# Pharmacokinetics and Efficacy of Albendazole in Deer

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## Introduction

Previous studies have shown differences in the susceptibility of subspecies of *Cervus elaphus* to parasite disease, including the Fading Elk Syndrome which appears largely due to abomasal parasitism (Waldrup and Mackintosh, 1992; Waldrup and Mackintosh, 1993a). There is also anecdotal evidence that the efficacy of anthelmintics may vary between different red and wapiti deer (Mackintosh unpub. data). The size of the abomasal parasite burden has also been shown to effect the efficacy of benzimidazole anthelmintics in sheep (Marriner, Evans and Bogan, 1985). Other factors which may affect the bioactivity and pharmacokinetics of benzimidazoles include solubility, absorption/retention in the gastro-intestinal tract (GIT) and faecal excretion. These are affected by diet, rumen degradation, binding to food particles and gut motility. The parent benzimidazole is usually metabolised by oxidation to the sulphoxide and the sulphide, and the activity of these metabolites may vary. Plasma binding and redistribution between body compartments including active excretion in the bile, may also affect efficacy (McKellar, 1997). The efficacy of benzimidazoles is thought to be a function of both concentration and duration.

Albendazole has been shown to be moderately effective in deer (Mackintosh *et al*, 1984; Anderson and Wilson, 1984) and has been used in New Zealand for some years. It was used in this study to determine if there were differences in it's efficacy and pharmacokinetics between red, wapiti and first cross ($F_1$) red/wapiti hybrid deer. Albendazole in ruminants is believed to be largely metabolised by "first pass" oxidation in the liver to sulphoxide and there are negligible amounts of the parent compound found in plasma (McKellar, 1997). Therefore the plasma samples were only analysed for the sulphoxide (thought to be active) and sulphone (thought to be relatively inactive).

## Materials and Methods

Eighteen red, 18 wapiti and 18 red/wapiti ($F_1$) hybrid newly weaned deer were brought together in April 1994 and 6 of each subspecies were randomly allocated to 3 treatment groups of (A) Parasitised Control (B) Parasitised Albendazole Treated and (C) Non-Parasitised Albendazole Treated (see Table 1). The intention was to treat the two Parasitised Groups (A and B) with 2 doses of albendazole[1] (10 mg/kg) 5 weeks apart, so that they would develop moderate lungworm and gastrointestinal burdens. They would be put indoors 5 weeks after the second dose and Group B would be treated with oral albendazole at 10 mg/kg and then all the weaners would be killed 18 days later to

[1] Albezol DC, SmithKline Beecham Animal Health

determine the efficacy of the anthelmintic. The Non-Parasitised Group C would be treated with 4 doses of moxidectin pour-on[2] (1 ml/10 kg) at 3 week intervals, put inside at the same time as Groups A & B, treated with albendazole (10 mg/kg) as for Group B and killed 14 days later. Immediately after treatment, the deer in Groups B and C would be bloodsampled at 0, 2, 4, 8, 12, 16, 24 and 48 hours and the plasma samples analysed for albendazole sulphoxide and sulphone, to compare their pharmacokinetics in parasitised and non-parasitised deer.

The design had to be modified when four of the elk deer in Groups A and B died of acute lungworm disease 7, 8, 15 and 16 days after the second treatment with albendazole. Also one red deer and one $F_1$ suffered fractured legs and were euthanased and one non-parasitised elk died of Malignant Catarrhal Fever. Therefore, it was necessary to disband the Parasitised Elk Control Group and continue the trial as shown in Table 2. All other procedures were carried out as originally planned.

Parasitological methods: Total worm counts were made on lung, abomasum, abomasal digest, small intestine and large intestine using standard MAFQual Laboratory methods.

