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Full Length Research Paper

Histological and molecular identification of *Sarcocystis* sp. species from pig carcasses slaughtered at abattoirs in Dakar, Senegal

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Abstract

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Sarcosporidiosis is a cosmopolitan protozoan disease caused by Sarcocystis, which is present in the form of cysts in the muscle tissue of several animal species, including pigs. Often asymptomatic, this disease can only be detected at the slaughterhouse, as its diagnosis relies on histological, enzymatic and molecular techniques. This study aimed to identify the *Sarcocystis* species present in the carcasses of pigs slaughtered at slaughterhouses in Dakar, Senegal. Samples were taken from different muscles (oesophagus, masseter, diaphragm, myocardium, gluteal) of 300 pig carcasses. These samples were subjected to histological sectioning techniques stained with haematoxylin and eosin (HE) on the one hand, and molecular biology analyses on the other, in order to precisely identify the species concerned. According to the results obtained by histology, the prevalence of *Sarcocystis* infection in pigs was 91.7%. The most infested muscle was the oesophagus. Molecular biology enabled us to identify *Sarcocystis miescheriana* in pigs for the first time in Senegal. In this context, strengthening measures against parasitic diseases in pigs in Senegal and improving health control in abattoirs is essential for public health. New, easy-to-use techniques for researching and identifying the parasite must therefore be developed in order to significantly reduce the risk of disease transmission to human populations.

Keywords: Slaughterhouses - identification - molecular - pigs – sarcosporidiosis.

INTRODUCTION

Meat from slaughtered animals and charcuterie products sometimes contain lesions or alterations that may render them unfit for human consumption (Ciui *et al.,* 2023). This is why preventive measures are implemented. These measures are based on monitoring the health status of livestock, ante-mortem inspection of animals for slaughter, post-mortem inspection of carcasses of animals for slaughter and charcuterie, and possibly microbiological and chemical analyses (Sénégal, 2009). Ante-mortem and post-mortem inspections are most commonly carried out

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in slaughterhouses. Unfortunately, there are lesions that cannot be identified by these methods, which are detrimental to the quality and safety of muscle tissue (Food Standards Agency, 2024). Detecting these lesions and/or the microorganisms responsible for their appearance requires the use of other methods such as microscopic examination, enzymatic digestion and molecular biology, which are not commonly used in everyday health control practices. This results in an underestimation of this type of lesion. Among muscle lesions are those related to muscle sarcosporidiosis, which is known worldwide because it has been studied in several countries (Seksenova *et al.,* 2020) ; (Euzeby, 1997) ; (O'Donoghue, 1978) ; (Flandrin, 2014) ; (Sela-Perez *et al.,* 1982) ; (Kich *et al.,* 2019) ; (O'Donoghue and Weyreter, 1984) ; (De Araújo *et al.,* 2018) ; (Thornton, 1972). Sarcosporidiosis is a parasitic disease caused by protozoa of the genus Sarcocystis, characterised by the presence of cysts in muscle tissue. This disease affects many vertebrate species, both domestic and wild. Recent studies (Chauan *et al.,* 2020 ; Rubiola et al., 2023 ; Shikweto *et al.,* 2024 ; Da Rosa *et al.,* 2024 ; Helman *et al.,* 2024 ; Shamsi et al., 2024 ; Hu *et al.,* 2025) have been conducted in South American, European and Asian countries to identify *Sarcocystis*. Unfortunately, studies conducted in Africa to search for Sarcocytis date back several years and are obsolete because the data has not been updated. The first molecular detection of Sarcocystis suihominis in Nigeria was carried out by Obadiah *et al.* in 2024. On the African continent, and particularly in Senegal, known prevalence in livestock has only been demonstrated in small ruminants (Vercruysse and Van Marck, 1981) ; (Gbati *et al.,* 2012) and cattle (Gbati *et al*., 2004). Furthermore, there appear to be no studies conducted on the search for Sarcocystis species in pigs in Senegal. In addition, the parasitic species involved have not been identified using new, more specific diagnostic techniques such as molecular biology. Pigs can be carriers of Sarcocystis suihominis, a zoonotic species that can cause quite serious clinical symptoms in humans. It is therefore important to screen carcasses of slaughtered animals in our countries' abattoirs for cysts and to accurately identify the Sarcocystis species responsible for sarcosporidiosis. It is in this context that our study here in Senegal was conducted, with the aim of identifying the species of Sarcocystis circulating in the carcasses of pigs slaughtered at Dakar abattoirs. This study, which was the first new research on Sarcocystis in pigs in Senegal, yielded conclusive results, which are presented in this manuscript.

