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COMPARISON OF THREE DRUGS TO CONTROL PANCREATIC FLUKE (EURYTREMA PANCREATICUM) IN SHEEP

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SUB-BALAI PENELITIAN TERNAK, SUNGAI PUTIH

BALAI PENELITIAN TERNAK

PUSAT PENELITIAN DAN PENGEMBANGAN PETERNAKAN

COMPARISON OF THREE DRUGS TO CONTROL PANCREATIC FLUKE (EURYTREMA PANCREATICUM) IN SHEEP

Ruth M Gatenby[1], Endang Romjali[2], Alan J Wilson[3], Melinda Hutauruk[2], John Glenn[4] and A Djoko Pitono[2]

ABSTRACT

An experiment was conducted to test the efficacy of nitroxynil, praziquantel and albendazole in the control of pancreatic fluke (Eurytrema pancreaticum) in sheep, and to gain more knowledge about the course of infection. Sumatra Thin-tail ram lambs were grazed for five weeks then confined in a sheep house with a raised slatted floor. Four months after grazing ceased, they were divided into groups of eight animals and given one of four treatments (N) Nitroxynil subcutaneous on days 0 (10 mg/kg) and 20 (30 mg/kg), (P) Praziquantel oral at 20 mg/kg on days 0 and 2, (A) Albendazole oral at 15 mg/kg on days 0 and 2, and (C) Untreated control. Thirty days after the drugs were first given the animals were slaughtered.

The mean value of pancreatic fluke eggs per gram of faeces (epg) preceeding treatment was 171, and mean nematode epg had risen to more than 4000. Samples taken at two-weekly intervals from individual animals exhibited great fluctuation in fluke epg, suggesting that there is not a steady release of eggs from the adult fluke in the pancreas. The average growth rate of lambs before the drugs were given was 30 g/d, and body weight on the day of treatment averaged 18.5 kg. Age at treatment averaged 11 months.

After treatment mean fluke epg of group N was high, similar to C, but that of P was consistently lower. Group A showed a peak in fluke epg two weeks after treatment, which then fell to a low level. Geometric mean values of the three final fluke epg (N 50, P 17, A 5 and C 45) were significantly ($P<0.05$) affected by treatment. Nematode epg remained high in groups C and P, and fell to very low levels in animals treated with N and A, confirming that nitroxynil and albendazole effectively control gastro-intestinal nematodes. Mean growth rates (g/d) were N 34, P 44, A 46 and C 23, showing that all drugs increased growth rate to some extent, although differences were not significant.

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At slaughter all pancreas contained fluke. Treatment had a significant (P<0.05) effect on geometric mean numbers of fluke, which were N 737, P 153, A 176 and C 408. There was a positive correlation (P<0.05) between number of fluke in the pancreas and fluke epg on the day of slaughter. Ninety-one per cent of animals showed fibrosis of the pancreatic ducts. Only three animals showed some necrosis in the liver confirming that infection was still at an early early stage. Inter-lobular fibrosis in the pancreas was less severe for A than for the other three groups (P<0.05), and intra-lobular fibrosis was more severe for C than for the other three groups (P<0.05).

Although albendazole and praziquantel significantly reduced the level of infection of pancreatic fluke, neither drug reduced it to a negligible level. More frequent doses of the drugs or higher dose rates may be more effective, but would almost certainly not give an economic response. Nitroxynil appeared to be ineffective in controlling pancreatic fluke.

ABSTRAK

Penelitian dilakukan untuk menguji kemanjuran dari nitroxynil, praziquantel dan albendazole di dalam mengontrol cacing pankreas (Eurytrema pancreaticum) pada domba, dan untuk meningkatkan pengetahuan tentang arah daripada infeksi. Domba jantan muda Sumatra ekor tipis digembalakan selama lima minggu kemudian dipelihara di dalam kandang dengan lantai dari kayu reng. Empat bulan setelah penggembalaan dihentikan, ternak dibagi dalam kelompok-kelompok yang terdiri dari delapan ternak dan diberi satu dari empat perlakuan (N) Nitroxynil subkutan pada hari 0 (10 mg/kg) dan 20 (30 mg/kg), (P) Praziquantel lewat mulut sebanyak 20 mg/kg pada hari 0 dan 2, (A) Albendazole lewat mulut sebanyak 15 mg/kg pada hari 0 dan 2, dan (C) tanpa perlakuan sebagai kontrol. Tiga puluh hari setelah pemberian obat seluruh ternak dipotong.

Nilai rata-rata dari telur cacing pancreas per gram feces (epg) dari perlakuan yang telah dilakukan adalah 171, dan rata-rata nematoda per gram feces (epg) naik lebih dari 4000. Sampel diambil dengan interval dua minggu dari ternak-ternak yang menunjukkan fluktuasi besar jumlah telur cacing per gram feces (epg), ditunjukkan bahwa tidak konstan telur-telur cacing yang dikeluarkan dari cacing dewasa di dalam pancreas. Rata-rata pertambahan bobot badan anak sebelum diberikan obat cacing adalah 30 gram/hari, dan rata-rata bobot badan pada hari diberikan perlakuan adalah 18,5 kg. Rata-rata umur pada saat diberikan perlakuan adalah 11 bulan.

Setelah perlakuan rata-rata jumlah telur cacing per gram feces dari kelompok N adalah tinggi, hampir sama dengan C, tetapi P secara konsisten lebih rendah. Kelompok A menunjukkan puncak telur cacing per gram pada dua minggu setelah perlakuan, kemudian

turun pada level yang rendah. Nilai rata-rata geometrik jumlah telur cacing per gram feses (epg) pada akhir dari ketiga perlakuan adalah (N 50, P 17, A 5 dan C 45) nyata ($P<0,05$) dipengaruhi oleh perlakuan. Telur cacing nematoda per gram feces (epg) selalu tinggi di kelompok C dan P, dan turun ke level yang sangat rendah pada ternak-ternak yang mendapat perlakuan N dan A, hal ini menunjukkan bahwa nitroxynil dan albendazole efektif dalam mengontrol nematoda didalam usus besar. Rata-rata pertambahan bobot badan (gram/hari) adalah N 34, P 44, A 46 dan C 23, menunjukkan bahwa semua obat meningkatkan pertambahan bobot badan, meskipun tidak nyata.

Pada saat pemotongan semua pankreas berisi cacing. Perlakuan mempunyai pengaruh nyata ($P<0,05$) pada rata-rata geometrik jumlah cacing, yaitu N 737, P 153, A 176 dan C 408. Ada korelasi positif ($P<0,05$) antara jumlah cacing di dalam pankreas dan telur cacing per gram feces (epg) pada hari pemotongan. Sembilan puluh satu persen dari ternak menunjukkan *fibrinosis* saluran-saluran pankreas. Hanya tiga ternak menunjukkan beberapa nekrosa pada hati, hal ini berarti bahwa infeksi baru pada stadium awal. *Fibrinosis inter-lobular* di dalam pankreas lebih sedikit untuk A daripada untuk ketiga kelompok lainnya ($P<0,05$), dan *fibrinosis intra-lobular* lebih banyak untuk C daripada untuk ketiga kelompok lainnya ($P<0,05$).