Analytical method: The analysis of the albendazole sulphoxide and sulphone was by HPLC using mebendazole as an internal standard as follows: 1 ml of plasma was adjusted to ca pH 9-10 with excess sodium bicarbonate and extracted with 5 ml of a solution of ethyl acetate-hexane (3:1). After centrifuging the organic layer was evaporated and reconstituted in 300 ul of a solution of 20% acetonitrile : water at pH 3.0. 25 ul of this was injected onto the HPLC. Quantitation was by a 7 point calibration curve of spiked plasma from 0.05 mg/L to 0.5 mg/L. A blank plasma was analysed as a control sample. The HPLC conditions were as follows: A Waters 600E pump and system controller and 700 series auto-injector, a Shimadzu SPD 10A UV detector and a HP 3396 reporting integrator with disc drive. The column was a 250 mm X 4.6 mm id Alltech Econosil C18 set at 30° C. The flow rate was 1 ml/min and the wavelength was 296 nm. Retention times were: albendazole sulphone 4.2 minutes, albendazole sulphoxide 5.8 minutes, mebendozole 11.3 minutes and albendazole 13.8 minutes. Slight variations on these retention times occurred but the elution order was always preserved.

Statistical analysis: Geometric means were calculated using log transformed data [ ie. $\log_{10}(x + 1)$] and back transformed. The efficacy and pharmacokinetic data were analysed using Genstat 5.

## Results

Group geometric mean counts of parasites are shown in Table 3. and estimated efficacies in Table 4. Note that a true assessment of the efficacy of albendazole in elk could not be made because there was no untreated control group. However, an estimate of the efficacy based on a comparison with the $F_1$ control group is shown.

[2] Cydectin Pour-on, Cyanamid of New Zealand Ltd

Bar graphs showing group mean concentrations of albendazole sulphoxide and sulphone in parasitised and non-parasitised red, $F_1$ and elk deer are shown in Figures 1 and 2 respectively. Figure 3 highlights differences between nonparasitised red, $F_1$ and elk deer, and Figure 4 shows parasitised red, $F_1$ and elk deer for sulphoxide concentrations.

The area-under-the curve (a-u-c) analyses showed that for sulphoxide, the non-parasitised red and elk deer had significantly greater a-u-c than parasitised red and elk, whereas the opposite occurred with $F_1$ deer. For sulphone the non-parasitised deer all had greater a-u-c than parasitised suggesting the $F_1$ sulphoxide result may be anomalous. Elk had a significantly greater a-u-c than red or $F_1$ deer.

## Discussion

### Efficacy

Geometric means were used in preference to arithmetic means for worm counts due to the non-normal distribution. This made large differences to the estimated efficacy of albendazole in the three subspecies of deer against the parasites, especially the lungworm. The estimated efficacy in elk was calculated using the control $F_1$ deer as a comparison and this is likely to have resulted in an underestimation, because previous studies indicated that elk are likely to have heavier parasite burdens than $F_1$ and red deer (Waldrup, unpub). Nevertheless, albendazole at 10 mg/kg appears moderately to highly effective against *Ostertagia*-type parasites in all three subspecies of deer, although the poorest results were seen against adult parasites in $F_1$ deer and $L_5$ parasites in red deer. The 100 % efficacy against *Trichostrongylus* species is not a robust result because only two treated deer had burdens while all the controls were negative.

The poor efficacy against lungworm in all three deer subspecies is surprising. However, this apparent poor efficacy against adult lungworm may be partly due to albendazole sulphoxide not killing immature or migrating lungworm larvae. When the deer were killed 18 days after treatment, a substantial number of these immature larvae may have matured into adults. The zero estimates for the elk are because the numbers of adult and immature lungworm in the treated elk were higher than in the control (untreated) $F_1$ deer. It appears from this estimate, and from the fact that four of the original control elk weaners died of very high lungworm burdens 7 to 14 days after treatment, that this drug has poor efficacy in elk. Anderson and Wilson (1984) also found that albendazole had variable efficacy, did not eliminate faecal larval shedding in all red deer weaners in a group which were treated with 10 mg/kg by oral drench, and 85 % were shedding by day 21 after treatment. Similarly, Mason and Beatson (1984) showed that albendazole did not eliminate faecal larval shedding in all treated red deer weaners, and appeared to have poorer efficacy and result in lower weight gains than oxfendazole. Another study in red deer weaners which compared the efficacy of oral albendazole (10 mg/kg) at 21 day intervals with an intra-ruminal albendazole bolus showed that both treated groups had significantly higher liveweight gains than control deer treated with albendazole at 5 weekly intervals. However, some of the albendazole treated deer had high faecal larval

