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*Full Length Research Paper*

# *In-vitro* efficacy evaluation of amitraz 0.025% and diazinon 0.06% against *Rhipicephalus pulchellus* and *Amblyomma gemma* in Borena pastoral area, Southern rangeland of Ethiopia

**Eyob Eshetu[1], Getachew Dinede[2], Matios Lakew[2] and Tadele Tolosa[3]***

[1]Department of Parasitology, Yabello Regional Veterinary Laboratory, Oromia Regional State, Yabello, Ethiopia.
[2]Oromia Pastoral Areas Technical and Vocational Education and Training College, Oromia Regional State, Yabello, Ethiopia.
[3]Department of Veterinary Public Health and Veterinary Microbiology, Jimma University College of Agriculture and Veterinary Medicine, Jimma, Ethiopia.

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**This study was conducted in Borena pastoral area, southern rangeland of Ethiopia to determine the *in vitro* efficacy of amitraz 0.025% and diazinon 0.06% against *Rhipicephalus pulchellus* and *Amblyomma gemma* using modified adult immersion. A total of 180 engorged adult female ticks (ninety ticks of each *A. gemma* and *R. pulchellus* species) were immersed in amitraz 0.025% or diazinon 0.06% at field recommended concentration (treated groups), or in distilled water (control groups) for 10 min and then incubated at 27 ± 1°C for 7 days. The oviposition response of each tick species in both groups was followed. The mean mass of eggs laid by each *A. gemma* and *R. pulchellus* in the treated group and those of untreated groups was compared to estimate the efficacy of each tested acaricide. Amitraz 0.025% has significantly higher (P < 0.05) in overall mean percent oviposition control (C% = 95.47) of *A. gemma* and *R. pulchellus* than diazinon 0.06% (C% = 80.9). At this recommended concentration, both amitraz 0.025% and diazinon 0.06% were less efficient in *R. pulchellus* oviposition control (C%= 90.94 for amitraz and C% = 71.41 for diazinon) than *A. gemma* oviposition control (C% = 100 for amitraz and C% = 88.85 for diazinon). The results of the study suggested that amitraz at field recommended concentration provides better efficient *R. pulchellus* and *A. gemma* oviposition inhibition than diazinon.**

**Key words:** Amitraz, diazinon, Borena pastoral area, cattle, efficacy, *in vitro* testing, *Amblyomma*, *Rhipicephalus.*

## INTRODUCTION

In tropical Africa, tick and tick borne diseases (TBDs) are economically very important diseases next to trypanosomosis (Belew and Mekonnen, 2011). Among 60 tick species found infesting both domestic and wild animal of Ethiopia, 30 species have been widespread and are important parasites of livestock (Solomon et al.,

*Corresponding author. E-mail: tadeletolos@yahoo.com. Tel: ++251 917 804070. Fax: ++251 471 110934

2004), and causes significant economic losses to the livestock industry. The economic losses incurred from downgrading of hides and skins are enormous; its export yields foreign earnings of the country, second only to coffee (Sileshi et al., 2001).

*Rhipicephalus pulchellus (R. pulchellus)* and *Amblyomma gemma (A. gemma)* are the predominant species in arid and drier land (Walker et al., 2000). Similarly, their predominance is reported in the arid and drier land of Borena pastoral area (Solomon and Kaaya, 1998; Regassa, 2001; Solomon and Kaaya, 2001).

The currently available tools for tick control consists of acaricides relying on treatment with different application methods and/or formulations, tick resistant animals, tick vaccines, TBD vaccines and management interventions. The successful implementation of rational and sustainable tick control programme in grazing animals is dependent upon a sound knowledge of the ecology or epidemiology of the parasite as it interacts with the host in specific climatic, management and production environments (Alanr, 2011). Nevertheless, not all developing countries and those in transition may have such information available, due to a lack of human, economic and infrastructural resources (Food and Agriculture Organization (FAO), 2004).

In most situations, however, efficient and reliable control of ticks and TBDs are still based primarily on intensive use of acaricides, often without the local understanding of those responsible factors for tick distribution dynamics (Brito et al., 2011). Since acaricide introduction in South Africa around 1890, tick treatment relying on different application methods have been the main method of tick control in Africa, leading to numerous problems; environmental pollution, development of resistant tick strains and escalating costs (Alanr, 2011; Brito et al., 2011). To alleviate these problems, the most frequently used techniques to detect resistance in cattle tick are: the adult immersion test (AIT), larval packet test (LPT), and larval immersion test (LIT) (Castro-Janer et al., 2009). However, for the success of any tick management strategy, it is necessary to use a test that is practical, quick, economical and reliable to detect presence of resistance in target population (FAO, 2004). Enthusiasm for the development of standard protocols for modified AITs has been driven by the ease with which the test can be done, lack of any special equipment, and the fact that the test can be completed within only 7 days and this test mimicked the field condition better than the Shaw Larval Immersion Test (SLIT) (Jonsson et al., 2007).

Likewise, in Ethiopia, over the past decades ticks are mainly controlled by using variety of acaricides; including organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids (Sileshi, 2001; Yilma et al., 2001). However, with the most widespread, under or over concentration and frequent use of organochlorines and organophosphates compounds, ticks are likely to develop resistance in Ethiopia (Adamu, 1996; Yilma et al., 2001; Sileshi et al., 2004). In Borena pastoral areas, where amitraz 0.025% and diazinon 0.06% were mostly used, in various circumstances, animal health personnels and livestock herders complained of failure of this two acaricides to kill ticks and toxicity associated with diazinon usage (Borena Zone Pastoral Area Development Office (BZPADO), 2009, 2010). Therefore, continuous studies on dynamics of tick population (Alanr, 2011) with the efficacy status of acaricides against the most abundant and important tick in particular area are necessary to carry out efficient tick control and/or tick burden reduction (Solomon, 2001). The objectives of this study were to evaluate the efficacy of amitraz 0.025% and diazinon 0.06% against field population of engorged adult female *R. pulchilus* and *A. gemma* ticks under *in vitro* condition using AIT.

## MATERIALS AND METHODS

### Characterizations of study area

The study was conducted between March and April, 2012 at Borena pastoral area of Oromia regional state, Southern rangeland of Ethiopia, located at 565 km south of Addis Ababa, the capital city of Ethiopia. The region has predominantly a semi-arid climate, and physiographically, it is dominated by Savannah vegetation. Animal husbandry in the area is characterized by extensive livestock productions system and seasonal mobility. Cattles are the dominant livestock species (Cooperative for Assistance and Relief Everywhere (CARE), 2009). Cattle are heavily infested with *R. pulchellus* and *A. gemma*. Acaricide application is the main tick control methods in the area and their application seems to be regulated primarily by their availability. Cattle are treated for tick infestation whenever the farmers bring their animals to the veterinary clinics for other complaints; otherwise the pastoralists are treating by themselves. The organophosphates (diazinon 0.06% and amitraz 0.025%) are the two acaricides used by the pastoralist communities in the area to control ticks.

### Study methodology and procedures

The fully engorged adult female ticks were collected from 17 different cattle herds at Borena pastoral areas, located roughly between 3 to 5°N and 37 to 42°E, covering an area of 120,000 $km^2$. The herds were selected on the history basis of communities' complaints on acaricides failure. From each herd, 6 or 8 cattle, based on their infestation level with engorged adult female ticks, a total of 134 cattle, were selected. At each collecting site, cattle were restrained, and a maximum of five adult engorged female ticks of any species (as it is difficult to identify the tick species at collection site) were collected from the sampled cattle (Ducoenez, 2005). The ticks were placed in labeled plastic flasks with small holes and free of acaricide.

The choice of acaricides used was based on their commercial availability and patronage by farmers and veterinary clinic in Borena pastoral area. Thus, amitraz 0.025% was manufactured by Laboratorios Microsules Uruguay S.A. and diazinon 0.06% EC was

**Table 1.** Mean oviposition of adult A. gemma and R. pulchellus after immersion in amitraz 0.025% and diazinon 0.06% EC at recommended concentration and 7 day incubation.

| Treated ticks | Treatment | N | $M_1$ | S | $M_2$ | C% |
|---|---|---|---|---|---|---|
| *A. gemma* | Amitraz 0.025% | 10 | 2.63 | 0 | 0.000 | 100.00 |
| | Water control | 10 | 2.45 | 8.67 | 0.333 | 0.000 |
| *R. pulchellus* | Amitraz 0.025% | 10 | 2.83 | 0.67 | 0.033 | 90.94 |
| | Water control | 10 | 2.75 | 9.67 | 0.373 | 0.00 |
| *A.gemma* | Diazinon 0.06% EC | 10 | 2.56 | 1.0 | 0.037 | 88.85 |
| | Water control | 10 | 2.45 | 8.67 | 0.333 | 0.000 |
| *R. pulchellus* | Diazinon 0.06% EC | 10 | 2.79 | 1.33 | 0.12 | 71.41 |
| | Water control | 10 | 2.75 | 9.67 | 0.373 | 0.00 |

N = Number of immersed female ticks; M1 = engorgement weight (g); S = number of tick survived after 7 days incubation; $M_2$ = average egg mass per treatment group (g).

manufactured by Shandong Luxi Animal Medicine Share Co. Ltd (China). The reconstitution of acaricides was done incognizance to the manufactures recommended concentrations to be used on infested animals using distilled water. The indicated concentration for diazinon is 0.06% while that of amitraz is 0.025%. The formula, $V_1C_1 = V_2C_2$ was used to prepare the concentration of acaricides, where $V_1$ and $V_2$ are the volume of the acaricide to be drawn from the stock product and the final volume after reconstitution, respectively, $C_1$ and $C_2$ are the stock product concentration and the required final concentration after preparation, respectively. For all the preparations, the final volume was 1000 ml.

The bioassay technique used was the modified AIT, a laboratory protocol first described by Drummond et al. (1973) and modified by FAO (2004). At the laboratory, from the total collected engorged adult female ticks, a sample comprising of 180 ticks (ninety ticks of each *R. pulchellus* and *A. gemma*) with no signs of injury was selected. Both species were carefully washed on a sieve using a clean tap water and dried at room temperature on absorbent paper and each species divided into groups according to their size. Three groups of ten ticks of each species were randomly formed (Group I for amitraz, Group II for diazinon and Group III for control/water), and all ticks were pasted onto double sided adhesive strips on glass petridish, with their ventral sides facing up wards. This set up was then covered and incubated in a dessicator maintained at 72% relative humidity (RH) and temperature of 27°C. Three replicates were used for each acaricide and species of tick as described.

