



## *In vitro* Efficacy of Albendazole against Strongyle eggs recovered from Trade Goats slaughtered at the Nsukka Abattoir: A Preliminary Survey of Resistance to Albendazole

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### SUMMARY

The efficacy of Albendazole against trichostrongyle nematode parasites in goats presented for slaughter at the Nsukka municipal abattoir was evaluated using the *In vitro* Egg hatch assay (EHA) model. The abattoir was visited once every week for 4 consecutive months during which a total of 240 goats were sampled. Faecal samples were collected per rectum from a minimum of 15 goats on each day of the visit. Egg Hatch Assay was performed on strongyle eggs recovered from pooled faecal sample on each day of sampling with a 2.5% W/V Albendazole. Faecal culture was also set up from the pooled faecal sample on each sampling day to recover and identify the nematode parasites present in the goats. Among the 240 goats sampled, the prevalence of trichostrongylosis as observed by the presence of strongyle eggs was 94.6% (227/240). Faecal culture and larval identification revealed 69.8% of the strongyles as *Haemonchus contortus*, while 25.5 and 4.8% were *Trichostrongylus colubriformis* and *Oesophagostomum* species respectively. In the EHA, Albendazole had mean LC50 value of 0.16 µg/ml which is slightly in excess of the discriminating dose of 0.1µg/ml as prescribed by the World Association for the Advancement of Veterinary Parasitology (WAAVP) as an indication of anthelmintic resistance. There is therefore an urgent need to screen the nematode parasite population in the Nigeria for the presence Albendazole resistance genes.

**Key words:** GI nematode; egg hatch assay; goat; Albendazole; Resistance; Nigeria.

### INTRODUCTION

Gastrointestinal (GI) trichostrongyloid parasites are the most commonly encountered causative agent of parasitic gastroenteritis and among the major causes of ill health and production loss in

goats all over the world (Chiejina, 1987; Mbaya *et al.*, 2009). Control of trichostrongylosis just like most other parasitic GI nematodes rely primarily on chemotherapy with anthelmintics (Probert, 2001). However, the continually

increasing threat of anthelmintic resistance (AR) challenges on the sustainability of the use of anthelmintics in small ruminants. Anthelmintic resistance has been reported almost in all parts of the world, against almost all classes of anthelmintics (Kaplan, 2004; Kaminsky *et al.*, 2008). The threat of AR is more in areas with climatic conditions that favour an all year-round development of the pre-parasitic stages resulting in several cycles of infection in a year and frequent treatment with anthelmintic, as is the case in the humid tropical regions of the world (Waller, 1997).

Available data show that AR is most severe and widely reported in small ruminants compared to other livestock (Van Wyk *et al.*, 1999; Chandrawathani *et al.*, 2003; McKenna, 2010; Sargison *et al.*, 2010; Kaplan and Vidyashankar, 2012; Torres-Acosta *et al.*, 2012). For instance, over 90% of small ruminant farms sampled in 3 different surveys in South Africa had *Haemonchus* strains that were resistant to no less than 1 of the 4 tested anthelmintics, and 60 to 78 % of the strains were resistant to 3 anthelmintic groups (Van Wyk *et al.*, 1999). A similar pattern of resistance was also reported by Boersema and Pandey (1997) on commercial sheep farms in Zimbabwe. Also, Chartier *et al.*, (2001) reported that 80 to 100% of dairy goat flocks surveyed in France, had nematode populations that were resistant to Benzimidazole compound. Anthelmintic resistance is therefore, a potent threat to profitable small ruminant enterprise, as resistant parasitic nematodes become uncontrollable. Kaplan (2004) noted that resistance to different classes of anthelmintics, were reported to have forced some farmers to stop sheep and goat farming in countries like South Africa, New Zealand and Australia.

The spread of AR particularly against benzimidazole drugs is common and has impacted severely on the productivity of small ruminant farming industries worldwide (Kaplan, 2004; Papadopoulos *et al.*, 2012; Falzon *et al.*, 2013; Ramünke *et al.*, 2016). Benzimidazoles are usually the first choice of anthelmintics for sheep and goat deworming because of their low cost, availability and broad spectrum of activity (Iliev, 2014). This is also the case in Nigeria, where benzimidazoles, particularly, albendazole is one of the most commonly used anthelmintics in small ruminant production Onuorah (2016).