MATERIALS AND METHODS

1) Study type and location

This cross-sectional study was conducted between june 2012 and December 2013. It was conducted in two phases : a muscle sampling phase at slaughterhouses in Dakar, followed by an analysis and research phase in the laboratories of the Inter-State School of Veterinary Science and Medicine (EISMV) in Dakar and the Swiss Centre for Scientific Research in Abidjan, Côte d'Ivoire. Histological sections of the muscle samples were prepared at the EISMV's Veterinary Histology and Histopathology Laboratory. Molecular biology tests to identify the species of Sarcocystis circulating in Senegal were carried out at the Molecular Biology Laboratory of the Swiss Scientific Research Centre (CSRS) in Abidjan.

2) Sampling and collection of muscle samples

To determine the prevalence of *Sarcocystis* infection in the muscles of pigs slaughtered at Dakar abattoirs, muscle samples were taken from half the average number of pigs slaughtered daily, i.e., five pigs per day. Thus, samples were taken from the carcasses of 300 pigs over a two-month period, including 150 of each sex from various regions of Senegal. Pigs from these localities are most often raised in an extensive and semi-intensive manner, which forces these animals to roam in search of food. Fifty grams of five muscles (the masseter, oesophagus, diaphragm, gluteal and heart) were taken from the carcasses. For each sample taken, a 2 cm² piece was preserved in a 10% formalin solution for histological examination, while the rest was stored in a freezer at -80°C for molecular biology. In total, the study was carried out on 1,500 samples taken from 300 pig carcasses, including 150 boars and 150 sows.

3) Laboratory examination and analysis

To identify *Sarcocystis* species from pig carcasses, it was necessary to use the classic haematoxylin-eosin histological technique and classic PCR for the diagnosis of Sarcocystis miescheriana. For the haematoxylin-eosin histological technique, one square centimetre (1 cm²) of each muscle sample was processed using histological techniques involving paraffin sectioning to a thickness of 5 microns and stained with haematoxylin-eosin-saffron. Examination of the preparations under a LEICA ICC 50 HD photonic microscope with integrated camera at 400X magnification enabled the detection of Sarcocystis cysts, assessment of their topography and counting per slide, i.e. per square centimetre. Each cyst was then photographed using LAS EZ version 2.0 image capture software, and the length, width and thickness of the cyst wall were measured using MOTIC Images Plus version 2.0 image processing software. Thus, on each positive histological section, the cysts are carefully counted and the results are listed according to the muscle for each carcass. The molecular diagnosis of *Sarcocystis* species involved searching for *Sarcocystis miescheriana* DNA using conventional PCR. Molecular analyses were performed on heart (myocardium) samples that were positive on histology. We

worked on one hundred (100) pig heart samples that had been previously frozen at -80°C. A small amount (1 gram) of heart tissue, carefully cut into thin strips, was placed in a 1.5 ml Eppendorf tube containing 400 µl of 2% CTAB (cetyl trimethyl ammonium bromide), then crushed and incubated for 15 minutes at 70°C in an oven. After incubation, the addition of 400 µl of chloroform and refrigerated centrifugation at 12,000 rpm for 5 minutes precipitates the DNA, which is recovered from the supernatant and placed in another 1.5 ml Eppendorf tube. To purify the DNA, 400 µl of isopropanol is added to the supernatant, which, after thorough mixing by inversion, is centrifuged at 12,000 rpm for 5 minutes. After emptying the isopropanol, 400 µl of 70° ethanol is added and centrifuged one last time at 12,000 rpm for 5 minutes. The pellet is then left to dry after removing the ethanol. Finally, the DNA is solubilised in 30 µl of sterile H2O, the pellet is vortexed and stored at -20°C.

