

Evaluation of IFAT reliability in diagnosing *Sarcocystis* spp. in Egyptian water buffalo (*Bubalus bubalis*)

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Abstract

The aim of the present work is to evaluate the diagnostic reliability of the indirect immunofluorescence antibody technique (IFAT) in *Sarcocystis* spp. from Egyptian water buffaloes (*Bubalus bubalis*) and determine the current *Sarcocystis* spp. incidence rates in Egyptian water buffalo in the Sohag area. Using traditional and immunological strategies, in 2017, a total of 145 samples were collected from neighborhood abattoirs in the territory from the esophagus, diaphragm, tongue, and masseter muscles, *S. buffalonis*, *S. leavenii* and *S. fusiformis* were recognized and the infestation rates were determined to be 26.9% by gross examination at the abattoir, 52.4% by microscopic examination, and 64.8% by IFAT. It was concluded that traditional gross exam at the abattoirs is not adequate to present safe meat for human utilization and *Sarcocystis* examination must be consolidated with other serological tests such as IFAT. In this study, IFAT found to have moderate specificity and high sensitivity in diagnosing sarcocystosis, in contrast, it had a mild capacity to predict the presence of macroscopic cysts, and it exhibited high efficacy in predicting the absence of the microscopic cysts.

Keywords: Egyptian water buffaloes, *Sarcocystis*, IFAT, Sohag, *S. buffalonis*.

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Introduction

Sarcocystis spp. are intracellular apicomplexan tissue cyst-forming coccidian protozoan parasites, they are widely distributed and they have two obligate hosts, they infect the muscle of several species of domestic animals, birds, reptiles, and humans as an intermediate host to form the cyst; dogs, cats, and humans act as the final host. *Sarcocystis* spp. is exceptionally common in animals and is thought to be host specific [1]. Thus, they are viewed as standout parasites among the most vital zoonotic parasites and they have public health significance in terms of meat utilization.

Some *Sarcocystis* species have a pathogenic effect on their host causing severe economic losses due to decreased weight, low feed conversion ratio, fever, weakness of muscle, decreased milk output, late abortion and even death of carrier animals, these species include *S. cruzi* in cattle, *S. tenella* in sheep, *S. capricanis* in goats and *S. miescheriana* in swine [1-3]. The gross visible cysts, such as those of *S. fusiformis*, *S. buffalonis* and *S. gigantea*, make the meat unaesthetic, thereby leading to partial or total rejection of infected carcasses. At present, there are four characterized species of *Sarcocystis* that have been distinguished in water buffalo as *S. fusiformis*, *S. buffalonis*, *S. levinei* and *S. dubeyi* [4].

Water buffalo are usually slaughtered in local abattoirs in Sohag province, so flawless meat inspection and identification of zoonotic parasites are essential steps in preventing danger arising from contaminants; to our knowledge, however, only one scientific work has been published on the incidence of

Sarcocystis spp. in Egyptian water buffalo in Sohag province [5]. Thus, the aim of the present work is to illustrate the current *Sarcocystis* spp. incidence rate and evaluate the indirect immunofluorescence antibody technique (IFAT) test's capacity to identify *Sarcocystis* with a view to decreasing the zoonotic potential of this parasite.

Material and Methods

Research area and sample collection

Sohag province is located in the southern part of Egypt, with coordinates of 26°33'N, and 31°42'E. It has longitude of 31.694780, and latitude of 26.556950, and it is elevated 67 m above sea level.

From January to May 2017, 145 blood and muscle samples were collected from the esophagus, diaphragm, masseter and shoulder of freshly slaughtered buffaloes in local abattoirs in the province. Samples were labeled and submitted to the department of Parasitology, Faculty of Veterinary Medicine, Sohag University, Egypt and they were kept refrigerated until use [6].

Pathological examination

The collected muscle samples from different parts of the carcass were grossly examined by the naked eye for the presence of any cysts; positive tissue specimens were fixed in neutral buffered formalin, dehydrated in ascending grades of ethyl alcohol, cleared and embedded in paraffin blocks.

Sections of 5-7 μ m were generated and stained with hematoxylin and eosin (H&E) stain [7]; they were then examined using a light microscope.

The Indirect Immunofluorescence Antibody Technique (IFAT)

Collection of serum samples

Blood samples from 145 buffaloes were collected after slaughter in test tubes without anticoagulant [8] and centrifuged at 2500 rpm for 10 minutes. Serum samples were stored in a freezer at -20°C until they were used for IFAT. Positive control reference sera were taken from water buffaloes naturally infected with *S. fusiformis*, while control negative reference sera were collected from new born calves (20 days old).

