

SOME STUDIES ON BUFFALO SARCOCYSTOSIS WITH REFERENCE TO THE BIOCHEMICAL ALTERATIONS

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SUMMARY

Two hundred and seventy buffalo's oesophagus muscle samples collected from Giza abattoir were examined macroscopically and microscopically for the presence Sarcocystis. The results indicate the presence of macroscopic Sarcocystis fusiformis at the rate 88.9% and microscopic Sarcocystis at the rate of 85.5% by the compressorium method and 93.3% by the use of tyrosine digestion technique. The incidence of infection with both species was 93%. The morphological features of the demonstrated Sarcocystis species and the liberated merozoites were described.

At the same time blood samples were taken from the same animals and sera were separated for the estimation of total protein, urea, uric acid, calcium, inorganic phosphorus and magnesium levels together with alkaline phosphatase and

alanine amino-transpherase activities. The results indicate significant decrease in total protein level and significant increase in both alkaline phosphatase and ALT activities. Minor insignificant changes were observed in the other investigated parameters.

INTRODUCTION

Water buffaloes are the most important farm animals in Egypt, as they constitute one of the main sources of high quality meat, milk and hinds for Egyptians.

Parasitic infestation is one of the ill health problems affecting the general condition of water buffaloes causing great economic losses in productivity or may even lead to death (Nassar, 1982).

Sarcocystis is one of the prevalent parasitic

infections of water buffaloes all over the world (Nassar, 1982; Manuel et al., 1983; Ghoshal et al., 1986; XIAO et al., 1988 and Nguyen, 1995). It causes loss of weight, weakness, anaemia, recumbence and death in calves and in dairy heifers (Frelief et al., 1979).

Giles et al. (1980) observed grey white foci in skeletal muscles and thickened fibrous, generalized lymphadenopathy, erosions in oral cavity and oesophagus and laminitis in cows.

Many studies were done on experimental infection in most species of animals to determine the morphology (Tongson and Molina, 1979), life cycle (Dubey et al., 1981) and effect of Sarcocystis (Fayer and Lunde, 1977).

The current study was conducted to investigate the incidence of Sarcocystis in water buffaloes, morphological picture of demonstrated parasites with the estimation of some serum constituents in naturally infected buffaloes.

MATERIAL AND METHODS

Collection of samples:

Oesophagus muscle samples were collected from each of 270 freshly slaughtered water buffaloes over 5 years old at the main slaughter house in Giza. At the same time about 15 ml blood was collected from each animal in a dry clean stoppered MacCartiny bottle during slaughtering of these animals and left to clot. Clear sera were separated and stored at 20° C unless they were used immediately for analysis.

Examination of samples:

Each oesophagus muscle sample was examined macroscopically and microscopically for the presence of Sarcocystis. Dimensions of the dissected macroscopic Sarcocystis fusi- cysts were measured under stereobin microscope. The detected cysts were crushed on slides and their contents were diluted with drops of saline, dried and fixed by methyl alcohol for 3 minutes then stained with Giemsa and examined under microscope for merozoites.

Two methods were used for the detection of microscopic cysts:

- 1) By the use of compressorium (El-Affifi, 1963), from each sample 10 small pieces the size of a rice seed were pressed between two plates of compressor and examined under microscope.
- 2) By the use of trypsin digestion technique (Erber, 1977). In which 10 grams of muscle samples were added to 100 ml of trypsin solution in phosphate buffer (2.5g/L), stirred with magnetic stirrer for two hours, sieved and centrifuged at 3000 rpm for 10 minutes. Fine smears were done from the sediment and diluted with equal amount of saline, stained with Giemsa to be examined under the microscope for detection of the liberated merozoites.

Serum analysis:

The collected serum samples were used for

estimation of total protein according to the method described by Weichselbum (1946), uric acid as mentioned by Artiss and Entwistle (1981), urea as recorded by Patton and Crauch (1977), inorganic phosphorus according to the method described by Kilchling and Freiburg (1951), magnesium according to the method mentioned by Niel and Neely (1956), calcium as recorded by Baron and Joyce (1957), alkaline phosphatase as recorded by Belfield and Graldbery (1971) and alanine aminotransferase (ALT) was carried out according to Reitman and Frankels (1957).

Statistical analysis and evaluation of the obtained results were carried out according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The present study showed that 252 out of 270 (93.33%) buffaloes slaughtered in Giza abattoir were found to be infected with both macroscopic and microscopic sarcocystis cysts (Table 1). Similar findings at Cairo abattoir were recorded by Nassar (1982) who recorded that the incidence of both macroscopic and microscopic infection in buffaloes was 97%. At Giza abattoir the reported macroscopic Sarcocystis in buffaloes over 5 years old were 88.9% (240 out of 270) while it was 90.8% at Cairo abattoir (Nassar, 1982). Regarding the incidence of microscopic sarcocystis species, it was found to be 231 (85.5%) with compressorium method and 252 (93.3%) when trypsin digestion technique was applied as shown in Table (1). Nassar (1982) stated that microscopically, it was 87.3% by trichinoscope