The efficacies of the acaricides were evaluated using the egg laying test (ELT). Thus, the overall mean efficacy was then estimated from all the three replica of trials. ELT method involves the comparison of the egg mass of ticks treated with acaricide and the egg mass of untreated ticks, and finally estimates the percentage control value, using the following formula:

$$\text{Percent control} = \frac{\text{MEC - MET}}{\text{MEC}} \times 100$$

Where, MEC and MET are mass of eggs laid by control ticks and treated ticks, respectively.

**Data management and analysis**

All collected data were entered into Microsoft Excel 2007 computer program. All statistical analyses were performed using statistical package for social science (SPSS)-Version 19 for windows 2007. Percent control (C%) for each acaricide, obtained with ELT, was used to evaluate effectiveness. Independent sample t-test was used to observe the mean C% difference between the two acaricides. A P-value less than 0.05 at 95% confidence intervals was considered for significance.

## RESULTS

The oviposition response of *A. gemma* and *R. pulchellus*, after immersion in amitraz 0.025% and diazinon 0.06% in three replicates was determined and presented on (Table 1). In the trial, none of *A. gemma* treated with amitraz laid eggs, while few of the treated *R. pulchellus* laid small batch of eggs with mean weight of 0.033 g. In contrast, both *A. gemma* and *R. pulchellus* immersed in diazinon 0.06% laid eggs with mean weight of 0.037 and 0.12 g, respectively. However, there was no statistical significance variation ($P > 0.05$) between the two acaricides in the overall oviposition control of each tick species (Table 2). Both tick species in the control group laid relatively large batch of eggs with mean weight of 0.33 g by *A. gemma* and 0.373 g by *R. pulchellus*.

The overall mean C% of amitraz 0.025% and diazinon 0.06%, and their respective standard deviations as well as their minimum and maximum mean efficacy during the three replica of the trial is presented in Table 3. Therefore, although, amitraz showed evidence of greatest effect on oviposition on *A. gemma*, the statistical comparison between the overall mean C% of each acaricide revealed no significant differences ($t = 2.438$, $df = 4$, 95% confidence interval (CI) = -1.5498 to 23.8498, $P > 0.05$). Moreover, the overall mean C% analysis of the two acaricides on the inhibition of *R. pulchellus* oviposition also showed no statistical significant variation ($t = 2.26$, $df = 4$, 95% CI = -4.48 to 43.71, $P > 0.05$).

**Table 2.** T-test analysis of mean percent *A. gemma* and *R. pulchellus* oviposition control between amitraz 0.025% and diazinon 0.06% EC at recommended concentration.

| Ticks | Acaricide | C% | N | Mean | SD | t-value | df | 95% CI | S |
|---|---|---|---|---|---|---|---|---|---|
| *A.gemma* | Amitraz | 100 | 3 | 100.0 | 0.0 | 2.438 | 4 | -1.55-23.85 | NS |
| | Diazinon | 88.85 | 3 | 88.85 | 7.9 | | | | |
| *R. pulchellus* | Amitraz | 90.94 | 3 | 90.94 | 11.3 | 2.26 | 4 | -4.48-43.71 | NS |
| | Diazinon | 71.41 | 3 | 71.33 | 9.9 | | | | |

C% = Percent control; N = number of trails; SD = standard deviation; NS = not significant; df = difference.

**Table 3.** Overall mean percent oviposition control of amitraz 0.025% and diazinon 0.06% EC at field recommended concentration against adult female *A. gemma and R. pulchellus*

| Acaricide | Min. efficacy (%) | Max. efficacy (%) | Mean efficacy (±SD%) |
|---|---|---|---|
| Amitraz 0.025% | 78.38 | 100 | 95.47±8.663 |
| Diazinon 0.06% EC | 61.11 | 97.06 | 80.09±12.537 |

Min = minimuim, Max = maximum.

## DISCUSSION

Several authors have studied the efficacy of amitraz on different tick populations using AIT. The results have shown different susceptibility levels. In most of the studies, amitraz revealed high degree of tickicidal efficacy that was agreed with the present finding. Similarly, a closely comparable finding, C% of 98 to 100, was reported by Sileshi (2001) at Sebeta, Ethiopia. In South Africa, Sileshi et al. (2002) also reported 100% efficacy for amitraz at the same dilution rate. Moreover, Souza et al. (2003) in Southeast Brazil obtained mean amitraz efficacy of above 95%. The minimum mean efficacy of amitraz observed in the present study (78.38%) is in accordance with that of Mendes et al. (2001) finding and he found an average efficacy of 77.44%.

In contrast, Furlong et al. (2007) found mean efficacy of 47.9% for amitraz. Camillo et al. (2009) also observed the presence of resistance with a low efficacy of Amitraz in some tick populations of RioGrande do Sul State, Brazil. In Northeast region of Brazil, a low efficacy of amitraz, with a control of 40.5 and 30.95% was reported by Santana (2000) and Campos and Oliveira (2005), respectively. In our case, we can assume that the tick populations were susceptible to amitraz.

The efficacy variation between the two presently tested acaricides might be associated with the high sterilization effect of amitraz compared to diazinon when applied at field recommended concentration (Sileshi et al., 2003). The oviposition response variation of each tick species is also most probably associated with prior exposure of these tick species to diazinon, as its usage began over the past 10 years in the study area. Sileshi et al. (2003) in South Africa observed relatively higher level of resistance to diazinon than amitraz.

The use of an acaricide at incorrect concentration is also one of the prime factors which affect the efficacy of an acaricide and causes of tick control failure (Natala et al., 2005; Kirby, 2010; Alanr, 2011). It has been observed during the study period that the pastoralists in the area believed that they needed to increase the concentration of acaricides during the peak tick season to control the excessive tick burdens infesting their cattle. This type of increased acaricide concentration can lead to a higher selection pressure for tick resistance (Brito et al., 2011; Pegram et al., 2000). In the present study, the two acaricides have conserved their efficacy on both tested tick species. However, a right tick control management needs to be pursued in order to avoid any resistance.

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*Full Length Research Paper*

# Cryptosporidium oocysts in *Anodonta* sp. (bivalve mollusc) as indicators of pollution of Tiga Lake ecosystem in Kano State, Nigeria

**Ebere V. Ugwoke[1], Jarlath U. Umoh[1]*, Emmanuel C. Okolocha[1] and Idris A. Lawal[2]**

[1]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[2]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

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**Bivalve molluscs are filter feeders and can bioaccumulate oocysts of Cryptosporidium. Tiga Lake in Kano State Nigeria is used for recreational, domestic and agricultural purposes by humans and also serves as a source of drinking water for animals. Bivalve molluscs from the lake are consumed by people. This study was conducted to assess the occurrence of *Cryptosporidium* spp. in Tiga Lake using edible *Anodonta* sp., (fresh water mussels) a bivalve mollusc as sentinel. The samples were examined using modified acid fast staining technique and micrometery of the oocysts. 169 and 150 samples of the molluscs were collected from two locations namely Tashan Idi and Rurum, respectively. The organs examined from each of the molluscs were the gastrointestinal tracts (GIT), gills and haemolymph. The mean oocysts load was higher in the GIT (192.50 ± 173.03) than in the other organs, although the difference was not statistically significant (P > 0.05). Of the two sampling sites, 60 (35.50%) and 40 (26.67%) of the molluscs from Tashan Idi and Rurum respectively were positive for the oocysts. However, the difference was not statistically significant (P > 0.05).The micrometry of the oocysts showed that most of them fell within the size range of 4.0 to 4.3 µm and 4.4 to 4.7 µm suggesting that the oocysts encountered in this work might be those of *Cryptosporidium parvum* and *C. meleagridis* which infect a wide range of animals and also humans. The result of this study reveals that 27.59% of the bivalve molluscs harboured Cryptosporidium oocysts and this may have public health implications if undercooked molluscs are consumed by humans.**

**Key words:** Cryptosporidium, bivalve molluscs, Tiga Lake, bioaccumulate, public health.

## INTRODUCTION

Cryptosporidium is a water-borne zoonotic coccidian protozoan parasite. It invades and then replicates within the microvillus region of epithelial cells, lining the digestive and respiratory organs of vertebrates (Nimri and Hijazi, 1994; Fayer et al., 1999; Xiao et al., 2002). Cryptosporidium is transmitted faeco – orally by ingestion of the sporulated oocysts, and most human cryptosporidiosis outbreaks have been associated with water-borne routes of transmission (Solo-Gabriele and Neumeister, 1996). Cryptosporidium parasites are endemic in many domestic and wild life populations, with young animals often shedding over a million oocysts during initial infection. The infective dose of *C. parvum* in humans can be as low as 10 to 100 oocysts (Fayer et al., 1998). The oocyst stages of *Cryptosporidium* spp. are shed in the faeces of animals and humans which may then enter sewage facilities via agricultural runoffs, wastewater discharges and faecal contamination by wild-

*Corresponding author. E-mail: juumoh@yahoo.com. Tel: 08037866550.

life or persist for a long time in the environment. These oocysts can contaminate surface water, water used for drinking, recreational activities and shellfish production (Graczyk et al., 1997). This can pose a serious public health problem especially on aquatic life intended for human consumption. Environmental monitoring for *Cryptosporidium* spp. Can be problematic, partly because of the dilution effect that occurs as oocysts are disseminated from terrestrial to aquatic ecosystems, and also because particulate matter can inhibit or interfere with Cryptosporidium detection methods (Feng et al., 2003). Filter-feeding invertebrates such as bivalve molluscs, which can filter over 2 L of water/h/shellfish, can act as a natural concentration system (McMahon, 1991). These bivalves can then be collected and tested for pathogens, providing an indication of water quality (Freire-Santos et al., 2000; Graczyk, 2003; Miller et al., 2005; Tamburrini and Pozio, 1999).