counts 21 days after treatment suggesting poor clearance of adults and or immature lungworms (Waldrup *et al.*, 1993c).

**Pharmacokinetics**

It appears that, like cattle, deer have a lower and shorter duration of peak plasma concentrations of albendazole sulphoxide following albendazole administration than sheep which have similar plasma concentrations of sulphoxide after receiving half the dose rate (5 mg/kg) of albendazole (McKellar *et al.*, 1995). This may account for reduced efficacy, especially against lungworm. In red, $F_1$ and elk deer the sulphoxide concentrations peaked earlier (8 hours vs 8 to 12 hours) and fell away quicker in parasitised than non-parasitised deer. This suggests that the presence of parasites in the abomasum affects the uptake and pharmacokinetics of albendazole, and the lower peak concentration and shorter duration of plasma levels may result in lower efficacy, as has been reported in sheep. The differences between nonparasitised (Fig. 3) and parasitised (Fig. 4) deer was greater in the elk and the low peak plasma concentrations of sulphoxide may account for the poor efficacy of albendazole in elk against lungworm. Similarly sulphone peaked earlier in parasitised red (12 hours) than non-parasitised (16 hours) red deer whereas in $F_1$ deer the sulphone peaked at 16 hours in both parasitised and non-parasitised animals and in elk sulphone peaked later (16-24 hours) in parasitised than non-parasitised (16 hours) animals. These findings suggest that the redistribution of sulphoxide and it's oxidation to sulphone differs between the three subspecies of deer. This is supported by the differences in area-under-the curve seen between them. The lower sulphoxide peak and the greater sulphone a-u-c for parasitised elk suggest that they metabolise albendazole and the sulphoxide to the inactive sulphone more rapidly than red or $F_1$ deer and this may explain the lower efficacy against lungworm in elk.

The higher efficacy of albendazole against gastro-intestinal parasites than against lungworm may be partially due to the direct activity of albendazole passing unaltered from the rumen down through the abomasum and intestines. Differences between the three subspecies and between parasitised and non-parasitised could also be influenced by the flow rate of ingesta from the rumen to the abomasum and beyond. Benzimidazoles, which are fairly insoluble, appear to associate strongly with particulate material in the rumen and a number of factors can influence the flow rate of rumen digesta including the quality and type of feed (Ali and Chick, 1992), quantity of food and the genotype of the animal. Animals with higher intakes have faster flow rates resulting in earlier plasma peak levels of oxfendazole but shorter duration and reduced a-u-c values than for animals on lower food intakes. Benzimidazoles and their metabolites appear to be more soluble in the more acidic environment of the abomasum than the rumen and higher levels may be found in the abomasum than in the plasma. Albendazole and its metabolites are also reversibly exchanged between plasma and gastrointestinal compartments, and may be greatly concentrated in the abomasum due to ion trapping (Pritchard, Gascon and Lanusse, 1991). The presence of excessive numbers of *Ostertagia*-type parasites in the abomasum, which have been shown to raise the pH (Waldrup and Mackintosh, 1993b), may reduce the uptake of benzimidazoles thus reducing their efficacy. This effect appears to be greatest in elk which appear more susceptible to abomasal parasites than red deer. In sheep, the

presence of high parasite burdens have been shown to reduce the efficacy of fenbendazole and febantel (Marriner, Evans and Bogan, 1985; Vynckier *et al*).

