Sudan J. Vet. Res. (2013), **28**: 37-42 *sudanjvr.net*
With 2 figures in the text

The Efficacy of Triclabendazole and Oxyclozanide Against Natural *Fasciola gigantica* Infection in Cattle

Osman, O.M. and Ibtisam A. Goreish
Veterinary Research Institute, P.O. Box 8067, Al amarat, Khartoum, Sudan

ملخص البحث

أُختبرت فعالية كل من عقارى أوكسيكلوزانايد (Oxyclozanide) و ترايكلابندازول (Triclabendazole) ضد دودة المتورقة العملاقة (*F. gigantica*) في تسعة عجول مصابة طبيعياً تتراوح أعمارها بين سنتين إلى ثلاث سنوات. تم تقسيم الحيوانات إلى ثلاث مجموعات ، ثلاث حيوانات لكل مجموعة وتم علاج المجموعة الاولي بعقار ترايكلابندازول بجرعة 12 ملجم/كلجم والمجموعة الثانية بجرعة 12ملجم/كلجم من عقار أوكسيكلوزانايد. تركت المجموعة الثالثة كمجموعة ضبط وتحكم. تم قياس أثر العلاج بحساب تركيز خضاب الدم و نسبة الخلايا المتكدسة و عدد بيض الدودة فى كل واحد جرام روث إسبوعياً. ذبحت الحيوانات بعد مرور ثمانيه أسابيع من العلاج وتم قياس فعالية الأدوية بحساب نسبة الديدان التي تم التخلص منها حيث وجدت بنسبه 100% عند إستعمال عقار ترايكلابندازول بينما كانت نسبة التخلص 80% عند إستعمال عقار أوكسيكلوزانايد.

Summary

Triclabendazole and Oxyclozanide were tested for the treatment of cattle naturally infected with *F. gigantica*. Nine calves naturally infected with *F. gigantica*, aged between two to three-year old were used. They were divided into three groups, three animals each; group one was treated with Triclabendazole at dose of 12 mg/kg, group two treated with Oxyclozanide at dose of 12 mg/kg and the third group was left untreated as a control group. At weekly intervals, haemoglobin concentration and PCV were measured, and faecal samples were collected for egg count per gram of faeces. The animals were slaughtered 8 weeks after treatment and the efficacy of the drugs was assessed according to worm burden reduction. The reduction of worm in the Triclabendazole-treated group was 100%, while in the Oxyclozanide-treated group was 80%.

Introduction

At present, there is no commercial vaccine available for the prevention of fasciolosis (McManus and Dalton, 2006) and hence its control is based largely on chemotherapy. A benzimidazole derivative, triclabendazole is the drug of choice as it is safe and efficacious against both juvenile and adult flukes (Fairweather and Boray, 1999) and has been marketed since 1983 as a veterinary drug (Fasinex1) (Keiser *et al*, 2005). The sulphoxide metabolite of triclabendazole (TCBZ-SX) is the active form of this drug (Hennessy *et al*, 1987). A number of morphological studies have been carried out to elucidate the mechanism of action of TCBZ and the combined data is in agreement with the targeting of microtubules; an action that is typical of benzimidazole anthelmintics (Fairweather, 2005).

The oxyclozanide is a member of Salicylanilides group and it is highly active against adult flukes and shows good activity against immature flukes aged 6–8 weeks and older, but not effective against younger stages (Boray, 1986).

Triclabendazole has been widely tested as a fasciolicide in sheep and cattle, both in controlled experimental infections (Boray *et al*, 1983; Richards *et al*, 1990) and in field tests using natural infections (Rapic *et al*, 1988). In all cases it has been reported to be highly effective against adult forms, and effective against immature forms.

Recent studies confirmed the occurrence of triclabendazole resistance in sheep and cattle from different parts of the world, e.g. Australia, Ireland, The Netherlands, Spain and the UK (Alvarez-Sanchez *et al*, 2006; Keiser *et al*, 2005). However, triclabendazole has not been assayed in *F. gigantica* infection in cattle in Sudan.