Bivalve molluscs belong to the Phylum Mollusca, Class Bivalvia, Order Unionoida, Family Unionidae and Genus *Anodonta* (Nedeau et al., 2005). They use their gills to capture particulate food from the water. The water current enters the shell from the posterior ventral surface of the animal and then passes upward through the gills and the filtered particles are transported to the mouth. Food entering the mouth is passed to the stomach via cilliary action. Oocysts can be removed by bivalve molluscs from contaminated water and retained on their gills and haemolymph (Freire-Santos et al., 2000; Giangaspero et al., 2007). An approximation of the parasite load of shellfish contaminated naturally indicated that each shellfish could transport more than $10^3$ oocysts (Fayer et al., 1999). Numerous edible bivalve species live burrowed in sand or mud and respire by means of siphons which reach to the surface of water. They are eaten as a delicacy in many parts of the world and are also served in place of red meat (www.wikipedia.org.seafood, 1999; Gomez-Bautista et al., 2000). In developing countries, cryptosporidiosis occurs mostly in children younger than 5 years, with peak occurrence of infections and diarrhoea in children younger than 2 years. Fatalities of this disease occur primarily among patients with Acquired Immune Deficiency Syndrome (AIDS) or other system disorders (Bern et al., 2000; Fox and Lytle, 1996; Levy et al., 1997).

Tiga Lake of Kano State, Nigeria, receives agricultural run-offs and is used by livestock and wildlife. These may bring Cryptosporidium into the lake. The lake also has a lot of molluscs that are harvested for human consumption.

### Statement of the problem

In Nigeria, there is paucity of literature on the status and prevalence of Cryptosporidium infection from bivalve molluscs. Globally, there is an increase in sourcing of food especially protein and consumption of less cholesterol meat. Varying consumer vogue like roasting, frying and undercooking of molluscs to retain natural taste; pre-contamination of infected molluscs with *Cryptosporidium* oocysts on hands of the handlers and cooking utensils can pose a risk for food borne transmission of Cryptosporidium from bivalve molluscs intended for human consumption (Doyle, 2003).

The paper assessed the occurrence of Cryptosporidium oocysts in Tiga Lake of Kano State, Nigeria using *Anodonta* sp. as sentinel to determine: the occurrence of Cryptosporidium oocysts in *Anodonta* sp., the load of Cryptosporidium oocysts in *Anodonta* sp., and the sites where Cryptosporidium oocysts are mostly found in the molluscs (gills, haemolymph and gastrointestinal tract).

## METHODOLOGY

Tiga lake is situated in Kano State between Latitude 11° 151' to 11° 29' North and Longitude 8° 16' and 8° 38' East. It is a man-made lake in Northern Nigeria with a surface area of 17,806 hectares and was the second largest man-made lake before the creation of the Jebba and Shiroro Reservoirs in 1983 in Nigeria (Ita et al., 1983; Galkowski and Galkowski, 1980). The lake was formed primarily for irrigation purposes with hydroelectric power generation as a secondary consideration and with recreation and conservation as other objectives. Other activities include, cattle grazing and supply of drinking water and human activities including washing, bathing, swimming and fishing (Galkowski and Galkowski, 1980). Sampling sites in the lake was Tashan Idi and Rurum which are tributaries of the lake. Bivalve molluscs are some of the fauna found in the lake.

A total of 319 *Anodonta* sp. were collected by hand picking between the periods of December 2009 through April 2010 by convenience random sampling from the two sampling sites in Tiga Lake (169 molluscs from Tashan Idi and 150 from Rurum). Fifty samples were collected weekly. Each collection was kept on ice and transported to the Parasitic Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, Nigeria immediately for examination. The anterior and posterior muscles of each mollusc were cut with a scalpel inserted gently below the anterior and posterior ends of the molluscs (Graczyk et al., 1997; Rowett, 1970). The shell was opened. The haemolymph, approximately 0.5 ml, was collected with a pipette from the adductor muscle into a clean well labeled test tube. The gills and GIT were excised from each bivalve and each of these organs was placed individually in 2 ml of phosphate buffered saline (PBS) pH 7.4 in an appropriately labeled test tube, washed and left for 30 min. The organs were then vortexed using a vorter mixer and removed. A drop of the sediment from each mollusc was carefully smeared on a properly labeled clean glass slide. The slides were air-dried overnight and stained using modified acid fast staining technique. Oocysts from the positive samples were further measured with a micrometer.

The modified acid fast staining technique was conducted as Nielson and Ward (1999). The prepared slides were examined by covering about 8 to 12 fields with a binocular microscope using x40 objective under a bright field.Measurement of the oocysts from the positive samples was estimated by using a calibrated microscope, knowing the size of Cryptosporidium oocysts to be between 4 to 6 µm (Sréter et al., 2000). A calibrated eye piece was inserted into the micrometer; the slide containing the oocysts was placed on the stage of the microscope and focused while viewing from the eye piece. The oocyst was moved towards the calibration with help of the adjustment knob of the microscope until the calibration was in alignment with the oocyst. The zero point was placed at the tip of the oocysts and measurement was taken from one end of the

**Table 1.** Frequency of *Cryptosporidium* oocyst and the Mean oocyst load in the GIT, gills and haemolymph of *Anodonta* spp in Tiga Lake of Kano State, Nigeria.

| Parameter | Number of oocysts examined | Number positive | Percentage positive (%) | Mean | Standard deviation | Lower bound | Upper bound |
|---|---|---|---|---|---|---|---|
| GIT | 319 | 40 | 12.54 | 92.50 | ±173.03 | 137.16 | 247.84 |
| Gills | 319 | 31 | 9.72 | 67.74 | ±132.63 | 119.09 | 216.40 |
| Haemolymph | 319 | 29 | 9.02 | 41.38 | ±111.86 | 98.83 | 183.93 |
| Total | 957 | 100 | 31.28 | 70.00 | ±145.30 | 141.17 | 198.83 |

95% confidence interval for mean.

**Table 2.** Single and mixed organ contamination with *Cryptosporidium* oocysts in *Anodonta* spp in Tiga Lake of Kano State, Nigeria.

| Parameter | Number positive | Percentage (%) |
|---|---|---|
| Single organ contamination | - | - |
| GIT only | 33 | 10.35 |
| Gills only | 25 | 7.83 |
| Haemolymph only | 20 | 6.27 |
| Double organ contamination | - | - |
| Gills and Haemolymph | 2 | 0.63 |
| Gills and GIT | 2 | 0.63 |
| Haemolymph and GIT | 4 | 1.25 |
| Triple organ contamination | 2 | 0.63 |
| Total | 88 | 27.56 |

oocyst to the other end. Each calibration represented a micrometer.

This was done in the Helminthology Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, NigeriaOne drop (approximately 0.02 ml) of the prepared sediment was placed on a clean slide. An approximate estimate of the oocysts was calculated as follows: Total volume of solution divided by 0.02 ml multiplied by number of oocysts present in a drop of the sample expressed in mills; as modified by Mc master oocysts counting technique (Soulsby, 1968).

The oocysts from the samples of the gills, GIT and haemolymph were enumerated separately to determine the concentration and where the oocysts are trapped most, in the different organs.One-way analysis of variance was used to compare the mean number of oocysts detected in the different organs. The t test was used to determine the difference in mean oocysts load according to the site of collection and values of $P < 0.05$ were considered significant.

## RESULTS

The overall infection rate of 319 *Anodonta* sp. obtained from Tiga Lake of Kano State was 88 (27.59%). Table 1 shows that 100 of the 957 organs of the molluscs examined were infected with Cryptosporidium oocysts. Out of 319 gastrointestinal tracts (GIT) of molluscs examined, 40 (12.54%) were positive. Thirty one (9.72%) of 319 gill samples were positive while 29 (9.02%) of the 319 samples of haemolymph examined were positive for *Cryptosporidium* oocysts.The contamination rate in the GIT (12.54%) was higher than in the other organs (Table 1). However, the difference was not statistically significant ($P > 0.05$). The mean oocyst load was higher in GIT (192.50) than in any of the organs, though the difference was not statistically significant ($P > 0.05$) (Table 1). Table 2 shows that the single organ contamination with Cryptosporidium oocysts was morecommon than the multiple organ contamination.Out of 169 samples of the *Anodonta* sp. from Tashan Idi examined, 60 (35.50%) were contaminated with Cryptosporidium oocysts, and of the 150 samples of molluscs collected from Rurum, 40 (26.67%) was contaminated with the oocysts. The difference in contami-nation rate between the two locations (Tashan Idi and Rurum) was not statistically significant ($P > 0.05$) (Table3). Oocysts with the size range of 4.0 to 4.3 µm were more in number 35 (45.45%), followed by 17 (22.08%) oocyst that fell in the range of 4.4 to 4.7 µm (Table 4).

## DISCUSSION

The presence of Cryptosporidium oocysts in the bivalve molluscs examined (27.59%) in Tiga Lake of Kano State showed that the molluscs are indi-cators of faecal contamination as seen with the findings of Gomez-Couso (2006) in which 184 mussel sample (29.30%) were positive for Cryptosporidium oocysts, though

**Table 3.** Distribution of *Cryptosporidium* oocysts in *Anodonta* sp. according to the site of sampling in Tiga lake of Kano state, Nigeria.

| Site of collection | Number of molluscs examined | Number positive | Percentage positive (%) | Mean oocysts load (per 2 ml) | Standard deviation |
|---|---|---|---|---|---|
| Tashan Idi | 169 | 60 | 35.50 | 166.67 | ±145.75 |
| Rurum | 150 | 40 | 26.67 | 157.50 | ±127.88 |

**Table 4.** Size range of *Cryptosporidium* oocysts in *Anodonta* sp. in Tiga lake of Kano State, Nigeria.

| Oocyst size range (µM) | Frequency of oocyst | Percentages (%) |
|---|---|---|
| 3.6 – 3.9 | 4 | 5.2 |
| 4.0 – 4.3 | 35 | 45.45 |
| 4.4 – 4.7 | 17 | 22.08 |
| 4.8 – 5.1 | 8 | 10.39 |
| 5.2 – 5.5 | 4 | 5.2 |
| 5.6 – 5.9 | 8 | 10.39 |
| 6.0 – 6.3 | 1 | 1.3 |
| Total | 77 | 100 |

this was statisti-cally significant, isolation and detection some-times play a significant role in the recovery and enumeration of the oocysts (Downey and Graczyk, 2007).The gastrointestinal tracts (GIT) of the molluscs examined were more contaminated with the oocysts than other organs. This is probably because most of the filtered particles from the surface water end up in the GIT for digestion before being circulated to other parts of the body (Sreter et al., 2000). However, Downey and Graczyk (2007) found that haemolymph exa-mined harboured more oocysts. They attributed this to the fact that shortly after exposure, oocysts may be caught in the gills and other tissues of the digestive system but with time, the haemocytes scavenge these particles in an attempt to clear them from the gills and digestive tracts. Though the recovery of the oocysts from the GITs was higher than the haemolymph and gills, this was not statistically significant ($P > 0.05$) indicating that these organs have an equal chance of being contaminated. Multiple-organ contamination observed in this study agrees with the findings of Fayer et al. (1997) in which oysters removed *Cryptosporidium parvum* oocysts from artificially contaminated water and retains them in hae-mocytes, gills and within the body for at least one month. Multiple organ contamination indicates a higher chance of contracting cryptosporidial oocysts since these oocysts are trapped all over the body of the molluscs. Also filter feeders such as bivalve molluscs may be of value as biological monitors for the presence of Cryptosporidium oocysts and other water borne pathogens in river and marine water and in particular contaminating bivalves used for consumption which has obvious public health implications (Chalmers et al., 1997).