During this trial the deer were held indoors and fed on dry lucerne hay for 2 days prior to treatment and during the pharmacokinetic study. This may have reduced the plasma levels of albendazole sulphoxide and sulphone and thereby reduced it's efficacy compared with treated deer grazing at pasture, as has been demonstrated with sheep dosed with oxfendazole while on dry forage (Ali and Chick, 1992; Ali and Hennessy, 1995). These latter authors found the peak plasma concentrations were similar but the duration and a-u-c were lower for sheep on dry oats, chopped lucerne and wheaten chaff, compared with sheep given fresh clover pasture.

## Acknowledgements

We would like to thank the staff at AgResearch Invermay including Julie Newman (now at Texas A & M University), MAFQual Invermay Animal Health Laboratory and MAFQual Wallaceville for the assistance in conducting this trial and the Elk and Wapiti Society of New Zealand for providing some of the animals.

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**Table 1: The original trial design with 3 treatments across three subspecies of deer and 6 animals per group.**

| | Parasitised *Control* | Parasitised *Alb treated* | Non-parasitised *Alb treated* |
|---|---|---|---|
| Red | 6 | 6 | 6 |
| F1 | 6 | 6 | 6 |
| Elk | 6 | 6 | 6 |

**Table 2: The modified trial design with the elimination of the elk control group and a summary of pretrial animal losses.**

| | Parasitised *Control* | Parasitised *Alb treated* | Non-parasitised *Alb treated* |
|---|---|---|---|
| Red | 6 | 5 | 6 |
| F1 | 6 | 5 | 6 |
| Elk | 0 | 8 | 5 |
| Animal losses | | Red deer | # leg |
| | | F1 | # leg |
| | | Para-Elk | lungworm dis |
| | | | 7, 8, 15 and 16 |
| | | Non-para Elk | MCF |

**Table 3: Total worm counts for all the trial animals together with group arithmetic and geometric means and the standard error of the geometric means.**

