

HISTOLOGICAL IDENTIFICATION OF MUSCULAR *SARCOSYSTIS SPP.* IN MANDARIN DUCK FROM ROMANIA. A REPORT OF THREE CASES

IDENTIFICAREA HISTOLOGICĂ A PROTOZOARULUI *SARCOSYSTIS SPP.*

ÎN MUSCULATURA RAȚEI MANDARINE DIN ROMANIA. RAPORTAREA A TREI CAZURI

Narcisa MEDERLE¹⁾, Jelena SAVICI^{1)*},
I. MARINCU²⁾, Luminița COSTINAR¹⁾,
Isidora RADULOV³⁾, Ana-Maria MARIN¹⁾,
V. HERMAN¹⁾

ABSTRACT | REZUMAT

Sarcocystosis is a protozoan parasitic zoonosis that affects mammals, birds, and humans, also. The infection is characterized by cysts formation in the muscular tissues of the intermediate hosts and colonization of the lamina propria of the intestines of the definitive hosts. Microscopic examination is usually used to confirm the presence of sarcocysts and to provide information about the thickness of the capsule wall (thick or thin). Striated muscle tissue samples taken from three mandarin ducks were processed for histological examination by embedding in paraffin by standard technique and stained with Haematoxylin-Eosin. The histological examination revealed the presence of *Sarcocystis spp.* with a thin wall. Leucocyte infiltrates, present both in the wall of the cysts and endomysium, appears as the response of the organism to the presence of parasites. Bradyzoites, present as the „swarm of worms” in the structure of the cysts, are the ones responsible for the cyst's irregular compartmentation. Future molecular studies are needed to complete the histological identification of sarcocysts in the duck muscles, intermediate hosts of this protozoan.

Keywords: histological identification, *Sarcocystis spp.*, Mandarin duck, Romania

Sarcocistoza este o zoonoză parazitară care afectează mamiferele, păsările și oamenii. Infecția se caracterizează prin formarea de chisturi în țesuturile musculare ale gazdelor intermediare, precum și prin colonizarea lamei proprii a intestinelor gazdelor definitive. Examenul microscopic este folosit pentru a confirma prezența sarcocistului și pentru a oferi informații despre grosimea peretelui capsulei. Probele de țesut din mușchii striati, prelevate de la trei rațe mandarine, au fost prelucrate pentru examenul histologic prin includere în parafină prin tehnica standard și colorate cu Hematoxilină-Eozină. Examenul histologic a evidențiat prezența protozoarului *Sarcocystis spp.* cu peretele subțire. Infiltratul leucocitar, prezent atât în peretele chisturilor, cât și în endomisium, apare ca răspuns al organismului la prezența paraziților. Bradizoitii, grupați ca „un roi” în structura chisturilor, sunt responsabili de compartimentarea neregulată a chisturilor. Sunt necesare viitoare studii moleculare pentru a finaliza identificarea histologică a sarcocisturilor din musculatura raței, gazdă intermediară a acestui protozoar.

Cuvinte cheie: identificare histologică, *Sarcocystis spp.*, rață mandarină, România

Sarcocystosis is an intracellular protozoan parasitic zoonosis caused by the *Sarcocystis spp.*, belonging to the phylum *Apicomplexa* and family *Sarcocystidae*. The infection is characterized by cysts formation in the muscular tissues (muscular sarcocystosis) or colonization of the lamina propria of the intestines (intestinal sarcocystosis) of the intermediate hosts or the definitive hosts respectively (2).

Microscopic examination is usually used to confirm

the presence of sarcocysts and to provide information about the thickness of the capsule wall (thick or thin). In general, sarcocysts can be in all striated muscles such as the tongue, diaphragm, oesophagus, even in the myocardium, and sometimes even in smooth muscles (6).

Cattle are long-known intermediate hosts of *S. cruzi*, *S. hirsute*, and *S. hominis*, followed by *S. suihominis* in pigs and *S. horvathi* and *S. wenzeli* in poultry. *Sarcocystis spp.* has public health importance, as they cause an array of opportunistic infections in the immunocompromised individual (1).