In Tiga Lake where large scale fishing activities takes place, bivalve molluscs which are in abundance are used as baits to catch fish. Contaminated molluscs when used for such purposes may serve as a route for cryptosporidial oocysts infection in fishes. This is possible as Graczyk et al. (1996) reported an infection in fishes, amphibians and reptiles. Thus aquatic animals other than bivalve molluscs might be potential sources of waterborne Cryptosporidium oocysts.

The two sampling sites investigated, showed that molluscs harvested at Tashan Idi had a higher number of molluscs positive for the oocysts than Rurum. This may be attributed to the activities of the surrounding villages such as swimming, bathing, farming and fishing. Also cattle owners around bring their herds for grazing and drinking of water. These activities may serve as routes in which Cryptosporidium oocysts are discharged into the water body. These agree with findings of Fayer et al. (1997) and CDC (2000) in which oocysts can conta-minate surface water directly from human faeces, urine, recreational activities and indirectly from runoffs.

In micrometery of the oocysts most of them fell within the range of 4.0 to 4.8 µm, suggesting that the oocysts might be *C. parvum* or *Cryptosporidium meleagridis.* These species are of zoonotic importance and their sizes fall in line with that of Sreter et al. (2000) and Xiao et al. (2004).

To date there are no published reports of human cryptosporidiosis cases resulting from the consumption of bivalve molluscs, despite a growing body of scientific literature on the recovery of Cryptosporidium oocysts

(Gomez-Bautista et al., 2000; Schets et al., 2007). The reason for this discrepancy is unclear. One explanation could be that infection caused by consumption of contaminated molluscs are not being diagnosed or reported. The long incubation period, that is, 7 to 10 days makes it difficult to associate the infection with a particular exposure (Graczyk and Schwab, 2000).

## Conclusion

This study has established the presence of Cryptosporidium oocysts in *Anodonta* sp. in Tiga Lake of Kano State. It is established that these molluscs can effectively remove and retain oocysts of Cryptosporidium from contaminated water bodies. Due to the zoonotic potentials of the infection, threats to human life should not be underestimated especially when considering susceptible groups such as newborn infants, the elderly, patients on immunosuppressive drugs, people infected with HIV who are at a higher risk of infection. There is a need for a more in depth molecular investigation to highlight and identify the Cryptosporidium species recovered from *Anodonta* sp.

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*Full Length Research Paper*

# Two years impact of single praziquantel treatment on urinary schistosomiasis in the Barombi Kotto focus, South West Cameroon

**L. Nkengazong[1]*, F. Njiokou[1] and T. Asonganyi[2]**

[1]Laboratory of General Biology and Physiology, Faculty of Science, P. O. Box 812, Yaounde, University of Yaounde I, Cameroon.
[2]Faculty of Medicine and Biomedical Science, University of Yaounde I, P. O. Box 1364, Cameroon.

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**To evaluate the impact of a single dose praziquantel on urinary schistosomiasis in the Barombi Kotto focus, urine samples were collected from 306 participants (279 school children and 27 volunteer parents) of the Barombi Kotto health area from May, 2007 to May, 2009 and were examined using the filtration technique. A malacological survey was conducted to identify and follow up the infection rates of the intermediate snail host. The overall prevalence (69.17%) of *Schistosoma haematobium* was significantly different between boys (73.22%) and girls (66.66%; P = 0.03), same as between the island (84.3%) and mainland (60.45%; P = 0.0001) quarters. The prevalence reduced significantly by 55.03% (from 69.17 to 31.10%; P = 0.0001) and intensity of infection by 76.16% (from 212.1 to 50.56 eggs/10 ml urine; P = 0.01) at 2 years post treatment. Heavy *S. haematobium* infections in school children decreased from 23.31 to 2.12% at 2 years post treatment. The infection rates of the intermediate snail hosts (*Bulinus truncatus* and *Bulinus camerunensis)* identified reduced from 3.7 to 0.9%. These results show a significant impact of a single dose praziquantel in reducing *S. haematobium* infections after two years. The general uptrend of the prevalence and intensity of infection observed requires a continued monitoring of the disease transmission, repeated treatment and availability of adequate sanitation facilities in the Barombi Kotto focus.**

**Key words:** *Schistosoma haematobium*, prevalence, intensity, praziquantel, Barombi Kotto focus, Cameroon.

## INTRODUCTION

Three main forms of human schistosomiasis exist in Africa (intestinal, rectal and urinary), caused by the species *Schistosoma mansoni*, *Schistosoma intercalatum*, and *Schistosoma haematobium,* respectively. Schistosomiasis remains a significant health burden for many parts of the world, especially where health resources are limited (King, 2010).

Out of the 239 million people with active *Schistosoma* infection in 2009 (King et al., 2011), 85% lived in sub-Saharan Africa, where about 112 and 54 million are infected with urinary and intestinal schistosomiasis, respectively and the number of subjects at risk of infection stands superior to 600 million (Martyne et al., 2007). Estimated data show that 200.000 deaths/year where caused by schistosomiasis (Zhang et al., 2007).World Health Organization (WHO) recommendation on schistosomiasis control is based on morbidity control through chemotherapy with praziquantel (WHO, 2002, 2006). Schistosome morbidity is mainly caused by eggs trapped in various parts of the human body; therefore, the fundamental aim of morbidity control is to reduce intensity o f infection by drug treatment

*Corresponding author. E-mail: nkenglu@yahoo.com. Tel: (237)77324562/94190714.

(Touré et al., 2008). Although praziquantel has been available as an effective treatment for *Schistosoma* infection for nearly 30 years; treatment may not be fully curative and questions remain about the best possible timing and frequency of praziquantel dosing for optimal control of infection and morbidity (King et al., 2011). It has been observed in some studies that preventive chemotherapy using praziquantel has resulted in significant reduction of infection and morbidity in one year post treatment (Koukounari et al., 2006; Zhang et al., 2007; Nkengazong et al., 2009) and two years post treatment (Saathoff et al., 2004; Nsowah-Nuamah et al., 2004; Touré et al., 2008). Previous findings suggest that the WHO recommended treatment strategies of schistosomiasis should be adopted with some degree of flexibility according to the different epidemiological and geographical settings (Nsowah-Nuamah et al., 2004; Touré et al., 2008).

In Cameroon, the three forms of human schistosomiasis exist with unequal distribution foci. Intestinal and urinary schistosomiasis is more prevalent in the northern part of the country, while only a few foci are found in the southern part (Ratard et al., 1990). Schistosomiasis due to *S. haematobium* is sparsely distributed in the South West Cameroon. Lake Barombi Kotto in Barombi Kotto village and Barombi Mbo in Kumba serve as the main transmission foci, where transmission is assured by *Bulinus truncatus* and *Bulinus camerunensis* (lake Barombi Kotto) and *B. truncatus* (lake Barombi Mbo). Multiple outbreaks of the disease have been observed in the Barombi Kotto focus despite the control done in the mid-1970s (Duke and Moore, 1976) and the treatment given after each survey, with the highest prevalence and infection intensity observed in children living around the transmission site (Moyou-Somo et al., 1987; Ratard et al., 1990; Ndamukong et al., 2001; Nkengazong et al., 2009). Past control done in this focus has mainly focused on the treatment of school-age children (Moyou-Somo et al., 1987; Ndamukong et al., 2001), but it has not been well defined for adults resident in the same vulnerable area.

The study reported here aimed to evaluate the parasitological impact of a single treatment on urinary schistosomiasis in the Barombi Kotto health area after two years by: (1) assessing the prevalence and parasite intensity at base line, prevalence and intensity reduction at one and two years post treatment among school children of 3 to 22 years; (2) identifying and following-up the infection rates of the intermediate snail hosts following the treatment of the human population. The results obtain will be vital in determining the timing of treatment in the area and other endemic areas.

## MATERIALS AND METHODS

### Study area

The study was conducted in the Barombi Kotto health area, where the principal transmission site (Lake Barombi Kotto) was found. The Barombi Kotto village (4° 28' 4''N; 9° 15', 2''E) is located in Mbonge subdivision (South West Cameroon). The Crater Lake Barombi Kotto (altitude of 400 m) is situated at about 41 km from Kumba, the head quarter of the division (Moyou-Somo et al., 1987). This village belongs to the equatorial forest zone, Cameroon-type climate having one long rainy season (9 months) and one short dry season (3 months). The total annual rain fall varies between 2000 and 10000 mm. Most water bodies are perennial, while few others dry up for only brief periods (Greer et al., 1990; Ratard et al., 1990). The Crater Lake Barombi Kotto has a width of about 1 km on its largest diameter. Two small streams meet in the lake, one empting into it (the inlet) and the other flowing out of it (outlet). Some of the inhabitants in Barombi Kotto village live in a small island situated in the middle of the lake, while the majority lives around the lake and along the road leading to Kumba through other villages in the middle of the equatorial forest. This village is characterised by the absence of good water sources (wells, taps, forages), poorly disposed garbage and inadequate sanitation facilities; this means that most human activities necessitating water (laundry, bathing, fishing) are done only in the lake.