Meskipun albendazole dan praziquantel secara nyata menurunkan level infeksi daripada cacing pankreas, tetapi tidak satupun obat cacing yang dipakai menurunkannya ke level yang layak. Peningkatan frekuensi pemberian obat atau meningkatkan dosisnya mungkin lebih efektif, tetapi mungkin tidak memberikan respon yang ekonomis. Nitroxynil menunjukkan tidak efektif dal m mengontrol cacing pankreas.

INTRODUCTION

In the past three years, it has become clear that a large proportion of sheep in North Sumatra are infected with pancreatic fluke, Eurytrema pancreaticum. This conclusion has been reached from inspection of animals that have died naturally, those that have been slaughtered because they were emaciated and apparently healthy animals slaughtered for meat (Arasu et al., 1991; Carmichael, 1991; Carmichael et al, 1992). Some observers have commented that the problem is particularly serious among imported sheep and their crosses.

pancreatic fluke appear to be confined to parts of East and South-east Asia and Latin America (Urquhart et al, 1987). Eurytrema pancreaticum has been reported in sheep in several parts of South-east Asia, including Malaysia (Basch, 1965; Sani et al, 1986), China (Tang, 1950; Shu et al, 1987). E pancreaticum is also found in goats and cattle (Wiroreno et al, 1987), and occasionally in man (Ishii, 1983). A related parasite Eurytrema coelomaticum is found in cattle but not in sheep.

The pancreatic fluke has two intermediate hosts - land snails (Tang, 1950) and grasshoppers (Basch, 1965). In North Sumatra it has been confirmed that the species of grasshopper which are the second intermediate host are long-horned green grasshoppers of the family Tettigonidae (Arasu et al, 1991).

Sheep are infected by eating grasshoppers. It is unlikely that healthy grasshoppers would allow themselves to be eaten, but those infected with the metacercariae of fluke are probably sick and do not jump away from the sheep's mouth. The fluke migrate from the small intestine of the sheep to the pancreas, and can be seen in the pancreatic ducts. Maturity of fluke in the pancreas is achieved about seven weeks after ingestion, but the life-span of adult fluke in the sheep is not known.

Although some authors (Hammond and Sewell, 1991) state that the disease is usually of little pathogenicity, in North Sumatra pancreatic fluke appear to be causing morbidity and probably are contributing to mortality. Of nineteen adult sheep which died at Sei Putih in 1991, post-mortem examinations suggested that in seven animals pancreatic fluke contributed towards death (Wilson, 1992a). In these seven sheep, there were very severe necrotic lesions in the pancreas tissue in conjunction with severe degeneration of the liver hepatocytes. In other animals in which pancreatic fluke was not thought to be a factor contributing to death, there was normal pancreas tissue with fluke in the pancreatic ducts which showed severe fibrosis.

To assess the magnitude of infection we must be able to measure fluke burdens in live animals and to kill the fluke. In 1991 a simple method was developed to count the eggs of E pancreaticum in faecal samples (Arasu et al, 1991). This is a flotation method using calcium nitrate, in which flotations for a 30-minute period are achieved by gravity.

By taking faecal samples of sheep before slaughter, Arasu et al (1991) found that there is a positive correlation between the number of adult fluke in the pancreas of an animal and its faecal egg count. Thus infection levels can be roughly predicted from examination of faecal samples. However, a close linear relation was not established. Unknown factors such as the frequency of egg release by adult fluke in the pancreas, the integrity of fluke eggs in faeces, and limitations of the flotation method, may obviate direct correlations.

Chemotherapy of Eurytrema pancreaticum

There is not yet a recognised chemical for the treatment of Eurytrema pancreaticum. In 1985 Sewell reviewed the control of many intestinal trematodes and concluded that there have been relatively few accounts of studies on the chemotherapy of Eurytrema spp and these have mostly been unsuccessful, using such drugs as bithionol sulphoxide, thiabendazole, hexachloro-paraxylene, hexachlorethane, and chlorophos. However, he noted a report by Kono et al (1981) that nitroxynil can be effective against E coelomaticum in cattle.

Sakomoto et al (1985) screened a large number of chemicals for possible activity against E coelomaticum in cattle; potential chemicals were screened in vitro, in experimentally infected mice and goats, and in naturally infected cattle. Several drugs were effective in vivo, but gave no decrease in faecal egg count (epg) when used with cattle. The two most effective drugs in vivo were nitroxynil and praziquantel. Suh (1983) reported that nitroxynil had some effect in reducing epg of E pancreaticum in goats.

Praziquantel is a chemical which is effective against schistosomes, namely blood flukes responsible for the disease bilharzia (Gonnert and Andrews, 1977). It was reported to be effective against pancreatic fluke in sheep in China (Li et al, 1983). Kono et al (1986) reported that in two goats treated with praziquantel, pancreatic fluke epg increased remarkably 1-2 days after the drug was given, and then continued to fall.

In addition to nitroxynil and praziquantel, albendazole was included in the study. This drug is commonly used in the control of gastro-intestinal nematodes and liver fluke in sheep. It is a benzimidazole derivative, and Sakamoto et al (1985) found that several benzimidazole derivatives have in vivo cercaricidal effects on E coelomaticum.

High and repeated doses of expensive chemicals would not be economically acceptable for the treatment of sheep in Indonesia. Therefore we confined our studies to give two doses of each of the three drugs tested.

Table 1. Details of lambs at start of trial.

	Lamb number	Date of birth (d/m/y)	Body weight (kg)
1	01159	31/12/90	24.6
2	10045	12/3/91	19.0
3	10046	12/3/91	15.5
4	10066	18/3/91	10.9
5	10076	20/3/91	16.2
6	10082	21/3/91	16.0
7	10084	22/3/91	15.2
8	10102	25/3/91	12.2
9	10103	25/3/91	11.9
10	10109	26/3/91	13.5
11	10110	26/3/91	15.0
12	10117	27/3/91	10.0
13	10118	27/3/91	8.0
14	10119	27/3/91	13.6
15	10122	27/3/91	11.3
16	10131	28/3/91	13.2
17	10139	29/3/91	14.2
18	10142	30/3/91	20.6
19	10144	30/3/91	16.0
20	10149	31/3/91	14.7
21	10152	1/4/91	22.6
22	10156	6/4/91	7.2
23	10158	6/4/91	18.7
24	10161	7/4/91	13.2
25	10162	8/4/91	14.5
26	10164	11/4/91	9.8
27	10165	3/5/91	11.6
28	10169	5/5/91	16.0
29	10178	27/5/91	10.2
30	11028	18/2/91	12.3
31	11031	15/3/91	13.1
32	11037	22/3/91	9.8
33	11041	25/3/91	18.0
34	11047	28/3/91	10.7
35	11048	28/3/91	11.0
36	11050	28/3/91	9.0
37	11056	4/4/91	7.2
38	11057	6/4/91	14.8
39	11060	23/4/91	15.5
40	11070	27/5/91	9.9

The aim of the study reported in this paper was to test the efficacy of nitroxynil, praziquantel and albendazole in the control of Eurytrema pancreaticum in sheep, and to gain more knowledge about the course of infection.

