

Abattoir monitoring of liver lesions caused by *Ascaris suum* larvae: A longitudinal study conducted in northern Italy

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ABSTRACT

Introduction: Data supplied by the abattoir monitoring system offer a useful tool both for tracing the prevalence of *Ascaris suum* milk spot lesions and for conducting large-scale epidemiological studies.

Materials: In this study, livers of 754833 slaughtered pigs from 5835 batches and 399 farms located in northern Italy, were evaluated in one of the largest national abattoirs during a year of survey. From each farm data concerning the breeding region and province, the season of post-mortem inspection, the farm size and the type of farm production were collected and the probability of finding a positive animal, i.e. with at least one milk spot lesion, in relation to these factors was estimated by a generalised linear mixed model (GLMM).

Results: 368 out of 399 farms tested positive (92.2 %), out of 5835 batches of animals, 4395 (75.3 %) had at least one positive pig, and out of 754833 carcasses, 198964 showed milk spots, with an overall prevalence of 26.4 %. The breeding province and the season of post-mortem inspection were two significant risk factors: a higher risk of finding animals with milk spots was recorded in some Italian provinces and in spring.

Conclusions: This study provides data on the prevalence of milk spots caused by *A. suum* migrating larvae in an area characterised by a high density of pig farms. Given the high prevalence recorded, it is necessary to promote effective communication between abattoir, veterinarians and farmers, monitoring the circulation of *A. suum*, developing specific control plans and enhancing animal health surveillance.

1. Introduction

Ascaris suum is a common migratory intestinal parasite affecting pigs of all ages. Adult ascarids live in the pig intestine, where they may provoke chronic malabsorption due to progressive atrophy of intestinal villi, while the migrating larvae may damage several organs. In the liver parenchyma, the larval migration is responsible for the characteristic lesions known as "milk spots", which appear as white, round and fibrous areas distributed over the surface of the organ and disappear approximately three weeks after infection (Dold and Holland, 2011; Martínez-Pérez et al., 2017; Lassen et al., 2019). On histopathological examination, recent milk spots are characterised by a necrotic centre surrounded by eosinophils, neutrophils, and macrophages that progress to granulomatous foci, whereas older lesions show fibrosis with

infiltration of lymphocytes and plasma cells (Pérez et al., 2001; Sanchez-Vazquez et al., 2010). In the lungs, migrating *A. suum* larvae can cause dyspnoea, pleuritis, pneumonia and allergic asthma, and promote secondary bacterial and viral infections (Dold and Holland, 2011; Vlaminck et al., 2015; Lassen et al., 2019).

Epidemiological surveys conducted in Europe reported prevalence rates of *A. suum* in fattening pigs raised in intensive systems ranging from 0.9 % to 10.5 %, employing copromicroscopic techniques (Joachim et al., 2001; Schubnell et al., 2016; Symeonidou et al., 2020; Pettersson et al., 2021a). According to these studies, the structural and management characteristics of farms, including the type of floor, farm size and application of an all-in/all-out system, as well as the diagnostic method used and season, are the main factors that could influence the circulation of *A. suum* (Boes et al., 2010; Dold and Holland, 2011; Kochanowski

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et al., 2017; Pettersson et al., 2021a). Despite the moderate prevalences observed, ascarid infection could have a major economic impact due to the reduction in weight gain and feed intake and the negative feed conversion index, which, together, are responsible for an increase in the daily feeding costs and organ wastage (Kipper et al., 2011; Martí-nez-Pérez et al., 2017). Recent cost estimates in the US showed an annual loss of up to \$60.1 million for supplementary feeding of pigs infected with *A. suum*, and an annual loss of \$17.5 million for the discarding of livers (Stewart, 2001; Boes et al., 2010; Massaglia et al., 2018). In European countries, along the slaughter line, livers of all pig carcasses are observed and if milk spots are confirmed, the organ is discarded and not intended for human consumption, as stated in EU Regulation 627/2019 of 15 March 2019. In addition, recent studies have shown that *A. suum* infection could indirectly affect meat quality, causing carcass depreciation (Knecht et al., 2011; Massaglia et al., 2018). Particularly in northern Italy, where almost all pig farms adhere to PDO (Protected Designation of Origin) consortia, the impact of *A. suum* infection on carcasses may lead to the withdrawal from PDO circuits, exacerbating the economic losses. In fact, these consortia aim to produce cured meats from pigs with a live weight of 160–170 kg: pigs that do not reach this weight are discarded and the price of their meat is lower than that set for PDO pigs (Bottacini et al., 2018; Guardone et al., 2020).

