DEVELOPMENT OF A NOVEL EQUINE COMBINATION ANTHELMINTIC AND A STUDY OF ANTHELMINTIC SUSCEPTIBILITY USING A LARVAL DEVELOPMENT ASSAY

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN VETERINARY SCIENCE AT MASSEY UNIVERSITY

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Abstract

The occurrence of anthelmintic resistance to benzimidazoles and avermectin in cyathostome nematodes in New Zealand horses was investigated using a larval development assay (LDA) to conduct a small survey. For the benzimidazoles the analogue, thiabendazole was used and for avermectin the analogues, ivermectin and ivermectin aglycone were used in the LDA. The normal range of LD_{50} values was estimated by assaying eggs from Kaimanawa feral horses (n = 22) for each analogue used. From these the mean LD_{50} values + 2×standard deviation was taken as the upper limit of normal. The survey involved domestic horses (n = 47) from several locations around New Zealand. For ivermectin and ivermectin aglycone 12% had LD_{50} values higher than normal with resistance factors up to 5.3 and 6.8 respectively. This represents horses from three separate farms. For the benzimidazoles 43% of domestic horses had higher than normal LD_{50} values with resistance factors up to 4.8. These results suggest that some nematodes in these domestic horses had an increased tolerance to avermectins and as well as to benzimidazoles.

Two studies were conducted to assess the efficacy of a combination of abamectin (0.2mg/kg), oxibendazole (10mg/kg) and bithionol (5mg/kg or 7mg.kg). In the first study bithionol was included at 5mg/kg and in the second study it was included at 7mg/kg. These studies showed the efficacy of the combination was >98% against adult luminal stages of *S. vulgaris, S. edentatus*, cyathostomes, migratory stages of *S. edentatus* and third instar stages of *Gasterophilus intestinalis*. Efficacy against mucosal stages of cyathostomes (about 64%) and arterial stages of *S. vulgaris* (71%) was poor. Inclusion of bithionol at 7mg/kg achieved an efficacy of 100% against *A. perfoliata* whereas at 5mg/kg the efficacy was only 84.6%.

In these studies the following species were identified: two species of *Strongylus* (*S. vulgaris* and *S. edentatus*); three species of *Triodontophorus* (*T. serratus, T. minor* and *T. tenuicollis*); and twelve species of cyathostomes (*Cyathostomum coronatum, Cyathostomum labiatum, Cyathostomum catinatum, Cylicocyclus nassatus, Cylicocyclus leptostomus, Cylicocyclus radiatus, Cylicocyclus insigne, Cylicostephanus poculatus, Cylicostephanus minutus, Cylicostephanus calicatus, Cylicostephanus longibursatus* and *Cylicostephanus goldi)*.

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µg	microgram
µl	microliter
µm	micrometer
µM	micromolar
AM	arithmetic mean
AOB	abamectin, oxibendazole, bithionol
AVM	Avermectin
BZ	Benzimidazole
C	Control
Ca	Canterbury
Ch	Christchurch
cm	Centimeter
CMA	Cranial mesenteric artery
D.	dorsal
DCS	Dose confirmation study
DMSO	Dimethyl sulphoxide
Dy	Drury
E	Exported horse
ED_{50}	The dose that prevents 50% of the eggs to hatch
ELC	External leaf crown
epg	eggs per gram
F	Farm
FEC	faecal egg count
g	gram
GM	geometric mean
H	Hastings
HCl	hydrochloric acid
HE	highly effective
ILC	Internal leaf crown
IVM	Ivermectin
K	Kaimanawa
kg	kilogram
L	liter

L_1	First stage larvae
L_2	Second stage larvae
L_3	Third stage larvae
L_4	Fourth stage larvae
LD_{50}	The dose that prevents 50% of the eggs develop into L_3 larvae
LDA	Larval development assay
LE	less effective
mg	milligram
$MgSo_4$	magnesium sulphate
ml	milliliter
mm	millimeter
NaCl	sodium chloride
ND	not done
NE	not effective
NM	not mentioned
No.	number
NTR	Not recorded
P	Palmerston North
p.p.m.	Parts per million
PES	Principal efficacy study
r^2	coefficient of determination
RF	Resistance factor
SD	Standard deviation
SF	susceptibility factor
SOP	Standard Operating Procedure
T	Treated
Ta	Takanini
TBZ	Thiabendazole
V.	ventral
wt	weight

CHAPTER ONE

General Introduction and Literature Review

1.1 Introduction

Parasitic infestations are one of the most common health problem in horses. In recent decades regular use of anthelmintics has been the main method of control employed especially when groups of horses are kept together.

Anthelmintic resistance is a recognised problem in nematode parasites in ruminants and horses around the world. Benzimidazole resistance in horse nematodes has been reported from many countries including New Zealand, Australia, England, Canada, Denmark, Norway and USA. Resistance has also been reported to pyrantel. To date there have been no reports of avermectin resistance in horse nematodes. To investigate the current situation in New Zealand larval development assays were carried out to investigate the occurrence of anthelmintic resistance in horse nematodes to the benzimidazoles and avermectins.

In recent years horse owners have favoured the use of one combination product to control all internal parasites. It has also been difficult to achieve good control of tapeworms except when using one particular combination product, which is the only one in New Zealand that has a registered claim against nematodes, cestodes and *Gasterophilus*. Therefore, two studies were conducted to investigate a combination of anthelmintics containing abamectin (0.2mg/kg), oxibendazole (10mg/kg) and bithionol (7mg/kg) for treatment of nematodes, cestodes and *Gasterophilus*.

This investigation was conducted through the Graduate in Industry Fellowships (GRIF) programme in conjunction with Virbac Laboratories Ltd. (NZ). It was done with a view to registering this combination anthelmintic with the New Zealand Animal Remedies Board to use in New Zealand. Although the component anthelmintics in this combination have been used in horses before they have not been used in this combination, especially (bithionol) which has not been used in New Zealand. These studies were necessary to achieve registration of this combination anthelmintic for Virbac Laboratories Ltd. (NZ).

This is only a limited knowledge of which nematode parasites of horses are present in New Zealand. These two efficacies studies also provided an opportunity to investigate which species of nematodes in the subfamily Cyathostominae are present in New Zealand.

1.2 Literature review

1.2.1 Anthelmintics

Anthelmintics are drugs which are used for prophylactic or therapeutic purposes against parasitic infestation in man or animals. Anthelmintics can be classified into several groups on the basis of their mode of action, range of activity against different parasites or their chemical structure. They can also be classified on the basis of their range of activity into either broad-spectrum or narrow-spectrum. According to their mode of action, the broad spectrum anthelmintics are divided into three classes, (i) the benzimidazoles (ii) the imidothiazoles and (iii) the avermectin groups.

Definition of anthelmintic resistance

Prichard *et al*., (1980) defined anthelmintic resistance as "present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and it is a heritable character". Side-resistance is defined as occurring where resistance of a nematode to one drug of a chemical class also renders it resistant to others in the same class. It is true for the benzimidazoles, and the avermectins. Cross resistance is defined as occurring when resistance develops between unrelated chemicals. An example is resistance between the imidothiazoles and the organophosphates (Sangster,1996). Multiple-resistance occurs when the same nematodes are resistant to two or more anthelmintic classes with different modes of action.

The three classes of anthelmintics used in horses are described below including their mode of action, efficacy and mechanism of resistance.

1.2.1.1 Benzimidazole

1.2.1.1.1 Mode of action

The mode of action of benzimidazole (BZ) anthelmintics is to bind to nematode β-tubulin to prevent microtubule formation (Prichard, 1986; Martin *et al*., 1997). It is selectively more highly bound to nematode β-tubulin than the host microtubule β-tubulin (Lacey, 1988).

Microtubules comprise a series of protofilaments made up of cytoskeletal proteins (tubulin), and are in active equilibrium where they are gaining and losing soluble tubulin units at opposite terminals of the developing microtubule. This involves the

polymerization of tubulin at one end, known as the positive pole and the depolymerization at the other end, known as the negative pole (Lacey, 1988). The formation of microtubules results from the combination of two sequence length of 450 and 445 amino acids proteins known as α-tubulin and β-tubulin respectively. Microtubules that form are made up of thirteen tubulin molecule rings (6 α-tubulin plus 7 β-tubulin or 7 α-tubulin plus 6 β-tubulin rings). Microtubule formation can be prevented or inhibited by substances that bind to the positive pole of polymerization. Growth of microtubules depends on the rate of addition (polymerization) or loss of tubulin units.

Microtubules are involved in the formation of mitotic spindles during cell division, maintenance of cell shape, cell motility, cellular secretion, nutrient absorption and intracellular transport (Lacey, 1988). Interference with them leads to a disruption of these functions.

1.2.1.1.2 Efficacy of Benzimidazoles

The first BZ drug, thiabendazole, at the standard recommended dose rate (44mg/kg), was highly effective against intestinal stages of many nematodes in horses, however it was not effective at this dose rate against *Parascaris equorum* and larval stages of cyathostomes. To improve its efficacy against these it was necessary to increase the dose rate and/or give repeat doses. For example, for treatment of *Parascaris equorum* , it has been recommended that 88mg per kg should be given (Drudge *et al.*, 1981). However, thiabendazole is no longer marketed as an equine anthelmintic and it has been replaced by later generations of BZ anthelmintics and pro-benzimidazoles which have improved efficacy against a range of parasites. These are remarkably safe in most horses with a safety index ranging from 10 to 100 (Herd, 1992). At recommended dose rates, they are all effective against *P. equorum, O. equi*, adult cyathostomes and adult *Strongylus* spp.

Oxibendazole was the product of the research stimulated by the introduction of thiabendazole (Theodorides *et al.* 1973). A number of authors have demonstrated that oxibendazole is highly effective (91-100%) against adult luminal *Strongylus* spp. and (94-100%) effective against adult luminal cyathostomes in horses (Tables 1.1 and 1.2).

Table 1.1 Reports of the efficacy of oxibendazole at 10mg/kg against susceptible nematodes in horses using faecal egg counts and larval cultures:

***Strongylus* spp.**	**Cyathostomes**	***Oxyuris equi***	***Parascaris equorum***	**References**
100%	100%	100%	100%	Lock *et al.*, 1979
94 -100%	94 -100%	ND	94 -100%	Drudge *et al.*, 1979
97-100%	97-100%	ND	ND	Drudge *et al.*, 1980
100%	97%	ND	ND	Drudge *et al.*, 1981
99.9%	99.9%	ND	ND	DiPietro *et al.*, 1986
ND	100%	ND	ND	Manners, 1989
100%	100%	ND	ND	Herd and Gabel, 1990
98.6%	98.6%	ND	ND	Burr *et al.*, 1994
ND	98.3%	ND	ND	Kivipelto and Asquith, 1997

ND = Not done

Table 1.2 Efficacy of oxibendazole at 10mg/kg against different parasites in horses in controlled slaughter studies by different authors

***Strongylus* spp.**		**Cyathostomes**				***Tri.* spp.**	***Hab.* spp.**	***T.axei***	***Gast.* spp.**	***P. equorum***	*O. equi*	References
Luminal	Migrating	Adult	Luminal larvae	Mucosal L3	Mucosal L4	All stages	All stages	All stages	2nd , 3rd & 4th instar	All stages	All stages	
96%	NE	99%	66-70%	ND	LE	98%	ND	ND	NE	HE	99%	Kates *et al.*, 1975
96.6%	ND	100%	99%	ND	ND	100%	NE	ND	NE	ND	100%	Nawalinski and Theodirides, 1976
100%	ND	100%	62-90%	ND	ND	100%	100%	ND	NE	ND	100%	Nawalinski and Theodirides, 1977
100%	NE	99%	ND	ND	ND	ND	ND	NE	NE	100%	100%	Lyons *et al.*, 1981
94.2%	ND	94.2-100%	91.7%	ND	ND	100%	97.6%	100%	ND	20%	99.9%	Theodorides *et al.*, 1982
100	ND	99%	71%	ND	ND	ND	ND	ND	ND	ND	ND	Drudge *et al.*, 1984
91-100%	ND	96.9%	ND	ND	ND	100%	ND	ND	ND	100%	100%	Stromberg *et al.*, 1985
100%	ND	99%	89%	ND	ND	100%	ND	ND	ND	ND	ND	Tolliver *et al.*, 1993

ND = not done; HE = highly effective; NE = not effective; LE = Less effective

Tri. Spp. = *Triodontophorus* spp.; *Habr.* Spp. = *Habronema* spp.; *T. axei* = *Trichostrongylus axei*; *Gastero* spp. = *Gasterophilus* spp.;

P. equorum = *Parascaris equorum*

However, oxibendazole is reported to be less effective (62-90%) against luminal larval stages of cyathostomes and it has no effect against migrating larvae of *Strongylus* spp. and mucosal stages of cyathostomes (Kates *et al.*, 1975; Lyons *et al.*, 1981).

1.2.1.1.3 Mechanism of resistance

The mechanism of benzimidazole resistance has been investigated in the past few years and it is now known that the resistance is based on alterations in benzimidazole-tubulin interaction. Sangster *et al.*, (1985) and Lacey, (1988) have observed that therapeutic doses of a benzimidazole anthelmintic cause microtubules to disappear from intestinal cells of benzimidazole-susceptible, but not from benzimidazole-resistant nematodes.

Although there has been no specific research on horse parasites to determine the mechanism of BZ-resistance, there are extensive studies with BZ-resistant trichostrongyles of small ruminants.

Lacey and Prichard (1986) found that binding of benzimidazoles to tubulin extracted from benzimidazole-resistant strains of *H. contortus* was reduced compared with binding to tubulin from susceptible strains. The binding of BZ's to the β-tubulin protein fraction of *H. contortus* indicated that tubulin from resistant parasites had reduced binding affinity for BZ's compared with that from susceptible parasites. Lubega and Prichard (1990) showed that binding of various BZ compounds to nematode tubulin could be resolved to high-affinity and low-affinity components and they associated resistance in *H. contortus* with a loss of high affinity BZ receptors on tubulin. Roos *et al.*, (1990) extended this concept by using restriction mapping with cloned α-tubulin and β-tubulin genes from *H. contortus* as probes and they found no differences in α-tubulin from BZ-susceptible and resistant populations but found differences in β-tubulin.

Roos *et al.*, (1995) have shown that two types of β-tubulin genes are present in *H. contortus*: isotype 1 and isotype 2. Each isotype has a number of alleles. There are up to 6 alleles for isotype 1 and up to 12 alleles for isotype 2. In both isotype 1 and isotype 2, BZ resistance was associated with a loss of allelic diversity. Roos (1997) showed that the resistance of *H. contortus* appeared due to a progressive loss of alleles for isotype 1 and a total loss of alleles for isotype 2. In conclusion, BZ resistance develops due to a change in β-tubulin in two-ways: (1) at lower

resistance levels there is selection of a specific isotype 1 gene, (2) at higher levels of resistance there is elimination of worms with isotype 2 genes.

Molecular study has shown that isotope 1 β-tubulin gene is most involved in BZ resistance. In *H. contortus* this was associated with the amino acid phenylalanine (Phe) being in place of tyrosine (Tyr) at position 200 on a derived amino acid sequence of the gene (*Geary et al.*, 1992; Roos *et al.*, 1995). A similar change of Phe for Tyr at position 200 in the amino acid sequence of the corresponding gene has been shown to confer resistance to BZ anthelmintics in the free living nematode *Caenorhabditis elegans* and in the fungus *Aspergillus nidulans* (Kwa *et al.*, 1993; 1994). This has also been found in BZ-resistant *O. circumcincta* (Elard *et al.*, 1996).