METHOD

The experiment was conducted at the Research Institute for Animal Production at Sei Putih in North Sumatra between September 1991 and March 1992.

Animals and management

Forty Sumatra Thin-tail ram lambs were grazed for five weeks in an old rubber plantation which had previously been grazed by ewes and lambs. It was intended that in this period they would become infected with pancreatic fluke. The animals grazed from 0800 h to 1600 h each day, and at night were confined in a sheep house with a raised slatted floor.

The average age of the lambs at the beginning of the trial on September 17th was almost 6 months (mean 171 d, SE ±4 d), and their average weight was 13.7±0.61 kg (Table 1). Previously, from the age of two weeks to three months these lambs had grazed, but after weaning at three months of age they had been confined and stall-fed.

After the five-week period of grazing in September and October 1991 the animals were confined in the house until the completion of the trial.

During the whole of the trial, concentrate was given at a rate of about 200 g/d. During the periods when the animals were not grazed, they were given grass cut from an ungrazed area. No anthelmintic of any sort was given during the initial part of the trial, and four animals died.

Administration of drugs

In February 1992, four months after the animals ceased grazing, they were divided into four similar groups on the basis of body weight and the average of the previous three measurements of fluke and nematode eggs in the faeces (Table 2). Each group contained eight animals; four animals (numbers 10139, 10158, 11047 and 11048) with very low levels of fluke eggs in the faeces were not included. The treatments were randomly allocated to the groups.

Table 2. Groups of animals showing the mean of the final three egg counts in the initial period, body weights before the drugs were first given, and quantities of anthelmintic.

Lamb number	Fluke (epg)	Nematode (epg)	Body weight (kg)	Amount of anthelmintic	
				Day 0 (ml)	Day 20 (ml)
Nitroxynil					
10046	110	1180	22.5	0.90	2.7
10066	40	1600	13.0	0.52	1.6
10084	160	5160	23.2	0.93	2.8
10109	70	4440	15.6	0.62	2.0
10117	60	2760	17.0	0.68	2.1
10142	180	2330	26.0	1.04	3.2
10164	100	30200	13.5	0.54	1.7
11037	170	4540	14.8	0.59	1.9
Mean	111	6520	18.2	0.73	2.2
Praziquantel				Days 0 and 2 (mg)	
10103	45	6390	18.2	364	
10110	30	1860	21.8	436	
10118	150	2860	12.0	240	
10119	90	3320	18.5	370	
10131	90	1140	19.0	380	
10165	150	5520	20.8	416	
11028	170	15500	19.2	384	
11057	300	3340	19.8	396	
Mean	128	5000	18.7	373	
Albendazole				Days 0 and 2 (ml)	
01159	80	2480	28.0	22.1	
10045	150	3820	24.5	19.3	
10102	50	5540	19.2	15.2	
10144	200	2500	18.0	14.2	
10149	180	7580	22.0	17.4	
10169	30	6780	24.5	19.3	
11031	150	4580	19.7	15.6	
11056	90	35060	8.0	6.3	
Mean	116	8540	20.5	16.2	
Control					
10076	180	14020	19.8		
10082	200	6080	16.5		
10152	70	7300	27.7		
10162	120	3060	18.8		
10178	60	18920	13.5		
11041	160	7760	25.2		
11050	100	4180	13.5		
11060	30	4180	25.2		
Mean	115	8180	20.0		

The drugs were administered as recommended by each manufacturer. The four treatments were

1. Nitroxynil subcutaneous on days 0 (10 mg/kg) and 20 (30 mg/kg).
2. Praziquantel oral at 20 mg/kg on days 0 and 2.
3. Albendazole oral at 15 mg/kg on days 0 and 2.
4. Untreated control.

All three drugs were first administered on 22nd February 1992 (day 0). Praziquantel and albendazole were given a second time on day 2, and nitroxynil on day 20. The dose rates were calculated for the current weight of each individual lamb, not according to the average weight of the group.

Nitroxynil is an anthelmintic which is sold as a 250 mg/ml solution (Dovenix, Rhone Meriux). It was given by subcutaneous injection into the woolless skin at the top of each hind leg, and using three sites of injection to avoid the formation of large blisters. The sites were gently rubbed after injection. It was noted that small abscesses developed in some animals at the site of injection.

The praziquantel (Bayer) was given orally. The powder was weighed into small plastic tubes, and tipped into the back of the mouth of each lamb. Immediately afterwards a fingerful of molasses was given so the lamb licked its lips.

Albendazole is sold as a 19 mg/ml suspension (Valbazen, SmithKline). It was given from large (20 ml) syringes which were slowly squeezed into the back of the mouth of the animal, as in normal drenching. Each lamb was then given a little molasses.

Measurements

Every two weeks during the initial part of the trial, and every week after the drugs were given, the animals were weighed and samples of faeces collected from the anus. The faecal samples were examined for eggs of pancreatic fluke and nematodes.

For faecal analysis, a saturated solution of technical grade calcium nitrate was prepared. The specific gravity of the solution is between 1.47 and 1.49. The method for counting eggs using calcium nitrate was devised by Prema Arasu, and is as follows:

1. Mix 2 g faeces with water, and make up to 60 ml.

2. Filter the mixture twice, with a coarse seive and a fine seive.

3. Shake and place 1 ml in a graduated centrifuge tube.

4. Add calcium nitrate solution until 0.5 cm below the top of the tube. Cover the tube with parafilm, and shake three times (not too hard or you make bubbles).

5. Remove the parafilm and put the tube in a test-tube stand. Fill the tube right to the top with calcium nitrate solution, and gently place a coverslip on top.

6. Leave the tube undisturbed for 30 minutes.

7. Remove the coverslip by lifting it quickly upwards. Place it on a microscope slide.

8. The number of eggs of (i) strongyles and (ii) pancreatic fluke are counted under the microscope, and multiplied by 30 to give estimates of eggs per gram of faeces.

On March 22nd and 23rd 1992, all 32 animals in the experiment were slaughtered. The complete pancreas were removed from the carcasses, and samples of pancreas and liver were later subjected to histological examination. All organs in the body were studied, and abnormalities recorded.

In the laboratory the numbers of fluke in each pancreas were counted. The fluke were removed from the pancreas by cutting the tissue into chunks and squeezing it to expel all fluke from the ducts. The fluke were counted by naked eye in a flat dish, 14 cm in diameter. The percentages of small immature fluke and of live fluke were estimated.

In order to avoid any possibility of bias, the persons counting the eggs in the faeces and the fluke in the pancreas and examining the histological slides were unaware of which animals had been treated and which were the controls.

RESULTS

Of the 40 animals, four died in the initial period and 36 survived until the end of the trial.

Initial period

The initial part of the experiment included the grazing period (158 to 123 days before the drugs were given), and the subsequent four-month incubation period terminating on day 0 when the drugs were first administered.

