Field trials to establish the clininical equivalence of two 34% injectable solutions of nitroxinil in sheep, cattle and buffalo naturally infected with *Fasciola hepatica* and certain gastrointestinal nematodes

Metodi Petrichev

University of Forestry, Department of Plant Pathology and Chemistry – Sofia

Corresponding author: metodi.biochemystri@gmail.com

Citation: Petrichev, M. (2021). Field trials to establish the clininical equivalence of two 34% injectable solutions of nitroxinil in sheep, cattle and buffalo naturally infected with *Fasciola hepatica* and certain gastrointestinal nematodes. *Zhivotnovadni Nauki*, *58*(6), 74-81 (Bg).

Abstract

The clinical equivalence in the field of two 34% injectable solutions of nitroxynil - Nitroxinil 34% solution for injection - ”Investigation product” and Trodax 34% inj. sol. - ”Control product” in sheep, cattle and buffaloes naturally infected with *Fasciola hepatica* and some gastrointestinal nematodes. In each experiment three groups were formed – two experimental and one control. The experiments were performed to compare and possibly confirm the clinical equivalence between the preparation Nitroxinil 34% inj. sol. and the original Trodax product 34%. A total of 126 sheep of the Local Improved breed were used, with a body weight of 40–50 kg, mostly female and of different ages. In the experiments with cattle, 60 cattle from the cross X Local Chernoshareno and 30 buffaloes of the breed ”Murrah”, of female sex with different live weight, were used. The test and control product was administered at a dose of 10 mg/kg bw. or 1 ml for 34 kg m.p. Sheep, cattle and buffaloes were injected once subcutaneously. In terms of its effectiveness, Nitroxinil 34% solution for injection does not differ from the original product Trodax ($P > 0.05$), which leads to the conclusion that the two preparations are equivalent in terms of their clinical efficacy.

The aim of the present study was to conduct a comparative clinical study of these two veterinary medicinal products in terms of their efficacy.

Key words: clininical equivalence, two 34% injectable solutions of nitroxinil, sheep, cattle, buffalo

Introduction

Nitroxynil (4-cyano-2-iodo-6-nitrophenole or 4-hydroxy-3-iodo-5-nitrobenzonitrile) is an antifascione agent active mainly against *Fasciola hepatica* and *Fasciola gigantica* (over 6 weeks of age) and some adult forms of gastrointestinal nematodes-chemotophages *Haemonhus spp.*, *Bunostomum spp.*, *Oesophagostomum spp.* and others. It is administered in doses of 10 mg/kg bw. against imaginary forms of fasciols over 12 weeks of age, while in younger invasion the dose may be increased to 15 mg/kg bw. It is known under the trademarks Dovenix – 25% solution for injection and Trodax – 34% solution for injection in both cases as the N-ethylglucamine salt of nitroxinil.

Materials and metods

In essence, the study is based on the formation of parallel groups in which the animals were randomized based on the intensity of the invasion with *F. hepatica*. In each separate experiment three groups are formed – two experimental and one control. The experiments were performed to compare and possibly confirm the clinical equivalence between the veterinary medicinal product Nitroxinil 34% inj. sol. and the original Trodax product 34%. The experiments are performed with naturally infested animals. In essence, they are defined as the so-called "Dose-confirming experiments".

Randomization: In preliminary studies, animals were grouped into triplets depending on the established invasion. In descending order, the members of each three are divided into three groups – two experimental and one control.

Experimental animals: A total of 126 sheep of the Local Improved breed were used, with a body weight of 40–50 kg, mostly female and of different ages. In the experiments with cattle, 60 cattle from the cross X Local Chernoshareno and 30 buffaloes of the breed "Murrah", of female sex with different live weight, were used.

Clinical signs and diagnosis: only healthy animals without clinical signs of *fasciolosis* were included in the experiments. The criterion for inclusion in the experiments is the intensity of fasciol invasion, determined in the laboratory.

Animal husbandry: sheep are privately owned. In spring, summer and autumn the sheep are raised on pasture, and in winter – on a stable. The feed during the stable period consists mainly of hay and feeding with cereals – barley, wheat and oats. The agility campaign is in the period January–March. Cattle and buffaloes are also owned by private farmers. Usually one owner has 2–3 cows. The feeding of cattle and buffaloes is pasture in summer and with hay and silage in autumn and winter. They are fed with concentrated fodder – barley, wheat and corn all year round. The water is from natural and tap sources.