and 97.7 by trypsin digestion. The higher incidence of sarcocystis in case of trypsin digestion technique compared to that of compressorium method can be attributed to the undetectable small sized cysts of Sarcocystis which escaped diagnosis by compressorium method. The macroscopic examination of the sarcocystis revealed that they were opaque white in colour, spindle shaped measuring 0.3-2.8cm in length and running parallel to longitudinal axis of the muscles (Fig.1). The same morphology was recorded by Chauhan et al. (1978) and Nassar (1982). The microscopic sarcocystis reported here were spindle shaped, running parallel to the muscle fibers. Their walls were thin and smooth, internally they were divided by septae. They varied greatly in size from 117 to 430um in length and from 26.5 to um in breadth (Fig.2). Similar description was given by Dissanaikie and Kan (1978) and Nassar (1982).

The merozoites observed here in both macroscopic and microscopic sarcocystis were banana shaped with one end more pointed than other. Their diameters were (15.5-17.4) x (3.7-5.9)um (average 16.5 x 4.8 micron) and (17.1 - 17.8) x (4.1-4.3)um average 17.4x4.2 respectively. The nucleus was located near the broader end and the cytoplasm contained some fine granules (Fig.3 and 4). These findings were in consistence with the findings of Abdel Ghaffar et al. (1978), Dissanaikie and Kan (1978) and Nassar (1982).

Table (2) shows the estimated values for serum total proteins, uric acid, urea, inorganic phosphorus, magnesium and calcium levels

together with alkaline phosphatase and ALT activities. The tabulated results revealed significant decrease in total protein level and significant increase in both alkaline phosphatase and ALT activities while the other parameters were insignificantly changed compared to that recorded in the uninfected animals. The significant decrease in total protein level compared to control non infected buffaloes was similar to the results recorded by Fayer and Lunde (1977); Leek et al. 1977 and Dubey et al. (1981) in experimentally infected lambs, calves and goats. However, Mahrt and Fayer (1975) and Dessouky et al. (1984) recorded minor changes in the level of total protein which was significant.

Regarding the uric and urea concentrations insignificant elevations were recorded in their levels (Table 2). The results were recorded by Mahrt and Fayer (1975) who found insignificant increase in urea levels in experimentally infected calves and they attributed the elevation to the degenerative changes observed in liver and kidney. Also Dessouky et al. (1984) recorded minor changes in urea level, while Prass and

Fayer (1981) demonstrated increase in urea ascribed these changes to destruction of red cells.

The insignificant elevation in serum inorganic phosphorus magnesium and calcium levels be attributed to destruction of some muscle cells, due to infection and the leak of contents into the serum.

No available literature dealing with measurements were found:

The obtained data revealed significant rise alkaline phosphatase and ALT activities. elevation can be attributed to liver and spleen affection (Coles, 1974). Similar findings recorded by Dubey (1981) who recorded increase in serum alkaline phosphatase and ALT activity in experimentally infected dogs, while in experimentally infected goats Dubey et al. (1981) found elevation in ALT and no change in alkaline phosphatase activity. On the other hand, Dessouky et al. (1984) recorded minor change in the activity of both alkaline phosphatase and ALT.

Table (1) Incidence Of Macroscopic Visible Cyst And Microscopic Sarcocystis Species Cysts Among Buffaloes In Giza Abattoir.

No. of animals	Age	% of animal infected with both species	% of animals infected with Macroscopic cysts	% of animals infected with microscopic cysts	
				by compressorium	by trypsin digestion
270	> 5 years	93 %	88.9 %	85.5 %	93.3 %



Fig. (3) Merozoites of *S. fusiformis* x 1250

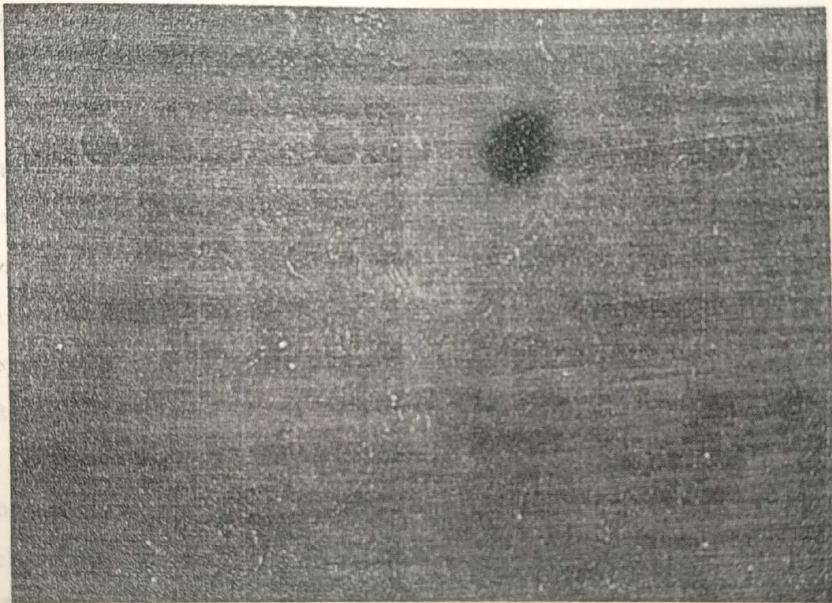


Fig. (4) Merozoites of *Sarcocystis* spp liberated from the digested oesophagus samples (40 x 12.5)

