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Title: The Anthelmintic Activity of SCH 32481 (Netobimin^{R}) Against Gastrointestinal Nematodes in Cattle and Sheep and Fasciola Hepatica in Sheep.

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Dr. Gary L. Zimmerman

The efficacy of a new broad-spectrum anthelmintic, Netobimin^{R} (coded SCH 32481-Schering Corporation) was evaluated in two trials conducted during either the spring or fall grazing seasons of 1984 in Western Oregon using 20 cross-bred yearling beef heifers and 30 cross-bred spring lambs, respectively. Percent efficacies were determined in both bovine and ovine hosts harboring naturally acquired gastrointestinal nematode infections and were reported with respect to genera and species of nematode and morphological stage of life cycle when recovered. Fasciolicidal efficacy was concurrently evaluated in the sheep with experimentally induced mature Fasciola hepatica infections. An oral formulation of netobimin was administered in both studies via a modified oral drenching gun to animals randomly divided into groups based on body weight and egg per gram (EPG) counts. Ten heifers received a dose level of 7.5 mg/kg (concentration 150 mg/ml) and

10 remained untreated as controls. Sheep were divided into three groups of ten and received either 7.5 mg/kg or 20 mg/kg of netobimin (concentration 50 mg/ml) or a tap water drench placebo which was given to the control group. All heifers were necropsied two weeks post-treatment and sheep were necropsied either one or two weeks post-treatment. Parasitic gastrointestinal helminths were recovered using standard techniques. Fecal samples were taken throughout the trial and EPG counts monitored. Fecal samples taken on trial termination dates revealed EPG counts (excluding F. hepatica eggs) were reduced in treated heifers by 98% and in treated sheep groups by 62% (7.5 mg/kg) or 100% (20 mg/kg). The overall efficacy against all species of adult nematode found (excluding Trichuris spp.) in the bovine study was 67% ($p \leq 0.05$). The overall efficacy in the ovine study against all nematode species including all life stages present was 88% ($p \leq 0.0002$) at 7.5 mg/kg and 99% ($p \leq 0.0002$) at 20 mg/kg; the respective efficacies against F. hepatica were 62% ($p \leq 0.05$) and 91% ($p \leq 0.01$). No adverse reactions or signs of toxicosis were observed in either trial.

The Anthelmintic Activity of SCH 32481 (Netobimin®) Against Gastrointestinal Nematodes in Cattle and Sheep and *Fasciola Hepatica* in Sheep

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Redacted for Privacy

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Introduction

Control of parasitic helminth infections in both the cattle and sheep industry continues to be a major concern for producers, consulting veterinarians, and ultimately the consumers. Controlling internal parasites since the late 1930's has largely centered around the use of anthelmintics. Economic losses resulting from parasitism in livestock continue to be staggering; production losses are due to impaired digestion and decreased feed conversion efficiency which results in decreased fat and protein deposition. Other factors which have been implicated include: cost of medicaton (anthelmintics and antibiotics) and labor required to administer them; altered immune status with increased susceptibility to viral, bacterial or protozoan infections; hyperplasia and/or destruction of gastric and intestinal mucosa; liver involvement associated with Fasciola hepatica infections and losses due to altered function and liver condemnation; decreased milk and wool production; decrease in skeletal calcium deposition resulting in osteoporosis with severe infestation; decreased reproductive efficiency has been reported; and lastly increased mortality rates when animals have been severely compromised (Armour, 1970; Black and Froyd, 1972;

Brundson, 1975; Hope-Cowdery et al., 1977; Hawkins and Morris, 1978; Sykes, 1978; Morris and Meek, 1980; Randell and Bradley, 1980; Bradley and Sand, 1982; Foreyt, 1982).

Since the introduction of phenothiazine in the late 1930's, anthelmintics have undergone extensive changes in terms of chemical composition, mode of action, toxic effects associated with some, and methods or routes of application. Older anthelmintics were usually administered as oral drenches or by stomach tubing partially due to their unpalatable nature or because products were so irritating to the oral mucosa. Although drenching suspensions are still being used effectively, newer formulations include tablets, boluses, pastes, gels, or either parenterally or intraruminally injectable solutions. Some innovations for herd or flock treatments include products available as top dressings in pellet or crumble form to be added to feed or drinking water. In addition, medicated feed blocks, feed premixes, "pour on" compounds, and slow-release drugs are becoming more readily available.

The chemotherapeutic approach to parasitic helminth control in ruminants began with phenothiazine, which had limited efficacy and potency against gastrointestinal nematodes in cattle and sheep (Page, 1949; Roberts and Keith, 1959; Thomas, 1959; Colglazier et al., 1967). In time, due to inappropriate or overzealous use, drug resistant strains of sheep nematodes began appearing in the U.S. (Leland et al., 1957; Drudge et al., 1964). Side effects related to phenothiazine also became apparent with reports of photosensitivity and corneal opacities in cattle. Unthrifty animals were also found to be highly sensitive to

phenothiazine , and this drug was contraindicated in later stages of pregnancy.

Early in the 1960's, a new class of anthelmintics were introduced, the benzimidazoles, possessing a broader spectrum of efficacy against parasitic helminths. Thiabendazole was the first marketed in the U.S. but within three years of its introduction, strains of sheep nematodes resistant to it were reported both here and abroad (Drudge et al., 1964; LeJambre et al., 1976, 1978, 1979; Hall et al., 1979; Kelly and Hall, 1979; Sangster et al., 1979; Barton, 1980; Miller and Baker, 1980; Pritchard et al., 1980). Albendazole, another benzimidazole introduced recently, had promising broad-spectrum properties against nematodes, cestodes, and trematodes in cattle and sheep (Theodorides et al., 1976a, 1976b, 1976c; Benz and Ernst, 1977; Knight and Colglazier, 1977; Williams et al., 1977; Downey, 1978; Campbell and Hall, 1979; Van Schalkwyk et al., 1979; Wescott et al., 1979; Theodorides and Freeman, 1980; Williams et al., 1981a; Malone et al., 1982; Todd and Mansfield, 1982). It received investigational new animal drug (INAD) clearance and was available on a prescription basis for a relatively short period of time, but was withdrawn by the FDA early in 1985. As a known teratogenic compound, albendazole was contraindicated in the first 45 days of gestation and had a required withdrawal time of 180 days prior to slaughter.

Fenbendazole, also a benzimidazole, has excellent efficacy against the most common parasitic nematodes of ruminants (Kennedy and Todd, 1975; Kirsch and Düwell, 1975; Anderson, 1977; Benz and Ernst, 1978; Craig and Bell, 1978; Thomas, 1978; Gunawan et al., 1979; Callinan and

Cummins, 1979) and is approved for use in cattle. Just recently (spring 1986) it received INAD clearance for use in sheep. Unfortunately, to date, there has been many reports of cross-resistance between benzimidazoles and pro-benzimidazoles in sheep (included in this group are: thiabendazole, parbendazole, cambendazole, mebendazole, oxibendazole, oxfendazole, albendazole, thiophanate, and fenbantel) (Berger, 1975; Hall et al., 1978; Kelly and Hall, 1979; Pritchard et al., 1980; Green et al., 1981; Hall et al., 1982). Resistance in cattle nematodes has not been documented to date, but probably is occurring. It is likely that resistance problems reported in ovine helminths has occurred due to a greater selection pressure placed on parasites with regard to more frequent treatments with more varied anthelmintic products.

