, *T. gondii*) in pigs of different stages (sows, growers, and finishers) from a sample of 70 alternative farms in France, and (ii) describe the typology of farms according to their level of parasitism, characteristics, and management.

MATERIALS AND METHODS

Study sample and herd selection

This study was carried out in continental France on pig farms using bedding housing and/or with access to the outdoors (outdoor courtyard, free-range). Farms had to produce pigs under an alternative system for at least 18 mo to be included (Table I). If the farm included a farrowing section (farrow-to-finishers or breeders), at minimum 20 sows were required. If the farm included a fattening section (farrow-to-finishers or fatteners), at minimum 100 pigs were required. All or part of the farm had to offer the animals an alternative rearing system. In the case of farrow-to-finish farms, the alternative system had to concern all or part of the sows and at least 1 growing stage (post-weaning and/or fattening).

Twenty-six breeders' organizations or associations and veterinary offices were contacted. Fourteen of these organizations participated by contacting the farmers in their files who could be included in the study. Out of these files, 70 farms were contacted and agreed to take part in the study.

Data collection and sampling on farms

Each farm was visited once (cross-sectional study between June 2020 and June 2021). During this visit, a questionnaire was filled in with the farmer concerning the description, technical and health management of the farm, and the use of anthelmintics. At the same time, observations and measurements of the farm and the animals were carried out by other investigators (2–4), and samples were taken from the animals.

On each farm, according to the categories of animals present, 10 sows, 10 piglets between 10- and 12-wk of age (grower pigs), and 10 pigs over 22 wk of age (finisher pigs) were selected.

A blood sample was taken from each finisher pig or sow by a jugular vein puncture, using evacuated tubes without an additive (Vacuette, Dutscher SAS, Brumath, France).

Fresh fecal samples were taken at random, if possible from the selected animals, or directly from the ground immediately after defecation, to limit the risk of sampling the same animal several times. These samples were individually placed in 180 ml polypropylene coprology jars.

Transportation of samples

The samples were individually identified and sent to the laboratory for processing. The feces 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 3,500 g and stored at -20 C until further analysis.

Laboratory analyses

Coprology: Three operators were previously trained with the aid of samples, photographs, and an ocular micrometer. The fecal samples, refrigerated at 5 C until examination, were analyzed individually. After homogenization with a spatula, 3 g of each sample were mixed for 5 min with 42 ml of saturated sodium chloride solution. 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 $\times 100$ magnification. In case of uncertainty about the observed elements, an observation was made at $\times 200$ magnification, especially for coccidia oocysts. In the event of detection and identification, the number of coccidia oocysts or eggs of strongyles, *T. suis*, or *A. suum* were counted per species in the filled McMaster cell chamber. The number of oocysts or eggs per gram of feces was obtained by multiplying the number of oocysts or eggs counted in a McMaster chamber by 100. If there were too many eggs or oocysts to count properly, 9 ml of saline solution was added to 1 ml of the first mixture to obtain a second dilution to 1:10. The number of oocysts or eggs per gram of feces was then obtained by multiplying the number of oocysts or eggs counted in 1 chamber of the McMaster cell by 1,000.

Serological tests

***Ascaris suum*:** an individual serological analysis for antibodies to *A. suum* was carried out on all blood samples taken from the finisher pigs. After centrifuging the samples, the sera were sent to Ghent University. The total IgG antibodies for *A. suum* were detected using the SERASCA® ELISA, as described by Vlaminck et al. (2012), using the purified hemoglobin antigen of *A. suum*. This test can detect antibodies from 6 to 8 wk after infestation and has a higher sensitivity for the diagnosis of *A. suum* infestation than a fecal examination (Vlaminck et al., 2014). The analytical sensitivity and specificity of this test in experimentally-infested pigs are estimated to be 99.5% and 100%, respectively (Vlaminck et al., 2012).

***Toxoplasma gondii*:** An individual serological analysis was carried out on each of the blood samples taken from the sows and finisher pigs. Pigs' sera were analyzed by the Modified Aggluti-

nation Test (MAT) designed to detect *T. gondii*-specific immunoglobulin (IgG) using an antigen prepared from formalin-fixed whole RH tachyzoites as described by Dubey and Desmonts (1987). This was provided by the National Reference Centre for Toxoplasmosis in Reims, France (Villena et al., 2012). Serum samples were diluted 2-fold starting at a 1:6 dilution until 1:12,288 dilution. The threshold dilution was 1:6. This is a species-independent serological test, considered to be the gold standard for the detection of anti-*T. gondii* antibodies in animals and meat (Klun et al., 2006; Forbes et al., 2012; Pardini et al., 2012; Villena et al., 2012).

Statistical analysis

Description of parasitism: The status of each farm (infested or non-infested) for helminths (strongyles, *A. suum*, *T. suis*) and coccidia was determined from the examination of fecal samples. A farm was considered infested if at least 1 helminth egg or coccidia oocyst was observed. Similarly, the status of each animal category (sows, grower pigs, finisher pigs) was defined for each farm. The percentages of infected animals were calculated, the distributions of the quantities of parasites observed per gram of feces studied, and the position parameters determined in each category for each parasite and each farm.

A farm was considered serologically positive for *A. suum* if at least 1 of the 10 samples taken was positive. Similarly, a farm was considered serologically positive for *T. gondii* if at least 1 of the animals sampled was positive. In the case of *T. gondii*, positivity was defined at the farm level, but also by the category of animal (sows and finisher pigs). In addition, the distributions of positive results, as well as the Optical Density ratios (ODr) of the ELISA test for *A. suum* and the MAT titers for *T. gondii*, were studied.

The descriptive analysis of the results was performed using the free software R (R Development Core Team, 2008).

Relationship between parasites: Associations between the percentages of animals infested with at least 1 egg of strongyles, *A. suum*, *T. suis*, at least 1 coccidia oocyst in each category of each farm and the seropositivity rate for *T. gondii* and *A. suum* were investigated by principal component analysis (Rstudio® (RStudio Team, 2020), FactoMineR (Lê et al., 2008), and Factoshiny packages (Vaissie et al., 2021)). The diversity of farm types led us to carry out this work successively on 3 subpopulations: a study of the relationships (i) among farms with sows (breeders and farrow-to-finish farms), (ii) among farms with grower/finisher pigs (fatteners and farrow-to-finish farms) and finally (iii) among farms with both sows and grower/finisher pigs (farrow-to-finish farms only).

Three hierarchical ascending classifications (Rstudio® (RStudio Team, 2020), FactoMineR (Lê et al., 2008), and Factoshiny packages (Vaissie et al., 2021)) were performed to group farms with both sows and grower/finisher pigs into clusters according to their status concerning (i) helminths, (ii) coccidia, or (iii) *T. gondii*.

Relationship between parasitism, farm characteristics, and management: For each classification, each farm was assigned to a cluster describing the level of parasitism. We then compared these clusters with the variables describing the type of farm (5 variables), housing modalities (27 variables), animal management (3 variables), health management (8 variables), and control of parasites (10 variables). The comparison was performed using a Chi² test or Fisher's exact test if the conditions for application of

Table II. Proportion of farms per production phase according to housing type (70 farms). Please note that the sum of the percentage may be higher than 100% per column, as a farm may have several different types of housing per rearing stage.