For the molecular identification of *Sarcocystis miescheriana*, the technique developed by Kia *et al.* (2011) was used. Thus, for DNA amplification, a pair of primers SarcoF: 5'-CCA TAT TTT ATG GTG GTG GTG A-3' and SarcoR: 5'-AGT CTT TGG CAA TGC TTT C-3' (Eurogentec) were used. For each sample, PCR was performed with a final volume of 25 µl containing 16.8 µl of ultra-pure H2O, 2.5 µl of 2X Mg2+ buffer, 0.5 µl of MgCl2 (25 mmol), 1 µl of dNTPs (10 mmol), 1 µl of each SarcoF and SarcoR primer at 10 picomoles, 0.2 µl of Kappa Taq Polymerase and 2 µl of the DNA sample. It was performed in a thermal cycler (LongGene A 200 Thermal Cycler) according to the following programme : 1 cycle of 5 minutes at 95°C (primary denaturation) followed by 30 cycles of 45 seconds at 94°C (denaturation), then 1 minute at 55°C (annealing) and 1 minute at 72°C (extension), and finally 7 minutes at 72°C for final extension.

Following amplification, we verified the PCR products by agarose gel electrophoresis. This verification of the PCR product was performed on agarose gel, which was weighed and prepared according to the following protocol: 1.5% agarose, 100 ml pure water, 0.5X Tri Borate EDTA (TBE) and 6 µl Ethidium Bromide. The agarose gel thus prepared was poured into a migration plate and then placed in a 10 cm x 15 cm electrophoresis tank. Next, 5 µl of PCR product from each sample, plus 2 µl of loading buffer and the molecular weight marker, were used to fill the wells cut into the 1.5% agarose gel using a comb for electrophoretic migration. During migration, the agarose gel was immersed in a migration buffer composed of: triborate, EDTA pH 8.2, tribase, boric acid, Na_2EDTA pH 8 and pure water. Migration was carried out for 2 hours 30 minutes at 60 volts.

4) Ethical approval

This study was non-invasive as it involved taking meat samples from animals slaughtered at the Dakar Abattoir. The search for pathogens in slaughtered animals is one of the sovereign duties of the authorities at slaughterhouses. Thus, sampling of animals for the purpose of strengthening disease diagnosis at slaughterhouses in order to protect public health is authorized. It was in this context that the management of the Dakar Abattoir authorised us to take these samples in order to contribute to the diagnosis of parasites found in meat inspected at slaughterhouses.

5) Statistical analyses

The data were entered using Epidata software. Prevalence was determined by dividing the total number of carcasses infected with *sarcocystis* by the total number of animals sampled during the study period and then multiplying by 100. Using R-Commander 2.15.2 software, the variability and disparities within the samples studied were assessed using χ2 tests and analysis of variance (ANOVA), with a significance threshold set at 5%.

RESULTS

The search for possible macroscopic lesions on the carcasses yielded no positive results. Indeed, after observation with the naked eye and observation of muscle samples under a binocular magnifying glass, no macroscopic lesion were found. Microscopic observation of histological slides prepared from muscle samples yielded positive results. *Sarcocystis* cysts were found in the muscles sampled: gluteal, myocardium, diaphragm, masseter and oesophagus. At low magnification (40X and 100X), in longitudinal section, elongated cysts of varying sizes were observed inserted into the muscle fibres (Fig 1). Thus, of the 300 carcasses examined, 275 carcasses harboured *Sarcocystis* cysts, representing a prevalence of 91.7%.

According to gender, the infestation rate in females was slightly higher at 92.7% compared to 90.7% in males, with no significant variation ($p>0.05$).

Depending on the muscle, *Sarcocystis* cysts were found in the muscles sampled at varying infestation rates. The most infested muscle was the oesophagus, with a prevalence of 79.3% (238 positive samples out of 300), followed by the myocardium with 70.30% (210 positive samples). The muscle least affected by the parasite was the masseter, with only 46.0% (138 positive samples) (Fig. 2).