Unfortunately, the diagnosis of *A. suum* infection in pigs is challenging due to its subclinical nature and non-specific symptoms (Thamsborg et al., 2013). The copromicroscopy can give false negative results due to the long pre-patent period of this parasite or to the presence of a parasite population consisting of individuals of the same sex or immature stages; at the same time, the serological screening is sensitive but also costly (Martínez-Pérez et al., 2017; Zheng et al., 2020; Joachim et al., 2021; Delsart et al., 2022; Tassis et al., 2022). Indirect diagnosis may include the assessment of liver milk spots at the abattoir, which reflect a recent exposure to *A. suum* (Joachim et al., 2021), allowing the evaluation of temporal and seasonal trends (Goodall et al., 1991; Sanchez-Vazquez et al., 2010, 2012). However, in Italy, their occurrence during the post-mortem inspection is scarcely investigated (Poglayen et al., 2008; Scollo et al., 2017; Vismarra et al., 2023).

Considering the great potential of monitoring these lesions and the lack of current data on *A. suum* circulation, a large-scale retrospective observational study was planned in northern Italy, where most of the Italian pig production is concentrated (Gazzonis et al., 2018; Allievi et al., 2024). Thus, the objectives were to investigate the prevalence of milk spots in carcasses slaughtered over a one-year period and assess the plausible influence of several variables, namely the breeding region and province, the season of post-mortem inspection, the farm size, the type of farm production and their interaction, on the risk of detecting this type of lesion.

2. Materials and methods

2.1. Data source and collection

In the present study, we retrieved data on milk spot liver lesions recorded between October 2020 and September 2021 during meat inspection of pigs slaughtered in one of the largest abattoirs located in northern Italy (Lombardy region, Mantua province) with a speed of the slaughter line of around 380–400 animals per hour. Within the reporting year, livers of 754833 pigs from 399 farms and 5835 batches, with an average of 129.4 pigs per batch, were evaluated. A range of batches (1–170) were brought from each examined farm to the abattoir and a batch was defined as a group of animals from the same herd and presented at the abattoir on a given date. The slaughtered pigs, commercial hybrids of Duroc, Landrace and Large White breeds and used to produce cured meats, were about nine months old and weighed 170 kg on average. During carcass inspection at the abattoir, all livers within each batch of animals were evaluated and the milk spot lesions were assessed

as a binary variable, present (at least one lesion) or absent, by trained veterinarians. A single milk spot present on the liver surface was enough to exclude the organ from human consumption (EU Regulation 627/2019).

2.2. Study population

The farms were located in different regions of northern Italy, specifically 250 in Lombardy (including the provinces of Bergamo, Brescia, Cremona, Lodi, Mantua, Milan and Pavia), 51 in Emilia-Romagna (including the provinces of Ferrara, Forlì, Modena, Parma, Piacenza, Ravenna, Reggio-Emilia), 47 in Piedmont (including the provinces of Alexandria, Asti, Biella, Cuneo, Novara and Turin), 38 in Veneto (including the provinces of Padua, Rovigo, Treviso, Venice, Verona and Vicenza) and 13 in Friuli-Venezia Giulia (including the provinces of Pordenone and Udine). Most farms were concentrated in the area between Lombardy and Emilia-Romagna, where the largest number of fattening pigs is raised (Fig. 1).