There is a pressing need for testing the fasciolicidal drugs used in Sudan, for this reason we have selected two drugs, the triclabendazole which is recently introduced in Sudan and it is not well known among cattle owners, and oxyclozanide which has been used for treatment of fasciolosis for several years. This work presents the results of a controlled test in naturally infected cattle, which establish the efficacy of triclabendazole and oxyclozanide against infection of *F. gigantica.*

Materials and Methods

Experimental design and treatment

Nine males and females Kenana calves naturally infected with *F. gigantica,* were purchased from Rabak animal market; they were selected following confirmation of their infection with *F. gigantica* by sedimentation test. They were kept in for six weeks for adaptation. Animals were allotted to three groups of 3 animals each, according to bodyweight. Treatment procedures were carried out as follows:

The first group was treated orally with oxyclozanide at a dose rate of 12 mg/kg, and the second group was treated orally with triclabendazole at a dose of 12 mg/kg (plus levamisole at a dose of 7.5 mg/kg). The third served as infected non-treated control.

Haematological tests

Blood samples were taken from the jugular vein before treatment and once a week thereafter for eight weeks. Plain tubes were used for serum collection to perform biochemical tests while lithium heparin tubes were used to collect unclotted blood for haematological studies. Plain tubes were left to clot at room temperature and then centrifuged at 2600g for 10 min. The resultant serum was dispensed into 2 mL aliquots and stored at -20 °C until required for use

Microhaematocrit capillary tubes containing blood were centrifuged at 8000g for 5 minutes. The percentage PCV was then read with the help of a Hawksley microhaematocrit reader (Hawksley, Lancing, United Kingdom).

Haemoglobin concentration was measured using a kit (COD 11743; from BioSystems S.A. Costa Brava 30, Barcelona, Spain) which depends on the method described by Van Kampen and Zijlstra (1961).

Faecal Examination

Individual faecal samples were taken on the day of treatment (Day 0) and weekly till the end of the study at week eight. For the calculation of eggs count per gram of faeces (EPG), the simple sedimentation method (McMaster), according to Gonzalez-Lanza *et al* (1989) was performed as follows:

Ten gram of faeces were mixed with tap water in a large test tube and stirred well with a stirring rod. The sample was strained through a large mesh sieve into a conical 1000 ml flask, washed copiously and filled up with tap water. The sample was allowed to stand for 20 min and then the supernatant was discarded and the sediment was re-suspended in tap water. The sedimentation process was done four times. After the last sedimentation and decantation the sediment was recovered into a test tube and fill up to 50 ml volume with tap water. The tube was agitated to re-suspend the sediment, and both chambers of a McMaster slide were filled with a Pasteur pipette. A McMaster slide with two chambers of 0.15 ml each was used and all the *F. gigantica* eggs present on the bottom of the chambers were counted.

EPG was determined according to the following equation:

$$\text{EPG} = \frac{\text{Total number of observed eggs} \times 50 \text{ ml}/10 \text{ g}}{\text{Number of chambers} \times 0.15 \text{ ml}}$$

Post-mortem examinations

Post-mortem examinations were carried out on all calves soon after they were slaughtered after eight weeks. After slaughter, each calf was eviscerated and the liver with intact bile ducts was separated from the rest of the organs. The major bile ducts were then opened and

thoroughly examined for flukes. The liver parenchyma was sliced into small pieces and squeezed in warm physiological saline to collect the flukes. The total number and size of flukes in each animal was recorded. The percentage of efficacy was determined by the following formula:

$$\frac{\text{Mean no. of flukes in controls - Mean no. of flukes in treated group}}{\text{Mean no. of flukes in controls}} \quad X100$$

Results

There were no significant ($P > 0.05$) differences between the mean haematological values in the control group and the treatment groups on day 0. Weekly examination of blood of the three groups revealed changes in the hematological values. The mean Hb concentration (g/dl) started to increase in the first week post treatment in triclabendazole-treated group. A significant increase ($P < 0.05$) was reported at week five in oxyclozanide-treated group.

Significant increase in packed cell volume was reported in the first week in all groups and continues to increase in the treated groups, and the increase continued, to reach its highest value at week six.

There were no significant ($P > 0.05$) differences between the mean bodyweight (b.wt) in the control group and the treated groups on day 0. The mean b.wt for oxyclozanide-treated cattle and cattle treated with triclabendazole were significantly ($P < 0.05$) greater than the mean b.wt for the control group at the end of the study. The mean b.wt gain of triclabendazole-treated cattle (74 kg) was higher than those of cattle treated with oxyclozanide (56 kg) and the control group. Mean body weight gains of all treated groups were significantly greater than that of the control cattle (19 kg).

