

Some Studies on Filariasis and associated biochemical alteration in Egyptian buffaloes

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Abstract

Filariasis are vector-transmitted parasites, exclusively tropical, except for dirofilariosis, it is associated with a heavy burden of morbidity and mortality in ruminants. This investigation was carried on one hundred Egyptian buffaloes, 1.5 - 5 years, from different private farms located in Kaliobia province in Egypt, suffering from fever (40-41°C), anorexia, enlargement of superficial lymph nodes, cutaneous sub cutaneous and intramuscular connective tissues ulcers and skin necrosis causing a seasonal haemorrhagic dermatitis on skin. Laboratory examination indicated the presence of filaria in the blood. The serum investigations using some biochemical parameters measurements revealed some increase in lipid peroxidation product serum Malondialdehyd (MDA) and a decrease in serum total protein and albumin in diseased animals. Protein electrophoretic patterns showed declined values of α_2a , α_2b , Total α , β_1 , and γ_1 and total globulins concentrations in contrast γ_2 fractions.

Mass drug administration study was reported here when infected animals were treated with two doses of Ivermectin (1 ml/50kg b.w) in combination to levamesol (1 ml/20 kg b.w) with two weeks interval, a marked recovery was noticed and confirmed by the serum biochemical analysis of treated animals. [Report and Opinion. 2009;1(1):35-44]. (ISSN: 1553-9873).

Key words: Filariasis, Buffalo, Biochemical analysis, Disease control.

Introduction

Filariasis is widespread throughout the developing world and are associated with a heavy burden of morbidity and mortality resulting in considerable economic losses each year to beef and dairy buffaloes.

Filariasis is debilitating parasitic disease in many Tropical Countries. Despite the highly evolved immune system, The Filarial parasites Successfully evade host immunity to persist for sustained period of time. Filarial parasites achieve this long – Term Survival Through release of immunosuppressive materials in host. (Muthian *et al*, 2006).

Filariasis is vector-transmitted parasites, transmitted by arthropod vectors, they are endemic in tropical and subtropical regions of the world, their impact differs according to the type of filaria and the induced immune response.

Diagnosis of filaria is made based on the presence of dermatological or lymphatic manifestations, it can also be established following a laboratory examination revealing adverse effects on some biochemical parameters or correspond to the

incidental finding of microfilariae (blood or skin), the visualization of the adult parasite confirms the infection. (Soulsby, 1968)

The specific laboratory diagnosis of filariasis depends either on the demonstration of circulating microfilaria in the peripheral blood or various stages of the parasite in tissue sections. Various morphological characteristics of the parasite will normally assist in its identification. Concentration techniques, especially those using the polycarbonate membrane filtration of a ml or more of heparinised blood, can detect the parasite in those with very low microfilaria counts (Mak, 2004). Filariasis symptoms are usually caused by the adult worms, where microfilariae cause slight pathological alteration. The presence of the adult worm in lymphatics gives rise to inflammatory lesions and fibrosis which may result in some obstructions of lymphatics, overgrowth of fibrous tissues around dead worms and lymphatic vessels may also rupture this in association with different biochemical functions changes. The present report deals with alterations in malonaldehyde (MDA) and total protein Fraction in blood of *filariasis infected* buffaloes before and after treatment.

There is little doubt that combination therapy mass drug administration represents a significant advance in treatment of filariasis (Maged el setouhy *et al.*, 2004), numerous field trials evaluating the efficacy in the control of lymphatic filariasis have been conducted,

In this study, three trends of investigations were utilized, first is the clinical examination and diagnosis, second is the biochemical alteration in the infected and treated animals, concerning the MDA, serum protein and its electrophoretic pattern was performed, third concerns with the efficacy of utility of two drugs in particular, ivermectin (IVM) and Levamesole for effectiveness in the treatment of lymphatic filariasis.

Material and Methods

Animals

One hundred buffaloes 1.5-5 years from different private farms located in Kaliobia were clinically examined.

Samples

Venous blood samples were collected on anticoagulant for direct smear parasitological examination. and other blood Sample without anticoagulant were collected at clean plastic centrifuge tubes and allowed to coagulate, The Serum were separated by centrifugation at 300 r.p.m for 10 minute then clear supernatant serum aspirated carefully into dry and sterile labeled vials and used for serum analysis.

