



## Multiple Drug Resistance of Four Anthelmintics Common in Use in Sheep in Sudan

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### ABSTRACT:

Following several reports indicating reduced efficacy of some anthelmintics in sheep in different parts of the Sudan; faecal egg count reduction test was used to evaluate therapeutic efficacy of four anthelmintics common in use in sheep health care procedures. In the current study, 16 male sheep with confirmed gastrointestinal nematodes (GINs) infection were used to evaluate the efficacy of Albendazole 2.5% drench formulation, Ivermectin 0.8% drench formulation, Tetramisole 5%, and Levamisole 2.5%. Animals were divided into two groups each of eight animals; sheep in the first group (A) were drenched orally with Albendazole at 5mg/kg body weight (bwt) as single dose, while animals in the second group (B) received Ivermectin oral solution at dose rate of 0.2mg/kg bwt. Faecal samples were collected at the following intervals: 0 (before treatment), and then at 2, 3, 4, 7, 10, 14, and 21 days (post treatment). Samples of the drugs (the same batch) were randomly selected and tested before the start of the experiment to ensure satisfaction of finished product specification. Results obtained indicated that there is no evidence of therapeutic efficacy in the two treatment groups (Albendazole and Ivermectin). One week later same animals were re-arranged again into two treatment groups each of eight sheep. Animals in the first group (C) were treated with Tetramisole 5% at dose rate 3mg/kg bwt and animals in the second group (D) were treated with Levamisole 2.5% at dose rate 7.5mg/kg bwt. Results indicated that the two anthelmintics (Tetramisole 5% and Levamisole 2.5%) appeared equally ineffective as the first two drugs (Albendazole 2.5% and Ivermectin 0.8%). Likewise, there were no significant reductions in egg count in the four drugs used in the current study. These findings represent the first report in Sudan from the field of multiple anthelmintics resistance having developed in more than one drug after being administered to sheep. The study recommends further evaluation of the status of anthelmintic resistance in sheep using *in vitro* methods and molecular techniques.

**Keywords:** sheep, anthelmintics resistance, albendazole, ivermectin, levamisole

### Introduction

Parasitic gastroenteritis is a frequent problem in small ruminants, interfering with animal wellbeing, and causing considerable economic losses (Sargison, 2012). Over the

years, parasitic diseases in livestock have been controlled based on the use of broad-spectrum and widely available antiparasitic drugs (Coles *et al.*, 2006). The momentousness of anthelmintics is to control these parasitic nematodes in order to

allow welfare and productivity of animals broadly and effectively (Mckellar and Jackson, 2004). The use of anti-parasitic medicines in farm animals over the years offers benefits; however, the continuous and indiscriminate use of these medicines has resulted in the reduction and/or loss of effectiveness of many active ingredients (Molento, 2009, Salgado and Santos, 2016). Anthelmintics resistance has been a significant issue for sheep and goat's farmers worldwide for many years (Kaplan, 2004). Resistance to anthelmintics over years has been evaluated and defined by many scientists according to their findings or susceptibility reduction to anthelmintics, the earliest one was that of Prichard *et al.*, (1980) and later on has been adopted by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) as "Resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species, and is heritable".

Multiple resistances describe the type of efficacy reduction; regularly involve 2 or more different anthelmintic groups, arise from use each group alone or from cross resistance (Prichard *et al.*, 1980), recently the prevalence of Multiple Drug Resistance (MDR) increase to incredible limits worldwide to involve not only the small ruminant but extend to cattle and horses, except doesn't reach the same levels (Kaplan, 2004, Gelot *et al.*, 2016). Resistance to ivermectin has been established among ruminants by many researchers in different countries: UK by Echevarria *et al.*, (1992), in Denmark (Maingi *et al.*, 1996a), Spain (Álvarez -Sanchez *et al.*, 2006). Along Indian sub-continent many records have documented reduction of ivermectin efficacy (Ranjan *et al.*, 2002, Makvana and Veer, 2009; Jaiswal *et al.*, 2013).

