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Histopathological and morphometric characterization of *Sarcocystis* spp. cysts in cattle muscles in central Algeria

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Summary

Sarcocystis* spp. are intracellular protozoans that belong to the Apicomplexa phylum and cause sarcocystosis in many animals, especially cattle. This study focuses on a detailed morphometric analysis of sarcocysts found in skeletal and cardiac muscles of cattle examined in three abattoirs in the central part of Northern Algeria. In order to assess the infestation of bovine carcasses with *Sarcocystis* cysts, 643 muscle samples (diaphragm, oesophagus, and heart) were taken from 269 cattle slaughtered in abattoirs of El Harrach, Eucalyptus (Algiers), and Draâ Ben Khedda (Tizi Ouzou). A total of 149 carcasses tested positive, representing a prevalence of 55.4%. The tissues were examined histologically to characterize the morphology of *Sarcocystis* species: *S. cruzi*, *S. hirsuta* and *S. hominis*. A total of 746 sarcocysts were identified and measured using an ocular micrometer. For each cyst, length, width and shape index (length/width ratio) were recorded. Morphometric analyses were performed at the level of the cyst. However, as individual cysts could not be linked to specific animals, the results were considered exploratory due to a possible pseudoreplication. Thick-walled cysts (*S. hirsuta* or *S. hominis*) ranged from 30 to 95 µm in length (mean ± SE: 64.37 ± 7.74 µm) and 17.5 to 70 µm in width (41.25 ± 6.61 µm), with a shape index between 1.22 and 2.86 (1.70 ± 0.19). Thin-walled cysts, typical of *S. cruzi*, measured 12.5 to 675 µm in length (93.70 ± 2.58 µm) and 7.5 to 137.5 µm in width (43.69 ± 0.69 µm), with a shape index ranging from 0.47 to 20.0 (2.21 ± 0.58 µm). No statistically significant differences ($p > 0.05$) between thick-walled and thin-walled cysts were observed for length, width or shape index. Differences in morphometric parameters were observed between the three organs examined ($p < 0.001$). Thin-walled cysts showed similar trends across organs ($p < 0.001$) for the three measurements, whereas no statistically significant differences ($p > 0.05$) were observed for thick-walled cysts. The observed morphological features were consistent with previous reports, supporting the usefulness of morphometric analysis for preliminary species differentiation. Definitive identification, however, requires molecular confirmation. These findings also highlight the potential impact of *Sarcocystis* infection on animal health and meat quality.

Keywords: histopathological method, morphometric analysis, *Sarcocystis*, bovine

The genus *Sarcocystis* comprises intracellular protozoan parasites belonging to the phylum Apicomplexa. They are characterized by an obligate two-host life cycle involving a carnivorous definitive host and a herbivorous intermediate host. In cattle (*Bos taurus*), the most commonly reported species include *Sarcocystis cruzi*, *S. hirsuta* and *S. hominis* (10, 15, 33). These species differ significantly in their pathogenicity, zoonotic potential and morphological characteristics of their sarcocysts (9, 11, 16, 19, 22, 23, 26, 35, 40).

Cattle become infected by ingesting sporulated oocysts or sporocysts present in the environment or contaminated feed (5). The resulting sarcocysts develop in skeletal and cardiac muscles of the intermedi-

ate host (11). Species identification traditionally relies on histological and ultrastructural examination of cyst walls, particularly wall thickness, surface morphology and the presence of protrusions (22, 23). Thin-walled cysts, usually attributed to *S. cruzi*, are non-zoonotic and often cause asymptomatic infections with minimal economic impact (1). In contrast, thick-walled cysts, such as *S. hominis* or *S. hirsute*, may be associated with food safety concerns and economic losses due to carcass condemnation during meat inspection (9, 40).

Although molecular tools are increasingly used for species confirmation, histological and morphometric techniques remain valuable, especially in resource-limited settings (23). These methods make it possible to measure cyst dimensions (length, width, and wall thickness) and presumptively identify species with relatively high confidence (23, 26). Histology is also essential for evaluating tissue tropism, infection intensity and possible inflammatory responses in affected muscles (10, 12).

Recent studies have highlighted considerable morphological variability among *Sarcocystis* spp. in cattle, potentially influenced by host immunity, geographic location and environmental factors (8, 19). Despite the global occurrence of *Sarcocystis* in cattle, morphometric surveys in North Africa and the Mediterranean region remain limited (3, 4).

The present study aims to characterize the morphometric and histological features of *Sarcocystis* spp. in slaughtered cattle from central Algeria. By analyzing key parameters, such as cyst dimensions, wall structure and tissue distribution, this work contributes to improving morphological diagnosis and the understanding of the regional epidemiology of bovine sarcocystosis.

Material and methods

Study area and sample collection. The study was carried out between January 2021 and April 2022 in three municipal abattoirs in northern Algeria: El Harrach and Eucalyptus (Algiers) and Draa Ben Khedda (Tizi Ouzou). A total of 269 cattle (*Bos taurus*) were sampled. The animals, aged between 1 and 12 years, were of local and imported origin and clinically healthy.