There is a significant variation in infestation depending on the location of *Sarcocystis* cysts ($p < 0.05$). This difference is found between: the oesophagus and other muscles. However, no significant difference was found between the diaphragm and the gluteal muscles ($p>0.05$).

The average infestation density was 8 cysts per square centimetre of muscle. However, the most densely affected muscle was the oesophagus with 13.93±2.02 cysts/cm2 (Table I). A significant difference was found between the

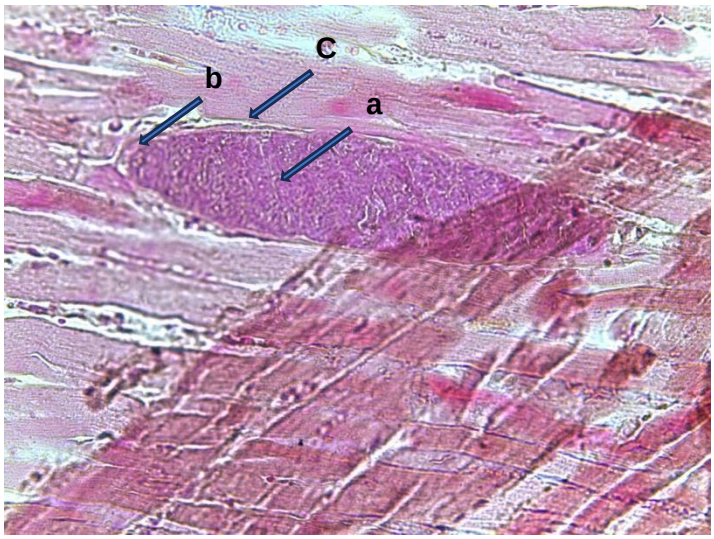


Fig. 1: *Sarcocystis* cyst in the longitudinal section of a pig's gluteal muscle (HE X400) a = Bradyzoites ; b = Cyst wall ; c = Muscle fibres.

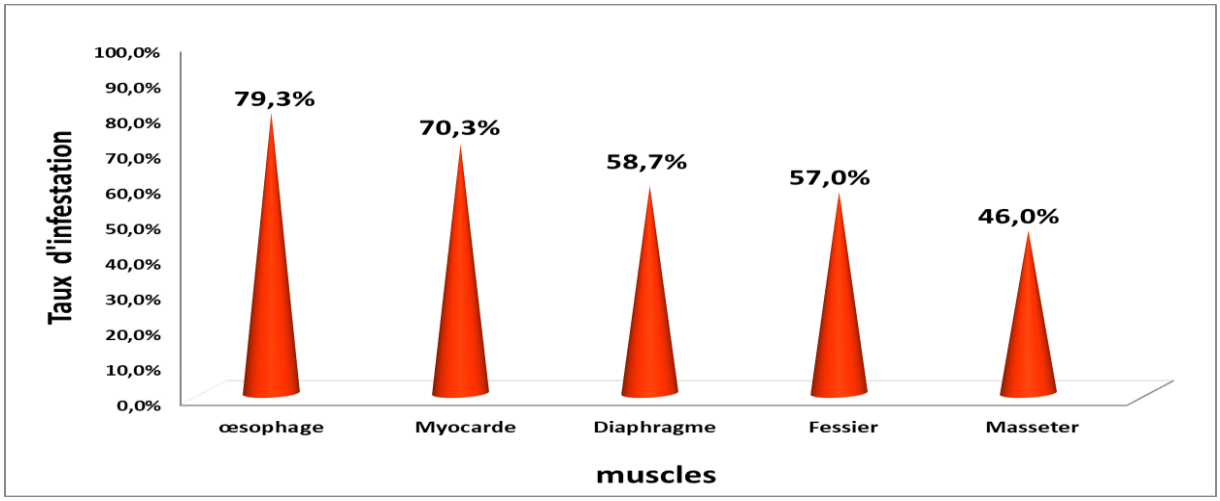


Fig. 2 : Infestation rates based on muscle samples taken from pigs at the Dakar slaughterhouse.

