***УДК** 616:576.8*

EFFICACY OF IVERMECTIN AGAINST GASTROINTESTINAL NEMATODES OF GOATS IN THE SUB-HUMID SAVANNA ZONE OF NIGERIA

© 2022 I. K. Idika[a], S. U. Asogwa[a], C. F. Obi[a*], C. O. Nwosu[a]

[a]Department of Veterinary Parasitology & Entomology, Faculty of Veterinary Medicine,
University of Nigeria,
Nsukka, Enugu State, Nigeria
*e-mail: chukwunonso.obi@unn.edu.ng

Received 25.01.2022
Received in revised form 03.04.2021
Accepted 08.04.2021

Faecal egg count reduction test (FECRT) was used to determine the efficacy of ivermectin in eight purposively selected goat farms with history of no anthelmintic treatment for a 2-month period. Faecal egg counts (FEC) were conducted on faecal samples collected per rectum from individual goats prior to treatment with ivermectin, and the egg per gram (EPG) of faeces recorded as the Pre-treatment FEC (PRFEC) for each animal. A second FEC was carried out on the same animals on day 14 post anthelmintic treatment and the EPG recorded as post treatment FEC (PTFEC). Ivermectin resistance was confirmed where the fecal egg count reduction (FECR) percentage was less than 95% and the lower 95% confidence limit (LCL) was less than 90% but if only one of the two criteria was met, resistance was suspected. Faecal samples were also pooled and cultured for larval identification and count. FECRT results showed that ivermectin treatment produced >95% reduction of the PRFEC in 5 farms and <95% reduction in 3 farms. Ivermectin resistance was suspected in two goat farms but was confirmed in one goat farm on the basis of 95% LCL and FECRT. Larval identification showed the occurrence of *Haemonchus contortus* (79%), *Trichostrongylus colubriformis* (17.5%) and *Oesophagostomum* (3.5%). The study revealed efficacy of ivermectin against GI nematode parasites of small ruminants in the study area at the level FECRT, resistance, however, was suspected and confirmed in 2 and 1 farms, respectively.

Keywords: resistance, GI nematodes, Nigeria, ivermectin, small ruminants

DOI: 10.31857/S0031184722030024, **EDN:** FFQLYS

Small ruminants are very important in poverty alleviation, especially in developing economies (Elzaki et al., 2019), contributing majorly to food security, in addition to their other cultural and religious values. Economically, small ruminant production compares favourably with other livestock, as they require little initial capital to set up. However, threats by diseases, particularly, gastrointestinal nematode (GIN) parasitism remain a major constraint to profitable small ruminant enterprise, especially in the developing countries with serious economic consequences (Biffa et al., 2006; Idika et al., 2012). Nevertheless, prophylactic and more so, therapeutic use of broad spectrum anthelmintics help in maximizing productivity of grazing livestock, including small ruminants as they diminish the effects of these parasites in these animals. However, overtime usage of anthelmintics could lead to emergence of worm strains that resist and survive exposure to therapeutic doses of the drugs. Resistance to anthelmintic drugs is a growing concern and threat to the beneficial and effective use of anthelmintics, as it militates against continued efficacy and sustainable use of the drug, and by implication, profitable livestock farming.

Anthelmintic resistance has been reported all over the world, under various climatic conditions, but more in areas with high annual number of circles of infection, requiring several anthelmintic treatments per year (Han et al., 2017; Arsenopoulos et al., 2021). Nevertheless, resistance to anthelmintics could be acquired by nematode parasites after only a few treatments (Ihler, 2010). The rapid rates of nucleotide sequence evolution seen in some parasitic nematodes of veterinary importance and their extremely large effective population sizes that give the worms an exceptionally high level of genetic diversity are genetic features that favour development of anthelmintic resistance in these parasites (Jimenez Castro et al., 2019). Anthelmintic resistance affects all classes of anthelmintics including macrocyclic lactones, of which ivermectin is the most popular.