| Animal No. | Group | *Ostertagia* - type | | | | *Trichs* | Lungworms | |
|---|---|---|---|---|---|---|---|---|
| | | *Adult* | *L5* | *LL4* | *EL4* | *Adult* | *Adult* | *Immature* |
| R1 | Red | 2900 | 0 | 2300 | 12500 | 0 | 10 | 29 |
| R2 | Control | 4700 | 300 | 300 | 9400 | 0 | 34 | 122 |
| R3 | | 4900 | 1200 | 1000 | 8400 | 0 | 1030 | 197 |
| R4 | | 2600 | 400 | 400 | 4400 | 0 | 42 | 6 |
| R5 | | 8400 | 3100 | 2800 | 41800 | 0 | 173 | 1060 |
| R6 | | 3100 | 500 | 1800 | 15400 | 200 | 1 | 54 |
| Mean | | 4433 | 917 | 1433 | 15317 | 33 | 215 | 245 |
| Geom mean | | 4063 | 246 | 1053 | 11848 | 1 | 42 | 80 |
| SE | | 0 9934 | 1 0816 | 0 8000 | 0 7551 | | 0 6641 | 0 4151 |
| R 8 | Red | 500 | 0 | 100 | 500 | 0 | 53 | 25 |
| R 9 | Treated | 0 | 0 | 0 | 0 | 0 | 76 | 32 |
| R 10 | | 2800 | 900 | 600 | 1300 | 0 | 48 | 64 |
| R 11 | | 0 | 500 | 0 | 0 | 0 | 17 | 10 |
| R 12 | | 300 | 500 | 900 | 200 | 0 | 14 | 68 |
| Mean | | 720 | 380 | 320 | 400 | 0 | 42 | 40 |
| Geom mean | | 52 | 46 | 34 | 41 | 0 | 34 | 32 |
| SE | | 1.0224 | 1 1848 | 0 8764 | 0 8272 | | 0 7275 | 0 4548 |
| BY31 | F1 | 8500 | 700 | 2600 | 13100 | 0 | 41 | 114 |
| BY32 | Control | 1800 | 600 | 700 | 4200 | 0 | 1 | 22 |
| BY33 | | 16200 | 300 | 2100 | 10700 | 0 | 32 | 87 |
| BY34 | | 13100 | 1200 | 4400 | 43500 | 200 | 10 | 181 |
| BY35 | | 9100 | 900 | 3600 | 10000 | 0 | 27 | 278 |
| BY36 | | 4500 | 300 | 1200 | 3800 | 0 | 1433 | 152 |
| Mean | | 8867 | 667 | 2433 | 14217 | 33 | 257 | 139 |
| Geom mean | | 7114 | 589 | 2038 | 9996 | 2 4 | 32 | 109 |
| SE | | 0 9334 | 1 0816 | 0 8000 | 0 7551 | | 0 6641 | 0 4151 |
| BY 38 | F1 | 400 | 0 | 0 | 100 | 0 | 18 | 101 |
| BY 39 | Treated | 1800 | 500 | 100 | 500 | 0 | 15 | 56 |
| BY 40 | | 700 | 0 | 0 | 100 | 0 | 179 | 254 |
| BY 41 | | 900 | 100 | 0 | 1700 | 0 | 9 | 62 |
| BY 42 | | 1100 | 600 | 100 | 600 | 0 | 12 | 79 |
| Mean | | 980 | 240 | 40 | 540 | 0 | 47 | 110 |
| Geom mean | | 870 | 31 | 5 4 | 350 | 0 | 23 | 94 |
| SE | | 1 0224 | 1 1848 | 0 8764 | 0 8272 | | 0 7275 | 0 4548 |
| P 61 | Elk | 0 | 100 | 0 | 100 | 0 | 1373 | 101 |
| P 63 | Treated | 0 | 100 | 0 | 100 | 0 | 216 | 502 |
| P 65 | | 100 | 0 | 0 | 100 | 0 | 97 | 0 |
| P 66 | | 1200 | 100 | 0 | 200 | 0 | 553 | 399 |
| P 67 | | 0 | 0 | 0 | 200 | 0 | 45 | 68 |
| P 68 | | 300 | 200 | 0 | 200 | 0 | 1385 | 303 |
| P 70 | | 0 | 0 | 0 | 0 | 0 | 142 | 351 |
| P 71 | | 300 | 100 | 100 | 400 | 0 | 76 | 305 |
| Mean | | 238 | 75 | 13 | 163 | 0 | 486 | 254 |
| Geom mean | | 17 | 19 | 1 7 | 86 | 0 | 239 | 223 |
| SE | | 0 8083 | 0 9367 | 0 6928 | 0 6539 | | 0 5751 | 0 3595 |

SE = standard error of geometric mean

**Table 4: Estimated efficacies (with 95 % confidence interval) for albendazole against deer parasites in the three subspecies of deer.**

| Group (n) | *Ostertagia*-type | | | | *Trichostr.* | Lungworm | |
|---|---|---|---|---|---|---|---|
| | *Adult* | *L5* | *LL4* | *EL4* | *Adult* | *Adult* | *Immature* |
| Red deer (6) | 98 7 (99.9-77 4) | 81 (99 3-0) | 96 7 (99 7-61 5) | 99 6 (99 9-96 4) | 100 (ND) | 17 3 (89-0) | 58 9 (88-0) |
| F1 (6) | 87 8 (99 3-0) | 94 7 (99 8-0) | 99 7 (99 9-96 4) | 96 5 (99 7-64 8) | 100 (ND) | 28 1 (90-0) | 13 9 (75-0) |
| Elk(8)* | 99 7 (99 9-96 8) | 96 7 (99 8-37) | 99 9 (99.9-99 2) | 99 1 (99 9-93 2) | 100 (ND) | [0] | [0] (34-0) |

