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COMPARATIVE EFFICACY EVALUATION OF MOXIDECTIN AND IVERMECTIN INJECTABLE FORMULATION AGAINST HELMINTHES INFESTATION OF DONKEYS (*EQUUS ASINUS*) IN SUDAN

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ABSTRACT

A total of 24 male and female donkeys of local breed, 4-10 years of age and naturally infected with helminth parasites was utilized in a controlled study to evaluate the efficacy of Moxidectin injectable formulation at the dosage of 0.2 mg/kg of live weight used either subcutaneously or intramuscularly and an ivermectin injectable formulation at the dosage of 0.2 mg per Kg applied subcutaneously. Animals were allocated randomly into four groups of six donkeys each, and treatments were randomized among the groups. One group was kept as untreated control. The experiment extended for 14 days. Faecal samples were collected at day 0 (before treatment), and then at 1, 2, 3, 7 and 14 days post treatment. The number of parasites remaining in each animal was determined after necropsy of the animals at day 14 post treatment for all donkeys. Both Moxidectin subcutaneously or intramuscularly and ivermectin administered subcutaneously reduced initial EPG from a mean of 550, 700, and 600 to 50 (90.9%), 150 (78.5%), and zero (100%) at the end of trial on day 14. Efficacy percentages of Moxidectin and ivermectin against immature and adult nematodes were as follows: *Trichostrongylus axei*, *Parascaris equorum*. *Habronema muscae* 100% for both products; respectively. *Cyathostomes spp.; Strongyloides westeri and Oxyuris equi* 100%, for the two products. No Gasterophilus larvae were found in donkeys from treated groups, while all the control donkeys had infections with this parasite. Ivermectin showed greater efficacy (99.8%) than moxidectin (74.4 and 98%) against *Strongylus vulgaris* larvae found in the mesenteric artery aneurisms. No adverse reactions were observed during the experimental period.

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INTRODUCTION

The importance of helminthes infestation in donkeys in Sudan has been documented by the work of several authors (Seri *et al*., 2004a, Sawsan *et al*., 2008). Recent reports concerning the treatment of donkeys helminthiasis was demonstrated by the work of Seri *et al*., (2004b and 2005) and Sawsan *et al*., (2010) using doramectin, ivermectin and Albendazole, respectively.

Moxidectin is a macrocyclic lactone with structural similarities to ivermectin and milbemycin oxime (Carter *et al*., 1987; Shoop, 1993). Such similarities suggest that these compounds may share activity towards important parasites of man and animals, while structural differences may impart variations in dosage levels or the spectrum of susceptible parasites, therefore, these compounds warrant evaluation as alternatives to ivermectin treatment.

Many authors have demonstrated the endoectocidal efficacy of Moxidectin in horses harbouring natural infections of nematodes and Gasterophilus larvae (DiPietro *et al*., 1992; Lyons *et al*., 1992; Reinemeyer and Tineo, 1993; Bello and Laningham, 1994; Xiao *et al*., 1994; Cobra *et al*., 1995; Jacobs *et al*., 1995; Monahan *et al*., 1995 and Gulanber *et al*., 1998).

The present study was designed to compare therapeutic efficacies of injectable formulation of Moxidectin (either injected intramuscularly or subcutaneously), used at dosage of 0.2 mg per Kg of body weight against larval and adult stages of helminthes in naturally infected donkeys under Sudanese conditions. Also to report on side and/adverse effects, if any. An ivermectin injectable formulation injected subcutaneously at a dose of o. 2

mg per Kg was included in the assay for comparative purposes.

MATERIALS and METHODS

Experimental animals. In this study we utilized 24 male donkeys (3-10 years). Before starting the study, animals were examined to prove infestation with gastrointestinal helminthes. The animals were kept at a private farm located at the periphery of Elgedarif town. They were provided with tap water and allowed to graze freely in pasture.