1.2.1.2 Avermectin

The avermectins are a group of macrocyclic lactones derived from mycelia of related fungi. Ivermectin was the first commercial product and was introduced in the early 1980s. Available anthelmintics in this groups include ivermectin, abamectin, doramectin and moxidectin. Abamectin or avermectin B1 is the naturally occurring precursor of ivermectin. Abamectin differs from ivermectin in only having a double-bond at the C22-C23 position (Campbell *et al.*, 1983), whereas in ivermectin that linkage has been reduced by selective hydrogenation.

1.2.1.2.1 Mode of action

The mode of action of avermectins in nematode parasites has not been fully determined but the group of drugs appears to share a common mode of action. Only one of these drugs, ivermectin, has been studied in any detail. Ivermectin potentiates the opening of glutamate-gated chloride ion channels in cell membranes which increases the chloride ion permeability of nerve and muscle membranes of nematodes leading to a hyperpolarization which cannot subsequently transmit an action potential (Shoop, 1993; McKellar and Benchaoui, 1996; Martin, 1996). Gill *et al.*, (1998) reported that ivermectin appears to have two sites of action in parasites: one in the pharynx and another in the somatic muscle.

Recently, Laughton *et al.*, (1997) suggested that glutamate-gated chloride ion channels, which are present in the pharynx of *C. elegans*, may be the site of ivermectin action in nematodes. A similar type of action described by Geary *et al.*, (1993) that pharynx function may be the actual site of anthelmintic action as

opposed to somatic musculature function, since ivermectin inhibits pharyngeal pumping more potently than motility in *H. contortus*. Bottjer and Bone (1985) also described a similar pattern of ivermectin sensitivity in the pharynx versus somatic muscle in *T. colubriformis*. Although, ivermectin paralyses the somatic musculature of larval (Gill *et al*., 1991) and adult (Geary *et al*., 1993) parasitic nematodes, this site is less sensitive than the effect on feeding.

Therefore, ivermectin potentiates the opening of glutamate-gated chloride ion channels, increases the chloride ion permeability of nerve and muscle membranes and especially inhibits pharyngeal pumping in nematodes.

There is no specific information describing the mode of action of ivermectin on cyathostomes or *Strongylus* spp, but it is assumed the drugs act in similar way in all nematodes.

1.2.1.2.2 Efficacy of avermectins

Ivermectin has a broad spectrum of activity against internal and external parasites in many animals (Leaning, 1983). A number of studies (Tables 1.3 and 1.4) have demonstrated that ivermectin is highly effective against luminal *Strongylus* spp., luminal cyathostomes, *T. axei, Parascaris equorum*, O*xyuris equi* and larval *Gasterophilus* spp.

Studies with moxidectin, another avermectin, have shown it is highly effective against luminal stages of *Strongylus* spp., cyathostomes, *T. axei, Parascaris equorum* and O*xyuris equi* but is less effective against *Gasterophilus* spp. larvae. The efficacy of moxidectin in horses from various studies is summarised in Table 1.5. Moxidectin demonstrates a greater efficacy than ivermectin against encysted cyathostome larvae but is less effective against *Gasterophilus* spp. larvae (Tables 1.4 and 1.5).

It should be noted that in horses ivermectin has a recommended dose rate of 0.2mg/kg and moxidectin 0.4mg/kg, which is in contrast to ruminants, where the recommended dose rates are the same for both active ingredients.

Table 1.3 Reports of the efficacy of ivermectin at 0.2mg/kg against nematodes in horses using faecal egg counts and larval cultures:

***Strongylus* spp.**	**Cyathostomes**	***Oxyuris equi***	***Parascaris equorum***	**References**
99.7%	99.7%	ND	100%	DiPietro *et al.*, 1982
100%	100%	ND	ND	DiPietro *et al.*, 1986
>97%	>97%	ND	ND	Bauer *et al.*, 1986
100%	100%	ND	ND	Ryan *et al.*, 1987
100%	100%	ND	ND	Herd and Gabel, 1990
100%	100%	ND	ND	Mogg *et al.*, 1990
100%	100%	ND	100%	Bell and Holste, 1990
100%	100%	ND	ND	Piche *et al.*, 1991
100%	100%	ND	ND	Boersema *et al.*, 1991
100%	100%	ND	ND	Mair and Cripps, 1991
100%	100%	ND	ND	Pereira *et al.*, 1991
100%	100%	ND	ND	Taylor and Kenny, 1995
100%	100%	ND	ND	Cobra *et al.*, 1995
100%	100%	ND	ND	Ihler, 1995
100%	100%	ND	ND	Woods *et al.*, 1998
100%	100%	ND	ND	Boersema *et al.*, 1998
100%	100%	100%	100%	Craven *et al.*, 1998

ND = Not done

Table: 1.4 Efficacy of ivermectin at 0.2 mg/kg against different parasites in horses in controlled slaughter studies by different authors

***Strongylus* spp.**		**Cyathostomes**				***Tri.* spp.**	***Hab.* spp.**	***T. axei***	***Gast.* spp.**	***P. equorum***	***O. equi***	**References**
Luminal	Migrating	Adult	Luminal larvae	Mucosal L3	Mucosal L4	All stages	All stages	All stages	2nd, 3rd & 4th instar	All stages	All stages	
>97%	ND	97%	97%	ND	ND	97%	HE	97%	97%	HE	97%	Klei and Torbert 1980
>99%	ND	99%	96%	ND	ND	>99%	HE	HE	96%	96%	>95%	Egerton *et al.*, 1981
ND	98.9%	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Slocombe *et al.*, 1982
>98%	ND	>98%	HE	ND	ND	HE	>98%	>98%	>98%	HE	100%	Torbert *et al.*, 1982
ND	HE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Slocombe *et al.*, 1984
ND	99%	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Klei *et al.*, 1984
ND	ND	100	100	ND	ND	ND	ND	ND	ND	ND	ND	Burger and Bauer, 1987
ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	HE	ND	French *et al.*, 1989
ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	98.2%	ND	Austin *et al.*, 1991
ND	ND	HE	HE	NE	NE	HE	ND	ND	ND	ND	ND	Eysker *et al.*, 1992
HE	ND	HE	HE	ND	ND	HE	ND	ND	ND	ND	ND	Lyons *et al.*, 1992
100%	100%	99.9%	87%	LE	35%	100%	ND	ND	99.7%	100%	100%	Klei *et al.*, 1993
100%	ND	100%	98.8%	10%	0%	100%	100%	ND	95.4%	ND	100%	Xiao *et al.*, 1994
HE	ND	HE	HE	ND	ND	HE	HE	HE	ND	HE	HE	French *et al.*, 1994
ND	ND	>99%	>99%	77%	77%	ND	ND	ND	ND	ND	ND	Love *et al.*, 1995
100%	100%	99.8%	99.8%	0	0	100%	100%	100%	99.7%	100%	100%	Monahan *et al.*, 1996
100%	67.8%	99.7%	99.7%	ND	ND	100%	99.6%	100%	ND	100%	100%	Costa *et al.*, 1998

ND = not done; HE = highly effective; NE = not effective; LE = Less effective

Tri. Spp. = *Triodontophorus* spp.; *Habr.* Spp. = *Habronema* spp.; *T. axei* = *Trichostrongylus axei*; *Gastero* spp. = *Gasterophilus* spp.; *P. equorum* = *Parascaris equorum*; *O. equi* = *Oxyrus equi*.

Table 1.5 Efficacy of moxidectin at 0.4mg/kg against different parasites in horses in controlled slaughter studies by different authors

***Strongylus* spp.**		**Cyathostomes**				***Tri.* spp.**	***Habr.* Spp.**	***T. axei***	***Gastro.* spp.**	***P. equorum***	***Oxyuris equi***	**References**
Luminal	Migrating	Adult	Luminal larvae	Mucosal L3	Mucosal L4		All stages	All stages	2nd, 3rd & 4th instar	All stages	All stages	
100%	ND	100%	99.9%	ND	90.7%	ND	100%	ND	>96.4%	NE	100%	Bello and Laningham, 1994
100%	ND	100%	99.9%	NE	62.6%	100 %	100%	ND	20.4%	ND	100%	Xiao *et al.*, 1994
ND	100	ND	ND	ND	ND	ND	ND	ND	ND	100%	ND	Monahan *et al.*, 1995
100%	100%	99.9%	100%	36.96	50%	100 %	100%	100%	75.4%	100%	100%	Monahan *et al.*, 1996
100%	ND	97.8%	93.3%	24.2%	76.5%	99.9 %	100%	100%	65%	100%	100%	Eysker, *et al.* 1997
100%	62%-*S.edentatus* 92% -*S. vulgaris*	99.9%	ND	ND	ND	99.9 %	ND	100%	92%	100%	98%	Dorchies *et al.*, 1998
100%	ND	100%	100%	ND	ND	100 %	ND	ND	ND	ND	ND	Boersema *et al.*, 1998
98.4- to 100%	ND	100%	ND	ND	ND	100 %	ND	ND	99.9%	ND	ND	Coles *et al.*, 1998
100%	84.9%	99.7%	99.7%	ND	ND	100 %	99.5%	100%	ND	100%	99.7%	Costa *et al.*, 1998
100%	ND	97.2%	97.2%	ND	ND	ND	100%	ND	91%	100%	99.9%	Rolfe *et al.*, 1998

ND = not done; HE = highly effective; NE = not effective; LE = Less effective
Tri. Spp. = *Triodontophorus* spp.; *Habr.* Spp. = *Habronema* spp.; *T. axei* = *Trichostrongylus axei*; *Gastero* spp. = *Gasterophilus* spp.; *P. equorum* = *Parascaris equorum*

There are no published studies on the efficacy of abamectin in horses except for one brief report by Martin *et al.*, (1999) which claimed more than 99% efficacy against arterial and luminal stages of *Strongylus* spp., cyathostomes, *Oxyuris equi, Parascaris equorum*, *Gasterophilus* spp. larvae and *Anoplocephala* spp.

1.2.1.2.3 Mechanism of resistance

The mechanism of ivermectin resistance is not yet determined. However, a few studies have reported some findings about the potential mechanism of ivermectin resistance.

Gill *et al.*, (1995) reported that IVM resistance may be related to the putative sites of action in pharyngeal muscle. Compared with the susceptible worms, feeding in IVM-resistant *H. contortus* is less sensitive to IVM, assuming that the pharynx is an important target of IVM (Sangster, 1996).

Another approach to investigate IVM resistance in parasitic nematodes has been to use studies with the free living nematode *Caenorhabditis elegans*, which is susceptible to ivermectin and in which it has been shown that mutations in more than 35 independent genes are responsible for various levels of resistance (Schaeffer and Haines, 1989).

Another experiment showed that several ivermectin receptor isotypes have been found in *C. elegans*, at least two of which are located on the pharynx (Martin and Pennington, 1989). If a change in ivermectin receptors occurs in resistant worms, the change must be common to the morphologically separate receptor types or alternatively, changes must occur in drug transport mechanisms for ivermectin at somatic and pharyngeal sites.

Another report suggested that ivermectin resistance of *H. contortus* appears to be inherited on a single gene which is fully dominant (Dobson *et al.*, 1996).

1.2.1.3 Bithionol

Bithionol is a compound with the formula 2,2'-dihydroxy-3,3', 5,5' terachloro-diphenyl sulphide. It is an anthelmintic agent effective against cestodes in animals. A bithionol preparation (Bitin ®, Tanabe Pharmaceutical Co., Ltd.) is marketed for use in horses in Japan. It was launched in August 1983. Initially bithionol was supplied in a powder form. It has been reported with this formulation, it is difficult to measure the optimal dose, difficult to administer, easily scattered in the wind and

occasionally causes transient diarrhoea. Therefore, bithionol was not widely prescribed by veterinarians in Japan (Takahashi, K. unpublished reports).

In one experiment (Takahashi, K. unpublished reports) bithionol was formulated as a paste preparation in a syringe with scale marks to facilitate accurate measurement of the optimal dose but this does not appear to have been marketed.

1.2.1.3.1 Mode of action

Very little has been published about its mode of action. Logachev and Koshkina (1979) showed that bithionol causes changes in the interstitial processes in the parenchyma of cestodes. After administration of bithionol, the first change in parasites is an absence of eggs in the uterus, then degeneration in the vitellarium tissue, the ovary and testes and finally a complete degeneration of the uterus with subsequent death of the cestode (Anonymous, 1964).

1.2.1.3.2 Efficacy

There are not many reports published on the efficacy of bithionol in any animal and only a few in horses. These are summarised in Table 1.6. There is one unpublished report of 8 experiments where bithionol was administered at 5mg/kg, 7mg/kg and 10mg/kg with feed Japan (Takahashi, K. unpublished reports). From these one experiment showed that the *Anoplocephala* fecal egg count results came to be negative by 42 days after administration in the 7mg/kg and 10mg/kg of bithionol. Another experiment showed that a paste formulation containing 5mg/kg or 10mg/kg bithionol was 86.7% and 100% efficacy against tapeworms in horses respectively and in powder form 5mg/kg and 10mg/kg were 100% effective against tapeworm in horses (Takahashi, K. unpublished reports). Although faecal examination is not a definitive method for diagnosis of tapeworm infection, it is useful procedure where no other diagnostic technology is currently available.

In another experiment (Fukui,1960) bithionol was administered at the single dose of 50mg/kg, 30mg/kg, 20mg/kg, 15mg/kg and 10mg/kg of body weight respectively to different groups of horses. The dose of 10mg/kg was elucidated to be satisfactory for removing all worm burdens without any serious side reactions.

Table 1.6 Efficacy of bithionol against cestodes in different animals by different authors:

Host	Parasite	Dose rate mg/kg	Efficacy	References from abstract
Horse	*Anoplocephala* spp.	5 paste form	86.7%	Takahashi, (not published)
		10 paste form	100%	
		5 powder form	100%	
		10 powder form	100%	
Horse	*Anoplocephala* spp.	10	100%	Fukui, 1960
Horse	*Anoplocephala* spp.	150	Highly effective	Akbaev, 1979
Sheep	*Moniezia spp.*	85	96%	Didenko, 1971
Sheep	*Fasciola hepatica*	100	100%	Ayupov *et al.*, 1972
Sheep	Cysticercosis	N.M.	effective	Daniyarov and Bekirov, 1973
Sheep	*Moniezia* and *Avitellina* spp.	125-150	Fully effective	Vibe *et al.*, 1974
Sheep	*Dicrocoelium* and *Fasciola hepatica*	200	effective	Ayupov and Khaziev. 1974
Sheep	*Anoplocephala* spp.	N.M.	effective	Smychkov, 1975
Sheep	*Moniezia* spp.	150	100%	Kadenatsii *et al.*, 1975
Sheep	*Moniezia* spp.	150-200	effective	Akbaev, 1979
Sheep	Cestodes (Species not stated)	N.M	effective	Medeubaev and Orynbaev, 1979
Sheep	*Moniezia* spp.	120	90%	Mustafin and Demidov, 1981
Cattle	Tape worm	20 paste form	80%	Takahashi, (not published)
		30 paste form	100%	
Cattle	*Paramphistomum and Fasciola* spp.	90	81-100	Mereminskii and Gluzman, 1972
Cattle	Cestodes (Species not stated)	70	99.7	Ruziev, 1972
Cattle	*Paramphistomum* spp.	70	99.8	Hovorka *et al.*, 1973
Dog	Cestodes (Species not stated)	200	effective	Zhuravets, 1978
Dog	*Echinococcus* and *Taenia*	100	Fully effective	Ramazanov, 1979

N.M = Not mentioned

1.2.1.4 Combination drugs

The administration of two or more actives in a combination may be more effective and useful than a single active used alone because of: i) the ability to treat mixed parasitic infections, ii) to achieve additive anthelmintic activity against parasites especially if resistance is developing to one component but not another in the combination, iii) the prevention or slowing of the emergence of drug resistance.