Also Sargison *et al.* (2001) reported that in the United Kingdom resistance to benzimidazole, imidazothiazole and macrocyclic lactones has appeared on a sheep farm infected with *Ostertagia* spp. Multi reduction of efficacy has been well-established in Brazil on sheep farm for albendazole, Levamisole and combination of Leva with ABZ and ivermectin (Echevarria *et al.*, 1996).

In Sudan, the use of anthelmintic treatment in the control of helminths infection in sheep was popular among animal owners. Several products are in use, among them: Albendazole, Ivermectin, Levamisole, and Tetramisole were the most commonly used according to importation records (NMPB, 2017). There is immense and/or indiscriminate use of Albendazole and Ivermectin by sheep owner's which may affect wide in the whole map of the treatments in the country and may lead to emergence of anthelmintic resistance, this of course will justify the current work.

Sporadic reports concerning the status of anthelmintics resistance in sheep in Sudan are available; most of these research works indicated the presence of treatment failure in Sudan throughout decades. Eldabbagh, (2009), used *in-vitro* tests viz.: larval paralysis assay (LPA) to report on anthelmintic resistance; results obtained indicated the presence of resistance for Levamisole and no resistance was detected for abamectin. While in egg hatch assay (EHA), the ED<sub>50</sub> showed resistance for Ivermectin, Doramectin, and for Levamisole. But Albendazole and Abamectin showed no resistance.

In the current study we attempted to evaluate therapeutic efficacy of Albendazole, Ivermectin, Levamisole and Tetramisole against helminths infections in sheep naturally infected with gastrointestinal

nematodes using faecal egg count reduction test (FECRT).

### Materials and Methods

**Study area:** This study was conducted in the farm of the College of Veterinary Medicine, Sudan University of Science and Technology. The farm is located in Hillat Kuku, East Nile Locality, Khartoum State, Sudan.

**Experimental animals:** A total number of 16 male sheep (local breed) was used. They were 8-12 months of age. Animals were purchased from *Elaaelafoon* Market, East Nile Locality, Khartoum State, Sudan.

**Animal housing and feeding:** Animals were housed in individual pens with dimensions of 2X1.5 meters, during the experimental part of the study. Animals were supplemented with sorghum straw and calculated amount of Dura maize, and tap water *ad libitum*.

**Experimental drugs:** Four different drugs were used in the current study viz.:

1. Albendazole 2.5% drench formulation: Albendazole® 25 mg Suspension from Kela Laboratoria NV, Belgium.
2. Ivermectin 0.08 % drench formulation: Intermectin®-0.8 mg oral solution from Interchemie werken “De Adelaar”, Holland.
3. Tetramisole 5% drench formulation: Tetrapam-L® 5% oral solution from Bash Pharma Pharmaceutical, Sudan.
4. Levamisole 2.5% drench formulation: Levozide® Drench 2.5% Suspension from Punjab Drugs House (P. D. H) Laboratories, Pakistan.

**Drugs testing:** All drugs utilized in the current study were subjected to chemical analysis before the start of the experimental part of the study to detect finished product quality (assay) and assure complying with specification. Methods used were either

Pharmacopeia methods (BP) or manufacturer methods (in house). All drugs were analyzed using High Performance Liquid Chromatography (HPLC) (Shimadzu vaphy Shimadzu, liquid chromatography with UV/visible and decide ray-detector, isocratic and low pressure gradient pump and pc control; Japan). Drugs analysis was conducted in the National Medicines Quality Control Laboratory, Khartoum, Sudan.

**Sample selection and collection:** Drug Samples (three samples from each drug) were selected according to National Medicines and Poisons Board (NMPB) importation rate along the year of the study, and collected from different private Veterinary pharmacies in Khartoum state.

**HPLC Method for analysis of Albendazole (BP):** 0.0208g of Albendazole working standard was weighed and dissolved in 100 mL mixture of Sulphoric acid: Methanol (1:100) (w/v). 5mL of the sample (equivalent to 125mg) was transferred to 100mL volumetric flask were dissolved in a mixture of Sulphoric acid: Methanol (1:100) (w/v), stirred for 15 minutes and sonicated for 10minutes to be mixed, then allowed to stand and the supernatant was taken to make another dilution at (1:5) with the same above solvent. As a mobile phase Ammonium dihydrogen orthophosphate (Mobile phase A) 3.4g and Methanol HPLC-Plus gradient (Mobile phase B) 600mL were used, with flow rate of 0.7 mL/minute. The liquid chromatography was equipped with 292 nm and a column C18: (25cm\* 4.6mm). The injection volume was 20µL of standard and sample solutions (BP, 2016).

