

ISSN 1996-3343

Asian Journal of  
**Applied**  
Sciences



## Research Article

# *Strobiloestrus clarkii* in *Redunca redunca* at Dinder National Park

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## Abstract

**Background and Objective:** Myiasis is the infestation by dipterous fly larvae in humans and animals. The larvae can infect living or necrotic tissue involving the skin, nasopharynx, genitourinary and gastrointestinal tracts. **Materials and Methods:** This study was carried out at Dinder National Park. Larvae were collected from skin and subcutaneous tissue of 20 dead or devoured reedbucks. Also 10 reedbucks were randomly captured. The larvae were removed by simple extraction, sorted out depending on stage and preserved in a container filled with 10% formalin until identification. Blood samples were collected for Hb, WBCs and RBCs count and PCV, in addition to albumin, total protein and glucose levels were also determined. **Results:** According to the founded morphological features the species responsible for the current infestation was found to be *Strobiloestrus clarkii*. The WBCs ( $4.800 \pm 1.5$ ), RBCs ( $14.2 \pm 0.65$ ) and Hb ( $57 \pm 0.73$ ) were higher at weaned than adult female and male reedbucks. WBCs value ( $4.500 \pm 0.75$ ) at female was higher than adult male reedbucks ( $2.140 \pm 0.65$ ), while PCV value ( $62 \pm 0.65$ ) at weaned and female reedback ( $62 \pm 0.76$ ) was similar. The average of biochemical parameters were  $6.7 \pm 4.4$  mmol L<sup>-1</sup> for glucose,  $4.5 \pm 1.73$  mg dL<sup>-1</sup> for total protein and  $2.24 \pm 0.65$  mg dL<sup>-1</sup> for albumin. **Conclusion:** *Strobiloestrus clarkii* is a causative agent of myiasis in reedback. A sound decrease of some biochemical parameters was observed namely glucose and total protein. In regard to WBCs count, it was remarkably low, while PCV and Hb values were almost normal.

**Key words:** Myiasis, *Redunca redunca*, dinder national park, *Strobiloestrus clarkii*, larvae

**Citation:** Abubakar Ahmed Saaid, Aisha El Fai Mohammed, A.M. Sara and H.A. Samia, 2019. *Strobiloestrus clarkii* in *Redunca redunca* at Dinder national park. Asian J. Applied Sci., 12: 7-14.

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Myiasis is the infestation with dipterous fly larvae of humans and other mammals, typically livestock. Myiasis is a natural infection of livestock causing a significant burden on this industry. Occasionally, humans exposed to endemic areas suffer from zoonotic fly larvae infestations. The fly larvae feed on living or necrotic host tissue, bodily fluids or ingested food<sup>1</sup>.

There are two species of reedbucks in the Sudan Bohor Reedbucks and mountain reedback (*Redunca fulvovufula*). The Mountain Reedback species occurs in three separate populations in east and southern Africa and in a restricted area of eastern Nigeria and north-central Cameroon<sup>2</sup>. Bohor reedback is Endemic to Africa, ranging north of the forest zone from Senegal, Gambia, N Guinea and SW Mauritania through the woodlands and flood-plain grasslands of the Savanna zone of West Africa through N Cameroon (Adamaoua Plateau northwards), S Chad, the savanna woodlands of the Central African Republic, extreme N and NE DR Congo, C and S Sudan, to W and C Ethiopia and south to L. Tanganyika and the Rovuma R. in Tanzania<sup>2</sup>.

Problem of myiasis is a matter of great concern among medical and veterinary fields. At the same time it is of great economic importance in an agriculture based country like India, where the economic status of a big chunk of the population depends on the livestock industry. Myiasis has long been recognized as a cause of decreased productivity in the livestock industry due to pathological effects and management costs. The need of the hour is to spread awareness among the masses about the actual cause and factors responsible for the occurrence of myiasis, so that this menace can be controlled among domestic animals. The preventive measures like maintenance of neat and clean surroundings control of fly populations and use of screens and covering of wounds can be helpful in protecting livestock animals from myiatic infestations<sup>3</sup>.