Another class of drugs in current use are the imadazothiazoles, which include levamisole and morantel tartrate. Levamisole has exhibited high antiparasitic activity against most sheep trichostrongyloids (Colglazier et al., 1969; McKenna, 1974; Reid et al., 1976; Callinan and Barton, 1979; Herd et al., 1984). In addition, efficacy has been reported against hypobiotic and benzimidazole-resistant sheep nematodes (Herd et al., 1984). However, reports of resistance to levamisole have been documented as well (LeJambre et al., 1976, 1978; Sangster et al., 1979; Whitlock et al., 1980; LeJambre, 1981; Barton, 1983). Efficacy against cattle nematodes has been well established, as well as a report published indicating a possible resistance phenomenon associated with Ostertagia ostertagi (Lyons et al., 1981). Reported signs of toxicity with oral levamisole include:

muzzle foaming, excessive salivation and lacrimation, head shaking, ataxia, and muscle tremors which can be induced at relatively low doses. Tissue reactions and necrosis have been observed with the injectable form.

Morantel tartrate has been shown to have effective activity, as well as reports of resistance to parasitic helminths, in ruminants (LeJambre et al., 1976, 1978; Sangster et al., 1979; Whitlock et al., 1980). This anthelmintic is now cleared for use in cattle in a slow-release bolus form, but at this time has not been cleared for use in sheep.

The avermectins, the most recent group of anthelmintics to be marketed in the U.S., are produced by fermentation of the actinomycete Streptomyces avermitilis. Excellent antiparasitic activity against a number of gastrointestinal nematodes, including various life stages and some activity against ectoparasities, have been reported in cattle (Benz and Ernst, 1979; Williams et al., 1981b; Yazwinski et al., 1981) and sheep (Armour et al., 1982; Wescott and LeaMaster, 1982; Yazwinski et al., 1983). Efficacy reports include activity against some benzimidazole-resistant strains of Haemonchus contortus, but limited efficacy against Nematodirus spp. (Wescott and LeaMaster, 1982). Ivermectin is presently available for use against gastrointestinal nematodes and ectoparasites of beef cattle, but requires a 35-day withdrawal period prior to slaughter. It is contraindicated in lactating dairy cows and, as of this writing, has not been cleared for use in sheep.

Anthelmintics produced for use against Fasciola hepatica have been employed with varying degrees of efficacy against immature and mature liver flukes (reviewed by Boray, 1982). Many fasciolicidal compounds are available in other contries, but as of this writing, only one compound is available in the United States. Clorsulon (Curatrem^{R}-Merck, Sharp and Dohme) received FDA clearance for oral administration in beef cattle only, early in 1985, within weeks after the INAD status of albendazole was withdrawn. This compound is highly efficacious (Wyckoff and Bradley, 1983; Malone et al., 1984; Fetterer et al., 1985; Yazwinski et al., 1985; Zimmerman et al., 1986) but has not been cleared for use in dairy cattle or sheep.

Clorsulon must be combined with a nematocidal compound for broad-spectrum coverage of nematodes and trematodes in beef cattle (Courtney et al., 1985). Levamisole and thiabendazole, are current anthelmintics with label approval for use in sheep. Use of clorsulon for F. hepatica infections in sheep is currently on an extra-label basis.

Recently, Schering Corporation developed a new broad-spectrum anthelmintic, netobimin, which has both nematocidal and fasciolicidal activity (Williams et al., 1985; Richards and Zimmerman-unpublished data and Schering Corporation-unpublished data, 1985). This compound has exhibited activity against some drug-resistant nematodes (Schering Corporation data, 1985) and has properties which would allow for either parenteral or oral administration.

The purpose of this study was to evaluate the activity of netobimin against gastrointestinal nematodes in cattle and sheep and Fasciola hepatica infections in sheep at different dosage levels.

Anthelmintic Efficacy of Netobimin Against Naturally Acquired Gastrointestinal Nematodes in Yearling Heifers

L.S. Richards,[1] G.L. Zimmerman,[1] D.W. Weber,[2] S.W. Dawley,[3] M.J. Nelson,[1] and D.J. Schons[1]

[1]College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331-4802 (USA)

[2]Department of Animal Science, Oregon State University, Corvallis, Oregon 97331-6702 (USA)

[3]Schering Corporation, Animal Health Division Galloping Hill Road, Kenilworth, New Jersey 07033 (USA)

Address to which proofs should be sent:

Dr. Gary L. Zimmerman
College of Veterinary Medicine
Oregon State University
Corvallis, OR 97331-4802

ABSTRACT

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The efficacy of netobimin (Coded SCH 32481-Schering Corporation) in removing naturally acquired gastrointestinal nematode infections was evaluated in 10 treated and 10 untreated (control) yearling beef heifers. The anthelmintic was administered as an oral drench at a dose level of 7.5 mg/kg body weight. EPG counts were reduced with netobimin by 98% ($p \leq 0.01$) at both one and two weeks post-treatment. The compound was highly effective in removing Oesophagostomum radiatum (100% at $p \leq 0.01$), Cooperia spp. (97.66% at $p \leq 0.01$) and Nematodirus helvetianus (100%, although not significant) but was ineffective against immature Ostertagia ostertagi (3.19%) and only moderately effective against adult forms (66.14% at $p \leq 0.05$). The low efficacy against adult O. ostertagi was partially attributed to the maturation of fourth-stage larvae during the 14-day treatment-slaughter interval not removed by the 7.5 mg/kg treatment. Efficacy against Trichuris spp. could not be evaluated due to low levels and unequal numbers of worms recovered in the groups. The overall efficacy against adult species,

excluding Trichuris spp., was 67.40% ($p \leq 0.05$). No adverse reactions or signs of toxicosis were observed in heifers treated with netobimin.

INTRODUCTION

Many anthelmintics are used in the United States for control of gastrointestinal helminth infections in cattle. However, with the exception of albendazole (no longer available), none have anthelmintic properties for control of nematodes, cestodes and trematodes (Theodorides et al., 1976a; Theodorides et al., 1976b; Benz and Ernst, 1977; Williams et al., 1977; Downey, 1978; Westcott et al., 1979; Theodorides and Freeman, 1980; Williams et al., 1981a; Malone et al., 1982; Todd and Mansfield, 1982).

The recently developed broad-spectrum anthelmintic, netobimin (Schering Corporation, Kenilworth, N.J.), has activity similar to that of albendazole against a wide variety of parasitic helminths (Williams et al., 1985; Richards and Zimmerman-unpublished data, and unpublished studies conducted outside the U.S. for Schering Corporation). Netobimin also has properties which allow for either oral or parenteral administration.

The purpose of this study was to evaluate the activity of oral netobimin at 7.5 mg/kg against naturally acquired gastrointestinal nematodes of mixed species in cross-bred yearling beef heifers.

MATERIALS AND METHODS

Animals and Allotment

A group of 36 mixed breed beef yearling heifers were obtained from and maintained at the Oregon State University Campus Beef Center. All had been grazed on pastures shown previously to be contaminated with infective larvae of mixed species of gastrointestinal nematodes. Patent infections were confirmed by fecal egg per gram (EPG) counts which demonstrated trichostrongyloid type, Nematodirus spp. and Trichuris spp. eggs. A modified salt flotation-centrifugation technique was used for EPG counts in which nematode eggs were recovered on glass coverslips placed over centrifuge tubes.