A number of drugs are available to control gastrointestinal nematode infestation in small ruminants in Nigeria, however, ivermectins are among the most commonly used. Ivermectins are preferred owing to their effectiveness against a wide range of nematodes and ectoparasites such as lice, mites, and ticks (Campbell, 1989) as well as their wide safety margin (González Canga et al., 2008).

The present study was therefore designed to determine the ivermectin resistance status of GIN of goats in Nsukka Local Government Area (LGA) of Enugu State, Nigeria using the Faecal Egg Count Reduction Test (FECRT). The study has become necessary considering the economic importance of GINs in small ruminants and the widespread use of ivermectin both in treatment and prophylaxis in small ruminant production in the study area and Nigeria in general.

MATERIALS AND METHODS

Study area

The study was conducted in Nsukka Local Government Area, a sub-humid derived savannah zone in Enugu State, lying approximately between longitude 6°52′–7°53′ E and latitude 6°38′–7°8′ N. Small ruminant farming, especially goats is very common in the area, where they are reared mainly as a subsidiary rural agricultural activity.

Study design

The study was conducted using 80 West African Dwarf (WAD) goats from eight purposively selected farms. Ten goats were randomly selected in each of the farms designated 1-8. Farms with faecal egg counts above 100 eggs per gram of faeces (epg), and whose goats did not receive any anthelmintic treatment for at least two months prior to the study were included in the study. Pregnant and nursing animals, as well as suckling kids were excluded. Ivermectin susceptibility or resistance was based on the faecal egg count reduction (FECR) percentage and the confidence limits.

Anthelmintic susceptibility test

The individual body weights (kg) of the randomly selected goats per farm were determined using a weigh band (we-bo®). Egg counts were conducted on faecal samples collected per rectum from individual goats in each farm prior to treatment with ivermectin, and the egg per gram (EPG) of faeces recorded as the Pre-treatment FEC (PRFEC) for each animal. Thereafter, the goats were given 1% ivermectin (Ivermectine®) at the dose of 0.02 mg/Kg as recommended by the manufacturer. A second FEC was carried out on the same animals on day 14 post anthelmintic treatment and the EPG recorded as post treatment FEC (PTFEC). All faecal egg counts were carried out by the modified McMaster counting technique (MAFF, 1997). Left over faecal samples for the pre- and post-treatment FECs were pooled and routinely cultured for larval identification and count (Hansen, Perry, 1994). The FECR (%) was thereafter calculated using the formula 100 (1-[T2/T1]) (Mckenna, 2006), where T1 and T2 represent the pre- and post-treatment FEC respectively. The confidence limits were calculated as described by Dobson et al. (2012). Ivermectin resistance was confirmed where the FECR percentage was less than 95 % and the lower 95 % confidence limit was less than 90 % but if only one of the two criteria was met, resistance was suspected.

Ethical Approval

The University of Nigeria, national and international guidelines for the ethical care and use of laboratory animals were fully adhered to. Also, appropriate ethical clearance/approval was gotten from the Ethics Committee for Medical and Scientific Research of the University of Nigeria, Nsukka (UNN/FVM/VPE/2592). The informed consent of the farmers were duly sought for and obtained.

RESULTS

An overall GIN prevalence of 67.5% among the 8 selected goat farms has been demonstrated (Tab. 1). Culture and larval identification of pooled pre-treatment faecal samples (Tab. 2) indicate that 79.0% of the recovered worm eggs were those of *Haemonchus contortus* (Rud.) 1915 with a mean larval count of 700 ± 49.01 L_3/ml. The proportion of the recovered eggs that belonged to *Trichostrongylus colubriformis* (Giles, 1892) and *Oesophagostomum* spp. were 17.49 and 3.45 % respectively with mean counts of 155 ± 19.0 and 31.0 ± 4.3 L_3/ml, respectively. The culture and larval identification of post treatment faecal samples showed that 100 % of recovered strongyle eggs were those of *H. contortus* (Tab. 2).