Results of faecal examinations

The results of faecal examinations are illustrated in Fig 1. There were no significant ($P > 0.05$) differences between the mean EPG in the control group and the treatment groups on day 0, whereas on day 7, the mean EPG in the triclabendazole- and oxyclozanide-treated groups showed a significant decrease ($P < 0.05$) due to treatment effect. All triclabendazole-treated animals were negative on week six, while animals of oxyclozanide group had still positive faecal egg counts on week 7. The relatively high faecal egg output was seen in oxyclozanide-treated group, and the low egg count was observed in the triclabendazole-treated group.

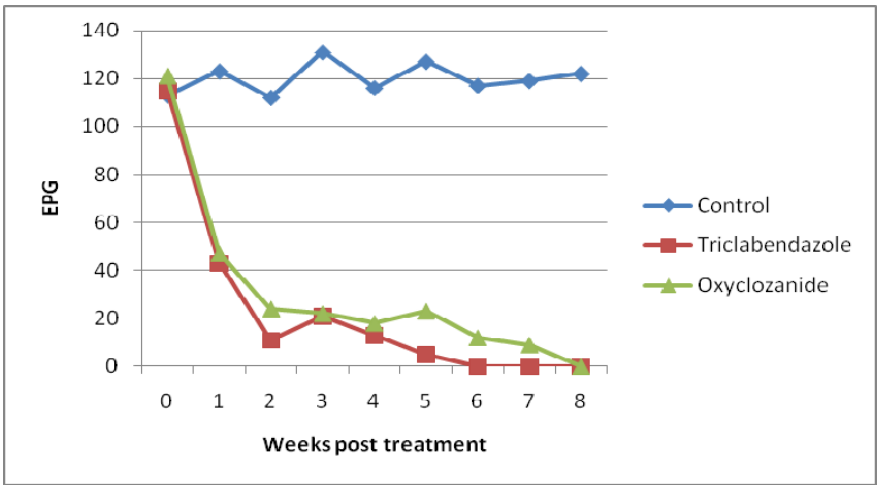

Fig. 1: Mean egg per gram counts before and after treatment of naturally infected cattle with triclabendazole and oxyclozanide for eight weeks period.

Fluke collection and efficacy of anthelmintics used

At eight weeks after treatment, all animals were slaughtered and flukes were collected. Table 1 summarizes the mean numbers of flukes collected and the efficacy percentages of the anthelmintics treatment. The control group had an average of 25 flukes/animal. No fluke was collected from the triclabendazole-treated group and the percentage of drug efficacy was 100%. In oxyclozanide group, there was a complete freedom from the fluke burden in one animal, and 15flukes were obtained from the other two animals, but they were smaller in size (0.9 – 1.6mm) than the flukes (4.6 – 5.8 mm) obtained from the control group (Fig. 2). The percentage efficacy of the oxyclozanide was 80%.

Table 1: Fluke collection and efficacy of treatments with oxyclozanide and triclabendazole in cattle naturally infected with *F. gigantica*.

Group	Fluke collected (mean)	Fluke collected (range)	Efficacy %
Control group	25	20 – 29	
Oxyclozanide-treated group	5	6 – 9	80
Triclabendazole-treated group	0	0	100