On 19 March 1984 twenty heifers were selected and allocated into treatment and control groups based on weights and fecal worm EPG counts. The animals were removed from pastures and housed indoors on concrete floored pens and maintained under standard management conditions. Animals were allotted into principle and control groups based on EPG counts such that group mean EPG counts were nearly equal on 19 March 1984. Heifers were also examined and weighed at that time, with weights ranging from 184.1 kg to 243.6 kg ($\bar{x}$ = 217.6 kg). Rectal fecal samples for EPG counts were again collected on 17 April 1984 and a week later on 23 April 1984 to monitor infections. On 1 May 1984 (treatment day), heifers were weighed and fecal samples again taken.

The oral formulation of netobimin was reconstituted with water to a concentration of 150 mg/ml. The animals of the treatment group were each given netobimin at a dosage level of 7.5 mg/kg body weight (b.w.) by means of a modified oral drenching gun. After treatment all animals were observed on a daily basis for adverse reactions or signs of toxicosis. Fecal EPG counts were performed 7 and 14 days post-treatment.

Necropsy Procedures

One half of the heifers of each group were necropsied on 14 May 1984 (day 13 post-treatment) and the remainder on 15 May 1984 (day 14 post-treatment). The abomasum, small intestine and large intestine-cecum were ligated at both ends and removed. The abomasum was opened longitudinally, contents collected by hand stripping the mucosal surface three times and washed with tap water. Duplicate samples (5% of pooled contents and wash) were then washed through a 12 inch 400 mesh (37.5 µm aperature) stainless steel screen and preserved with a 70% alcohol-iodine solution. After hand stripping, each abomasum was incubated in tap water for 24 hours at room temperature, stripped again and washed with tap water. The incubate material and rinse water were pooled, sieved (400 mesh screen), divided in half and preserved with the alcohol-iodine solution. The small and large intestines-cecum were prepared in the same manner as the abomasal contents. The aliquots (5%)

were sieved through either a 400 mesh screen (small intestines) or a 100 mesh 150 µm aperature screen (large intestines-cecum) and preserved.

Nematodes were recovered with aid of dissecting microscopes (20x) and then preserved in a 10% buffered formalin solution. Enumeration and identification as to genera, species, and morphologic stage of development was with a compound microscope. Numbers of parasites recovered from control and treated groups were compared and calculations of efficacy were based on the aliquots taken from various gut sections. (See Table I.1).

Statistical Analysis

The intensity of infection per species and/or developmental stage as well as total numbers of parasites were determined for animals in the control and treatment groups. Calculations of the total worms contained in the animals were based on the actual numbers of worms recovered from 5% aliquots taken from various gut sections. Data was then tested for normality using the Chi-square Goodness of Fit Test and for homogeneity of variance using the Bartlett Test. Both were found to be significantly non-normal and/or lacking in homogeneity of variance. Consequently, the Mann-Whitney Two Sample Test was used to compare group means (see statistical summary Table I.2). Results were ultimately reported in terms of efficacy using the formula:

$$\frac{\text{control worm burden-treated worm burden}}{\text{control worm burden}} \times 100 = \% \text{ efficacy}$$

Animal EPG counts were also subjected to homogeneity of variance and normality tests and were significantly non-normal and therefore also subjected to the non-parametric Mann-Whitney Test.

RESULTS

Allocation of heifers into control and treated groups on 19 March 1984 resulted in mean weights of 216.9 kg and 218.4 kg, respectively; pre-treatment mean EPG counts for both groups were equal (416 EPG). On 1 May 1984 (treatment day), the mean EPG for the control and treatment groups were 138 and 155, respectively. Post-treatment (PT) mean EPG counts for the treated animals (one and two weeks PT) were 4 and 11, respectively. The final mean EPG counts for the control group was 586 (two weeks PT time). The EPG reduction by netobimin at 7.5 mg/kg was 98% ($p \leq 0.01$) at weeks one and two PT.

The numbers of worms recovered and the efficacy of netobimin at 7.5 mg/kg dose level are shown in Tables I.1 and I.2. The compound was highly effective in removal of *Oesophagostomum radiatum* 100% ($p \leq 0.01$), *Cooperia* spp. 97.66% ($p \leq 0.01$) and *Nematodirus helvetianus* 100%, although not statistically significant due to low burdens of this species.

Netobimin at 7.5 mg/kg dose level was ineffective in removal of immature forms (early and late fourth stage) of Ostertagia ostertagi (3.19%). The efficacy demonstrated against adult forms of O. ostertagi was 66.15% ($p \leq 0.05$). Efficacy against Trichuris spp. was not evaluated due to insufficient numbers of worms recovered. The efficacy against total adult worms in all gut sections excluding Trichuris spp. was 67.4% ($p \leq 0.05$). No signs of toxicosis nor any adverse reactions were noted during this trial with netobimin.

DISCUSSION

In naturally infected yearling beef heifers netobimin was highly effective at 7.5 mg/kg b.w. against Oesophagostomum radiatum (100%; $p \leq 0.01$), Cooperia spp. (97.66%; $p \leq 0.01$) and Nematodirus helvetianus (100%; although not statistically significant). Williams et al. (1985) also reported high efficacy of netobimin at 7.5 mg/kg against Trichostrongylus axei (adults 99.7%, immatures 100%), Haemonchus spp. (adults 95.1%) and slightly lower efficacy against Cooperia spp. (89.5%). In this trial, burdens of Haemonchus and T. axei were inadequate for evaluation.

In this trial (Oregon) and trials in Louisiana (Williams et al., 1985) there are variable efficacies of netobimin against all stages of Ostertagia ostertagi. Efficacy against early (E_4) and late (L_4) larvae was extremely low (3.2%) as compared to results reported by

Williams et al., (1985) against developing L_4 (83.3%) and inhibited E_4 larvae of 60.2% at the same dose level. Extreme variability of dose response in which numbers of inhibited or developing larvae numbers in treated animals exceed those found in controls have been frequently reported (Lancaster and Hong, 1977; Lancaster et al., 1981; Williams et al., 1981a; Williams et al., 1981b; Williams et al., 1984). Possible explanations for lack of anthelmintic activity against inhibited or hypobiotic larvae include hypotheses that relate conditioning of larvae with respect to environmental effects or the host's immune response (Lancaster and Hong, 1977). Seasonal differences in the depth of larval hypobiosis (Duncan et al., 1977) and the rate of passage of anthelmintic through the upper alimentary tract and effects such as esophageal groove closure with the use of benzimidazole compounds were suggested by Lancaster et al., 1981.

With respect to the low efficacy of netobimin against adult O. ostertagi (67.14%; $p \leq 0.05$) as compared to a 94.9% efficacy reported by Williams et al. (1985) at the same dose level, we propose the following explanation. In Western Oregon, the emergence of O. ostertagi from the hypobiotic state has routinely occurred during the early to mid-spring. The current study was conducted under the assumption that the hypobiotic larvae from fall inhibition had matured and therefore, we expected few fourth stage larvae remaining in our trial animals. Our data suggest that emergence from hypobiosis was a late season development phenomenon in 1984, which resulted in hypobiotic O. ostertagi (not removed by netobimin at 7.5 mg/kg) resuming development to adult 5th stage during the treatment-slaughter interval. Because the 7.5 mg/kg dosage was only

partially effective against immature forms, a large portion likely continued development to adult forms. This explanation is based on average life cycles of O. ostertagi in which resumption of development from inhibited to adult stages is usually around 13 to 15 days. Had we necropsied the animals one week earlier, we could have ruled out that adults found in our aliquots were derived from the hypobiotic larvae pool. However, with shorter time between treatment and necropsy dates, worms killed by the drug may not yet have passed from the animals. Because O. ostertagi made up such a large percentage of the helminth population (99.78% in the treated group and 97.76% in the control group) in this trial, the overall efficacy of netobimin against adult gastrointestinal nematodes (excluding Trichuris spp.) was only 67.4% ($p \leq 0.05$) as compared in the overall adult worm efficacy of 98.78% reported by Williams et al., (1985).