Table 1. Prevalence of GI trichostrongyloid nematodes in 8 selected goat farms in Sub-humid Savanna Zone of Nigeria

No. Examined	No. Infected	Prevalence
80	54	67.5%

Table 2. Species of nematodes determined by culture of pooled faeces obtained from selected goat farms in Sub-humid Savanna Zone of Nigeria

Nematode species	Larval count (Mean ± SEM) (x 10^2)	Proportion of recovery (Mean ± SEM), %
Pre-treatment faecal samples		
Haemonchus contortus	7.00 ± 0.49	79.00
Trichostrongylus colubriformis	1.55 ± 0.19	17.49
Oesophagostomum spp.	0.31 ± 0.04	3.50
Post treatment faecal samples		
Haemonchus contortus	0.40 ± 0.09	100
Trichostrongylus colubriformis	Nil	
Oesophagostomum spp	Nil	

Table 3 shows the mean pre- and post- treatment FEC of the goats in different farms. The animals possesed mean pre-treatment FEC that ranged between 179.00 ± 69.29 and 490.00 ± 165.03 epg and post-treatment FEC of 0.67 ± 0.67 and 37.67 ± 37.17.25%. The results of the FECRT showed that a single dose treatment with ivermectin produced >95% reduction of the PRFEC in 5 farms, namely, farms 1, 3, 4, 5, and 7 with their 95% LCL above 90%. Consequently, the ivermectin produced <95% reduction in 3 farms, namely, farms 2, 6, and 8. However, the 95% LCL was less than 90% in farm 6 and above 90% in farms 2 and 8. Therefore, resistance was suspected in farms 2 and 8 but present in farm 6 on the basis of the FECRT. Consequently, 62.5% of the farms sampled were susceptible to ivermectin, 12.5% were resistant while 25% had GIN parasites that suspected of resistance to ivermectin.

Table 3. Mean faecal strongyle-egg count reduction percentage in WAD ruminants treated with ivermectin 0.02 mg/Kg in Sub-humid Savanna Zone of Nigeria

Farms	PREFEC	PTFEC	FECR	LCL	UCL	Resistance status
1	335.00 ± 70.30	4.00 ± 2.12	98.81	96.30	99.68	Susceptible
2	490.00 ± 165.03	27.33 ± 27.33	94.42	91.21	96.60	Suspected
3	186.67 ± 107.29	1.00 ± 1.00	99.46	96.11	99.94	Susceptible
4	393.33 ± 158.36	0.67 ± 0.67	99.83	98.30	99.99	Susceptible
5	316.67 ± 192.73	3.00 ± 3.00	99.05	96.59	99.79	Susceptible
6	433.33 ± 197.01	37.67 ± 37.17	91.31	87.26	94.26	Resistant
7	179.00 ± 69.29	2.33 ± 1.20	98.70	94.64	99.75	Susceptible
8	400.00 ± 165.03	24.33 ± 24.33	93.92	90.18	96.40	Suspected

DISCUSSION

The present study showed that goats in Nsukka LGA of Enugu State, Nigeria are commonly infested with GI trichostrongyloid nematodes with 67.5 % prevalence rate. Larval identification revealed the predominance of *Haemonchus contortus* above other GI nematode parasites in goats in the study area. These findings collaborate with prevalence rates of 70–90 % of GI nematode infections of small ruminants, been reported by previous studies (Chiejina, 1986; Idika et al., 2012) in the study area, of which *Haemonchus contortus* has consistently been predominant. Nsukka is situated within the derived Savannah zone of Nigeria, with annual temperature range of 16 to 30°C (rarely below 12 and above 32°C) and 9.3 months (mid February to late November) of rainfall that support survival and development of pre-parasitic stages of nematodes almost all year round. Hence, grazing livestock in the area is usually associated with high annual number of circles of infection per year, with several anthelmintic treatments per year. The implication of such frequent treatment especially with a particular type of anthelmintics resulted in the unwanted emergence of nematode populations with heritable ability to resist anthelmintic doses. In the study area, ivermectin is the most commonly used anthelmintic for treatment of small ruminants. Campbell (1989) reports that ivermectin is a drug of choice in small ruminants, due to its wide margin of action, been effective against endo- and ecto-parasites, as well as its wide safety margin.