Whilst there are, and have been, many combination products marketed for use in horses, there would appear to be no published reports on the efficacy of a combination product containing oxibendazole and abamectin. Oxibendazole has been combined with trichlorfon to obtain efficacy against nematodes and *Gasterophilus* spp. (Erdmann, 1990) and with dichlorvos and morantel tartrate to remove both *Gasterophilus* spp. and presumably Benzimidazole resistant nematode (Rolfe and Dawson, 1994). The only report of abamectin used in combination with praziquantel to remove *Anoplocephala* spp. as well as nematodes (Martin *et al.*, 1999).

1.2.2 Anthelmintic resistance in horses

Anthelmintic resistance involving benzimidazole, levamisole/morantel and the avermectins has emerged as a problem in nematode parasites of sheep and goats. Benzimidazole resistance has also been reported in nematode parasites of cattle especially in New Zealand (Jackson *et al.*, 1987). The prevalence of anthelmintic resistance in the world is still increasing and more and more cases of multiple resistance to the three classes of broad-spectrum anthelmintics have occurred. Resistance to ivermectin was first reported in *H. contortus* from sheep in South Africa (Carmichael *et al.*, 1987; Van Wyk and Malan, 1988) and subsequently has been reported from Brazil (Echevarria and Trindale, 1989), USA (Craig and Miller, 1990), New Zealand (Badger and McKenna, 1990; McKenna *et al.*, 1990), and Australia (LeJambre, 1993).

In the horse, anthelmintic resistance in cyathostomes has been reported to phenothiazine, benzimidazoles and pyrantel, but so far there are no reports of ivermectin resistance. There are no reports of resistance in any other groups of horse parasites.

Poynter and Hughes (1958) were the first to describe anthelmintic resistance to phenothiazine in cyathostomes of horses in the United Kingdom. Further, in 1960, Gibson (1960) also observed phenothiazine resistance in cyathostomes in the United Kingdom. Similar results were later reported by Drudge and Elam (1961) in the USA.

The first report of BZ resistance in cyathostomes in horses was from Kentucky, USA by Drudge and Lyons, (1965). Subsequently resistance to benzimidazole anthelmintics in cyathostomes has been reported from many countries around the world including New Zealand (Hope and Kemp, 1980), Australia (Barger and Lisle 1979; Kelly *et al.*, 1981), England (Round *et al.*, 1974; Britt and Clarkson, 1988, Fisher *et al.,* 1992), Scotland (Ryan *et al.*, 1987; Love *et al.*, 1989), Canada (Slocombe and Cote, 1977; Piche *et al.*, 1989), USA (Herd *et al.*, 1981; Baker *et al.*, 1984), Germany (Bauer *et al.*, 1986; Ullrich *et al.*, 1988), Ireland (Parr *et al.*, 1993), Belgium (Domy *et al.*, 1988), Norway (Helle, 1986; Ihler, 1995), Sweden (Nilsson *et al.*, 1989), Netherlands (Boersema *et al.*, 1991), Denmark (Bjorn *et al.*, 1991) and South Africa (Van Wyk and Van Wijk, 1992). Since the initial report, the prevalence of BZ resistance has increased rapidly in England and in the early 1990's more than 80% of horse farms were found to harbour benzimidazole-resistant parasites (Fisher

et al.,1992). Similarly it has recently been reported that 72% of the horse farms in Denmark now have benzimidazole resistance (Craven *et al.*, 1998).

Pyrantel pamoate-resistance in cyathostomes has been reported from USA (Drudge *et al.*, 1988) and recently was found to be moderately common in north central Florida (Woods *et al.*, 1998). It has also been reported in horses from Norway (Ihler, 1995) and to pyrantel embonate in horses from Denmark (Craven *et. al.*, 1998).

In Denmark, Craven *et. al.* (1998) also observed that ivermectin resistance was possibly identified on one of 16 horse farms. They used the faecal egg count reduction test and after treatment with ivermectin, one horse had eggs present 14 days after treatment but by 19 days it was negative and he concluded it was not a case of resistance to ivermectin.

1.2.3 *In vitro* test for detection of Anthelmintic resistance

Anthelmintic resistance has been detected by *in-vivo* and *in-vitro* techniques in most parts of the world. Generally, *in-vivo* tests have been used. These include the faecal egg count reduction test and the controlled slaughter test which are both suitable for all types of anthelmintics, but can be costly in terms of labour usage and animal requirements.

Different types of *in vitro* tests have been developed and are briefly reviewed below.

1.2.3.1 Egg hatch assays for Benzimidazoles

This test is used for the diagnosis of benzimidazole resistance and relies on the ovicidal activity of benzimidazoles. The principle of this test is to incubate undeveloped eggs in serial dilutions of thiabendazole for 24 hours at 26^{0}C and record the percentage of eggs that have failed to hatch at each concentration (Le Jambre, 1976; Hall *et al.*, 1978; Whitlock *et al.*, 1980). The percentage of eggs that hatch is corrected for natural mortality from control wells and is then plotted against drug concentration. Transformation of data either by log-probit (Healy, 1988) or arcsin (Cawthorne and Whitehead, 1983) is required to obtain a linear regression from which an ED_{50} (the concentration of anthelmintic required to kill 50% of the eggs) can be calculated after back transformation. By maintaining reference strains of known susceptible parasites, the responses of test isolates can be compared and a resistance ratio calculated. The incubation of first stage larvae to third stage larvae allows the genus of nematode involved to be determined.

Whitlock *et al.* (1980) first used this assay to detect thiabendazole resistance in cyathostomine infections of horses.

A number of variations of the egg-hatch/embryonation assay have been developed for detection of resistance to benzimidazole anthelmintics (Le Jambre, 1976 ; Coles and Simpkin, 1977 ; Whitlock *et al.*, 1980). Others reviewed these variations in the methodology of the egg hatch assay and summarised the different approaches as either to prevent embryonation or to prevent hatching.

Coles and Simpkin (1977) counted only the number of hatched larvae, which is a faster procedure. However, when only hatched larvae are counted variation can occur in dose-response lines and calculated ED_{50} values, as compared to counting both hatched larvae and eggs containing larvae.

Lacey and Prichard (1986) reported that other benzimidazoles such as fenbendazole, are unsuitable for use in this test because of their poor solubility and low ovicidal effects. However, the use of thiabendazole as the test drug in this assay to detect anthelmintic resistance has been justified because side-resistance occurs within this group of anthelmintics (Martin *et al.*, 1985).

A major disadvantage of *in vitro* egg-hatch assays in routine diagnosis, has been the requirement for undeveloped eggs (Coles and Simpkin, 1977). Whitlock *et al.* (1980) recommended recovery of nematode eggs by sugar floatation and their incubation with a range of concentrations of anthelmintic in McCartney flasks before transportation back to laboratory. Another method to prevent the development of nematode eggs in transit to the laboratory that was found to be satisfactory is to store faecal samples at 4^0C for up to 3 days (Smith-Buijs and Borgsteede, 1986). Comparable results have been obtained by storing the faecal samples in polythene bags with the air excluded (Presidente, 1985). Similar results were described by Hunt and Taylor (1989) who stored faeces under anaerobic conditions by immersing them in water for up to 7 days from the date of collection.

The egg hatch assay (BZ) is probably better suited to research than for primary screening, because it is technically demanding, presents difficulties with interpretation when mixed infections are involved and requires either freshly collected or well stored eggs (Donald, 1985). Nevertheless, it has been used on many occasions to characterize resistance of nematode parasites of sheep, goats and horses in the field (Le Jambre, 1976; Hall *et al.*, 1978; Presidente, 1985; Scott *et al.*, 1989). One advantage is that this test is fast, with results being obtained within 1-3 days.

With regard to horse nematodes, the egg hatch assay has been claimed to be a practical test for confirming benzimidazole resistance. Whitlock *et al.*, (1980) incubated eggs at 27-30^0C for 20-24 hours in thiabendazole solution ranging from 0.05 to 0.5 p.p.m. They found that eggs of susceptible strains of horse strongylid nematodes rarely hatched at 0.1 p.p.m. but resistant strain eggs hatch at 0.1 p.p.m. or above which is similar to ruminant strongylid nematodes.

1.2.3.2 Egg hatch assay for Levamisole

Dobson *et al.*, (1986) first reported this technique. This test quantifies the difference between resistant and susceptible strains in the rate of recovery from paralysis of unhatched larvae in different concentrations of levamisole. Fresh undeveloped eggs

are recovered, concentrated, then incubated at 26^{0}C in microtitre plates until one hour before hatching when anthelmintic is added. Following the addition of the drug, another 6 hrs incubation is required before the assay is terminated by snap freezing for 5 minutes prior to adding a drop of chilled formaldehyde to each well. The number of hatched larvae are then counted.

Waller (1986) used morantel instead of levamisole in this assay to determine specific resistance to this drug but toxic effects on eggs unrelated to resistance occur at concentrations exceeding 64 µg/ml of morantel.

For sheep, the levamisole egg hatch assay is accurate and sensitive but the timing, both of the addition of the drug and of the termination of the experiment, is critical. It is more labour intensive than the benzimidazole egg hatch assay but the equipment is the same. However, it has been noted that it is difficult to compare results between different laboratories and it is not recommended for field diagnosis of resistance (Dobson *et al.*, 1986; Johansen, 1989).

1.2.3.3 Larval Paralysis and Motility test

Martin and Le Jambre (1979) first developed this method to detect levamisole and morantel resistance. The principle of these assays is to measure the ability of anthelmintics to paralyse infective third stage larvae. The third stage larvae are recovered from faecal cultures and incubated in serial dilutions of anthelmintic for 24 hours. The larvae are then observed under a microscope and classified as normal (moving) or paralyzed (not moving) over a 5 second observation period. The percentage of paralyzed larvae is then calculated and the LD_{50} values calculated, using log dose-probit lines (Le Jambre, 1976). The procedure is generally rapid and simple to carry out.

This test has the advantage of using third stage larvae, which are readily available. However, there are a number of disadvantages with this technique. Firstly, Barton (1983) found that paralysis at high concentration of levamisole was reversible. Secondly, there is some subjectivity in judging whether or not a larva is paralyzed. Thirdly, the assay cannot be preserved for counting at a later time and the test is time-dependent.

A modification of the larval paralysis assay, suitable for detecting thiabendazole resistance, was described by Sutherland and Lee (1990). Infective larvae were incubated in the acetyl cholinesterase inhibitor, easerin. They found that

thiabendazole-resistant larvae were paralyzed more slowly than susceptible counterparts. They indicated that these differences are probably related to the presence of higher levels of acetyl cholinesterase in the thiabendazole-resistant strains than susceptible strains.

A micromotility meter which is an instrument for measuring the motility of larvae and adult nematodes after incubation with anthelmintics, has been developed by Bennett and Pax (1986). The instrument uses microprocessor technology to measure light reflection at the meniscal interface. Movement of larvae in the solution is claimed to alter the angle of light refraction entering the photodiode. The changes in light refraction are then measured by a computer to give a motility index.

Folz *et al.*, (1987; 1988) reported a different motility meter which they used to test the motility of *H. contortus* infective larvae to various anthelmintics (albendazole, cambendazole, fenbendazole, ivermectin, and levamisole) and observed differences in motility between benzimidazole-resistant and susceptible strains but not between levamisole-resistant and susceptible ones (Coles *et al.*, 1989). Folz *et al.*, (1988) also used the motility assay to determine the resistance of ivermectin, dichlorvos and pyrantel pamoate in horses of *S. edentatus* and he found this test is effective and accurate. Use of instruments to detect motility has some limitations in that if only a few larvae are present they produce insufficient movement to register any significant effect whilst too many motile larvae move the dead ones thus leading to an exaggerated or misleading result. This technique is not suitable for testing more than one sample at a time.

Gill *et al.*, (1991) have described the inhibition of larval motility by ivermectin. The overall principal of this assay is to incubate L3 larvae in the dark on an agar matrix containing serial dilutions of ivermectin for 24 hours at 25^{0}C before exposing them to light sufficient to activate more than 90% of larvae in the control wells (1-2) minutes. The numbers of non-motile larvae as a proportion of the total larvae present at each concentration are calculated and a log concentration-logit model is fitted to estimate LD_{50} values. Generally third stage larvae of *H. contortus* isolates resistant to ivermectin show a decreased sensitivity to the induction of paralysis. The technique is sensitive, precise, quick and cheap to perform. Gill *et al.*, (1991) also suggested that it could be used in the detection of ivermectin resistance in other trichostrongylid nematodes *T. colubriformis* and *O. circumcincta.*

1.2.3.4 Larval development assay

The larval development assay (LDA) is an *in vitro* assay which has been used for the detection of resistance to benzimidazoles, levamisole, benzimidazoles/levamisole combinations and avermectin anthelmintics in gastrointestinal nematode parasites (Lacey *et al.*, 1990 and Gill *et al.*, 1995). The general principle of the larval development assay is to incubate eggs in serial dilutions of anthelmintics through to infective larvae. The number of eggs developing to third stage larvae are counted, the proportion of development estimated and the LD_{50} values are calculated from a dose-response curve. Variations of this assay have been described by Waller and Lacey (1986), Coles *et al.*, (1988), Giordano *et al.*, (1988), Stringfellow (1988), Lacey *et al.*, (1990), Taylor (1990), Hubert and Kerboeuf (1992), Gill *et al.*, (1995), Amarante *et al.*, (1997) and Gopal *et al.*, (1999).

These variations differ in the media used (whether liquid or agar culture), in the volume of the culture media, the culture apparatus and in egg recovery procedures but the basic principle remains the same.

The technique was originally described by Ibarra and Jenkins (1984). In this description the media used for cultivation of parasites consisted of one part distilled water and one part faecal suspension of rat faeces rich in bacteria on which the developing larvae feed. They found the development of larvae was effective for assessing activity of broad spectrum anthelmintics including benzimidazoles but unsuitable for narrow spectrum anthelmintics. In the same year Hubert and Kerboeuf (1984) described the use of a nutritive medium consisting of Earle's balanced salt solution and Yeast extracts. They found the development of larvae was better than using rat faeces. Another variation of the nutrient media was reported by Coles *et al.*, (1988), who used heat-treated lyophilized *Escherichia coli* to which the anthelmintic under test was added. This test successfully differentiated between benzimidazole-resistant and susceptible strains of *H. contortus*.

In a later report, Hubert and Kerboeuf (1992) developed a microlarval development assay for the detection of benzimidazole resistance in *H. contortus* in which eggs were cultured in the presence of Earle's balanced salt solution and Yeast extracts together with lyophilized *E. coli* with serially diluted anthelmintic in tubes and incubated at 23^0C for 7 days. Further, Amarante *et al.*, (1997) followed the Hubert and Kerboeuf, (1992) procedures, to detect ivermectin, benzimidazole and

levamisole resistance in *Ostertagia* spp. This also successfully differentiated between resistant and susceptible strains of *Ostertagia* spp.

Taylor (1990) was the first to report the use of an agar matrix. He incubated nematode eggs in a nutritive medium consisting of Earle's balanced salt solution and yeast extract on top of a layer of agar containing anthelmintic (thiabendazole, levamisole and ivermectin) in 96-well microtitre plates. The plates were incubated at 27^{0}C for 7 days. Lacey *et al.*, (1990) also reported the use of an agar matrix containing the drug but did not report details on the overlaying nutritive media. Ihler and Bjom (1996) followed the methodology of Lacey *et al.*, (1990) with horse nematodes and found resistance to benzimidazoles but not avermectins. In horses, Craven *et al.*, (1999) utilized the LDA to detect anthelmintic resistance using the drugs pyrantel citrate, levamisole, TBZ and mebendazole but didn't find any evidence of resistance using this assay.

