

The Effect Of Freezing And Heating On The Infectivity Of *Sarcocystis fusiformis* To Cats and Evaluation Of ELISA For Its Diagnosis In Water Buffaloes (*Bubalus bubalis*)

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Abstract: One hundred and thirty oesophageal muscles of slaughtered water buffaloes (*Bubalus bubalis*) were examined macroscopically for detection of *Sarcocystis fusiformis* cysts. The prevalence of *S. fusiformis* cysts in the examined buffaloes (3-6 years old) was 65 (50%), their dimensions ranged from 10.0–45.0 mm length & 1.5-7.0 mm width. Cats fed on fresh *S. fusiformis* cysts or those exposed to temperature at 50°C for 15 minutes were infected and shed *Sarcocystis* oocysts and sporocysts. Boiling of *S. fusiformis* cysts for 15 minutes, refrigeration for 3 days at 4°C and freezing for 2-3 months at -18 °C were effective in inactivating the cysts in buffalo meat. The antibodies against *S. fusiformis* were detected in 77 (59.23%) sera of the examined buffaloes by ELISA using bradyzoites of the same species. [Journal of American Science 2011; 7(7):55-57]. (ISSN: 1545-1003). <http://www.americanscience.org>.

Key words: Adjustment, Self-Esteem, Adolescence, Gender, Inventory

Key words: *Sarcocystis fusiformis*, heating, freezing, ELISA.

1. Introduction

Sarcocystis spp. are common cyst-forming coccidian parasites with a heteroxenous life cycle and wide range of hosts. *Sarcocystis spp.* are oval, whitish cysts that vary in size from microscopic to visible. Each intermediate and definitive host may harbor more than one *Sarcocystis* species (Dubey et al., 1989).

Water buffaloes (*Bubalus bubalis*) in Egypt harbour four *Sarcocystis spp.* (El-Sayed, 2010). Two macroscopic (*S. fusiformis* and *S. buffalonis*) with cats as definitive hosts and two microscopic (*S. levinei* with dogs as definitive hosts and *S. dubeyi* with unknown definitive host but thought to be zoonotic). The infection of water buffaloes with macroscopic *Sarcocystis* cyst renders the meat unmarketable leading to downgrading and condemnation of the carcass. In addition, the propagation of these *S. fusiformis* cysts in their final host (cats) will induce contamination of the environment due to excretion of the infective sporocysts (Dubey et al., 1989).

Sarcocystis fusiformis infecting water buffaloes in Egypt were studied by a number of authors (reviewed by El-Sayed, 2010). Its prevalence varied from 17.2 % (age 2-3 years old) and 68.1 % (over 5 years old). However, research studies on the effect of heating and freezing on its infectivity to the final host (cats) were not studied in Egypt and few data are available from other countries on other *Sarcocystis spp.* from water buffaloes and sheep (Srivastava et al., 1986 and Collins and Charleston, 1980). Furthermore, importation of water buffalo meat from India with possible introduction of *S. fusiformis* cysts has promoted our interest in studying this topic. In

addition, this study aimed at evaluation of serodiagnostic test (ELISA) for detection of *S. fusiformis* antibodies before slaughtering using crude antigen prepared from bradyzoites of the same species of cysts.

2. Materials and Methods

I-Collection of samples:

One hundred and thirty oesophageal muscles of slaughtered buffaloes ranged from 3 to 6 years old at El-Bassatin abattoir were macroscopically examined and those found infected with *S. fusiformis* cysts were collected and placed in properly labeled plastic bags, then immediately transported to the Parasitology laboratory. Upon arrival to the laboratory, *S. fusiformis* cysts were collected from water buffaloes and *S. fusiformis* cysts were also dissected out from imported meat (from India). Both cysts were used for experimental infection of cats.

II- Collection of sera for serological examination:

Serum samples were obtained from the same examined animals (hundred and thirty buffaloes). Sera were separated by centrifugation at 1500 rpm and stored at -20 °C until used.