It was a core objective of the present study to provide information on the state of ivermectin resistance by GI trichostrongyloid nematodes of goats in the study area, using the Faecal egg count reduction test (FECRT). The study therefore, identified varying degrees of efficacy and possible presence of ivermectin resistance. The results of the present study showed that at the recommended therapeutic dose of 0.02 mg/kg, ivermectin produced over 95% reduction of the pre-treatment FECs in five farms. Therefore, ivermectin was efficacious in these farms and their gastrointestinal nematode (GIN) parasite population judged susceptible to ivermectin. On the other hand, the GIN population in farm 6 was assumed resistant to ivermectin on the basis of a less than 95% FECR produced by the ivermectin with a 95% LCL of <90%. However, ivermectin resistance was suspected in farms 2 and 8 given that the FECR percentage in these farms were less than 95% (94.42 and 93.92% respectively) but the 95% LCLs were >90% (96.6 and 96.4% respectively).

Frequent use and misuse of antiparasiticides, especially diminazene, ivermectin, and albendazole by farmers and animal attendants in the study area was reported following a study by Obi et al. (in press). The ivermectin resistance noted in this study was attributed to frequent and over usage of the drug, given that ivermectin serves dual purposes been effective against nematode- and ecto-parasistes. Farmers and veterinarians often use ivermectins against ectoparasites in animals including without any iota of regard to their nematode parasites in these animals. Ectoparasitism, particularly mite infestation is very common among small ruminants in the study area, requiring frequent treatment with acaricides of which Ivermectin is widely used due it its efficacy, wide safety margin, cost effectiveness and ease of administration. This invariably imposes continuous selection pressure on nematode strains in favour of resistant genes. Such frequent and often unnecessary uses of anthelmintics with respect to nematode parasites are known risk factors for the development of anthelmintic resistance (Shalaby, 2013).

Culture of pooled post treatment faecal samples suggests that the observed ivermectin resistance was demonstrated by *H. contortus.* This worm is known to have a high propensity to develop resistance (Redman et al., 2012). *Haemonchus contortus* is the most important GI nematode of goats in the study area, with very high daily egg output, rapid build-up of infective L_3 on pasture under suitable climatic conditions, high establishment rate and very short latent period (Chiejina, 1986; Mghomga et al., 2012). Therefore, the result of the present study calls for great caution with the use of ivermectins in goats. Coles et al. (2006) report that early stages of anthelmintic resistance usually go unnoticed as the anthelmintics may still be effective, however, complete efficacy is lost when resistance reaches higher levels either in individual host or proportion of affected worm population. It is also important to note that resistance to an anthelmintic by GI nematodes could lead to resistance to other compounds with similar mode of action, irrespective of whether or not, the nematode has been exposed to that particular anthelmintic. Resistance to ivermectin in small ruminants has been reported in so many countries under different farm management. Adediran, Uwalaka (2015) reported a low level of resistance against ivermectin in West African Dwarf goats. Likewise, Dey et al. (2020) reported resistance to ivermectin as well as to levamisole and albendazole in sheep and goats in Bangladesh.

CONCLUSION

This study confirmed and suspected ivermectin resistance in 1 and 2 farms respectively, out of the 8 farms studied at the level of FECRT. The importance of this finding in the study area cannot be over-emphasized given that many veterinarians and animal handlers in the area rely heavily on ivermectin for treatment against GI nematode- and ectoparasites in small ruminants. The FECRT, though highly recommended for detecting anthelmintic resistance in farm animals, (Coles et al., 1992), lack sensitivity as it only detects anthelmintic resistance in populations were more than 25% of worms are resistant (Domke et al., 2012). This, therefore, calls for great caution on the use of ivermectin in the study area to limit the pressure imposed on the selection for resistant genes. Molecular detection technique is however required for further confirmation of the ivermectin resistant status of small ruminants in the area.