Experimental design. In post mortem, muscle samples were taken from three anatomical sites commonly recognized as predilection sites for *Sarcocystis* spp: oesophagus, diaphragm and heart (10, 33). A total of 643 samples were taken (269 oesophagi, 269 diaphragms and 105 hearts). At each site, 20 grams of muscle tissue were aseptically excised, placed in sterile plastic bags, and immediately transported at 4°C in coolers to the Laboratory of Parasitology and Mycology in the Higher National Veterinary School, Algiers (19, 23).

Histopathological and morphometric analysis. Tissue samples were fixed in 10% neutral buffered formalin for at least 48 hours. Subsequently, they were dehydrated through a graded ethanol and xylene series, embedded in paraffin wax, sectioned at 4-5 μm^2 and stained with hematoxylin and eosin (H&E), following standard protocols (11, 15). Upon arrival at the laboratory, all samples were initially examined macroscopically under direct light to detect visible sarcocysts, typically appearing as white, rice grain-like structures embedded within muscle fibres (22).

Histological preparations were conducted in the Laboratories of Anatomy and Cytopathology at the University Hospitals of Nafissa Hamoud (ex-Parnet) and Beni Messous, and at the Laboratory of Pathological Anatomy of the Higher National Veterinary School, Algiers. Microscopic examinations were carried out at different magnifications (×100, ×400, ×1000 with oil immersion) to identify microscopic sarcocysts. Cysts were identified and classified based on morphological characteristics of the cyst wall and internal structure, according to criteria established by Dubey et al. (23).

Morphometric analysis was performed using an ocular micrometer. The following parameters were recorded: cyst length, width, shape index (length/width) and wall thickness (6, 18, 21).

Statistical analysis. Descriptive statistics, including means, standard errors and 95% confidence intervals, were calculated for all morphometric parameters. To assess normality, the data distribution was evaluated by the Kolmogorov-Smirnov test. Therefore, comparative analysis was performed using non-parametric approaches, using the Mann-Whitney U test and the Kruskall-Wallis test. Graphical representations (histograms and boxplots) were used to identify potential outliers. Individual animal identity was not available for the dataset analysed. Differences were considered statistically significant at $p < 0.05$. Data were processed using the IBM SPSS Statistics software, version 27.

Results and discussion

Macroscopic examination. No sarcosporidian cysts were observed macroscopically in the 643 samples, and no eosinophilic myositis lesions were detected in the 269 cattle carcasses inspected in the three abattoirs.

Microscopic examination for *Sarcocystis* spp. Microscopic examination for *Sarcocystis* was carried out using the histological technique and micrometry to measure the cysts detected. This combined approach makes it possible to presumptively differentiate between cyst types and provides a basis for subsequent morphometric analysis (3) https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-024-06628-4?utm_source=chatgpt.com.

Distribution of infested bovine carcasses by organ. To assess the infestation of bovine carcasses by sarcocystosis cysts, we identified a total of 149 positive carcasses (Tab. 1). In ninety-six carcasses (64.43%), infestation was limited to a single organ. Among these, only the heart was infested in 22 carcasses (14.8% of the total), only the diaphragm was infested in 30 carcasses (20.1%), and only the oesophagus was infested in 44 carcasses (29.5%). Simultaneous infestation of two organs was observed in 38 carcasses (25.5%): 7 carcasses (4.7%) with heart and diaphragm involvement, 17 carcasses (11.4%) with heart and oesophagus involvement, and 16 carcasses (10.7%) with diaphragm

Tab. 1. Distribution of infested bovine carcasses by organ

	One infested organ			Two infested organs			Three infested organs
Number of carcasses (%) 95% CI	96 (64.43%) (56.47-71.67)			38 (25.5%) (19.18-33.06)			13 (8.7%) (5.17-14.35)
Organ infested	Heart	Diaphragm	Oesophagus	Heart + Diaphragm	Heart + Oesophagus	Diaphragm + Oesophagus	
Number of carcasses (%)	22 (14.8%)	30 (20.1%)	44 (29.5%)	7 (4.7%)	17 (11.4%)	16 (10.7%)	

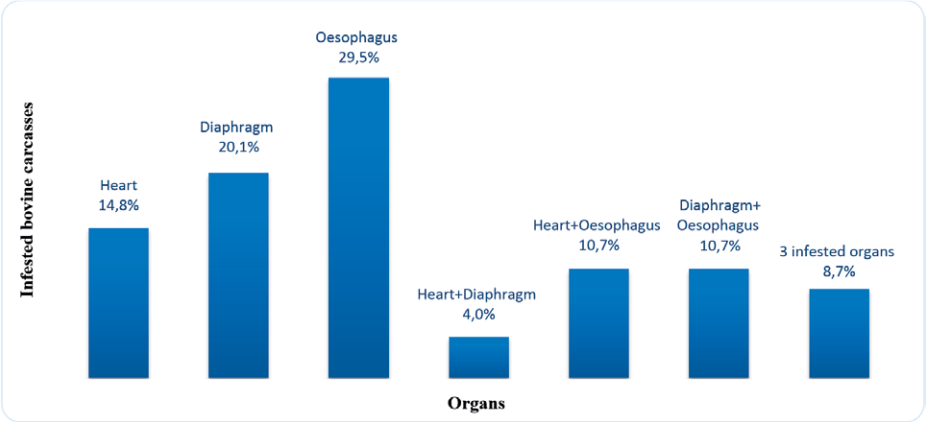

Fig. 1. Infested bovine carcasses by organ

and oesophagus involvement. Finally, 13 carcasses (8.7%) showed concurrent infestation of all three organs: heart, diaphragm and oesophagus (Fig. 1).