Microscopic examination

Identification of microfilariae by microscopic examination is the most practical procedure. Examination of blood samples will allow identification of adult filaria and microfilariae. It is important to time the blood collection with the known periodicity of the microfilariae. Venous blood samples were collected on anticoagulant before and after the treatment for direct smear parasitological examination. The detection of microfilaria was made by direct smear (the simplest method described by (Soulsby, E.J.L.1968) as follow:

- 1- Place one drop of venous blood onto a clean microscope slide.
- 2- Place a coverslip over the drop of blood.
- 3- Examine the coverslip area under low magnification (X 100) of microscope, look for the undulating of larvae.

Serum analysis

Blood samples without anticoagulant for serum analysis during infection and 4 weeks after treatment were collected.

Serum was used for determination of total protein and its electrophoretic pattern, this was carried out according to *SonnenWirth and Jaret (1980); Davis (1964) and Ornstein (1964) respectively*. Also, MDA was measured by thiobarbituric acid method, a modified form of the procedure described by (*Ohkawa 1979*).

Treatment

Infected animals were treated with two doses, Ivermectin manufactured by Arab Company for Medical Products), S/C injection in a dose of (1 mg/50Kg body weight in addition to Levamesole 7.5% manufactured by El. Nasr pharmaceutical chemicals co . Hydrochloride S/C injection in a dose of (1 mg/ 20Kg body weight) with 2 weeks intervals for the two drugs

Statistical analysis:

Data obtained were statistically computed for ANOVA test and using least significant difference (LSD) for comparison between means at $p < 0.05$, using SPSS 14 (2006).

Results

Clinical signs of infected animals

The clinical investigation of infected animals revealed that the different infected buffaloes were suffering fever (40-41°C), anorexia, enlargement of superficial lymph nodes, cutaneous subcutaneous and intramuscular connective tissues ulcers, nodules and skin necrosis causing a seasonal haemorrhagic dermatitis on skin (filarial dermatitis), and abscesses in some cases. The nodules open spontaneously and produced a haemorrhagic exudates.(Souls by , 1968)

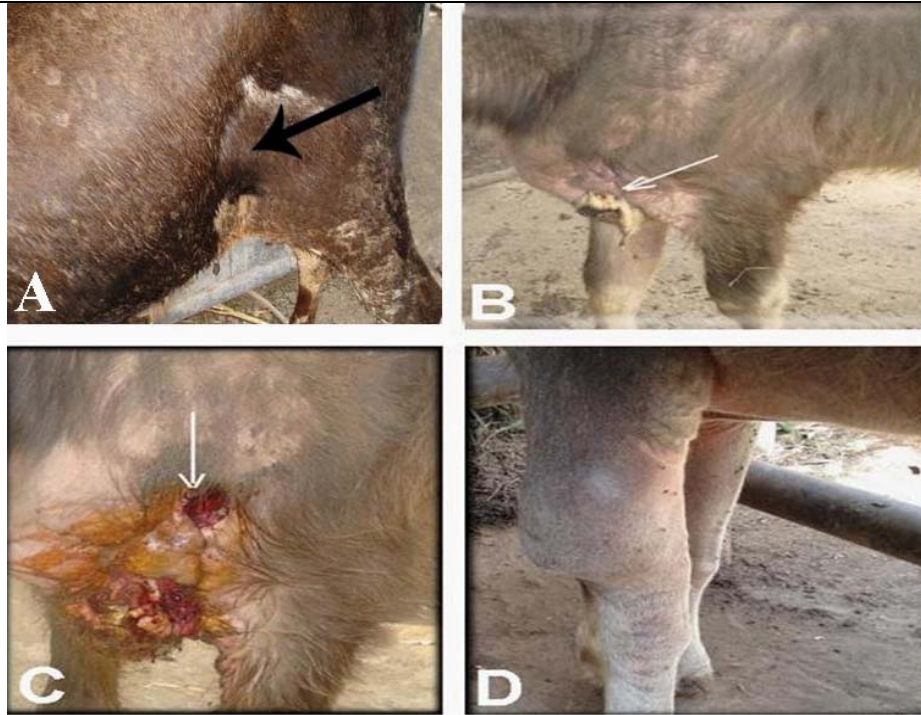


Figure 1: Different lesions of filariasis;

A, superficial lymph node enlargement.

B, spontaneously opened nodule.