Fluke egg counts. The mean value of pancreatic fluke epg in the samples preceeding day 0 was 171 per gram. The absolute maximum recorded during the initial period was 1900 per gram, and the minimum was zero.

The frequency distribution of fluke epg is shown in Figure 1. The distribution is extremely skewed. Most animals had levels of between 0 and 100 per gram, but a few observations were very high.

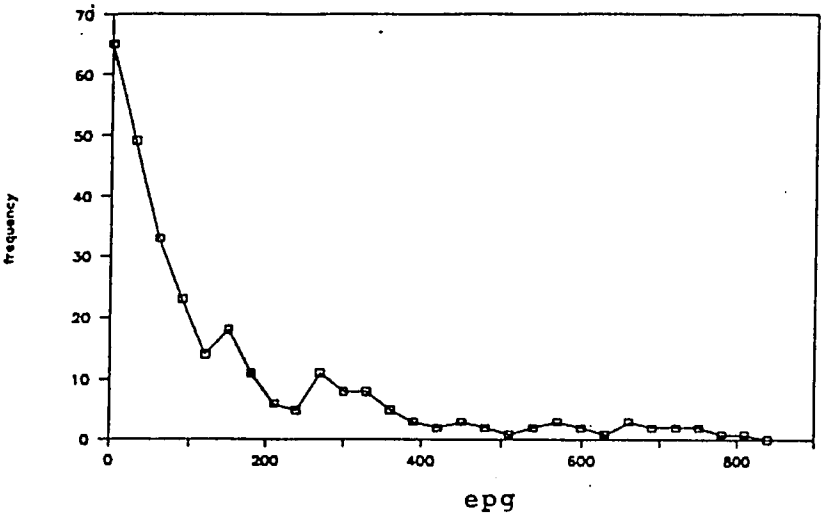

Figure 1. Frequency distribution of fluke epg.

Individual animals exhibited great fluctuation in fluke epg between samples (Figure 2). This suggests that there is not a steady release of eggs from the adult fluke in the pancreas.

The average values of fluke epg are shown in Figure 3. All averages show a similar pattern, though the median and geometric mean values are below the arithmetic mean as is typical for a skewed distribution. References in this report to "mean values" of epg refer to arithmetic means as these are the easiest to calculate.

Soon after the start of the experiment (days -105 to -75) fluke epg increased. We first thought this might be because the fluke ingested during the five-week grazing period had already started producing eggs. Subsequently fluke epg fell again, and we realised that the initial rise must have resulted from infection during the pre-weaning phase approximately 5 months previously. We therefore conclude that all our animals were already contaminated with pancreatic fluke at the start of the experiment.

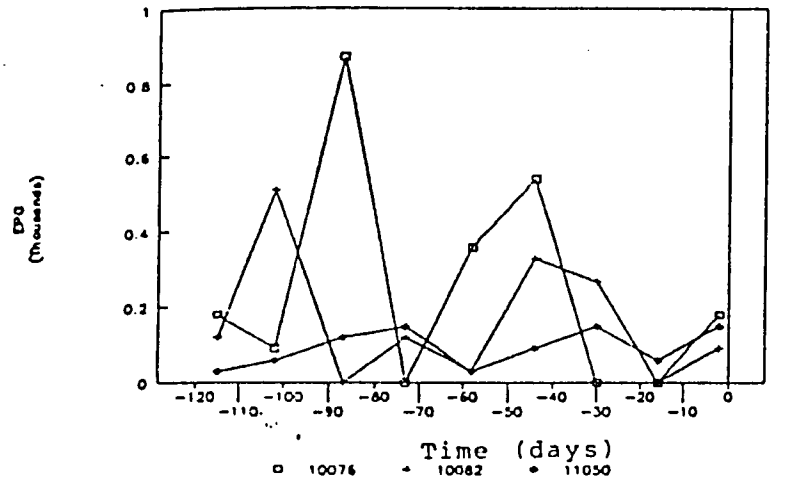

Figure 2. Fluke epg of three individual sheep.

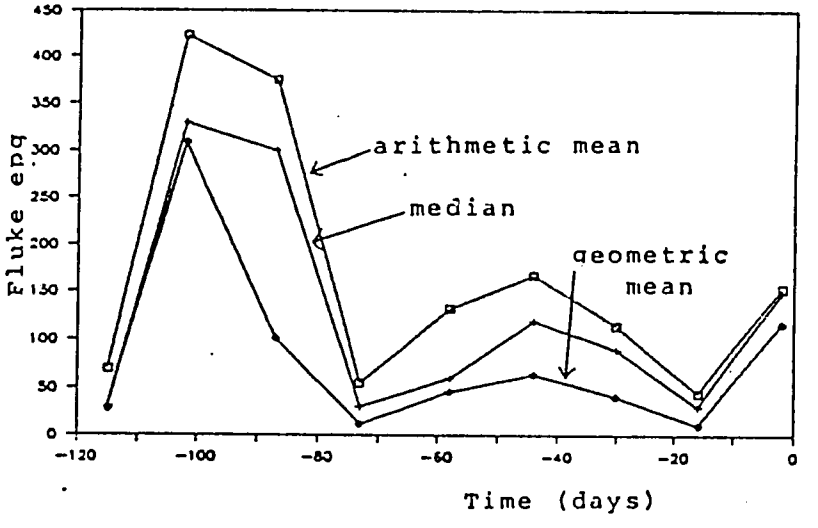

Figure 3. Changes in average fluke epg during the intial phase.

The four animals that died had only slightly higher values of fluke epg (average 130 per gram) than the average for the surviving animals (171 per gram).

Nematode egg counts. Average values of nematode epg during the first part of the trial are shown in Figure 4. No anthelmintic had been given to the lambs since weaning, so that nematode burdens progressively became more severe. As for fluke epg, there was considerable fluctuation in the values for each individual animal (Figure 5), and the distribution of nematode epg was severely skewed (Figure 6).

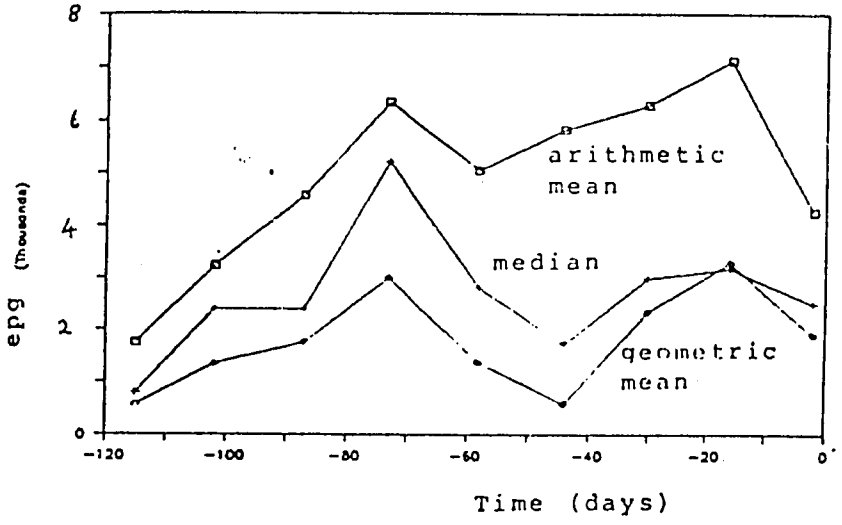

Figure 4. Changes in average nematode epg during the intial phase.