L5 - fifth stage larvae
LL4 - late fourth stage larvae
EL4 - early fourth stage larvae
*Trichostr* - *Trichostrongylus* spp

Fig 1 Group mean plasma concentrations of albendazole sulphoxide in parasitised and non-parasitised red, F1 and elk deer over the 48 hour period after treatment

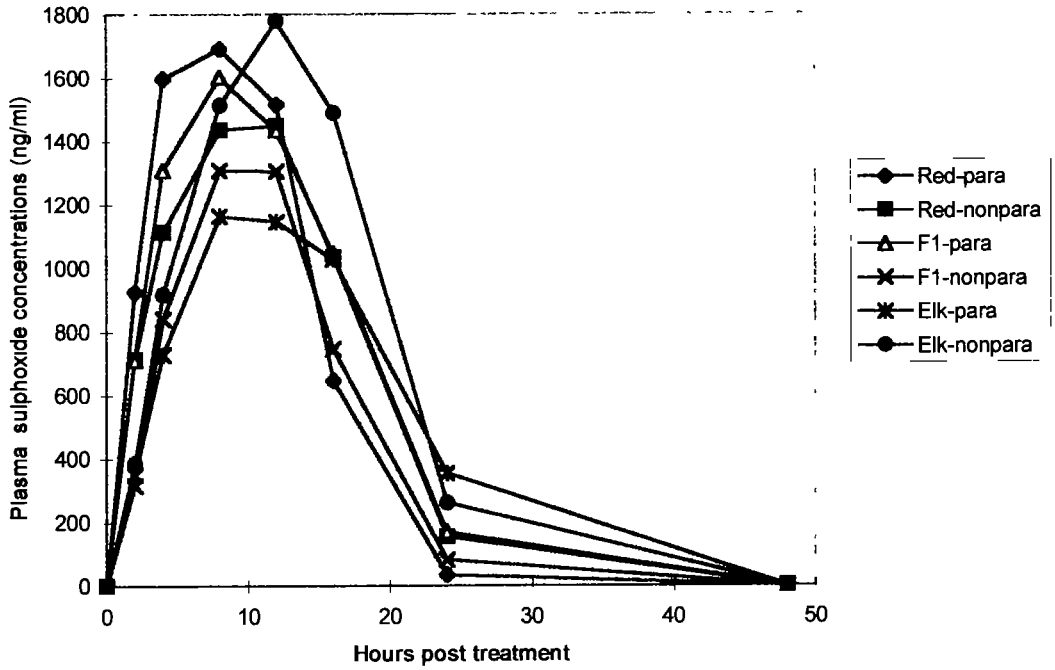


Fig 2 Group mean plasma concentrations of albendazole sulphone in parasitised and non-parasitised red, F1 and elk deer over the 48 hour period after treatment