Building and floor types	Farrowing rooms, 57 farms (%)	Mating, 47 farms (%)	Pregnant sows, 57 farms (%)	Nursery, 65 farms (%)	Fattening phase, 65 farms (%)	Quarantine, 41 farms (%)
Closed building	54	28	16	44	26	39
On slatted floor	18	13	2	8	6	9
On litter	36	15	14	35	20	30
Semi-open building WITHOUT outdoor access	5	21	19	24	23	24
On slatted floor	0	2	0	0	0	0
On litter	5	19	19	24	23	24
Semi-open building WITH outdoor access	12	38	28	37	49	15
Open building WITH outdoor access	0	2	2	0	2	0
Concrete floor	0	2	0	0	2	0
Natural floor	0	0	2	0	0	0
Outdoors	35	11	40	2	6	24

the Chi² test were not met. Items with a *P*-value for this test below 25% were retained for multivariate analysis. The collinearity between the selected items was then tested (*P* < 0.05). When variables were too redundant, we kept those that best described the set of variables involved. Relationships between these selected items and parasite status were then investigated by multiple correspondence analysis (MCA) (Rstudio® (RStudio Team, 2020), FactoMineR (Lê et al., 2008), and Factoshiny packages (Vaissie et al., 2021)) taking the cluster variable (level of parasitism) as a supplementary variable.

RESULTS

Description of farms

The sample of farms studied, whose geographical distribution is shown in Figure 1, included 52 farrow-to-finish farms, 5 breeders, and 13 fatteners. Of these 70 farms, 68 produced animals under officially certified schemes identifying quality and origin: 45 under organic production, 5 under the French “Label Rouge” and 10

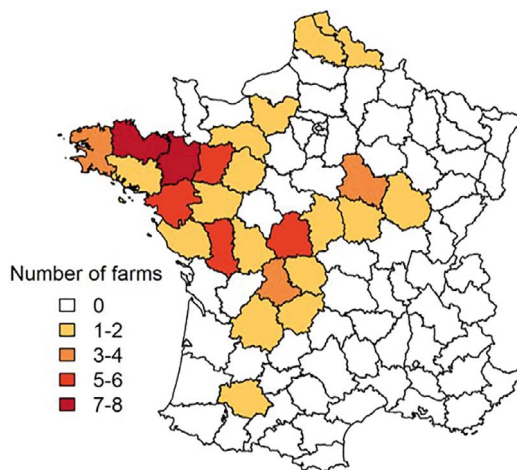


Figure 1. Geographical distribution of the 70 farms included in the study. Color version available online.

under the “Label Rouge Fermier” programs. The rest produced animals under other quality specifications.

The average size of the breeding stock was 118, with a median of 72 sows ($\sigma = 141$; min = 24; max = 882). The average age at weaning was 37.2 days ($\sigma = 7.2$; min = 21; max = 49). An average of 697 grower/finisher pigs were present on the day of the visit in the farrow-to-finisher or fatterer structures (median = 602; $\sigma = 513$; min = 53; max = 3,160).

The grower pigs sampled (feces) were on average 10.8 wk old ($\sigma = 0.9$; min = 10; max = 13), while finisher pigs (serology and feces) were 25.4 wk old ($\sigma = 4.0$; min = 21; max = 52).

At least some of the sows were kept outdoors in 40% of the farms, while this was the case in only 6% of the farms for grower/finisher pigs. Table II describes the distribution of housing according to animal categories.

Deworming was practiced on 62 of the 70 farms, on at least 1 category of pig. Only 19% of farms did not deworm sows and 17% did not deworm grower pigs (Table III). The molecules most often used were benzimidazoles, although unweaned piglets were treated mainly with an oral or injectable anticoccidian (toltrazuril) (Table IV).

Table III. Distribution of farms according to the number of treatments against internal parasites and physiological stages (70 farms).

	No deworming (%)	Number of internal deworming treatments (annual for breeding stock and per animal for piglets and pigs)		
		1 (%)	2 (%)	≥3 (%)
Gilts in quarantine (57 farms)	58	33	9	0
Sows (57 farms)	19	4	70	7
Boars (57 farms)	35	12	49	4
Unweaned piglets (57 farms)	82	18	0	0
Growing pigs (after weaning, 65 farms)	17	23	35	25

Table IV. Distribution of molecules used to control internal parasites by production phase (65 farms).

	Gilts in quarantine, 22 farms (%)	Sows, 46 farms (%)	Boars, 37 farms (%)	Unweaned piglets, 10 farms (%)	Grower/finisher pigs (after weaning), 54 farms (%)
Fenbendazole	36	39	43	0	40
Flubendazole	50	52	49	10	26
Ivermectin	9	4	5	0	0
Levamisole	5	2	3	0	35
Toltrazuril	0	0	0	90	0
Phytotherapy	0	2	0	0	0

Very few farms used alternative approaches to antiparasitic treatments (homeopathy, phytotherapy). Only 1 farm used a specialty based on plant extracts on sows to control coccidiosis in piglets.

Coprological analyses

Of the 70 farms, only 5 had neither helminth eggs nor coccidia oocysts. Coccidia oocysts were found in 79% of the farms, while eggs of *Oesophagostomum* spp./*H. rubidus* (hereafter referred to as strongyles), *A. suum*, and *T. suis* were observed in 47%, 16%, and 36% of the farms, respectively. The proportion of farms infested per parasite and per production phase is detailed in Figure 2. Other parasites observed included *Strongyloides* spp. eggs in the grower pigs of 1 farm, and eggs of unidentified parasites on another farm.

All the coccidia oocysts observed were of the genus *Eimeria*. No *Cystoisospora* oocysts were observed in the samples. At least 2

different species of coccidia oocysts were found on 47% of the farms (32%, 15%, and 29% of the farms for sows, grower pigs, and finisher pigs, respectively).

On each infested farm, on average 56.8% of sows, 23.8% of grower pigs, and 38.9% of finisher pigs were parasitized. Table V details all these results, parasite by parasite. Helminth eggs were found in greater quantities in infested sow samples (Fig. 3), while coccidia oocysts were more numerous on average in the infested feces of finisher pigs.

***Ascaris suum* serological results**

The SERASCA® serological test was carried out on pigs from 65 farrow-to-finish or fatter farms. Forty-three percent of the ODr (optical density ratio) results were above 0.5 (cut-off). Ninety-one percent of farms had at least 1 positive sample, with

Table V. Rate of infested animals per farm, per parasite and per production phase (70 farms, 57 with sows and 65 with grower/finisher pigs).

	Rate of infested animals on the farm (%)			
	Mean	Median	Min	Max
All parasites included				
Sows*	56.8	60	0	100
Growers†	23.8	10	0	100
Finishers†	38.9	20	0	100
<i>Oesophagostomum</i> spp./<i>Hyostromylus rubidus</i>				
Sows*	35.3	10	0	100
Growers†	7.1	0	0	100
Finishers†	10.0	0	0	100
<i>Ascaris suum</i>				
Sows*	1.8	0	0	60
Growers†	0.5	0	0	10
Finishers†	2.9	0	0	60
<i>Trichuris suis</i>				
Sows*	2.6	0	0	20
Growers†	5.1	0	0	80
Finishers†	4.9	0	0	70
Coccidia				
Sows*	29.1	20	0	100
Growers†	14.2	0	0	100
Finishers†	31.2	10	0	100

* Fifty-seven farms.

† Sixty-five farms.