Since resistance is an unavoidable consequence of anthelmintic usage (Prichard, 1994) and a common occurrence in small ruminant farms, particularly goat farms, it will be needful to investigate the resistance status of GI nematodes to commonly used anthelmintics in small ruminants. Therefore, the present study was designed to assess the efficacy of Albendazole against GI trichostrongyloid nematodes of trade goats slaughtered at the Nsukka municipal abattoir using the *in vitro* egg hatch assay (EHA). Given that trade goats slaughtered at the Nsukka abattoir are mainly sourced from goats in the Northern part of the country, an area that holds more than 70% of goat population in Nigeria, such epidemiological study will provide reliable information on the status and spread of AR in goats in the country.

## METHODS

### Study Area

The study was conducted at the Nsukka municipal abattoir, in the Nsukka Local Government Area of Enugu State, Nigeria. Nsukka is located in the Derived Savannah zone of Eastern Nigeria lying approximately between

longitude 6°52' - 7°53'E and latitude 6°38' - 7°8'N.

Albendazole Shanuzole® (Jawa International Limited, Lagos, Nigeria) containing 25mg/ml (2.5% W/V) of Albendazole was used in this study. Shanuzole® was chosen because it is a commonly used brand of albendazole within the study area by sheep and goat farmers and has recorded very good efficacy.

### Study Design

Faecal samples were collected per rectum from goats presented for slaughter at Nsukka abattoir while laboratory procedures were performed at the Department of Veterinary Parasitology and Entomology laboratory at the University of Nigeria, Nsukka. The abattoir was visited once every week for 4 consecutive months (March to June) given a total of 16 visits. Fifteen goats were sampled on each visit given a total of 240 goats of varying sex and age. The goats were Kano Brown breed sourced from the northern region of Nigeria. The faecal samples were placed in labeled leak-proof containers and taken to the laboratory immediately for faecal analysis.

Faecal egg count of each goat was determined using the McMaster counting technique where appropriate as described in MAFF (1977). Samples with up to 100 eggs per gram of faeces were pooled on each sampling day and used for the EHA. Strongyle eggs were then harvested from the pooled faecal samples as described by Idika *et al.* (2016) and the eggs used to assess the efficacy of Albendazole. Faecal cultures were also set up with left over strongyle eggs on each day of the assay using sterile faeces for larval isolation and identification as described by Hansen and Perry (1994). The proportions of the

different species that make up the strongyles eggs were determined descriptively.

### Egg Hatch Assay (EHA)

Strongyle eggs used for the EHA were harvested from freshly collected goat faeces by initial floatation of the eggs using the saturated NaCl salt floatation method in a 45 ml test-tube to collect suspended eggs, followed by repeated (4 times) washing of the eggs in distilled water. Following the methods of Coles *et al.* (1992) with minor modifications, the strongyle eggs obtained from freshly collected goat faeces were incubated with twelve different concentrations (0.012 to 25ug/ml) of a 2.5% albendazole stock. The 12 concentrations of albendazole were prepared by 2-fold serial dilutions of the stock solution using 0.1% NaCl constituted with de-ionized water, in a 96-well flat-bottomed plate (Dynatech Immulon). The control wells received only the diluents (0.1% NaCl). Thereafter, an estimated 50 strongyle eggs in 20 µL of 0.1% NaCl were added to each of the 200 µL of the 12 concentrations and the control wells. The eggs were incubated in the albendazole at 27° C for 48 h after which a drop of Lugol's iodine was added into each well to terminate further development of the eggs if any. The wells were observed under the microscope for dead or embryonated eggs and hatched out larvae were counted, and each category enumerated. The experiment was at each time of sampling set up in triplicates. Thereafter, Probit analysis (Finney, 1971) in SPSS version 20 for windows was carried out to determine the concentration of Albendazole (LC50) that prevented 50% of the eggs in a particular setup from hatching. Eggs having LC50 values of 0.1 µg/ml were suspected of anthelmintic resistance as described in Le Jambre (1976) and Coles *et al.* (1992). The probit

percentage egg hatch in each well was plotted against respective Log<sub>10</sub> concentration of albendazole in that well to obtain a drug response curve.