The mean nematode epg for the four lambs which died was 7500. This very high value, together with post-mortem findings of many worms in the abomasum, suggests that these animals died from helminthiasis.

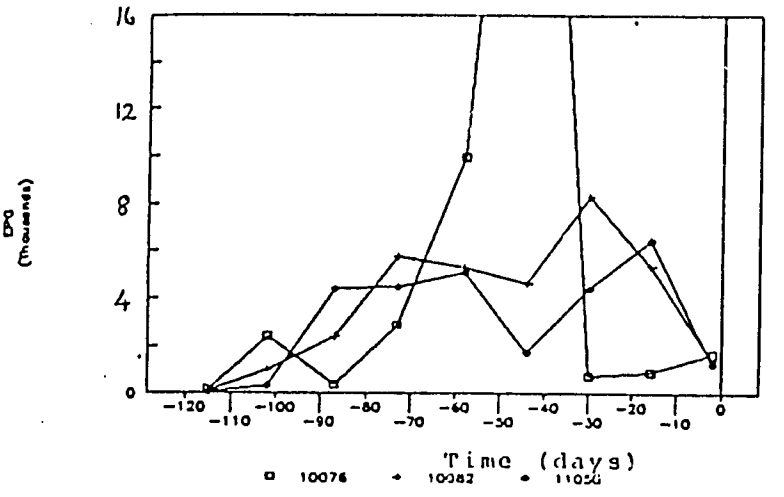

Figure 5. Nematode epg of three individual sheep.

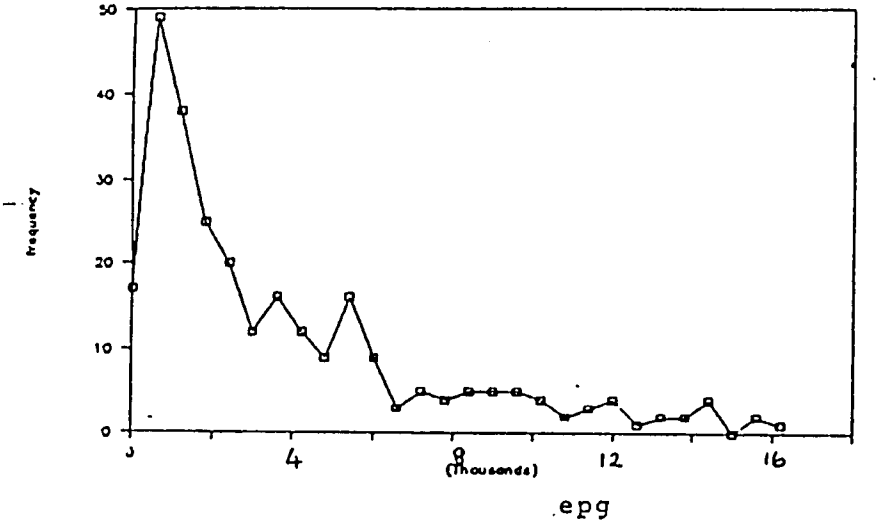

Figure 6. Frequency distribution of nematode epg.

Body weight with time. Figure 7 shows the increase in mean body weight with time. The fitted linear regression of the 36 survivors is

$$W = 18.5(\pm 0.6) + 0.030(\pm 0.003)t$$

where W is body weight in kg, and
t is time in days.

The slope of this regression shows that the average growth rate of lambs during the intitial period was 30 g/d. This is a low growth rate, but is typical of growth rates for small breeds of lambs with heavy parasite burdens given only moderate quality food.

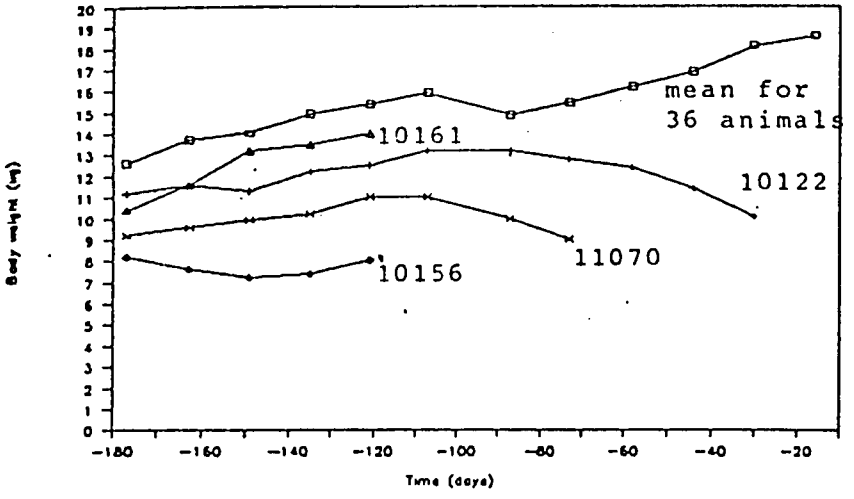

Figure 7. Increase in body weight during the intial phase.

The average body weight on day 0 was 18.5 kg. The four animals that died had below-average body weights, and the two that survived the longest had been steadily losing weight for two months.

Final period

This section reports the observations recorded on 32 animals in the final month of the trial, from the time when the drugs were first given on day 0, until they were slaughtered on days 29 and 30.

Fluke egg counts. As was observed in the initial part of the trial, there was much variability in fluke epg after the drugs were given. The mean values for each group are shown in Figure 8.

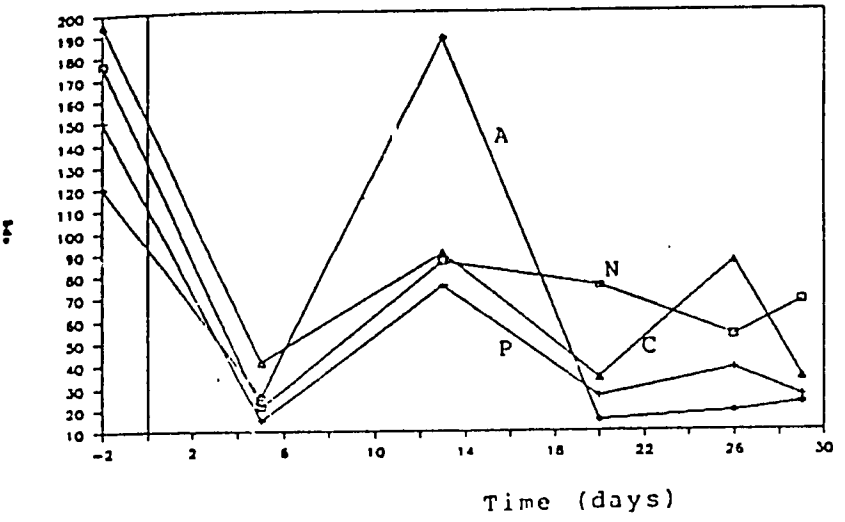

Figure 8. Changes in fluke epg after the drugs were given. Mean values for each group.

