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Diagnosis of microfilaria using modified Knott's technique in buffalo and its treatment

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Abstract

Filariasis is a vector-transmitted parasitic disease caused by *Filarioidea nematode* with world wide spread especially in tropical and sub-tropical countries. This is highly prevalent in hot and humid areas of India mainly hilly parts of India like Tarai region of Uttar Pradesh and coastal areas of Andhra Pradesh. The tentative diagnosis was carried out by direct faecal smear examinations, wet blood film observation and blood smear examinations, and modified Knott's technique, further case history was reviewed. The collected blood was subjected to different staining techniques including modified Knott's technique. On modified Knott's method examination detected microfilaria is long slender anterior blunt and tapering posterior ends. Ivermectin 200 µg/kg body weight subcutaneous route (orally microfilaricide 50 ug/ kg) is used as a curative drug in present clinical case and animal was responded well with the treatment. Hematological parameters of buffalo includes decreased hemoglobin (6.5 grs /dl), packed cell volume (20%), and total erythrocytic count ($4.5 \times 10^6/\text{ul}$) were recovered within 1 week after treatment.

Keywords: Microfilaria, mosquito, modified Knott's technique, buffalo, Ivermectin

Introduction

Infections with parasitic filarial worms cause disease conditions generically known as filariasis. It is a vector-transmitted parasitic disease caused by *Filarioidea nematode* with world wide spread especially in tropical and sub-tropical countries ^[10]. These larval stages of microfilaria include variety of genera setaria, onchocerca, dipetalonema stephanofilaria and parafilaria contribute to Microfilariasis in bovines ^[5, 8]. These parasites after reaching into blood stream and tissue spaces they taken up by mosquitoes and fleas while feeding in these intermediate host larvae develop into infective stage, reach proboscis by body cavity, later that again sucks the blood and infective larvae reaches the final host for complete their development ^[3]. Most of microfilarias circulate in peripheral blood in night time compare to day time this is called as nocturnal periodicity ^[11]. Transmitted by hematophagous arthropods like biting flies and mosquitoes (*Aedes*, *Culex*, and *Anopheles*).

This is highly prevalent in hot and humid areas of India mainly hilly parts of India like Tarai region of Uttar Pradesh ^[10] and coastal areas of Andhra Pradesh ^[5]. Microfilaria shows great impact on general body condition and performance of affected animals causing, anaemia, lethargy, loss of appetite, reduced milk yield ^[4, 1]. Microfilariasis is associated with economic losses in beef and dairy buffaloes as they cause high morbidity and mortality. In dairy buffaloes, it leads to sudden drop in milk production. Filariasis induce haemolytic anaemia, with alternation in antioxidant enzymes and liver, cardiac function. Inflicts economic loss gradually by deteriorating health, marked decline in milk yield, prolonged convalescent period and treatment costs.

Materials and Methods

Chemicals and stains

EDTA, 2% Formalin (2ml of 37% formaldehyde solution in 98 ml of distilled water), Methylene blue (1%) and Methylene Green (0.2%)

Equipments and other materials

Microscope, Centrifuge, Centrifuge Tubes, Microscopic slides, coverslips and Pipettes

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Case history and observations

A Nine year old female buffalo with case registration number 6346 was presented to the district veterinary hospital Khammam, Telangana with the history of watery, mucoid or mucopurulent discharges from nostrils, dyspnea, lustreless and rough hair coat, facial and pedal edema with the anorexia symptoms since 4 days (Fig-1). There was no history of any insect or snake bites. Animal was six months pregnant. Urination and defecation were normal and milk production was decreased. The buffalo was treated 3 days before with Inj. Meloxicam 10 ml IM, Inj. Avil 10 ml IM and Inj. Enrofloxacin 10 ml IM, but animal health was not improved.



Fig 1: Symptomatic Buffalo

Clinical Observation

On clinical examination, the rectal temperature of Buffalo was 104.1 °F, pulse rate of 70/min and respiratory rate of 32/min. Conjunctival and Buccal Mucous Membranes were pale pink, ruminal motility was 3/2 min and there was pitting on pressure on dependent parts of the body on haematological examination decreased haemoglobin (6.5 grs /dl), packed cell volume (20%), and total erythrocytic count (4.5×10^6 /ul) and increased total leukocytic count (TLC), eosinophilia, lymphocytosis observed.

Diagnosis

Tentative diagnosis was carried out by direct faecal smear examinations, wet blood film observation, blood smear examination and modified Knott's technique [12].

- On direct faecal smear examinations – No parasitic ova detected
- Wet blood film observation-we have collected the blood from ear vein and made a thin smear on glass slide and overlay it coverslip and examined under low power microscope (10x and 40x objectives) – No hemoprotozoans detected
- Modified Knott's technique –we have mixed 1 ml of EDTA blood with 9 ml of 2% formalin in a 15 ml tube and inverted several times before closing the tube to mix properly, centrifuge the tube for 5 min at 2000 rpm, then discarded the supernatant, later we added 0.1% methylene blue solution to the sediment and mixed well [12].
- Taken 1 drop of stained sediment with a pipette, spread it on a slide and examined under microscope on whole blood examination by modified Knott's technique and it revealed that the presence of microfilaria.