**RESULTS**

The study recorded an overall prevalence rate of 94.6% for GI trichostrongyloid parasitic infection in the goats with 95% confidence interval between 91.0 and 96.8% respectively as shown in Table 1. The results of the larval identification and count as recorded in Table 2, indicate that 67.79 % of the recovered larvae were those of *H. contortus* with a Mean count of 1200 ± 129.00 L3/ml. The proportion of larvae that belong to *Trichostrongylus colubriformis* and *Oesophagostomum* spp were 25.52% and 4.79% respectively with Mean counts of 430 ± 0.48 and 87 ± 0.19 L3/ml respectively.

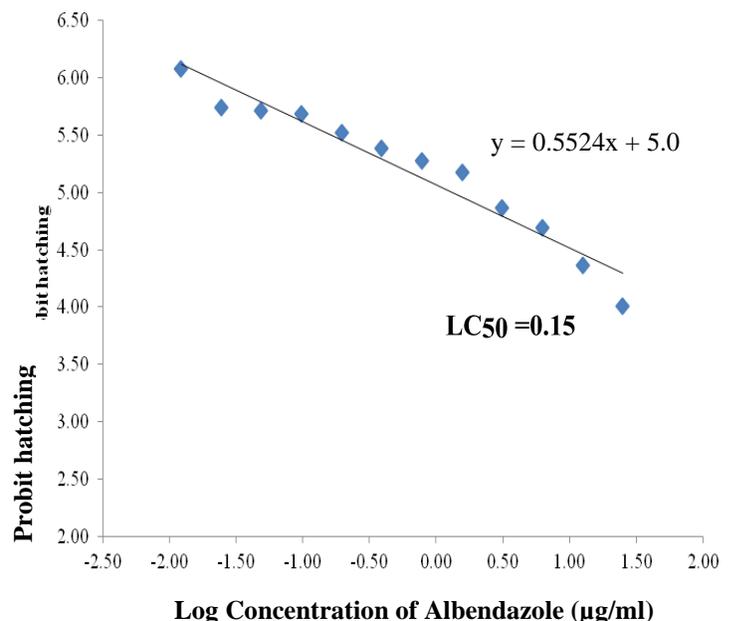
**Table 1: Prevalence of GI trichostrongyloid nematode parasites of goats presented for slaughter at the Nsukka municipal abattoir.**

No examined	No positive	Prevalence (%)	Lower 95% CL	Upper 95% CL
240	227.0	94.6	91.0	96.8

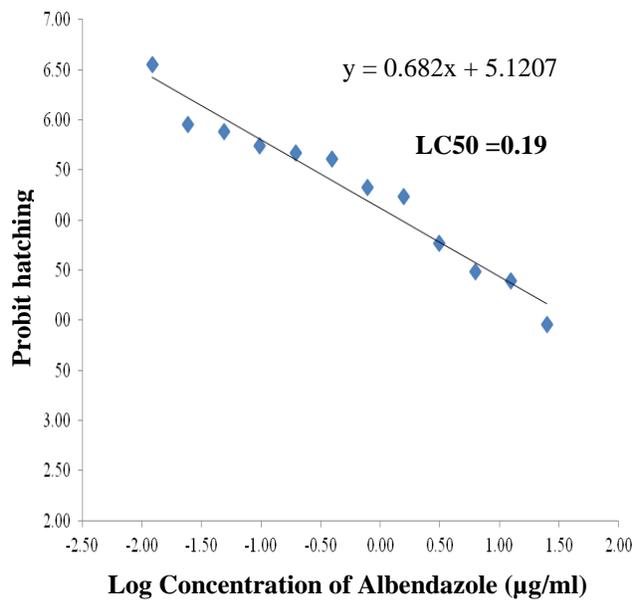
In the EHA, Albendazole had an overall mean LC50 value of 0.16 ± 0.01µg/ml, following a Probit analysis of the Log-dose response of albendazole on the strongyle eggs. Specifically, on monthly bases, albendazole had LC50 value of 0.15, 0.19, 0.11 and 0.18 µg/ml of albendazole for the assays performed in the months of March, April, May and June respectively as shown in Figures 1- 4