In conclusion, we believe that netobimin is a highly effective compound but the actual effectiveness of this compound was not realized due to this late season emergence of hypobiotic O. ostertagi.

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TABLE I.1
Post-mortem worm counts for treated and control heifers

Treatment Group	Animal No.	Ostertagia ostertagi Early Fourth	Late Fourth	Total Fourth	Fifth	Total	Cooperia spp.	Nematodirus helvetianus	Oesophagostomum radiatum	Trichuris spp.	Totals
Netobimin (7.5 mg/kg)	049	2,000	260	2,260	240	2,500	0	0	0	0	2,500
	131	1,220	120	1,340	540	1,880	0	0	0	20	2,000
	159	7,000	580	7,580	2,400	9,980	0	0	0	0	9,980
	167	9,220	400	9,620	7,020	16,649	20	0	0	40	16,700
	169	12,280	860	13,140	3,160	16,300	0	0	0	0	16,300
	171	2,620	180	2,800	2,080	4,880	40	0	0	0	4,920
	172	27,840	3,400	31,240	14,040	45,280	0	0	0	0	45,280
	190	960	20	980	140	1,120	0	0	0	0	1,120
	206	2,680	140	2,820	820	3,640	0	0	0	20	3,660
	207	8,120	200	8,320	720	9,040	0	0	0	0	9,040
	Totals	73,940	6,160	80,100	31,160	111,260	60	0	0	80	111,500
	$\bar{x}$	7,394	616	8,010	3,160	11,126	6	0	0	8	11,150
Controls	023	500	80	580	4,840	5,420	20	0	20	0	5,460
	048	5,680	280	5,960	6,500	12,460	360	0	140	0	12,960
	077	5,640	720	6,360	2,680	9,040	280	0	20	20	9,360
	112	260	20	280	2,440	2,720	200	0	140	0	3,060
	134	80	40	120	1,280	1,400	400	40	40	0	1,880
	184	360	200	560	5,020	5,580	100	0	20	20	5,720
	201	21,880	2,000	23,880	12,020	35,900	500	0	0	0	36,400
	212	8,140	800	8,940	16,600	25,540	200	0	140	20	25,900
	213	17,420	3,000	20,420	27,240	47,660	340	0	780	0	48,780
	215	13,840	1,800	15,640	16,200	31,840	160	0	100	0	32,100
	Totals	73,800	8,940	82,740	94,820	177,560	2,560	40	1,400	60	181,620
	$\bar{x}$	7,380	894	8,274	9,482	17,756	256	4	140	6	18,162

TABLE I.2
Statistical Summary Table for Yearling Beef Heifers Treated with Netobimin

Nematode Species	Treatment (mg/kg)	Mean	S.E.[a]	Efficacy[b] (%)	p-value
O. ostertagi E4	Control	7380	2492.2	-	
	7.5	7394	2581	?	.6501
O. ostertagi L4	Control	894	325.5	-	
	7.5	616	319.3	31.10	.6501
O. ostertagi 5	Control	9482	2648	-	
	7.5	3116	1378.2	67.14	.0156
O. ostertagi (E4 + L4)	Control	8274	2796.6	-	
	7.5	8010	2886.5	3.19	.6501
O. ostertagi (E4 + L4 + 5)	Control	17756	5142.9	-	
	7.5	11126	4195.8	37.34	.3075
Cooperia Spp.	Control	256	46.46	-	
	7.5	6	4.27	97.66	.0002
N. helvetianus	Control	4	4	-	
	7.5	0	0	100	.7055
Oe. radiatum	Control	140	73.33	-	
	7.5	0	0	100	.0007
Trichuris Spp.	Control	6	3.06	-	
	7.5	8	4.42	?	.9097
Unidentified[c]	Control	16	7.77	-	
	7.5	6	4.27	62.50	.2899
Adults (excluding Trichuris & O. ostertagi E4 & L4)	Control	9884	2712.3	-	
	7.5	3222	1374	67.40	.0233
Total (excluding Trichuris)	Control	18156	5205.4	-	
	7.5	11132	4195.4	63.10	.2730

[a] Standard error of the mean

[b] % efficacy = $\left[\frac{\text{control-treated}}{\text{control}} \times 100\right]$

[c] Incomplete worms
E_4 Early 4th stage
L_4 Late 4th stage
5 Adult stage

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The Anthelmintic Efficacy of Netobimin Against Naturally Acquired Gastrointestinal Nematodes in Sheep

L. S. Richards[1], G. L. Zimmerman[1], E. P. Hoberg[1], D. J. Schons[1], and S. W. Dawley[2]

[1]College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331-4802 (USA)

[2]Schering Corporation, Animal Health Division, Galloping Hill Road, Kenilworth, New Jersey 07033 (USA)

Address to which proofs should be sent:

Dr. Gary L. Zimmerman
College of Veterinary Medicine
Oregon State University
Corvallis, Oregon 97331-4802

ABSTRACT

Richards, L. S., Zimmerman, G. L., Hoberg, E. P., Schons, D. J., and S. W. Dawley, 1985. The Anthelmintic Efficacy of Netobimin Against Naturally Acquired Gastrointestinal Nematodes in Sheep. Vet. Parasitol., : - .

The broad-spectrum anthelmintic efficacy of netobimin (SCH 32481--Schering Corporation) was evaluated using 30 cross-bred spring lambs with naturally acquired infections of gastrointestinal nematodes. Three groups of ten animals each were allotted into either control (given a tap water drench as a placebo) or 7.5 mg/kg and 20 mg/kg dosage groups (given the netobimin as an oral drench). Seven to fourteen days post-treatment, animals were necropsied and nematodes recovered by standard techniques. Examination of fecal samples taken on dates of necropsy showed median egg production was reduced in treated animals (61.98% with 7.5 mg/kg and 100% with 20 mg/kg). The compound was highly effective in removal of adult nematodes representing a number of genera and species of trichostrongyloids at the 7.5 mg/kg and 20 mg/kg dose levels (shown respectively below). These included Ostertagia spp., with O. circumcincta, O. trifurcata, O. ostertagi, and Teladorsagia davtiani (96.20%; 100%), Trichostrongylus spp., with T. axei, T. vitrinus, and T. colubriformis (100%; 98.72%), Nematodirus spp., with N. spathiger, N. filicollis, and N. battus (100% both levels), and Haemonchus contortus

(100% both levels). High efficacies against other species of nematodes, (at both dose levels) were not statistically significant (Cooperia spp., Chabertia ovina and Oesophagostomum venulosum). At 20 mg/kg, netobimin significantly reduced populations of early and late fourth stage larvae of Ostertagia spp. by 100%. The overall efficacy (all life stages included) was 88.27% at 7.5 mg/kg and 98.77% at 20 mg/kg dose level. No adverse reactions or signs of toxicosis were observed.