Histological examination. Infection with *Sarcocystis* spp. was confirmed in 149 of the 269 cattle examined (55.4%). A total of 746 sarcocysts were measured, including 738 thin-walled cysts (*S. cruzi*) and 8 thick-walled cysts (*S. hominis* or *S. hirsuta*). All cysts were located within muscle fibres.

Histological analysis under a light microscope (400× magnification) made it possible to detect and count *Sarcocystis* cysts in muscle tissue. Two distinct types of cysts were identified based on the morphology of their cyst walls. The majority had thin, smooth walls, typical of *Sarcocystis cruzi* (Fig. 2), whereas a smaller number displayed thicker, striated walls, indicative of either *Sarcocystis hirsuta* or the zoonotic *Sarcocystis hominis* (Fig. 3).

In longitudinal sections, the cysts appeared elongated and oriented parallel to the muscle fibres

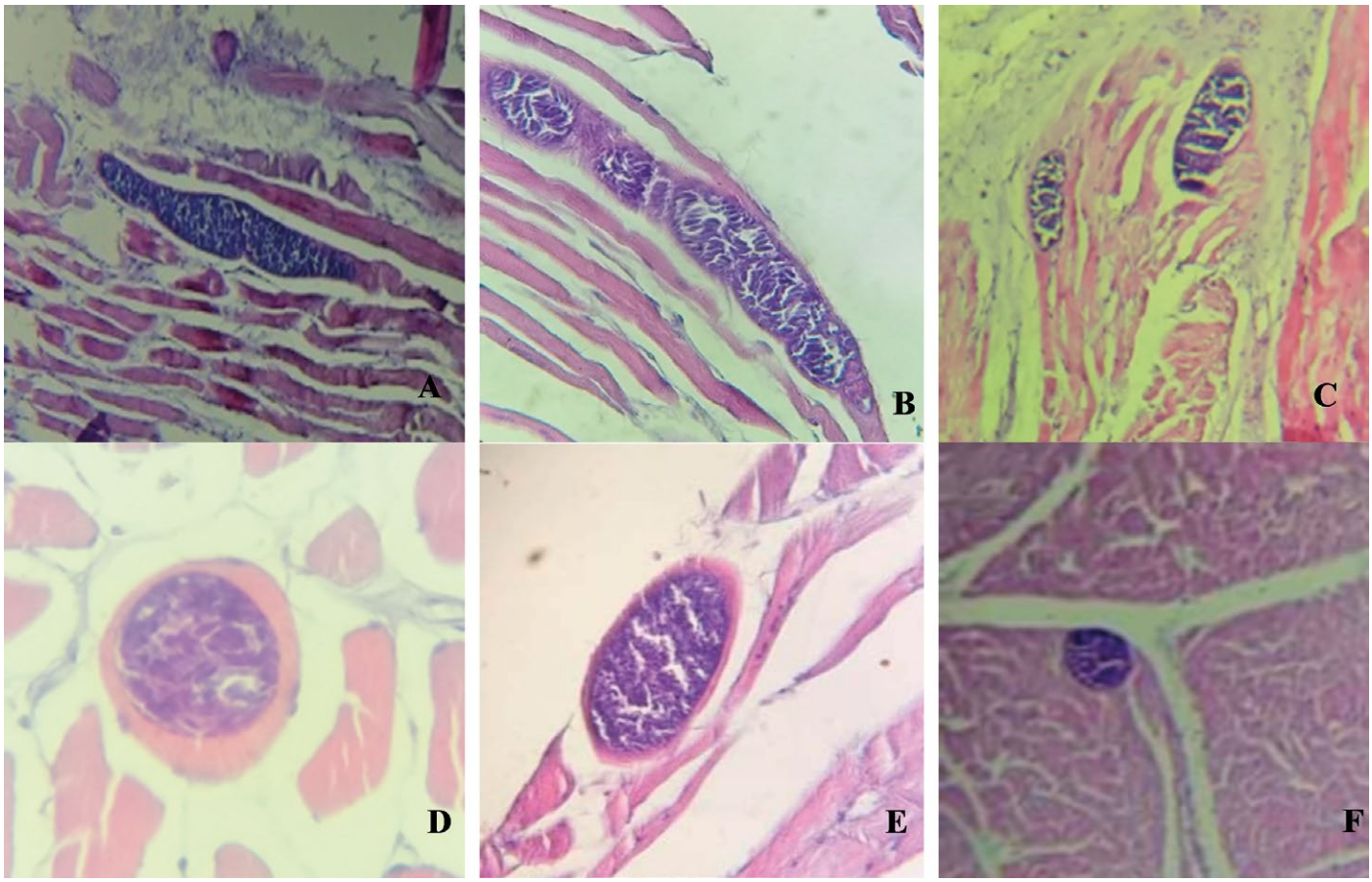

Fig. 2. Thin-walled cysts of *Sarcocystis* observed in longitudinal sections of the diaphragm (A), oesophagus (B) and heart (C), and in transverse sections of the diaphragm (D), oesophagus (E) and heart (F). Light microscopy, magnification ×400