Concomitant treatments: no other animal treatment drugs were administered during the experiments in the experimental and control groups.

Veterinary medicinal products: Dosage forms Nitroxinil 34% solution for injection – "Investigation product"

VMP is manufactured by the company Dopharma to order. Contains 34% Nitroxinil as N-Ethylglucamine salt. Batch No.03G299, Manufacturing date 07-2019. Expire date 07-2021.

Trodax 34% inj. sol. – "Control product"

Trodax 34% inj. sol., Commercially available, with Batch No. R077321A, Expire date 11-2021, was used.

Doses, method and frequency of administration: The test and control products were administered at a dose of 10 mg/kg bw. or 1 ml per 34 kg bw. The sheep were injected once subcutaneously behind the elbow joint, following the rules of asepsis and subcutaneous medication. Cattle and buffaloes were also injected once subcutaneously, but in the neck area, in an amount of 1 ml per 34 kg body weight.

Methods / Analytical procedures

Fasciola hepatica: the semi-sedimentation method was used to determine the amount of eggs in grams of faeces (Gonzales-Lanza et al., 1989).

Gastrointestinal nematodes: The classical McMaster method was used to count eggs in grams of faeces in sheep, cattle and buffaloes.

The helminths from the rennet, the duodenum, the small, large and caecum of the autopsied 18 sheep were collected after repeated washing with tap water and settling of the intestinal contents, and preserved in Barbagal's solution.

They were listed and assigned to a genus. The procedures described in section 4.6 have been used. Necropsy procedures (Veterinary Parasitology, 58 (1995), 181-213).

Statistical methods

Working with raw data. When processing the data from the study of the intensity of the inva-

Table 1. Intensity of invasion in cattle and buffaloes before inclusion in the experiments

Experiment №	Populated place	Number of animals in a group			Invasion before treatment (eggs in grams of faeces)					
		Nitroxinil 34% n	Trodax n	Control group n	Nitroxinil 34%	Trodax	Control			
					F. hepatica	*GI nematodes*	*F. hepatica*	*GI nematodes*	*F. hepatica*	*GI nematodes*
1	A	9	9	9	37.03 ± 2.45	88.89 ± 27.36	37.03 ± 3.93	122 ± 11.79	35,1 ± 1,85	83.33 ± 23.57
2	B	11	11	11	48.47 ± 8.24	93.18 ± 23.86	46.6 ± 10.05	86.36 ± 30.16	46,9 ± 8,65	50.0 ± 15.08
3	C	10	10	10	98.32 ± 20.25	70.00 ± 13.33	99.98 ± 19.72	100,0 ± 25.82	98,3 ± 11,36	70.0 ± 12.72
	Total:	30	30	30						

Table 2. Intensity of invasion in sheep before inclusion in the experiments

Experiment №	Populated place	Number of animals in a group			Invasion day 0 (before treatment) eggs in grams of feces					
		Nitroxinil 34% n	Trodax n	Control n	Nitroxinil 34%	Trodax	Control			
					F.hepatica	*GI nematodes*	*F. hepatica*	*GI nematodes*	*F. hepatica*	*GI nematodes*
1	A	10	10	10	104.99 ± 28.98	155.0 ± 28.34	104.99 ± 20.41	155.0±28.51	104.98 ± 28.00	130.0 ± 22.61
2	B	9	9	9	127.76 ± 31.3	216.67 ± 20.41	127.76 ± 31.3	222.2 ± 44.96	122.2 ± 26.5	144.4 ± 28.2
3	C	6	6	6	86.09 ± 7.95	141.67 ± 15.36	86.09 ± 7.95	141.67 ± 27.13	77.76 ± 9.29	150.0 ± 28.86
4	D	6	6	6	88.87 ± 15.31	116.67 ± 10.54	83.31 ± 10.53	125.0 ± 11.18	86.09 ± 7.95	141.67 ± 32.7
5	E	11	11	11	257.6 ± 16.44	245.45 ± 31.95	257.61 ± 16.44	254.55 ± 37.82	255.92 ± 15.73	181.82 ± 34.56
	Total:	42	42	42						

sion, the detected invasion was transformed into a geometric mean for the whole group.