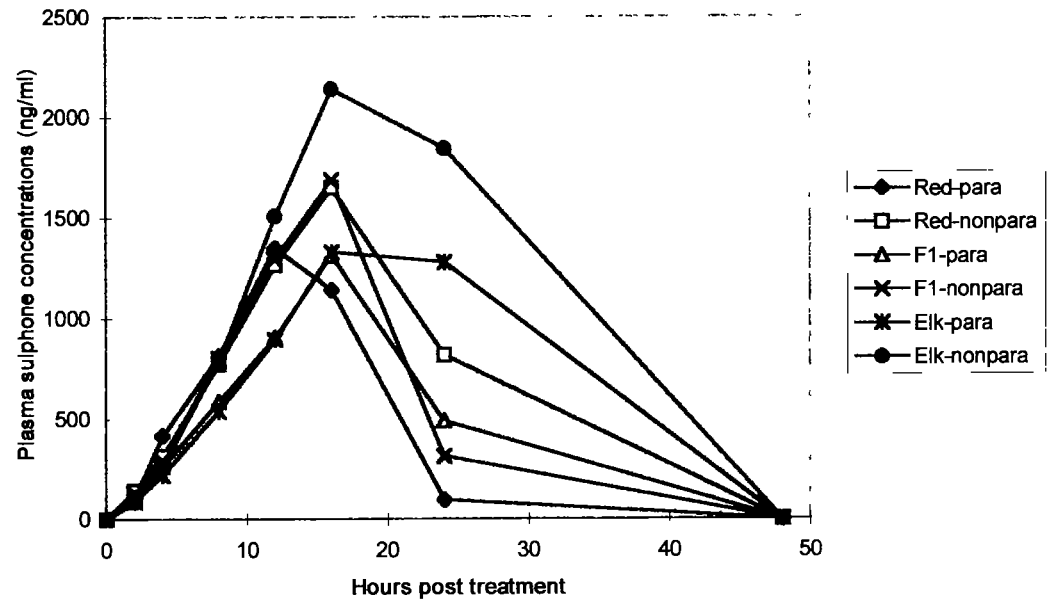

Fig 3 Group mean plasma concentrations of albendazole sulphoxide in non-parasitised red, F1 and elk deer

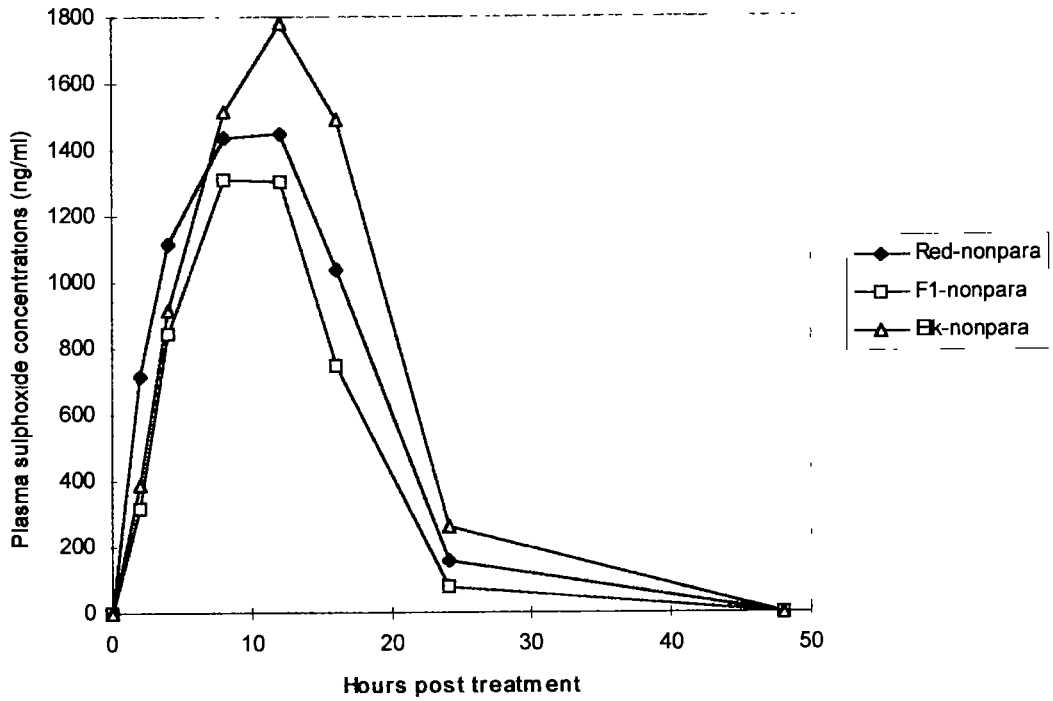


Fig 4 Group mean plasma concentrations of albendazole sulphoxide in parasitised red, F1 and elk deer