For each animal, the specific season of post-mortem inspection was recorded: spring (March to May), summer (June to August), autumn (September to November) winter (December to February). Data regarding farm management were obtained using the National Zootechnical Database (www.vetinfo.it). In our study, it was only possible to collect information on the farm size and the type of farm production. Specifically, the farm size was grouped into five classes: 1) up to 1500 fatteners; 2) 1501–3000 fatteners; 3) 3001–6000 fatteners; 4) 6001–9000 fatteners and 5) more than 9001 fatteners; and the type of farm production was divided into two categories: feeder-to-finish farms, which are specialised fatteners producers, where pigs of about three months old and weighing 30 kg are housed after weaning, and farrow-to-finish farms, which include all phases of the production cycle: breeding, farrowing, weaning, growing and fattening units.

2.3. Statistical analysis

The presence of at least one milk spot lesion was considered equivalent to positivity. Farms were considered positive if they had at least one positive batch, and batches were considered positive if they included at least one slaughtered pig with milk spot lesions. Accordingly, the prevalence was expressed as the percentage of positive cases at different levels, by number of farms, batches and pigs slaughtered in different regions of northern Italy. Moreover, the average probability of extracting a positive animal from a specific farm (indicated as “Risk”), was calculated considering 5 classes with upper limits included in each class: $\leq 5\%$, between 6 % and 10 %, between 11 % and 30 %, between 31 % and 50 %, and $> 50\%$.

The probability of finding a positive animal in relation to certain factors was analysed by a generalised linear mixed model (GLMM) applied to binomial distribution, using the procedure (PROC) GLIMMIX of SAS statistical software. Only farms with at least three batches were included, since in a large dataset as this one, the model may not converge due to the structure of data and the presence of groups with only one or two observations. Thus, a total of 5628 batches related to 258 farms were considered. The response variable, i.e., the number of pigs with at least one milk spot out of the total number of slaughtered pigs, was analysed by a mixed model with the random effect of farm and the fixed effects of breeding region and province, post-mortem inspection season, farm size, type of farm production, and interaction between farm size and type of farm production. The estimates were at the logit scale and were converted into probability scale by applying the ILINK function of SAS software. The model, applied to binomial distribution, showed the least square mean (LSM) and the relative confidence interval (CI) on a probabilistic scale. The reported LSM values represent the probability of extracting a positive animal in the specific levels of factors included in the model; the Tukey–Kramer method was used to adjust multiple comparisons among classification groups. The differences

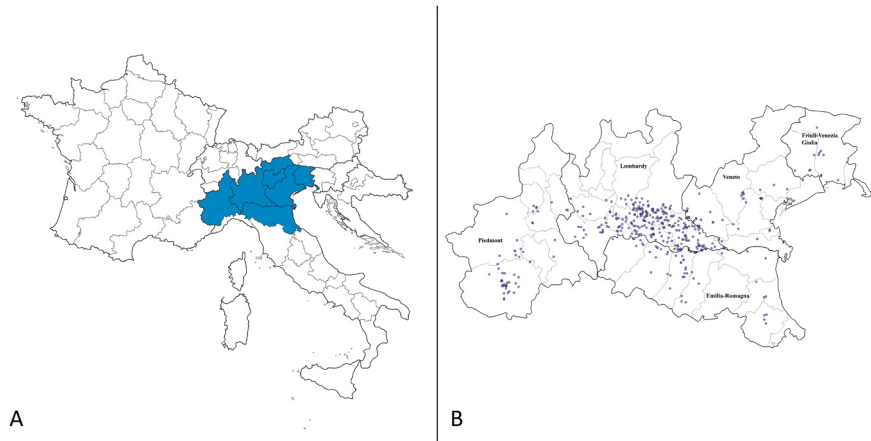


Fig. 1. Geographical distribution of surveyed pig farms in northern Italy using R Studio, version 2023.09.1. A) Map of Italy with neighbouring countries, from left to right: France, Switzerland, Austria and Slovenia. Regions where pig farms are located are indicated in blue. B) The blue dots indicate the location of pig farms in regions of northern Italy. The map lines delineate the study area and do not necessarily depict accepted national boundaries.

between least square means were also reported in terms of odds ratio (OR) with relative confidence interval (CI).