Meat that is heavily infected may be condemned as unfit for human consumption. Therefore, eating raw or undercooked beef, poultry, and pork containing mature sarcocysts of *Sarcocystis hominis*, *S. suihominis*, and *S. horvathi* respectively, can result in humans a-

1) University of Life Sciences „King Mihai I”,
Faculty of Veterinary Medicine, Timisoara, Romania

2) „Victor Babeș” University of Medicine and Pharmacy,
Timisoara, Romania

3) University of Life Sciences „King Mihai I”,
Faculty of Agriculture, Timisoara, Romania

*) Corresponding author: jelenasavici@usab-tm.ro

cquiring intestinal Sarcocystosis (1).

Dubey et al. (2004) performed a redescription of sarcocysts structure from an experimentally infected shoveler duck (*Anas clypeata*) and a naturally infected duck, unanimous recognized to be the intermediate hosts for *Sarcocystis rileyi*. By light microscopy, the sarcocyst wall was 3-5 microns thick with indistinct villar protrusions. Ultra-structurally, the sarcocyst wall was a type-23 cyst wall with anastomosing villar protrusions that were up to 7.5 microns long. The villar projections contained filamentous structures. The bradyzoites were 12-14 microns long. Structurally, the sarcocyst from the naturally infected and experimentally infected ducks appeared similar (5).

This current study aimed to histological identification of *Sarcocystis spp* in the muscle of Mandarin duck from a private household in Romania.

MATERIALS AND METHODS

In the spring season, there was an episode of diarrhoea in Mandarin ducks that came from a private household where a herd of 100 domestic and wild Anseriformes (swans, geese, and Mandarin ducks) lived. The bird shared an artificially created pond. The bodies of one mandarin duck were transported in accordance with the sanitary-veterinary law to the Faculty of Veterinary Medicine Timisoara (ULST) to perform laboratory examinations. Tissue samples from striated muscles were processed for standard histological paraffin embedding. Fixation was done for 7 days in ethanol 80%, then samples were washed in distilled water and dehydrated by immersing in increasing concentrations of ethanol from 80% to 100%. After dehydration, the samples were embedded in paraffin (Paraffin pastilles for histology Merck, Germany). Sample tissue included in paraffin pieces was cut at 5µm thickness on a Cut 4062 microtome (Slee Mainz, Germany), and put on a glass slide.

The slide stained by the usual Haematoxylin - Eosin method was examined on a CX41 Olympus microscope (Olympus, Germany), provided with a digital camera Olympus life and material science CX41RF and software for image analysis QuickPHOTO Micro 2.2. (Promicra, Czech Republic).

RESULTS AND DISCUSSIONS

The bodies of one mandarin duck showed lesions of catarrhal enteritis, and in the chest, muscles were visible cystic formations, elongated. Histological sections from duck striated muscles evidenced the presence of sarcocysts represented by basophilic structures, with spherical or elongated shapes depending on the section plane (cross or longitudinal). The cyst measured between 0.6-1.2 mm (Fig. 1 and 2).

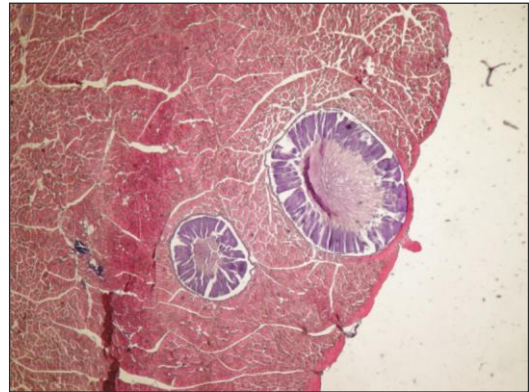


Fig. 1. Sarcocyst in skeletal muscle of duck, cross-section, Haematoxylin & Eosin stain, ob. 4X

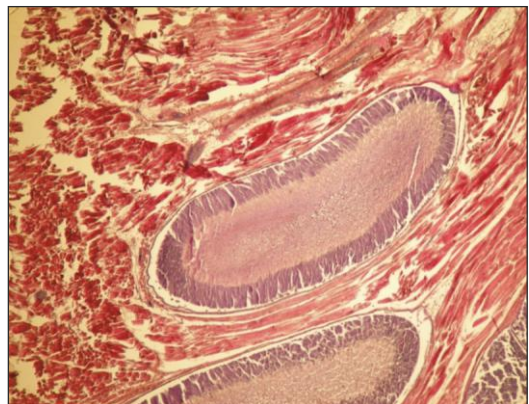


Fig. 2. Sarcocyst in skeletal muscle of duck, longitudinal section, Haematoxylin & Eosin stain, ob. 4X