C, surgical incision.

D, knee abscess and oedma.

Microscopic examination

Microscopic examination before treatment confirmed the presence of the adult and the microfilaria in the infected animal's blood, the microscopic examination of the same animals after treatment confirmed the disappearance of the filarial parasites in the blood.



Figure 2: An impression smear of the filaria and the RBC in the capillaries

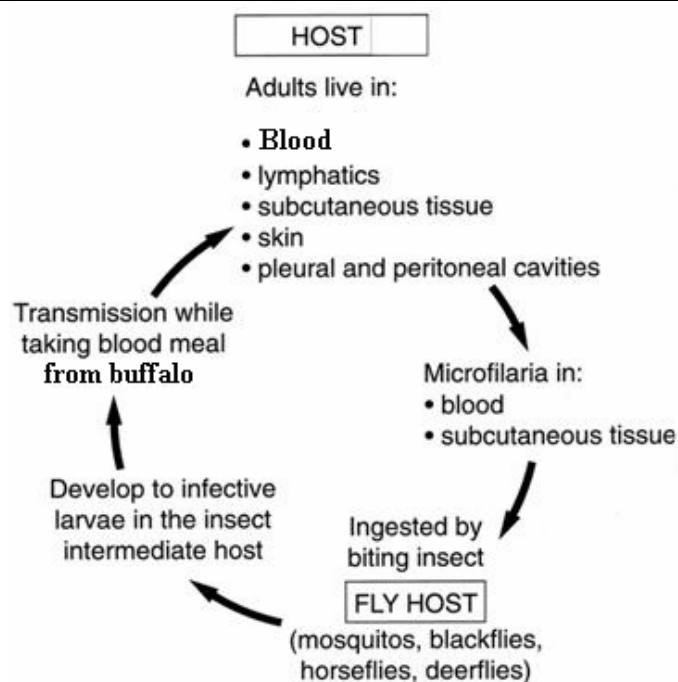


Figure 3: Basic life cycle of filarial

Biochemical Analysis

From gel electrophoresis (Fig. 3) and from the statistical analysis exemplified in table 1 , It is evidenced that, diseased buffaloes with filaria reported hypoproteinemia hypoalbuminemia , hypoglobulinemia (α_2a ; α_2b β_1 and γ_1 .) and reduced A/G ratio was significantly increased as noticed after treatment. There was a significant drop in albumin value and consequently total protein.

Table (1): Total serum protein and its electrophoretic patterns concentrations (gm/dl) in control, diseased and treated buffaloes.

Parameter	Control	Diseased	Treated	LSD
T.P	8.478±0.043 ^a	6.724±0.1745 ^c	8.028±0.1026 ^b	0.3683***
Albumin	3.427±0.018 ^b	2.439±0.039 ^c	3.817±0.199 ^a	0.363***
α_1	0.280±0.005 ^b	0.250±0.016 ^b	0.437±0.014 ^a	0.0386***
α_2a	0.413±0.004 ^a	0.282±0.007 ^b	0.243±0.003 ^c	0.01649***
α_2b	1.076±0.014 ^a	0.496±0.058 ^b	0.988±0.017 ^a	0.10917***
Total α	1.769±0.017 ^a	1.028±0.080 ^b	1.668±0.020 ^a	0.1507***
β_1	0.144±0.002 ^a	0.063±0.003 ^c	0.084±0.002 ^b	0.0066***
β_2	0.407±0.004 ^a	0.459±0.028 ^a	0.537±0.078 ^a	0.1476ns
Total β	0.551±0.006 ^a	0.522±0.029 ^a	0.621±0.078 ^a	0.1471ns
γ_1	2.452±0.028 ^a	1.452±0.070 ^b	1.213±0.094 ^c	0.21407***
γ_2	0.279±0.007 ^c	1.284±0.033 ^a	0.709±0.106 ^b	0.1973***
Total γ	2.731±0.034 ^a	2.735±0.053 ^a	1.922±0.143 ^b	0.2776***
Total globulin	5.051±0.040 ^a	4.285±0.153 ^b	4.211±0.217 ^b	0.476**
A/G	0.679±0.006 ^b	0.572±0.019 ^b	0.925±0.098 ^a	0.176**

Each value represents mean ± S.E.; n = 5

*: Significant variation between groups by one ways ANOVA at $P \leq 0.05$.