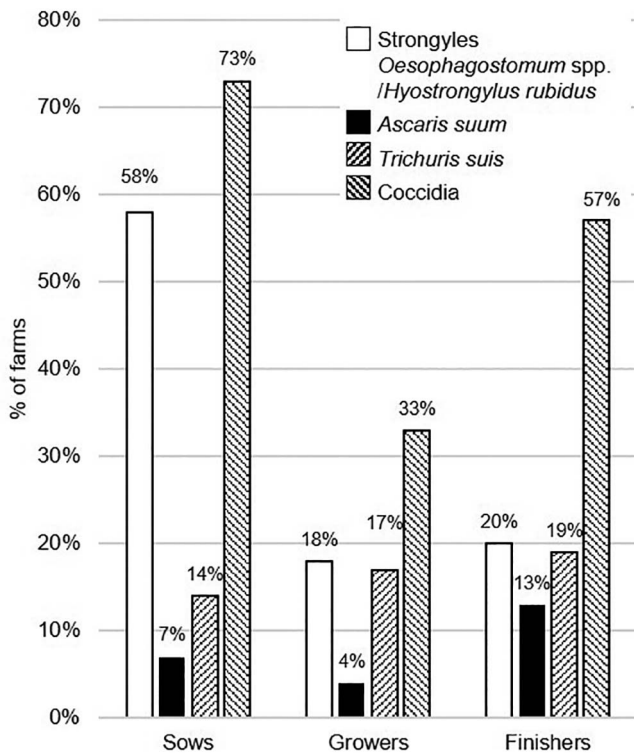


Figure 2. The proportion of farms infested per parasite and per production phase (70 farms, 57 with sows, and 65 with fattening pigs).

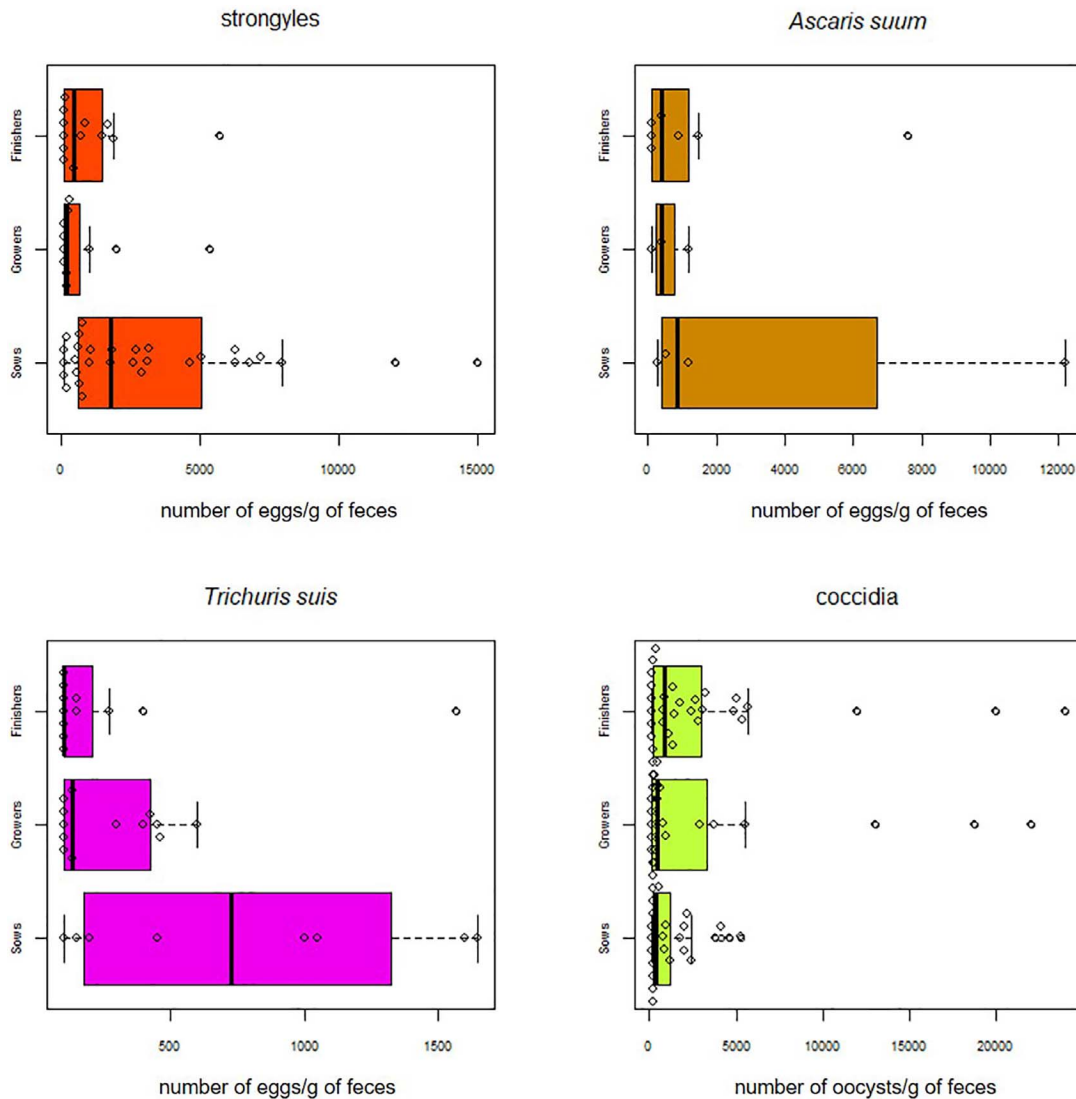


Figure 3. Distributions of average parasite quantities in infested samples per farm and per production phase (n = 65 farms). Strongyles = *Oesophagostomum* spp./*Hyostrongylus rubidus*. Color version available online.

an average of 43.0% [35.8;52.2]_{95%} seropositive animals per farm. Forty-five percent of the farms had an average ODr value of more than 0.5. The distribution of serological values per farm is shown in Suppl. Figure S1.

Toxoplasma gondii serological results

Serological tests for *T. gondii* were carried out on both sows (57 breeder or farrow-to-finish farms) and grower pigs (65 breeder or post-farrow-to-finish farms). Forty-two of the 70 farms (60%) had at least 1 seropositive animal. At least 1 sow was seropositive in 63% of the breeder and farrow-to-finish farms, while at least 1 pig was seropositive in 26% of the farrow-to-finish and fattener farms.

The average percentage of seropositive animals per farm was 11.9% [8.1; 15.8]_{95%}, with a maximum of 70%. On average, 21.9% [15.4; 28.5]_{95%} of sows and 4.5% [1.7; 7.3]_{95%} of pigs were seropositive per farm (with a maximum of 80% and 70%,

respectively). The distribution of positivity rates per farm is shown in Figure 4.

The serological titers of positive samples ranged from 1:6 to 1:12,288, with a modal value of 1:24, and a median of 1:36 (1:48 and 1:24 for sows and finishers, respectively). The distribution of serological titers per animal type is shown in Figure 5, and per farm and animal type in Figure S2.

