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Diagnostic aspects of cattle sarcocystosis in Ukraine

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Abstract. *Sarcocystosis of cattle remains a relevant parasitological problem in the system of post-mortem veterinary and sanitary control due to the predominantly asymptomatic course of infection and the limited diagnostic value of macroscopic examination. The aim of this study was to investigate the level of sarcocystosis infestation in cattle by means of macroscopic examination of muscle tissues and compression microscopy of stained sections, to determine the degree of involvement of different anatomical muscle groups, and to study the morphological and morphometric characteristics of Sarcocystis spp.*

A total of 473 muscle samples (esophagus, myocardium, masticatory muscles, hind limb muscles, diaphragm, and intercostal muscles) collected during post-mortem veterinary inspection in December 2025 and January 2026 were examined. Diagnostic procedures included macroscopic inspection with serial incisions and compression microscopy of stained preparations, followed by morphometric analysis using digital microscopy and ImageJ software.

No macroscopic cysts of Sarcocystis spp. were detected during visual inspection. In contrast, compression microscopy revealed microsarcocysts in 105 out of 473 examined samples, corresponding to an overall prevalence of 22.2%. A pronounced anatomical unevenness of infection was established, with the highest infestation rates observed in the esophagus and hind limb muscles (up to 72%). Morphometric analysis revealed two main morphological types of microsarcocysts: oval and spindle-shaped, which differ visually.

The obtained results confirm the latent course of sarcocystosis in cattle and demonstrate the high diagnostic value of compression microscopy of stained preparations. The use of this method is essential for an objective assessment of infestation levels and for improving the effectiveness of veterinary and sanitary control of meat safety.

Keywords: sarcocystosis, cattle, compression microscopy, muscle tissues, post-slaughter diagnosis

Sarcocystosis is one of the most widespread parasitic diseases of farm animals, particularly cattle, with a pronounced zoonotic potential (Dubey, 2016). The causative agents of this disease are protozoa of the genus *Sarcocystis*, which infect the muscular tissues of animals (Fayer, 2004). According to numerous studies, the prevalence of *Sarcocystis* infection in cattle in different regions of the world may reach 70–100%, highlighting the significant epizootic importance of this parasitosis (Dubey, 2006).

The disease not only reduces animal productivity but also poses risks to public health through the consumption of contaminated meat (Moré, 2011). Zoonotic species of the pathogen, such as *S. hominis* and *S. heydorni*, can be transmitted to humans through insufficiently heat-treated meat, causing gastrointestinal disorders (Gjerde, 2013; Fayer, 2015). The parasite has a heteroxenous life cycle with alternation of definitive hosts (carnivorous animals: dogs, cats, and humans) and intermediate hosts (cattle) (Dubey, 2016, Hu, 2017). Definitive hosts excrete sporocysts with feces, contaminating the environment, feed, and water, thereby ensuring stable circulation of the parasite in the external environment (Dubey, 1989). In intermediate hosts, the parasite forms sarcocysts in the muscles, which become a source of infection for definitive hosts when raw, insufficiently heat-treated, or frozen meat is consumed (Gjerde, 2014, Dubey, 2015).

The causative agents of sarcocystosis demonstrate high resistance to environmental factors (Oryan, 2010). Sporozoites and sporocysts of *Sarcocystis spp.* are able to retain invasive

properties for months at low temperatures (down to $-20\text{ }^{\circ}\text{C}$), in moist soil, or on contaminated surfaces (European Food Safety Authority (EFSA), 2015). This contributes to long-term contamination of pastures, feed, water, and animal housing facilities (Fayer R, 2019). Tissue stages of the pathogen in meat are relatively resistant to cooling, freezing, and salting: sarcocysts survive at $-4\text{ }^{\circ}\text{C}$ for several weeks but die during deep freezing ($-20\text{ }^{\circ}\text{C}$ for 3 days) or cooking ($70\text{ }^{\circ}\text{C}$ for 20 min) (World Organisation for Animal Health (WOAH, ex-OIE), 2021) Such resistance is critically important for veterinary-sanitary safety of meat products, as insufficient processing may lead to outbreaks of zoonotic infections (European Food Safety Authority (EFSA), 2015).