**HPLC Method for analysis of Ivermectin (non-Pharmacopeia):** 0.0202g of ivermectin working standard was weighed and dissolved in 5mL of methanol, 2mL of the solution was taken and completed to 10mL with the same solvent to obtain concentration equivalent to 8µg/mL. A

volume of the sample equivalent to 8mg was transferred to 10mL volumetric flask where dissolved with 10mL of Methanol, stirred for 15 minutes and mixed with the aid of ultrasound for 10 minutes, a solution with concentration equivalent to 8µg/mL was obtained. As a mobile phase a mixture of (Water: Methanol: Acetonitrile) 120:240:640 (v/v/v) with a flow rate of 1.0mL/minute was used. The liquid chromatography was equipped with 254 nm and C18 (150\*4.6) mm. was heated to 30 °C and the flow rate was 1.0mL/minute for standard and sample solutions (Interchemie werken "De Adelaar", Holland).

**HPLC Method for analysis of Tetramizole content (non-Pharmacopeia):** 0.0312 g Tetramisole HCl working standard was weighed and dissolved in 25 mL of methanol, another dilution was made with solvent of (water: methanol) (50:50) at (2:100); to obtain a solution with a concentration equal to (25µg/mL) of Tetramizole base. 5 mL of the sample (equivalent to 250 mg Tetramizole HCl) was transferred to 50 mL volumetric flask and diluted with 50 mL methanol and shake. 5mL of the solution was transferred to 50 mL volumetric flask and diluted with the same solvent (50 mL). Another dilution was made with other solvent (water: methanol) (50:50) at (1:100), to obtain a solution with concentration (25µg/mL) of Tetramizole base. As a mobile phase a mixture of Methanol, Water, Heptane, sulfonic acid and Phosphoric acid (300:250:0.75:2.5) (v/v/w/v) was used with a flow rate of 1.0mL/minute. The liquid chromatography was equipped with 225nm and a column C18: (250\*4.6) mm and 5µ particle size. The injection volume was 10µL of standard and sample solutions (Bash Pharma Pharmaceutical, Sudan).

**HPLC Method for analysis of Levamisole HCl (non-Pharmacopeia):** A quantity of

Levamisole HCl working standard was weighed and transferred to a volumetric flask where dissolved with distilled water to obtain a solution with concentration of 30µg/mL.

A volume of the sample equivalent to 8mg of Levamisole HCl was transferred to volumetric flask where dissolved with distilled water to obtain a solution with concentration 30µg/mL.

As a mobile phase a mixture of phosphate buffer Ph 8: Acetonitrile (70:30) % with flow rate of 1.0 mL/minute was used. The liquid chromatography was equipped with 215 nm and column C8 was used. The injection volume was 10 µL. The flow rate was 1.0 mL/minute for standard and sample solutions (Amal, 2018).

**Experiments description:** Two experiments were conducted in the current study

**The First Experiment:** In this experiment therapeutic efficacy of Albendazole 2.5% was evaluated and compared with that of Ivermectin drench formulation in sheep naturally infected with gastrointestinal nematodes (GINs). Sixteen male sheep, 8-12 months of age were used in a faecal egg count reduction test (FECRT). Animals were selected based on positive GINs infection. On day zero, faecal samples were collected and the sheep were assigned into two treatment groups each of eight animals. Animals in the first group (A) were drenched according to body weight with Albendazole (Albendazole 2.5%) orally using syringe at 5mg/kg body weight as single dose. While animals in the second group (B) were treated with Ivermectin 0.2mg/kg body weight. To avoid under dosing, dose volumes were rounded up to the nearest 0.5ml. Further faecal samples were collected at days 2, 3, 4, 7, 10, 14, and 21 post-treatments.