New World screwworm, *Cochliomyia hominivorax*, Old World screwworm, *Chrysomya bezziana*, blowflies, *Lucilia sericata* and *L. cuprina* (Calliphoridae) are some of the common flies that cause traumatic/wound myiasis of great medical and veterinary importance. Although myiasis is known to occur in wild animals, not much information is available to describe the clinico-pathology or epidemiology of the infestation especially in African free ranging wildlife<sup>4</sup>.

## MATERIALS AND METHODS

**Area of study:** Dinder National Park is the oldest natural park in northern Sudan. It is located in the southern part of the central region, adjacent to the Ethiopian border with the Blue Nile Province<sup>5</sup>.

**Ethical approve:** The ethical approve document was obtained from the general administration of wildlife, via direct contact from wildlife research center, Khartoum-Sudan.

**Temperature and humidity:** Daily temperature and humidity at DNP were recorded using powerrnix digital temperature and humidity controller. Temperature and humidity during February ranged between 25-30.4°C and 27.1-35.7%, respectively.

**Animals study and the larvae collection:** Larvae were collected from 20 dead animals and 10 captured different age reedback.

**Dead animal's investigation:** Skin and subcutaneous tissues of dead or devoured reedbucks were investigated thoroughly for the presence of dipterous larvae at DNP. Ten reedback *Redunca redunca cottoni* carcasses were examined and the larvae were removed by simple extraction. The collected larvae similar in stage were counted and preserved in separate containers in 10% formalin.

**Observation and capture of reedback (*Redunca redunca*) investigation:** Ten reedback *Redunca redunca cottoni* (6 weaned, 2 adult male their age between 8-10 years and 2 adult females, their age between 4-5 years old) were randomly captured from the park by using net capturing and gun anesthesia. The capturing was done according to an observed swelling on the skin of the reedbucks in through binocular glasses.

The animals were anesthetized with a combination of xylazine and Ketamine to perform the clinical examination. Immediately after capturing animals were examined for the presence of larvae. On observation and the palpation of the skin, pendulant mass of tissue was seen hanging from the back of the animals. Fingers were used to pressure the larvae from the animal skin swelling and then collected in a bottle for identification. The larvae were collected in two types of bottles, one with 70 % ethyl-alcohol and other with 10% formalin.

Two weaned reared under captivity at the premises of the wildlife research center for a period 3-9 months to continue the development of the larvae to the third instars.

### Identification and analysis

**Larval identification:** Larvae were identified under the dissecting microscope for morphological inspection. During microscopic examination, the larvae were identified as the second and third larvae. The collected third mature instars was preserved in 70% alcohol for morphological identification of the anterior spiracles, posterior spiracles and Cephalopharyngeal skeleton.

The anterior and posterior parts of each larva were dissected out using a scalped blade. Anterior part transferred to tube containing 3 mL HCL 38% for 48 h. Then the part placed on a clean slide, covered with cover slip and examined under light microscope at 40X for cephalopharyngeal skeleton identification. The posterior part was examined under light microscope. Larval instars were characterized according to the number of spiracle openings and the presence or absence of anterior spiracles. The cephalopharyngeal skeletons as well as the first and last segments were severed from their structure and spiracle structure for easier observation. The analysis and drawings were performed under the light microscope and digital camera was used. The terminology used for the description of larval instars was that of Lopes<sup>6,7</sup> and McAlpine *et al.*<sup>8</sup>.

**Breeding site pupa:** Dry and wet sand, sand-clay and clay soils were investigated under the laboratory conditions as breeding site for the maggots.

### Hematology and serum analysis

**Blood heamogram:** Blood samples were collected from jugular vein using a 5 mL disposable syringe into clean dry bottles containing the disodium salt of ethylene diamine tetraacetic acid (EDTA) as anti-coagulant for hematological samples which were evaluated on the same day of collection<sup>9</sup>. Blood was analysis for hemoglobin (Hb) concentration, red blood cells (RBC), packed cell volume (PCV) and white blood count was done according to methods described by Turgeon<sup>10</sup>.

**Determination of serum constituent:** Jugular blood samples were collected from the animals after capturing and allowed to clot. Serum was separated by centrifugation (3000 rpm for 5 min) and stored at -20°C until analyzed.

**Blood sugar:** Serum glucose values were determined using enzymatic kit provided by Plasmatic Laboratory Product, Ltd. Glucose level was measured according to the method described by Pospisil *et al.*<sup>11</sup>.