INTRODUCTION

Control of parasitic helminths in sheep has largely depended upon chemotherapeutic methods. Factors resulting in the ineffective control of helminths include: 1) development of resistance to anthelmintics by economically important helminths in sheep; 2) differences in drug efficacy against adults and larvae of particular species (including hypobiotic larvae); 3) variations in parasite populations and their response to treatment due to the phenomenon of periparturient rise and relaxation of immunity; 4) increased costs of drugs and labor required for their administration; and 5) limitations in approved use.

Among the benzimadazole anthelmintics used in ruminants, thiabendazole has limited efficacy due to drug-resistance by nematodes (Drudge et al., 1964; Le Jambre et al., 1976, 1978, 1979; Hall et al., 1979; Kelly and Hall, 1979; Sangster et al., 1979; Barton, 1980; Miller and Baker, 1980; Pritchard et al., 1980). INAD (Investigational New Animal Drug) approval for emergency use of a related drug, albendazole, effective against nematodes and platyhelminths (Theodorides et al., 1976a, 1976b; Knight and Colglazier, 1977; Campbell and Hall, 1979; Van Schalkwyk et al., 1979) was recently withdrawn by the Food and Drug Administration (FDA). Fenbendazole, also in this group of compounds, has exhibited good efficacies against nematodes (Kennedy and Todd, 1975; Kirsch and Düwel, 1975; Thomas, 1978; Gunawan et al., 1979) but has not yet been approved for use in sheep in the United States.

The imadazothiazoles, including levamisole and morantel tartrate, have shown excellent activity against trichostrongylids in ruminants (Colglazier et al., 1969; McKenna, 1974; Reid et al., 1976; Callinan and Barton, 1979; Herd et al., 1984). However, nematodes, resistance to the former have been reported (Le Jambre et al., 1976, 1978; Sangster et al., 1979; Whitlock et al., 1980; Le Jambre, 1981; Barton, 1983) while the latter has not been cleared for use in sheep in the United States.

The avermectins, specifically ivermectin, are effective anthelmintics against gastrointestinal nematodes in cattle (Benz and Ernst, 1979; Williams et al., 1981; Yazwinski et al., 1981), and in sheep (Armour et al., 1982; Wescott and LeaMaster, 1982; Yazwinski et al., 1983). They show high efficacy against benzimidazole-resistant strains of Haemonchus contortus, but limited efficacy against Nematodirus spp. (Wescott and LeaMaster, 1982) and no activity against trematodes. Ivermectin is presently available for use in cattle but not sheep in the United States.

Thiabendazole and levamisole have label clearance and are used for routine control of nematodes in sheep, while many other anthelmintics are used on an extra-label basis by veterinarians. Resistance to benzimidazoles and imadazothiazoles by nematodes and the inactivity of these compounds against Fasciola hepatica limit their application as broad-spectrum anthelmintics.

Recently, Schering Corporation developed netobimin (SCH-32481, Schering Corporation), a broad-spectrum anthelmintic with activity against nematodes (including some inhibited forms) and liver flukes in ruminants (Williams et al., 1985; Richards and Zimmerman, unpublished

data; Schering Corporation, unpublished data). Netobimin has good activity against drug-resistant nematodes (Schering Corporation unpublished data) and it has properties which allow for either oral or parenteral administration. The objective of this study was to evaluate netobimin, administered orally at 7.5 mg/kg and 20 mg/kg body weight, against naturally acquired infections of gastrointestinal nematodes in sheep. Concurrently, the fasciolicidal activity of netobimin in these sheep was also examined (Richards and Zimmerman unpublished data).

MATERIALS AND METHODS

Experimental animals, housing and allocation

Thirty cross-bred spring lambs were purchased from a local producer in the Willamette Valley of Western Oregon. They were transported to the Veterinary Medical Isolation Laboratory (VMAIL) at Oregon State University on 14 June 1984 and maintained there throughout the trial. On that date animals were examined, weighed, sampled for rectal-feces, and placed on pastures at VMAIL. Samples of rectal-feces were taken every four weeks (beginning 6 July 1984 until termination of the trial) to monitor infections of nematodes. Patent infections of gastrointestinal nematodes were confirmed by using a salt-flotation and centrifugation procedure to determine counts of eggs per gram (EPG) in

each fecal sample. All experimental animals remained on pastures until 5 October 1984 when they were moved to indoor isolation stalls (with concrete floors) at VMAIL until the end of the trial. On 5 November the sheep were allotted into three groups of ten each. To ensure equal representation among groups, distribution of sheep was based on body-weight and EPG counts of feces.

Anthelmintic Treatment

On 5 November 1984, the ten animals, selected as controls, were each given a placebo of tap water as an oral drench. The remaining twenty sheep were divided into two treatment groups and netobimin was administered orally by syringe at either 7.5 mg/kg or 20 mg/kg body weight. The oral formulation of netobimin was supplied by Schering Corporation in powder form which was reconstituted with water to a concentration of 50 mg/ml. Following treatment, all animals remained at VMAIL until necropsy.

Parasitological Techniques

Counts of EPG in rectal-feces were performed as above. The eggs were identified at the generic level (Strongyloides, Nematodirus spp.,

Trichuris spp., and Capillaria spp.) or classified as trichostrongyles and others (Ostertagia spp., Trichostrongylus spp., Cooperia spp., Marshallagia, Haemonchus, Chabertia, and Oesophagostomum).

Necropsies were conducted on 12 and 19 November 1984. Half of the animals from each group were necropsied seven and 14 days post-treatment. The abomasum, small intestine and large intestine-cecum were double ligated and removed from each animal. The abomasum was opened longitudinally, and the mucosa stripped. All washings and contents were then brought to a known volume from which two 5% aliquots were saved. Aliquots were sieved through a 400 mesh (37.5 µm) screen and preserved with a 70% alcohol-iodine solution. Each abomasum was then incubated in tap water at room temperature for 24 hours. After incubation, the abomasum was again stripped and washed. This material and rinse water was sieved as specified above, divided in half (one kept as backup sample) and preserved. Samples from the small and large intestines were prepared in the same manner as the abomasal contents (no incubation). Aliquots were sieved through either a 400 mesh screen (small intestines) or a 100 mesh (150 µm) screen (large intestine-cecum) and preserved. Nematodes were recovered from aliquots, with the aid of dissecting microscopes (20x), preserved in 10% buffered-formalin, counted, and identified as to genera, species and morphologic stage of development. Anthelmintic efficacies were calculated based on the following formula:

$$\frac{\text{number in controls} - \text{number in treated}}{\text{number in controls}} \times 100 = \% \text{ efficacy}$$

Statistical Analysis

Data for the number of parasites recovered were found to be significantly non-normal (using the Chi-Squared Goodness of Fit Test) and/or lacking in homogeneity of variance (using Bartlett's Test). Consequently, non-parametric techniques were used in the analysis, and efficacies based on medians are reported in the results.

The Kruskal-Wallis Test (the non-parametric analog of the one-way analysis of variance) was used to detect overall differences among medians of treatment groups using an overall alpha = 0.05. Pairwise comparisons of these medians were then performed using the Mann-Whitney Test (the non-parametric analog of the unpaired t-test). (For each of these three tests [control vs. 7.5 mg/kg; control vs. 20.0 mg/kg; 7.5 vs 20.0 mg/kg] an alpha = 0.05/3 = .0167 was used to ensure the overall alpha = 0.05.) (The p-values reported with efficacies are those from the associated pairwise comparison of medians; the efficacies themselves could not be tested for statistical significance.)