Total Protein was significantly decreased in diseased animals then partially restored in treated ones, albumin also significantly decreased in diseased buffalos where it was increased in treated animals.

α_1 significantly increased in treated buffalos, α_2 significantly decreased in diseased and more decreased in treated. α_2 significantly decreased in diseased animals where the total α significantly decreased in diseased buffalos.

In concern to β_1 , it was significantly decreased in treated and more decreased in treated animals. It also noticed that total β and β_2 were non significantly changed in all groups.

γ_1 was significantly decreased in diseased and more decreased in treated

γ_2 significantly increased in diseased and more increased in treated, but total γ was significantly decreased in treated, concerning the A/G we can notice the significant increase in the treated animals

Utilizing the MDA measurement in serum of diseased and treated animals, there was an elevated values of MDA in case of the infected animals, this value was declined after the treatment with ivermectin and levamisole

Fig (3) serum protein electrophoresis in different experimental buffalo groups: 1-2 control; 3-5 diseased; 6-8 treated

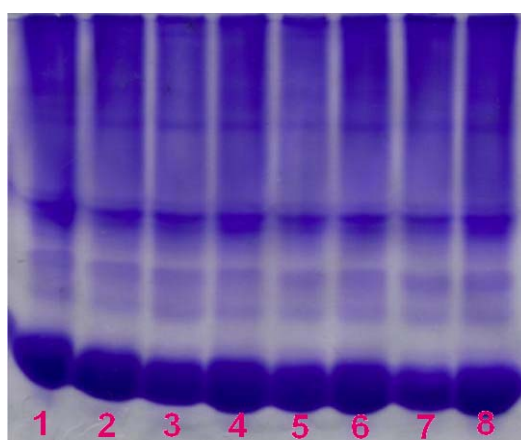


Table (2): Serum MDA (nmol/ml) in control, diseased and treated buffaloes.

Parameter	Control	Diseased	Treated	LSD
MDA	0.058±0.0018	0.081±0.0034	0.0624±0.0065	0.0135**

Each value represents mean ± S.E.; n = 5

*: Significant variation between groups by one ways ANOVA at $P \leq 0.05$.

Discussion

Screening for the filariasis has traditionally been difficult, requiring a microscopic examination of a blood sample. Often, this blood sample had to be collected in the middle of the night in order to correspond with the time of peak microfilariae abundance. Parasitic infections causes some biochemical alterations which could be recognized using some biochemical parameters investigations exemplified here by total serum protein and MDA with protein electrophoretic analysis.

It is evidenced from table (1) that diseased buffalo with filaria reported hypoproteinemia hypoalbuminemia, hypoglobulinemia (α_2 ; β_1 and γ_1) and reduced A/G ratio. The significant drop in albumin value and consequently total protein might be the result of inflammation caused by filarial disease, Taylor *et al* (2006). In inflammatory process, fluids and proteins move into the tissue fluids, resulting in edema and contributing to the decrease in albumin (Kaneko, 1989).

However, the significant alterations of globulins fraction table (1) may be attributed to immunosuppression effect of filarial host's (Kamalu 1991) and the increase of prostaglandin E(2) which can affect the host s metabolism and immune response Brattig *et al* (2006). These results may reflect the decrease in α_1 -globulin due to decrease in alpha (1)-acid glycoprotein (Matsumoto *et al*,

2007). Also filaria can produce immune deficient in mice by development of lymphedema in the absence of T cells and opportunistic infection (Vincent, et al 1984).

Kuchbaev *et al*, 2001 showed that a significant increase of lysophosphatidylcholine and arachidonic acid as well as a decrease of docozagexaenic acid in PL of sheep infected with filaria have been recorded. That points out to structural and functional disorders of cellular membranes during the infection

Levamisole is a broad spectrum anthelmintic effective against the nematode infections including filaria. The combination of levamisole with ivermectin was neither macrofilaricidal nor more effective against the microfilariae and the adult worms Awadzi *et al* (2004). In this study, the alteration of serum t. protein and its fraction, induced by filarial infectious corrected by levamisole and ivermectin (anathematic treatment), treatment, which possibly protection against infection by enhance the immune system Kumari and Sahoo (2006). Also levamisole increase serum albumin level of Atessahin *et al* (2004) and consequently the level of total protein increase.

levamisole has consider an immune enhancer and reported to increase cell mediate, cellular and humeral immunities. Wang *et al* (2008).