Animals treated with nitroxynil had a high level of fluke eggs in the faeces, usually at least as high as the control group. The mean fluke epg for the praziquantel group was lower than that of the control group.

Animals given albendazole showed a peak in fluke epg two weeks after treatment, then fluke epg fell to a low level. The peak may merely be random variation, or it may have arisen because the drug caused the fluke in the pancreas to release eggs.

For each animal the mean of the final three values (days 20, 26 and 29) was calculated. These means (F) were transformed for analysis using the transformation ln(F+1), and analysis of variance demonstrated that treatment had a statistically significant effect on level of fluke eggs in the faeces (Table 3).

Table 3. Analysis of variance of transformed fluke and nematode epg.

Source	df	Fluke ms	Fluke variance ratio	Nematode ms	Nematode variance ratio
Treatment	3	7.8	4.74	58.7	34.2
Error	28	1.7		1.7	
Total	31				

The mean transformed values and geometric means are shown in Table 4. This analysis confirms the visual impression that nitroxynil had no effect on F, whereas both praziquantel and albendazole both lowered F. Albendazole was the most effective drug in this respect, but none of the drugs completely eradicated the fluke.

Table 4. Mean values of the three final fluke epg.

	ln(F+1)	F
Nitroxynil	3.9	50
Praziquantel	2.9	17
Albendazole	1.8	5
Control	3.8	45
SE	±0.3	

Nematode egg counts. The drugs had clearly observed effects on nematode epg. Faecal egg counts remained high in the control and praziquantel groups, and fell to very low levels in animals treated with nitroxynil and albendazole (Figure 9). Thus, nitroxynil and albendazole are effective anthelmintics against gastro-intestinal nematodes, but praziquantel has no effect.

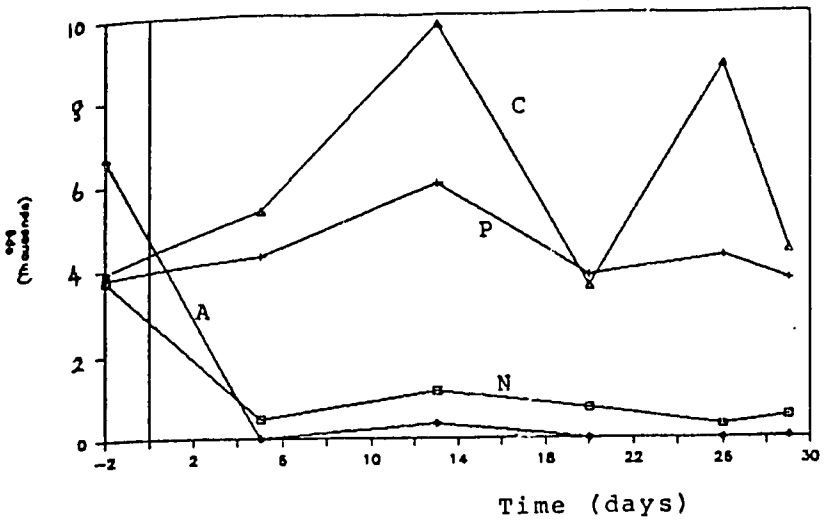

Figure 9. Changes in nematode epg after the drugs were given. Mean values for each group.

An analysis of variance was carried out using the average of the five values collected for each animal in the last phase of the trial. The values of nematode epg (N) were transformed, and the value of ln(N+1) used in the analysis (Table 3).

The values in Table 5 show that praziquantel had no effect on N. Nitroxynil reduced N to an acceptable mean of about 500, and albendazole was the most effective drug in this respect, reducing N almost to zero.

Table 5. Mean values of nematode epg after giving the drugs.

	ln(N+1)	N
Nitroxynil	6.3	526
Praziquantel	8.1	3256
Albendazole	2.4	8
Control	8.1	3284
SE	±0.5	

Body weight. Changes in body weight during the second phase of the trial are shown in Figure 10. The slopes of linear regressions fitted to the data shown in Figure 10 give an estimation of growth rate (Table 6).

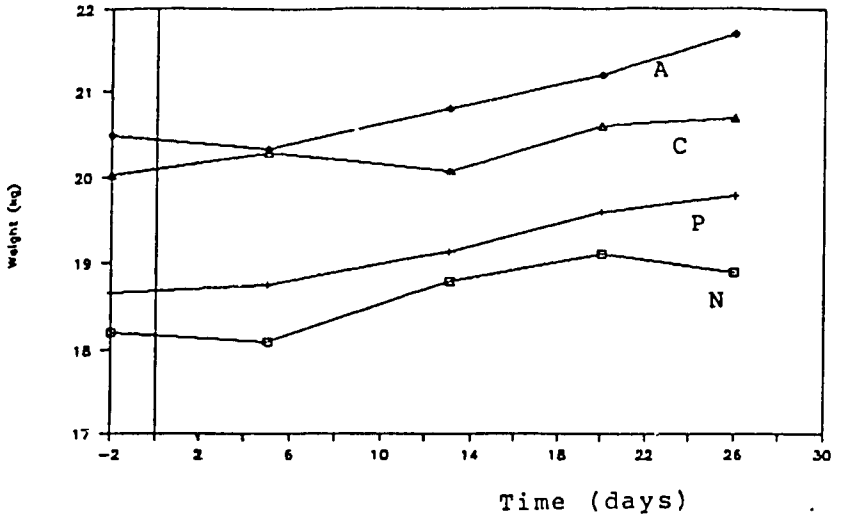

Figure 10. Changes in body weight after the drugs were given. Mean values for each group.

Table 6. Mean growth rates (g/d) after giving the drugs.

Nitroxynil	34 ± 11
Praziquantel	44 ± 5
Albendazole	46 ± 10
Control	23 ± 8

Analysis of variance of growth rates of individual animals showed no statistically significant effect of treatment. However, the growth rate of the control group was the lowest and all drugs increased growth rate to some extent. We had expected that nitroxynil and albendazole which are effective against nematodes would increase growth rate, and this was observed. There was also a surprisingly large benefit from praziquantel which had little or no effect on nematode burden, suggesting that alleviating the fluke burden increases growth.

Post-mortem findings

Number of fluke in pancreas. All the pancreas contained fluke in the pancreatic ducts. The range was from 2 to 1926, with a mean of 473. In those pancreas containing more than about 100 fluke the colour of the exterior of the pancreas was dark.

Treatment had a statistically significant effect on number of fluke in the pancreas (Table 7). The highest values were recorded for the nitroxynil and control groups, and the lowest values for the praziquantel and albendazole groups. However, even in the animals treated with praziquantel and albendazole there were large numbers of fluke.

Table 7. Mean values at slaughter.

