

Case report

UNIQUE REPORT OF TWO SARCOCYSTIS SPECIES FROM EGYPTIAN DOMESTIC CHICKEN (GALLUS GALLUS) – NEW HOST AND LOCALITY

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Summary

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Sarcocystis is an intracellular protozoan parasite in the phylum Apicomplexa. It is widely distributed all over the world. There are scarce reports about chicken Sarcocystis. From February 2016 to January 2018, a total number of 630 chicken carcasses, intestines and viscera were collected from different chicken markets in Menoufia and Gharbia Governorates, Middle region of the Nile Delta, Egypt and carefully inspected. Macroscopic and microscopic cysts of Sarcocystis spp. were found in the intestinal wall and mesentery of 5 birds. Histopathological sections revealed the presence of two shapes of the macroscopic cysts (oval and kidney shape). Their wall was striated and characterised by the presence of radial septa. It had compartments mostly of hexagonal shape, containing both bradyzoites and metrocytes in the periphery. The bradyzoites were banana-shaped and measured $20-30 \times 8-10 \mu m$ with centrally or posteriorly located nuclei. Microscopic cysts of Sarcocystis spp. were detected inbetween muscle bundles, with variable shapes (spindle and oval).

Key words: domestic chicken, Egypt protozoa, Sarcocystis

Sarcocystis parasites are obligatory, twohost, intracellular coccidian parasites. They require two separate hosts for completion of their life cycle: a definitive host (in which the sexual stage develops, usually a carnivorous predator) and an intermediate host (often herbivorous prey) containing the meronts (sarcocysts) (Olias *et al.*, 2010a). *Sarcocystis* spp. are widely distributed as parasites of wild mammals and birds. German chickens (19%) were reported to be affected with Sarcocystis horvathi and S. wenzeli (Wenzel et al., 1982) and also, the parasite was said to cause nervous signs in German domestic pigeons (Olias et al. 2010b). Obviously, the birds' muscles were infected with Sarcocystis species. Kutkiene et al. (2008, 2009) reported Sarcocystis from ducks and crows, respectively. Prakas et al. (2011) detected the wood pigeon sarcocyst (Sarcocystis columbae). Kutkienė et al. (2012) recovered S. wobeseri and S. turdusi from the barnacle goose and the common black bird, respectively. Prakas & Butkauskas (2012) found Sarcocystis from goose and duck muscles. Recently, Prakas et al., (2013) identified S. corvusi from the jackdaw.

Domestic chickens are reared in Egypt for egg and meat production by private farms and housewives.

Infection with *Sarcocystis* may cause serious extraintestinal problems. Smith *et al.* (1989) recorded extrapulmonary sarcocystosis in a budgerigar infected with *Sarcocystis falcatula*.

This study recorded a novel infection of domestic chickens (*Gallus gallus*) with *Sarcocystis* spp., with morphological identification and description of the revealed *Sarcocystis* using light microscopy.

A total number of 630 carcasses of free ranging Baladi domestic chickens (*Gallus gallus*), aged from 4 weeks to 4 months were collected from two regions centrally located in the Middle region of the Nile Delta, Egypt; Menoufia (30°35'50.09"N; 30°59'15.48"E; elevation above sea level: 10.07 m or 33.03 ft and Gharbia (30°47'18" N; 31°00'06" E; elevation above sea level: 18 m or 59 ft). Over two years from February 2016 to January 2018, routine examination of internal organs (heart, liver, lung & intestines) was done by naked eye for the presence of macrocysts and histologically for microcysts of *Sarcocystis* species.

From the different parts in the suspected infected birds, cuts of lesions were firstly fixed in 10% neutral buffered formalin and gradually processed for histopathology. Five μ m sections were stained using hematoxylin and eosin (H & E) and carefully examined using light microscope at magnifications ×40, ×100, ×400, and ×1000.

Macroscopic and microscopic sarcocysts of Sarcocystis spp were detected only in 5 (0.79%) out of 630 domestic chickens (G. gallus). Macroscopic sarcocysts of Sarcocystis spp. were recorded in 4 cases and mixed infection of macroscopic and microscopic cysts was detected in one case. The observed cysts of Sarcocystis spp. were present only in the external wall of the small intestine and the mesentery. Morphologically, macroscopic Sarcocvstis cysts collected from the infected chickens belonged to Sarcocystis horvathi (Rátz, 1908) and its definitive host is the domestic dog (Canis lupus familiaris).

Some were oval in shape and others were kidney-shaped. These cysts were macroscopic and measured 1000-2000 μ m in length × 600–650 μ m in width. The sarcocyst wall thickness was approximately 2.7-3 µm. The wall was striated (n=15). Radial striations produced hexagonal shaped compartments. The sarcocysts were packed heavily with bradyzoites, centrally located (Z; Fig. 1). The bradyzoites were banana-shaped with a curvature and measured 10–20 \times 3–5 μm with centrally or posteriorly located nuclei (Fig. 2). Metrocytes of Sarcocystis horvathi in this study were with unidentified shape, and were located in the periphery (Fig. 3).