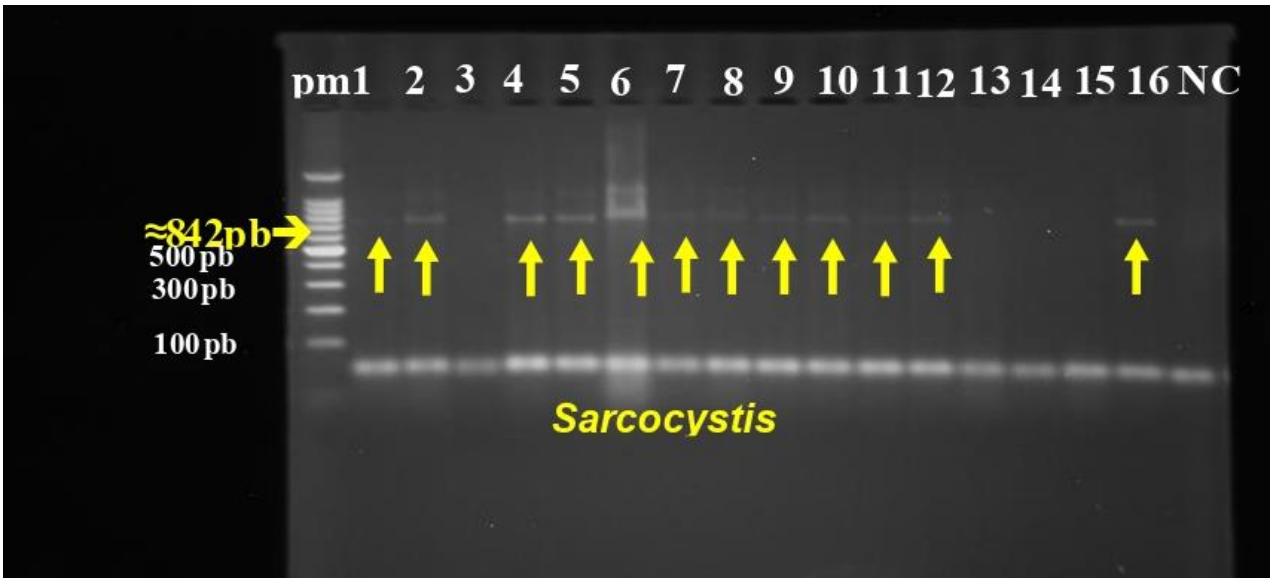
different cyst densities depending on the muscles studied ($p<0.05$).

For cyst size, the average length was 313.48±19.01 µm, with extremes ranging from 113.35 µm for the shortest cyst found in the oesophagus to 892.67 µm for the longest cyst found in the masseter muscle. The average thickness of the cyst wall was 5.28±0.33 µm (2.04 µm to 10.97 µm) (Table II). This average wall measurement corresponds to that found in cysts caused by *Sarcocystis miescheriana*.

Molecular analyses were carried out to identify more precisely the *Sarcocystis* species responsible for the cysts observed. Of the 100 samples submitted for PCR testing, 89 were positive, representing a rate of 89%. The PCR reaction revealed the presence of DNA from *Sarcocystis miescheriana* or S. *suicanis* (Fig. 3). This was the first time this parasite had been identified in pigs in Senegal.

DISCUSSION

This cross-sectional study on the histological and molecular identification of *Sarcocystis* sp. species from pig carcasses slaughtered at abattoirs in Dakar, Senegal, was



Pm: size marker; NC = Negative Control
Fig. 3: DNA profiles of Sarcocystis miescheriana

Table 1 : Cyst density (in cysts/cm²) according to the muscles sampled from pigs at the Dakar slaughterhouse.

	Average number of cysts/cm² (95% CI)						
Porcs	Oesophagus	Myocardium	Gluteal muscle	Diaphragm	Masseter	**Middle**	***p-values***
	13.93±2.02	8.83±1.76	6.92±1.60	6.44±1.11	3.91±0.83	**8.00±1.46**	P<0.05

conducted with the aim of accurately identifying the *Sarcocystis* species circulating in Senegal. Health checks in slaughterhouses are essential for seizing unfit meat but remain limited for the diagnosis of certain parasites, which requires more complex identification techniques. The meat slaughtering and inspection system continues to face enormous difficulties. In fact, there is only one slaughterhouse in the Dakar region. Slaughterhouses in inland regions receive almost no pigs for slaughter for possible health inspections. This phenomenon can be explained by the rise in illegal slaughter in the pig industry. These findings lead us to admit that the results of our study may not reflect reality. Thus, the prevalence rates obtained in our work may be significantly lower than the actual figures.