Experimental drugs. The following drug formulations and trade marks were used as experimental drugs: Moxidectin: Cydectin® injection, Fort Dodge Veterinaria, S.A. (Spain). Ivermectin: kelamectin® 1% injection, KELA Laboratoria N.V., Sint Lenaart-seweg48, B 2320 Hoogstraten, BELGIUM.

Experimental design. The animals were allocated into four groups and penned according to treatment groups. The first three groups were treated and the last group remained untreated as a control group. The animals in the three treatment groups received treatment as follows:

Moxidectin treated group 1 (Mox1) received a single subcutaneous dose of Moxidectin at the manufacturer's recommended dose of 0.2 mg/kg body weight.

Moxidectin treated group 2 (Mox2) received a single intramuscular dose of Moxidectin at the manufacturer's recommended dose of 0.2 mg/kg body weight.

Ivermectin treated group (IVMT) received a single subcutaneous dose of ivermectin injectable at the manufacturer's recommended dose of 200 µg/kg body weight.

Then donkeys were monitored for possible adverse or unwanted reactions for 2 hours after administration of each drug.

The experiment was extended for 14 days. Faecal samples were collected at 0 (before treatment), 1, 3, 7, and 14 days post treatment. Necropsy of the animals was done at day 14 post treatment for all donkeys.

Animals were euthanized for worm recovery as described by Reinecke and Le Roux (1972). After the donkeys were euthanized, the thoracic and abdominal cavities were opened by making an incision along the ventral line of the animal and the left half of the thorax and the abdominal wall was removed. The organs from the thoracic and the abdominal cavities were removed from the carcass. The different organs from the gastrointestinal tract were then isolated by tying double ligatures around the gut to separate it in the stomach, small intestine, caecum, colon and rectum. The contents of the different organs were removed and then sieved through a 150 mm sieve to obtain residue samples. The residues preserved in 10% formalin. Residue samples of ingesta were examined macroscopically. Nematodes present were placed in a specimen bottle containing 10% formalin. Helminthes were identified at a later stage by placing them on a glass slide, examining them microscopically and classifying them according to Lichtenfels (1975).

The anthelmintic efficacy of Moxidectin and ivermectin was estimated using a faecal egg count reduction test (FECR) for helminthes burden. The arithmetic mean of the egg count and helminthes burden was calculated to determine the mean percentage reduction within each group, according to the following formula:

$$\text{FECR\%} = \frac{\text{Pre-treatment EPG - Post-treatment EPG}}{\text{Pre-treatment EPG}} \times 100$$

A modified McMaster technique (Anonymous, 1986) was used to count the egg per gram (epg) of faeces.

RESULTS

The results of mean egg per gram of faeces and the range in addition to the reduction percentage of egg per gram of faeces for the three treated groups from day zero to day 21 are presented in the Tables 1, 2 and 3.

On day 3, Moxidectin (S/C) showed reduction of 81.8% egg per gram count (EPGC), while Moxidectin (IM) showed 71.4%. In ivermectin treated group 100% of egg per gram count (EPGC) was reported. The three groups reported 90.9%, 78.5% and 100% reduction of egg per gram of faeces on day 14 for Moxidectin (S/C), Moxidectin (IM) and ivermectin respectively.

Table 1: Mean faecal egg counts and reduction for moxidectin treated donkeys (subcutaneous injection).

Day	Arithmetic Mean (EPG)	Range	Mean Reduction %
0	550	300-1500	-
1	550	300-1500	0
2	800	300-3600	-31.2
3	100	0-600	81.8
7	100	0-300	81.8
14	50	0-300	90.9

Table 2: Mean faecal egg counts and reduction for moxidectin treated donkeys (intramuscular injection).

Day	Arithmetic Mean(EPG)	Range	Mean Reduction %
0	700	300-900	
1	550	300-2400	21.4
2	100	0-900	85.7
3	200	0-600	71.4
7	0	0-0	100
14	150	300-600	78.5

Table 3: Mean faecal egg counts and reduction for ivermectin treated donkeys (subcutaneous injection).