Relationships between parasites

Principal component analysis was performed on 3 populations. On the 57 farms with breeding stock, 3 main groups of associations between variables describing infestation frequencies per farm were revealed (Fig. 6A). One group of positively correlated variables (GrpS1, upper right corner of the map) included the percentage of sows with a coprology result positive for *T. suis* and strongyles. The second group of positively-correlated variables, located in the lower right part of the map

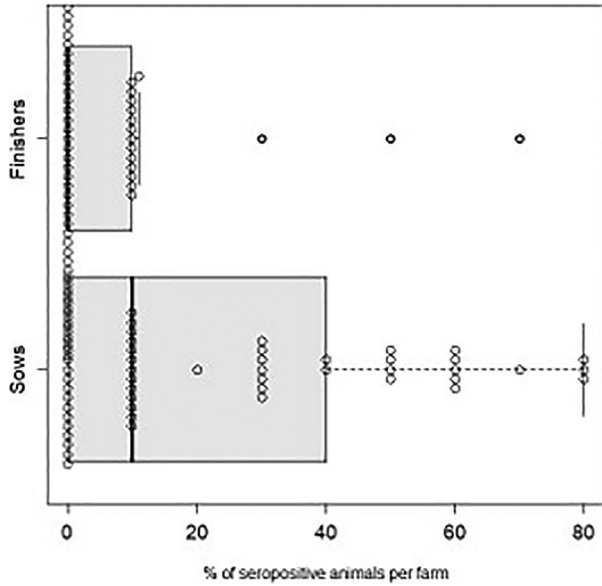


Figure 4. Rate of *Toxoplasma gondii*-positive animals per farm and production phase (70 farms, 57 with sows, and 65 with finishers). The Modified Agglutination Test was used to define these rates.

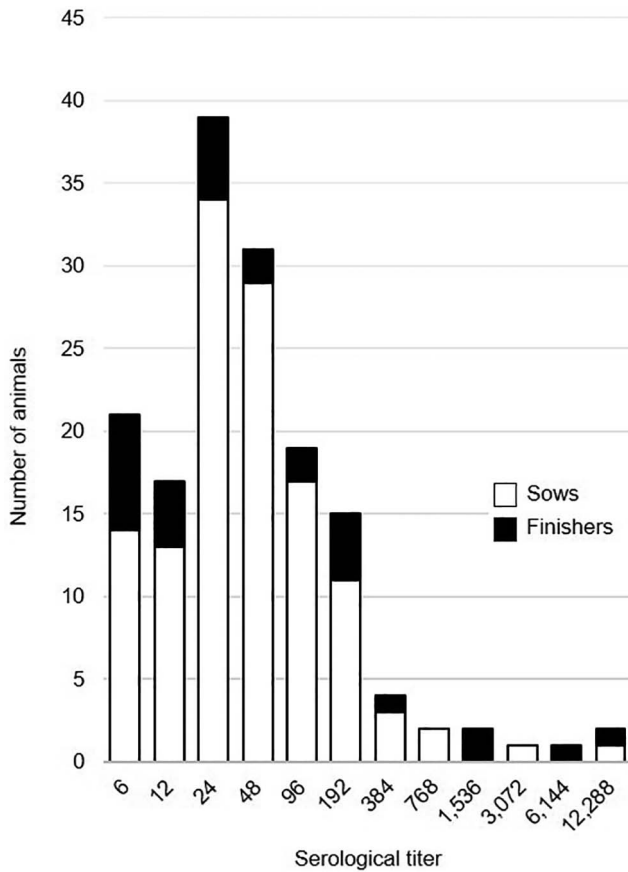


Figure 5. Distribution of serological titers for *Toxoplasma gondii* for all samples (70 farms, 57 with sows, and 65 with finishers). The Modified Agglutination Test was used to define these rates.

(GrpS2), was related to the percentage of sows infected with coccidia and the percentage of sows seropositive for *T. gondii*. Finally, the variable related to the percentage of sows infected with *A. suum* was not correlated with any of the variables in the first 2 groups (GrpS3).

The second analysis performed on the 65 farms with grower/finisher pigs (Fig. 6B) also showed 3 main groups of associations. The bottom right group (GrpP1) included variables highlighting a higher percentage of animals infested late (after 22 wk of age) for strongyles, *T. suis*, and coccidia, as well as the percentage of piglets infected with coccidia at 10–12 wk of age, and to a lesser degree the percentage of *A. suum*-seropositive animals at the end of fattening. A second group, top right (GrpP2), included variables associated with a higher percentage of animals infested earlier (at 10–12 wk of age) for *A. suum*, *T. suis*, and, to a lesser degree, strongyles. These variables were also positively correlated with the percentage of *T. gondii*-seropositive finisher pigs. The variable associated with the percentage of pigs with a coprology result positive for *A. suum* at the end of fattening was not correlated with either of these 2 groups (GrpP3).

The same groups of associations between variables were found in the third analysis, performed on the 52 farms with both sows and grower/finisher pigs (Fig. 6C). Here the variables in groups GrpS1, GrpS2, and GrpS3 were correlated with the variables in groups GrpP1, GrpP2, and GrpP3, respectively.

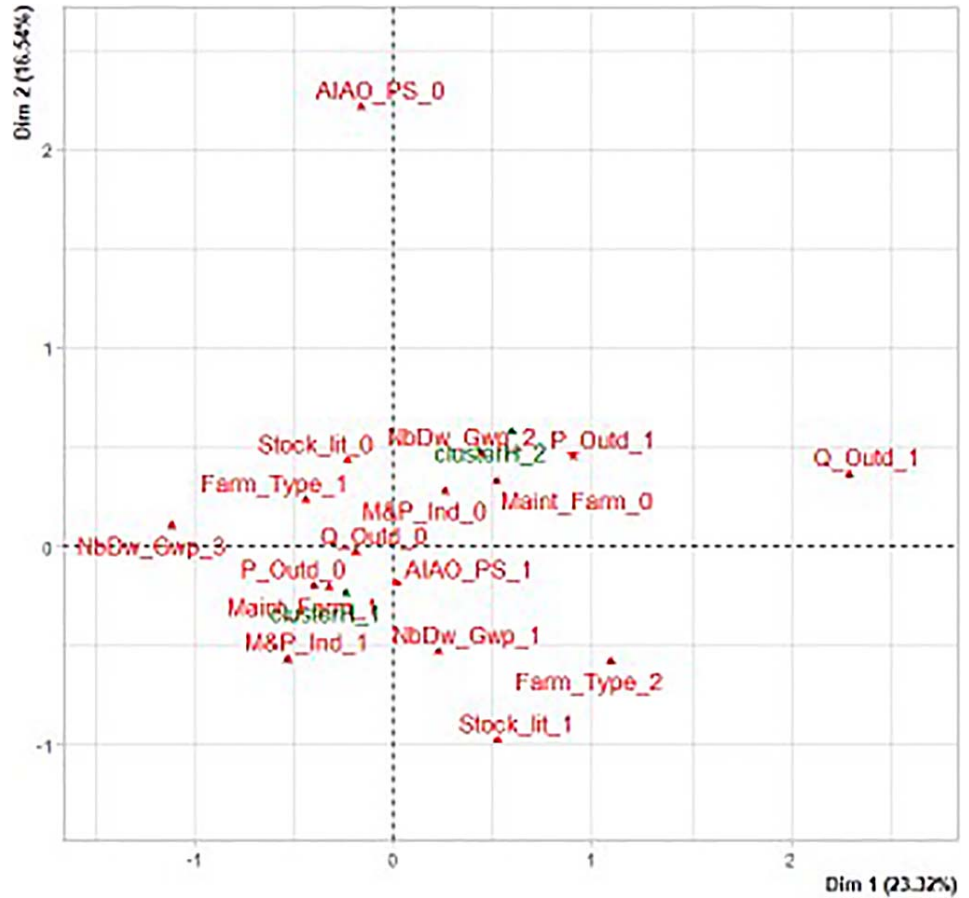
Relationship between parasitism, farm characteristics, and management

Helminths: The hierarchical ascending classification used to group farms according to their helminth parasite status revealed 2 groups of farms (Table VI). The first group of 37 farms was characterized by a lower level of parasitism concerning strongyles and *T. suis* than the second group of 15 farms, both in sows (average percentage of sows infested by strongyles or *T. suis*, respectively, 20.3 and 1.4% in group 1 vs. 75.3 and 5.3% in group 2), as well as in piglets of 10–12 wk of age (average percentage of animals infected by strongyles or *T. suis*, respectively, of 1.4 and 1.6% in group 1 vs. 25.3 and 14.7% in group 2) or on finishers (average percentages of animals infested by strongyles or *T. suis*, respectively, of 0.5 and 1.6% in group 1 vs. 40.7 and 14.7% in group 2).