The identification of *Sarcocystis* species from pig carcasses was carried out using two techniques, namely the classic histological technique with haemalun-eosin and classic PCR.

From a macroscopic point of view, all samples were negative for *Sarcocystis* spp. cysts. The same finding was made by Falzy et al. (2017) in Peninsular Malaysia. In the present study, the prevalence using the histological technique was 91.7%. These prevalence rates (91.7%) are well above what has been described in the literature to date. It is higher than the 23.4% and 58% found respectively by Kálmán et al. (2017) in Romania and Falzy et al. (2017) in Peninsular Malaysia. It is also higher than the 73% and 85% found in India (Saleque and Bathia, 1991) and Rhodesia (Thornton, 1972), respectively. The high prevalence obtained in our study may be related to the pig production system. Indeed, in Africa in general and Senegal in particular, most of the pigs slaughtered in Dakar slaughterhouses come from extensive farms where the animals are left to fend for themselves, roaming the streets with stray dogs and feeding on rubbish dumps. Their coprophagous behaviour also increases the risk of infestation. From a macroscopic point of view, all samples

Table II : Morphometry of *Sarcocystis* cysts according to muscle type in pig carcasses at the Dakar slaughterhouse

Measurement of cysts	Muscles sampled					
	Diaphragm	Oesophagus	Myocardium	Gluteus	Masseter	Average
Length (µm)	**402,47±32,79[a]** (158,85 – 892,67)	**342,92±22,02[b]** (113,35 – 854,77)	**299,06±15,92[c]** (165,36 – 661,83)	**284,16±14,54[c]** (165,30 – 678,95)	**238,78±10,79[d]** (143,57 – 892,65)	**313,48±19,01** (113,35 – 892,67)
Width (µm	**101,72±3,78[a]** (39,02 – 182,91)	**93,14±2,59[b]** (19,31 – 161,12)	**90,53±2,46[b]** (52,21 – 188,90)	**93,95±2,40[b]** (52,264 – 131,26)	**92,80±2,33 [b]** (57,55 – 117,65)	**94,43±2,71** (19,31 – 188,90)
Length/Width	**3,89±0,25**	**3,71±0,25**	**3,33±0,17**	**3,02±0,12**	**2,57±0,10**	**3,30±0,17**
Thickness (µm)	**5,13±0,34[a]** (2,04 – 10,01)	**5,59±0,32[a]** (2,09 – 10,97)	**5,52±0,33[a]** (2,11 – 13,19)	**5,19±0,31[a]** (2,15 – 9,88)	**4,99±0,38[a]** (2,22 – 9,98)	**5,28±0,33** (2,04 – 10,97)

NB: For each measurement, the difference is statistically significant if the subscript letters are different (p<0.05).

were negative for *Sarcocystis* spp. cysts. The same finding was made by Falzy et al. (2017) in Peninsular Malaysia. In terms of muscles, this study found that the oesophagus (79.3%) and myocardium (70.3%) were the most frequently affected by infestation. Although our infestation rates are much higher than theirs, Falzy et al. (2017) found that the oesophagus (36%) and heart muscles (30%) were more affected by *Sarcocystis* infestations. It follows, therefore, that in the diagnosis of sarcosporidiosis in pigs, the oesophagus and myocardium can be used as reference samples.

In terms of cyst density per muscle, the oesophagus (13 cysts/cm²) is the most affected, followed by the myocardium (8 cysts/cm²). In terms of food, the risk of human contamination would be higher when consuming these organs.