$$IE\% = \frac{(GM\ e.p.g\ SG - GM\ e.p.g.\ TG) \times 100}{GM\ e.p.g.\ CG}$$

$$IE\% = \frac{(GM\ No.\ of\ parasites CG - GM\ No.\ of\ parasites\ TG) .100}{GM\ No.\ of\ parasites\ CG}$$

Where:

IE – Intensity Efficiency%

GM e.p.c TG – geometric mean (number of eggs per gram of faeces) in the experimental group

GM e.p.g C – geometric mean (number of eggs per gram of faeces) in the control group

GM No. of parasites TG – geometric mean (number of parasites) in the experimental group

GM No. of parasites C – geometric mean (number of parasites) in the control group

Statistical processing of results

One-way analysis of variance (Regular ANOVA test) and Tukey's Multiple Comparison Test (post test) were used for data processing. GraphPad Prsm program, version 5.0 was used

Results and discussion

Experiments with Large Ruminants (Intensity of invasion after treatment)

Tables 3 and 4 present the results of coprooscopic examinations of faecal samples from the experimental and control groups of Large Ruminants on the 14th and 21st after treatment with the two compared preparations Nitroxinil 34% and Trodax 34%.

No fasciol eggs were found in cattle in both experiments (populated place A and B) in both studies on the indicated dates.

In the case of buffaloes (populated place B) on the 14th and 21st day, an arithmetic mean of 6.67 ± 4.44 eggs per gram of faeces was found in those treated with Nitroxinil 34%, and in the group treated with Trodax 34% – 3.33 ± 3.33 eggs per gram of feces

The same tables show the results of a study of the clinical efficacy of the two compared veterinary medicinal products Nitroxinil 34% and Trodax 34% against natural invasion by gastrointestinal nematodes in large ruminants on days 14 and 21 after treatment.

It is known that nitroxynylate has a proven activity in the usual dose of 10 mg/kg body weight only against *Haemonchus contortus* and some blood-sucking nematodes – *Oesophagostomum spp.* and *Bunostomum spp.*

During the statistical processing of the results, no statistically significant difference in the number of eggs was found between the groups treated with the test preparations and the control group ($P > 0.05$).

Calculation of the effectiveness of the compared preparations Nitroxynil 34% and Trodax against invasion with *F. hepatica.*

The table shows that in cattle, 100% efficacy against *F. hepatica* invasion was reported on both the 14th and 21st day after treatment, while in buffaloes it was 97.7% and 97.9%, respectively.

According to the recommendations of the W.A.A.V.P., based on the registered efficacy, it can be assumed that both preparations are highly effective.

No efficacy was reported against mixed infestation with gastrointestinal nematodes due to the inability to determine the type of infestation only in eggs found in the faeces of treated animals.

Experiments with sheep

Intensity of invasion after treatment (copro ovoscopic examinations).

Tables 6 and 7 present the results of coprooscopic examinations of faecal samples from the experimental and control groups in sheep of the 14th and 21st after treatment with the two compared products Nitroxinil 34% and Trodax 34%.

The tables show that the intensity of the invasion in the groups treated with the tested products decreased sharply compared to the control group at 14 days after treatment ($P < 0.001$).

Table 3. Intensity of invasion with *Fasciola hepatica* and gastrointestinal nematodes in Large Ruminants on day 14 after treatment with Nitroxinil 34% and Trodax

Experiment №	Populated place	Number of animals in a group	Intensinvasion Day 14 after treatment eggs in grams of feces							
		Nitroxinil 34% n	Trodax n	Control Group n	Nitroxinil 34%	Trodax	Control Group			
					Fasciola hepatica	*GI nematodes*	*Fasciola hepatica*	*GI nematodes*	*Fasciola hepatica*	*GI nematodes*
1	A	9	9	9	0	66.67 ± 14.43	0	66.67 ± 23.57	40.73 ± 2.93	72.22 ± 8.78
2	B	11	11	11	0	81.82 ± 23.62	0	72.73 ± 30.16	51.51 ± 12.2	43.18 ± 3.52
3	C	10	10	10	6.67 ± 4.44	60.00 ± 14.53	3.33 ± 3.33	75.0 ± 17.08	99.98 ± 15.72	70.0 ± 10.55
	Total:	30	30	30						

Table 4. Intensity of *Fasciola hepatica* and gastrointestinal nematode invasion in Large Ruminants on day 21 after treatment with Nitroxinil 34% and Trodax