The study carried out on bovines was conducted in slaughterhouses in Algeria, using samples from the oesophagus and diaphragm. A proportion of 95% of the samples from the oesophagus and 80% of samples from the diaphragm were positive by the enzymatic digestion method. The main species identified was *S. cruzi*, 94.2% in the diaphragm and 100% in the oesophagus. Histological examination of the samples highlighted cyst of *Sarcocystis cruzi* with a thin wall, *Sarcocystis hominis* or *Sarcocystis hirsute* with a thick wall (15). The presence of sarcocysts in the muscles of the oesophagus and heart were described also in domestic chickens from Kenya. Mbuthia et al. (2000) believe that *Sarcocystis rileyi* was responsible for the infestation of chickens, which are the source of infection for the duck population (10).

In the case of ducks, sarcocyst can be observed macroscopically, but the taxonomic status of sarcocyst is considered uncertain since ducks are intermediate hosts for the genus *Sarcocystis*. Only to *Sarcocystis rileyi* shoveler duck (*Anas clypeata*) and the striped skunk (*Mephitis mephitis*) were attributed as intermediate and definitive hosts (4).

The sarcocysts presented a thin wall that was infiltrated with inflammatory cells (Fig. 3). Similar findings were presented in a study from Turin's province (Piedmont region, Italy), regarding the presence of *Sarcocystis* spp. in minced meat from bovines. The samples were collected from butcher shops and retail stores, representing the meat destined for raw consumption. PCR and histology examination of the samples identified 80% *Sarcocystis cruzi*, 68% *Sarcocystis hominis*, 4% *Sarcocystis hirsuta*, with thin and thick wall cysts, respectively (11).

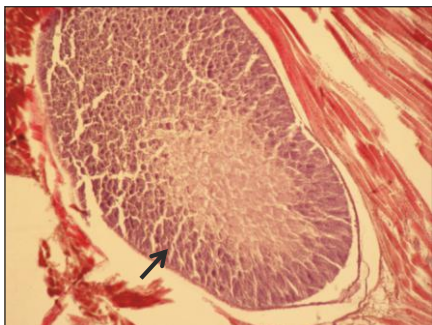


Fig. 3. Sarcocyst, longitudinal section, internal septation by groups of bradyzoites, Haematoxylin & Eosin stain, ob. 10X

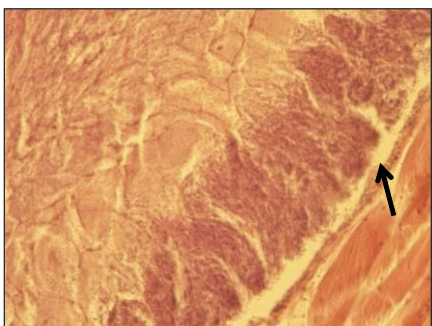


Fig. 4. Sarcocyst, longitudinal section, radial processes on the outer surfaces - cytophaneres, a thin wall with lymphocytic infiltrate (←), Haematoxylin & Eosin stain, ob. 40X

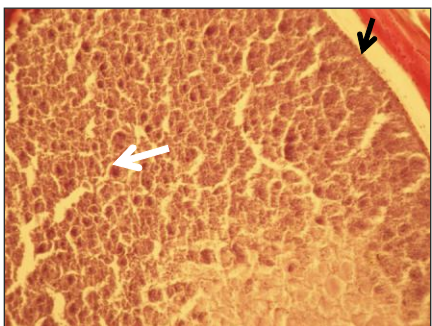


Fig. 5. Sarcocyst, longitudinal section, a thin wall with lymphocytic infiltrate (←), bradyzoites separated by irregular internal septations (←), Haematoxylin & Eosin stain, ob. 20X

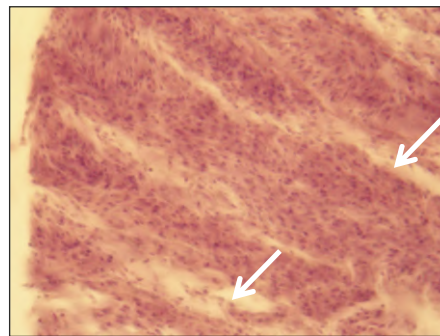


Fig. 6. Sarcocyst, longitudinal section, a cyst filled with groups of bradyzoites separated by irregular internal septations (←), Haematoxylin & Eosin stain, ob. 100X

Also, toward the wall radial (finger-like) processes called cytophaneres were observed (Fig. 4). The internal structure of the cyst is septate into irregular compartments, where bradyzoites are located (Fig. 5, 6).