The result of the multiple correspondence analysis related to helminth parasite pressure, including the descriptive elements of the farm characteristics, is presented in Figure 7. The distribution of farms is shown in Figure S3. The first 2 dimensions explain 39.8% (23.3 and 16.5%, respectively) of spatial variation in the data. The variables that contributed most to the variability in dimension 1 were the type of farm (22.9%), outdoor gestation (17.3%), outdoor quarantine (20.9%), and the number of parasite treatments performed during the growth phase after weaning (18.3%). Those that contributed most to the variability of dimension 2 were all-in-all-out (AI-AO) management in post-weaning rooms (27.4%), indoor mating and pregnancy (10.6%), and proper storage of the litter in terms of biosecurity (28.4%).

The farms with a higher level of helminth parasitism (cluster H_2) appear to be associated with the presence of outdoor quarantine and gestation, with the absence of a building for the mating-gestating phase. They also appear to be associated with the absence of AI-AO management in the post-weaning period

Figure 7. Graphical representation of the results of the multiple correspondence analysis of the descriptive variables of the farm, including housing, animal management, and health and antiparasitic management, and the classification of the farms according to the proportion of animals infected by helminth eggs or seropositive for *Ascaris suum* on 52 farrow-to-finish farms. Abbreviations: Farm-Type, Type of farm (1, Farrow-to-finish; 2, Partial farrow-to-finish); M&P_Ind, indoor mating and pregnancy (0, No; 1, Yes); P_Outd, Pregnancy outdoors (0, No; 1, Yes); Q_Outd, quarantine outdoors (0, No; 1, Yes); AIAO_PS, all-in-all-out management in post-weaning rooms (27.4%); Stock_lit, correct condition of litter storage in terms of biosecurity (0, No; 1, Yes); Maint_Farm, correct maintenance of farm building (0, No; 1, Yes); NbDw_Gwp, number of deworming treatments on grower/finisher pigs (1, ≤1; 2, >1 and ≤2; 3, ≥3). Color version available online.



interval between batches, and the number of sows per Man Work Unit (MWU) contributed to the construction of dimension 1 (respectively, 34.8%, 26.5%, 18.9%, and 19.8%). The variables related to the number of sows per MWU, the systematic washing of the farrowing room between batches and the interval between batches contributed to the construction of dimension 2 (38.1%, 38.0%, and 18.1%, respectively).

The clusters of farms were mainly projected on dimension 1. Farms with a higher frequency of feces infected with coccidia oocysts (cluster Co_2) appear to be associated with the presence of straw in the farrowing pens but also with systematic washing of the farrowing room between batches. Farms with long intervals between batches (>6 wk) or where there are fewer than 50 sows

per MWU appear to be associated with a higher frequency of animals carrying coccidia oocysts.

Toxoplasma gondii: The hierarchical ascending classification used to group farms according to their serological status regarding *T. gondii* revealed 3 groups of farms (Table VIII). The first group of 32 farms (Cluster Tx_1) is characterized by a lower average seroprevalence level in both sows (4.4%) and finisher pigs (1.9%). The second group (Cluster Tx_2) has few seropositive finisher pigs on average (2.9%), but a high average percentage of seropositive sows (45.9%), while the third group (Cluster Tx_3) is characterized by a high average seroprevalence in both sows and finisher pigs (76.7% and 50.0%, respectively).

The result of the multiple correspondence analysis linked to the percentages of *T. gondii*-seropositive animals, including the

Table VII. Description of the variables related to coccidia that characterized the 2 clusters obtained by hierarchical ascending classification (52 farrow-to-finish farms). The variable (bold) characterized the cluster. Coprology is the diagnostic test used.

Variable (coprology)	General sample		Cluster Co_1		Cluster Co_2	
	52 herds		37 herds		15 herds	
	Mean	SD*	Mean	SD	Mean	SD
Percentage of positive sows	26.0	28.8	19.7	24.1	41.3	33.4
Percentage of positive grower pigs	15.8	29.3	1.6	6.8	50.7	34.0
Percentage of positive finisher pigs	27.7	36.2	7.0	12.3	78.7	23.1

* SD, standard deviation.

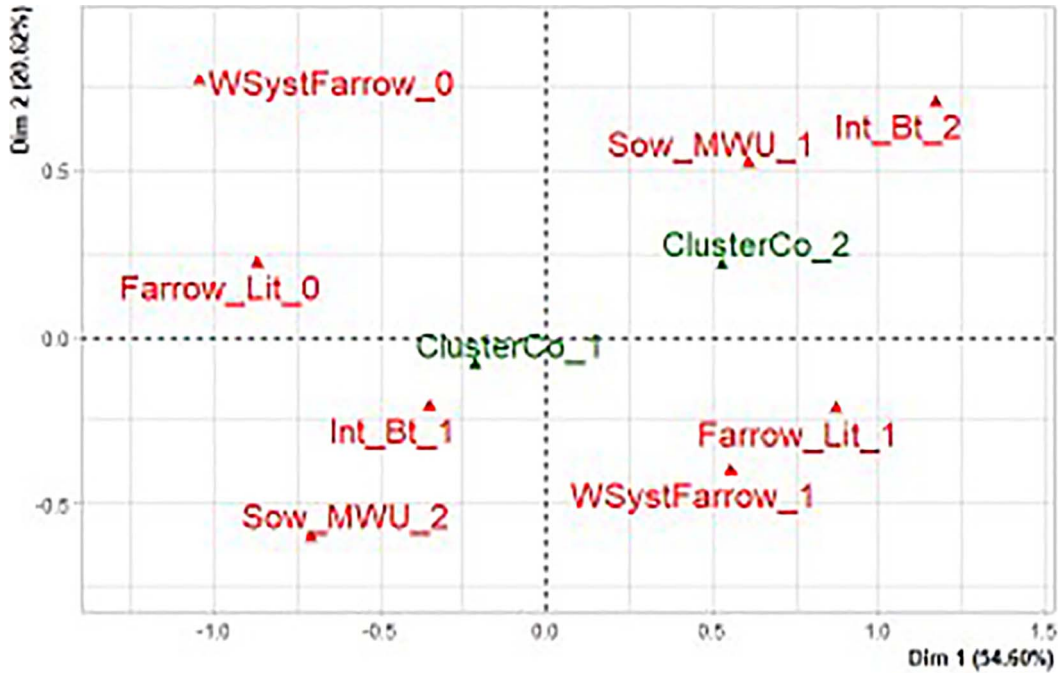


Figure 8. Graphical representation of the results of the multiple correspondence analysis of the descriptive variables of the farm, including housing, animal management, and health and antiparasitic management, and the classification of the farms according to the proportion of animals infected by coccidia oocysts on 52 farrow-to-finish farms. Abbreviations: WSystFarrow, farrowing rooms washed systematically between each batch (0, No; 1, Yes); Farrow_Lit, presence of litter in farrowing pens (0, No; 1, Yes); Int_Bt, interval in weeks between batches of animals (1, ≤6; 2, >6); Sow_MWU, number of sows per man work unit (MWU) (1, ≤50; 2, >50). Color version available online.

descriptive elements of the farm, is presented in Figure 9. The distribution of farms is shown in Figure S5. The first 2 dimensions explain 48.7% (30.7 and 18.0%, respectively) of spatial variation in the data.