**The Second Experiment:** After accomplishing of the first experiment (the

experiment extended for 28 days), and following the failure of the treatment to eliminate GINs infection, animals were allowed for 7 days before rearrangement of the previous groups into two treatment groups (C) and (D) each of eight animals, according to previous procedure adopted in the first experiment. Animals in group (C) were drenched orally with Tetramizole at dose rate of 3mg/kg bwt, while animals in group (D) were treated orally with Levamisole at 7.5mg/kg bwtt according to manufacturer recommended dose.

Animals were sampled for epg count (egg per gram) before treatment at day 0, and then following treatment at days 2, 3, 4, 7, 10, 14, 21 and 28.

**Faecal samples collection:** Faecal samples were collected directly from the rectum in clean plastic containers and labelled and transported immediately to the diagnostic laboratory, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine (SUST). Samples were collected in the morning from 8:00 to 9:00 AM.

**Parasitology techniques:**

**Coproculture:** faecal samples were collected 7 days before the start of the experimental part of the study using sterile disposable plastic gloves directly from the rectum in labelled clean plastic containers, and then dispatched to the laboratory. Pooled faecal samples were mixed with water and kept at

37°C for approximately 14 days to allow development to the third larval stage. For the larval culture 100 larvae were later identified based on morphology. Larvae were identified using the keys provided by Anon. (1986).

**Egg count:** Egg counts were performed using modified McMaster method where each egg counted represents 50 eggs per gram of faeces (Stafford *et al.*, 1994)

**Calculations:** Faecal egg count reduction (FECR) was calculated using the method endorsed by the World Association for the Advancement of Veterinary Parasitology (Coles *et al.*, 1992).

$$\text{FECR \%} = \frac{(\text{Pre-treatment epg} - \text{Post-treatment epg})}{\text{Pre-treatment epg}} \times 100$$

Resistance is considered to occur when the FECR was <95%.

**Statistical analysis:** No statistical analysis was done on the data from the egg count reduction test.

**Results**

**The first experiment**

**Drug testing:** the two drugs utilized in the first experiment (Albendazole and Ivermectin) showed assay% values that comply with the specifications for registration at the Directorate of Veterinary Medicines Registration, in the National Medicines and Poisons Board (NMPB), Sudan (Table 1).

**Table 1: Assay (%) of the albendazole and ivermectin**

Generic name/Drug	Type	Average retention time	area	Sample			RSD %
				Reference	Specification	Sample Content % (N=3)	
Albendazole oral suspension	Standard	21.867	14277591	BP 2016	90-110 %	108.9	0.543
	Sample	21.937	16530224				
Ivermectin oral solution	Standard	H <sub>2</sub> B1b	12.43	Interchemie werken	90-110 %	100	0.1071
		H <sub>2</sub> B1a	15.376				
	Sample	H <sub>2</sub> B1b	12.5187	525623			0.0242
		H <sub>2</sub> B1a	15.498	17731147			

**Larval identification:** *Strongyloides papillosus* and *Haemonchus* spp. comprised 84% of the larvae identified in the infected sheep (Table 2)

**Table 2: larval identification of nematodes obtained from pooled faecal samples of sheep**

Type	Number	Percentage
<i>Haemonchus contortus</i>	1300	26
<i>Oesophagostomum</i> spp	100	2
<i>Strongyloides papillosus</i>	2900	58
Hookworms,	50	1
<i>Dictyocaulus</i> spp.	150	3
Protostrongylids	100	2
<i>Nematodirus</i> spp	400	8

**Faecal egg count reduction:** the results of day zero to day 21 epg values are presented in Table (3), together with the mean faecal egg count reductions. At day 14, Albendazole and Ivermectin produced 33.8% and 48.5% reduction in faecal egg count, respectively. It is worth to mention that up to the end of the experiment all animals had epg equal to or greater than 300 epg.