- Experimental infection of the final host:

Eighteen young cats (4-6 weeks old) were used for experimental infection. All cats were coccidian free at the time of the experiment since neither oocysts nor sporocysts were detected in their feces after daily examination for five consecutive days. Then, cats were assigned into six groups 3 cats each. Each group was caged individually and fed only commercial dry cat food. Each cat in each experimental group was inoculated with 20 cysts whether fresh or treated. The cats of 1st group were

fed on freshly collected *S. fusiformis* cyst. The 2nd group was infected with *S. fusiformis* cyst exposed to temperature of 50°C for 15 min. The 3rd group was inoculated with the cysts after boiling for 15 min. The cats of the 4th group were inoculated with *S. fusiformis* cysts kept in refrigerator (4 °C) for three days. The 5th group was inoculated with *S. fusiformis* cysts obtained from imported buffalo's meat preserved for about 2-3 months at -18°C. The last group of cats was kept as non-infected negative control. Starting from 1st day post inoculation, faeces was regularly collected daily and thoroughly examined for the presence of sporulated oocysts by direct smear and the flotation concentration technique for 50 days post infection (Soulsby, 1982).

IV Serological diagnosis:

IV.a-Preparation of *S. fusiformis* antigen:

Freshly collected *S. fusiformis* cysts (40 cysts/ml PBS pH 7.2) were frozen and thawed for six times followed by sonication 2 times, each for 20 seconds and centrifugation at 14,000 rpm for 45 minutes. The supernatant was collected and aliquoted. The protein concentration of the antigen was determined (Lowry et al., 1951).

IV.b- Enzyme- linked immunosorbent assay (ELISA) (Iacona et al., 1980):

Specific antibody against *S. fusiformis* antigens was detected in the buffalo's sera by ELISA. Sera of water buffaloes infected with macroscopic *S. fusiformis* cysts (65 in number) and those negative for these cysts (65 in number) were subjected to serological analysis. Wells in ELISA plates were coated with 100 ul of *S. fusiformis* antigen at the rate of 40 ug /ml and sera were tested at dilution 1:100 in PBS. The optical density (O.D) was measured at 405 nm against blank control well. The tested sera were considered positive when the absorbency values were as more than the cut off values (0.36).

3. Results

1- Results of Macroscopical examination

The result of macroscopical examination of oesophageal muscles revealed that 65 (50%) of 130 buffaloes (3-6 years old) were infected with *S. fusiformis* cysts. Macroscopic cysts appear grossly as spindle shaped white or creamy in color. *Sarcocystis fusiformis* cysts measured 10.0–45.0 mm length & 1.5-7.0 mm width. Stained smears of *S. fusiformis* contents revealed banana shaped bradyzoites (9-14um ×4.5-5.5um).

2-Results of experimental infection:

Feeding cats with *S. fusiformis* cysts in the six groups revealed that only cats fed on fresh *S. fusiformis* cyst (group1) and cysts subjected to temperature of 50°C (group2) were infective since the cats excreted *Sarcocystis* oocysts and sporocysts. Cats in the other groups (3-5) were negative for *S. fusiformis* infection during experiment (50 days post infection). The prepatent period was 10-14 days and cats were still excreting sporocysts until the end of the experiment (50 days). The sporocysts were shed intermittently; they were ellipsoidal measuring 9.5-14.5 µm×8.5-12.4µm (12.0×10.45 µm), containing four sporozoites and a granular residuum.

3-Result of serological diagnosis (ELISA):

Examination of sera of 130 water buffaloes by ELISA using *S. fusiformis* antigen revealed that 77 (59.23%) had antibodies against *S. fusiformis* (O.D. Error! Not a valid link.). Fifty three (40.07 %) serum samples were negative (O.D. ranging between 0.125- 0.355) (Table 1). All the 65 water buffaloes infected with macroscopic *S. fusiformis* were ELISA positive (100%) while only 12 (18.46 %) out of 65 macroscopically negative had *S. fusiformis* antibodies.