The residents of the island cross the lake every day for their activities (Farming, marketing, schooling, etc) in the other villages. This village was qualified as a highly endemic area, because of the great number of patients' diagnosed (Moyou-Somo et al., 1987; Ndamukong et al., 2001; Nkengazong et al., 2009).

### Malacological study

Three water points were chosen for the collection of snails: Point I, situated at the mainland shoreline of the lake; Point II, with multiple sub-points situated at the island shoreline of the lake; and Point III, situated at about 1.5 km away from the lake in a cocoa plantation (Figure 1). These water points were the ones used by the population for multiples activities (laundry, bathing, fishing, and farming).

Snails were collected at each point following the method described by Njiokou et al. (2004) during 7 surveys (first, second, third, forth, fifth, sixth and seventh), respectively in the months of May and October, 2007, January, February, March, September and November, 2008. Snails collected were brought to the laboratory for test of natural infestation and for species identification following the morphology of their shells (Mimpfoundi and Ndassa, 2005). The test of cercarial shedding and observation was done during one month according to Njiokou et al. (2004).

### Study subjects

The study was conducted from May, 2007 to May, 2009. Out of the 380 school children contacted, 279 (153 girls and 126 boys), 197 (109 girls and 88 boys) and 151 (81 girls and 70 boys) of ages between 3 and 22 years, participated, respectively for the initial, one and two year post treatment surveys. An additional 21 volunteer parents participated in the longitudinal survey. The infection status in these parents should give information on the quality of community-based treatment in the focus.

### Subject consent

Administrative authorities (Chief of health District, school directors and traditional leaders) were informed about the project and they gave their verbal consent for the study to be undertaken. A written informed consent that met the standards of the National Ethical Commission was obtained from the pupils or the guardians of the young children that accepted to participate in the study.

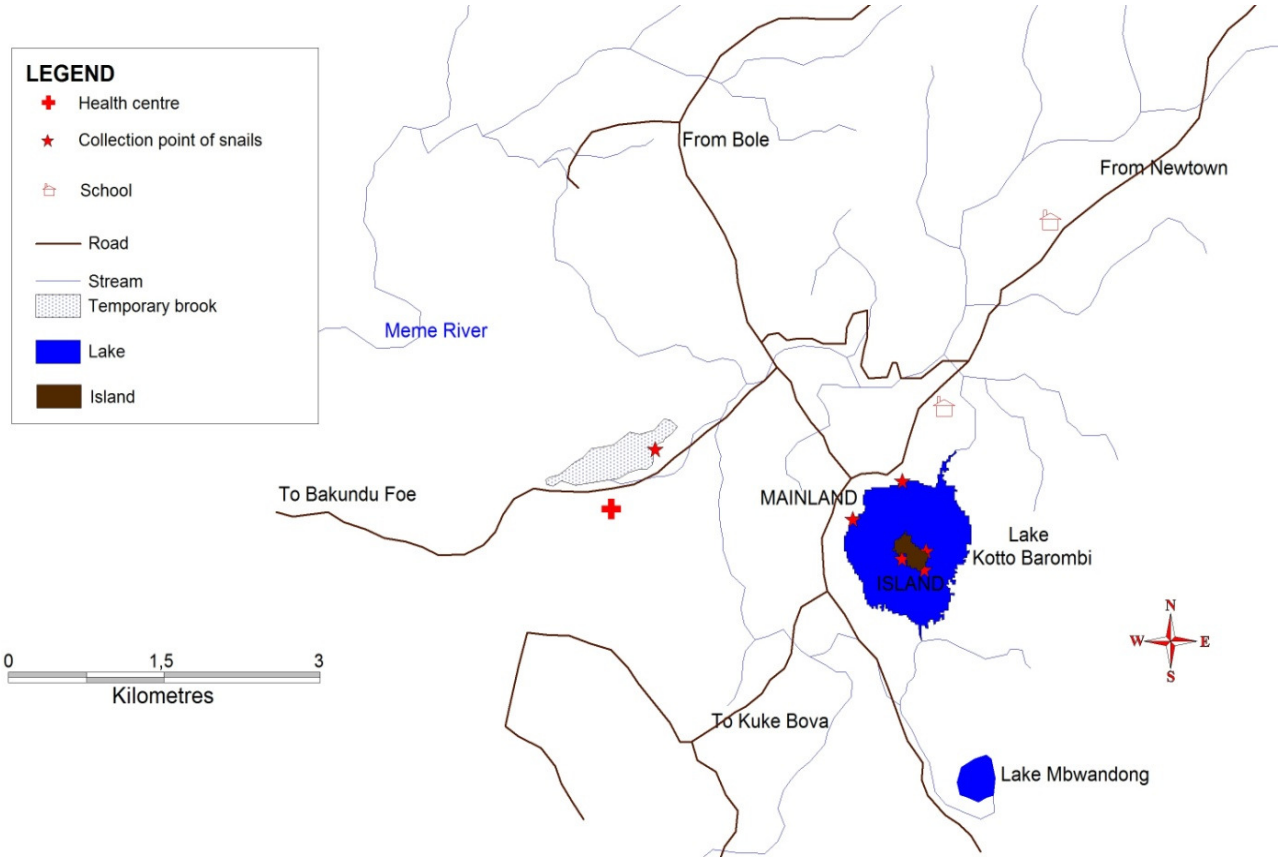


**Figure 1.** Map showing the different collection points of snails.

### Parasitological study

One urine sample was collected from each participant in a 10 ml plastic screw-cap vials between 10 am and 2 pm. The samples were transported to the General Biology Laboratory (Faculty of Science) at the University of Yaounde I and were examined using the filtration technique (Plouvier et al., 1975). The urine was filtered, stained with 1% Lugol solution, eggs counted under a light microscope at 10× magnification and their number in 10 ml urine recorded (e/10 ml). The entire population (children and parents: ≥ 3 years) living in the Barombi Kotto health area were treated two weeks after the initial survey with a single dose of praziquantel (40 mg/kg of body weight). The drug impact was evaluated one and two years later by collecting urine samples from all those who participated during the initial survey. The samples were examined using the same method described earlier

### Data analysis

The parameters assessed during the study were the prevalence (Margolis et al., 1982), egg load, egg reduction rate (Touré et al., 2008; Nkengazong et al., 2009), the level of infection intensity (Kihara et al., 2007) and the natural infection of intermediate snail hosts.

The Chi-square test was used to compare the prevalence in relation to sex, quarters and between the different survey periods, while one way analysis of variance (ANOVA) or Kruskal-Wallis (Sokal and Rohlf, 1981) tests were used to compare the egg load in relation to sex, quarters, egg load and the level of infection intensity during the different survey periods. The level of statistical significance was at 95% ($P < 0.05$). Participants who dropped out or missed either of the two follow-up surveys were not included in the longitudinal analysis.

## RESULTS

### Malacology

During the malacological survey of a total of 2847 snails, all intermediate snail hosts of *S. haematobium* were collected. This number included 1309 (46.0%) *B. truncatus* and 1538 (54.02%) *B. camerunensis*. Snails collected during the first (May 2007) and the fifth (March 2008) surveys from the Barombi Kotto lake emitted *S. haematobium* forked-tailed cercariae with their respective infection rates being 3.7 and 0.9%. Those collected during the other surveys did not emit forked-tailed cercariae during one month period of exposure in the laboratory (Table 1).

### Parasitology

#### *Baseline results*

The global prevalence of *S. haematobium* among 279 children examined at baseline was 69.17%. Children

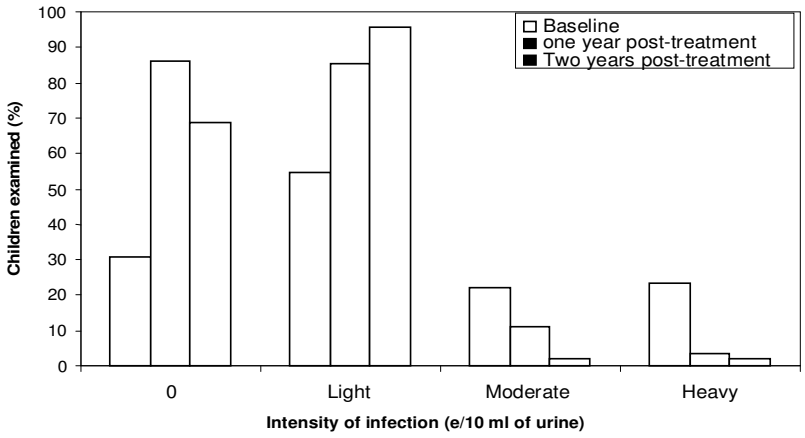


**Figure 2.** Categories of *S. haematobium* intensity before (n = 279), one year post treatment (n = 197) and two years post treatment (n = 151): 0 = number of persons uninfected.

from the island (84.3%) were significantly more infected than those from the mainland (60.45%; P = 0.0001). A significant difference of prevalence was observed between boys (73.22%) and girls (66.66%) (P = 0.03). The global intensity of infection was 212.1 e/10 ml urine. This value was significantly higher in children from the island (280.1 e/10 ml urine) than the mainland (130.2 e/10 ml urine) (P= 0.01). The intensity of infection did not vary significantly between boys and girls (Table 2).

The proportion of light (≤ 99 e/10 ml urine), moderate (100 to 399 e/10 ml urine) and heavy (≥ 400e/10ml urine) infections of the school children examined were 54.4 %, 22.27 % and 23.31 % respectively (Figure 1). The level of heavy infection did not vary significantly by sex and quarter of residence. Of the 27 volunteer parents examined, six (22.22%) were positive for *S. haematobium* eggs among whom four (14.81%) were heavily infected.

### *Post treatment results*

One round of mass Praziquantel treatment significantly reduced the prevalence in the cohort children from 69.17% at baseline to 13.7 and 31.10% at one year and two years post treatment, respectively (P = 0.0001) with the corresponding overall reduction rates of 80.19 and 55.03%.

A significant decreased of prevalence was observed at baseline as compared to one and two years post treatment, with a significant increase from one to two years post treatment (P = 0.01). The overall intensity of infection significantly reduced from 212.1 at baseline to 32.65 and 50.56 e/10 ml urine at one and two years post treatment, respectively (P = 0.01), with the corresponding overall reduction rates of 84.60 and 76.16% (Table 2). No significant difference, though a high prevalence was observed in females, and two years post treatment with a high intensity observed in males. At one and two yearspost-treatment,nosignificantdifferencewasobserved.