Experiment №	Populated place	Number of animals in a group	Intensinvasion Day 21 after treatment eggs in grams of feces							
		Nitroxinil 34% n	Trodax n	Control group n	Nitroxinil 34%	Trodax	Control group			
					Fasciola hepatica	*GI ematodes*	*Fasciola hepatica*	*GI nematodes*	*Fasciola hepatica*	*GI nematodes*
1	A	9	9	9	0	61.11 ± 11.11	0	66.67 ± 11.79	40.73 ± 2.93	77.78 ± 14.7
2	B	11	11	11	0	81.82 ± 22.64	0	68.18 ± 45.24	63.62 ± 9.75	63.64 ± 11.86
3	C	10	10	10	6.67 ± 4.44	70.00 ± 13.33	3.33 ± 3.33	80.0 ± 15.28	109.98 ± 19.41	85.0 ± 7.89
	Total:	30	30	30						

Table 5. Efficacy against *F. hepatica* of Nitroxinil 34% and Trodax 34% in Large Ruminants

Experiment №	Populated place	Number of animals in a group			IE (%) 14th day		IE (%) 21th day	
		Nitroxinil 34%	Trodax	Control	Nitroxinil 34%	Trodax	Nitroxinil 34%	Trodax
					Fasciola hepatica	*Fasciola hepatica*	*Fasciola hepatica*	*Fasciola hepatica*
1	A	9	9	9	100	100	100	100
2	B	11	11	11	100	100	100	100
3	C	10	10	10	97.7	97.81	97.9	98.001

Table 6. Invasion rate of *Fasciola hepatica* and gastrointestinal nematodes in sheep on day 14 after treatment with Nitroxinil 34% and Trodax

Experiment №	Populated place	Number of animals in a group	Intensinvasion Day 14 after treatment eggs in grams of feces							
		Nitroxinil 34% n	Trodax n	Control n	Nitroxinil 34%	Trodax	Control			
					F. hepatica	*GI nematodes*	*F. hepatica*	*GI nematodes*	*F. hepatica*	*GI nematodes*
1	A	10	10	10	8.33 ± 4.48	105.0 ± 13.85	10.00 ± 5.89	100.0 ± 16.67	103.32 ± 18.05	145.0 ± 11.67
2	B	9	9	9	11.11 ± 7.35	111.11 ± 13.86	11.11 ± 6.21	144.44 ± 22.74	166.6 ± 28.9	133.3 ± 18.6
3	C	6	6	6	8.33 ± 5.69	141.67 ± 23.86	11.11 ± 8.24	116.67 ± 27.88	83.31 ± 10.53	125.0 ± 11.18
4	D	6	6	6	8.33 ± 5.69	108.33 ± 15.36	11.11 ± 8.24	116.67 ± 27.88	83.31 ± 10.54	116.67 ± 10.54
5	E	11	11	11	24.24 ± 6.10	172.73 ± 31.89	23.46 ± 6.03	131.82 ± 12.20	263.50 ± 17.06	186.36 ± 29.46
	Total:	42	42	42						

Table 7. Intensity of invasion with *Fasciola hepatica* and gastrointestinal nematodes in sheep on day 21 after treatment with Nitroxinil 34% and Trodax

Експ. №	Populated place	Number of animals in a group		Intensinvasion Day 21 after treatment eggs in grams of feces							
		Nitroxinil 34% n	Trodax n	Control n	Nitroxinil 34%		Trodax		Control		
					F. hepatica	*GI nematodes*	*F. hepatica*	*GI nematodes*	*F. hepatica*	*GI nematodes*
1	A	10	10	10	13.33 ± 5.98	135.0 ± 16.73	13.33 ± 5.76	130.0 ± 19.00	119.98 ± 15.28	185.00 ± 25.88
2	B	9	9	9	27.77 ± 8.78	138.89 ± 23.24	25.92 ± 8.38	133.33 ± 22.05	161.1 ± 23.2	144.4 ± 30.6
3	C	6	6	6	22.22 ± 10.24	116.67 ± 21.08	22.22 ± 10.24	133.33 ± 27.88	108.31 ± 15.36	150.0 ± 28.86
4	D	6	6	6	27.77 ± 7.02	133.33 ± 16.66	27.77 ± 9.29	125.0 ± 21.40	136.04 ± 18.92	183.33 ± 24.72
5	E	11	11	11	21.21 ± 6.39	172.73 ± 32.59	21.21 ± 6.39	154.55 ± 15.75	265.07 ± 15.13	163.64 ± 27.88
	Total:	42	42	42						

No differences were found in the effectiveness of the studied products (the decrease of eggs in grams of feces in the two experimental groups is the same – ($P > 0.05$). The data are similar in the study of coprobes from the groups on the 21st day after injection.