Gill *et al.*, (1995) reported in detail on their use of an agar matrix containing the anthelmintic. This is probably the same as that reported earlier by Lacey *et al.*, (1990). They cultured nematode eggs in a nutritive medium layered on an agar matrix containing serial dilutions of various avermectins, thiabendazole and levamisole in the wells of 96-well microtitre plates. The nutritive medium was similar to that described by Hubert and Kerboeuf (1992) but included amphotericine-B (to prevent fungal growth). Development was allowed to proceed for 6 days at 25^{0}C by which time larvae in the control wells had reached the infective stage (L_3). The number of eggs, first-, second- and third- stage larvae at each drug concentration were counted after staining with Lugol's iodine to determine the proportion of ensheathed L_3s. This was corrected against the number in the control wells and a log-dose response curve was fitted to calculate the LD_{50} values. Water soluble drugs were serially diluted in distilled water and insoluble compounds were serially diluted in 2% dimethyl-sulphoxide (DMSO) (Gill *et al.*, 1995).

The LDA technique is generally simple to carry out, several anthelmintics can be tested simultaneously and nematode genera can be differentiated when mixed infections are being tested (Coles *et al.*, 1988; Taylor, 1990). The most important attribute of the LDA as described by Gill *et al.*, (1995) assay is its ability to detect resistance to the three main broad-spectrum anthelmintic classes: benzimidazoles, levamisole/morantel and the avermectins. The technique has proved to show good repeatability, therefore it is very useful for both field screening and research work because eggs are easily obtained in large numbers from faeces. Ihler and Bjom

(1996) used this technique in horses for the detection of anthelmintic resistance and successfully determined the anthelmintic resistance in horses to benzimidazole. It has been developed to the point that a commercial kit called "DrenchRite™" is now available, essentially using the technique described by Gill *et al.*, (1995) although, for commercial reasons, the anthelmintic analogues included and their concentrations are not known. The DrenchRite™ larval development test was developed by Commonwealth Scientific & Industrial Research Organization in Australia. Young *et al.*, (1999) successfully used this commercial assay plate to evaluate the resistance of anthelmintics in horses in Texas, USA. They determined the resistance ratio of a domestic herd compared with a feral herd using benzimidazole, pyrantel pamoate and avermectin, and found resistance to benzimidazole but not to avermectins or pyrantel.

A critical issue with the LDA would appear to be the volume of the nutritive medium. Hubert and Kerboeuf, (1992) optimized the technique by testing several factors in the assay, such as concentration of *E. coli*, Earle's balanced salt solution and yeast extract, volume of media as well as number of eggs per tube and temperature. They also indicated that relative volume of nutritive medium in the cultures appeared to be the main factor influencing the development of eggs. A critical requirement for larval development is oxygen. If the medium is too deep this may limit oxygen uptake and hence larval development.

Other difficulties with the LDA include bacterial overgrowth of the agar in the wells and a poor dose response with levamisole causing problems with interpretation (Johansen, 1989).

1.2.3.5 Adult development assay

Taylor and Hunt (1989) have developed nematode culture techniques to permit the development of infective third stage larvae to adult parasites *in vitro* for several species of nematodes. They used susceptible and resistant strains of *O. circumcincta* and *H. contortus*, and incorporated an anthelmintic into the growth medium at the early fourth stage of development which suppressed further development of the susceptible strains of the parasites whereas the resistant parasites were able to complete their development to late fourth and adult stages. Stringfellow (1988) also described an *in vitro* adult development assay for detecting benzimidazole resistance in *H. contortus* based on these *in vitro* techniques.

1.2.3.6 Tubulin binding assay

This assay is based on the binding of benzimidazoles to tubulin from susceptible and resistant nematodes (Lacey and Prichard, 1986). In this assay, a crude extract of tubulin is made from infective larvae and then incubated with tritiated labeled thiabendazole until equilibrium is reached (Lacey and Snowdon, 1988). The free unbound drug is then removed with charcoal leaving tritium-benzimidazole-tubulin complex to be estimated with a liquid scintillation spectrometer. Bound radio-activity was calculated and standardized to p-mole mg^{-1} for each of the isolates by dividing by the protein concentration assayed. The extent of resistance was expressed as Susceptibility Factor (SF) i.e. ratio of resistant isolate binding to susceptible binding using McMaster susceptible strains as a standard (SF = 1.00). Different susceptible and resistant isolates of *H. contortus*, *T. colubriformis* and *O. circumcincta* were examined and the assay showed lesser SF for resistant isolates and higher SF for susceptible isolates.

1.2.3.7 Colorimetric assay

Sutherland and Lee (1989) used a colorimetric assay which compares the levels of non-specific esterase's and acetyl cholinesterases of benzimidazole-resistant and susceptible trichostrongylid strains. In this assay, greater esterase or acetyl cholinesterase activity occurs in the benzimidazole-resistant strain which is assessed by visual examination or through the use of a densitometer.

This assay showed significantly more non-specific esterase or acetyl cholinesterase activity in infective larvae of benzimidazole-resistant strains than susceptible strains.

1.2.3.8 Genetic assays

Today these are used only to detect benzimidazole resistance where resistant populations have been identified using cloned β-tubulin probes with restriction mapping (Le Jambre, 1990; Roos *et al*, 1990) or by iso-enzyme analysis using isoelectric focussing (Sutherland *et al*, 1988). Isoelectric analysis could be useful in identifying ivermectin-resistant strains of nematodes (Echevarria *et al*, 1992). However, this is not possible if benzimidazole resistance is also present as the difference between ivermectin-susceptible and resistant strains is masked by elevated levels due to the benzimidazole resistance.

1.2.4 Parasites of Horses

Parasites are one of the most common equine health problems in the world. The major types of internal parasites of equines are nematodes of the family Strongylidae, *Trichostrongylus axei*, *Parascaris equorum*, *Oxyuris equi*, *Gasterophilus* spp. and *Anoplocephala* spp.

Different parasites are located in different areas of the gastrointestinal tract. In horses, *Trichostrongylus axei* is found in the stomach, *Parascaris equorum* is found in the small intestine, *Anoplocephala* spp. are found in the small intestine and caecum, *Strongylus vulgaris, S. edentatus, S. equinus, Triodontophorus* spp., *Oesophagodontus* spp., *Cyathostomum* spp., *Cylicocyclus* spp. and *Cylicostephanus* spp. are found in the caecum, ventral colon, dorsal colon and small colon. *Oxyuris equi* is found in the colon and rectum, *Dictyocaulus arnfieldi* in the lungs and *Gasterophilus* spp. larvae in the mouth and stomach.

1.2.4.1 *Strongylus*

The genus *Strongylus* contains three species: *Strongylus vulgaris, Strongylus edentatus* and *Strongylus equinus.* The first two are prevalent in many countries like UK, USA, Australia, Germany, Denmark and have also been reported in New Zealand (Tetley, 1948). However, *Strongylus equinus* is relatively less common in the world, apparently including New Zealand, and sporadic in its occurrence.

1.2.4.1.1 *Strongylus vulgaris*

The adult worms live in the caecum, ventral colon and dorsal colon. The male is 14-16 mm long and the female 20-24 mm long and 1.4 mm thick. The buccal capsule is large and contains two ear-shaped teeth at its base.

Duncan and Pirie (1974) described development and migration of larvae as follows. After ingestion by the horse the infective larvae exsheath and the infective larvae penetrate the mucosa of the small and large intestine within 1-3 days where they can be demonstrated within the lumen of sub-mucosal arteries. By 7 days they moult to the 4th stage larvae. From here they travel on the intima against the blood stream reaching the caecal and colic arteries within 14 days and reaching the cranial mesenteric artery by about 3 weeks. There they develop to mature fourth stage

larvae and by day 120 most have moulted to immature adults. At about this time they return to the large intestine by travelling down the arteries and lodging in arterioles. At the serosal surface of the large intestine the larvae, still within the arterioles, are each surrounded by a nodule which later ruptures into the intestine releasing the young adults into the lumen of the intestine (Drudge *et al.*, 1966). The time taken for these young adults to reach maturity once they enter the lumen of the intestine is 6-8 weeks (Duncan and Pirie, 1972). The minimum prepatent period required for this species is about 5-7 months (Round, 1969; Duncan, 1973).

Disease resulting from the infection may be considered as being caused by larval worms or caused by adults in the intestinal lumen.

The larvae penetrating into the mucosa and entering into submucosal arterioles and migrating to the cranial mesenteric arteries, cause inflammation and arteritis, thrombosis and thickening of the cranial mesenteric and ileocaeco-colic arteries (Ogboume and Duncan, 1984). Thrombosis and embolism of branches of the ileo-caeco-colic artery may lead to inflammation and infarction of the intestinal walls (Enigk, 1951). The clinical signs of infected horses are pyrexia, anorexia and colic. Other lesions have been associated with migration of larvae within the arterial system beyond the predilection site. For example, Cronin and Leader (1952) reported sudden death occurring in a thoroughbred horse due to arteritis and thrombosis of the right coronary artery .

The effect of the adult worm has not been studied in detail. Although adult worms are not considered very pathogenic, heavily infected horses are often in poor condition, with a staring coat, sometimes anaemic and losing weight.

1.2.4.1.2 *Strongylus edentatus*

The adult worms live in the caecum, ventral colon and dorsal colon. *Strongylus edentatus* is a large worm with males being 24-28 mm, and females being 33-44 mm in length. It has a large buccal capsule, which is wider anteriorly than at the middle and contains no teeth. The buccal capsule contains two rows of leaf crowns and a dorsal gutter reaching the anterior edge.

McCraw and Slocombe (1974) described the life cycle as follows. After ingestion the infective third stage larvae exsheath and penetrate the caecum and right ventral

colon and reach the liver via the portal blood stream within 2 days where they form nodules. The third stage larvae migrate in the liver for about 7 days and then moult between 11-18 days after infection. Following the third moult, the fourth stage larvae grow rapidly and cause fine tortuous tracks 4-5 mm in length in the liver from about one month after infection.

After about 7-9 weeks of burrowing and tracking in the liver, the larvae pass via the hepato-renal ligament to the sub-peritoneal tissue where they cause the formation of haemorrhagic nodules. After about three months the larvae moult to the immature adult stage and migrate, probably via the root of the mesentery and between the layers of the mesocolon, to the walls of the caecum and colon, where they form large haemorrhagic nodules on the serosa. These finally break and release immature adults into the lumen of the intestinal tracts. Many larvae go astray and can be found in unexpected sites such as the pleural cavity, kidney and testis. The minimum prepatent period is about 11 months (Russell, 1948).

Clinical disease can be divided into that caused by adult worms and that caused by the migrating larvae.

The larval stages cause adhesions involving the intestine and disruption of the omental architecture. This occurs several weeks after infection (McCraw and Slocombe, 1974). Clinically, the acute condition causes toxaemia, pyrexia, anorexia, jaundice and peritonitis. The larvae cause haemorrhagic nodules in the subperitoneal region and these are reported to cause colic (Wetzel, 1952). Similar lesions may occur under the capsule of the liver and spleen.

The adult worms ingest a plug of mucosa, digest the intestinal cells and rupture the associated blood vessels. Heavy feeding of this type produces intestinal damage, blood and protein loss into the intestine. Intestinal damage causes diarrhoea, anorexia, anaemia and depression (Archer and Poynter 1957).

1.2.4.1.3 *Strongylus equinus*

The adult worms live in the caecum, ventral colon and dorsal colon. *Strongylus equinus* is the largest of the *Strongylus* spp. There are two rows of leaf crowns, the buccal capsule is oval and has a dorsal tooth with two points and two sub-ventral teeth. It is not a common parasite in the world.

The life cycle has been described by Wetzel (1942) as follows: Infective larvae exsheath and penetrate the walls of the caecum and colon. They then migrate to the subserous tissues of the intestine where they induce the formation of haemorrhagic nodules. After 11 days 4th stage larvae are formed within the nodules. These migrate into the peritoneal cavity then move to the liver where they wander for about 4 months and moult to form immature adult. Then they leave the liver and migrate back to the large intestine. After returning they develop to mature adults (Wetzel & Vogelsang,1954). The minimum prepatent period is about 8.5-9 months.

The pathogenesis of larval forms causes haemorrhagic nodules on the intestinal wall. Wetzel (1941) found that experimental infections of 4000 larvae caused severe illness and death while 1000 larvae caused pyrexia, anorexia and colic. The adult stages causes ill thrift and anaemia.

1.2.4.2 Cyathostomes

This sub-family Cyathostominae is composed of 8 genera and more than 40 species (Lichtenfels 1975), although there is continuous debate about the taxonomy. They are commonly referred to as "cyathostomes" and this terminology will be used throughout this document. The most common genera are *Cyathostomum, Cylicocyclus* and *Cylicostephanus*. In *Cylicocyclus* the posterior margin of the buccal capsule has a ring like hoop-shaped thickening and the lateral papillae are usually large and broad; elements of external leaf crowns much longer, broader and fewer than the internal leaf crowns, whereas, in *Cyathostomum* the buccal capsule is generally short and thick walled; lateral papillae are not prominent; elements of external leaf crowns much longer, broader and fewer than the internal leaf crowns. In *Cylicostephanus* the buccal capsule is not greatly elongated and is usually narrower anteriorly; mouth collar depressed; elements of internal leaf crowns shorter; usually narrower and more numerous than elements of external leaf crowns. In *Cyathostomum*, an extra-chitinous support of the external leaf crowns is present but is absent in *Cylicocyclus* and *Cylicostephanus*. Cyathostomes are mostly about 5-20 mm long.

As a group they are common parasites of horses throughout the world including New Zealand. Though more than 40 species of cyathostomes are described less than

12 species are abundant and common (Reinemeyer *et al.*, 1984). The caecum and large colon are the preferred sites for the cyathostomes (Bucknell *et al.*, 1995).

All cyathostomes have direct life cycles. Eggs are passed in the faeces, then hatch and develop to infective larvae under favourable conditions. When the horse has swallowed the infective larvae, they exsheath and penetrate the wall of the caecum and colon. Migration of the invading infective larvae is limited to the mucosa and submucosa particularly of caecum and ventral colon (Reinemeyer and Herd, 1985). There, many become encysted within fibrous capsules to form nodules but others, particularly those located superficially in the mucosa of the caecum and large colon do not induce a noticeable tissue reaction. Eysker *et al*, (1984) showed that early third stage larvae become inhibited and are abundant in late autumn. The period of inhibition may be quite prolonged in parasitised horses. After a period of time for development in the mucosa, cyathostome larvae emerge into the intestinal lumen as adults or 4th stage larvae (Eysker *et al.*, 1984). In the intestinal wall they may remain dormant for several months, especially in the winter season, before becoming adults. The minimum time required for maturation from invading larvae to sexually mature worms varies between the cyathostomine species. Round (1969) gave the minimum prepatent period of various species as from 35-70 days.

If large numbers of larvae emerge into the caecum and colon, considerable blood loss and severe diarrhoea may occur, with large numbers of 4th stage larvae and immature adult cyathostomes present in the faeces (Mirck, 1977). Heavy infections of the encysted larvae may seriously impair digestive function and may produce intermittent spells of diarrhoea and constipation (Eysker *et al.*, 1984). When the larvae emerge, bacteria invasion may cause areas of ulceration.

At post-mortem the intestinal worms are found free or attached to mucosa and the intestinal mucosa may shows small red-bite marks or ulcers.

1.2.4.3 *Trichostrongylus axei*

This parasite is found in the stomach of the horses, ruminants and pigs. The adult worms are tiny being 2.5-8 mm long. The male has two dissimilar spicules of unequal length. This worm is prevalent in horses in most areas around the world including Australia (Waddell and McCosker, 1969), UK (Taylor & Kenny, 1995), USA (Monahan *et al.,* 1995) and has been recorded in New Zealand (McKenna, 1976).