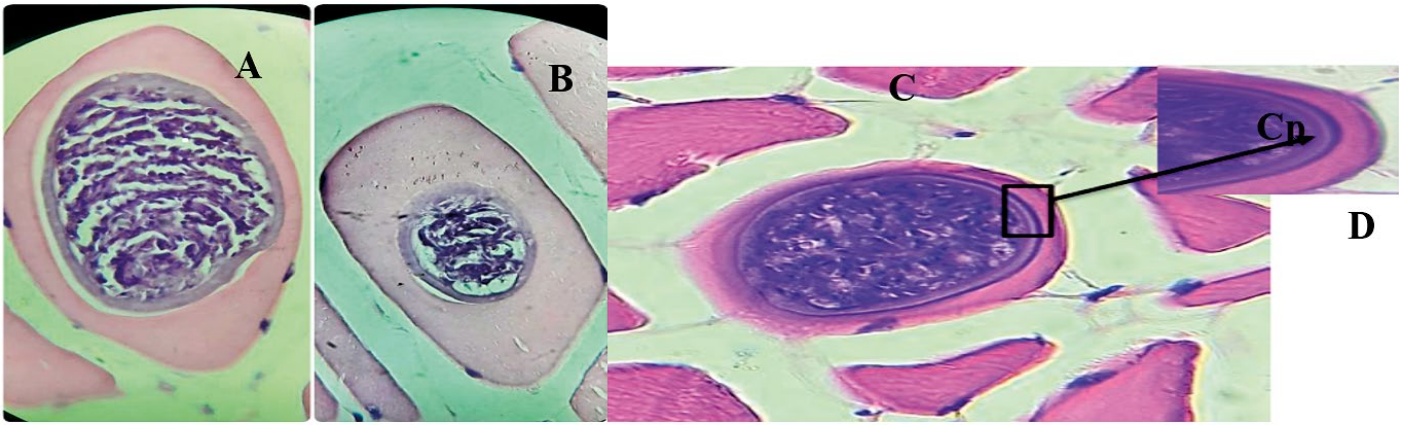

Fig. 3. Thick-walled cysts of *Sarcocystis hominis* or *Sarcocystis hirsuta* observed in cross-sections of the diaphragm (A, B) and in longitudinal section (C). Cytophaneres (Cp), characteristic of *S. hominis*, are shown in detail (D). All images were obtained by light microscopy (H&E staining, magnification ×400 and ×1000)

(Fig. 2 A-B). In transverse sections, the cysts showed internal compartmentalization, with numerous alveolar structures filled with bradyzoites (Fig. 2 C-D).

Micrometry. For each cyst, dimensions were recorded in longitudinal sections, ensuring consistency by aligning the measurement axis with the direction of muscle fibres.

• Morphological characteristics of thick-walled and thin-walled cysts (length, width, shape index) (Tab. 2).

A total of 746 cysts were measured with an optical micrometer at 400× magnification. Measurements included cyst length and width, recorded for both thin-walled and thick-walled types.

The mean length of these structures was 93.38 ± 2.56 µm. The mean width was 43.66 ± 0.68 µm. The mean value of the shape index amounted to 2.20 ± 0.058. (Fig. 4, 5 and 6).

Tab. 2. Morphological characteristics of thick-walled and thin-walled cysts (length, width, shape index)

	Parameters	Length (µm)	Width (µm)	Shape index (µm)
All cysts (n = 746)	n	746	746	746
	Mean ± SE	93.38 ± 2.56	43.66 ± 0.68	2.20 ± 0.058
	95% CI	(88.35-98.41)	(42.31-45.01)	(2.09-2.32)
	Min	12.50	7.50	0.47
	Max	675	137.5	20
Type of cysts				
Thin-walled cysts (n = 738)	n	738	738	738
	Mean ± SE	93.70 ± 0.58	43.69 ± 0.69	2.21 ± 0.058
	95% CI	(88.62-98.78)	(42.33-45.04)	(2.09-2.32)
	Min	12.50	7.50	0.47
	Max	675	137.50	20.00
Thick-walled cysts (n = 8)	n	8	8	8
	Mean ± SE	64.37 ± 7.74	41.25 ± 6.61	1.70 ± 0.19
	95% CI	(46.06-82.69)	(25.60-56.89)	(1.24-2.15)
	Min	30.00	17.50	1.22
	Max	95.00	70.00	2.86
P-value		0.376	0.84	0.661

Thin-walled cysts had a mean length of 93.70 ± 2.58 µm, a mean width of 43.69 ± 0.69 µm and a mean shape index of 2.21 ± 0.58. In contrast, thick-walled cysts showed a mean length of 64.37 ± 7.74 µm, a mean width of 41.25 ± 6.61 µm and a mean shape index of 1.70 ± 0.19. Length, width and shape index did not differ significantly between thin- and thick-walled cysts ($p > 0.05$).