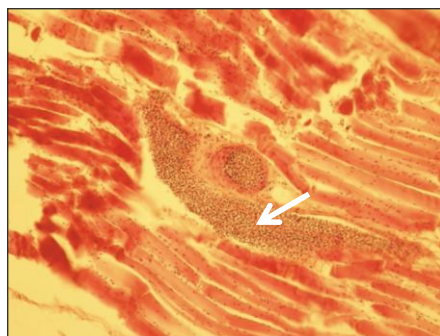


Fig. 7. Skeletal muscle, longitudinal section, endomysium dilation due to inflammatory infiltrate (←), Haematoxylin & Eosin stain, ob. 20X

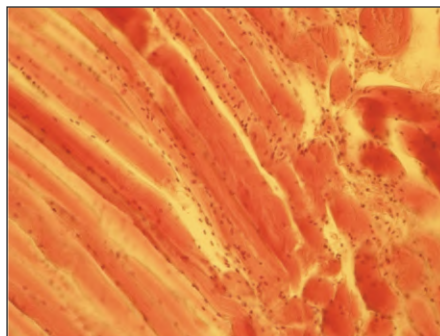


Fig. 8. Skeletal muscle, longitudinal section, multifocal lymphocytic infiltrate, Hematoxylin & Eosin stain, ob. 40X

Examination of the slides under the immersion oil (high magnification) showed the cyst full of bradyzoites, with spindle / elongated shape giving the appearance of a "swarm of worms", as it was also described by Makhija (2012) in humans. Mainly, in a 20-year-old

boy from Patna (Bihar, India) and a 50-year-old man from Varanasi (Uttar Pradesh, India) cysts of 1-2 mm in length were identified by histological examination in samples taken from skeletal muscles (9).

All the samples with one or more identified cysts in one section were considered positive.

Muscular fibres presented no pathological changes, but the endomysium was dilated and filled with inflammatory cells (Fig. 7). On all sections, multifocal lymphocytic infiltrate was present (Fig. 8). Because of the parasite's capacity to enclose in a cyst, in some cases inflammatory response, which is observed in our study as in literature (6), can be absent (9).

Samples taken from the heart and oesophagus of slaughtered chickens (Nigeria) revealed the presence of sarcocysts on microscopic examination. The infestation was present in both males and females, not correlated with age either (1).

In Mazandaran province, a study regarding infestation in native birds with *Sarcocystis* was carried out using samples from breast muscles. The samples were collected from native ducks and native chickens and examined by the digestion method. Results pointed out an infestation with *Sarcocystis* bradyzoite of 100% to the local duck and 94.78% to the native chickens (16). The Chinese authors described sarcocystis in chickens like microscopics. They exhibited palisade-like villar protrusions measuring 1.5-2.8 μm . Ultra-structurally, the sarcocyst wall contained numerous stubby hill-like villar protrusions. The protrusions included scattered microtubules, which extended from the tips of the protrusions into the ground substance (12).

Four specimens of mallard (*Anas platyrhynchos*) shot by local hunters along the eastern coastline of the island of Bornholm in the Baltic Sea were diagnosed with a heavy load of sarcocysts in the musculature (17). Elongate cysts (mean length 5.25 (SD 0.6) mm, width 1.37 (SD 0.2) mm) were recorded in all parts of the striated skeletal musculature of the birds. The main part (72%) of the 2585 cysts in one female mallard was in the outer superficial pectoral musculature, with 11% in the inner pectoral musculature. Minor but significant parts were found in the dorsal, ventral abdominal, neck and head, legs, hand and arm (wing) musculature. No cysts were found in the smooth musculature. Each of the cysts contained a median number of 3.2 millions of bradyzoites (range 1.9-5.0 millions) and with a total of 2585 cysts in one single host, the bird represents an infective potential of more than 8 billion bradyzoites. Bradyzoites had a median length of 13.5 μm (range 12.1-14.5) and a median width of 2.66 μm (range 2.1-3.3) (17).