Eggs are typical strongylid types being 79-92μ by 31-41μ. The life cycle of *Trichostrongylus axei* is typical of all trichostrongylid nematodes, the infective 3rd stage larvae moult to form fourth stage larvae then develop to form adults and the infection becomes patent in 25 days (Leland *et al.*, 1961).

T. axei does not usually produce serious pathogenic infections in horses but exceptionally heavy infestations are debilitating (Leland *et al.*, 1961). The worms burrow into the gastric mucosa of the stomach causing hyperaemia or an acute catarrhal gastritis and may produce irregular, circumscribed wart-like thickenings with a finely verrucose surface.

After necropsy of horses with a light infestation, no visible signs are seen but during heavy infestation the glandular tissues are swollen.

1.2.4.4 *Parascaris equorum*

This is a common and important parasite of young horses throughout the world including Australia (Bain, 1954; Wiltshire, 1954), UK (Comwell *et al.*, 1973), USA (Lyons *et al.*, 1990), Zambia (Islam, 1986) and Brazil (Costa *et al.*, 1998) and it has been recorded in New Zealand (Tetley, 1948) where it is also common. The males are 15-28 cm long and females up to 50 cm by 5-8 mm diameter. Adults are located in the small intestine. They are stout worms with a mouth that has 3 main lips, which are separated by interlabial swellings. The eggs are thick walled and covered by a thick albuminous layer with an irregular surface.

Foals become infected after ingesting eggs containing larvae. These may stick to the mare's udder or legs, or to grass or water or other objects that are licked and nibbled. The eggs hatch in the small intestine and the larvae burrow into the wall of the gut. Within 24 hours after the eggs have been ingested, the larvae pass through the portal circulation to the liver (Srihakin and Swerczek, 1978). The larvae then migrate from the liver to the lungs from 7 to 14 days after infection and then pass up the bronchi and trachea to the pharynx and are swallowed with most arriving in the small intestine by 23 days after infection. After returning to the small intestine they develop and moult to 4th stage larvae between 2-3 weeks after infection. They become adult in the small intestine 3-4 weeks after infection (Clayton and Duncan, 1979). The minimum prepatent period in experimental infections varies widely from

72-115 days (Clayton 1978). Thus, foals are passing out eggs of these parasites by about 3 months of age.

When the larvae migrate, they cause destruction of the tissue resulting in haemorrhage in the liver and lungs. The effects of this may not be serious but growth may be retarded. Early larval invasion of the liver causes narrow tracks particularly under the capsule, along the interlobular septa and in the interlobular tissue between the portal triads (Brown and Clayton, 1979). Heavy experimental infections, either as single or repeated infections, cause coughing 2 to 3 weeks after infection (Clayton and Duncan, 1979; 1978; Srihakin and Swerczek, 1978). In severe infections, the foals may become weak, emaciated and have poor growth rates. Intestinal disturbances are common and diarrhoea, sometimes alternating with constipation, is seen. Gastric motility may be reduced (Clayton *et al.*, 1980). The adult worms in the intestine are most dangerous, sometimes they obstruct the intestine, then cause perforation and the horse finally dies due to peritonitis (Wiltshire, 1954).

1.2.4.5 *Strongyloides westeri*

This nematode occurs in the small intestine of the horse. It has been reported in New Zealand (Dewes, 1972) and is found throughout the world. It is small being about 9 mm long and 0.08-0.095 mm in diameter. The female produces thin-shelled transparent eggs, slightly longer than broad, approximately 45 X 38 µm, which contain a larva. Large number of eggs may be found. Russell (1948) explained the life cycle that the hatched larvae develop to the third stage, which is either infective or non-infective. The infective larvae have no protective sheath and invade the host either *per-os* or percutaneously. These can penetrate the skin of a new host but the non-infective larvae are destined to produce free-living adult worms. The infective larvae pass through the blood stream to the lungs. Thereafter they ascend the respiratory tract and on reaching the pharynx are swallowed to proceed to the small intestine.

Infection by the transmammary route has also been described (Lyons *et al.*, 1973). Infective larvae entering a mare will accumulate in the tissues and migrate to the mammary gland, when a foal is born. The foals become infected when suckling the mare. Larvae are passed in the milk from 4 days after parturition and patent infections of foals are developed from about 10-14 days of age. Egg production

increases to about 20,000 epg of faeces by 3 weeks of life and declines from about 6 weeks (Bello *et al.*, 1973) usually reaching zero by about 16 weeks. Thereafter foals are resistant to infection. Russell (1948) reported peak egg production at 8-9 weeks of age and this then fell away, and all except one foal was free of infection by 15-23 weeks.

If the foals are heavily infected, diarrhoea may occur and this is usually seen in the first few weeks after birth.

1.2.4.6 *Oxyuris equi*

Oxyuris equi is a common parasites of horses throughout the world and it also has been reported in New Zealand (Tetley, 1948). The adult male is small 9-12mm long whilst the adult female is much longer being up to 150 mm long. The oesophagus is divided into several parts; the anterior half being expanded and is followed by a short constricted part and then a posterior bulb. The male is white, has one pin-shaped spicule and has a tail which is short bearing two pairs of large and a few small papillae. The females are a whitish or yellowish colour, slightly curved with a tail then this is 3 times the length of the body. The eggs are elongated, slightly flattened, operculate and measured about 90 μm by 42 μm (Arundel, 1985).

Adult *Oxyuris equi* lives in the caecum and large colon. The gravid female worms migrate to the rectum and crawl through the anus to deposit their eggs on the perineum and then die. The eggs are attached to the skin with a gelatinous cement-like material that is very irritating. Females may burst after passing through the anus and scatter their eggs in the peri-anal area (Levine, 1968). Then the infective larvae are formed within the egg and fall on the ground. The eggs do not hatch. The host is infected by ingestion of infective eggs containing the L_3. The eggs hatch in the small intestine and the larvae migrate to the caecum and ventral colon and are found in the mucosal crypts. There they moult to form the 4th stage larvae about 8-10 days after infection. The immature adult stage is reached in about 50 days. These feed on intestinal contents. The prepatent period is about 5 months.

The adults are not pathogenic but the egg laying female causes intense irritation when laying its eggs on the perineum. This produces anal pruritis, which disturbs the horse from rest and feeding and results in loss of condition and a dull coat. The animal rubs the base of the tail against any suitable object, causing broken hairs

around the tail-base (Levine, 1968). Heavy burdens of fourth stage larvae cause small erosions and enteritis.

1.2.4.7 *Dictyocaulus arnfieldi*

Dictyocaulus arnfieldi is the only nematode in the lung of the horse. Lungworm infection occurs in equines throughout the world and has been reported in New Zealand (Campbell *et al.*, 1971) and Australia (Rickard and James, 1976), but is uncommon. The worm is 2-4 cm long in the male and up to 7 cm long in female. The female passes oval eggs, 90 by 55μ that are embryonated when passed.

Dictyocaulus arnfieldi has a direct life cycle. The eggs contains larvae which are usually coughed up, swallowed and voided in the faeces of the host. Then the larvae hatch and develop on the pasture to reach the infective larval stages in about a week. The minimum prepatent period following infection is usually stated to be about 35-40 days but epidemiological data suggest that it is probably 11 to 14 weeks (Round 1976; Clayton and Duncan, 1981).

Nicholls *et al.*, (1978) described the lesions seen in a horse with a severe multiple helminth infection. The lungs had isolated groups of swollen lobules, which consisted of over-inflated pulmonary tissue above bronchi plugged with a greenish mucus. The main lobar bronchi contained small amounts of frothy mucus.

1.2.4.8 *Gasterophilus* spp.

Stomach bots are the larval stages of *Gasterophilus* spp. and are quite different from other intestinal parasites of the horse. The common *Gasterophilus* spp. are *G. intestinalis, G. nasalis* and *G. haemorrhoidalis*. *G. intestinalis* is the most common in New Zealand (Tetley, 1948) but *G. nasalis* and *G. haemorrhoidalis* are also found in New Zealand (Tetley, 1948) although only in light infestations. The adults are large, yellow to dark coloured hairy flies, like a bumble bee, but they have one pair of wings. Their mouth parts are not developed, so they do not bite or feed. Zumpt (1965) gives a key to the adults, eggs, 1st and 2nd and 3rd instars to identify various stages. The third larval stages of the various species are 14-18 mm long and broad. They have large mouth hooks with which they attached to the stomach of the host. There are 12 segments and each have 1 or 2 rows of spines. In *G. nasalis* the spines on the ventral segments arranged in one row but in *G. intestinalis* the spines

on the ventral segments are arranged in two rows and the mouth hooks have a saddle-like depression. In case of *G. haemorrhoidalis* the spines are smaller, with spines arranged in two rows and mouth hooks uniformly bent.

1.2.4.8.1 *Gasterophilus intestinalis*

Zumpt (1965) describes the life cycle as follows: Female flies deposit eggs on the hairs of the horse, mainly on the forelimbs, chest, neck and mane. The eggs are ready to hatch by the fifth day but require moisture and friction, which is produced by the horse licking at the area. Development is delayed in some eggs, particularly in cool weather, and larvae remain alive for weeks or even months. Once inside the mouth the larvae penetrate the anterior end of the tongue and burrow in the buccal mucosa for 24-25 days. They then invade pockets between teeth or between the gum and molars. After about one month, the larvae moult and the 2nd instar may attach for a few days at the root of the tongue or to the pharynx and the sides of the epiglottis before passing to the stomach. The migration of the larvae in the mouth has been examined by Cogly *et al.*, (1982). After about 5 weeks in the stomach they moult to 3rd instar. They attach to the mucosa of all parts of the squamous oesophageal region, less than 1% are found in the glandular region (Waddell, 1972) and remain there until maturity. The mature larvae are excreted with the faeces and pupate in the soil in 3-4 weeks but at low temperatures this may be prolonged to 3 months. There is only one life cycle per year.

The first instar *G. intestinalis* may cause pus pockets and excessive irritation between or alongside the molar teeth, and result in pain on mastication and poor growth (Bello and Seger, 1972). Blood *et al.*, (1983) reported a non-specific syndrome of unthriftiness, poor coat, mild colic and poor appetite ascribed to bot infestations. There are many reports that the L_1 of *G. intestinalis* produces gingivitis (Tolliver *et al.*, 1974), periodontal ulceration (Cogley, 1989) and stomatitis (Cogley *et al.*, 1982). In the stomach, larvae produce ulcers (Pandey *et al.*, 1980), subserosal abscesses (Waddle, 1972) or stricture of the oesophagus-intestinal junction (Soulsby, 1982).

1.2.4.8.2 *Gasterophilus nasalis*

Gasterophilus nasalis, the throat bot fly, lays a yellow-brown or black egg on hairs in the space between the rami of the mandible. The egg is attached along its whole

length, usually one egg per hair, although up to 5 may be seen. Each female produces 300-500 eggs, which hatch spontaneously in 5-6 days. The larvae migrate to the lips and invade the gum in pockets around the teeth and in the interdental spaces. Larvae develop in the oral and pharyngeal tissue after 3-4 weeks. During development in the mouth, the first stage larvae moult to the L_2 and L_3 stages. L_3 larvae then develop in the pyloric part of the stomach and in the first part of the duodenum (Blagbum *et al.*, 1991). About 10-11 months from hatching of the egg, the third instars are passed with the faeces and pupate. The pupal stage lasts from 16-64 days depending on the temperature.

The first instar *G. nasalis* penetrate gum tissue between or alongside the molar teeth and produce gingivitis (Tolliver *et al.*, 1974). They may also cause pus pockets and excessive irritation, which can result in pain on mastication and poor growth (Bello and Seger, 1972).

1.2.4.9 *Anoplocephala* spp.

There are three species of cestodes in the family Anoplocephalidae that can infect the equine gastrointestinal tract but only one *Anoplocephala perfoliata,* has been reported from New Zealand (McKenna, 1976). Prevalence rates of *A. perfoliata* were 82% in New Zealand (Bain & Kelly,1977), 80% in Australia (Dunsmore and Jue Sue, 1985), 69% in England (Owen *et al.*, 1988), 54% in Kentucky (Lyons *et al.*, 1984), 47% in Louisiana (Torbert *et al.*,1986) and 18% in Ohio (Reinemeyer *et al.*,1984). Severe infections can occur in all ages of horses.

The parasite is found attached on either side of the ileocaecal valve (the junction between the large and small intestine), with smaller numbers in the distal small intestine, caecum and proximal ventral colon (French and Chapman, 1992). It measures up to 8 by 1.2 cm. The scolex contains four suckers but no rostellum or hooks. Behind the sucker there are prominent swellings, known as lappets. Most of the body is made up of a series of narrow segments. These segments contain the reproductive system of the parasites and eggs. The eggs are normally discharged as the segment breaks up. The eggs are about 65-80µm diameter and have an angular appearance and contain a chitinous structure called the pyriform apparatus (Arundel, 1985).

The life cycle of these species requires an acarine intermediate host. Mites of the family Oribatidae may ingest these eggs. In these, they develop into a cysticercoid in 2-4 months. The horse becomes infected by accidentally eating infected mites when grazing. The tapeworms then take about 2 months to mature in the horse and start passing eggs (Soulsby, 1982).

A large burden of *Anoplocephala perfoliata* can produces a thickening of the mucosa, oedema, ulceration, excess granulation tissue and catarrhal enteritis at the site of attachment around the ileocaecal valve (Jubb *et al.*, 1985; Lyons *et al.*, 1984). There are reports of horses affected by *Anoplocephala perfoliata* that describe such lesions as ileac obstruction and ileocaecal or caecocolic intussusception (Barclay *et al.*, 1982; Edwards, 1986). Roberts (1953) has reported death due to rupture of the intestine near the ileocaecal valve. These occasionally produce mild colic, unthriftiness and diarrhoea. However, whether these lesions are directly attributable to *Anoplocephala* is difficult to prove.

CHAPTER TWO

Dose Confirmation Study

Efficacy of an Anthelmintic Paste against Natural Nematode and Tapeworm Infections in Horses:

2.1 Introduction

This study was designed to assess the efficacy of an anthelmintic paste formulation (AOB 198) which contains abamectin, oxibendazole and bithionol, against a mixed natural infection in horses with nematodes, *Anoplocephala* and also *Gasterophilus* larvae. All three actives in this combination have been used in horses but not in this combination, especially the inclusion of bithionol. The efficacy of bithionol is poorly documented. This study was undertaken mainly to measure the efficacy of 5 mg/kg bithionol in the intended combination product but also to confirm efficacy of oxibendazole and abamectin against nematodes and *Gasterophilus* larvae. The Registration authority require a Dose Confirmation Study (DCS) to use the final or nearly-final formulation, dosage rate and route of administration of the product.

2.2 Materials and methods

2.2.1 Animals

Kaimanawa horses were used in this experiment because these are feral horses which have never been drenched with anthelmintics and it was expected that all sorts of parasites were present.

Twelve sexually mature, mixed age, male horses were obtained from the Kaimanawa region in July 1997. All were wild herd captures, grazed together on their feral range and on a holding farm after capture. Their weight varied from 249 to 371 kg. They were in good condition and were healthy animals. None had ever received anthelmintics.

The horses were identified with secure numbered neckbands. Bands were attached 2 weeks prior to the study commencing, to confirm that this was a reliable and secure means of identification.

Secure yards, a crush and scales were provided on the holding property. As the horses were unbroken and shy, this means of handling gave least stress and

greatest safety to horses and personnel.

2.2.2 Test Site

The trial animals were grazed at the property of Mr Paul Synes, Springhall Rd, Te Kauwhata. Mr Synes runs a property of ~200 hectares on which both horses and cattle are grazed. Separate holding paddocks were provided for horses held prior to treatment and after treatment. The paddock used for the horses following treatment had only been grazed by cattle for the previous 12 months and was assumed to be free of horse parasite contamination with the exception of *T. axei*. Manipulations were performed in the yards on this property.