• Morphological characteristics of thick-walled cysts and thin-walled cysts (length, width, shape index, wall thickness) according to the organ (Tab. 3)

In the oesophagus, the overall mean cyst length was 97.09 ± 5.06 µm, the mean width was 43.63 ± 1.24 µm,

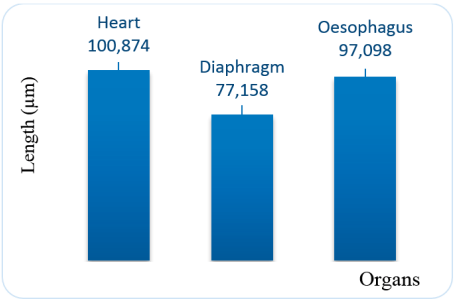

Fig. 4. Average cyst length by organ

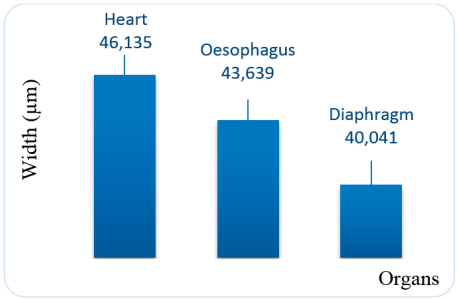

Fig. 5. Average cyst width by organ

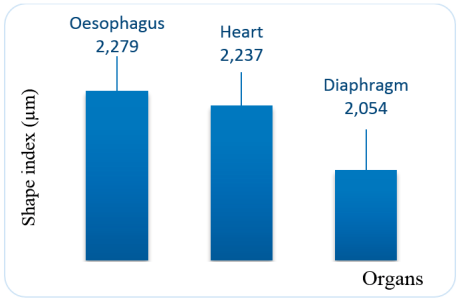

Fig. 6. The cyst shape index by organ

Tab. 3. Morphological characteristics of thick-walled cysts and thin-walled cysts (length, width, shape index, wall thickness) according to the organ

Organ	Cysts	Parameters	Length (µm)	Width (µm)	Shape index (µm)
Oesophagus cysts (n = 267)	Total	Mean ± SE	97.09 ± 5.06	43.63 ± 1.24	2.28 ± 0.10
		95% CI	(87.12-107.07)	(41.19-46.08)	(2.06-2.48)
		Min	15	10	0.78
		Max	675	137.50	12
	Thin-walled cysts (n = 264)	Mean ± SE	97.49 ± 5.12	43.66 ± 1.25	2.28 ± 0.10
		95%CI	(87.81-107.57)	(41.20-46.11)	(2.07-2.49)
		Min	15	10	0.78
		Max	675	137.50	12
	Thick-walled cysts (n = 3)	Mean ± SE	62.50 ± 12.50	41.66 ± 15.29	1.81 ± 0.52
		95% CI	(8.71-116.28)	(0-107.48)	(0-4.06)
		Min	50	17.50	1.25
		Max	87.50	70	2.86
P-value		Between cysts	0.588	0.809	0.724
Diaphragm cysts (n = 193)	Total	Mean ± SE	77.16 ± 4.47	40.04 ± 1.20	2.05 ± 0.14
		95% CI	(68.33-85.98)	(37.67-42.41)	(1.77-2.33)
		Min	12.50	7.50	0.47
		Max	500	112.50	20
	Thin-walled cysts (n = 189)	Mean ± SE	77.46 ± 4.56	40.00 ± 1.21	2.06 ± 0.14
		95%CI	(68.47-86.46)	(37.60-42.39)	(1.78-2.35)
		Min	12.50	7.5	0.47
		Max	500	112.50	20
	Thick-walled cysts (n = 4)	Mean ± SE	62.50 ± 13.61	41.87 ± 9.26	1.53 ± 0.10
		95% CI	(19.16-105.83)	(12.39-71.35)	(1.18-1.87)
		Min	30	17.50	1.22
		Max	95	62.50	1.71
P-value		Between cysts	0.957	0.577	0.758
Heart cysts (n = 286)	Total	Mean ± SE	100.87 ± 3.52	46.13 ± 1.08	2.23 ± 0.063
		95% CI	(93.93-107.81)	(44.00-48.26)	(2.11-2.36)
		Min	20	17.50	0.75
		Max	450	125	5.86
	Thin-walled cysts (n = 285)	Mean ± SE	100.95 ± 3.54	46.16 ± 1.08	2.24 ± 0.063
		95% CI	(93.99-107.91)	(44.03-48.30)	(2.11-2.36)
		Min	20	17.50	0.75
		Max	450	125	5.86
	Thick-walled cysts (n = 1)	Value	77.50	37.50	2.07
P-value		Between cysts	0.867	0.706	0.916
P-value between organs (thin-walled cysts)			< 0.001	0.001	< 0.001

and the mean shape index was 2.28 ± 0.10. Thin-walled cysts showed comparable measurements, while thick-walled cysts tended to have lower mean values, but no significant differences were observed between cyst types for length, width or shape index ($p > 0.05$).