The variables that contributed the most to the variability of dimension 1 were the type of housing in the farrowing room (indoor, outdoor, indoor with outdoor courtyard) (34.9%), the systematic washing of the farrowing room between batches (33.3%), outdoor quarantine (16.4%), and the presence of an outdoor courtyard in mating and pregnancy pens. It was mainly the variables related to safe litter storage (34.6%), type of housing in the farrowing room (33.9%), and size of the farm (21.7%) that contributed to the construction of dimension 2. The farms with lower seroprevalences in both sows and finisher pigs (Cluster Tx_1) appear to be associated with the absence of outdoor farrowing and quarantine units, but with the presence of an outdoor courtyard in mating and pregnancy pens. It appears that

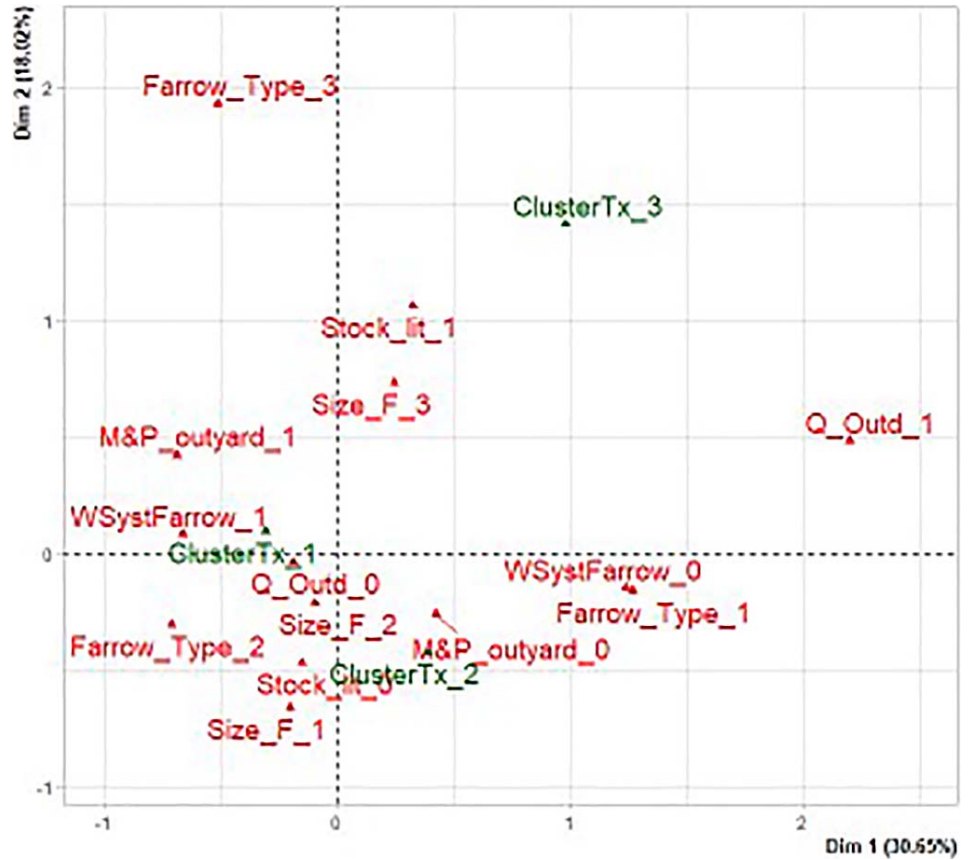
these farms are of medium size (between 50 and 100 sows), with systematic washing of the farrowing rooms between batches and proper storage of the litter in terms of biosecurity. In contrast, the farms in Cluster Tx_2 with higher seroprevalences in sows appear to farm with outdoor farrowing and quarantine units. The absence of an outdoor courtyard in mating and pregnancy pens on these farms may indicate the presence of either exclusively indoor or strictly outdoor mating and pregnancy pens. These farms are quite small (<50 sows) and appear to be associated with poor litter storage in terms of biosecurity. Unlike Cluster Tx_2 farms, Cluster Tx_3 farms appear to be larger (≥100 sows), and litter is stored properly. As with Cluster Tx_2, they appear to be associated with the implementation of outdoor quarantine and the absence of an outdoor courtyard in mating and pregnancy pens. They appear to have either outdoor farrowing pens (dimension 1) or an outdoor courtyard in farrowing units (dimension 2).

Table VIII. Description of the variables (bold) related to *Toxoplasma gondii* that characterized the 3 clusters obtained by hierarchical ascending classification (52 farrow-to-finish farms).

Variable	General sample		Cluster Tx_1		Cluster Tx_2		Cluster Tx_3	
	52 herds		32 herds		27 herds		3 herds	
	Mean	SD*	Mean	SD	Mean	SD	Mean	SD
Percentage of seropositive sows	22.1	25.4	4.4	5.6	45.9	15.4	76.7	4.7
Percentage of seropositive finisher pigs	5.0	12.5	1.9	3.9	2.9	4.6	50.0	16.3

* SD, standard deviation.

Figure 9. Graphical representation of the results of the multiple correspondence analysis of the descriptive variables of the farm, including housing, animal management, and health and antiparasitic management, and the classification of the farms according to seroconversion for *Toxoplasma gondii* on 52 farrow-to-finish farms. Abbreviations: Size_F, farm size (1, ≤50 sows; 2, >50 and ≤100 sows; 3, ≥100 sows); Farrow_Type, type of farrowing room (1, Outdoor; 2, Closed building; 3, Indoor with outdoor courtyard); WSystFarrow, farrowing rooms washed systematically between each batch (0, No; 1, Yes); M&P_outyard, mating and pregnancy indoors with an outdoor courtyard (0, No; 1, Yes); Q_Outd, quarantine outdoors (0, No; 1, Yes); Stock_lit, litter stored properly in terms of biosecurity (0, No; 1, Yes). Color version available online.



DISCUSSION

Internal parasitism remains a major concern in alternative farming systems, including non-organic farms (Rousing, 2011; Delsart et al., 2020). Other studies have already investigated parasitism in alternative pig farms (Carstensen et al., 2002; Baumgartner et al., 2003; Eijck and Borgsteede, 2005), but this study is the first to our knowledge to include so many pig farms in alternative systems (organic and non-organic) and to use both coprological and serological analyses. Another originality is the inclusion of serological investigations into *T. gondii*, a parasite whose infections are usually fully asymptomatic in pigs, but whose zoonotic impact can be significant (Lindsay et al., 2019). However, we did not search for other parasites of zoonotic interest, such as *Trichinella spiralis*. Given the epidemiological situation in France concerning this parasite, research was not warranted. Indeed, according to European regulations, all pigs with outdoor access are tested for *Trichinella* spp. at the slaughterhouse. There has been no evidence of the parasite in pig carcasses since 2007 in continental France (Vallée et al., 2019).

The large number of structures to which the selected farms belonged, as well as their diversity, ensured variability. A total of 70 farms were visited, which was the maximum number of farms that could be included during the study period (June 2020 to June 2021). There are few numerical data on alternative farms in France. However, in 2018, 721 French pig farms were adhering to organic agriculture specifications (FNAB, 2018). Forty-five were included in our study, their geographical distribution being very

close to that of organic pig production in France as a whole (FNAB, 2018).