2.2.3 Test Substance

The test substance was supplied by Virbac accompanied by a certificate of analysis, batch number, manufacture date and Material Safety Data Sheet. The anthelmintics were combined in a paste with a total volume of 30mls and coded AOB 198. The composition of AOB 198 was as follows:

a.i.	**Dosage**	**Total formula content per tube**
abamectin	0.2 mg/kg	120 mg abamectin
oxibendazole	10 mg/kg	6 g oxibendazole
bithionol,	5 mg/kg	3 g bithionol
total actives		9.12 g
excipients to		30 g

Each 30ml tube contains treatment for a 600 kg horse. Syringes of the test product were marked in 2.5 ml gradations with each of these gradations equivalent to a dose for 50 kg body weight which gives a dose of 1g AOB 198 per 20kg body weight.

2.2.4 Study Design

The animals were weighed on an electronic scale before the commencement of the study (Day –25 and Day –10), and again just prior to dosing with anthelmintic paste (Day 0).

This experiment was a controlled study with 6 animals per group. Six animals were allocated to the Treated Group, and six to the Control Group. Allocation was performed on paired mean faecal egg counts from Day –25 and Day –10 and individuals within each pair were randomised to either the Treated Group or the

Control Group. As age could not be accurately determined, this was not taken into account.

The Treated Group animals were treated with the anthelmintic paste AOB 198 on Day 0. Following treatment, all horses were confined on the cattle pasture which had not been previously grazed by horses for at least 12 months.

On Day 8 all animals were transported on a horse float to Bombay Pet Foods facility for slaughter and necropsy. Six horses were killed on both Day 9 and Day 10 (3 control and 3 treated on each day).

The efficacy of the compound was determined by comparing the number of nematodes and tapeworms in the control animals with the numbers in the treated animals.

No drug or vaccine in addition to the experimental treatments was administered during the study period.

2.2.5 Treatment Regime

Each horse was treated once intra-orally from the 30 ml syringes of test substance. The dose to each Treated Group horse was calculated from the weight recorded immediately prior to treatment. Total dose measured (in ml) along with any spillage observed, was recorded.

During administration of these test products, the syringe was fitted with a piece of 20 cm long and 0.5 cm diameter plastic tube used as an extension. To further aid delivery and acceptance of the test product, apple pulp was given before and following treatment, also by syringe. The apple pulp given after dosing with the test product was delivered through the same extension tube which ensured no test product remained in the extension tube. This formulation (AOB 198) was not particularly liked by the treated horses, presumably as it has a persistent bitter taste to humans.

After use, syringes were weighed to determine loss of weight due to product expressed. From this, individual and average dose rate of active ingredient was calculated.

The Control Group animals remained untreated.

2.2.6 Collection of Samples and Observations

Faecal samples were collected directly from the rectum on Days –25, -10, 0, 9 and 10 of the study (Table 2.1). Faecal egg counts were performed with a modified McMaster method. Faecal flotation technique was also performed on each sample to detect *Anoplocephala* eggs. Both techniques are described in Appendix I.

Each animal was observed at least 10 minutes after each treatment and then approximately hourly for four hours. Thereafter, it was also observed once daily throughout the course of the trial (9-10 days).

All necropsy and worm count procedures are also described in detail in Appendix I. In brief, at necropsy the abdomen was opened and the gastro-intestinal tract was removed in its entirety. Then the retro-peritoneal fat was trimmed off from the hanging carcass. The cranial mesenteric artery with associated branches within the mesentery were separated from the intestinal tract and placed in a labelled plastic bag. The gastro-intestinal tract was ligated at the oesophagus-stomach junction, at the pylorus, 30 cm proximal to the ileo-caecal junction, the junction of the caecum and ventral colon, the ventral colon 30 cm distal from the caecum, the junction of the ventral and dorsal colon, the junction of the dorsal and small colon and the rectum. Each of these segments was then separated. Each section was separately bagged, labelled and stored in 50L (plastic rubbish) containers and placed in frozen storage within 12 hours of death. Note that in this study the ventral colon was collected in 2 sections with the view that *Anoplocephala* could be found in the proximal segment.

At a later time and on a planned basis, single organs were thawed, washed and examined separately. Two 5% aliquots were collected separately from each of these segment's contents. These were stored in 10% formalin, to be subsequently examined under a dissecting microscope. One of the 5% aliquots was kept as a reserve.

The remaining 90% of the washings and contents from each segment plus the reserve 5% aliquot were sieved through a 1.25mm aperture sieve and all large strongylid species were collected and stored in 10% formalin. All *Gasterophilus* spp. larvae and *Anoplocephala* spp. were also counted and collected into 10% formalin.

From each of the caecum, ventral colon and dorsal colon 6-14 pieces of 12cm × 12cm square samples of mucosa were saved. This was approximately 5% by weight of each organ. Details of the total weight of each organ and the weight of portions examined of each organ are given in Appendix III. These sections of

mucosa were then transilluminated and digested. Details of both techniques are described in Appendix I. No attempt was made to identify mucosal larvae to genus or species.

The cranial mesenteric artery with its branches and the retro-peritoneal fat (the area beneath the parietal peritoneum of the abdominal wall) were examined for migratory stages of nematodes which were counted. Nodules on the walls of the caecum and ventral colon were examined and migrating stages of nematodes were counted and recovered. Any recovered parasites were stored in 10% formalin with records of their location and condition.

2.2.7 Statistical Evaluation

Total nematode counts for animals in the DCS were calculated by multiplying the number of worms actually counted in each alimentary location by the aliquot factor, and summing over all locations for the specific parasite.

Efficacy was calculated with arithmetic means and also $\log_{10}$ transformed geometric means. The arithmetic mean is the sum of results observed in one group of horses divided by the number of observations. Unless stated otherwise, discussion of efficacy has been based on arithmetic means.

The geometric mean was calculated by converting all single horse data to $\log_{10}(x+1)$ calculating a mean (y) and then expressing the mean as the $(y^{10} - 1)$.

The percentage efficacy of the anthelmintic AOB 198 for specific genera, species and stages of endoparasites recovered was calculated by comparing the arithmetic mean number of parasites found in control animals to arithmetic mean number of parasites in the treated animals as follows:

$$\text{Proportion} = \frac{\text{Mean number of worms in controls} - \text{Mean number of worms in treated animals}}{\text{Mean number of worms in controls}}$$

Statistical analysis was done with the Rank Sum test using the Mann-Whitney U statistic for efficacy comparison between the Treated Group and the Control Group horses. Interpretation was done as a one-tailed test. The comparison between the transillumination and digestion techniques of both groups of horses was made with the paired t-test after converting all single horse data to $\log_{10}(x+1)$. This analysis was made with the Software Program Statistix Version 4.1 (Analytical Software, Florida, USA).

Another statistically analysis was done with the ANOVA using the Proc glm procedure of SAS (SAS, USA) for comparison of the numbers of mucosal larvae in the caecum, ventral colon and dorsal colon after converting all single horse data to $\log_{10}(x+1)$.

2.3 Results

2.3.1 Anthelmintic drenched dose (Dose given)

After nominal allowance for spillage, the average AOB 198 dose rate appears to be 1.08g/20kg body weight compared to the target 1g/20kg body weight. In spite of all measures taken, there was some difficulty in ensuring that these uncooperative horses swallowed all treatment. An allowance of 2 ml was made when spillage was observed. Actual average dosage in therefore likely to be not greater than target (5mg/kg of bithionol). Details of doses are given in Appendix III.

2.3.2 Clinical observations:

After being treated on Day 0, the Treated and Control Groups of horses were feeding well and their behavior was normal, except for one treated horse. Horse 17 staggered immediately on release from the crush, which suggested disorientation due to some nerve pressure, and/or muscle spasm. This horse also showed some agitation in the evening on the day of treatment.

On Day 1, all horses from both groups appeared normal when observed in the yards and the paddock. Faeces of normal consistency were found, including some which contained large numbers of worms.

On Day 2, again all horses appeared normal when observed in the yards and the paddock. Large numbers of *Gasterophilus* spp. larvae and nematodes were observed in freshly passed faeces of treated animals. Some loose faeces, like cowpats, were observed in the paddock but could not be associated with individual horses. It is possible that all the worm burdens and loose faeces observed came from treated horses.

When observed in the paddock on Day 3 and subsequent days, both groups of horses appeared normal and feeding well.

No other adverse reactions were observed. In particular, no signs of irritability or skin irritation were seen in any horses, either around the muzzle immediately following treatment, or over the body during the succeeding days.

2.3.3 Parasitological findings

2.3.3.1 Strongylid Egg Counts

These are shown in Table 2.1. On Day 0, roughly equivalent strongylid egg counts were estimated in faecal samples from both the Control and the Treated Groups. On Day 9, faecal egg counts were zero for all treated horses, whereas for all control horses strongylid eggs were not significantly changed from Day 0 ($p>0.01$), indicating excellent potency of AOB 198 against adult ova-producing strongylid nematodes. Efficacy of egg count reduction after 9 days was 100% for the treated horses ($p<0.001$).

Table 2.1 Mean numbers of faecal strongylid egg counts of the Control Group and the Treated Group horses.

Horse No.	**Eggs Per Gram**				
	Day -25	**Day –10**	**AM♦**	**Day 0**	**Day 9/10**
Control Horses					
16	1250	450	850	600	500
18	500	400	450	NTR	1600
20	400	400	400	300	650
14	100	1000	550	800	1600
27	700	500	600	950	650
23	1000	450	725	600	800
AM	658	533		650	967
Treated Horses					
17	700	900	800	1600	0
2	NTR	450	450	700	0
24	400	300	350	900	0
4	550	450	500	500	0
21	500	600	550	700	0
11	900	600	750	800	0
AM	610	550		867	

AM = Arithmetic mean of group; AM♦ = Arithmetic mean of Day –25 and Day –10 by horse; NTR = Not recorded. Statement sequence within groups is as allocated from pairs.

2.3.3.2 Parasite Counts

2.3.3.2.1 Non-cyathostome luminal parasite counts

Table 2.2 shows non-cyathostome luminal parasite numbers found in Control and Treated Group horses with a summary of effectiveness show in Table 2.3.

Adult and immature luminal stages of *S. edentatus* (151-522), *S. vulgaris* (69-771) and *Triodontophorus* spp (28-87) were found in moderately high numbers in all control horses but were absent in treated horses. Therefore, 100% ($p<0.01$) efficacy was achieved for *S. edentatus, S. vulgaris* and *Triodontophorus* spp.

Table 2.2 : Non-cyathostome parasites recovered at necropsy from the Control Group and the Treated Group horses.

Horse No.							
	***Strongylus edentatus* adults**	***Strongylus vulgaris* adults**	***Triodontophorus* spp. adults**	***Trichostrongylus axei* adults**	***Oxyuris equi* 4th stage larvae**	***Anoplocephala perfoliata***	***Gasterophilus intestinalis* larvae**
Control							
16	281	771	43	4644	0	394	66
18	151	492	71	17554	236	86	111
20	242	207	63	3568	0	331	110
14	522	508	38	12020	0	272	313
27	224	425	87	832	210	772	215
23	285	69	28	5049	3740	105	254
Treated							
17	0	0	0	0	0	1	0
2	0	0	0	0	0	49	0
24	0	0	0	0	0	61	0
4	0	0	0	0	0	30	0
21	0	0	0	0	0	13	0
11	0	0	0	117	0	76	5

Table 2.3 Means numbers of luminal non-cyathostome parasites recovered from the Control Group and the Treated Group horses; efficacy by species.

Parasites	**Control**		**Treated with AOB198**		**Efficacy**		***p*-value**
	AM	GM	AM	GM	%AM	%GM	
Anoplocephala perfoliata	327	251	38	23	88.4	90.8	0.001
Strongylus edentatus	285	265	0	0	100	100	0.001
Strongylus vulgaris	412	325	0	0	100	100	0.001
Triodontophorus spp.	55	51	0	0	100	100	0.001
Trichostrongyles axei	7278	4949	20	2	99.7	99.96	0.001
Oxyuris equi	698	24	0	0	100	100	0.092
Gasterophilus intestinalis 3rd instar larvae	178	155	1	1	99.4	99.4	0.001

Large numbers of *T. axei* were recovered from all control horses, whereas in treated horses, *T. axei* was not found except for 117 in one horse (horse No. 11). The efficacy of AOB 198 was 99.7% ($p<0.01$) against *T. axei*.

Oxyuris equi fourth stage larvae were obtained from 3 horses in the Control Group, but none were seen in the Treated Group horses. Efficacy of AOB 198 was 100% against *Oxyuris equi* 4th stage larvae. No adult *Oxyuris equi* were seen in this experiment in any horse.

Gasterophilus intestinalis third instar stages were found in all the Control Group horses, but only found in one treated horse (no. 11) in low numbers (5). The efficacy of AOB 198 was 99% ($p<0.01$) against *Gasterophilus intestinalis* third instar larvae. No *Gasterophilus nasalis* larvae were found in any horse.

Anoplocephala perfoliata were recovered from all control horses in large numbers; and also from all treated horses, but in low numbers. As a result, AOB 198 gave an efficacy of 88.2% ($p<0.01$) against *Anoplocephala perfoliata*.

2.3.3.2.2 Luminal cyathostome nematode counts

Table 2.4 Numbers of luminal cyathostome nematodes recovered at necropsy from the Control Group and the Treated Group horses.

Horse no.	Name of the Parasite	No. of adult Parasites	Number of L4
Control Horses			
16	*Cyathostomum* spp.	1156	200
	Cylicocyclus spp.	1961	230
	Cylicostephanus spp.	2743	470
18	*Cyathostomum* spp.	8225	1110
	Cylicocyclus spp.	15355	840
	Cylicostephanus spp.	8130	820
20	*Cyathostomum* spp.	7299	620
	Cylicocyclus spp.	8258	2520
	Cylicostephanus spp.	7023	1080
14	*Cyathostomum* spp.	4230	840
	Cylicocyclus spp.	8683	1420
	Cylicostephanus spp.	7367	650
27	*Cyathostomum* spp.	1872	550
	Cylicocyclus spp.	8718	1240
	Cylicostephanus spp.	7450	470
23	*Cyathostomum* spp.	1768	470
	Cylicocyclus spp.	2262	530
	Cylicostephanus spp.	1550	320
Treated Horses			
17	*Cyathostomum* spp.	0	0
	Cylicocyclus spp.	0	0
	Cylicostephanus spp.	0	0
2	*Cyathostomum* spp.	20	44
	Cylicocyclus spp.	0	0
	Cylicostephanus spp.	3	33
24	*Cyathostomum* spp.	0	0
	Cylicocyclus spp.	0	0
	Cylicostephanus spp.	0	0
4	*Cyathostomum* spp.	0	0
	Cylicocyclus spp.	0	0
	Cylicostephanus spp.	0	0
21	*Cyathostomum* spp.	0	0
	Cylicocyclus spp.	0	0
	Cylicostephanus spp.	0	0
11	*Cyathostomum* spp.	0	1
	Cylicocyclus spp.	0	0
	Cylicostephanus spp.	0	0

Table 2.4 shows the number by genus of adult cyathostome (*Cyathostomum* spp., *Cylicocyclus* spp. and *Cylicostephanus* spp.) nematodes and developing stages of larvae recovered from the gut lumen of both the Control and Treated Group horses.

To define the numbers of each genus within the Subfamily Cyathostominae, at least 100 adult cyathostomes were differentiated from each horse.

The efficacy of the test formulation against different cyathostomine nematodes is summarised in Table 2.5. After treatment with AOB 198, luminal cyathostomes were not found in treated horses, except in two (horses no's 2 & 11) in very small numbers.