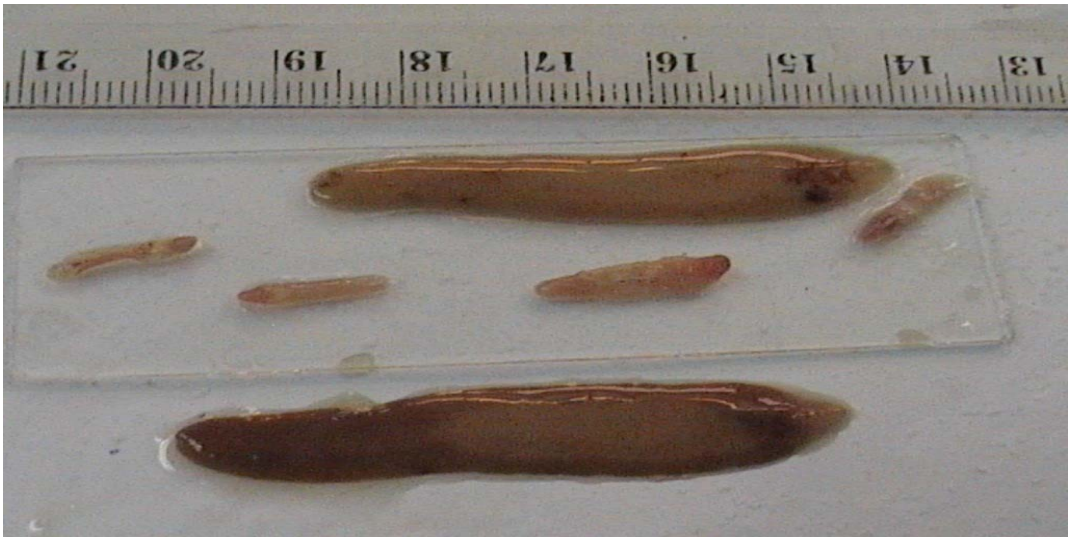

Fig. 2: Comparison between liver flukes obtained from the control group (large in size) and those obtained from the oxyclozanide-treated group (small in size).

Discussion

The most reliable method for determining the anthelmintics activity of a drug in ruminants is a controlled test, where efficacy is calculated by comparing parasite populations in groups of treated and untreated animals (Wood *et al*, 1995). It is clearly better than faecal egg counts, often used in the evaluation of the efficacy of fasciolicides, which can lead to erroneous conclusions due to factors such as the effect of the drug on the development of surviving flukes, the prolongation of the prepatent period and the time of collection of faecal samples (Rapic *et al*, 1988; Richards *et al*, 1990).

Controlled tests have been carried out in cattle and sheep using triclabendazole and many other drugs (albendazole, clorsulon, nitroxynil, oxyclozanide, rafoxanide) to test their efficacy against *F. hepatica*. In goats, albendazole has been tested on mature flukes (Foyret, 1988) and triclabendazole on late immature flukes (Kinabo and Bogan, 1988). The present work reports a controlled test for efficacy evaluation of triclabendazole and oxyclozanide against *F. gigantica* in naturally infected cattle in the Sudan.

Triclabendazole at a dose rate of l2 mg/kg l.b.wt proved highly effective against *F. gigantica* in this study. There was a complete elimination of flukes from all animals in the triclabendazole-treated group with a 100%drug efficacy. Faecal egg count for triclabendazole group scored zero at week 6 and the majority of triclabendazole -treated cattle had nearly negative faecal egg counts at week 2. Wood *et al* (1995) reported that fecal egg reduction test affords valuable information, even though the number of flukes removed is unknown. In addition, these authors suggested that the suitable time to evaluate the efficacy of a compound is at day 14 or 21 post-treatment.

The results for triclabendazole-treated cattle, in the present study, show that this drug is highly effective as a liver fluke therapy. Similar results were reported by Rapic *et al* (1988) and Lecuyer *et al* (1985) who found efficacies of 96.5% and 90% in cattle, respectively. Moll *et al* (2000) treated sheep and cattle with triclabendazole in the Netherlands; they reported very low levels of efficacy. Their results were highly indicative of presence of triclabendazole resistant *F. hepatica* in sheep and cattle. However, in our study this drug is newly introduced to the Sudan; it is not widely used and this may explain why no resistant *F. gigantica* strains were observed.

The efficacy of the oxyclozanide was 80%, and small-sized flukes were obtained from two animals of its treated group at the end of the experiment (Fig 2); this indicates that the parasite has begun to develop some sort of resistance to the drug. The reduction in size of the flukes in the oxyclozanide group can be attributed to the actions of the salicylanilides which cause significant disruption of energy metabolism and paralysis leading to detachment and cessation of feeding (Fairweather and Boray, 1999). Consequently, the fluke enters a state of starvation which will impose a severe metabolic stress on it. The fluke has to draw on its energy reserves in an attempt to survive.

The present study has also examined the influence of triclabendazole and oxyclozanide treatment on the clinical course of cattle fasciolosis. The results obtained from all animals groups before treatment indicate the development of a moderate anaemia. Treatment with triclabendazole and oxyclozanide eliminated most of the flukes, reduced haematoiogical alterations and helped rapid return to normal haemoglobin and PCV values after treatment.