The pathogenic effect of *Sarcocystis spp.* is caused by mechanical damage to muscle fibers, toxic-allergic effects of parasite metabolites, and immunopathological reactions of the host organism (Lindsay, 2020). During the acute phase (schizogony), endothelial damage to blood vessels occurs, which may lead to hemorrhages, anemia, and neurological symptoms (Dubey, 2023). The chronic phase is characterized by the formation of sarcocysts in the muscles and often proceeds subclinically (Yang, 2018). In most cases, sarcocystosis in cattle does not manifest with pronounced clinical signs (latent course), complicating diagnosis during the life of animals (Araujo, 2025, European Food Safety Authority (EFSA), 2015).

The absence of specific symptoms contributes to underestimation of the invasion and preservation of infection sources within animal populations (Vangeel, 2021). Economic losses caused by sarcocystosis are complex and include reduced body weight gain (up to 20% in infected animals), deterioration of meat quality (reduced tenderness and flavor), Carcasses affected by sarcocysts are subject to technical disposal, which leads to direct losses for farms and meat processing plants (European Food Safety Authority (EFSA), 2015). The disease also requires additional costs for control and prevention, including improvement of animal husbandry hygiene and meat processing measures (Imre, 2019).

Timely laboratory diagnosis is a key aspect of sarcocystosis control, especially considering its latent course (Gjerde, 2015, Yang, 2018). Traditional post-mortem examination methods (visual inspection and macroscopic examination of muscles) detect only macroscopic forms of the parasite, missing microscopic sarcocysts (Oryan, 2010). Therefore, more sensitive diagnostic methods are commonly used, including compression microscopy, histological staining (hematoxylin–eosin), enzyme-linked immunosorbent assay (ELISA) for antibody detection, and PCR for molecular identification of species (Gjerde, 2015, Yang 2018).

Control measures involve preventive strategies such as restricting access of carnivorous animals to pastures, maintaining feed hygiene, and adequate heat treatment of meat (World Organisation for Animal Health (WOAH, ex-OIE), 2021). Further research is required to develop effective vaccines and antiparasitic drugs (Dubey, 2023, Zaib, 2024). Sarcocystosis remains a significant problem for livestock production and public health, requiring integrated approaches to diagnosis, prevention, and control (Lindsay, 2020).

Aim of the study. To determine the degree of infection of different anatomical groups of muscle tissues with *Sarcocystis spp.* using compression microscopy of stained sections for the detection of microcysts, and to investigate the morphological and morphometric characteristics of microsarcocysts.

Materials and methods. The material for the study consisted of samples taken from cattle, namely: esophagus, myocardium (heart muscle), masticatory muscles, hind limb muscles, diaphragm, and intercostal muscles. Sampling was carried out during post-mortem veterinary-sanitary inspection in accordance with generally accepted rules for the collection of biological material.

One of the first stages of diagnosis was the visual detection of macrocysts, which are larger than 2.0 cm. Examination was performed according to generally accepted methodological guidelines for the diagnosis of sarcocystosis, in compliance with standards of veterinary-sanitary meat inspection.

Each anatomical specimen was subjected to external visual inspection under sufficient lighting to evaluate the surface structure and detect pathological inclusions. Particular attention was paid to the detection of macroscopic sarcocysts in the form of whitish or grayish, elongated or oval formations located between muscle fibers or under serous membranes.

To increase diagnostic sensitivity, each examined organ was subjected to a series of longitudinal and transverse incisions with a scalpel at intervals of 0.5–1.0 cm, which allowed

visualization of deeper layers of muscle tissue. The cuts were examined with the naked eye and using a magnifying glass ($\times 2.0$ – 5.0) to detect macrocysts.