Importantly, heavy infections decreased to 3.7% at 1 year post treatment and remained at 2.12% at two years post treatment. Moderate infection was maintained at 11.10 and 2.12% and light infections at 85.20 and 95.74%, respectively at one and two years post treatment (Figure 2). Four parents were found infected at one and two years post treatment among which one who was negative at base line was heavily infected during the two follow-up periods.

## DISCUSSION

The malacological survey conducted enabled us to identify two water points in the Barombi Kotto village harbouring the intermediate snail hosts of S. *haematobium* (*B. truncatus* and *B. camerunensis*) known since the discovery of the focus. *B. camerunensis* was more abundant than *B. truncatus* during our survey confirming the results observed by Duke and Moore (1976), Moyou-Somo et al. (1987) and Greer et al. (1990). This shows stability in the Gastropod population in the Barombi Kotto focus. The cercariae observed from snails collected during the first and fifth survey from the lake confirm that the lake remains the main transmission site of urinary schistosomiasis in the Barombi Kotto health area. The absence of cercariae during the second, third and forth surveys could likely be due to the fact that both children and parents were treated, leading to a reduction in the level of snail infection. The sixth and seventh surveys were conducted during the rainy season, when the human population had another water source (rain water), that could limit the frequency of contact with the transmission site. This is in conformity with the result of Sturrock et al. (1979) who showed that the rate of natural infestation of snails in a transmission focus of schistosomiasis could be linked to the pressure of snail infestation in a transmission site or to the frequency of water contact by infected people. However, most water

**Table 1.** Distribution and infection rates of *Bulinus* species from lake Barombi Kotto, South West Cameroon.

| Collection period | Species and sites* | | | | | | Total* | | | Overall total | Infection rate (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *B. truncatus* | | | *B. camerunensis* | | | | | | | |
| | I | II | III | I | II | III | I | II | III | | |
| May 2007 | - | - | - | 52 (2) | 112 (4) | - | 52 (2) | 112 (4) | - | 164 (6) | 3.7 |
| October 2007 | 5 | 117 | 2 | 25 | 187 | 6 | 30 | 304 | 8 | 342 | 0.0 |
| January 2008 | 2 | 298 | - | 51 | 48 | 10 | 53 | 346 | 10 | 409 | 0.0 |
| February 2008 | - | 6 | - | 81 | 256 | 21 | 81 | 262 | 21 | 364 | 0.0 |
| March 2008 | 18 (1) | 43 (2) | - | 49 | 312 (2) | 32 | 67 (1) | 355 (3) | 32 | 454 (4) | 0.9 |
| September 2008 | 11 | 489 | 3 | 9 | 171 | 15 | 20 | 660 | 18 | 698 | 0.0 |
| November 2008 | - | 315 | - | 26 | 68 | 7 | 26 | 383 | 7 | 416 | 0.0 |
| Total | 36 | 1268 | 5 | 293 | 1154 | 91 | 329 | 2422 | 96 | 2847 | 0.4 |

I, II and III: Collection sites for mainland, island and in cocoa plantation, respectively. *Values in parentheses indicate the number of snails infected.

**Table 2.** Prevalence and intensity of *S. haematobium* infection before and after treatment in Barombi Kotto focus, South West Cameroon.

| Variable | Baseline | 1 year post-treatment | 2 years post-treatment | Overall reduction (%) | |
|---|---|---|---|---|---|
| | | | | 1 year post-treatment | 2 years post-treatment |
| **Prevalence (%)** | | | | | |
| Overall prevalence | 69.17 (193: n = 279) | 13.7 (27: n = 197) | 31.10 (47: n = 151) | 80.19 | 55.03 |
| **By quarter** | | | | | |
| Mainland | 60.45 (107: n =177) | 8.8 (11: n = 125) | 27.05 (n = 85) | 85.44 | 55.33 |
| Island | 84.3 (86: n = 102) | 22.2 (16: n = 72) | 33.36 (n = 66) | 73.66 | 60.42 |
| **By sex** | | | | | |
| Boys | 73.22 (91: n = 126) | 14.80 (13: n = 88) | 28.60 (20: n = 70) | 79.80 | 60.93 |
| Girls | 66.66 (102: n = 153) | 12.84 (14: n = 109) | 33.33 (27: n = 81) | 80.73 | 50.0 |
| **Egg load (e/10 ml urine)** | | | | | |
| Overall mean | 212.1 (n = 193) | 32.65 (n = 27) | 50.56 (n = 47) | 84.60 | 76.16 |
| **By quarter** | | | | | |
| Mainland | 130.2 (n = 107) | 41.8 (n = 11) | 20.96 (n = 23) | 67.90 | 83.90 |
| Island | 280.1 (n = 86) | 24.31 (n = 16) | 61.41 (n = 24) | 91.32 | 78.08 |
| **By sex** | | | | | |
| Boys | 213.7 (n = 91) | 36.07 (n = 13) | 62.61 (n = 18) | 83.12 | 70.70 |
| Girls | 210.53 (n = 102) | 29.23 (n = 14) | 29.13 (n = 29) | 86.11 | 86.16 |

n: Total.

periods, as contamination is determined by human behaviour for specific purposes. This makes snails infestation to be a discontinuous process, taking place only at particular moments in such a way that a given snail population can be uninfected completely. This may explain the case of snails collected in the third water point located in a cocoa plantation where people come into contact with water point only during cocoa harvesting and clearing of the farm. In line with previous studies, high prevalence and intensity of *S. haematobium* was found at baseline in the Barombi Kotto health area (Moyou-Somo et al., 1987; Ndamukong et al., 2001) with the prevalence rate of 76.0 and 75.9%, respectively. Even though our prevalence obtained was slightly lower as compared to that of previous authors, it confirms the high endemicity of schistosomiasis in this area. These differences may reflect the difference in the number of years spent since the last mass treatment before each of the three surveys.

Our results are similar to those of King (2006) who showed that villages without pipe-borne water access maintained a high level of infection. Our results confirm the well documented observation that children living near the transmission site of schistosomiasis are more infected (Moyou-Somo et al., 1987; Ndamukong et al., 2001; Saathoff et al., 2004; Kabatereine et al., 2011), and that boys are generally more active in water collection than girls (Tchuem Tchuenté et al., 2003; Njiokou et al., 2004).

Following treatment, the proportion of heavy infections was greatly reduced, which is particularly important as high intensity of *S. haematobium* infection has been shown to contribute to morbidity, including anaemia in children (Koukounari et al., 2007; Green et al., 2011; Sousa-Figueiredo et al., 2012). In previous studies conducted on *S. haematobium* control in Eastern Africa, an annual treatment strategy was predominantly used, with varying results (Magnussen, 2003; Satayathum et al., 2006). However, Nsowah-Nuamah et al. (2004) in Southern Ghana and Touré et al. (2008) in Burkina Faso observed an important reduction of prevalence and parasite intensities at two years post treatment, while Garba et al. (2004) in Niger observed a significant reduction of prevalence and parasite intensities after three years post treatment. Our results are in line with the results of these studies, suggesting that a more spaced treatment strategy is highly effective on *S. haematobium* infections, even in highly endemic areas. However, the effectiveness of such strategy may depend on the local epidemiological and geographical settings of each area.

Though one dose of praziquantel reduced the level of infection significantly at one and two years post treatment; it has been shown that between infection intensity and morbidity risk, even light infection may impose risk of serious disease (Wamachi et al., 2004). Given the association between schistosome infection and anaemia and other disability-related outcomes, infection at any level may impose a significant burden on local health (King et al., 2005). Our results showed that one parent was found heavily infected at one and two years post treatment.

This may be a reflection of the observation that targeted school-age treatment can suppress transmission in some, but not all *S. haematobium* infected communities, because one individual usually uses multiple water sites, and a single infected person can maintain local transmission for an indefinite period (Woolhouse et al., 1997, 1998).

The significant increase of prevalence observed from one to two years post treatment as compared to baseline and one year post treatment may reflect the period of treatment which was in the rainy season (June); the availability of rain water probably reduces human contact with the transmission site during the first year post treatment as compared to the second year. Nevertheless, the general uptrend of the prevalence and intensity of infection showed that two years post treatment could be a sign of a potential rebound of *S. haematobium* should drug distribution be interrupted, as reported by Touré et al. (2008); it could also be a reflection of the fact that multiple villages interact with multiple water contact sites, thus favouring transmission of infection or contamination of water sites (King, 2006). In general, our results highlight the importance of continued effort in monitoring schistosomiasis transmission and repeated treatment in the Barombi Kotto focus, as already shown in some studies (Mduluza et al., 2001; King et al., 2011).

## Conclusions

This study has shown: (1) a high prevalence and intensity of *S. haematobium* infection in the Barombi Kotto health area, (2) a significant impact of a single dose of praziquantel in reducing prevalence and intensity of *S. haematobium* after two years, and (3) a high pace of re-infection and the presence of other water sites harbouring the intermediate snail hosts of *S. haematobium* beside the main transmission site (lake Barombi Kotto). It is therefore necessary to: (1) carry out a continuous monitoring of the disease transmission to maintain infection prevalence and intensity at a low level, (2) repeat treatment once every year at the start of the dry season, involving both children and parents with high coverage, (3) organise a survey regarding knowledge, attitude, and practices (KAP) of the people living in the area, and (4) improve the socio-economic conditions of the area (availability of good water sources in all villages).

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*Full Length Research Paper*

# Camel hydatidosis: Prevalence and economic significance in pastoral regions of Ethiopia

**Bayleyegn Gizachew*, Fikadu Kibru and Biruhtesfa Asrade**

College of Veterinary Medicine, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia.