REFERENCES

Adediran O.A., Uwalaka C.E. 2015. Effectiveness evaluation of levamisole, albendazole, ivermectin, and Vernonia amygdalina in West African Dwarf Goats. Journal of Parasitology Research 5 p. https://doi.org/10.1155/2015/706824

Arsenopoulos K.V., Fthenakis G.C., Fthenakis E.I., Papadopoulos E. 2021. Haemonchosis: a challenging parasitic infection of sheep and goats. Animals 11: 363. https://doi.org/10.3390/ani11020363

Biffa D., Jobre Y., Chakka H. 2006. Ovine helminthosis, a major health constraint to productivity of sheep in Ethiopia. Animal Health Research Reviews 7 (1-2): 107–118. https://doi.org/10.1017/S1466252307001132

Campbell W.C. 1989. Ivermectin and Abamectin. New York, Springer-Verlag, 363 pp.

Chiejina S.N. 1986. The epizootiology and control of parasitic gastroenteritis of domesticated ruminants in Nigeria. Helminthological Abstracts 55: 413–429.

Coles G.C., Borgsteede F.H., Geerts S., Klei T.R., Taylor M.A., Walle P.J. 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology 44: 35–44. https://doi.org/10.1016/0304-4017(92)90141-U

Coles G.C., Jackson F., Pomroy W.E., Prichard R.K., von Samson-Himmelstjerna G., Silvestre A., Taylor M.A., Vercruysse J. 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology 136: 167–185. https://doi.org/10.1016/j.vetpar.2005.11.019

Dey A.R., Begum N., Anisuzzaman Alim A., Alam M. 2020. Multiple anthelmintic resistance in gastrointestinal nematodes of small ruminants in Bangladesh. Parasitology International 77 : 102105. https://doi.org/10.1016/j.parint.2020.102105

Dobson, R.J., Hosking, B.C., Jacobson, C.L., Cotter, J.L., Besier, R.B., Stein, P.A., Reid, S.A. 2012. Preserving new anthelmintics: A simple method for estimating faecal egg count reduction test (FECRT) confidence limits when efficacy and/or nematode aggregation is high. Vet. Parasitol. 186, 79–93. https://doi.org/10.1016/j.vetpar.2011.11.049

Domke A.V.M., Chartier C., Gjerde B. et al. 2012. Prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep and goats in Norway. Journal Parasitology Research 111: 185–193. https://doi.org/10.1007/s00436-012-2817-x

Elzaki R., Abdalla S., Al-Mahish M. 2019. Small ruminants as a pathway to reduce urban poverty: An empirical analysis of Sudan. Veterinary world 12 (12): 2017–2024. https://doi.org/10.14202/vetworld.2019.2017-2024

González Canga A., Sahagún Prieto A.M., Diez Liébana M.J., Fernández Martínez N., Sierra Vega M., García Vieitez J.J. 2008. The pharmacokinetics and interactions of ivermectin in humans--a mini-review. The AAPS Journal 10 (1): 42–46. https://doi.org/10.1208/s12248-007-9000-9

Han T., Wang M., Zhang G., Han D., Li X., Liu G., Li X., Wang Z. 2017. Gastrointestinal nematodes infections and anthelmintic resistance in grazing sheep in the Eastern Inner Mongolia in China. Acta Parasitologica 62 (4): 815–822. https://doi.org/10.1515/ap-2017-098

Hansen, J., Perry, B. 1994. The epidemiology, diagnosis and control of helminth parasites of ruminants a hand book. International Library of Research in Animal Diseases, Nairobi, Kenya, 62–72.