During the macroscopic examination, no cases of macrocyst detection were recorded in the examined anatomical organs.

The second stage of the study was based on the detection of microcysts. From each anatomical sample, four sections approximately the size of a rice grain were prepared. The sections were stained for 40 minutes using methylene blue with the addition of concentrated acetic acid (1:1), which provided contrast staining of muscle fibers and intracellular parasitic structures. After staining, the sections were washed twice with distilled water.

The experimental sections were placed in the wells of the lower part of a compressorium, covered with the upper plate, and evenly fixed by tightening the screws until a thin transparent preparation suitable for microscopic examination was obtained. Microscopic examination was performed using a light microscope.

Primary examination was carried out at magnification: objective $\times 5$, eyepiece $\times 10$ to detect inclusions morphologically similar to sarcocysts. When suspicious forms were detected, further examination was performed at magnification: objective $\times 10$, eyepiece $\times 10$ for detailed assessment of the shape, size, and localization of the parasite in muscle fibers.

To establish the final diagnosis and detect developmental stages of the parasite, individual sections were transferred to a glass slide, covered with a coverslip, and examined at high magnification: objective $\times 40$, eyepiece $\times 10$. Diagnostic criteria included the presence of a clearly defined sarcocyst, the characteristic structure of its wall, and detection of bradyzoites.

The final stage of diagnosis involved the study of morphological and morphometric characteristics of microsarcocysts by isolating the parasite from muscle fibers through mechanical homogenization. To isolate sarcocysts, muscle samples from different anatomical locations (heart, diaphragmatic pillars, esophagus, masticatory, intercostal, and striated muscles) were used, obtained from one animal. Samples were cleaned of fat and fascia and mechanically ground twice by passing through a meat grinder with holes 3.0 mm in diameter. For examination, 10.0 g of minced muscle mass was selected, to which 40.0 ml of phosphate-buffered saline (PBS) (pH 7.4) was added, thoroughly mixed, and filtered through a medium-density sieve to remove coarse tissue fragments.

The obtained filtrate was transferred to centrifuge tubes and centrifuged at $600 \times g$ for 5 min, after which the supernatant was removed and the sediment resuspended in 10.0–20.0 ml PBS. A portion of the suspension (1.0–2.0 ml) was transferred to a Petri dish and examined under a light microscope at low magnification ($\times 4$ – 5) with further clarification at $\times 10$. Sarcocysts were identified by their characteristic elongated shape with rounded ends and absence of an internal fibrous structure; detected cysts were isolated under microscopic control and transferred into tubes with PBS for further storage in a refrigerator at 5 ± 1 °C.

Microscopic examinations were carried out using a ZEISS light microscope (Carl Zeiss, Germany) equipped with a standard digital video camera and proprietary ZEISS ZEN software. Preparations were examined in transmitted light using magnifications of $\times 50$, $\times 100$, and $\times 400$, which allowed the study of microsarcocyst structure, shape, wall thickness, and the nature of internal contents, including the presence of bradyzoites.

Morphometric analysis was performed using the ImageJ software (National Institutes of Health, USA) after preliminary calibration using a micrometric scale. The length, width, and wall thickness of microsarcocysts were measured; for each morphological type, at least 20–30 measurements were performed followed by calculation of minimum, maximum, and mean values as well as standard deviation.

Results. During the examination for the presence of macrocysts, a visual inspection of anatomical organs was performed, including transverse and longitudinal incisions and the use of magnifying devices. No macrocysts were detected in the examined beef samples obtained from cattle, indicating the predominance of microscopic forms of sarcocyst invasion.

Diagnostic investigations aimed at detecting microcysts were carried out in december 2025 and january 2026. In total, 473 muscle tissue samples collected from cattle carcasses were examined. Positive results for the presence of sarcocysts were obtained during microscopic examination using the compression diagnostic method with preliminary staining of the samples.