Data for EPG counts were also found to be significantly non-normal (using the Chi-Squared Goodness of Fit Test) and/or lacking in homogeneity of variance (using Bartlett's Test). Consequently, non-parametric techniques were used in the analysis, and reductions in EPG count were calculated using medians rather than means.

Within each treatment group, the Wilcoxon Matched Pairs Test (the non-parametric analog of the paired t-test) was used to detect differences between medians of EPG counts before (10/25/84 sample) and after (trial termination sample) netobimin treatment. The p-values reported with EPG reductions are those from the associated Wilcoxon Matched Pairs Test; the reductions themselves could not be tested for statistical significance.

RESULTS

Mean weights of sheep allocated to each study group (control, 7.5 mg/kg and 20 mg/kg) were 49.2 kg, 49.1 kg and 50.4 kg, respectively. Median counts of EPG for each group prior to treatment (based on fecal samples from 25 October) were 930, 960, and 1028, respectively, whereas at the termination of the trial, median EPG's were 1300, 365 and 0 in the three groups. Reductions in median counts of EPG were 61.98% ($p \leq 0.004$) and 100% ($p \leq 0.008$) in animals treated with netobimin at dose levels of 7.5 mg/kg and 20 mg/kg. Netobimin was highly effective at both dose levels in removal of adult nematodes representing a number of genera and species of trichostrongylids (Table II.1) including: Ostertagia spp. with O. circumcincta , O. trifurcata, O. ostertagi, and Teladorsagia davtiani; Trichostrongylus spp. with T. axei, T. vitrinus, and T. colubriformis; Haemonchus contortus and Nematodirus spp. with N. spathiger, N. filicollis, and N. battus. High efficacies against other

species of nematodes were not statistically significant (Cooperia spp., Chabertia ovina and Oesophagostomum venulosum). Netobimin at 20 mg/kg was effective against Strongyloides papillosus but not against Capillaria sp. and Trichuris sp. Efficacy against Marshallagia marshalli could not be evaluated due to low levels of worms recovered.

At 20 mg/kg netobimin significantly reduced populations of early and late fourth-stage larvae of Ostertagia spp. Data were insufficient to evaluate activity against larvae of Trichostrongylus spp. and Haemonchus contortus (Table II.1). Of interest was the recovery of ensheathed third stage larvae (L_3) of H. contortus in the abomasal contents and incubates of 10 animals (irrespective of treatment group). The occurrence of these L_3's was not apparently influenced by the treatment regime (see Hoberg and Zimmerman in press). The overall efficacy of netobimin against all life stages present was 90.16% ($p \leq 0.01$) at 7.5 mg/kg and 98.77% ($p \leq 0.01$) at 20 mg/kg. Mean comparison tests indicated overall differences between 7.5 mg/kg and 20 mg/kg groups to be highly significant ($p \leq 0.01$).

DISCUSSION

The efficacy of netobimin in controlling naturally acquired infections of gastrointestinal nematodes in sheep had not previously been reported. Results indicated that netobimin administered at 20 mg/kg was highly effective in removal of a variety of trichostrongyles

and other helminths in ovine-hosts (overall reduction 98.77%, $p \leq 0.01$). Additionally, this compound exhibited excellent activity against Fasciola hepatica in sheep (Richards and Zimmerman, unpublished data).

Netobimin at both dose levels was effective in limiting infections of Nematodirus spp. Nematodes of this genus are typically the dose-limiting parasites for many anthelmintics. It is notable that N. battus, a pathogenic nematode previously known only in Great Britain and Western Europe, was found for the first time in North America during this drug trial (Hoberg et al., 1986). Currently there are no drugs available in the United States with labeled-approval for use against N. battus (Hoberg et al., 1985).

Unlike the benzimidazoles, imadazothiazoles and avermectins, netobimin is a broad-spectrum anthelmintic with proven activity against nematodes and trematodes. Due to limitations in availability and approval such drugs as thiabendazole and levamisole are the only anthelmintics currently labeled for control of nematodes in sheep. The development of drug-resistance by nematodes and the poor fasciolicidal activity of these compounds limits their applications as broad-spectrum anthelmintics. Thus the use of a compound such as netobimin to concurrently reduce populations of flukes and nematodes could result in a less labor intensive and more economical approach to the control of parasitism in ovine-hosts.

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TABLE II.1
Statistical Summary of Nematodes Recovered From
Control and Netobimin-Treated Sheep

Parasite	Treatment (mg/kg)	Mean	S.E.[a]	Median	Efficacy[b] using means	%Efficacy[c] using medians	p-value for pairwise[d] comparison of control vs. treat. medians	p-value for pairwise[d] comparison of 7.5 vs. 20 mg/kg treatment medians
Ostertagia E4	Control	48.8	14.60	32	—	—		
	7.5	18.0	9.86	0	63.11	100	.0494	
	20	5.2	4.38	0	89.34	100	.0036	.5708
Ostertagia L4	Control	4.4	3.98	0	—	—		
	7.5	.4	.4	0	90.91	—	.6776	
	20	.4	.4	0	90.91	—	.6776	1.0
Ostertagia E4 + L4	Control	53.2	17.11	32	—			
	7.5	18.4	9.98	0	65.41	100	.0539	
	20	5.6	4.43	0	89.47	100	.0036	.5453
Ostertagia Adults	Control	807.2	185.56	790	—	—		
	7.5	62.4	23.94	30	92.27	96.2	.0005	
	20	1.6	.88	0	99.80	100	.0002	.0058
Total Ostertagia	Control	860.4	182.80	892	—	—		
	7.5	80.8	28.13	62	90.61	93.05	.0004	
	20	7.2	4.29	2	99.16	99.78	.0002	.0156
Haemonchus[e] L3	Control	144.8	100.4	0				
	7.5	959.2	913.7	2	-84.90	—	.6501	
	20	114.0	114.0	0	21.30	—	.3258	.1620
Haemonchus E4	Control	46.0	18.75	30	—	—		
	7.5	15.6	8.55	0	66.09	100	.2899	
	20	.4	.4	0	99.13	100	.0343	.1988
Haemonchus L4	Control	4.0	4.0	0	—	—		
	7.5	0	0	0	100	—	.7055	
	20	0	0	0	100	—	.7055	1.0
Haemonchus E4 + L4	Control	50	21.65	30	—	—		
	7.5	15.6	8.55	0	68.80	100	.2899	
	20	.4	.4	0	99.20	100	.0343	.1988
Haemonchus Adults	Control	77.2	26.71	58	—	—		
	7.5	4.4	4.4	0	94.30	100	.0017	
	20	0	0	0	100	100	.0007	.7055
Total Haemonchus	Control	127.2	44.41	68	—	—		
	7.5	20.0	11.82	0	84.28	100	.0173	
	20	.4	.4	0	99.69	100	.0009	.1988

TABLE II.1 (Cont.)