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**Camel hydatidosis was studied at Addis Ababa abattoir, Ethiopia to determine the prevalence and financial losses associated. From 501 camels slaughtered, 328 (65.47%) were found harboring hydatid cyst. The prevalence between females and males was statistically significant ($\chi^2$=35.74; P=0.000). Additionally, the disease was significantly different among the age groups ($\chi^2$=18.71; P=0.00) revealing higher prevalence in older animals. In respect to origin, the highest prevalence was observed from Borena (65.67%). The lung (47.90%) was the most frequently affected organ. Majority of the cysts identified were non calcified cysts. 57.78 and 39.10% of the cysts were found to be fertile in the lung and liver, respectively. Of these fertile cysts 68.27 and 60% were viable in the lung and liver, respectively. 212 lungs, 209 livers, 21 spleens and 2 hearts were totally condemned and these results in financial loss of 1089758.8 ETB (61222.4 US Dollar) annually. In conclusion, hydatidosis is highly prevalent in camels slaughtered at Addis Ababa Abattoir resulting to high economic loss due to organ condemnation. Thus, an effort should be made to control and prevent echinococcosis in the camel herding areas.**

**Key words:** Akaki abattoir, camel hydatidosis, financial loss, prevalence.

## INTRODUCTION

Pastoralism accounts for the livelihood of 50 to 100 million people in developing countries, while 60% of this population lives in more than 21 African countries confined to the most arid regions of the continents (Sheik-Mohamed and Velema, 1999; UNDP, 2007). Pastoralists are migratory people whose livelihood largely depends on livestock rearing. Among the East Africa countries, Ethiopia has the largest pastoralist population accounting for 10 to 12% of the total population (USAID, 2005). Ethiopian pastoralists virtually depend on livestock for their livelihood, moving seasonally from place to place in search of water and pasture for their animals (Nori, 2005). The dromedary camel (*Camelus dromedarius*), which is adaptable and capable of living in desert areas serves as a source of milk, meat, and draft power for the subsistence of the pastoralists. Ethiopia possesses an estimated amount of 2.3 million of *C. dromedaries* (Central Statistical Agency (CSA), 2008) mainly distributed in the Southern, Eastern and Northeast pastoral regions (Workneh, 2002; Ministry of Information, 2005).

In spite of the great ecological and economical value of the dromedary camel, there is scarce research information on camel diseases when compared with that of other domestic animals (Zeleke and Berkeley, 2000). This reveals that camels may be either carrier, susceptible or suffering from several diseases(Demeke, 1998). In

*Corresponding author. Email: gzenew@gmail.com. Tel: +251911747923. Fax: +251255530102.

connection to these, parasitic diseases like hydatidosis appear to be the major constraints that are hampering the potential performances of the camels (Getahun and Kassa, 2002; Megersa, 2010). In farm animals, it causes considerable economic losses due to condemnation of edible organs, decreased meat and milk production (Polydorous, 1981; Romazanvoc, 2001). In Ethiopia no work has been done on camel hydatidosis except a prevalence report by Muskin et al. (2011). In this regards, as Ethiopian dromedaries are primarily reared by pastoralists, camel hydatidosis could have significant economic and public health consequences in the regions. To this effect, abattoir based epidemiological studies are needed to show the real picture of the disease and to know its impact on economy. Therefore, the objectives of the present study are to investigate epidemiology and assess economic significance of camel hydatidosis in pastoral regions of Ethiopia.

## MATERIALS AND METHODS

### Study area and subjects

The study was conducted at Akaki (Addis Ababa) abattoir on apparently healthy slaughtered camels. The camels slaughtered at the abattoir were both male and female that originated from pastoral parts of the country mainly from Borena (Southeastern part of Oromia region) and Afar (eastern regions of Ethiopia).

### Post mortem examination

Thorough post mortem examination was carried out by visual inspection, palpation and systematic incision on visceral organs; lung, liver, heart, kidney and spleen according to procedures recommended by Food and Agricultural Organization (FAO, 1994). All organs harboring cysts were partially or totally condemned and were judged according to the guidelines on meat inspection for developing countries (FAO, 1994).

Whenever and wherever the hydatid cysts appeared, the number and the size of the cysts per organ and per animal were counted, measured and recorded. Accordingly, the size of cysts were measured systematically and classified into three based on their diameter as small (<4 cm), medium (4 to 8 cm) and large (>8 cm) (Oostburg et al., 2000). The cysts were randomly selected and collected from different organs and were taken to regional laboratory (Shola, Addis Ababa) for fertility and viability tests (Figure 1).

### Cyst viability tests

Non-calcified cysts were randomly selected and collected from different organs and were taken to regional laboratory (Shola, Addis Ababa) using ice box for fertility and viability tests. The cyst was put on clean petri-dish and incised by sterile scalpel blade and the fluid part was poured in another clean petri-dish. The supernatant was discarded and resultant sediment was finally examined for the presence of protoscolesces that appear as white dot under the microscopic field at 40X magnification power. Fertile cyst was subjected to viability test. A drop of 0.1% eosin solution was added to equal volume of protoscolisces in hydatid fluid on petri-dish with principle that viable protoscolisces should completely or partially avoids the dye, while the dead once take it up. Furthermore, infertile cysts were classified as sterile or calcified. Sterile hydatid cysts were characterized by their smooth inner lining usually with slightly turbid fluid in its content. Typically calcified cysts produced a gritty sound feeling upon incision (Soulsby, 1982).

### Data management and analysis

The data were entered and coded into Microsoft excel program. Pearson $\chi^2$ was used to test the existence of association between sex, body condition, origin and age groups. P-value less than 0.05 ($P<0.05$) was considered as statistically significant.

Estimation of the financial losses was analyzed on the basis of secondary data (annual slaughter rate) from the slaughter house and the mean retail market price of the offal which was obtained from the abattoir workers and butchers, and associated economic loss was calculated on the annual basis according to Sariözkan and Yalcin (2009).

## RESULTS AND DISCUSSION

There are few epidemiological studies published on camel hydatidosis in Ethiopia as well as in other countries. Based on post mortem inspection, the prevalence of camel hydatidosis was 328/501 (65.5%). In this study, the prevalence of hydatidosis in camel is relatively higher when compared to the previous reports in camel, cattle and small ruminants in Ethiopia by Ahmed (1998) (18.6%) in Eastern Ethiopia, Weldemeskel (2001) (18.86%), Mulatu (2013) (30.5%) in Dire Dawa and Jijiga Bitsat (2009) (28.5%) and Muskin et al. (2011) (22.6%), and elsewhere in the world: 39.65, 48.5, 35.25, and 32.85%, were reported in Kuwait (Abdul-Salam et al., 1988), Libya (Ibrahim, 1998), Iran (Ahmadi, 2005), and Saudi Arabia (Mohamed, 2010), respectively. The disease was highly prevalent in female animals 300/328 (91.5%) and was statistically significant ($\chi^2=35.74$; $P=0.00$). The result of the association of the different risk factors to the hydatid cyst is presented in Table 1. Among the age groups, the disease was also found to be in higher prevalence in older animals. The age dependent variation could be due to high probability of the exposure of older animals to the infection during their long existence in life for many years.

This is also true for higher prevalent record in female animals, because during inspection, these animals were found old due to the fact that most pastoralists sell the female animals after they finished their productive age. These results agree with the previous reports by Cleaveland et al. (2007) and Inangolet et al. (2008). In this case, it is also good to consider the chronic nature of cyst development in organs. Since camel can live around 40 years (Kinne et al., 2006), they are more affected than other domestic animals. Regarding the origins, camels from Borena area were more affected with disease than

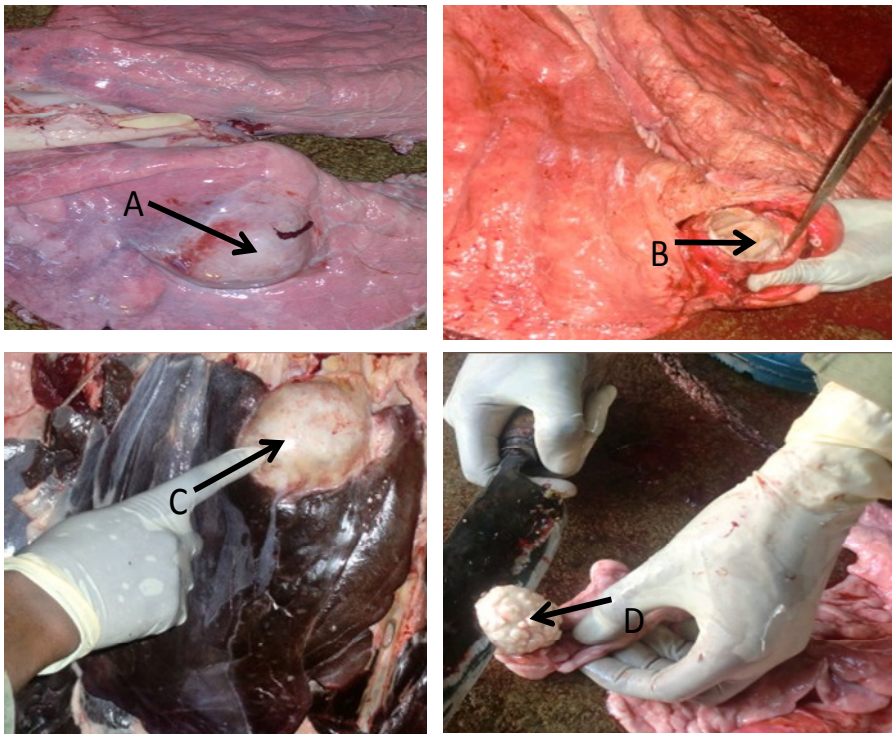


**Figure 1.** Hydatid cysts on different organs of slaughtered camels. A: None calcified hydatid cyst on the surface of camel lung; B: None calcified hydatid cyst in the lung exposed after incision; C: None calcified hydatid cysts on the surface camel liver; D: calcified hydatid cyst on camel lung.

**Table 1.** Association host related variables with camel hydatidosis.

| Variable | No. of camels slaughtered | No. of positive Animals | % of positive animals | $\chi^2$ | P-value |
|---|---|---|---|---|---|
| **Sex** | | | | | |
| Female | 423 | 300 | 91.5 | 35.74 | 0.00* |
| Male | 78 | 28 | 8.54 | | |
| **Age** | | | | | |
| < 5 | 48 | 21 | 6.40 | | |
| 5-10 | 172 | 103 | 31.40 | 18.2 | 0.00* |
| >10 | 281 | 204 | 62.20 | | |
| **BCS** | | | | | |
| Score 1 | 81 | 60 | 18.29 | | |
| Score2 | 38 | 29 | 8.84 | | |
| Score3 | 193 | 129 | 39.36 | 9.28 | 0.054 |
| Score4 | 124 | 73 | 22.26 | | |
| Score5 | 65 | 37 | 1.28 | | |
| **Origin** | | | | | |
| Borena | 329 | 233 | 71.04 | | |
| Afar | 81 | 49 | 14.94 | 17.24 | 0.001* |
| Meiso | 66 | 37 | 11.28 | | |
| Minjar | 25 | 9 | 2.74 | | |

*Statistically significant.