Table1: Results of serological examination of 130examined buffaloes using ELISA.

| Total No. of examined animals by ELISA | NO. of seropositive animals | % | Average O.D. | NO. of seronegative animals | % | Average O.D. |
|--|-----------------------------|--------|----------------------|-----------------------------|--------|-----------------------|
| 130 | 77 | 59.23% | 1.26 (0.37-2.150) | 53 | 40.07% | 0.24 (0.125-0.355) |

4. Discussion

Sarcocystis spp. is one of the most common parasites affecting of water buffaloes meat. The present study revealed that 65 (50%) of 130 buffaloes were infected with macroscopic *S. fusiformis* The high incidence of *S. fusiformis* cyst infection in

buffaloes is associated with the fact that the buffaloes are in close contact with the final host (cats). In Egypt, Nassar, 1982 reported the incidence of *S. fusiformis* in buffaloes of 2-5 years was 94.0%. El-Sayed (2010) also, mention that the prevalence of *S. fusiformis* was 17.2 % in buffaloes 2-3 years old and

68.1 % in buffaloes over 5 years old. Low incidences (28%) was recorded in Sohag governorate, Egypt by Khalifa et al. (2008) and in Iran 20% of the examined buffaloes were infected with *S. fusiformis* by macroscopic examination (Ghorbanpoor et al., 2007). The difference in the incidence of *Sarcocystis* infections may be attributed to the difference in the ecological conditions and hygiene in the different localities where water buffaloes are raised. The difference in the age groups of the sampled animals contributes also to different incidence.

Concerning the effect of heating on the infectivity of *S. fusiformis* cysts, it was found that cats fed on fresh cysts and those subjected to 50°C for 15 minutes excreted viable oocysts and sporocysts of *Sarcocystis*. The obtained results revealed also that boiling for 15 minutes, refrigeration (for 3 days at 4°C) and freezing (for 2-3 months at -18 °C) were effective in inactivating *S. fusiformis* in buffalo meat. In sheep, *Sarcocystis gigantea* macrocysts were viable after 10 minutes at 52.5°C but not at 60°C. Macrocysts survived 60 days at -14°C and infected cats (Collins and Charleston, 1980). *Sarcocystis levinei* cysts infecting water buffaloes lost its infectivity to dogs when heated at 65-75°C while those cysts heated between 40-60°C were still infective to dogs (Srivastava et al., 1986). Also, cysts stored at -4°C for 2 days when fed to dogs didn't shed sporocysts but those kept at -2°C for 1 day shed sporocysts.

Conventional methods of diagnosing *Sarcocystis* spp. infections, involve time consuming and labour-intensive examinations of host muscle tissue for the presence of cysts or cystozoites are neither suitable for use in large-scale screening programmes, nor for use in diagnosing infections in livestock. In addition, diagnosis of *S. fusiformis* in living animals is needed specially for importing water buffaloes. Therefore, serological tests detecting specific antibodies to *S. fusiformis* have been assumed to be important in the diagnosis of sarcocystosis. A Few attempts have been made to evaluate the accuracy of these tests in diagnosis of sarcocystosis in buffaloes. In this study, antigen derived from *S. fusiformis* in buffaloes was utilized in ELISA procedure. Seventy seven (59.23%) out of 130 serum samples of examined buffaloes were positive for Sarcocystosis. Twelve samples (18.46%) out of 65 negative samples macroscopical examination for *S. fusiformis* were considered positive by ELISA which was most likely due to early stage of infection in these animals. This may be due to that these serologically positive water buffaloes harbour *S. fusiformis* cysts which were not detected macroscopically. *Sarcocystis fusiformis* antibodies were detected by the ELISA in 54.6% of the examined buffaloes in Iran (Ghorbanpoor et al.,

2007). Antibodies against *Sarcocystis* spp. were also detected in 88.6% of buffaloes from Iraq by IFAT (Latif et al., 1999). The obtained result revealed that sero-epidemiological surveys on livestock using ELISA can be carried out without the need to examine the animals after slaughtering.

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