In the diaphragm, the mean cyst length was 77.16 ± 4.47 µm, the mean width was 40.04 ± 1.20 µm, and the mean shape index was 2.05 ± 0.14. Measurements for thin-walled cysts were similar to overall values, whereas thick-walled cysts showed lower mean length and shape index, but differences between cyst types were not statistically significant ($p > 0.05$).

In the heart, cysts exhibited a mean length of 100.87 ± 3.52 µm, a mean width of 46.13 ± 1.08 µm and a mean shape index of 2.23 ± 0.063. Measurements for thin-walled cysts were nearly identical. Only one

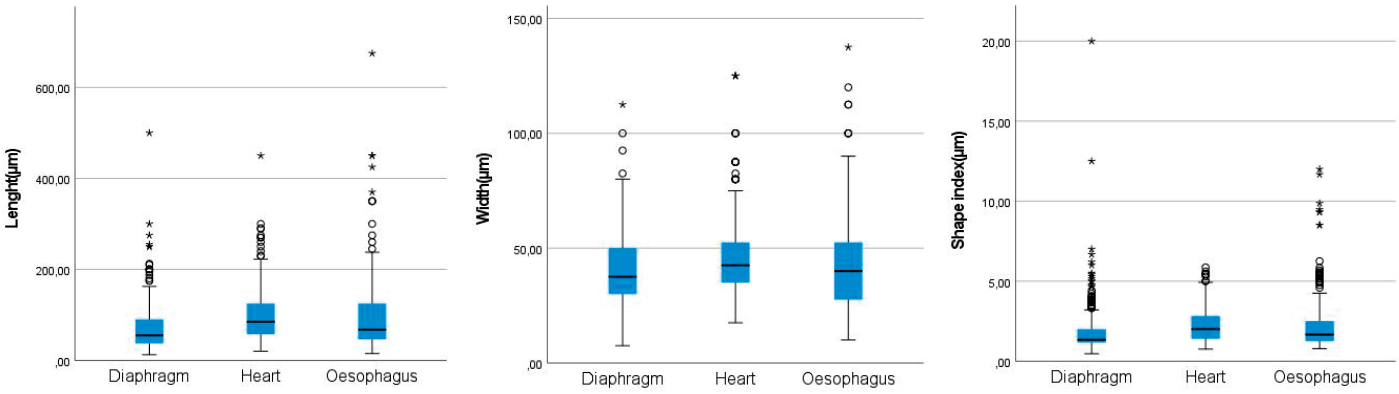

Fig. 7. Morphometric variation of sarcocysts among different organs (diaphragm, heart, and oesophagus)

thick-walled cyst was observed, precluding meaningful comparison; no significant differences were detected between cyst types ($p > 0.05$).

Kruskall-Wallis test analysis showed that organ type had a significant effect on cyst length, width and shape index for thin-walled cysts ($p < 0.001$), whereas no significant effect on these parameters ($p > 0.05$) was observed for thick-walled cysts.

The boxplots show that the morphometric parameters of the cysts vary among the organs examined (diaphragm, heart, and oesophagus). Cyst length tends to be greater in the heart and oesophagus compared with the diaphragm, with considerable variability, particularly in the oesophagus, as indicated by the wide dispersion and the presence of several outliers. In contrast, cyst width shows a more moderate variation among organs, with relatively similar median values, but a slightly greater dispersion in the heart and oesophagus. The shape index also displays some variability, broadly comparable across organs (Fig. 7). Overall, these results indicate that cyst morphometry, particularly length, varies according to anatomical site, while substantial intra-organ variability is also evident, as reflected by the wide interquartile ranges and numerous outliers.

Mean and standard deviation of the wall thickness of thick-walled cysts. The wall thickness of thick-walled cysts had a mean value of 3.00 ± 1.11 µm, with measurements ranging from 2.5 to 5.0 µm.

Sarcocystosis is an important zoonotic disease found worldwide. It causes considerable economic losses and affects a wide range of animal species, including cattle and humans. Numerous studies have examined its prevalence and epidemiological importance in various intermediate hosts in different geographical regions (25, 27, 28, 31, 36, 38, 41).