Day	Arithmetic Mean(EPG)	Range	Mean Reduction %
0	600	300-600	-
1	150	0-300	75
2	100	300-3900	83.3
3	0	0	100
7	0	0	100
14	0	0	100

The results of post-mortem findings are presented in the Tables 4, 5 and 6 for the three groups. Moxidectin administered either subcutaneously or intramuscularly showed efficacy of 100% and Ivermectin administered subcutaneously also showed 100%, but the efficacy against L4 *Strongylus vulgaris* found in the cranial mesenteric arteries was 74.4% for Moxidectin S/C and 98% for Moxidectin (IM), as shown in tables (4 and 5). On the other hand, ivermectin showed 99.8% efficacy against L4 *Strongylus vulgaris*.

Figures (1, 2 and 3) show Gasterophilus larvae, *Parascaris equorum*, and *Strongylus vulgaris* larvae, in different parts of GIT and cranial mesenteric artery aneurisms of a donkey in the control group (not treated).

Table 4: Summary of worms recovered from control and animals treated with Moxidectin (S/C) at necropsy.

Organs examined	Control	Moxidectin Sc	
		No.	Reduction %
Cranial mesenteric artery			
Strongylus vulgaris	148	38	74.4
Stomach			
Gasterophilus spp.	157	0	100
Habronema spp.			
Trichostrongylus axei			
Small Intestine			
Parascaris equorum	15	0	100
Caecum			
Gasterophilus spp.	3315	0	100
Strongylus spp.			100
Cyathostomes spp.			100
Colon			
Strongylus spp. *Cyathostomes spp.* *+ Strongyloides westeri* *+ Oxyuris equi*	26203	0	100

Table 5: Summary of worms recovered from control and animals treated with Moxidectin (IM) at necropsy.

Organs examined	Control	Moxidectin IM	
		No.	Reduction %
Cranial mesenteric artery			
Strongylus vulgaris	148	3	98
Stomach			
Gasterophilus spp.	157	0	100
Habronema spp.			
Trichostrongylus axei			
Small Intestine			
Parascaris equorum	15	0	100
Caecum			
Gasterophilus spp.	3315	0	100
Strongylus spp.			100
Cyathostomes spp.			100
Colon			
Strongylus spp. *Cyathostomes spp.* *+ Strongyloides westeri* *+ Oxyuris equi*	26203	0	100

Table 6: Summary of worms recovered from control and animals treated with Ivermectin (S/C) at necropsy.

Organs examined	Control	Ivermectin S/C	
		No.	Reduction %
Cranial mesenteric artery			
Strongylus vulgaris	148	1	99.4
Stomach			
Gasterophilus spp.	157	0	100
Habronema spp.			
Trichostrongylus axei			
Small Intestine			
Parascaris equorum	15	0	100
Caecum			
Gasterophilus spp.	3315	0	100
Strongylus spp.			100
Cyathostomes spp.			100
Colon			
Strongylus spp. *Cyathostomes spp.* *+ Strongyloides westeri* *+ Oxyuris equi*	26203	0	100