**Table 3: mean faecal egg count (arithmetic) and reductions for albendazole and ivermectin-treated sheep**

Days	Albendazole		Ivermectin	
	epg (Arithmetic Mean)	Reduction %	epg (Arithmetic Mean)	Reduction %
0	981.3	-	1625	-
2	250	74.5	725	55.4
3	475	51.6	975	40.0
4	262.5	73.2	975	40.0
7	100	89.8	1725	-6.2.0
10	412.5	58.0	1413	13.1
14	650	33.8	837.5	48.5
21	837.5	14.6	1400	13.8

**The second experiment:**

**Drug testing:** Tetramisole and Levamisole, used in the second experiment are within the

acceptance limits of assay% by Directorate of Veterinary Medicines Registration (NMPB), Sudan.

**Table 4: Assay (%) of the Tetramisole and Levamisole**

Generic name/Drug	Type	Average retention time	Area	Sample			
				Reference	Specification	Sample Content % (N=3)	RSD %
Tetramisole HCl oral solution	Standard	5.605	883674.3	Bash Pharma	90-110 %	98.0	0.537
	Sample	5.499	1057544				
Levamisole HCl oral solution	Standard	6.888	1730133	BP, 2016	92.5-107.5 %	103.2	0.181
	Sample	6.884	1723912				

**Faecal egg count reduction test:** In the second experiment, neither of the two drugs resulted in any significant reduction ( $\geq 95\%$ )

of the overall number of eggs being released by the sheep. At day 14 Tetramisole showed

only 62.8% while Levamisole exhibited 91% reduction in egg count.

**Table 4: mean faecal egg count (arithmetic) and reductions for tetramisole and Levamisole-treated sheep**

Days	Tetramisole		Levamisole	
	epg (arithmetic mean)	Reduction %	epg (arithmetic mean)	Reduction %
0	3325	-	3325	-
2	250	92.5	450	86.5
3	475	85.7	462.5	86.1
4	1063	68	600	82
7	1225	63.2	762.5	77.1
10	962.5	71.1	612.5	81.6
14	1238	62.8	300	91
21	2013	39.5	1450	56.4

### Discussion

The current study signifies the presence of multiple drug resistance for the three major anthelmintics groups' common in use in sheep in Sudan. Resistance has been characterized by Shoop *et al.*, (1995) who determined the resistance of whatever medicine by comparison the previously effectiveness status with 95% elimination of the target parasite and become less. Also resistance considers present if the percentage reduction in faecal egg count (FEC) after treatment was less than 95%, and the lower limit of the 95% confidence interval was less than 90%. If only one of the two criteria was met, resistance was suspected (SR) to be present (Coles *et al.* 1992, and Domke *et al.*, 2012).

The compatibility of the four medicines with the registration guidelines (NMPB, 2017), following chemical analysis supports accuracy of dosing and stability of drugs under investigation.

In the first experiment both Albendazole and Ivermectin failed to eliminate nematode helminths from sheep with average 33.8% and 48.5% reduction in faecal egg count, respectively. The development of benzimidazoles efficacy reduction has been

reported worldwide (Bauer, 2001, Love and Coles, 2002, and Bartley *et al.*, 2003,).

A study in Ethiopia conducted to estimate the efficacy of albendazole in goats by using the recommended sheep dose; revealed considerable change in the efficacy of albendazole in goats, while doubling and tripling the recommended dose (3.8, 5.7 and 7.6 mg/kg) gave a near results (65.5, 81.4 and 84.1%) respectively. In the other hand, using the recommended dose in sheep showed reduction of efficacy which was 62% near to the result obtained in goats at the same dose level (Egualé *et al.*, 2009).

In south Africa the results of efficacy by using (FECRT) for Albendazole and Levamisole and Closantel were over 80% in 12 farms, when was considered as the cut point for the efficacy reduction and most of the farms respected as effective with few cases out of the cut point (Bakunzi, 2008), in their study they used different rate of efficacy than that recommended by Coles *et al.*, (1992). Also in South Africa a combined survey has been conducted on 52 farms aimed to target *Haemonchus* spp. to estimate efficacy of four medicines (Albendazole, Levamisole, Ivermectin and Rafoxanide), < 60% susceptible to three of the four anthelmintics tested, and 8 % of the strains

were < 40 % susceptible to all four of the anthelmintics (Van Wyk *et al.*, 1999)

Reduced efficacy of ivermectin obtained in the current study could be attributed to the continuous and indiscriminate use of that drug in the field and correlates with the justification that resistance development to ivermectin follows the excessive using in the field due to its wide spectrum activity against ecto-endo parasite's (Adediran and Uwalaka, 2015).