To be included in the study, the farm had to have at least 20 sows and/or 100 grower/finisher pigs. This selection criterion was imposed by the protocol, which required 10 samples from breeding stock and 20 grower/finisher pigs (10 grower pigs and 10 finisher pigs). At minimum 20 sows were therefore needed to be able to perform all the samples during a single visit, without having to sample animals in critical periods (farrowing, beginning, and end of gestation). Without having guaranteed its representativeness through random selection, our sample is globally close to the nationwide population of French alternative farms with more than 20 sows and/or more than 100 grower/finisher pigs, according to the different elements available (FNAB, 2018).

According to the categories of animals present, 10 sows, 10 grower pigs, and 10 finisher pigs were sampled on each farm to detect a parasitic infestation with a minimum prevalence of 26% ($\alpha = 0.05$) within each category of animals sampled and for each type of sample, for a test with an assumed sensitivity of 100%. For the farrow-to-finish farms (52 farms), 30 fecal samples were taken to detect a parasitic infestation with a minimum prevalence of 10% ($\alpha = 0.05$). Concerning serological analyses, 10 animals were sampled per farm for *A. suum* and a maximum of 20 for *T. gondii*. The minimum prevalence for detecting *T. gondii* infestation at the farm level was 14% ($\alpha = 0.05$) for farrow-to-finish farms. Given the results obtained, our numbers appear to be sufficient for detecting strongyles and coccidia (at least for sows

for strongyles and finisher pigs for coccidia) as well as for detecting antibodies to *A. suum* (43% seropositive animals on average). On the other hand, the average rates of infested animals per farm are much lower than the prevalence limits, i.e., the prevalence detection thresholds for *A. suum* and *T. suis*, but also for *T. gondii*-seropositive pigs at the end of the fattening period, which may have led to an underestimation in the rate of farms infested by these parasites.

This is especially true because the tests used are not perfectly sensitive. This is particularly the case for coprology (Roepstorff and Nansen, 1998; Vlamincx et al., 2012), the sensitivity of which is difficult to estimate because it depends on both the number of parasite eggs or oocysts excreted (sensitivity of 100 eggs per gram of feces (Pereckiene et al., 2007)) and the type of parasite: sensitivity decreases with the length of the parasite's prepatent period (Roepstorff and Nansen, 1998).

Of the 70 farms, only 5 had neither helminth eggs nor coccidia oocysts, confirming that internal parasitism is nearly omnipresent in alternative systems, especially production systems with outdoor access (Baumgartner et al., 2003; Rousing, 2011; Früh et al., 2018). Furthermore, the diversity of parasites found in our study is consistent with previous studies (Carstensen et al., 2002; Baumgartner et al., 2003; Eijck and Borgsteede, 2005), which mainly identified the eggs of strongyles, *A. suum*, *T. suis*, and *Strongyloides* spp., as well as coccidia oocysts.

Coccidia oocysts were found in almost 4 out of 5 farms (79%). Proportionally more farms with sows were found to have coccidia oocysts than farms with grower/finisher pigs. The rate of positive animals was slightly lower in sows (29.1%) than in finisher pigs (31.2%), but still significantly higher than in grower pigs (14.2%). These levels may be related to the animals' greater exposure to the parasite with age, due to their rearing conditions. The reduction in the number of oocysts per gram of feces could be explained by a stronger immunity in sows induced by more frequent and numerous contacts with coccidia.

In our study, all the coccidia oocysts observed belonged to the genus *Eimeria*, as described in many other studies (Carstensen et al., 2002; Eijck and Borgsteede, 2005; Prunier, 2010). The real role of *Eimeria* spp. coccidia remains to be evaluated, but several studies suggest that some species of *Eimeria*, such as *Eimeria deblickei* or *Eimeria spinosa*, may be responsible for digestive disorders or even mortality in growing or breeding pigs (Lindsay et al., 2002; Yaeger et al., 2003). Clinical coccidiosis can occur in finishing animals and breeding stock exposed to contaminated facilities (Lindsay et al., 2019). In our study, *Eimeria* species were not defined, but at least 2 different species coexisted (based on the morphology and size of oocysts) in at least 1 sample, on nearly 1 farm out of 2.

The eggs of strongyles are represented by *Oesophagostomum* spp./*H. rubidus*, and were found in a large proportion of farms (47%). The similarity of *Oesophagostomum* spp. eggs to *H. rubidus* eggs makes differentiation difficult. The proportion of infested farms, the proportion of infected animals, and the average number of strongyles eggs per infested sample increases with the age of the animals, in accordance with what is observed in many studies (Carstensen et al., 2002; Baumgartner et al., 2003; Eijck and Borgsteede, 2005; Prunier, 2010).

Trichuris suis was the second most common helminth in terms of the proportion of farms infested. At least 1 fecal sample contained a *T. suis* egg on 36% of farms. These results are close to

those of Eijck and Borgsteede (2005), who observed *T. suis* eggs in 10 (37%) out of the 27 alternative farms in their study, but are slightly lower than those obtained by Carstensen et al. (2002), who isolated *T. suis* eggs from 6 out of the 9 farms studied. Within-farm prevalence was generally low. The rate of infected animals decreased with age, as already shown in a previous study in France on 20 organic farms (Prunier, 2010). This seems logical, as pigs develop strong protective immunity against whipworms (Thamsborg et al., 1999). However, other studies show different results with higher prevalence in older animals (Carstensen et al., 2002; Eijck and Borgsteede, 2005). In conventional farming, this parasite is only present quite sporadically and without preference for a specific age group (Roepstorff and Nansen, 1994).

Ascaris suum eggs were found on 16% of farms, which is significantly lower than results from other studies (Baumgartner et al., 2003; Eijck and Borgsteede, 2005). In our study, sows and finisher pigs were predominantly infested, which is in line with the observations of Eijck and Borgsteede (2005). As with *T. suis*, the within-farm prevalence was extremely low, averaging less than 3% in any animal category. Again, as with *T. suis*, infested sows had the highest quantities of *A. suum* eggs per gram of feces.

To complement the coprology results, blood samples were taken from finisher pigs to test for antibodies to *A. suum*. Forty-three percent of the animals sampled were positive, and 91% of the farms had at least 1 positive sample. In many studies, the average titer of the ODr is calculated to define the level of infestation pressure. A mean titer greater than 0.5 characterizes a medium to high infestation pressure. In our study, 45% of the farms had an average ODr value greater than 0.5. To our knowledge, these are the first data obtained specifically for alternative pig farms. However, their range of values is quite close to those available for conventional farms (Vandekerckhove et al., 2014; Martínez-Pérez et al., 2017).

Coprology and serology results were thus significantly different for *A. suum*, whose eggs were found on only 16% of the farms, while 91% of them had at least 1 positive serology result. Other studies have previously shown that more animals were positive for *A. suum* when considering their serological rather than their coprological status (Roepstorff, 1998; Vlamincx et al., 2012). Egg counts, therefore, appear to underestimate the proportion and number of pigs exposed to *A. suum*. The SERASCA® test is used to detect antibodies to *A. suum* hemoglobin. It can thus determine whether a pig has been infested with larval or adult forms of the parasite, whereas coprology tests focus on parasite eggs in feces (Vlamincx et al., 2014). Boes et al. (1997) showed that *A. suum* eggs may not be observed in coprological samples despite the presence of adult worms in the gut. This can be observed when the adult worms infesting the pig are of the same sex. Furthermore, this can also be the case in the event of infestation by immature larvae, which do not produce eggs (Roepstorff and Nansen, 1998). In addition, in the event of a parasitic infestation, there is an immune response with a possible decrease in egg production (Roepstorff and Nansen, 1998). Still, serology has some limitations, such as the time to seroconversion, which is at least 6 wk (Vlamincx et al., 2012). Furthermore, the presence of antibodies at the end of fattening does not mean that the pig is still parasitized, but that it has been in contact with *A. suum* (larval or adult stage) during its life. Conversely, the presence of *A. suum* eggs in a coprological sample indicates the presence of adult females.