We have treated the buffalo with Inj. Ivermectin (200µg/kg body weight subcutaneously) 2 doses with 1 week interval, Inj. Meloxicam – 10ml, Inj. Tribivet – 10 ml IM, Inj. Ridema (furosemide) – 10 ml IM and Inj. DNS – 2 liters IV, Inj. Feritas – 5 ml /day for 3 days and oral haematinic mixture for 5 days as supportive therapy [7, 8].

Results

Using blood smear and modified Knott's method examination, microfilaria is detected. It has long slender anterior blunt and tapering posterior ends (Fig-2 & 3).

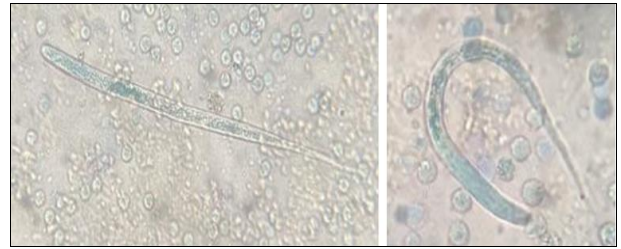


Fig 2: Methylene blue stain (40x)



Fig 3: Giemsa stain (40x)

Ivermectin 200 µg/kg body weight subcutaneous route (orally microfilaricide 50 ug/ kg) is used as a curative drug in present clinical case and animal was responded well with the treatment.

Temperature of buffalo decreased from 104 °F to 101.7 °F within 3 days after treatment. Appetite increased in 3-4 days after treatment. Edema of dependent parts cured in 2 weeks of treatment. Ivermectin is a macrolide antibiotic highly selective for glutamate gated chloride (cl) channels-in invertebrate cells and it increases the permeability of increase via cell membrane leads to hyper polarisation of nerve cells causes flaccid paralysis & death of parasite so it's called as microfilaricidal [2].

Haematological parameters of buffalo includes decreased haemoglobin (6.5 grs /dl), packed cell volume (20%), and total erythrocytic count (4.5×10^6 /ul) were recovered within 1 week after treatment.

Discussion

Microfilariae are thread like nematodes require intermediate host for completion of life cycle morphology of these nematodes consist of cylindrical pharynx with an anterior muscular portion and posterior glandular portion, males have well develop alae and spirally coiled tails sexually mature female worms release pre larval stage microfilaria into blood [6].

Microfilariae enter the buffalo host during a blood meal when the vector, a mosquito, punctures the skin. The infective larvae enter through the wound and migrate to the peripheral lymphatics where they grow to mature male and female worms. They can live there for several years. After mating, the gravid females release sheathed microfilariae into the peripheral blood where they can be detected 8-12 months after the initial infected bite [9]. The mosquito acquires the

infection by ingestion of the microfilaria in the blood meal. The microfilariae lose their sheath on arrival in the stomach of the mosquito due to gastric juices. The larvae migrate to the thoracic muscles and develop into infective larvae over a period of 6-14 days. The larvae then migrate to the mouthparts of the mosquito which infects the host during a blood meal. The blood stages of filariae, microfilarine, vary in the times when they are present in the peripheral blood, corresponding with the peak biting time of the vector. Thus, in nocturnally periodic forms the microfilaria are present in the peripheral blood is increased at night and during the day they reside in the deep tissues, particularly the lungs^[11].

These parasites meet their nutrients requirement from host tissue and fluids causes various deficiency symptoms in host. Significant decrease in albumin levels and increased values of globulins are due to the hepatic cell damage leads to affect the colloids osmotic pressure which contribute to the fluid leakage in dependent parts of body creates edema^[10].

Buffaloes affected by Microfilariasis display clinical signs like fluctuations in temperature, anorexia, increased pulse rate and respiratory rates, dyspnea, sudden drop in milk yield, watery, Mucopurulent nasal discharges, pale mucous membranes, swelling of dependent parts of body, weakness, lameness, paralysis of hind limbs, rough hair coat, nervous signs like head pressing against wall, scratching)

Microfilariasis in buffaloes treated by several drugs includes

- **Levamisole:** nicotinic agonist paralyzes worms by selectively activating nematode nicotinic acetylcholine (Ach) receptors, allowing entry of Na⁺, Ca²⁺, for excessive body muscle contraction, and thus induces paralysis.
- **Diethyl carbamazine:** acts by GABA agonist so prevents neural transmissions in worm causing muscular paralysis and expelling of worms alive
- **Lithium antimony tartarate:** inhibits excitatory neurotransmitters like glutamate and dopamine and promotes GABA mediated neuro transmission

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