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Fig. 1. Macroscopic Sarcocystis horvathi. A and C. oval shaped cyst; B and D. kidney shaped cyst; E and F. hexagonal departments with several bradyzoites (Z). H & E stain; bar= 50 μm.

Microscopic cysts were detected after histopathological examination on H & E sections measuring 200 μ m in length and 50 μ m in width. There were elongated spindle shapes, parallel to muscle fibres filled with numeorus blue dark stained bradyzoites in-between red coloured muscle bundles. There was no clear demarcation of the departments (Fig. 4). These microscopic sarcocysts were suspected to be one of pigeons' or wild bird's microsarcocysts. The presence of these microcysts may be due to the soil infection with oocysts of canine *Sarcosystis* species

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Fig. 2. Macroscopic *Sarcocystis horvathi* bradyzoites with central nucleus (black line). Other bradyzoites with peripheral nucleus. Bar = $50 \mu m$.

or it may be a new species infecting chicken requiring further molecular studies.

All examined sarcocysts were mature, occupied with high number of curved bradyzoites with few unidentified shaped metrocytes. Accurate measurement of bradyzoites was difficult due to the curvature of their bodies. So, there is no basic morphological identification depending on irregularly divided metrocytes (Dubey *et al.*, 1989).

Character of the macroscopic Sarcocystis was similar to that recovered by Ratz (1908, 1909) who recorded Sarcocystis horvathi in Hungarian chicken muscle with length 1 mm and the cyst wall was 1.8–2.7 µm thick. Also, Kruse & Go-



Fig. 3. Macroscopic *Sarcocystis horvathi* showing the striated wall and peripheral metrocytes. Bar = $50 \ \mu m$.

ranoff (1933) detected *S. gallinarum* from Bulgarian chicken which was 10 mm, filled with banana shaped bradyzoite (7– 13 × 2–4.5 μ m). Munday *et al.* (1977) described a case report of *Sarcocystis* resulting in myositis in 3 fowls in Papua New Guinea and 2 in Australia. The cyst was 2 mm long and 45 μ m in diameter with 2.5–3 μ m thickness of the striated wall. Moreover, Dubey *et al.* (2006) identified a mature *Sarcocystis* species from naturally infected African grey parrot. The cyst has a smooth wall and measured 2 mm in length and 750 μ m in width.

Kutkienė *et al.* (2011) detected macrocysts (*S. rielyi*) from the breast muscles of mallard ducks from USA and Lithuania. In China, (Chen *et al.*, 2012) identified macro thread-like cyst (*S. wenzeli*) in

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Fig. 4. Microscopic *Sarcocystis* sp. in-between muscle layer of the infected chicken intestine. A. cross section within the cyst. B. cross section. C–F. elongated, spindle shape cyst. Bar= $50 \mu m$.

chickens, that measured 334–3169 μ m × 41–117 μ m. Histologically they appeared multi-septated with dense, short finger-like protrusions that were radially striated. The cyst wall was 1.4–3.5 μ m thick.

Microscopic Sarcocystis were recovered mostly from different wild birds. Dubey *et al.* (2001) collected *S. lindsayi* which were 600 μ m long and 50 μ m wide with 2 μ m thickness of their wall. Also, Kutkiene *et al.* (2008) detected ribbon-shaped microscopic sarcocysts, from single infected mallard duck. Kutkiene *et al.* (2009) detected *S. cornixi* from the crows;

the cyst had radial spines with smooth wall whileOlias *et al.* (2010a) recorded microscopic *Sarcocystis calchasi* as a muscle cyst of the domestic pigeon.

In Egypt, the most extensive studies were done on ruminant *Sarcocystis* (El-Kady *et al.*, 2018). Only wild birds got some concern. El-Morsey *et al.* (2014) recovered a *Sarcocystis* species from the common moorhen. To best of our knowledge it is the first report recording avian sarcocysts in Egyptian domestic chickens.

Few *Sarcocystis* species can infect humans as intermediate or final host but on the other hand many studies confirmed that humans showed signs of toxicity after eating raw or undercooked meat infected with *Sarcocystis* species (Heydorn, 1977; Laarman & Tadros, 1982; Sam *et al.*, 1998, Kamata *et al.*, 2014) due to the ability of this parasite to produce a peculiar substance called sarcocystin which is highly toxic when injected into other animals; hence the parasite has a great importance concerning human health.

In conclusion, to our knowledge there are no data to determine the species of microscopic and macroscopic sarcocysts in bird meat in Egypt. This is the first time when multiple *Sarcocystis* spp. infections could be identified in the same infected birds. This case report put a spot for further studies on *Sarcocystis* spp. infection in Egyptian fowl. Excellent inspection and further investigation of the poultry *Sarcocystis* spp. must be undertaken by parasitologists as it was ignored in the previous decades.

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