**Total protein:** A commercially available kit (Plasmatec Laboratory Products Ltd., England) was used. Total protein content was determined using Biuret method as described by Weichselbaum<sup>12</sup>.

**Total albumin:** A commercially available kit (Plasmatec Laboratory Products Ltd., England) was used. Total albumin was determined by Bromocresol green method (BCG) as described by Webster<sup>13</sup>.

## RESULTS

**Larval identification:** The anterior part of the third instars has hook and sensory papillae antenna (Fig. 1). The Cephalopharyngeal Skelton in the third instars show mouth hock and intermediate sclerite and pharyngeal sclerite (Fig. 2), The end of the posterior third instar presents a pair of posterior spiracles with two openings each, peritreme closed, 19 slits some finger shape, some v shape, some u shape and some w shape and the button central located (Fig. 3).



Fig. 1: Mouth part of mature third larvae



Fig. 2: Cephalopharyngeal skelton

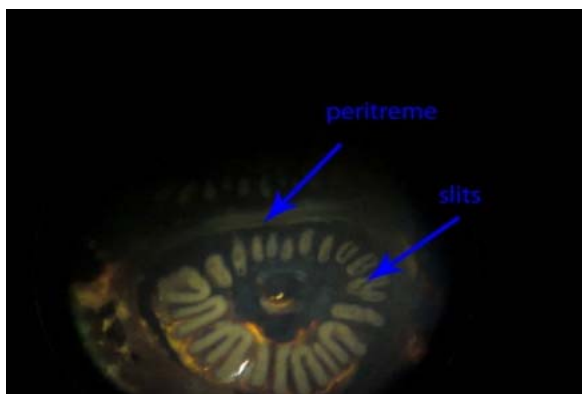


Fig. 3: Posterior spiracles of third instar



Fig. 7: Third instar in boil



Fig. 4: Young second instars abdominal side



Fig. 5: Weaned reed bucks infested



Fig. 6: Old second instar in skin

According to these morphological features the species responsible for the current infestation was found to be *Strobiloestrus clarkii*.

Investigation was performed on the captured reed buck and from fresh dead animals during the period of the study. The diagnoses of diseases and affections were based on clinical signs, isolation and identification of the larvae. The larvae expressed on the animals were identified as being in the early second stage of development (Fig. 4).

It was found that two females and six live weaned reed bucks were infested. Infestation was characterized by the presence of circumscribed, raised nodules in the skin of the upper halves of the shoulder and sides (Fig. 5). Approximately 20-30 nodules were present in the skin of six infested weaned reed bucks but 1-2 in two infested female reed buck, no infestation at two male reed bucks. A small opening was present in the centre of each nodule and single larva could be expressed through this opening. The larvae recovered from this opening. The larvae recovered from the nodules on this occasion were late second stage larvae. Also 20-30 larvae were recovered from the skin of the dead animals (Fig. 6).

Blister like lesion was observed on the skin of the back animals from March-May. The larvae recovered from the subcutaneous tissue and hides of reed buck during October-December. The skin lesion is typically described as resembling a boil (Fig. 7, 8) exuding serous fluid and draining bloody exudates surrounded by an area of tender indurations. A living third instar emerged from the skin lesion during December-January. All larvae drop from the reed buck to the soil. The lesion spontaneously ulcerated and larvae dropped out (Fig. 9). The larvae fall from the skin and ending in the emergence to developing pupa, Table 1 showed the incidences of myiasis in weaned, female and male reed bucks. All weaned captured reed bucks were found infected with the larvae of myiasis. The incidence of the larvae at live weaned reed buck was higher than adult female. No larvae found at adult male, while all the collected skin of dead animals was founded infected with the larvae of myiasis. Higher prevalence of myiasis was recorded at weaned reed buck.



Fig. 8: Bleeding boil skin



Fig. 11: Old second instar



Fig. 9: Third instars drill soil



Fig. 12: Third instar anterior side



Fig. 10: Young second instar abdominal side

**Larvae morphology**

**Young second instars larvae:** The color of the young second instars larvae was white ivory. The body lengths were 7-12 mm and have lobular protrusions long on the sixth segment (Fig. 10).

**Old second instars larvae:** The color of the second old instars was white, the body length 12-18 mm and the protrusions was stouter and become gradually shorter (Fig. 11).