**Table 2.** Distribution of hydatid cyst on different organs of slaughtered animals.

| Cyst location | No. of infected animal | Percent of infected animal |
|---|---|---|
| Lung | 94 | 28.66 |
| Liver | 83 | 25.30 |
| Spleen | 2 | 0.61 |
| Heart | 0 | 0.00 |
| Lung and liver | 128 | 39.02 |
| Lung and spleen | 5 | 1.52 |
| Lung and heart | 1 | 0.31 |
| Liver and spleen | 2 | 0.61 |
| Liver and heart | 1 | 0.31 |
| Lung, liver and spleen | 12 | 3.66 |

camels from other origins. Theses could be due to the absence of similar environmental and climatic situations in the different areas (Mohamed, 2010).

In this study, it was also possible to characterize the nature of the cyst. Most of the hydatid cysts were recorded in lung and liver of camel. Single and multiple organ pathology with hydatid cysts are depicted in Table 2. The infection rate of cystic echinococcosis in each organ with relation to the cyst size is summarized in Table 3. Majority of the identified cysts were non-calcified. The distribution of calcified and non calcified cysts in relation to age is summarized in Table 4. Regarding the fertility of the hydatid cysts, this study indicated that cysts from the lung showed a higher fertility proportion as compared to cysts collected from livers (Table 5).

The reason for the difference may be attributed to the age of animals. An increased number of fertile cysts tend to occur in older animals (World Health Organization/ Office International des Epizootics (WHO/OIE), 2011); this strongly suggest that fertile cysts, are important factors that can influence the transmission of *Echinococcosis granulosus*, and are most likely found in older animals while the sterile one is found in young animals (Grayesm, 1986).

Furthermore, the extensive practice and roadside slaughter of camels could have contributed to the maintenance of the parasite's cycle. In such a way, dogs have easy access to ingest the offal or organs harboring the cysts and become easily infected. This plays a crucial role in the transmission of the disease and enables the parasite to maintain its life cycle among the hosts. Moreover, pastoralists in east Africa move from place to place with their livestock including camels in search of feed where wild canids are found. This may favor the transmission of the infection between infected canids and susceptible camels and also be considered as risk factors in increment of the prevalence in this study. Moreover, Ethiopia has proclamation for animal disease prevention and control (Federal Negarit Gazeta (FNG), 2002). However, currently there is no documented and implemented rule and regulation for meat inspection in particular for camel meat.

The annual economic loss due to hydatidosis in camel slaughtered at Akaki abattoir was estimated by considering the main cost of organs (lung, liver, heart and spleen) (Sariözkan and Yalcin, 2009). The main price of respective organs at Addis Ababa city was obtained from abattoir workers and butchers during the study period.

These parameters were then fed to the following formula in order to compute the economic loss due to organ condemned, as unfit for human consumption due to cystic echinococcosis thus:

$$X = (AS \times C_{Lu} \times P_{Lu}) + (AS \times C_{Li} \times P_{Li}) + (AS \times C_{Sp} \times P_{Sp}) + (AS \times C_{Kid} \times P_{Kid}) + (AS \times C_{Hr} \times P_{Hr}).$$

where AS: estimated mean annual kill; $P_{Lu}$: percent involvement of the lung; $C_{Lu}$: local retail price of a lung; $P_{Li}$: percent involvement of the liver; $C_{Li}$: local retail price of a liver; $P_{Sp}$= percent involvement of the spleen; $C_{Sp}$: local retail price of a spleen; $P_{Kid}$: percent involvement of the kidney; $C_{Kid}$: local retail price of a kidney; $P_{Hr}$: percent involvement of the heart; $C_{Hr}$: local retail price of a heart; $P_{SC}$: percent of spleen condemned; $C_S$: local retail price of the spleen.

$X = (3201 \times 65.47\% \times 10) + (3201 \times 41.72\% \times 800) + (3201 \times 0.39\% \times 35) + (3201 \times 0.00\% \times 80) + (3201 \times 4.19\% \times 0) = 20957 + 1068365.8 + 437 + 0 + 0$

X = 1,089,758.8 ETB (61,222.4 US Dollar)

Conclusively, this study indicated that hydatidosis is a highly prevalent parasitic disease of slaughtered camels at Addis Ababa abattoir. In connection to this, significant

**Table 3.** Cyst size in different organs of infected camels.

| Organ | Small | Medium | Large | Total |
|---|---|---|---|---|
| | N (%) | N (%) | N (%) | N |
| Lung | 409 (39.10) | 850 (57.32) | 242 (63.35) | 1501 |
| Liver | 627 (59.94) | 613 (41.34) | 136 (35.60) | 1376 |
| Spleen | 10 (0.96) | 19 (1.28) | 3 (0.79) | 32 |
| Heart | 0 (0.00) | 1 (0.07) | 1 (0.26) | 2 |
| Total | 1046 (100.00) | 1483 (100.00) | 382 (100.00) | 2911 |

**Table 4.** Calcification of cysts.

| Age (Years) | No. cyst Examined | Non-calcified cyst | Calcified cyst |
|---|---|---|---|
| | | N (%) | N (%) |
| ≤5 | 19 | 10 (52.63) | 9 (47.37) |
| 5-10 | 152 | 115 (75.66) | 37 (24.34) |
| >10 | 217 | 183 (84.33) | 34 (15.67) |
| Total | 388 | 308 (79.38) | 80 (20.62) |

**Table 5.** Cyst fertility and viability in relation to organ affected.

| Cyst location | No. of cyst examined | N(%) | | | |
|---|---|---|---|---|---|
| | | Sterile | Fertile | Viable | Dead |
| Lung | 180 | 76(42.22) | 104(57.78) | 71(68.27 | 33(31.73) |
| Liver | 128 | 78(60.93) | 50(39.10) | 30(60.00) | 20(40.00) |
| Total | 308 | 154(50.00) | 154(50.00) | 101(65.58) | 53(34.42) |

financial losses were recorded due to condemnation of edible organs from the domestic markets. Therefore, an effort should be made to control and prevent camel hydatidosis and further identification and characterization of causative agent would be useful towards the efforts made to control the disease.

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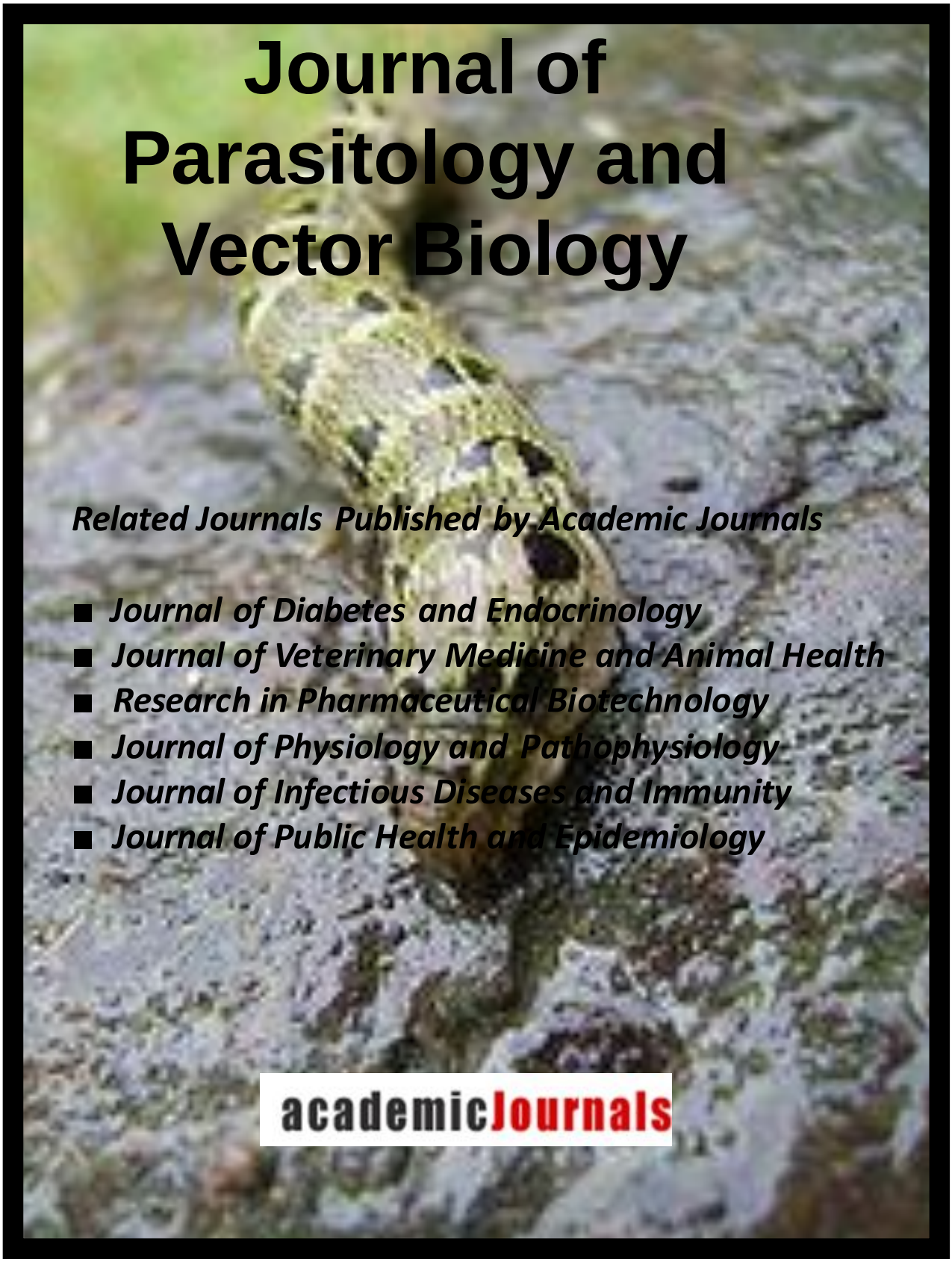
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