In this study, no macroscopic cysts were observed in 643 muscle samples from 269 bovine carcasses. This result is consistent with regional data showing that macroscopic cysts are generally rare or absent in cattle worldwide. Studies conducted in Algeria during post-mortem inspections of slaughtered cattle revealed no macroscopic cysts, suggesting that macrocyst-forming *Sarcocystis* species are rare or absent in North Africa (37, 38). However, a study conducted in a slaughterhouse in Egypt revealed a significantly higher prevalence of macroscopic cysts in females (62.73%) than in males (0.73%), highlighting the influence of host-related factors, such as age, sex and management practices, on the development of these cysts (28).

In Europe, studies conducted in Belgium and Lithuania indicate that macroscopic cysts are rarely detected in cattle (24, 41), whereas studies in Norway revealed the presence of predominantly macroscopically visible sarcocysts measuring 3 mm in length (mostly 5-6 mm) and approximately 0.5 mm in width. All macroscopic sarcocysts examined by molecular methods belonged to *S. hirsuta* (22). In Asia, macroscopic cysts have been observed at low frequencies: in north-western Iran, 8.2% of cattle had macroscopic cysts, while in Iraq prevalence rates of 6 to 27% were reported, reflecting local differences in husbandry and exposure to definitive hosts (25, 36). Collectively, these data confirm that macroscopic sarcocysts are exceptional in cattle, emphasizing the limited usefulness of gross inspection for assessing the prevalence of sarcocystosis across diverse regions.

In our samples, the absence of macroscopic sarcocysts may reflect the low circulation of the feline species (*Sarcocystis hirsuta*), probably due to limited cat–cattle contact and reduced environmental contamination resulting from feline defecation burying behaviour, which limits pasture contamination with infective oocysts (22).

In the present study, the oesophagus, heart and diaphragm were used, as previous investigations have shown these organs to be the most common sites for *Sarcocystis* spp. *Sarcocystis* infection was detected in 149 bovine carcasses, with infestation limited mainly to a single organ. The oesophagus was the most frequently affected organ, followed by the diaphragm and the heart, while multi-organ involvement accounted for 34.2% of infected carcasses. This distribution is comparable to that reported from North Africa, where studies from Egypt and Algeria showed higher infection rates in the oesophagus (70-100%) and diaphragm (40-90%) than in the heart, particularly when macroscopic or digestion-based methods were used (13, 37).

Similar patterns have been described in Asia, especially Iran and Iraq, where prevalence rates exceeding 90% were reported in oesophageal and diaphragmatic muscles, compared with lower and more variable cardiac involvement (40-60%) (2, 25). In contrast, European and Brazilian surveys reported a different organ distribution, with higher detection rates in the heart (40-50%) compared with the diaphragm (30-40%), reflecting both diagnostic sensitivity and the predominance of *Sarcocystis cruzi* forming thin-walled cysts widely distributed in striated muscles (20, 41).

Overall, these data indicate that, although bovine sarcocystosis is globally prevalent, organ-specific distribution varies geographically, largely influenced by parasite species composition and diagnostic methodology, which underscores the need for multi-organ sampling to avoid underestimation of infection burden.

Out of the 269 cattle examined, *Sarcocystis* spp. infection was confirmed in 149 animals, confirming that bovine sarcocystosis is predominantly subclinical and detectable mainly by microscopic methods (11, 34). The clear predominance of thin-walled cysts, consistent with *Sarcocystis cruzi*, agrees with recent studies indicating that this species accounts for more than 85-90% of bovine infections worldwide (34, 41). Thick-walled cysts compatible with *S. hominis* or *S. hirsuta* were rare, as reported in recent European surveys (32, 41).

All cysts were located within muscle fibres and showed a typical longitudinal orientation and alveolar compartmentalization, in accordance with established histological descriptions (11). Although infrequent, the detection of thick-walled cysts is epidemiologically relevant because of the zoonotic potential of *S. hominis*, which has been molecularly confirmed in cattle carcasses intended for human consumption in recent European studies (32).

In Algeria, no published data are available on the morphometry of *Sarcocystis* spp. cysts in cattle, with the exception of a study by Dahmani et al. on sheep (10). All of the work carried out worldwide focuses on histological studies of microscopic cysts found in bovine carcasses in organs most commonly affected (diaphragm, oesophagus, heart, tongue) and on their molecular characterisation (11, 12).

In our study, the morphometry of the microscopic cysts of *Sarcocystis* (length, width, shape and wall thickness) from naturally infected slaughtered cattle was carried out.

Histological examination in several studies confirms the predominance of microscopic, thin-walled sarcocysts in bovine tissues (11).

The results provide a detailed morphometric analysis of *Sarcocystis* cysts in cattle, distinguishing between thin-walled and thick-walled forms. Thin-walled cysts were predominant among the 746 cysts examined, which is in agreement with previous studies identifying *Sarcocystis cruzi* as the most frequent species in bovine muscles worldwide (11, 17).

Thin-walled cysts had greater mean dimensions compared with the few thick-walled cysts observed. Comparable morphometric ranges have been reported for Chinese cattle, where sarcocysts measured 20-400 µm in length and 30-100 µm in width, with thin-walled cysts predominating (39). Earlier studies also described a broad distribution of cyst lengths, with most sarcocysts falling within the 0-300 µm classes and only a small proportion exceeding 500 µm, regardless of the cyst wall type (18).