Table 1: Incidences of myiasis in weaned, female and male reed buck

Incidences animals examined	Number of animal	Incidences percent	Larvae a count in skin
Live weaned	6	33	20-30
Live female mature	2	11	1-2
Live males mature	2	0	0
Dead animal (skin)	10	56	20-30

**Young third instars larvae:** The color was yellow-white, the body length 18-22 mm. The larvae have patches of the scales. The anterior and posterior segments have about 10-12 patches, two or three patches united (Fig. 12).

**Pupa and parasitism for pupa in sit:** The mature larvae after burrowing into the soil undergo a process of stage during which the cuticle of the larva becomes heavily sclerotised, occurred pupal parasitism for pupa by the larvae that after 15 days buffer drilling (Fig. 13).

**Hematology and serum chemistry of infected reedbucks:**

Table 2-5, showed the hematological values of weaned, female and male and differential account of weaned reed buck. Values of WBCs ( $4.800 \pm 1.5$ ), RBCs ( $14.2 \pm 0.65$ ) and

Table 2: Blood hematological values and differential a count of reed buck

Parameters	Weaned	Female	Male
WBCs (cell mm <sup>-3</sup> )	4.800±1.5	4.500±0.75	2.140±0.65
RBCs (cell mm <sup>-6</sup> )	14.2±0.65	13.900±0.73	13.900±0.85
Hb (%)	57.0±0.73	55.000±0.72	52.000±0.65
PCV (%)	62.0±0.65	62.000±0.76	60.000±0.75

Table 3: Blood serum biochemical values of infected weaned reedbuck

Parameter	Glucose (mmol L <sup>-1</sup> )	Total protein (mg dL <sup>-1</sup> )	Albumin (mg dL <sup>-1</sup> )
Average	6.72±4.4	4.5±1.73	2.24±0.65

Table 4: Average temperature in DNP during February

Days	Time							Total	Average
	7 am	9 am	12 pm	3 pm	7 pm	9 pm	12 pm		
15	18.00	23.00	35.00	38.00	34	30.00	29.00	207	29.57
16	19.00	25.00	34.00	40.00	27	25.00	22.00	192	27.42
17	19.00	27.00	31.00	34.00	32	27.00	25.00	195	27.85
18	19.00	28.00	36.00	39.00	33	30.00	28.00	213	30.42
19	20.00	30.00	38.00	34.00	34	27.00	25.00	208	29.71
20	17.00	24.00	35.00	43.00	30	28.00	25.00	202	28.85
22	19.00	27.00	35.00	37.00	31	30.00	28.00	207	29.57
23	15.00	24.00	30.00	32.00	28	24.00	22.00	175	25.00
24	17.00	21.00	30.00	32.00	30	29.00	29.00	188	26.58
Total	163.00	229.00	304.00	329.00	297	250.00	233.00	1787	
Average	18.11	25.44	33.77	36.55	31	27.77	25.88		28.36

Table 5: Average humidity (%) in DNP during February

Days	Time							Total	Average
	7am	9am	12pm	3pm	7pm	9pm	12pm		
15	65.00	48.00	18.00	12.00	22.00	25.00	38	228	32.57
16	66.00	39.00	19.00	9.00	27.00	35.00	55	250	35.71
17	66.00	34.00	20.00	18.00	27.00	27.00	45	237	33.85
18	64.00	39.00	18.00	12.00	28.00	30.00	39	230	32.85
19	49.00	24.00	20.00	22.00	22.00	30.00	45	212	30.28
20	55.00	30.00	12.00	9.00	22.00	23.00	45	196	28.00
21	45.00	22.00	19.00	14.00	20.00	21.00	49	190	27.14
22	53.00	36.00	18.00	17.00	28.00	31.00	55	238	34.00
23	55.00	45.00	19.00	17.00	22.00	22.00	25	205	29.28
Total	518.00	317.00	163.00	130.00	218.00	244.00	396	1986	
Average	57.55	35.22	18.11	14.44	24.22	27.11	44		31.52

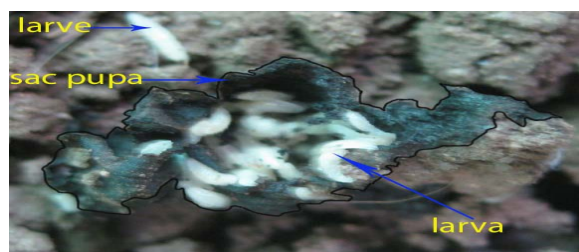


Fig. 13: Parasitism for pupa

Hb (57±0.73) were higher at weaned than adult female and male reed buck. The WBCs value (4.500±0.75) at female is higher than adult male reed buck (2.140± 0.65) while PCV value (62±0.65) at weaned and female reed buck (62±0.76) was similar.