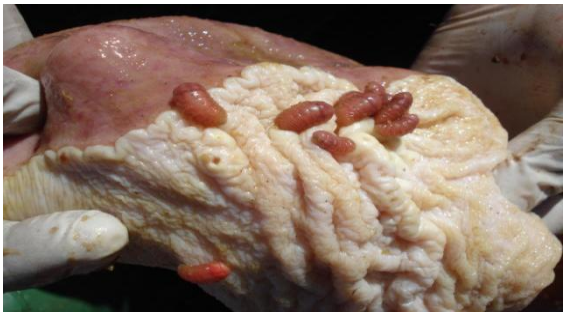

Figure 1: Gasterophilus larvae in the stomach of a non treated donkey

Figure 2: *Parascaris equorum* removed from intestine of a non treated donkey

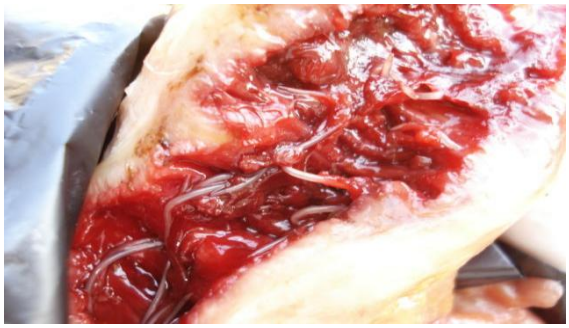

Figure 3: Larvae of *Strongylus vulgaris* in cranial mesenteric artery removed from control (non treated) donkey

DISCUSSION

The first MOX-containing product was an injectable formulation for cattle, approved for commercial use in Argentina in 1989. Subsequent formulations introduced worldwide for control of parasitosis include a tablet and a sustained release injectable for prevention of heartworm disease in dogs, injectable and oral drenches for sheep, a pour-on formulation for cattle and deer, and sustained release injectable formulations for cattle and sheep. These long-acting formulations provided significantly longer persistent activity than the earlier formulations, including season-long control against some parasite species.

For horses, MOX is formulated as unique, easy-to administer oral gel formulation that provides excellent and long-lasting efficacy against nematodes and gastrointestinal bots. A second gel formulation containing MOX in combination with praziquantel adds efficacy against cestodes.

Horse strongylids are known to be resistant to Benzimidazoles (BZ) and tetrahydropyrimidines (Kaplan 2002, 2004), while the only case of resistance in cyathostomins to macrocyclic lactones was reported in a donkey in the UK (Trawford *et al.*, 2005).

The therapeutic efficacy of Moxidectin in donkeys in this study, in both groups of animals treated either subcutaneously or intramuscularly, was 81.8 and 100% reduction in (epg) count on day 7 post treatment. Gulanber *et al*., (1998) obtained a similar result to that of intramuscular injection (100%) at day 7 post treatment in horses treated with Moxidectin injectable formulation administered orally. Following post-mortem, Moxidectin administered subcutaneously or intramuscularly resulted in high efficacy of 100% against *Habronema* sp., *Trichostrongylus axei*, *Strongyloides westeri*%; *Parascaris equorum* %; *Strongylus* sp.; and *Cyathostomum*. This result is in accordance to that of Lyons *et al.* (1992); French *et al.* (1992); DiPietro *et al*. (1993); Bello and Laningham (1994); Xiao *et al*. (1994); Monahan *et al*. (1995), Scholl (1997); Costa *et al*. (1998) and Dorchies *et al*. (1998).

Besides the strongylid nematodes, eggs of *Parascaris equorum, Oxyuris equi, Strongyloides westeri* and *Habronema* sp. were found in faecal samples a day before treatment. No eggs of these nematodes were found in horse faeces on the 10th and 14th days after treatment with both drugs.

In this study, ivermectin showed 100% faecal egg count reduction on day 7, which is in agreement with Seri *et al*. (2005) and Sawsan *et al*. (2010), who reported the same result when using ivermectin injectable formulation intramuscularly or subcutaneously, at a dose rate of 200 µg/kg body weight in donkeys in the Sudan, while DiPietro *et al.* (1982) revealed 100% efficacy.

When ivermectin was given intramuscularly to donkeys at a dose rate of 200 µg/kg, the efficacy was 100% against larvae of *Gasterophilus* sp. (Seri *et al*., 2005), and this result is in agreement with the results obtained in this study. Ivermectin successfully (100%) removed *P. equorum* from the small intestine when given orally as a paste formulation at 200 µg/kg. Ivermectin totally eliminated the passage of *P. equorum* in the naturally infected horses (Cobra *et al*., 1986). In the case of *T. axei*, ivermectin (100%) eliminated *T. axei* from the small intestine of donkeys when used at dose rate of 200 µg/kg in intramuscular formulation (Seri *et al*., 2005).