Preparation of antigen from macroscopic *Sarcocystis* cysts

S. fusiformis cysts were gathered from substantial esophageal muscles using clean forceps, placed in clean petri dishes, and washed with clean, sterile saline solution. Using a sterile surgical blade, the cysts were cracked open, and the liquid inside was released, each 1 ml of the liquid was diluted with 3 mL of clean physiological saline and utilized as antigen. The antigen (25 μ L) was added to eight-well immunofluorescent slides which were left to dry in air; at that point, the samples were fixed using cold acetone in three progressive passages 10 minutes each in a cooler at -20°C. The slides were then thoroughly washed using phosphate-buffered saline (PBS) and left to air dry, according to Azab [9].

Preparation of the antigen-antibody complex

Sera (primary antibodies) were weakened with sterile PBS to the prescribed dilution of 1:16 (25 μ L from serum+75 μ L from diluents); following this, 2 μ L of the diluted serum was added to the fixed antigen on the tissue slide. The antigen-antibody complex was placed under humid conditions in the incubator for 30 minutes at 37°C slides were then washed three times using PBS for 3 minutes each time.

Preparation of the conjugate and slides mounting

10 μ L from the anti-human conjugate was added to 2 mL of 1% BSA, and 10 μ L from the anti-bovine conjugate was added to 2 mL of 1% BSA, then 25 μ L of each diluted conjugate was added to the slides. The slides left in the incubator for 30 minutes at 37°C and rinsed 3 successive times in PBS, 3 minutes each then left to air drying, then mounting buffer was added and cover slips were applied to slides, slides were examined with the fluorescent microscope at magnification 20 and positive slides were photographed.

Ethical considerations

The Study protocol was reviewed and approved by the ethics committee of the Faculty of Veterinary Medicine, Sohag University on October 14, 2014.

Statistical analysis

Savini et al. described through his research.

Results

Macroscopic and histopathological examination

The results of gross examination by eyes of 145 samples showed 39 positive cases (26.9%) with *Sarcocystis*, while histopathological examination showed 76 positive cases (52.4%) and 94 (64.8%) were positive by (IFAT). Two visible cysts: *S. buffalonis*, *S. fusiformis* and one microscopic *S. levinei* were distinguished, as follow:

S. fusiformis: For the most of them we found it located subserosal parallel to longitudinal axis between muscle filaments, visibly; it is fusiform or spindle shape, white to creamy in colour, grossly, the size ranged from (4.4-37 mm) length and (2.3-7.3 mm) width, it is spherical or sub spherical in histopathological cross section and measured from (219.9 \times 223.11 μ m), the wall thickness was ranged from (1.82-2.73 μ m), formed from a long striated projections in a palisade-like manners measured from (8.32-8.90 μ m) and the cyst was partitioned with septa into several sporadic irregular compartments loaded with highly condensed bradyzoites measured from (9.44-13.46 μ m). Based on all above-mentioned criteria, it was distinguished as *S. fusiformis*.

S. buffalonis: They were long and curved in shape, milky white colored cysts, and located immersed in the connective tissue along the longitudinal axis of muscle fiber. Grossly, the size ranged from (2.0-8.5 mm) length and (0.25-0.55 mm) width, by cross section masured from (84.82-104.45 μ m length \times 41.59-43.47 μ m width) with thick walls (3.44-4.11 μ m), but of no outwards projections and the center was free from bradyzoites.

S. levinei: Not seen by naked eyes, they are spherical in cross section or ovoidal in longitudinal section, and have a slight compartment with condensed bradyzoites all over the center and periphery under the wall, those have fine septa. Size was ranged from (43.5-44.72 \times 23.14-35.27 μ m). It has thin walls ranged from (1.22-1.28 μ m), bradyzoite length ranged from (8.9-11.49 μ m), based on these, it was identified as *Sarcocystis levinei*.

Histopathological examinations revealed the presence of myositis were in form of degenerated and necrosed muscle fibers and diffuse mononuclear cell infiltration of lymphocytes and eosinophils in the connective tissue of the skeletal muscles where *Sarcocystis* were explained.

IFAT

Results of IFAT revealed that 64.8% of serum samples had antibodies against *S. fusiformis*. All samples with macroscopic *S. fusiformis* were IFAT positive (100%), which showed diffuse fluorescent pigment in the trophozoites in the dark background. Sensitivity of the test was (100%), specificity (48.11%), positive predictive value was (41.49%) which means the ability of the test to correctly predict the presence of the disease, negative predictive value was 100% which means the ability of the test to correctly predict the absence of the disease and predictive validity value was (62%), which means the combined ability of the test to predict the presence or absence of the disease.