While many studies have demonstrated deterioration in the efficacy of Levamisole in the control of GIT parasites in goats (Yadav and Uppal, 1992; Yadav *et al.*, 1995 and Ram *et al.*, 2007), but Jaiswal *et al.*, (2013) indicated contradictory results, where Levamisole is still maintained its efficacy, this maintaining could refer for the less regular applying of Levamisole. In the current study the situation for Levamisole is much better when compared with Albendazole, Ivermectin and Tetramisole with 91% reduction in epg count in sheep. The popularity of Albendazole and Ivermectin among sheep owners as effective, economic and easy to administer anthelmintics justifies the less regular application of Levamisole in sheep health care management.

Considering efficacy <95% as cut point for resistance occurrence, Tetramisole showed only 62.8% reduction in epg count, a result which many indicate reduced efficacy of Tetramisole against sheep gastrointestinal nematodes.

Multi- drug efficacy reduction has been reported worldwide, where in goat has been documented in 15 Danish goat herds by using faecal egg count reduction test (FECRT), egg hatch assay (EHA) and larval development assay (LDA), where 6 farms have revealed reduction for BZs and LEV, and for IVM and BZs on one farm (Maingi *et al.*, 1996b). The results of multi- efficacy

reduction have been confirmed in goat for Albendazole, Levamisole and ivermectin where have shown (53%, 65% and 76%) at day 14 post treatment (Gelot *et al.*, 2016), a result that supports our findings.

Continuous and valuable follow up for the status of efficacy reduction must perform even in areas of sporadic cases of efficacy reduction (Dolinská *et al.*, 2014), where by tracing reports over world, the failure of anthelmintics medicines become a global issue extends to Europe when most AR records were for benzimidazole- or levamisole-resistance and with descriptive cases of macrocyclic lactones resistance, intensely for ivermectin (Papadopoulos, 2008). In South African *H. contortus* showed the highest level of resistance in the world that hasn't been recorded before to four types of anthelmintics medicines (Albendazole, Levamisole, Ivermectin and Rafoxanide) by using (FECRT) (Van Wyk *et al.*, 1999). Also in Kenya *H. contortus*, *Trichostrongylus*, *Oesophagostomum* spp. displayed multiple resistance to Levamisole, Ivermectin, Albendazole, Ivermectin and Rafoxanide (Waruiru *et al.*, 1998).

Another study conducted in Nigeria in sheep GINs, using FECRT it revealed well efficacy of Albendazole (99%), Ivermectin (96%) and Levamisole (96%), with lower 95% confidence interval (91,89 and 89) respectively, where it showed efficacy reduction to Ivermectin and Levamisole and suspicious to Albendazole (Adediran and Uwalaka, 2015).

There are many factors that encounter the animals to keep anthelmintics efficacy, could conclude as follow: updated status of efficacy of currently marketed anthelmintics is basic requirement and first barrier to encounter resistance particularly within regions where medicines still effective (Dolinská *et al.*, 2014). Unfortunately, the absence of new medicines with different



mechanism of action makes the AR challenge (Prichard, 2008), well management of the recent medicines is needed to keep efficacy of them (Coles *et al.*, 2006).

Strategies to Combine medicines from same or different anthelmintics groups to fortify and widen the spectrum; have also shown well advancing in efficacy reduction, as in New Zealand sheep, where benzimidazoles (BZ) displayed 60% in (9/15) of the farms, to Levamisole (LEV) 66% in (10/15) of the farms, combination drench (BZ+LEV) on 43% of farms (3/7) and avermectin on 1 of 8 farms (Sharma, 2004). Also combination (ABZ + TET), in addition to compare with ABZ, TET, and IVM in nematode of sheep and goats, have demonstrated well efficacy in eastern Ethiopia (Sissay *et al.*, 2006), but in another study some years later in Southern Ethiopia with the same medicines has defeated this (Sheferaw *et al.*, 2013).