**Table 2: Species of nematodes determined by culture of the faeces obtained from goats presented for slaughter at the Nsukka municipal abattoir.**

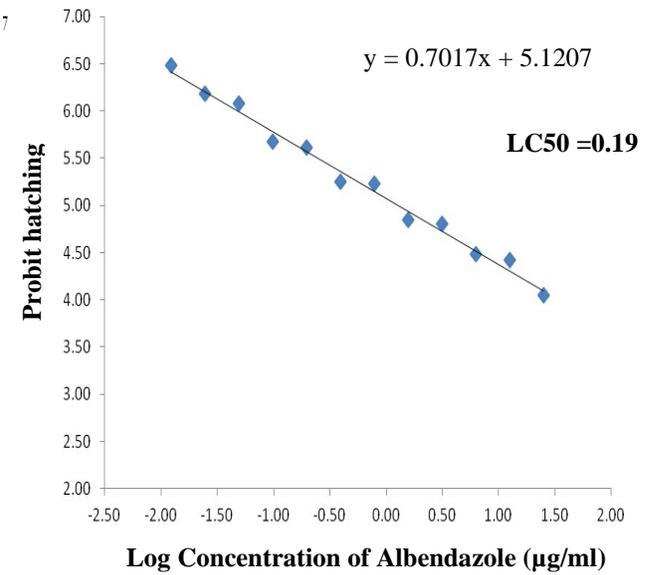
Nematode species	Mean larval count ± SEM (× 10 <sup>2</sup> )	Proportion of recovery (Mean% ± SEM)
<i>H. contortus</i>	12.00 ± 1.29	69.79 %
<i>T. colubriformis</i>	4.30 ± 0.48	25.52 %
<i>Oesophagostomum</i> sp	0.87 ± 0.19	4.79 %



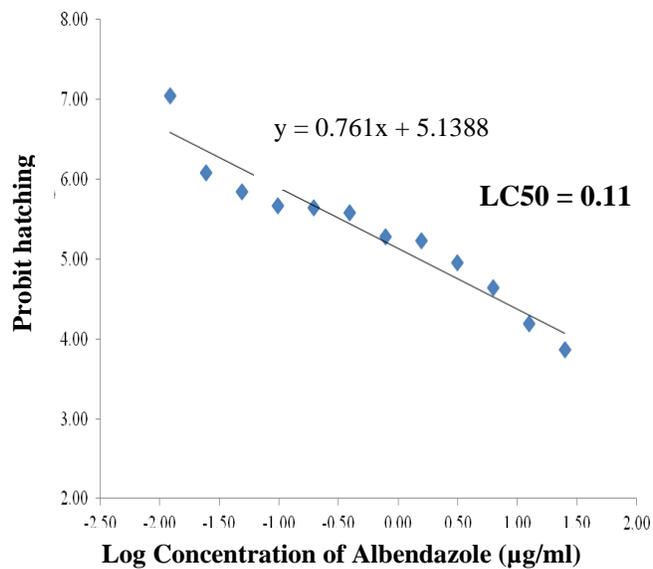
**Figure 1: Log-dose Probit response line of Albendazole on the strongyle eggs harvested from goats presented for slaughter at Nsukka municipal abattoir for the month of March.**



**Figure 2:** Log-dose Probit response line of Albendazole on the strongyle eggs harvested from goats presented for slaughter at Nsukka municipal abattoir for the month of April, 2016



**Figure 3:** Log-dose Probit response line of Albendazole on the strongyle eggs harvested from goats presented for slaughter at Nsukka municipal abattoir for the month of May, 2016.



**Figure 4:** Log-dose Probit response line of Albendazole on the strongyle eggs harvested from goats presented for slaughter at Nsukka municipal abattoir for the month of June, 2016.