	N	P	A	C	SE	
Fluke in pancreas (FP)	925	238	251	478	±125	P<0.05
ln(FP+1)	6.6	5.0	5.2	6.0	±0.32	P<0.05
geometric mean	737	153	176	408		
% live fluke	39	63	84	50	±12	ns
Weight of pancreas (g)	24	28	22	24	±3.5	ns
Body weight (kg)	18.9	19.8	21.7	20.7	±1.78	ns
Fluke epg	68	26	23	34	±14	ns
ln(F+1)	3.59	2.32	1.89	2.45	±0.68	ns
geometric mean	35.3	9.1	5.6	10.6		

It is interesting that in the animals treated with nitroxynil, there were more live fluke than in the control animals. This observation appears to completely rule out the thesis that nitroxynil effectively controls pancreatic fluke in sheep.

There did not appear to be any effect of treatment on the proportion of fluke alive at the time of counting, a few hours after the animals were slaughtered. The percentages of live fluke ranged from 40% for nitroxynil to more than 80% for albendazole.

In some pancreas there were substantial numbers of small light-coloured fluke, but there did not seem to be any relation between proportion of these apparently immature fluke and treatment.

Pancreas weight. Some of the pancreas were very small and difficult to find. Others were large and fibrosed. The variation in weight was from 6.4 to 42.8 g. This is similar to the weight of normal pancreas which ranges from 11 to 32 g in Indonesian sheep (Beriajaya, personal communication).

The larger pancreas tended to contain more fluke than the small pancreas (Figure 11), but the correlation between pancreas weight and number of fluke was not significant (r=0.24, n=32).

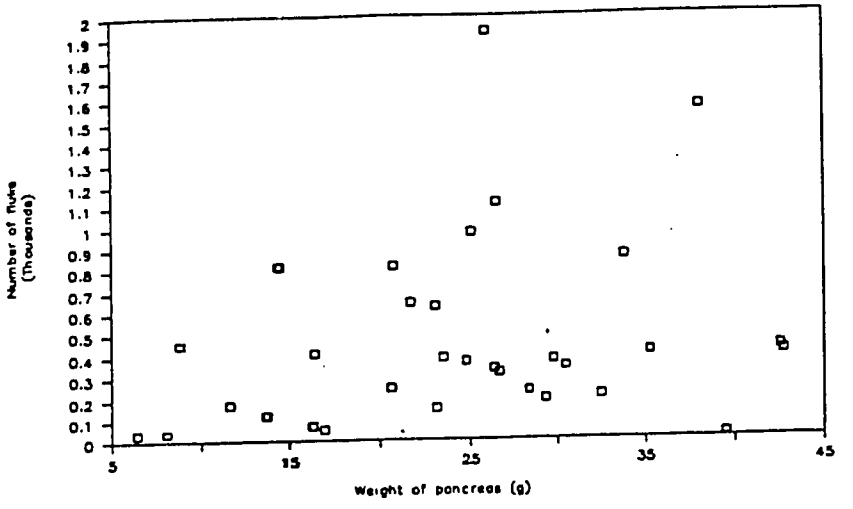

Figure 11. Relation between number of fluke in pancreas and weight of pancreas.

There was, however, a significant correlation between weight of pancreas (P, in g) and body weight (W, in kg):

$P = 10.3(\pm 9.2) + 0.70(\pm 0.34)\ W$ $\quad n=32,\ r=0.35,\ P<0.05$

These data are shown in Figure 12, and indicate that larger animals had larger pancreas than small animals.

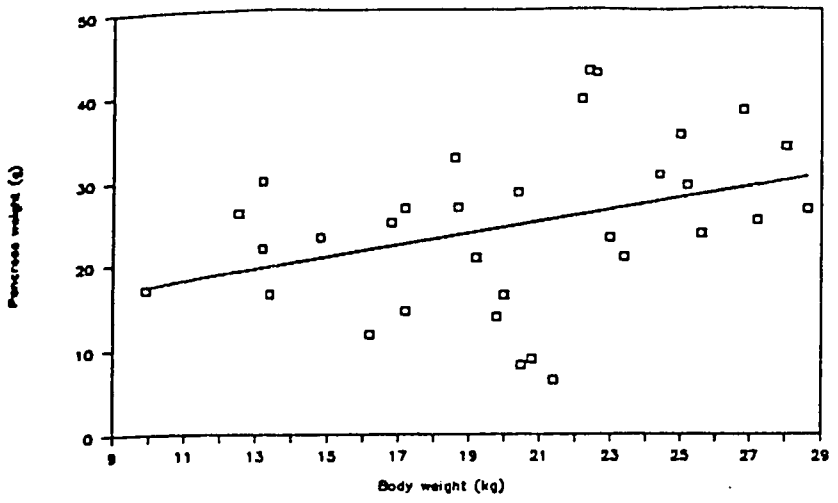

Figure 12. Relation between weight of pancreas and body weight.

Relation between F and number of fluke. There was a positive correlation between number of fluke in the pancreas (FP) and fluke epg (F) on the day of slaughter (Figure 13). A similar relation between these two measurements was reported by Arasu et al (1991). The calculated linear regression for our data is:

$F = 18(\pm 39) + 0.040(\pm 0.016)\ FP$ n=32, r=0.42, P<0.05

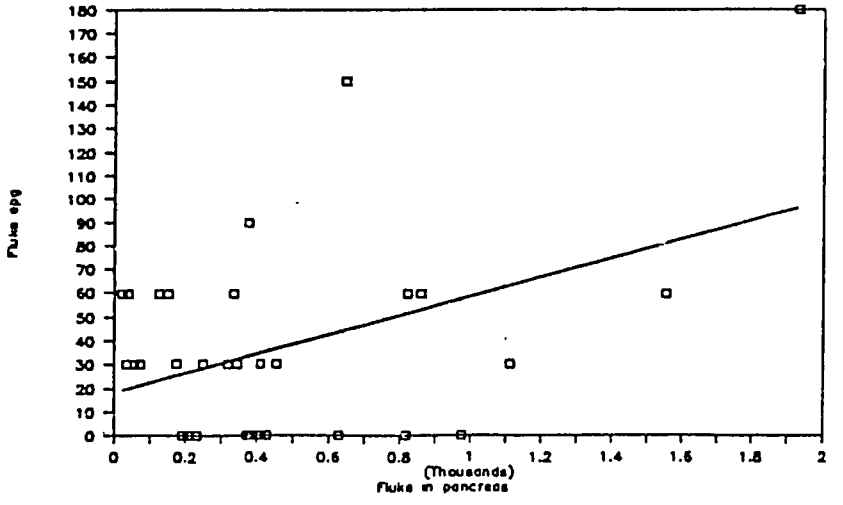

Figure 13. Relation between fluke epg on day of slaughter and number of fluke in pancreas.

Histological findings.

The histopathological findings in the pancreas and liver are summarised in Table 8. Previous work shows that the pathogenesis of Eurytrema pancreaticum can be categorised into three stages (Wilson, 1992b).