Statistical analysis was performed using SAS software (version 9.4, SAS Institute, Cary, NC, USA).

3. Results

3.1. Descriptive results

In the survey year, 368 out of 399 farms had at least one positive batch (92.2 %) and out of 5835 batches of animals, 4395 (75.3 %) had at least one positive pig. Considering the total number of slaughtered pigs, amounting to 754833, 198964 showed at least one milk spot, with an overall prevalence of 26.4 % (Table 1).

Considering the average probability of extracting a positive animal from a specific farm (indicated as “Risk”), 81 farms were grouped in class 1, 61 in class 2, 125 in class 3, 74 in class 4 and 58 in class 5. Thus, the highest number of farms was distributed in class 3, with a risk of finding pigs with at least one milk spot between 11 % and 30 % (Fig. 2).

3.2. Association between milk spots and risk factors

The breeding province and the post-mortem inspection season significantly affected the number of pigs with at least one milk spot over the total number of slaughtered pigs, while the breeding region, the farm

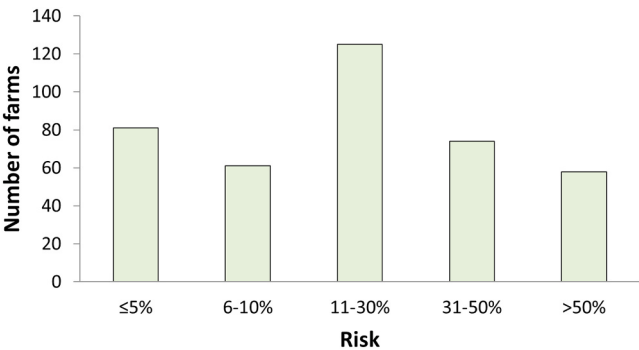


Fig. 2. Distribution of 399 pig farms into five classes based on the average probability of extracting an animal with at least one milk spot (indicated as “Risk”).

size, the type of farm production and their interaction did not have a significant influence on the response variable.

According to the breeding provinces, Forlì-Cesena (0.59 ± 0.20), located in Emilia-Romagna region, had a significantly higher ($p < 0.0001$) least square mean (LSM) than all others, except Rovigo (0.33 ± 0.23), located in Veneto region, and Alexandria (0.31 ± 0.31) and Biella (0.25 ± 0.16), located in Piedmont region.

Table 1
Prevalence of pigs with at least one milk spot out of farms, batches and pigs slaughtered by region of breeding.

Region of breeding	Positive farms		Positive batches		Positive pigs	
	Number of positives/Total number	P (95 % CI)	Number of positives/Total number	P (95 % CI)	Number of positives/Total number	P (95 % CI)
Emilia-Romagna	49/51	96.1 % (86.5–99.5)	798/995	80.2 % (77.6–82.6)	30645/127632	24 % (23.8–24.2)
Friuli-Venezia Giulia	13/13	100 % (96.4–100)	62/101	61.4 % (51.2–70.9)	1653/13253	12.5 % (11.9–13)
Lombardy	231/250	92.4 % (88.4–95.4)	2829/3753	75.4 % (74–76.7)	136112/487630	27.9 % (27.8–28)
Piedmont	41/47	87.2 % (74.3–95.2)	160/243	65.8 % (59.5–71.8)	5300/29393	18 % (17.6–18.5)
Veneto	34/38	89.5 % (75.2–97.1)	546/743	73.5 % (70.2–76.6)	25254/96925	26.1 % (25.8–26.3)
Total	368/399	92.2 % (89.1–94.7)	4395/5835	75.3 % (74.2–76.4)	198964/754833	26.4 % (26.3–26.5)

N°: Number of positives; P: Prevalence; CI: Confidence Interval

As for the post-mortem inspection season, the least square mean (LSM) was significantly higher ($p < 0.0001$) in spring (0.15 ± 0.15) than in other seasons (Table 2).