These results (Table 2.5), indicate that against luminal stages, AOB 198 was 100% ($p<0.001$) effective against adult and larval stages of *Cylicocyclus* spp., 99.99% ($p<0.001$) effective against adult *Cylicostephanus* spp. and 99.1% effective against larval stages of *Cylicostephanus* spp. and 99.9% ($p<0.001$) effective against adult *Cyathostomum* spp. and 98.7% ($p<0.001$) effective against larval stages of *Cyathostomum* spp.

Table 2.5 Arithmetic and geometric means of cyathostome nematode numbers recovered from the Control Group and the Treated Group horses.

Name of the Parasites	**Control**		**Treated with AOB 198**		**Efficacy**		***p* value**
	AM	GM	AM	GM	AM%	GM%	
Adult♦							
Cyathostomum spp.	4091	3147	3	1	99.9	99.9	0.001
Cylicocyclus spp.	7540	5910	0	0	100	100	0.001
Cylicostephanus spp.	5711	4870	0.5	1	99.99	99.98	0.001
Larvae							
Cyathostomum spp.	632	557	8	1	98.7	99.8	0.001
Cylicocyclus spp.	1130	877	0	0	100	100	0.001
Cylicostephanus spp.	635	587	6	1	99.1	99.7	0.001

♦ - Includes immature and mature adults.

2.3.3.2.3 Mucosal cyathostome nematode counts

Two different methods, viz. digestion and transillumination, were used to count mucosal stages of cyathostome nematodes. The digestion technique gave significantly ($P<0.05$) higher counts than the transillumination technique in all intestinal regions, for both treated and control groups.

After digestion the larvae were damaged, therefore, it was not possible to attempt to speciate the mucosal stages.

Table 2.6 Larvae recovered at necropsy after transillumination and digestion methods of counting.

Horse No.	Transillumination				Mucosal Digestion			
	Caecum	V. colon	D. colon	Total	Caecum	V. colon	D. colon	Total
Control								
16	3440	36110	8580	48130	5270	43870	12180	61320
18	1590	79240	5980	86810	2440	95560	6450	104450
20	2020	5680	640	8340	2500	7170	4340	14010
14	2410	74480	6160	83050	3540	96520	6890	106950
27	13140	96260	320	109720	15130	115860	480	131470
23	2800	36620	2540	41960	4510	69690	3310	77510
A. M.	4233	54732	4037	63002	5565	71445	5608	82618
Treated								
17	1400	8320	2880	12600	1560	11880	3260	16700
2	6310	37530	360	44200	10120	46920	680	57720
24	590	22720	4350	27660	960	27720	5620	34350
4	8640	31140	1240	41020	11380	34970	2870	49220
21	320	3720	80	4120	440	4830	140	5410
11	2980	5440	640	9060	3750	6480	930	11160
A.M.	3373	18145	1592	23110	4702	22133	2250	29085

A.M. = arithmetic mean of each organs in each group of horses.

Table 2.6 shows the results of mucosal counts of individual horses of both control and treated horses, by both counting methods. Significantly higher numbers of larvae ($p<0.01$) were present in the ventral colon than in either the caecum or dorsal colon for both the Treated Group and the Control Group.

A moderate efficacy against encysted (late) L_3 and L_4, of 64.8 %, was detected by the mucosal digestion technique. But a slightly smaller value (63.3 %) was shown by the transillumination method. There was a significant difference ($p<0.05$) in the numbers of encysted larvae recovered at necropsy between the Treated and the Control Groups of horses. The group results are given in Table 2.7.

Table 2.7 Mean numbers of cyathostome nematode larvae recovered from intestinal mucosa of the Control Group and the Treated Group horses by mucosal digestion or transillumination.

Method	Control		Treatment		Efficacy		p-value
	AM	GM	AM	GM	%AM	%GM	
Mucosal digestion	82618	68068	29085	21495	64.8	68.4	0.013
Transillumination	63002	48690	23110	16935	63.3	65.2	0.065

2.3.3.2.4 Peritoneal and arterial parasite stages

Table 2.8 shows the strongylid larvae recovered from the cranial mesenteric artery and sub-peritoneal sites in individual horses. Table 2.9 shows mean numbers of the *S. vulgaris* larvae recovered from the mesenteric artery and *S. edentatus* larvae recovered from the sub-peritoneal sites and their efficacy.

In this study, *S. vulgaris* larvae were present in all horses in the cranial mesenteric artery and all showed visual evidence of verminous arteritis at this location.

From 3 to 52 (arithmetic mean 28) of these larvae were recovered from the cranial mesenteric artery from the Control group horses, and 12-37 (arithmetic mean 20) were recovered from the treated Group horses. The small difference in average arterial larval counts could be interpreted as an efficacy of 28.8% for the test product against *S. vulgaris* larvae. However, there is no significant difference ($p>0.05$) in numbers of larvae recovered at necropsy between Treated and Control Groups.

Table 2.8 Numbers of *Strongylus* spp. larvae recovered from mesenteric arterial dissections and peritoneal sites of the Control Group and the Treated Group horses.

Horse No.	Organ	*Strongylus vulgaris*	Organ	*Strongylus edentatus*
Control				
16	CMA	38	Sub-peritoneal sites	5
18	CMA	52	Sub-peritoneal sites	1
20	CMA	32	Sub-peritoneal sites	3
14	CMA	14	Sub-peritoneal sites	5
23	CMA	3	Sub-peritoneal sites	2
27	CMA	28	Sub-peritoneal sites	18
Treated				
4	CMA	16	Sub-peritoneal sites	0
11	CMA	19	Sub-peritoneal sites	2
24	CMA	12	Sub-peritoneal sites	1
2	CMA	17	Sub-peritoneal sites	38
21	CMA	18	Sub-peritoneal sites	1
17	CMA	37	Sub-peritoneal sites	5

CMA = cranial mesenteric artery including the nodular *Strongylus.*

All horses were also infected with *S. edentatus* larvae, located just beneath the parietal peritoneum in the retro-peritoneal fat in the abdominal wall. From 1 to 18 (arithmetic mean 6) larvae were recovered from the sub-peritoneal sites of control horses, and 1-38 (arithmetic mean 8) were recovered from the treated horses.

Nodules were found in the walls of the caecum and ventral colon, some of which contained immature adult *S. vulgaris*, in both the Control and Treated Group horses. These parasites are included in the counts for *S. vulgaris* found in the cranial mesenteric artery as shown in Tables 2.8 and 2.9.

Table 2.9 Mean numbers of *Strongylus* spp. larvae recovered from arterial dissections and peritoneal sites:

Organ	Parasites	Control		Treated		Efficacy		*p* value
		AM	GM	AM	GM	AM%	GM%	
Mesenteric artery	*S. vulgaris*	28	21	20	19	28.8	9.35	0.268
Sub-peritoneal sites	*S. edentatus*	6	4	8	3	0	25	0.214

AM = arithmetic mean; GM = geometric mean

There was no apparent effect of the test product against *S. edentatus* in the subperitoneal sites and no significant difference ($p>0.01$) between the Treated and Control Groups.

2.4 Discussion

The test product AOB 198 was formulated to deliver dosages of 0.2 mg/kg abamectin, 10 mg/kg oxibendazole and 5 mg/kg bithionol. During administration of this product apple pulp was used to increase the acceptance by the horse. However, it was very difficult to assess the acceptance of this drug, because these were unbroken feral horses not used to being handled.

As far as can be determined after considering compensating inaccuracies, the average dose given to horses treated was 1.08g of paste per 20 kg bodyweight, i.e. very close to the target dose (1g per 20 kg bodyweight). Thus when administered, the target specific doses were largely achieved.

The treatment was highly effective in reducing strongylid faecal egg counts which were zero for all treated horses. This is consistent with the very low numbers of adults in treated horses at slaughter.

This dose rate of oxibendazole (10mg/kg) has been demonstrated to be highly effective (93-100%) against susceptible luminal stages of *Strongylus* spp., cyathostomes, *O. equi* and *P. equorum* in horses (Kates *et al.*, 1975; Nawalinski and Theodorides, 1977; Drudge *et al*, 1981; Lyons *et al.*, 1981;Tolliver *et al.*, 1993; Burr *et al.*, 1994; Kivipelto and Asquith 1997). Thus similar activity on these nematodes was expected from this formulation.

There is no published efficacy data for abamectin against horse parasites except for one brief report (Martin *et al.*, 1999) which does not provide specific data but claimed

comprehensive efficacy against all stages of all nematodes. There is considerable data for the related anthelmintic, ivermectin. Ivermectin paste used alone at 0.2mg/kg was found to be highly effective (>97%) against luminal stages of cyathostomes, *Strongylus* spp., *Triodontophorus* spp. and *Oxyuris equi* larvae and is also highly effective against *Gasterophilus intestinalis* larvae (Klei *et al.*, 1993; Xiao *et al.*, 1994; French *et al.*, 1994; Cobra *et al.*, 1995; Costa *et al.*, 1998). If the efficacy of abamectin is similar to ivermectin then a similar high efficacy could be expected with abamectin.

In this experiment, the combination of abamectin and oxibendazole was highly effective in eliminating the luminal stages of common gastrointestinal parasites of horses including adult *S. edentatus* 100%, *S. vulgaris* 100%, *Triodontophorus* spp 100%, *Trichostrongylus axei,* 99.7%, *Oxyuris equi* larvae 100% and *Gasterophilus intestinalis* larvae 99.4% which is consistent with the activity of either component when used alone.

Pooled efficacy against adult cyathostomes (*Cyathostomum* spp., *Cylicocyclus* spp. and *Cylicostephanus* spp.) was 99.9%, and for luminal larval stages of cyathostomes (*Cyathostomum* spp., *Cylicocyclus* spp. and *Cylicostephanus* spp.) the pooled efficacy was 98.7%. The small burden of intestinal larvae in treated horses may be related to the re-emergence of encysted cyathostomes in the 9-10 days period between treatment and necropsy. This level of efficacy is also consistent with the activity of either component when used alone.

Efficacy was poor against *S. vulgaris* and *S. edentatus* present in the cranial mesenteric artery and beneath the parietal peritoneum of the abdominal wall at the time of treatment. *S. vulgaris* were recovered from the cranial mesenteric artery in all horses, and all showed visible evidence of verminous arteritis at this location. Similarly, *S. edentatus* were recovered from the sub-peritoneal sites of all horses. However, ivermectin at 0.2mg/kg has been shown to be highly effective against migrating strongylids (Herd, 1992; Slocombe *et al.*, 1982). In contrast, oxibendazole at 10mg/kg was found to be inactive against migrating larvae of strongylids (Kates *et al.*, 1975; Lyons *et al.*, 1981). Therefore, the results of this suggest that abamectin is less effective against migrating larvae of *Strongylus* spp. than ivermectin but it is extremely difficult to assess the effect of the test product against the migrating larvae because necropsies were performed relatively early (9 - 10 days after treatment) and larvae killed by the anthelmintic drug may still have been found in their location. This is easier to understand for *S. edentatus* than *S. vulgaris* as it might have been

thought that killed *S. vulgaris* would have been removed from the cranial mesenteric artery by this time.

Lower numbers of encysted cyathostome larvae were recovered from treated horses compared with control horses, and the difference was significant ($p<0.05$). However, an efficacy of only 64.8% was obtained against these encysted mucosal cyathostomes when calculated using counts made by digestion. As for efficacy against luminal adults, there are no published studies reporting efficacy of abamectin against encysted mucosal stages of cyathostomes but there are several for ivermectin. This relatively low efficacy is similar to that reported by Love *et al.*, (1995) who showed that ivermectin (0.02mg/kg) was 77% effective against mucosal larval stages of cyathostomes in horses but is higher than some other studies which have shown that ivermectin (0.02mg/kg) has little or no efficacy against encysted cyathostome larvae (Herd, 1992; Eysker *et al.*, 1992; Klei *et al.*, 1993; Xiao *et al.*, 1994). There are very few reports of efficacy of oxibendazole against encysted cyathostome larvae. In one, Kates *et al.* (1975) showed that oxibendazole (10mg/kg) was only 66% effective against mucosal cyathostome larvae. The results in this current experiment suggest that this combination of anthelmintics is not very effective against encysted cyathostomes and the efficacy achieved was no greater with the combination than the expected activity of either active used alone. One potential problem in interpretation is that killed larvae may still be intact and *in situ* as the time between treatment and necropsy was relatively short, so that some larvae killed by the anthelmintic drug may still have been found and counted in the mucosa.

The numbers of developing mucosal stages were always higher after digestion than observed by transillumination, in both groups of horses. This is consistent with the reports of others (Murphy and Love, 1997). The main reason for this is that very small inhibited and developing stages cannot be seen by transillumination.

Bithionol was included to provide efficacy against *Anoplocephala*. One report from Japan showed that 5mg/kg of bithionol paste was 86.7% effective against *Anoplocephala perfoliata* in horses (Takahashi, K. unpublished report,1960). The efficacy of 88.4% in the present experiment using 5mg/kg bithionol is remarkably similar to this earlier Japanese report. The appearance of transient diarrhoea is consistent with other reports of studies with bithionol in horses (Takahashi, K. unpublished report).

In summary, the results of this experiment showed that the combination anthelmintic AOB 198 which included abamectin (0.2mg/kg), oxibendazole (10mg/kg) and

bithionol (5mg/kg) was highly effective against luminal stages of *Strongylus* spp., cyathostomes, *Triodontophorus* spp., *Trichostrongylus axei, Oxyuris equi* and *Gasterophilus* spp. but less effective against encysted cyathostomes and showed poor efficacy against migrating stages of *S. vulgaris* and *S. edentatus*.

It appears that the dose of 5mg/kg bithionol provides useful control against *Anoplocephala*, but is less than the dose required for a full efficacy claim (>95%).

Therefore it was proposed to use a revised formulation, to deliver a dose increased by 40% to 7.0mg/kg bithionol, in the subsequent Principal Efficacy Study.

CHAPTER THREE

Principal Efficacy Study

Efficacy of an Anthelmintic Paste against Natural Nematode and Tapeworm Infections in Horses:

3.1. Introduction

Following the efficacy study reported in Chapter 2 (Dose Confirmation Study; DCS), it was decided that the dose rate for bithionol should be increased to 7mg/kg. Consequently this study was undertaken with the same combination of oxibendazole and abamectin but with an increased dose of bithionol. It was also designed to determine the efficacy after a longer period of 20 days between treatment and slaughter than the interval of 9-10 days in the DCS. Another change from the DCS was the use of a more palatable paste.

3.2 Materials and methods

3.2.1 Animals

Twelve (12) horses were used for this study. They were sexually mature, mixed age males. Their health status was normal and their weight range was 321 to 395 kg. They were wild herd captures, grazed together on their feral range and since capture (in July 1997) and were part of the same group of horses used in the DCS. They had grazed together with these horses and continued to be grazed after that trial in the same paddocks. These horses also had never received anthelmintics.

3.2.2 Test Site

This was as described in section 2.2.2.

After treatment all horses were placed on pasture not previously grazed by horses for least one year except that the same paddock was used for the post-treatment period of the DCS, some 6 months earlier.

3.2.3 Test Substance

The test substance was supplied by Virbac Laboratories (NZ) Ltd. accompanied by a certificate of analysis, batch number, manufacture date, Material Safety Data

Sheet, storage conditions, handling and safety precautions. The anthelmintics were combined in a paste with a total volume of 30mls and coded AOB 698. The composition of AOB 698 is as follows:

a.i.	**Dosage**	**Total formula content per tube**
abamectin	0.2 mg/kg	120 mg abamectin
oxibendazole	10 mg/kg	6 g oxibendazole
bithionol	7 mg/kg	4.2 g bithionol
total actives		10.32 g
excipients to		30 g

Each 30ml tube contains treatment for a 600 kg horse. Syringes of the test product are marked with 2.5 ml gradations with each of these gradations equivalent to a dose for 50kg body weight which gives a dose of 1g/20kg AOB698.