Table (2) Serum Total Protein, Uric acid, Urea, Phosphorus, Magnesium, Calcium level and Alkaline Phosphatase and ALT Activities in Infested and Non infested Buffaloes

	Infested mean \pm SE	Control mean \pm SE
Total protein g / 100 ml	* 8.1 \pm 0.64	9.65 \pm 0.22
Uric acid mg / 100 ml	0.632 \pm 0.09	0.615 \pm 0.06
Urea mg / 100 ml	0.31 \pm 0.06	0.29 \pm 0.03
Phosphorus mg / 100 ml	5.348 \pm 0.31	4.86 \pm 0.9
Magnesium mg / 100 ml	2.616 \pm 0.34	2.45 \pm 0.4
Calcium mg / 100 ml	11.206 \pm 0.72	10.94 \pm 0.61
Alkaline Phosphatase i.u. / 100 ml	*** 4.16 \pm 0.16	2.65 \pm 0.22
ALT u / ml	*** 42.275 \pm 0.6	22.67 \pm 1.75

* $P \geq 0.05$

*** $P \geq 0.001$

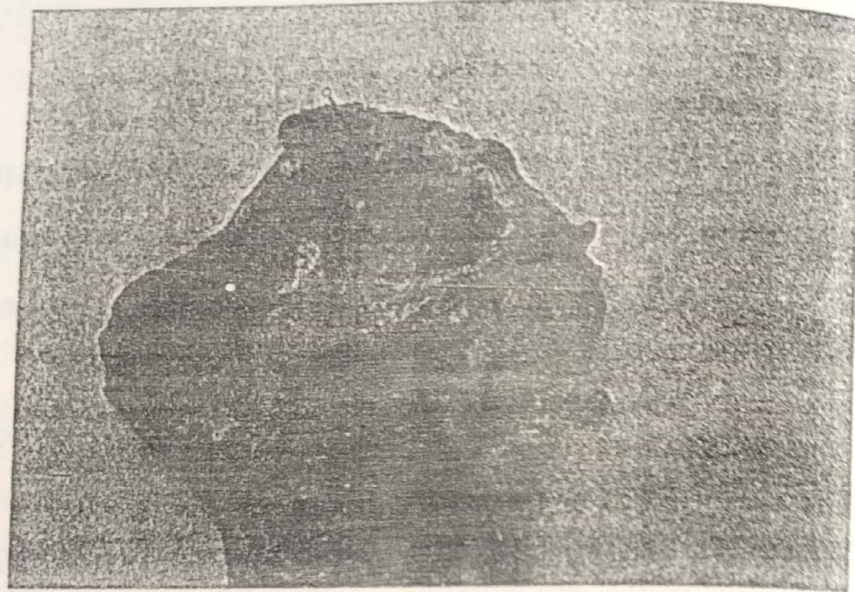


Fig. (1) Macroscopic Sarcocystis cyst in oesophagus of naturally infected buffaloes.



Fig. (2) Microscopic Sarcocystis species cyst in oesophagus of naturally infected buffaloes (40 x 12.5).

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A total of 411 sera (223 from cattle and 188 from buffaloes) 2 to 15 years old were collected from different localities from Egypt (Cairo, Delta and Upper Egypt). The sera were tested by ELISA for *Parafilaria bovicola*. Molecular weight of *Parafilaria bovicola* detected to 41 kDa protein (National Veterinary Institute, Uppsala, Sweden) has been used. All the tested sera recorded negative results. The inhibition rate was recorded to be 25-30% in cattle and buffaloes from Cairo. While, in Delta and Upper Egypt it was recorded to be 14.9 to 31.3% (the older ages 10-15 years) recorded higher inhibition rate (27.7-30.8%). The standard deviation was recorded as 75%.

INTRODUCTION

Parafilaria bovicola is one of Filarioidea which infects cattle and buffaloes in different countries.

The original description of the filarial worm *Parafilaria bovicola* was first recorded in 1934 by Takahashi (1934). Dr. Jezus (1934) noted the bleeding nodules on the skin of the live animals due to *P. bovicola* in Philippine. *P. bovicola* was recorded a tick in Romania (Metcalf, 1949), in Hungary (Falk and Deranics, 1949), South Africa (Strom and Van den Hoever, 1964), Costa Rica, 1968 and Webster and Wikens, 1973 and Sweden (Nilsson, 1978). Water buffalo was recorded to be infected with *P. bovicola* in India (Tiwari and Pand, 1963) in 1974. Choudhry et al. recorded the occurrence of *P. bovicola* parasitizing worm in the eye of