Calculation of the Efficacy of Nitroxynil 34% and Trodax (based on coprooscopic studies).

Table 8 shows that the found Efficacy against *F. hepatica* is above the required 90%, which shows that the tested products are effective against imaginary forms of the parasite. In some of the experiments an efficiency of over 98% was registered, which speaks of its high efficiency.

Statistical processing of the data showed that there was a significant difference between the experimental and control groups in all experiments ($P < 0.01$), while no significant difference was found between the two experimental groups ($P > 0.05$).

(Gupta et al., 2004) and (Islam KS, 1985) reported 100% efficacy of nitroxynil (Inj. Nitronex) against fasciolosis in cattle at a dosage of 10 mg/kg body weight. (Ponikarov, 1989) reported 100% efficacy of nitroxynil (Inj. Nitronex) at a dose of 12 mg/kg body weight when administered as a subcutaneous injection against fasciolosis in cattle. The results obtained for nitroxynil are in accordance with (Durrani et al., 2007), which determines the percentage efficacy for nitroxynil of 93.88% against fasciolosis without side effects after injection of the veterinary medicinal product. (Rapic et al., 1988) determined a 95.0% efficacy of nitroxynil in cattle against fasciolosis. (Dobbins et al., 1982) determined the efficacy of nitroxynil at 88.5% in cattle against *Fasciola sp.*

Conclusion

Based on the results of clinical field trials to establish the efficacy of the veterinary medicinal product Nitroxinil 34% solution for injection produced by Dopharma, the product was found to be highly effective against imaginary forms of *F. hepatica* (over 8 weeks of age) at the usual

Table 8. Efficacy (%) of Nitroxinil 34% and Trodax against *F. hepatica* in sheep

Experiment №	Populated place	Number of animals in a group			IE% 14th day		IE% 21st day	
		Nitroxinil 34%	Trodax	Control	Nitroxinil 34%	Trodax	Nitroxinil 34%	Trodax
					Fasciola hepatica	*Fasciola hepatica*	*Fasciola hepatica*	*Fasciola hepatica*
1	A	10	10	10	96.96	96.84	96.39	95.55
2	B	9	9	9	98.35	97.84	93.94	94.2
3	C	6	6	6	96.36	96.13	93.51	93.51
4	D	6	6	6	96.38	96.13	90.97	90.88
5	E	11	11	11	94.64	97.24	96.15	97.96

doses of 10 mg/kg body weight (1 ml – 34 kg/kg body weight).

With regard to invasion by gastrointestinal nematodes, the test products have activity only against *H. contortus.*

The efficacy of Nitroxinil 34% solution for injection does not differ from the original Trodax ($P > 0.05$), which leads to the conclusion that the two products are equivalent in terms of their clinical efficacy.

Adverse reactions associated with the use of test products: No side effects associated with the tested products were observed.

References

Durrani, A. Z., Khan, M. S., & Kamal, N. (2007). Prevalence of fascioliosis in buffaloes and comparative efficacy of Endoactiven and Nitroxynil against fascioliosis. *J. Anim. Pl. Sci*, *17*, 1-2.

Gupta, S. C., Ghosh, S., & Singh, B. P. (2004). Efficacy of triclabendazole against experimental Fascioliasis in cross breed cattle calves. *Indian Veterinary Journal. 81*,132-134.

Islam, K. S., & Jabbar, M. A. (1985). Present situation of livestock and Poultry Diseases in Bangladesh. In Bangladesh Poshushampad Unna-yan – Neiti O Kowshal. BARC, *Dhaka and ADC, N. Y. P.*, 48-128.

Ponikarov, A. V. (1989). Efficacy of Nitronex injection in Cattle. By Uleten-Vesesoyunogo-Instituta-Gelmintologi-im, -K-1-Kryabina, 52:86.

Rapic, D., Dzakula, N., Sakar, D., & Richards, R. J. (1988). Comparative efficacy of triclabendazole, nitroxynil and rafoxanide against immature and mature Fasciola hepatica in naturally infected cattle. *The Veterinary Record*, *122*(3), 59-62.