1. Invasion of the pancreatic ducts by immature fluke which subsequently become mature to the egg-laying stage. There is marked fibrosis around the ducts, but the glandular and islets tissue of the pancreas is normal.

2. Destruction of the islets of Langerhans (which produce insulin) and glandular tissue (which produces digestive enzymes). The mechanism for this destructive process is uncertain but in some cases is thought to be due to the movement of eggs into the tissue. This stage is associated with massive inter-lobular and intra-lobular fibrosis and generalised necrosis of all parts of the pancreas. These changes are also associated with invasion by inflammatory cells especially lymphocytes.

3. Development of toxic changes in the liver (hepatosis) as a direct result of metabolic disturbances caused by the destruction of the islets of Langerhans. The energy pathways in the body have been disturbed, leading to mobilisation of fat reserves to produce energy; the glucose source is excreted in the urine as insulin is not being produced. This leads to widespread degenerative changes in the hepatocytes of the liver, beginning with centro-lobular fatty degeneration and ending with generalised necrosis and hepatic failure.

All animals were infected with pancreatic fluke. Ninety-one per cent showed fibrosis of the pancreatic ducts (column 6 in Table 8). Only three animals, 9% of the total, showed some necrosis in the liver (score of 3 or more in column 8), suggesting that at this early stage of infection production was not seriously affected.

Although the albendazole group had the lowest mean number of cases of necrosis in the islets and glandular tissue of the pancreas (columns 3 and 4 in Table 8), the Chi-square test revealed no statistically significant differences between groups.

Table 8. Histopathological findings in the pancreas and liver.

Column 1	2	3	4	5	6	7	8
Nitroxynil							
10046	1	0	0	0	2	0	0
10066	w	0	0	1	3	1	0
10084	1	0	0	0	1	0	2
10109	1	1	1	3	3	3	2
10117	1	0	0	0	1	0	0
10142	1	0	0	1	3	1	1
10164	1	1	0	1	3	2	3
11037	1	0	0	0	1	0	0
% affected	100	25	13	50	100	50	50
Mean				0.75	2.13	0.88	1.00
Praziquantel							
10103	w	1	0	0	2	1	1
10110	1	1	1	2	3	2	2
10118	1	0	0	1	2	0	0
10119	w	0	0	0	3	0	0
10131	1	0	0	0	0	0	0
10165	1	0	0	0	2	0	0
11028	1	1	1	3	3	3	3
11057	1	0	0	1	2	2	1
% affected	100	38	25	50	88	50	50
Mean				0.88	2.13	1.00	0.88
Albendazole							
01159	1	0	0	0	1	0	0
10045	1	0	0	0	1	0	0
10102	1	0	0	0	1	0	0
10144	1	1	1	3	3	3	0
10149	1	0	0	1	2	0	3
10169	1	0	0	0	1	0	0
11031	1	0	0	0	0	0	0
11056	1	0	0	0	0	0	0
% affected	100	13	13	25	75	13	13
Mean				0.50	1.13	0.38	0.38
Control							
10076	w	0	0	0	2	0	1
10082	1	1	1	3	4	4	2
10152	1	1	0	1	3	3	1
10162	1	1	0	1	3	3	1
10178	1	0	0	0	1	0	0
11041	1	0	0	1	2	2	0
11050	1	1	1	3	3	3	1
11060	1	1	0	1	3	2	1
% affected	100	63	25	75	100	75	75
Mean				1.25	2.63	2.13	0.88

Columns in Table 8

1. Animal number
2. Presence of fluke eggs in female parasites. 0 = eggs not present in fluke; 1 = eggs present in fluke; w = fluke not seen in section.
3. Necrosis of islets of Langerhans in pancreas. 0 = normal; 1 = necrosis.
4. Necrosis of glandular tissue in pancreas. 0 = normal; 1 = necrosis.
5. Degree of necrosis of glandular tissuse in pancreas. 0 = normal; 1 = mild; 2 = moderate; 3 = severe; 4 = very severe.
6. Degree of interlobular fibrosis in pancreas. 0 = normal; 1 = mild; 2 = moderate; 3 = severe; 4 = very severe.
7. Degree of intralobular fibrosis in pancreas. 0 = normal; 1 = mild; 2 = moderate; 3 = severe; 4 = very severe.
8. Degree of degenerative changes in liver. 0 = normal; 1 = moderate centrolobular fatty degeneration; 2 = severe centrolobular degeneration; 3 = generalised centrolobular degeneration with some necrosis; 4 = generalised necrosis.

The Mann-Whitney rank test (Snedocor and Cochran, 1967) was used to compare the scores of the histological examinations. The only statistically significant findings were

(i) inter-lobular fibrosis in the pancreas (column 6) was less severe (mean 1.125) for the albendazole group than for the other three groups (mean 2.291). $T = 75$ ($P<0.05$).

(ii) intra-lobular fibrosis in the pancreas (column 7) was more severe (mean 2.125) for the control group than for the other three groups (mean 0.750). $T = 91.5$ ($P<0.05$).

The lower levels of pancreatic fibrosis and liver degeneration in the A and P groups than in the C and N groups suggest that if the fluke are controlled, the damaged tissues are able to regenerate. The drugs were given only one month before the animals were slaughtered, and it appears that during this period some degree of regeneration took place.

DISCUSSION

All the sheep in this study were superficially healthy, yet every one harboured fluke in the pancreas. It appears that these animals were infected while they were lambs at an age of less than three months.

Chronic infection is characterised by the presence of adult fluke in the pancreatic ducts, fibrosis around the pancreatic ducts and excretion of fluke eggs in the faeces. Severe infection is associated with massive inter- and intra-lobular fibrosis and necrosis of all parts of the pancreas, followed by centro-lobular fatty degeneration in the liver then necrosis of the liver and hepatic failure.

Chronic infection appears to continue in individual sheep for years without developing into the fatal form. We do not know what causes the fluke to turn from an apparently benign invader to a deadly parasite. Why can some animals cope with infection, and not others?

There was a positive correlation between number of fluke in the pancreas and fluke epg on the day of slaughter, confirming that we can use fluke epg as a rough guide to level of infection.

Treatment with albendazole and praziquantel significantly reduced the level of infection of pancreatic fluke. The animals treated with albendazole showed a peak in fluke epg two weeks after treatment, which then fell to a low level. This peak may be random variation, or it may have arisen because the drug caused the fluke in the pancreas to release eggs. Albendazole completely controlled gastro-intestinal nematodes, but praziquantel had no effect on nematode epg.

Nitroxynil appeared to be ineffective in controlling pancreatic fluke, but it reduced nematode epg. All three drugs increased growth rate to some extent, and this can be attributed to the reduced burdens of fluke and nematode parasites.

However, none of the drugs reduced levels of adult fluke in the pancreas or faecal egg counts to negligible levels. A moderate reduction in infection is unlikely to have any long-term biological or economic effect as the sheep would soon pick up more fluke if they were grazing.

More frequent doses of the drugs, or higher dose rates, may be more effective in reducing infection. But this would make the cost of control very high, and almost certainly not give an economic response.

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