Morphometry allowed us to note the absence of Toxoplasma cysts. The ratio between the length and width of the cyst was used as a criterion. For Toxoplasma cysts, which are globular in shape, it can be assumed that the length-to-width ratio is less than or equal to 1.2 (Soothwood and Henderson, 2009 ; Fassi-fehri, 1978). The length-to-width ratio found in our study is greater than 2, with extremes ranging from 2.57 to 3.89. The cysts found can be considered to be those of the genus Sarcocystis. As for the size of the cysts, on average, the longest were found in pig muscles at 313.48 µm (113.35 µm – 892.67 µm). These measurements are consistent with the observations of Tenter (1995) and Dubey and Lindsay (2006), who state that *Sarcocystis* cysts in pigs measure less than 1.5 mm, confirming the microscopic nature of the lesions. In our study, no cysts smaller than 100 µm were found in this species. These results are similar to those obtained by Kia et al. (2011) in Iran, who made the same observation. These sizes correspond to those of Sarcocystis miescheriana cysts, whose definitive host is canids, including dogs, and *Sarcocystis suihominis*, whose definitive host is humans (Tenter, 1995 ; Dubey and Lindsay, 2006). However, the average thickness of 5.28 µm (2.08 µm to 10.97 µm) points more towards the species *Sarcocystis miescheriana* or S. *suicanis* rather than *Sarcocystis suihominis*. Indeed, Chen et al. (2007) measured thicknesses of 2.5 µm to 6.1 µm on cysts found on pigs in Yunnan, China, infested with S. *miescheriana*. In pigs, our research focused on identifying *Sarcocystis miescheriana*. After reviewing the literature during our research, it was difficult to find a protocol for detecting S. suihominis. For S. miescheriana, the protocol was carried out as proposed by Kia et al. (2011), with the only difference being that we used CTAB as a tissue lysis buffer during DNA extraction instead of the 'Qiagen, Hilden, Germany' kit proposed by the authors. As mentioned above, the myocardium is therefore a good sample model for *Sarcocystis* research. Unfortunately, the heart is a highly prized organ in the diet of African populations, but also in the diet of dogs. The consumption of undercooked

or raw hearts by butchers poses a potential risk of human contamination by zoonotic species, particularly S. hominis from cattle and S. suihominis from pigs. Although these two species have not been identified in Senegal, it is highly likely that they exist.

Sarcocystis hominis has been reported in the subregion, particularly in Nigeria (Obijiaku, 2013). Feeding dogs and cats meat by-products such as oesophagus, diaphragm and chopped hearts contributes to the spread of the disease. However, molecular biology has enabled us to confirm the presence of *Sarcocystis miescheriana* (S. suicanis) in pigs. Although still in its infancy, this technique can be used in abattoirs to help diagnose *Sarcocystis* species in pigs in cases of suspected infection. However, it has confirmed the suspicion arising from histological examination of muscle samples. This is the first time that this parasite has been identified in pigs in Senegal. This work leads us to strengthen disease surveillance and control systems through the adoption and appropriation of new techniques. It is important to always bring the results of new studies to the attention of the authorities so that appropriate corrective measures can be taken to protect public health. In this way, researchers could work more closely together in the fight against often neglected diseases that affect public health.

CONCLUSION

This cross-sectional study identified the species of *Sarcocystis* circulating in pork meat at Dakar slaughterhouses. Of the 300 carcasses examined, 275 were found to harbour *Sarcocystis* cysts. Molecular biology analysis confirmed the presence of *Sarcocystis miescheriana*. Thus, pigs slaughtered in Dakar slaughterhouses are not free from sarcosporidiosis. Although this study did not show the presence of the zoonotic species (*Sarcocystis suihominis*), it is essential to strengthen measures to combat parasitic diseases affecting pigs in Senegal and to ensure better health control in slaughterhouses in order to reduce the impact of these diseases and protect public and animal health. To ensure the sustainability of animal health systems in Senegal and West Africa, It is imperative to strengthen research initiatives by adopting new techniques that are easy to use in slaughterhouses. Finally, awareness-raising and training programmes must be targeted at all stakeholders in order to raise awareness of the implications of porcine sarcocystosis for public health.

CONFLICTS OF INTEREST

There is no conflict of interest.

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