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Microscopy of the total number of samples examined (473) revealed 105 positive samples, which accounted for 22.2 % of the total number tested.

In December 2025, 376 samples were examined, of which 15 yielded positive results, representing 4 %.

In January 2026, 97 samples were examined, of which 50 yielded positive results, representing 51.5 %.

Thus, all data on the invasion of the examined cattle carcasses were obtained exclusively through compression microscopy of stained preparations. These findings confirm the low diagnostic informativeness of visual methods used during post-mortem veterinary and sanitary inspection and substantiate the necessity of mandatory microscopic diagnostics to obtain reliable information on the presence of sarcocysts in samples.

The overall detection rate of sarcocysts in 22.2% of the examined samples indicates a stable circulation of the pathogen among the cattle population, even in the absence of clinical manifestations.

The detection intensity of sarcocysts largely depends on the age of the animals, the duration of invasion, and the localization of the parasite within muscle fibers, as well as on the cyst density in the examined material. During the winter period, animals of older age groups are more frequently sent for slaughter, which potentially increases the probability of detecting sarcocystosis pathogens.

Thus, the obtained data confirm the key role of compression microscopy in the laboratory diagnostic system of sarcocystosis. The use of stained samples and stepwise microscopic analysis allowed to reveal the actual level of animal infestation, which was completely inaccessible using macroscopic examination methods. The analysis of the results suggests that the actual prevalence of sarcocystosis may be underestimated, since even the compression method has limited sensitivity in cases of low invasion intensity and uneven distribution of cysts in muscle tissue.

To determine the intensity of lesions in each anatomical group, 473 samples were examined from the esophagus, myocardium (cardiac muscle), masticatory muscles, hind limb muscles, diaphragm, cardiac muscle, and intercostal muscles.

According to the results, the intensity of muscle tissue infection ranged from 46.0% to 72.0%, indicating a significant prevalence of sarcocystosis among the examined cattle population. The obtained data made it possible to determine the percentage of infection for each organ according to the level of invasion. The highest level of invasion was observed in muscles with intensive blood supply, which creates optimal conditions for parasite migration and development. Based on the conducted studies, the following distribution of muscles according to the degree of pathogen accumulation was established:

Highly affected muscle groups:

esophagus – 72 %, myocardium (cardiac muscle) – 66 %, masticatory muscles – 61 %, hind limb muscles – 58 %

Moderately affected muscle groups: diaphragm – 52 %, intercostal muscles – 46 % (Fig. 1).

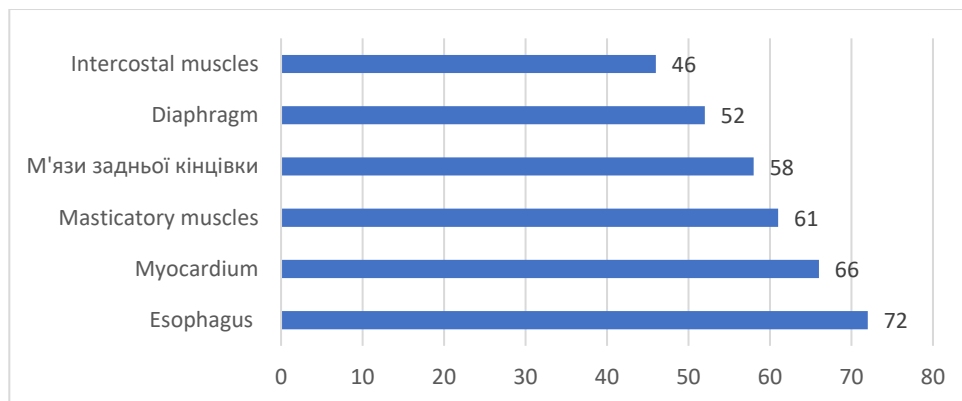


Fig. 1. Prevalence of Sarcocystis spp. infection in different muscle tissues of cattle

During microscopic examinations, microsarcocysts of *Sarcocystis spp.* were detected in the muscle tissues of cattle. These structures were predominantly localized intracellularly between muscle fibers, and less frequently arranged parallel to the direction of muscle bundles. Morphometric analysis performed using digital microscopy and measurement software (ImageJ) made it possible to classify the cysts according to their shape and size.