Discussion

There are several studies in Egypt which were carried out on the prevalence of *Sarcocystis* spp of water buffaloes, in Sohag by Khalifa et al. [5], in Assiut by Metwally et al. [10], Abdel Rahman [11], Said [12] and Fatma et al. [13]; in Qena by Fawaz [14]; in Beni-Suef, by El-Dakhly, et al. [15], in Dakahlia Province by Abu-Elwafa, et al. [16] in Cairo by Hilali et al. [4] and in Behera province by Hendawy [17]; in Kafr Elshiekh by Mahmoud El-Seify et al. [18].

The current work was done to illustrate the prevalence status of infection of Egyptian water buffaloes with *Sarcocystis* spp. at Sohag province due to the very low research work was carried out in the province on *Sarcocystosis* in buffaloes, to our knowledge; only one study was carried out by Khalifa et al. [5] and they revealed three species *S. cruzi*, *S. hominis*, and *Sarcocystis fusiformis*, in the present work, histopathological and IFAT test as serological test and sensitivity and specificity were calculated considering the IFAT test as the gold test. It was explored that, of a total 145 examined animals, there are 39 positive cases (26.9%) by macroscopic examination. While histopathological examination revealed 76 positive cases (52.4%) and 94 (64.8%) were positive by (IFAT) through using *S. fusiformis* antigen which had 48.11% specificity, and 100% sensitivity and is considered as the best sensitive antigen for serodiagnosis of *Sarcocystis* spp. but of mild specificity to detect the infection. On the other hand, the positive predictive value was (41.49%) which means the ability of the test to correctly predict the presence of the disease, negative predictive value was 100% which means the ability of the test to correctly predict the absence of the disease, and predictive validity value was (62%), which means the combined ability of the test to predict the presence or absence of the disease. So, the IFAT has mild ability to predict the presence but of high efficacy to predict the absence of the disease. Unfortunately, the available literature revealed little previous studies that used IFAT as a tool for diagnosis of *Sarcocystis* spp. These results come nearly in agreement with those of Ashmawy, who revealed the prevalence of *Sarcocystis* spp. infection was 67.6% in buffaloes aged 5-7 years old, and Wahba [19] who recorded 73% in buffaloes slaughtered at Cairo and Belbis slaughter houses, Egypt, and this suggests that buffaloes may be exposed to infection due to their close relationship with

dogs, cats and even wild animals that act as final hosts for these protozoa. A higher incidence (93%) was recorded among water buffaloes aged over 5 years old in Behera province, Egypt [17]. The difference in incidence may be due to the different methods of diagnosis, different localities, and different management.

The common serological test for *Sarcocystis* spp. detection by researcher is the ELISA test [10,17,20,21], this due to its high sensitivity and specificity values, the present study can prove that also IFAT test can be considered a reliable diagnostic test for *Sarcocystis* spp infection in water buffaloes, only one previous study was carried out by El Nazer and Abdel-Azem [22], they used *S. fusiformis* antigen in ELISA and IFAT for detection of extra intestinal sarcocystosis in human in attendants of rheumatology clinic in Sohag University Hospital, this may be due to the concept of Tadros et al. [23] who found a remarkable degree of cross reaction among *Sarcocystis* species from widely divergent host origins.

There is a high variation of infection rate at different localities in Egypt among examined animals, this high rate of infection may be due to the therapeutic courses against sarcocystosis is of little or no value either for the tachyzoites or muscle cyst, and several final hosts are incriminated in completion of the cycle, as main output of sporocysts (as infective form) for long periods, also the of oocysts or sporocysts resistance to sever hard environmental conditions as desiccation, drying, in addition to little or no immunity to shedding of sporocysts.

Conclusion

From the present study, we can conclude that, IFAT test has mild ability to predict the presence of macroscopic cyst but of high efficacy to predict the absence of the microscopic cyst, and three *Sarcocystis* spp. infecting water buffaloes in Sohag province, Egypt. In addition, this study proves that the routine exam at abattoirs is not sufficient to introduce safe food for human consumption, and must be combined with other serological tests as IFAT. Our findings prove the hypothesis that the antigen of *S. fusiformis* has cross antigenicity to other *Sarcocystis* spp. with mild specificity and very high sensitivity to detect sarcocystosis.

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Compliance with Ethical Standards

The cattle from which *Sarcocystis* spp. were collected were being processed at a local abattoir in Sohag Province, Egypt, as part of the normal work of the abattoir.

Conflicts of interest

"The Authors declares that there is no conflict of interest".

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