Previous studies have reported a wide morphometric variability of bovine sarcocysts observed under a light microscope (15, 26). Araujo et al. (5) described thick-walled sarcocysts measuring 154 µm in length, whereas Portella et al. (30) reported smaller cysts in cardiac tissue, reaching a maximum length of 60 µm. On the other hand, Obijiaku et al. (29) observed sarcocysts of much larger sizes, measuring 228.8 to 1215 µm in length and 46.93 to 114.40 µm in width. The majority of cysts (less than 500 µm long) were characterized by thin walls, which were attributed to *Sarcocystis cruzi*, while a smaller proportion of longer microscopic cysts with thick walls were identified as *S. hominis*.

The histological differentiation between thin-walled sarcocysts (*S. cruzi*) and thick-walled species (*S. hirsuta* and *S. hominis*) is relatively simple. However, distinguishing *S. hirsuta* from *S. hominis* based on histology alone is difficult. Notably, *S. hominis* forms are microscopic, whereas *S. hirsuta* typically presents as macroscopic cysts detectable during meat inspection (21, 29).

S. cruzi is the main species involved and may be related to the habitual coexistence of definitive hosts (dogs) and intermediary hosts (cattle), which favours the parasite's life cycle (20).

The mean shape index (2.20) in naturally infected cattle indicated a predominantly elongated morphology and was probably influenced by cyst age, tissue location and host-related factors.

Cyst morphology showed a variation according to anatomical site. Sarcocysts in the heart exhibited the largest mean dimensions, followed by those in the oesophagus and diaphragm, with differences in length, width and shape index among organs. Comparable organ-related morphometric patterns have been reported in cattle (22, 36, 40). The higher shape index values for cardiac and oesophageal cysts indicate a more elongated morphology, whereas diaphragmatic cysts were relatively more compact, which is consistent with previous observations (17, 22). Inter-organ p-values do not constitute evidence of independent biological differences, but suggest hypotheses for future studies.

Histopathological findings further corroborate these results, as oesophageal sarcocysts were predominantly fusiform or oval (96.7 µm × 326.9 µm), while cardiac cysts were smaller and ovoid (48.8 µm × 158.1 µm)

(14, 30). Detailed descriptions of cyst morphology, associated histopathological changes and molecular characteristics in bovine muscle and cardiac tissues have also been provided (7, 30). The frequent location of sarcocysts in the oesophagus has been consistently reported and is probably related to local conditions favourable for parasite development (2, 14, 16, 28, 37).

Thick-walled sarcocysts displayed a more rounded morphology than thin-walled forms across all bovine tissues examined. In the diaphragm, thick-walled cysts were shorter and had lower shape indices than thin-walled cysts, indicating a more compact structure. Comparable morphometric differences have been consistently reported in cattle, where thick-walled sarcocysts are less elongated than thin-walled forms (27, 36, 38). Although morphometric overlap exists, the recurrent association of rounded thick-walled cysts with *Sarcocystis hirsuta* or *S. hominis*, in contrast to the elongated thin-walled cysts, commonly attributed to *S. cruzi*, supports the utility of morphometry as a preliminary tool in bovine sarcocystosis studies (2, 38).

In the present study, thick-walled sarcocysts had an average thickness of 3.00 ± 1.11 µm (range: 2.5-5.0 µm), which confirms the importance of wall thickness as the main histopathological parameter (12). Comparable measurements were reported in cattle in China, where thick-walled cysts reached 5.8-1.2 µm (39). Previous studies indicate that the walls of sarcocysts of *S. hirsuta* and *S. hominis* typically measure 2-7 µm and are indistinguishable by light microscopy (12). In some cases, thicker walls measuring 7-9 µm, with palisade-like villar protrusions, were described and attributed to *S. hirsuta* or *S. hominis* (15).

Moreover, nearly perpendicular, cylindrical villar protrusions characteristic of *S. hominis* have also been observed in cattle (6). The similarity in cyst wall thickness among species means that the infested animal may host more than one species of *Sarcocystis* (18) and highlights the limitations of histopathology alone in differentiating between thick-walled species, particularly *S. hirsuta* and *S. hominis* (26).

This study provides a morphometric characterization of *Sarcocystis* cysts in Algerian cattle, showing a clear predominance of thin-walled forms, probably corresponding to *S. cruzi*, with a low occurrence of thick-walled cysts potentially attributable to *S. hominis* or *S. hirsuta*. Cyst morphology varied according to anatomical site, with sarcocysts in the heart being larger than those in the oesophagus and diaphragm, and thin-walled cysts generally exceeding thick-walled forms in size.

While the morphometric findings provide useful insights into the characteristics of *Sarcocystis* cysts in cattle, the results should be regarded as exploratory due to the cyst-level nature of the analysis and the potential for pseudoreplication.

These results highlight the importance of integrating histological approaches and complementing them with molecular analyses to confirm species identity and to assess the potential zoonotic risk associated with the consumption of raw or undercooked beef in Algeria.

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