## DISCUSSION

There are very few of data in *Strobiloestrus clarkii* as a causative agent of myiasis in reedbuck. *Strobiloestrus* sp. causing myiasis in the Sudan Bohor Reed-buck *Redunca redunca cottoni*<sup>14</sup>.

The present study is the first morphological and life cycle report of myiasis in reed buck (*Redunca redunca*) caused by *Strobiloestrus clarkii* at Dinder National Park. The result is in agreement with Zumpt<sup>15</sup>, who found *Strobiloestrus clarkii* in reedbuck that similar and supports this study. The genus *Strobiloestrus* and their larvae are found in the skin and subcutaneous tissue of reed buck<sup>15</sup>. El Bihari and Osman<sup>16</sup> reported *Strobiloestrus clarkii* from reedbuck in Khartoum zoo.

The current work studied the life cycle from March-May reared reedbucks showed developed swelling in the skin and the second instars young was found. From October-December, the second instars old was found. While the third instars was observed during the period of time between 25th December to 25th January. By February the reedback freed all the larvae, which emerged to pupate during February (winter). These observations are in agreement with, Zumpt (15). -with regard to the weather difference between northern and southern equatorial line- who described the life cycle. Zumpt reported that; the maggots becomes mature in October and the flies are on the wing in November, one or two generation in one year indicates that is the *Strobiloestrus* sp. flies probably attach their eggs to hair of the vaal rhebok during September and October (spring) and 1st stage larvae are found in the subcutaneous tissue of back during December and the 2nd stage during December and by February all are in this stage of development. The 2nd stage larvae moult to the 3rd stage during May and June (autumn and winter) the animal is free of infestation by August (winter) only 1 life cycle a year<sup>17</sup>. Another supportive study, one life cycle one year when to see the life cycle in the South Africa is differ from the south equator but the same free larvae observed on animals in winter. In the present study reported high prevalence in weaned animals than adult reedback, this observation agrees with the observation of Stafford<sup>18</sup> and Giterson *et al.*<sup>19</sup>, who suggested, that age has an influence on parasitic burdens in lechwe.

The hematological results obtained from this result was in agreement with what Ahmed *et al.*<sup>20</sup> reported, with slight high hemoglobin level observed in our current study.

There is shortage in information in regard to the biochemical profile in wildlife. In this study the values of total protein, albumin and glucose were determined. The extracted results showed almost similar result of glucose level in reedback reported by Vahala *et al.*<sup>21</sup>, while the total protein level was remarkably lower compared to the same reporter, this difference could be attributed to different kinds of reedbucks which subjected to study.

## CONCLUSION

This study investigated the *Strobiloestrus* spp. in wildlife (Reedback), in Sudan (Dinder National Park). The observation and examination of the randomly captured animals and the dead ones, had lead to a solid conclusion, that reedback at DNP is a host of the *Strobiloestrus clarkii*. The

tracking of the life cycle showed that the larvae remained about ten months in subcutaneous tissues of the host and the animal was free of larvae by February.

## ACKNOWLEDGMENTS

I thank my God firstly and finally for giving me the strength to accomplish this study. My special thanks to my supervisor Dr. Aisha Elfaki head of Wildlife Research Center, Animal Resources Research Corporation, Ministry of Animal Resources and Fisheries for help and advice. My thanks are extended to Prof M.J. Hall Department of Entomology, The National Museum UK. Special thanks to Prof. Ivan Horal, Department of Veterinary Tropical Diseases in University of Pretoria, South Africa. I would like express my deep thanks to Prof. Ahmed Hussain manager in the Veterinary Research Institute, Animal Resources Research Corporation, Ministry of Animal Resources and Fisheries. Special thanks to Prof. Shawgi Mohamed Hassan, Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum.

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