## DISCUSSION

The results obtained in this study have demonstrated that trade goats at Nsukka Municipal abattoir, in Enugu State, Nigeria are commonly infected with GI trichostrongyloid nematodes. The study also showed the predominance of *Haemonchus contortus* over other GI nematodes in the goats. These agree with a previous study by Idika *et al.* (2012) in the same study area which obtained a prevalence of 96.2% for GI trichostrongyles and identified *H. contortus* as the most prevalent nematode in goats presented for slaughter at the Nsukka municipal abattoir. *Haemonchus contortus* is often reported as the most important and predominant parasite in field outbreaks of parasitic gastroenteritis (PGE) in Nigeria (Schillhorn van Veen, 1973; Chiejina, 1987).

The EHA results showed that the albendazole had an overall mean LC50 value of 0.16 µg/ml (range: 0.11 - 0.19 µg/ml) against GI trichostrongyloid nematode of the goats. The *in vitro* Egg Hatch Assay (EHA) is widely used to detect resistant strongyle nematodes in different livestock species (Coles *et al.*, 1992). According to the guideline of the World Association for the Advancement of Veterinary Parasitology (WAAVP) on diagnosing AR, eggs having LC50 value in excess of 0.1 µg of a benzimidazole anthelmintic per ml following an EHA are indicative of AR (Coles *et al.*, 1992). It was therefore, assumed that the trichostrongyle worm population in the goats could be on the verge of developing resistance against Albendazole, as the LC50 values obtained in the present study was marginally in excess of the reference 0.1 µg/ml.

According to Dobson *et al.* (1996), development of AR by nematodes usually begins with an

initial phase of light insusceptibility to anthelmintics, characterized by a few numbers of resistant individual worms within the parasite population. This is usually followed by the intermediate phase, characterized by an increase in the frequency of heterozygous resistant individuals within the population, as a result of selection pressure imposed by continued exposure to the same drug group. Finally, the resistant phase, whereby sustained selection pressure results in the predominance of homozygous resistant individuals within the parasite population. The speed at which this process occurs is determined by the intensity of the selection pressure on the parasite population. The results obtained in the present study suggest that the trichostrongyloid nematode population in the goats could be in its early stage of AR development. It is feared that the situation could be more severe than assumed, given that GI nematodes develop AR more rapidly in goats than other livestock species (Waller, 1994; Domke *et al.*, 2011).

Albendazole is a frequently used anthelmintic in Nigeria due to its availability and affordability. In Nigeria, anthelmintics can be purchased without any prescription from a qualified veterinarian and thus prone to abuse and indiscriminate use by farmers. These farmers are also in the habit of instituting anthelmintic treatment without any association with a particular parasite or confirmed diagnosis of nematode infection. Therefore, with its frequent and sometimes haphazard use it is possible that the nematodes are being selected for resistance to albendazole. Such haphazard use of the drug either by too frequent treatment or under dosing are known factors that contribute to the development of benzimidazole resistance either

by increasing the pressure for selection of benzimidazole-resistant alleles or the mutation of genes that confer benzimidazole resistance (Humbert *et al.*, 2001; Sylvester and Humbert, 2002).

## CONCLUSION AND RECOMMENDATIONS

The data obtained in this study confirmed *H. contortus* as the most prevalent GI nematode of the trade goats in Nsukka. More importantly, the study suggests that the GI strongyle population of goats slaughtered at the Nsukka abattoir could be at a critical stage of development of AR. Nevertheless, more confirmatory tests such as the faecal egg count reduction test, controlled efficacy test and molecular assays are required for confirmation. It is therefore, recommended that careful consideration be given to worm control measures for sheep and goats, and regular monitoring of faecal egg output be conducted to evaluate the effectiveness of control programmes in small ruminant farms. Diagnosis and surveillance of AR are essential for its management (Sangster, 1999), hence the need to conduct routine and coordinated surveys to monitor and detect AR nematodes in the field using both basic and molecular parasitological tools.

**Conflict of Interest:** The authors hereby state that they have no conflict of interest regarding the content of the paper

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