According to Fairweather and Boray (1999), no new fasciolicides are expected to be marketed in near future, so different strategies have been proposed to slow down the development of anthelmintic resistance. Strategic treatment with anthelmintics based on the knowledge of liver fluke epidemiology and a good farm management can reduce infection to a low level and control disease with a low treatment frequency (Boray *et al*, 1985). Reduction of treatment frequency may also reduce the chance of drug resistance development, especially in high risk areas where several treatments appear to be necessary. In these areas, it may be necessary to develop strategic treatment regimes which minimise treatment frequency and monitor treatment efficacy. Obviously, the faecal egg count reduction test only provides an indication of efficacy during patency and cannot detect developing resistance amongst immature stages.

Although studies in Australia have demonstrated that the use of a combination of drugs with different modes of action can be effective against resistant strains of *F. hepatica* (Boray, 1993;1997), this approach has the risk of building up a multiple drug resistance.

Acknowledgements

Funding support for this work was provided by the International Atomic Energy Agency (IAEA) and The Veterinary Research Institute, Soba, Khartoum. We appreciate the technical assistance of the staff of Rabak Regional Veterinary Research Laboratory.

References

Alvarez-Sanchez, M.A.; Mainar-Jaime, R.C.; Perez-Garcia, J. and Rojo-Vazquez, F.A. (2006). *Vet. Rec.,* **159**: 424–425.

Boray, J.C. (1986). Trematode infections of domestic animals. **In**: Campbell, W.C. and Rew, R.S. (eds) *Chemotherapy of Parasitic Diseases*. 1st edn. Plenum Press, New York,Pp. 401–425.

Boray, J.C. (1993). *Proc. 14th Inter. Conf. WAAVP*, Cambridge, p. 370.

Boray, J.C. (1997). *Immunology, pathobiology and control of Fasciolosis*. MSD AGVET, Rahway, NJ, pp. 83–97.

Boray, J.C.; Crowfoot, P.D.; Strong, M.B.; Allison, J.R.; Schellembaum, M.; von Orelli, M. and Sarasin, G. (1983). *Vet. Rec.,* **113**: 315–317.

Boray, J.C.; Jackson, R. and Strong, M.B. (1985). *NZ Vet. J.,* **33**: 182–185.

Dargie, J.D. (1987). *Int. J. Parasitol.,* **17**: 453–463.

Fairweather, I. (2005). *J. Helminthol.,* **79**: 227–234.

Fairweather, I. and Boray, J.C. (1999). *Vet. J.,* **158**: 81–112.

Foyret, W.J. (1988). *Vet. Parasitol.,* **26**: 261-264.

Gonzalez-Lanza, C.; Manga-Gonzalez, Y.; Del-Pozo-Carnero, P. and Hidalgo-rguello, R. (1989). *Vet. Parasitol.,* **34**: 35–43.

Hennessy, D.R.; Lacey, E.; Steel, J.W. and Prichard, R.K. (1987). *J. Vet. Pharmacol. Therap.,* **10**: 64–72.

Keiser, J.; Engels, D.; Buscher, G. and Utzinger, J. (2005). *Expert Opin. Invest. Drugs,* **14**: 1513–1526.

Kinabo, L.D.B. and Bogan, J.A. (1988). *J. Vet. Pharmacol.Therap.,* **11**: 254-259.

Lecuyer, B.; Bordas, C.H.; Marchand, C.; Nguyen, H.N. and Sourd, C.H. (1985). *Bull. Soc. Vet. Prat. France,* **69**: 507–513.

McManus, D.P. and Dalton, J.P. (2006). *Parasitol.*, **133**: 43-61.

Moll, L.; Gaasenbeek, P.H.; Vellema, P. and Borgsteede, F.H.M. (2000). *Vet. Parasitol.,* **91**: 153–158.

Rapic, D.; Dzakula, N.; Sakar, D. and Richards, R.J. (1988). *Vet. Rec.,* **122**: 59-62.

Richards R.J.; Bowens, F.L.; Essenwein, F.; Steiger, R.F. and Biischer, G. (1990). *Vet. Rec.,* **126**: 213-216.

Van Kampen, E.J. and Zijlstra, W.G. (1961). *Clin. Chem. Acta*., **6**: 538-544.

Wood, I.B.; Amaral, N.K.; Bairden K.; Duncan, J.L.; Kassi, T.; Malone, J.B.; Pankavich, J.A.; Reincke, R.K.; Slocombe, O.; Taylor, S.M. and Vercruysse, J. (1995). *Vet. Parasitol.,* **58**: 181-213.