Compared to spring, all other seasons showed a significantly lower risk ($p < 0.0001$) of finding animals with at least one milk spot lesion during the post-mortem inspection. The only comparison that was not statistically significant was between summer and winter (Table 3).

4. Discussion

This study investigates the prevalence and determinants of milk spots in fatteners slaughtered in northern Italy, an area characterised by a high density of intensive farming.

368 out of 399 farms were positive (92.2 %) and the recorded individual pig prevalence was 26.4 %, with the highest value in the Lombardy region. Comparable values were recorded in two large-scale surveys carried out in northern (23.9 %) and central (28.3 %) Italy (Scollo et al., 2017; Guardone et al., 2020). Another study, although focusing on a small number of pigs, associated the results of the post-mortem inspection with serological screening for *A. suum* antibodies and still showed a prevalence of milk spots of 30.3 % (Vismarra et al., 2023). These data confirm a noticeable circulation of *A. suum* in the fattening units, underlining a possible failure of parasite control plans in several Italian farms. Conversely, in other countries, as England and Slovakia, there has been a general reduction in the incidence of milk spots over the years, probably due to the improved biosecurity and hygiene strategies applied at the farm level (Ondrejková et al., 2012; Sanchez-Vazquez et al., 2012).

The prevalence found in this study was high at both animal and farm level, and most of farms ranked in risk class 3, with a probability of extracting an animal with at least one milk spot predominantly between 11 % and 30 %. These findings suggest significant economic damage, as annual losses in the pig industry are closely linked to organ condemnation, and the presence of milk spots may be penalised in some European abattoirs, with an estimated loss of around €2 per discarded liver (Stewart, 2001; Ózsvári, 2018; Winkler, 2020; Joachim et al., 2021).

As for the results of the statistical analysis, the farm location at provincial level was an important risk factor and, considering that *A. suum* is a widespread parasite, the difference observed in Italian provinces may reflect differences in farm management rather than a different geographical distribution of the parasite (Sanchez-Vazquez et al., 2010). On the contrary, at regional level, no significant differences were evidenced.

Seasonality represented another factor that significantly influenced the risk of finding an animal with milk spots and in spring the risk was significantly higher than in the rest of the year. Considering an optimal temperature of 15 °C for larval development (Nansen and Roepstorff, 1999), a seasonal peak of condemned livers could occur in early spring in Mediterranean countries (e.g. Italy) and in summer in Nordic countries (e.g. the Netherlands), due to the different climate (Scollo et al., 2017; Horst et al., 2019). Several studies observed seasonal fluctuations in the prevalence of milk spots, reflecting temperature variations in pig herds that affect *A. suum* cycle. When temperatures are higher, eggs develop to the infective stage in a shorter time interval, resulting in an

Table 2
Least square means of pigs with at least one milk spot by post-mortem inspection season.

Risk factor	Category	LSM	SE	95 % CI
Post-mortem inspection season	Spring	0.15 ^a	0.15	0.11–0.21
	Summer	0.12 ^{bd}	0.02	0.09–0.17
	Autumn	0.10 ^c	0.02	0.07–0.15
	Winter	0.12 ^b	0.02	0.09–0.17

a, b, c Means with different superscripts are significantly different from each other ($p < 0.05$). LSM: Least Square Mean; SE: Standard Error; CI: Confidence Interval

Table 3
Odds ratios of the comparisons of different post-mortem inspection seasons in the risk of finding pigs with at least one milk spot. The odds ratio was considered statistically significant at 5 % level if the 95 % CI did not include 1.0.