#### **Conclusion and Recommendations**

All the four used medicines that descended from three groups have showed advanced status of efficacy reduction, which are less than recommended threshold, where medicines should have a minimum ability to reduce the percentage till 95% with arithmetic means  $\geq 90$ .

Further studies using *in vitro* methods as well as molecular techniques to characterise anthelmintics resistance up to the genus level are required. Alternative drugs, not common in use in Sudan such as Moxidectin and Monepantel may be used to overcome the problem.

#### **Conflict of interest**

All authors declare to have no conflicts of interest regarding the information provided in this manuscript.

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## المقاومة المتعددة لاربعة من ضادات الديدان الشائعة الاستخدام لعلاج الضان في السودان

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### المستخلص

عدة تقارير من انحاء متفرقة من السودان اشارت الي نقصان فعالية مضادات الديدان عند استخدامها في علاج الضان، تم استخدام اختبار الاختزال في عدد البيوض لقياس فعالية اربعة من مضادات الديدان شائعة الاستخدام وسط مربي الضان. في الدراسة الحالية؛ تم استخدام عدد (16) من ذكور الضان المصابة طبيعياً بالديدان الاسطوانية لتقويم فعالية عقار البندازول 2.5% المعد للتجريب، عقار اليفرمكتين 0.8% المعد للتجريب، عقار التتراميزول 5% و عقار الليفاميزول 2.5%. تم تقسيم الحيوانات الي مجموعتين بواقع ثمانية حيوانات لكلاً، المجموعة الاولى (أ) تم علاجها بعقار البندازول عن طريق الفم بجرعة واحدة مقدارها 5 مجم لكل كيلوجرام من وزن الجسم. بينما تم علاج الحيوانات في المجموعة الثانية (ب) بعقار اليفرمكتين عن طريق الفم بجرعة واحدة مقدارها 0.2 مجم لكل كيلوجرام من وزن الجسم. تم تجميع عينات الروث في الفترات الزمنية التالية: الزمن صفر (قبل اعطاء الدواء) ثم في اليوم الثاني و الثالث و الرابع و السابع و العاشر و الرابع عشر و الحادي والعشرون (عقب العلاج). عينات من المستحضرات الدوائية (نفس التشغيلات) تم اختيارها عشوائياً واختبارها لضمان مطابقة المنتج النهائي للمواصفات قبل بداية التجربة. النتائج التي تم الحصول عليها اشارت الي انه لم يك هناك دليل لوجود فعالية للعلاج في المجموعتين (البندازول و اليفرمكتين). بعد اسبوع من الدراسة الأولى تم إعادة توزيع وتقسيم الحيوانات لمجموعتين؛ ثمانية حيوانات لكل مجموعة، حيوانات المجموعة الأولى (ج) تم علاجها بعقار التتراميزول 5% عن طريق الفم بجرعة واحدة مقدارها 3 مجم لكل كيلوجرام من وزن الجسم، بينما تم علاج حيوانات بالمجموعة الثانية (د) بعقار الليفاميزول 2.5% عن طريق الفم بجرعة واحدة مقدارها 7.5 مجم لكل كيلو جرام من وزن الجسم. مره أخرى أوضحت النتائج عدم فعالية المستحضرين (التتراميزول 5% والليفاميزول 2.5%) لدرجة مقارنة لما حدث مع المستحضرين الآخرين (البندازول 2.5% واليفرمكتين 0.8%). لم يتم ملاحظة اي اختزال ذو دلالة معنوية في عدد البيوض عقب استخدام المستحضرات الاربعة. هذه النتائج تمثل أول تقرير من الحقل بالسودان لمقاومة متعددة لمضادات الديدان لأكثر من مستحضر بعد ان تم تجريبها للضأن. توصي الدراسة بمزيد من التقصي عن حالة مقاومة الديدان للأدوية باستخدام طرق مختبرية و طرق الاحياء الجزيئية.