Parasite	Treatment (mg/kg)	Mean	S.E.[a]	Median	Efficacy[b] using means	%Efficacy[c] using medians	p-value[d] for pairwise comparison of control vs. treat. medians	p-value for pairwise[d] comparison of 7.5 vs. 20 mg/kg treatment medians
Trichostrongylus E4	Control	9.2	5.50	0	—	—		
	7.5	0	0	0	100	—	.1306	
	20	0	0	0	100	—	.1306	1.0
Trichostrongylus Adults	Control	4088	740.46	3116	—	—		
	7.5	24.0	10.67	0	99.41	100	.0002	
	20	25.2	6.90	40	99.38	98.72	.0002	.6501
Total Trichostrongylus	Control	4097.2	741.68	3116	—	—		
	7.5	24.0	10.67	0	99.41	100	.0002	
	20	25.2	6.90	40	99.38	98.72	.0002	.6501
Nematodirus L3	Control	32.0	16.65	0	—	—		
	7.5	0	0	0	100	—	.1306	
	20	0	0	0	100	—	.1306	1.0
Nematodirus L4	Control	4.0	4.0	0	—	—		
	7.5	0	0	0	100	—	.7055	
	20	0	0	0	100	—	.7055	1.0
Nematodirus Adults	Control	580.0	238.53	360	—	—		
	7.5	0	0	0	100	100	.0007	
	20	0	0	0	100	100	.0007	1.0
Total Nematodirus	Control	616.0	236.88	460	—	—		
	7.5	0	0	0	100	100	.0007	
	20	0	0	0	100	100	.0007	1.0
Capillaria	Control	36.0	13.92	20	—	—		
	7.5	16.0	10.67	0	55.66	100	.2899	
	20	12.0	8.54	0	66.67	100	.2265	.9397
Cooperia	Control	32.0	14.36	0	—	—		
	7.5	0	0	0	100	—	.1306	
	20	0	0	0	100	—	.1306	1.0
Strongyloides	Control	428.0	87.44	380	—	—		
	7.5	588.0	144.72	460	-37.38	-21.05	.4963	
	20	16.0	12.22	0	96.26	100	.0002	.0012
Trichuris	Control	6.0	6.0	0	—	—		
	7.5	0	0	0	100	—	.7055	
	20	2.0	2.0	0	66.67	—	.9698	.7055

TABLE II.1 (Cont.)

Parasite	Treatment (mg/kg)	Mean	S.E.[a]	Median	Efficacy[b] using means	%Efficacy[c] using medians	p-value[d] for pairwise comparison of control vs. treat. medians	p-value for pairwise[d] comparison of 7.5 vs. 20 mg/kg treatment medians
Chabertia	Control	8.0	3.27	0	—	—		
	7.5	0	0	0	100	—	.1306	
	20	0	0	0	100	—	.1306	1.0
Oesphagostomum	Control	2.0	2.0	0	—	—		
	7.5	0	0	0	100	—	.7055	
	20	0	0	0	100	—	.7055	1.0
TOTAL[e]	Control	6212.8	912.33	6016	—	—		
	7.5	728.8	142.39	592	88.27	90.16	.0002	
	20	62.8	12.38	74	98.99	90.77	.0002	.0002

[a] Standard error of the mean

[b] % Efficacy = [(mean control count - mean treatment count)/mean control count] x 100

[c] % Efficacy = [(median control count - median treatment count)/median control count] x 100

[d] The p-values for the associated pairwise comparison of the medians can be used as an estimate of the significance level of the % efficacy using medians.

Pairwise comparisons performed using alpha pairwise = alpha overall/3

[e] Data for ensheathed L_3 Haemonchus contortus are not included in totals or in the statistical analysis.

E4 = Early fourth stage

L4 = Late fourth stage

L3 = Parasitic third stage, except for those of H. contortus

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The Anthelmintic Efficacy of Netobimin Against Experimental Infections of *Fasciola hepatica* in Sheep

L. S. Richards[1], G. L. Zimmerman[1], M. J. Nelson[1], D.J. Schons[1], and S. W. Dawley[2]

[1]College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331-4802 (USA)

[2]Schering Corporation, Animal Health Division, Galloping Hill Road, Kenilworth, New Jersey 07033 (USA)

Address to which proofs should be sent:

Dr. Gary L. Zimmerman
College of Veterinary Medicine
Oregon State University
Corvallis, Oregon 97331-4802 (USA)

ABSTRACT

Richards, L. S., G. L. Zimmerman, M. J. Nelson, D. J. Schons, and S. W. Dawley, 1985. The Anthelmintic Efficacy of Netobimin Against Experimental Infections of Fasciola hepatica in Sheep. **Vet. Parasitol.**, : - .

Netobimin--(coded SCH 32481--Schering Corporation), a new broad-spectrum anthelmintic having both fasciolicidal and nematocidal properties was evaluated for efficacy against mature Fasciola hepatica infections in sheep.

The trial was conducted with 30 cross-bred spring lambs, each experimentally infected with 250 F. hepatica metacercariae. A single treatment of netobimin was administered by oral drench at 7.5 mg/kg or 20 mg/kg of body weight while 10 animals remained as untreated controls. At necropsy, the mean number of adult flukes recovered from the control, 7.5 mg/kg and 20 mg/kg groups were 94.7, 35.9 and 8.8 respectively. The resulting efficacies were 62% ($p \leq 0.05$) and 90.7% ($p \leq 0.01$) respectively. No signs of toxicosis nor any adverse reactions were observed.

INTRODUCTION

Control of ovine fascioliasis has primarily depended upon chemotherapeutic methods. Outside of the United States, numerous fasciolicides have been employed with varying efficacies against infections of both immature and mature Fasciola hepatica (reviewed by Boray, 1982). In contrast, there have been relatively few compounds legally available to producers and veterinarians in the United States for use against liver flukes in livestock. Fasciolicidal compounds used in the past such as carbon tetrachloride ("Tetrawyn Red") exhibited marked toxicity in the host resulting in renal and hepatic calcification and in many instances mortality (Angus & Greig, 1979). Hexachlorethane ("Hexane" or "Hexavec"), another toxic chemical used with variable efficacy (Olson, 1946; Olson, 1947; Randell and Bradley, 1980), was determined to be carcinogenic resulting in approval of the compound being withdrawn in the early 1970's by the U.S. Food and Drug Administration (FDA). In the late 1970's, albendazole (Smith, Kline and French Co.) was approved for use by prescription only on a restricted status (Investigational New Animal Drug--INAD) in 14 states. The highest degree of efficacy of albendazole was against mature flukes and adult gastrointestinal nematodes (Theodorides et al., 1976; Knight and Colglazier, 1977; Campbell and Hall, 1979; Johns and Dickeson, 1979; Van Schalkwyk et al., 1979; Theodorides & Freeman, 1980; Malone et al., 1982; Todd and Mansfield, 1982). Within weeks after the INAD clearance was withdrawn and albendazole was no longer available (early 1985),

clorsulon (Curatrem^{R}--Merck, Sharp & Dohme) received FDA clearance for oral administration against F. hepatica in beef cattle. (Clorsulon is not yet cleared for use in dairy cattle of breeding age nor in sheep.)

Schering Corporation developed a new anthelmintic netobimin (SCH 32481) which has activity against both liver flukes and a number of gastrointestinal nematodes in ruminants (Williams et al., 1985; Richards and Zimmerman--unpublished data). Although the compound can be formulated for either parenteral or oral application, the purpose of this trial was to evaluate the oral formulation of netobimin against F. hepatica in sheep at 7.5 mg/kg and 20 mg/kg body weight.

MATERIALS AND METHODS

Experimental Animals

Thirty cross-bred spring lambs purchased from a producer in the Willamette Valley of Western Oregon were maintained at the Veterinary Medical Isolation Laboratory (VMAIL) at Oregon State University. They were examined and weighed on 14 June 1984, ranging from 33.6 to 50.9 kg; mean 41.3 kg). Rectal fecal samples were taken to confirm the presence of gastrointestinal helminth eggs (used for concurrent study) and to demonstrate the absence of eggs of F. hepatica.