Based on morphological characteristics, the microsarcocysts detected in cattle muscle tissue were clearly divided into two main types, differing in shape, size, and predominant localization within the host organism.

Oval (ellipsoidal) microsarcocysts were characterized by the following parameters:

- length — 120–280 μm ;
- width — 40–90 μm .

These cysts predominated in the myocardium (cardiac muscle) and esophageal muscles, which may indicate their adaptation to tissues with high functional activity and intensive blood supply.

Spindle-shaped (fusiform) microsarcocysts had the following dimensions:

- length — 180–350 μm ;
- width — 30–70 μm .

They were more frequently detected in skeletal muscles, particularly in the masticatory muscles, neck muscles, limb muscles, and lumbar muscles. Their elongated shape and smaller width facilitate better integration into the long muscle fibers of skeletal musculature, which may explain their predominance in these tissues.

The sarcocyst wall was clearly delineated and thin or moderately thickened, with a thickness of 1.0–3.0 μm . The internal cavity of the cysts was filled with numerous bradyzoites, which were densely packed and oriented parallel to the longitudinal axis of the cyst.

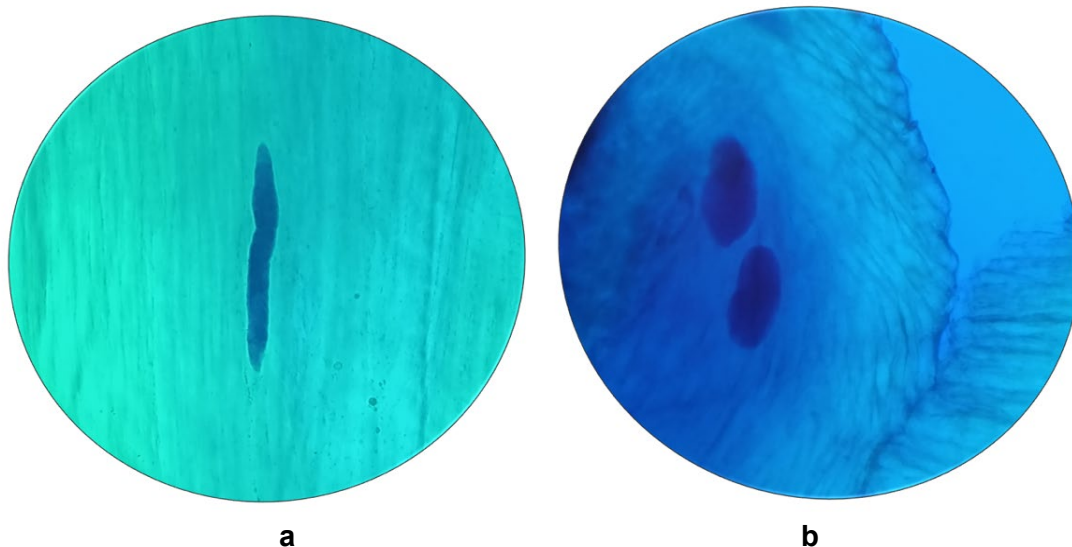


Fig. 2. Microsarcocysts of *Sarcocystis spp.* in cattle muscle tissues: (a) spindle-shaped (fusiform) microsarcocysts; (b) oval (ellipsoidal) microsarcocyst

Discussion. Thus, the identified morphological and morphometric heterogeneity of *Sarcocystis spp.* microsarcocysts indicates the presence of at least two morphotypes of the parasite, differing not only in shape and size but also in tissue tropism. The predominant localization of oval microsarcocysts in the myocardium and esophageal muscles, and spindle-shaped microsarcocysts in skeletal musculature, reflects the parasite's adaptation to the morphofunctional characteristics of different types of muscle tissue.