Toxoplasma gondii antibodies were detected using MAT, which is considered the gold standard test in animals (Djokic et al., 2016b). The sensitivity and specificity of this test were estimated to be 82.9 and 90.3%, respectively (Dubey et al., 1995). The proportion of farms with at least 1 seropositive animal (60%) is higher than that described in the study by Van der Giessen et al. (2007). However, in this study, only finisher pigs were sampled. Considering only the results obtained in our study on this category of animals, we obtained data of the same order of magnitude in terms of the frequency of positive farms. In terms of within-farm prevalence, our results are close to those of Djokic et al. (2016a) obtained on conventional farms.

We studied the relationships between the levels of parasitism on the investigated farms. For both sows and grower/finisher pigs, it is difficult to find a common rationale in the associations between variables related to coccidia positivity rates, *T. gondii* seroprevalences, and variables associated with helminth positivity rates. Given these results, it appeared difficult to characterize a farm based on its parasitism in general. This is why we tried to characterize the farms separately according to (i) coprology and serology results for helminths, (ii) coprology results for coccidia, and (iii) seroprevalences for *T. gondii*.

The multivariate analysis was performed on the 52 farrow-to-finish farms to have all the descriptive data of the farms, including housing, animal management, and health and antiparasitic management. The advantage of analyzing the farrow-to-finish farms lies in being able to couple the results from sows and grower-finisher pigs.

Among these 52 farms, 15 did not raise all their animals after weaning (partial farrow-to-finish farms). These 15 farms would appear to have a higher level of helminth parasitism. This result is probably related to the association observed between the number of anthelmintic treatments given after weaning and the higher level of helminth parasitism. Indeed, partial farrow-to-finish farms tended to deworm their pigs less frequently after weaning (1.3 anthelmintic treatments per animal for partial farrow-to-finish farms versus 2.1 treatments for other farrow-to-finish farms). Since current anthelmintic drugs do not have a persistent effect (Vandekerckhove, 2018), re-infestations are therefore possible after treatment through the absorption of new eggs still present on the farm.

The results of our study show that access to the outdoors for sows, and more specifically free-range livestock, is a characteristic of farms with the greatest helminth or *T. gondii* infestations. It is complicated in outdoor facilities to decontaminate the soil and break the parasite cycle (Carstensen et al., 2002; Lindgren et al., 2019). The persistence of helminth eggs in the environment facilitates infestation of animals from successive batches in the paddocks. For *T. gondii* in particular, access to outdoor facilities may promote contact with cats and/or rodents, increasing the probability of pigs ingesting oocysts and tissue cysts. Numerous studies have identified the presence of cats as a risk factor for *T. gondii* seropositivity (Assadi-Rad et al., 1995; Garcia-Bocanegra et al., 2010; Pablos-Tanarro et al., 2018). Although the outdoor housing of gilts in quarantine, or sows and piglets in farrowing facilities, characterized the farms with the highest *T. gondii* infestation in our study, the presence of outdoor courtyards in mating and pregnancy pens characterized farms with the lowest proportions of *T. gondii*-seropositive animals. The presence of outdoor courtyards in mating and pregnancy pens is statistically

related in our study to other variables, in particular to the systematic washing of farrowing facilities between batches. It has already been shown by Veronesi et al. (2011) that washing and disinfecting pens between batches can reduce the seroprevalence of *T. gondii*.

Descriptive parameters of the hygiene of farrowing and post-weaning rooms also contribute to defining the typology of farms according to their level of parasitism by helminths or coccidia. The farms with the highest levels of helminth parasitism in our study were more likely not to use an all-in-all-out postweaning system than those with the lowest levels of helminth parasitism, confirming the observations of Kochanowski et al. (2017). With such a continuous flow system, the rooms or pens cannot be systematically washed and decontaminated between batches, which could increase parasite pressure. Inter-batch contamination between pigs of different infestation and immune statuses is also more likely in this type of management, thus facilitating the intra-farm contamination cycle. Conversely, the absence of systematic washing of farrowing facilities between batches characterizes the farms with the lowest frequencies of feces infected with coccidia oocysts. Among the farms that do not systematically wash the farrowing units between batches, 17/18 have outdoor farrowing units. Outdoors, conditions are less favorable for sporulation of coccidia oocysts than indoors, which provides optimal conditions around farrowing for sporulation of some *Eimeria* (Graat et al., 1994). Oocysts are more susceptible to destruction when in the unsporulated state and during sporulation (Lindsay et al., 2019).

Regarding the housing conditions of animals in the farrowing unit, the presence of straw bedding rather than slatted floors characterizes farms where feces are more frequently infected with coccidia oocysts. Compared to bedding, a slatted floor allows for more rapid evacuation of feces and thus reduces the duration of exposure of pigs to the parasite by limiting the contact time with potentially infected excreta. The effect of soil type on the infection cycle of other pathogens has been previously described (Andres and Davies, 2015). The type of slatted floor is probably also important. It is generally accepted that concrete flooring tends to be porous and therefore difficult to clean. In our study, 10 farms had farrowing rooms with fully slatted floors. Slatted flooring material varied little from farm to farm, and often combinations of materials were used in the same pen. Molded plastic was present in all farrowing pens, combined with metal bars on 9 farms, stainless steel slats on 2 farms, and concrete on only 1 farm. The lack of diversity did not allow us to identify differences between types of slatted floors in our study.

One of the original outcomes of our study is how it shows that the level of biosecurity concerning the storage of straw used as bedding for pigs is another factor that discriminates among farms as it affects the level of parasitism by helminths and *T. gondii*. Storing straw under good biosecurity conditions could limit the possibility of it becoming contaminated with helminth eggs via mechanical or biological vectors, or—in the case of *T. gondii*—by dead rats, mice, or even birds that may be carrying the parasite (Dubey, 2002; Kijlstra et al., 2008). Good storage conditions thus limit the exposure of pigs to these pathogens via litter contaminated during storage.

Variables describing general farm characteristics such as farm size, number of sows per farmer, and the interval between batches also contribute to the characterization of the farms studied concerning their level of parasitism by *T. gondii* or coccidia. The

interval between batches and the number of sows per farmer is correlated with the size of the farm: in our study, a long interval between batches and a few sows per farmer is generally frequent on farms with a small number of pigs. The size of the farm determines many factors related to management, biosecurity practices, and measures as well as animal and human flows not considered here. Farm size should be considered in this work as a more general descriptor of a set of other unmeasured parameters that contribute more specifically to maintaining the cycle of contamination between animals.

In conclusion, this observational study confirms that parasitism is frequent on alternative farms. A specific study including even more farms and considering all risk factors could be useful to try to better understand the typologies described in this study, and to propose to farmers and veterinarians alike better ways of controlling parasitism on alternative farms. Finally, our study shows major differences in the results obtained for *A. suum* depending on whether the search is performed by coprology or serology.

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