Morphometric investigation results of *Sarcocystis* sp. from the barnacle goose (*Branta leucopsis*) and *Sarcocystis* sp. (cyst type IV) from the mallard duck (*Anas platyrhynchos*) are performed by Kutkiene L. et

al. No significant morphometric differences between the investigated *Sarcocystis* species were found (8). But, one year after, the same authors reported the first well-documented case of *S. rileyi* in Europe. According to ultrastructure results, macrocysts examined by light and electron microscopy and isolated from naturally infected mallard duck (*Anas platyrhynchos*) from Lithuania correspond to *S. rileyi* (7).

Reports of *Sarcocystis rileyi*-like protozoa ('rice breast disease') from anseriform birds had been rare in Europe until the last two decades when *S. rileyi* was identified in northern Europe and the UK. In 2019, Sándor Szekeres et al. described first time the evidence of *Sarcocystis rileyi* in Hungary (14).

The distribution and prevalence of infections with species of *Sarcocystis* in domestic fowl in Asia are poorly known. Ducks, pigeons, and chickens from Yunnan Province, China were examined for evidence of parasitic infection with *Sarcocystis* spp. One hundred and ninety-one chickens, 514 ducks, and nine pigeons from Yunnan Province, China were examined for evidence of parasitic infection with *Sarcocystis* spp. (3). Whereas the ducks and pigeons lacked tissue cysts in their muscle, brain or peripheral nervous system, cysts of *Sarcocystis wenzeli* were identified in 17 of 191 chickens (8.9%). The authors described the cysts from a morphological point of view: the cysts were thread-like, ranging in size from 334-3169 $\times$ 41-117 μm (mean 1093 $\times$ 65 μm). Cysts were septate with dense, short finger-like protrusions which appeared radially striated. The cyst wall was 1.4-3.5 μm (mean 2.4 μm) thick. The bradyzoites were lancet-shaped and measured 12.2-17.7 $\times$ 1.8-2.9 μm (mean 14.6 $\times$ 2.5 μm). Ultra-structurally, the primary sarcocyst wall had stubby villar protrusions, corresponding to the 'type 9' class previously designated. The protrusions measured 0.87-1.89 $\times$ 0.47-0.91 μm (mean 1.27 $\times$ 0.59 μm ; n = 57) (3).

Even though *Sarcocystis rileyi* is one of the earliest described species of the genus *Sarcocystis* forming macrocysts in ducks, the life cycle of this species is still unknown in Europe (13). *Sarcocystis* spp. oocysts/sporocysts were observed in feces and in small intestine mucosal scrapings of red foxes and raccoon dogs hunted in Lithuania. Using species-specific PCR and subsequent sequencing, the study performed by Prakas, P et al. provides strong evidence showing that the red fox and the raccoon dog can serve as final hosts of *S. rileyi* in Europe.

CONCLUSIONS

Microscopic examination of the slides from all samples of skeletal muscle taken from Mandarin ducks revealed the presence of *Sarcocysts* spp. with a thin wall. Leucocyte infiltrates, present both in the wall of

the cysts and endomysium, appears as the response of the organism to the presence of parasites. Bradyzoites, present as the "swarm of worms" in the structure of the cysts, are the ones responsible for cysts' irregular compartmentation. Other than leucocyte infiltrate, muscle cells presented normal histological structures. Future molecular studies are needed to complete the histological identification of sarcocysts in the duck muscles, intermediate hosts of this protozoan.

ACKNOWLEDGMENTS

This scientific paper was carried out in the Laboratory of Parasitology and Parasitic Diseases at the Faculty of Veterinary Medicine Timisoara, Laboratory within the Animal Hygiene and Pathology Research Center / ULS Timisoara.