Infections

On 6 July 1984, the sheep were inoculated with 250 F. hepatica metacercariae (Baldwin Enterprises, Monmouth, Oregon). Metacercariae were pipetted onto moistened pre-filter papers (Nucleopore Corporation), which were placed in gelatin capsules and administered via a balling gun. The sheep were turned out onto pastures at VMAIL allowing for fluke maturation.

Confirmation of Patent Infections

Rectal fecal samples and blood samples were taken from each animal every four weeks, beginning 6 July 1984 and ending on 12 November 1984 and 19 November 1984 (trial termination dates). The Telemann technique (Thienpont et al., 1979) was used to confirm patent F. hepatica infections. Serum from blood samples collected were subjected to ELISA and DOT-ELISA tests to detect presence of antibodies to F. hepatica (Zimmerman et al., 1982; 1985).

Anthelmintic Treatment

On 5 November 1984, 30 sheep were allotted into 3 groups of 10 based on weights and fecal egg counts (mean EPG for nematodes). Ten animals were selected as controls and given a placebo of tap water as an oral drench. The oral formulation of netobimin was supplied in a powder form which was reconstituted with water to a concentration of 50 mg/ml. Animals in the two treatment groups were given netobimin by a modified oral drenching gun at 7.5 mg/kg or 20 mg/kg body weight. Following treatment, all animals were housed in concrete stalls at VMAIL for the remainder of the trial.

Parasitological Procedures

Half of the animals from each group were necropsied on 12 November 1984, and the remaining half necropsied a week later on 19 November 1984.

The liver with gallbladder intact and a section of the duodenum extending 50 cm from the hepatic duct were removed from each animal, placed in separate buckets, and transported to the laboratory for examination. For recovery of intact flukes, the livers were processed by first opening the major bile ducts, gallbladders, and duodenum. The tissue was then sliced into sections (1 cm^3), manually squeezed to

express any flukes, and placed in warmed tap water and allowed to incubate 3-4 hours at 37.5°C. After incubation, the sections were again squeezed and examined. Water from the liver incubation and liver sections were then placed in a 12-inch, 200 mesh (150 μm aperature) stainless steel sieve and again macerated and examined under running water. All intact flukes and fragments were fixed in 10% buffered formalin. Specimens recovered from each liver were enumerated using an illuminated magnifying loop and relegated to one of four categories: entire mature flukes, anterior fragments with suckers, posterior fragments not matching any anterior portions, and nearly entire flukes without suckers. The total number (sum of all four groups) of flukes recovered was used in the statistical analysis.

Statistical Analysis of Data

The data on recovery of mature F. hepatica were found to be significantly non-normal using the Q-Q Plot Test for normality and/or lacking in homogeneity of variance using Bartlett's Test. Consequently, the Kruskal-Wallis Test (a non-parametric analog of the one-way ANOVA) was used to compare treatment groups. Significant differences between treatment groups were then subjected to Dunn's Rank Multiple Comparison Test. Results were ultimately reported in terms of efficacy.

RESULTS

Fecal Egg Counts

The initial and subsequent rectal fecal samples taken during the trial revealed animals to be passing helminth eggs including those of trichostrongyloids (Nematodirus, Trichostrongylus, etc.), Trichuris sp., Capillaria sp., and Moniezia. At 8 weeks post-infection (PI), fecal samples taken from all sheep were negative for fluke eggs. By 12 weeks PI, 21 sheep were passing F. hepatica eggs and by 16 weeks PI, patent F. hepatica infections were detected in all animals.

Serological Tests

Antibodies against F. hepatica were confirmed in all sheep at 4 weeks PI (DOT-ELISA) and 8 weeks PI (ELISA).

Fluke Recovery

Numbers of mature flukes recovered, animals weights, and anthelmintic dosages administered for individual animals are given in Table III.1. No immature F. hepatica were found in any animal at necropsy. The mean number of flukes recovered in the control group was 94.7. Mean numbers of flukes recovered from the 7.5 mg/kg and 20 mg/kg treated groups were 35.9 and 8.8 respectively; this resulted in an overall efficacies of 62% ($p \leq 0.05$) and 90.7% ($p \leq 0.01$) using dosages of 7.5 mg/kg and 20 mg/kg respectively. The difference in efficacies between the 7.5 mg/kg and 20 mg/kg treatments were not significant ($p > 0.05$).

DISCUSSION

The overall efficacy of netobimin at 20 mg/kg b.w. dosage level in sheep experimentally infected with F. hepatica, resulted in significant reductions of fluke populations (90.7%; $p \leq 0.01$). At 7.5 mg/kg b.w., the 62% against F. hepatica ($p \leq 0.05$) was considered unacceptable.

Although other fasciolicides legally available for use in Europe and elsewhere may have similar or greater efficacies against adult and/or immature stages of F. hepatica in domestic ruminants (reviewed by

Boray, 1982), none are currently available for use in the United States. Clorsulon has high efficacy against immature and mature stages of F. hepatica (Mrozik, et al., 1977; Wyckoff and Bradley, 1983; Malone, et al., 1984; Yazwinski, et al., 1985; Zimmerman, et al., 1986); however, unlike netobimin, it has no activity against gastrointestinal nematodes. Consequently, for complete coverage against nematodes and flukes, clorsulon must be used in concert with other anthelmintics. Recent studies have demonstrated the safety of clorsulon used concurrently with ivermectin (Courtney, et al., 1985). Although the safety and efficacy of such concurrently administered anthelmintics have been demonstrated, many producers will not use such combined products because of the increased cost.

Data from the present study indicate that netobimin administered at 20 mg/kg b.w. is an effective anthelmintic for the control of ovine fascioliasis. Additional benefits include its activity against most trichostrongyloid gastrointestinal nematodes and its solubility which allows for application in either oral or parenteral form.

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TABLE III.1
Activity of Netobimin Against Adult Fasciola hepatica Infections in Sheep

Necropsy Date	Animal Number	Weight (kg)	Treatment	Total Fluke Burden	Mean Fluke Burden	% Efficacy[a]
11/12/84	050[b]	52	20.0 mg/kg	19		
	041	50	16.4 mg/kg	3		
	035	40	20.0 mg/kg	5		
	257	49	"	2		
	205	54	"	4		
11/19/84	031	40	"	4		
	025	47	"	3		
	206	47	"	12		
	019	57	"	21		
	032	68		15		
		x 50.4		88	8.8	90.7% ($p \leq 0.01$)
11/12/84	037	49	7.5 mg/kg	39		
	202	42	"	57		
	214	43	"	12		
	026	46	"	52		
	028	60	"	23		
11/19/84	016	51	"	10		
	018	52	"	25		
	044	54	"	50		
	078	45	"	19		
	034	49		72		
		x 49.1		359	35.9	62% ($p \leq 0.05$)
11/12/84	258	39	Control	96		
	030	56	"	73		
	036	40	"	86		
	215	38	"	53		
	040	51	"	68		
11/19/84	208	49	"	129		
	049	50		98		
	046	53		86		
	022	51		138		
	209	65		120		
		x 49.2		947		

a) % Efficacy = $\frac{\text{Mean Control Burden} - \text{Mean Treated Burden}}{\text{Mean Control Burden}} \times 100$

b) Incorrect dosage given to this animal.

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