Conclusions

1. Macroscopic examination of cattle muscle tissues collected during post-mortem veterinary-sanitary inspection revealed no macroscopic sarcocysts (*Sarcocystis* spp.) in any of the examined anatomical samples, indicating the low diagnostic value of visual examination for sarcocystosis detection.
2. Compression microscopy of stained tissue sections allowed the detection of microsarcocysts of *Sarcocystis* spp. in 105 out of 473 examined samples, confirming the effectiveness of this method for identifying latent forms of invasion and determining the actual level of muscle tissue infection.
3. A pronounced anatomical unevenness of sarcocystosis infection was established. The highest infection rates were recorded in the esophagus and hind limb muscles (up to 72%), slightly lower in the myocardium and masticatory muscles (61–66%), while the intercostal muscles and diaphragm showed the lowest prevalence (46–52%).
4. Morphometric analysis made it possible to distinguish two main forms of microsarcocysts — oval and spindle-shaped, with lengths ranging from 120 to 350 µm and widths from 30 to 90 µm, indicating the morphological variability of tissue stages of the parasite and likely reflecting species-specific characteristics of the pathogen.
5. The obtained results confirm the latent course of sarcocystosis in cattle and justify the mandatory use of compression microscopy of stained preparations in post-mortem veterinary-sanitary control systems for ensuring the safety of meat products.

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Діагностичні аспекти саркоцистозу великої рогатої худоби в Україні

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Резюме. Саркоцистоз великої рогатої худоби залишається актуальною паразитологічною проблемою у системі післязабійного ветеринарно-санітарного контролю, з огляду на переважно безсимптомний перебіг інвазії та обмежену інформативність макроскопічних методів діагностики. Метою роботи було вивчити рівень інвазованості великої рогатої худоби саркоцистозом шляхом макроскопічного огляду м'язових тканин та компресорної мікроскопії пофарбованих зрізів, визначити ступінь ураження різних анатомічних груп м'язів, а також дослідити морфологічні й морфометричні особливості мікросаркоцист *Sarcocystis* spp.

Матеріалом для дослідження слугували 473 зразки м'язових тканин (стравохід, міокард, жувальні м'язи, м'язи задньої кінцівки, діафрагма, міжреберні м'язи), відібрані під час післязабійного ветеринарно-санітарного огляду у грудні 2025 та січні 2026 років. Діагностику проводили шляхом макроскопічного огляду з виконанням серійних розрізів та компресорної мікроскопії пофарбованих препаратів із подальшим морфометричним аналізом із використанням цифрової мікроскопії та програмного забезпечення ImageJ.

У ході макроскопічного дослідження макроцисти *Sarcocystis* spp. не були виявлені. Водночас компресоріумна мікроскопія дозволила встановити наявність мікросаркоцист у 105 із 473 досліджених зразків, що становило 22,2 %. Встановлено виражену анатомічну нерівномірність інвазії з найвищим рівнем ураження у стравоході та м'язах задньої кінцівки (до 72 %). Морфометричний аналіз виявив дві основні морфологічні форми мікросаркоцист, овальну та веретеноподібну, які відрізнялися за розмірами та тканинною тропністю.

Отримані результати підтверджують латентний перебіг саркоцистозу у великої рогатої худоби та доводять високу діагностичну цінність компресорної мікроскопії пофарбованих зразків тканин. Застосування даного методу є обов'язковим для об'єктивної оцінки рівня інвазованості та підвищення ефективності ветеринарно-санітарного контролю безпечності м'ясної продукції.

Ключові слова: саркоцистоз, велика рогата худоба, компресоріумна мікроскопія, м'язові тканини, післязабійна діагностика.

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