In this study, Moxidectin expressed moderate to excellent efficacy of 74.4 to 98% against *Strongylus vulgaris* larvae which were found in the cranial mesenteric arteries following subcutaneous and intramuscular injection, respectively. Ivermectin showed efficacy of 99.4% for the *S. vulgaris* larvae. This result is to be considered to be higher than that reported by Costa *et al*. (1998) and Seri *et al*. (2005) who reported 67.8%, 69.23% respectively.

No adverse reactions were observed within the 14 days experimental period, a result which is in line with that reported by Costa *et al.* (1998).

CONCLUSION

Although it is recommended to be used as oral gel, Moxidectin injected either subcutaneously or intramuscularly at 0.2 mg/kg was moderately effective against naturally acquired infections of adult *Cyathostomum* sp., *Strongylus* sp., *Trichostrongylus axei*, *Parascaris equorum*, *Oxyuris equi* and *Strongyloides westeri* gastrointestinal nematodes in donkeys.

These findings suggest that further research might be warranted into the use of new dosage regimens of Moxidectin with higher doses as an equine anthelmintic and to control *Strongylus vulgaris* in the cranial mesenteric artery.

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مقارنة الفعالية الدوائية لعقاري الموكسيديكتين والايفرمكتين عن طريق الحقن ضد الاصابة بالديدان في الحمير في السودان

محمد اسماعيل فنقاما ، هشام اسماعيل سري ، سهام الياس سليمان ، سوسن محمد أحمد امام ، ايمان عبد الوهاب مزمل

تم استخدام 24 من الحمير (ذكور واناث) من السلالة المحلية، تتراواح اعمارها بين 4-10 سنوات، والمصابين بشكل طبيعي بالديدان الطفيلية المعدية المعوية، وذلك لدراسة فعالية الموكسيدكتين المعطي عن طريق الحقن بجرعة مقدارها 0.2 ملغ لكل كغ من الوزن الحي (اما عن طريق تحت الجلد أو في العضل) ومقارنة النتائج بالايفرمكتين الذي اعطي بجرعة مقدارها 0.2 ملغ لكل كغ من الوزن الحي بالحقن تحت الجلد. وتم توزيع الحيوانات عشوائيا الي اربع مجموعات (كل منها من ست حمير) والعلاجات تم توزيعها بصورة عشوائية بين المجموعات. مجموعة واحدة ظلت من دون علاج كمجموعة تحكم (سيطرة). تم جمع عينات البراز في اليوم صفر (قبل العلاج) ثم في 1 و 2و 3و 7 و 14 أيام بعد العلاج. تم ايضا عد الديدان والاطوار اليرقية المتبقية في كل حيوان بعد التشريح في اليوم 14 بعد العلاج لجميع الحمير. العلاج بكل من الموكسيدكتين (تحت الجلد أو في العضل) والايفرمكتين (تحت الجلد) ادي الي انخفاض متوسط العد للبيوض من 550- 700 و 600 الي 50(90.9%)، 150 (78.5%) و صفر (100%)، علي التوالي، وذلك عند نهاية التجربة، في اليوم 14. كانت النسب المئوية لفعالية الموكسيدتكين والايفرمكتين ضد الاطوار اليرقية والديدان الناضجة علي النحو التالي: 100% *Trichostrongylus axei, Parascaris equorum, Habronema muscae* لكلا المركبين، 100% *Cyathostomes spp.; Strongyloides westeri and Oxyuris equi* لكلا المركبين. لم يتم العثور يرقات نغف الخيل في كل الحيوانات المعالجة بينما وجدت في كل الحمير في مجموعة التحكم. الايفرمكتين اظهر فعالية بنسبة 99.8% في علاج الاطوار اليرقية لديدان *Strongylus vulgaris* في الشريان المساريقي مقارنة مع الموكسيديكتين (74.4% و 98%). لم تلاحظ اي من الاثار الجانبية أو العلامات الظاهرية للتسمم علي الحيوانات المعالجة.