Filarial mediated oxidative stress in the host which to produce reactive oxygen species (ROS), resulting from cellular metabolism of hosts (**Pal .et.al , 2006**). Data revealed from this study shows a significant increased in lipid peroxidation in diseased group as compared with control. Thiobarbituric acid reactive materials including MDA is the most popular and easiest method used as an indicator of lipid peroxidation and free radical activity in biological samples (**Romero et al., 1998**). Filarial infection is displayed a significant increase of arachidonic acid. That points out to structural and functional disorders of cellular membranes during the infection. Kuchbaev and Bastarbekova (2001). The increase in arachidonic acid was accompanied by an increase in the quantity of thiobarbituric acid reactive substances (TBARS), Pompeia *et al* (2002) and Huang *et al* (2007).

As revealed from this study, levamisole and ivermectine co-administration with filarial infectious produced a significant reduction in serum malondialdehyde (MDA) level as compared with those treated with filaria alone(Table 2) These results are in agreement with those obtained by *Kumar et al (1980)* the author suggested that the inhibition of microsomal lipid peroxidation by levamisole is due to the generation of a sulfhydryl metabolite which had anti-oxidant properties. Also *Cam et al (2008)* ivermectine ameliorated the increase in lipid peroxide

Treatment of filariasis involves two components: (1) getting rid of the microfilariae in animal's blood, so that the transmission cycle can be broken and (2) maintaining careful hygiene in infected animals to reduce the incidence and severity of secondary (*e.g.*, bacterial) infections.

The operational efficiency of disease elimination programs in developing countries could be improved by integrating delivery of several interventions at local village and district areas endemic with filarial nematodes, the disease elimination strategy would be based on mass administration of a drug combination (WHO, 1998).

Anti-filariasis medicines commonly used include Levamisole, which kills adult worms, and ivermectin, which kills the microfilariae produced by adult worms, El-Tahtawy *et al.*, 2008.

ivermectin has a considerable direct macrofilaricidal action against female worms and that this lethal effect is supplemented by the drug's ability in some worms to increase the incidence, and the spread throughout the body of the worm, of the potentially fatal PN ovarian tumour. Duke BO, 2005 in his study confirmed that, in moribund and dead ivermectin-treated female worms that were heavily invaded by PN, it is probable that the neoplasm was chiefly responsible for their death, but the additional direct anthelmintic action of the drug, which by itself has been responsible for the death of many other female worms, cannot be excluded as having played a supplementary lethal rôle. Similar problems as to the exact means by which adult female worms are killed may arise now that ivermectin is used in Africa for the mass treatment of lymphatic filariasis; or if and when the macrofilaricidal actions on *O. volvulus* of other drugs, which are closely related to ivermectin, come to be investigated.

In this investigation, the treatment was carried out mass drug administration (multi dose combination therapy with ivermectin and levamisole) one targeting microfilariae and one targeting adult worms. Ivermectin is widely used for the control of filarial infections, particularly as a donated product for onchocerciasis and lymphatic filariasis (Edwards 2003). Ivermectin appears to work by paralyzing and then killing the offspring of adult worms, this may be a good drug administration strategy in the case of treatment of the microfilaria, it has long effective concentration time (2-4 week) after administration , this may be a good drug administration in the treatment of filarial , and serious adverse events (SAE) rarely occur (Brunton *et al*, 2006, especially that Levamisole has the capacity to enhance both the humoral and cellular immunity and kill the adult worms. Levamisole targeting on adult worms at low dose causing paralysis of the worms, it could be absorbed through the skin after dermal application and it will distribute through out the body , it also metabolize in liver, slow release and reach its peak concentration in blood after one hour from injection , so, levamisole efficiency depend on concentration not on the period contact. In this concern we utilized the

administration of one mixed dose which was repeated after 14 days. Levamisole also has an immunomodulatory activity as its immunostimulant effect by restoring suppressed host immunity and enhancing the interferon activity (Lullman *et al.*, 2005).

It could be concluded that, different biochemical alterations in buffaloes serum proteins were influenced as a result of filarial infection and from the drug administration point of view, it is recommended that, co-administration of ivermectin and levamisole as filaricidal drugs was the administration of choice in the treatment of filarial in buffalo.

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