Season		OR	95 % CI
Autumn	Summer	0.81	0.79–0.83
Autumn	Winter	0.82	0.80–0.84
Autumn	Spring	0.64	0.63–0.66
Summer	Winter	1.01	1.00–1.04
Summer	Spring	0.80	0.79–0.81
Winter	Spring	0.78	0.77–0.80

OR: Odds Ratio; CI: Confidence Interval

increase in both the number of infected animals and subsequent milk spots (Goodall, 1991; Nansen and Roepstorff, 1999; Wagner and Polley, 1999; Sanchez-Vazquez et al., 2010, 2012). However, it should be noticed that in the present study seasonality was evaluated during only one survey year, so this aspect could vary if other years were considered.

Regarding farm size, type of farm production, and the interaction between them, no statistically significant differences were found in the categories considered, probably due to the random effect determined by the farm factor. However, several studies have shown that farm size could represent a risk factor for the occurrence of milk spots (Gardner et al., 2002; Sanchez-Vazquez et al., 2010). On the one hand, in large herds the circulation of *A. suum* may be limited by the application of standardised all-in/all-out systems and regular cleaning and disinfection protocols in large herds. On the other hand, in small herds more efficient management focused on each animal may be ensured. Thus, medium-sized farms may have a higher risk of *A. suum* infection with subsequent development of milk spot lesions.

The correct recognition of liver lesions caused by *A. suum* larvae is essential to distinguish them from those caused by the larval migration of other nematodes, in particular belonging to the genus *Toxocara* spp. and *Gnathostoma* spp. Pigs, as paratenic hosts of *Toxocara* spp. may present granulomatous lesions in the liver similar to milk spots, although current studies concern experimental infections and incidental findings (Davidson et al., 2012; Poulsen et al., 2024). Likewise, the distribution of *Gnathostoma* spp. is geographically restricted and migrating larvae mainly cause abscesses (Brewer and Greve, 2019). Furthermore, it should be considered that current intensive farming limits the risk of infections by both *Toxocara* spp. and *Gnathostoma* spp. by reducing exposure to the outdoor environment (Davidson et al., 2012; Van Tuyen et al., 2019; Suzuki et al., 2025).

The ability to use the abattoir database allows access to a wealth of data, providing representative results of the studied population and identifying monthly and country-specific trends of different injuries (Sala and Cabrini, 2005; Sanchez-Vazquez et al., 2010, 2011; Correia-Gomes et al., 2017; Joachim et al., 2021). In this context, one study evaluated the detection of milk spots as a surveillance tool and found a good correlation between farms with multiple liver lesions and those with a high intra-farm prevalence of *A. suum*, derived from copromicroscopic analysis (Bernardo et al., 1990). Moreover, several studies have shown a positive correlation between the occurrence of milk spots at the abattoir and antibody positivity on serological screening; therefore, the risk of having both anti-*A. suum* antibodies and milk spots at the end of the fattening period is high (Vlaminck et al., 2015; Martínez-Pérez et al., 2017; Vandekerckhove et al., 2019; Vismarra et al., 2023).

Ultimately, it would be essential to combine the liver inspection, with regular faecal analysis and serological testing to diagnose the infection even in the pre-slaughter stages and determine the most appropriate time for anthelmintic treatment (Joachim et al., 2021). Depending on the management and prevalence of milk spot lesions on each farm, specific hygiene measures, such as cleaning, disinfection, daily removal of faeces from pens, and application of the all-in/all-out system, could be implemented to limit the circulation of *A. suum* and

the resulting economic losses (Pettersson et al., 2021b).

5. Conclusion

This retrospective observational study provides current data regarding the prevalence, risk factors and inter-farm distribution of pigs with at least one milk spot lesion on a national scale, considering that most pig farms in Italy are concentrated in the investigated area.

The recorded prevalence was rather high, both at the individual and farm level, demonstrating that ascariasis is a persistent problem in Italian pigs and suggesting the need to further investigate the economic impact of this type of lesions in a territory with several PDO consortia, that guarantee a high profit for producers.

CRedit authorship contribution statement

Manfredi Maria Teresa: Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Mortarino Michele:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Zanon Alessandro:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Rizzi Rita:** Writing – review & editing, Validation, Software, Methodology, Formal analysis, Data curation. **Lana Emilio:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Allievi Carolina:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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