Idika I.K., Iheagwam C.N., Ezemonye C.N., Nwosu C.O. 2012. Gastrointestinal nematodes and body condition scores of goats slaughtered in Nsukka, Nigeria. Nigeria Veterinary Journal 33 (1): 440–447.

Ihler C.F. 2010. Anthelmintic resistance. An overview of the situation in the Nordic countries. Acta Veterinaria Scandinavica 52: 24. https://doi.org/10.1186/1751-0147-52-S1-S24

Jimenez Castro P.D., Howell S.B., Schaefer J.J. et al. 2019. Multiple drug resistance in the canine hookworm *Ancylostoma caninum*: an emerging threat? Parasites and Vectors 12: 576. https://doi.org/10.1186/s13071-019-3828-6

MAFF 1977. Manual of veterinary laboratory diagnostic techniques. Bulletin Number 18. Ministry of Agriculture Fisheries and Food. London, HMSO, 5–50.

McKenna P.B. 2006. Further comparism of fecal egg count reduction test procedure: sensitivity and specificity. New Zealand Veterinary Journal 54: 365–366. https://doi.org/10.1080/00480169.2006.36726

Mhomga L.I., Nnadi P.A., Chiejina S.N., Idika I.K., Ngongeh L.A. 2012. Effects of dietary protein supplementation on the performance of West African dwarf (WAD) goats infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*. Turkish Journal of Veterinary and Animal Science 36 (6): 668–675. https://doi.org/10.3906/vet-1106-21

Redman E., Sargison N., Whitelaw F., Jackson F., Morrison A., Bartley D.J., Gileard S.J. 2012. Introgression of ivermectin resistance genes into a susceptible *Haemonchus contortus* strain by multiple backcrossing. PLOS Pathogens 8 (2): e1002534. https://doi.org/10.1371/journal.p11pat.1002534

Shalaby H.A. 2013. Anthelmintics resistance; how to overcome it? Iranian journal of parasitology 8 (1): 18–32.

ЭФФЕКТИВНОСТЬ ИВЕРМЕКТИНА ПРОТИВ НЕМАТОД ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА У КОЗ В СУБГУМИДНОЙ ЗОНЕ САВАННЫ В НИГЕРИИ

И. К. Идика, С. У. Асогва, Ц. Ф. Оби, Ц. О. Нвосу

РЕЗЮМЕ

Тест на уменьшение подсчитанных яиц в фекалиях (FECRT) был использован для определения эффективности применения ивермектина в восьми специально отобранных козьих фермах, в которых антигельминтные мероприятия не проводились, по крайней мере, в течение 2 месяцев. Подсчеты яиц в фекалиях (FEC) проводили в образцах, собранных per rectum от отдельных особей коз, и обозначенных как предшествовавшие опытам (PRFEC) для каждого животного отдельно. Повторный тест (FEC) был выполнен на тех же самых животных на 14-й день после использования антигельминтного препарата и обозначен как PTFEC. Резистентность к ивермектину считалась подтвержденной, когда коэффициент FECRT был меньше 95%, а нижний доверительный предел (LCL) составлял менее 90%, но если был выявлен только один из критериев, резистентность только подозревалась. Образцы, полученные из фекалий, были объединены и культивированы для определения личинок и их подсчета. Результаты теста FECRT показали, что обработка ивермектином привела к более чем 95% уменьшению PRFEC на пяти козьих фермах и менее чем 95% уменьшению на трех фермах. Резистентность к ивермектину находилась под подозрением на двух козьих фермах, но была подтверждена на единственной ферме, на основании 95% LCL и FECRT. Определение личинок показало, что среди гельминтов присутствовали *Haemonchus contortus* (79%), *Trichostrongylus colubriformis* (17.5%) и *Oesophagostomum* (3.5%). Исследование показало эффективность использования ивермектина против желудочно-кишечных нематод, паразитов мелкого рогатого скота на исследованной территории на уровне FECRT, при этом, однако, резистентность к ивермектину была обнаружена у животных с одной фермы, и предположительно подозреваема у животных с двух ферм.