REFERENCES

1. Asaga M.P., Musa I.T., Buba D.M., Ehi Airiohuodion P., Ambu S., (2018), Prevalence of *Sarcocystis* species in meat of cattle, pigs and birds slaughtered in North Central Nigeria. *J Vet Sci Med Diagn*, 7:1-6
2. Avwioro O.G., (2011), Staining reactions of microwave processed tissues compared with conventional paraffin wax processed tissues. *Eur J Exp Biol*, 1:57-62
3. Chen X., He Y., Liu Y., Olias P., Rosenthal B.M., Cui L., Zuo Y., Yang Z., (2012), Infections with *Sarcocystis wenzeli* are prevalent in the chickens of Yunnan Province, China, but not in the flocks of domesticated pigeons or ducks. *Exp Parasitol*, 131 (1):31-34
4. Dubey J.P., Rosenthal B.M., Felix T.A., (2010), Morphologic and molecular characterization of the *Sarcocystis* of *Sarcocystis rileyi* (Apicomplexa: *Sarcocystidae*) from the mallard duck (*Anas platyrhynchos*). *J Parasitol*, 96:765-770
5. Dubey J.P., Cawthorn R.J., Speer C.A., Wobeser G.A., (2004), Redescription of the sarcocysts of *Sarcocystis rileyi* (Apicomplexa: *Sarcocystidae*). *J Eukaryot Microbiol*, 50(6):476-482
6. Faye R., (2004), *Sarcocystis* spp. in human Infections. *Clin Microbiol Rev*, 17(4):894-902
7. Kutkiene L., Prakas P., Sruoga A., Butkauskas D., (2011), Identification of *Sarcocystis rileyi* from the mallard duck (*Anas platyrhynchos*) in Europe: cyst morphology and results of DNA analysis. *Parasitol Res*, 108(3):709-714
8. Kutkiene L., Prakas P., Sruoga A., Butkauskas D., (2010), The mallard duck (*Anas platyrhynchos*) as intermediate host for *Sarcocystis wobeseri* sp. nov. from the barnacle goose (*Branta leucopsis*). *Parasitol Res*, 107(4):879-888
9. Makhija M., (2012), Histological identification of muscular sarcocystis: A report of two cases. *Indian J Pathol Microbiol*, 55:552-554
10. Mbuthia P.G., Karaba W., Kuria J.N., Kagunya D.K., (2000), Sarcosporidiosis in domestic chicken in Kenya. *The Kenya Veterinarian*, 19:25-26
11. Meistro S., Peletto S., Pezzolato M., Varello K., Botta M., Richelmi G., Biglia C., Baioni E., Modesto P., Acutis P., Bozzetta E., (2015), *Sarcocystis* spp. prevalence in bovine minced meat: a histological and molecular study. *Ital J Food Saf*, 4(2):4626
12. Pan J., Ma C.L., Huang Z.M., Ye Y.L., Zeng H.X., Deng S.S., Hu J.J., Tao J.P., (2020), Morphological and molecular characterization of *Sarcocystis wenzeli* in chickens (*Gallus gallus*) in China. *Parasit Vectors*, 13(1):512
13. Prakas P., Liaugaudaitė S., Kutkienė L., Sruoga A., Švažas S., (2015), Molecular identification of *Sarcocystis rileyi* sporocysts in red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) in Lithuania. *Parasitol Res*, 114(5):1671-1676
14. Szekeres S., Juhász A., Kondor M., Takács N., Sugár L., Hornok S., (2019), *Sarcocystis rileyi* emerging in Hungary: is rice breast disease underreported in the region? *Acta Vet Hun*, 67(3):401-406
15. Taib M., Harhoura K.H., Aissi M., Chaouadi M., Djouhri Y., (2016), Study of the bovine sarcosporidiosis in the slaughterhouses of the north of Algeria: Case of the slaughterhouses of El Harrach (Algiers). *Front Cell Dev Biol*, 5:1
16. Vahedi Noori N., Salehi A., Razavi M., Masoumi M., (2020), Poultry carcasses investigation of parasitic sarcocystis infection in native in Mazandaran (North part of Iran). *Parasit Vectors*, 13(1):512
17. Zuo S.Z., Sorensen S.R., Kania P.W., Buchmann K., (2021), *Sarcocystis rileyi* (Apicomplexa) in *Anas platyrhynchos* in Europe with a potential for spread. *Int J Parasitol Parasite Wildl*, 15:270-275.