3.2.4 Study Design

This study was a controlled study with 6 animals per group. Six animals were allocated to the Treated Group, and six to the Control Group. Allocation was performed on paired means of pretreatment faecal egg counts from Day -14 and Day -3, and individuals within each pair were randomised to either the Treated Group or Control Group. As age could not be accurately determined, this was not taken into account. Egg counts were also estimated on Day 0 and Day 20 of the study.

Moreover, the animals were weighed on an electronic scale before the commencement of the study (Day –14 and Day –3), and again just prior to dosing with the anthelmintic paste AOB 698 on Day 0.

The Treated Group animals were treated with the anthelmintic paste AOB 698 on Day 0 at the rate of 1g per 20 Kg body weight.

On Day 19 all animals were transported on a horse float to Bombay Pet Foods facility, for slaughter and necropsy. All horses were killed on Day 20 post treatment at the same facility as in the DCS.

The efficacy of the compound was determined as for the DCS.

No drug or vaccine in addition to the experimental treatments had been

administered during the study period.

3.2.5 Treatment Regime

Each animal of the Treated Group was treated once orally from the 30 ml syringes of test substance. A piece of 20 cm long and 0.5 cm diameter pre-filled plastic tube fitted as an extension to the syringe was used for the administration of the test products. This extension tube held a constant volume before and after treatment thus its volume could be discounted for the dose give.

The dose for each Treated Group horse was calculated from the weight recorded immediately prior to treatment. Syringes were weighed before and after use, to determine loss of weight due to product expressed. Doses (ml) were recorded for each horse in the Treated Group.

To aid delivery and acceptance of the test product, apple pulp was given before treatment, by a separate syringe. This test formula (AOB 698) is strongly apple flavored, almost sickly sweet to humans, with a mildly sour aftertaste in an attempt to improve its palatability.

The Control Group of animals remained untreated.

3.2.6 Collection of Samples and Observations

This was as described in section 2.2.6 and in Appendix I except that faecal samples were collected directly from the rectum on Days –14, -3, 0 and 20 of the study time (Table 3.1).

Another minor change was that the proximal 30cm of the ventral colon was not ligated and separated from the rest of the ventral colon. So the whole of the ventral colon was examined together.

3.2.7 Statistical Evaluation

This was as described in section 2.2.7.

3.3 Results

3.3.1 Anthelmintic drenched dose (Dose given)

After nominal allowance for spillage, the average AOB 698 dose rate appears to be 1.04g/20kg body weight compared to the target 1g/20kg body weight. In spite of all

measures taken, there was some difficulty in ensuring that these uncooperative horses swallowed all treatment. The actual average dosage was therefore likely to be not greater than target (7mg/kg of bithionol). Details of doses actually are given in Appendix IV.

3.3.2 Clinical observations:

On collection of faecal samples per rectum immediately prior to treatment, it was apparent that most horses had relatively soft faeces, which may be related to the fresh grass on which they were feeding.

After being treated on Day 0, the Treated and Control horses as a group were observed to be feeding well and their behavior was normal.

On Day 1, both groups of horses looked normal when observed in the paddock, bearing in mind the typical nervous disposition of the group.

On Day 2, many relatively soft faeces were found, and a few that were splattered. Only about 20% of faecal deposits were of a reasonably formed, normal consistency.

In contrast to the previous study on similar horses, no parasites were apparent grossly on observing these faeces.

When observed on Day 3 and subsequent days, both groups of horses appeared normal, were feeding well and their faeces were also of normal consistency.

In evaluating the significance of the soft faeces observed, the fresh paddock, and thus even more fresh feed which these horses were obliged to graze following treatment, should be taken into account.

One incident on Day 0 is reported. Temporary in-coordination after being held in the crush was observed in one horse(A3), immediately after treatment. This is likely to be related to pressure on peripheral nerves whilst held in the crush and thus this is not considered to be an effect of the test drug.

In other respects all horses appeared normal and were feeding well.

No other adverse reactions were observed. In particular no signs of irritability or skin irritation were seen in any horses, either around the muzzle immediately following treatment, or over the body during the succeeding days.

3.3.3 Parasitological findings

3.3.3.1 Strongylid Egg Counts

Table 3.1: Faecal strongylid egg counts of the Control Group and the Treated Group horses.

Horse No.	Eggs per Gram				
	Day -14	Day –3	AM♦	Day 0	Day 20
Control Horses					
B13	700	800	750	1000	1100
Y10	700	800	750	550	750
A4	650	550	600	600	650
A2	550	400	475	350	450
A6	600	400	500	650	550
Y2	400	300	350	300	400
AM	580	500		490	560
Treated Horses					
A5	750	850	800	1000	0
Y7	700	600	650	750	0
Y1	500	600	550	450	0
A3	350	450	400	300	0
Y8	500	400	450	600	0
A1	450	350	400	350	0
AM	500	480		490	

AM = arithmetic mean of group; AM♦ = arithmetic mean of Day –14 and Day -3 by horse; Statement sequence within groups is as allocated from pairs.

Table 3.1 shows the faecal strongylid egg count results for Control and Treated Groups horses. On Day 0, roughly equivalent strongylid egg counts were estimated in faecal samples from both Control and Treated Groups.

On Day 20, strongylid faecal egg counts for all control horses were very similar to Day 0. In contrast egg counts were zero in all treated horses on Day 20.

Efficacy of egg count reduction after 20 days was 100% for the treated horses ($p<0.001$).

3.3.3.2 Parasites counts

3.3.2.2.1 Non-cyathostome luminal parasite counts

Table 3.2 shows non-cyathostome luminal parasite numbers found in Control and Treated Groups of horses with a summary of efficacy based on arithmetic mean counts shown in Table 3.3. Adult *Strongylus edentatus* were found in moderately high numbers in the lumen of all Control Group horses but were absent in the Treated Group horses. In addition, adult *S. vulgaris* were found in the lumen of all Control Group horses except in horse A2, whereas they were absent in all Treated Group horses, Therefore, 100% ($p<0.01$) efficacy was demonstrated against *S. vulgaris* and *S. edentatus* adults in the intestinal lumen.

Adult luminal *Triodontophorus spp.* were present in very low numbers in three Control Group horses but were not found in the Treated Group horses. Therefore it is considered that AOB 698 was 100% effective against adult *Triodontophorus spp.* but statistically it was not significant ($p= 0.09$) because only 3 control horses contained low numbers of this genus.

Large numbers of *T. axei* were recovered from all Control Group horses, whereas *T. axei* were not found in Treated Group horses. The efficacy of AOB 698 was 100% ($p<0.01$) against *T. axei*.

Fourth stage *Oxyuris equi* larvae were obtained from one horse in very low numbers (2) in the Control group, but none were seen in the Treated group horses. Although this is not a reasonable evaluation of efficacy, again it is consistent with 100% efficacy against *Oxyuris equi* larvae. This difference was not significant ($p>0.05$), because only one Control horse contained very low numbers of this species.

Table 3.2: Non-cyathostomine nematodes recovered from the intestinal lumen at necropsy from the Control Group and the Treated Group horses.

Horse No.	Name of the Parasites							
	***Strongylus edentatus* adults**	***Strongylus vulgaris* adults**	***Triodontophorus* spp. adults**	***Trichostrongylus axei* adults**	***Oxyuris equi* larvae**	***Anoplocephala perfoliata***	***Gasterophilus intestinalis* larvae**	***G. nasalis* larvae**
Control								
B13	188	227	0	63620	0	85	61	0
Y10	195	66	19	8530	2	128	110	1
A4	133	62	0	8070	0	84	109	0
A2	144	0	2	28930	0	181	110	0
A6	219	72	0	25370	0	200	161	0
Y2	36	140	21	17610	0	58	49	0
Treated								
A5	0	0	0	0	0	0	0	0
Y7	0	0	0	0	0	0	0	0
Y1	0	0	0	0	0	0	0	0
A3	0	0	0	0	0	0	0	0
Y8	0	0	0	0	0	0	0	0
A1	0	0	0	0	0	0	0	0

Table 3.3 Arithmetic and geometric means of luminal non-cyathostome parasites recovered from the Control Group and the Treated Group horses; efficacy by species.

Parasites	Control horses		Treated Horses	Efficacy		*p* value
	AM	GM		AM%	GM%	
Anoplocephala perfoliata	123	111	0	100%	100%	0.001
Strongylus edentatus	153	133	0	100%	100%	0.001
Strongylus vulgaris	94.5	46	0	100%	100%	0.007
Triodontophorus spp	7	3	0	100%	100%	0.092
Trichostrongyles axei	25355	19594	0	100%	100%	0.001
Oxyuris equi larvae	0.3	1	0	100%	100%	0.415
Gasterophilus intestinalis larvae	100	93	0	100%	100%	0.001

AM = Arithmetic mean; GM = Geometric mean

Gasterophilus intestinalis larvae were found in the stomach of all Control Group horses, but none were found in Treated Group horses. The efficacy of this compound was 100% ($p<0.01$) against gastric stages of *G. intestinalis*. One *Gasterophilus nasalis* larva was found in the stomach of one Control horse (Y10) but none were seen in the Treated Group horses. Although this is not a reasonable evaluation of efficacy, it is consistent with 100% efficacy against gastric stages of *Gasterophilus nasalis*.

Anoplocephala perfoliata (many mature as well as immature tapeworms) were recovered from all Control Group horses in large numbers, whereas these tapeworms were totally absent in Treated Group horses. As a result, AOB 698 gave an efficacy of 100% ($p<0.01$) against *A. perfoliata*.

3.3.3.2.2 Luminal cyathostome nematode counts

Table 3.4 shows the number of adult cyathostomes (*Cyathostomum* spp., *Cylicocyclus* spp., *Cylicostephanus* spp.) parasites and developing stages of larvae recovered after necropsy from the intestinal lumen of both Control and Treated Groups. To define the numbers of each genus within the Subfamily Cyathostominae, at least 100 adult cyathostomes were differentiated from each horse.

Table 3.4 Number of cyathostome (small strongyles) recovered at necropsy from the Control Group and the Treated Group horses.

Horse No.	Name of the Parasite	Number of Adult♦ Parasites	No. of L4
Control Horses			
B13	*Cyathostomum* spp.	7364	4734
	Cylicocyclus spp.	22616	6836
	Cylicostephanus spp.	7890	3156
Y10	*Cyathostomum* spp.	10518	3506
	Cylicocyclus spp.	40324	11396
	Cylicostephanus spp.	18408	3508
A4	*Cyathostomum* spp.	9168	5159
	Cylicocyclus spp.	22924	10316
	Cylicostephanus spp.	6876	2865
A6	*Cyathostomum* spp.	11030	5515
	Cylicocyclus spp.	48530	15442
	Cylicostephanus spp.	22060	7723
A2	*Cyathostomum* spp.	2036	674
	Cylicocyclus spp.	2910	1352
	Cylicostephanus spp.	2324	384
Y2	*Cyathostomum* spp.	9568	2733
	Cylicocyclus spp.	13668	5467
	Cylicostephanus spp.	10024	4100
Treated Horses			
A5	*Cyathostomum* spp.	447	1699
	Cylicocyclus spp.	625	3218
	Cylicostephanus spp.	268	2683
Y7	*Cyathostomum* spp.	139	800
	Cylicocyclus spp.	206	1256
	Cylicostephanus spp.	35	1044
Y1	*Cyathostomum* spp.	124	1360
	Cylicocyclus spp.	185	2712
	Cylicostephanus spp.	121	1668
A3	*Cyathostomum* spp.	85	767
	Cylicocyclus spp.	82	1835
	Cylicostephanus spp.	43	1448
Y8	*Cyathostomum* spp.	0	0
	Cylicocyclus spp.	0	0
	Cylicostephanus spp.	0	0
A1	*Cyathostomum* spp.	190	2090
	Cylicocyclus spp.	285	3895
	Cylicostephanus spp.	285	2755

♦ Includes immature and mature adults

Overall efficacy against luminal stages of cyathostomes is summarised in Table 3.5. Large numbers of luminal cyathostome adults were recovered from Control Group horses, while very low numbers were recovered from Treated Group horses. These results show that AOB 698 was highly effective (98.0 to 99.1%; $p<0.01$) against

adult luminal stages of cyathostomes (*Cyathostomum* spp., *Cylicocyclus* spp., *Cylicostephanus spp.*).

Table 3.5: Arithmetic and geometric mean numbers of cyathostomes recovered from the intestinal lumen of the Control and Treated Group horses.

Name of the Parasites	**Control**		**Treated with AOB 698**		**Efficacy**		***p*-value**
	AM	GM	AM	GM	AM%	GM%	
Adult♦							
Cyathostomum spp.	8281	7311	164	71	98.0	99.0	0.001
Cylicocyclus spp.	25162	18520	232	91	99.1	99.5	0.001
Cylicostephanus spp.	11264	8950	126	55	98.9	99.4	0.001
Larvae•							
Cyathostomum spp.	3721	3093	1119	379	69.9	87.7	0.020
Cylicocyclus spp.	8469	6721	2153	654	74.6	90.3	0.013
Cylicostephanus spp.	3623	2702	1600	515	55.8	80.9	0.020

♦ = Includes immature and mature adults. • = Includes L4.

High numbers of *Cyathostomum* spp., *Cylicocyclus spp.* and *Cylicostephanus* spp. developing fourth stage larvae were present in the Control Group horses while relatively low numbers of larvae were present in the Treated Group horses. The efficacy against these three genera was 69.9 %, 74.6 % and 55.8% respectively, which were all significantly different ($p<0.05$) from the control untreated group.

On the other hand, when the efficacy is calculated using the geometric mean reductions are in the range 80.9 % to 90.3%.

3.3.3.2.3 Mucosal cyathostome nematodes counts

Two different methods, viz. digestion and transillumination techniques, were used to count cyathostome larval stages in the mucosa. Table 3.6 shows the results of mucosal counts of individual horses for both the Control and Treated Group of horses, by both counting methods. Significantly higher numbers of larvae ($p<0.01$) were present in the ventral colon than in either the caecum or dorsal colon. The numbers of mucosal stages were lower in the Treated Group than in the Control Group.

Table 3.6: Larvae recovered at necropsy after transillumination and digestion methods of counting.

Horse No.	Transillumination				Mucosal Digestion			
	Caecum	Ventral colon	Dorsal colon	Total	Caecum	Ventral colon	Dorsal colon	Total
Control								
B13	18700	35300	5260	59260	21000	37940	5700	64640
Y10	11180	34840	6900	52920	12080	36340	7880	56300
A4	19600	37700	2920	60220	24940	40100	3100	68140
A6	20380	33120	3900	57400	22200	34140	4860	61200
A2	26240	36220	12340	74800	28020	37740	12580	78340
Y2	11840	16860	6740	35440	12320	22220	7160	41700
AM	17990	32340	6343	56673	20093	34747	6880	61720
Treated								
A5	8440	10620	140	19200	9420	12920	280	22620
Y7	10600	6420	2720	19740	11540	7160	2760	21460
Y1	8100	12900	1880	22880	9500	13340	1920	24760
Y8	3060	12600	220	15880	3980	13540	320	17840
A3	7200	9580	2160	18940	8240	11940	2200	22380
A1	9940	10720	1000	21660	11060	12060	1100	24220
AM	7890	10473	1353	19717	8957	11827	1430	22213

AM = arithmetic mean

Comparatively, the digestion technique gave higher counts than the transillumination technique in all intestinal regions in the Treated Group than in the Control Group and it was significant different ($p<0.05$). After digestion the larvae were damaged. Therefore, it was not possible to speciate the mucosal stages of larvae.

In Table 3.7 comparing arithmetic means, a moderate efficacy against encysted larvae, of 64.0% ($p<0.01$), was detected by the mucosal digestion technique where as an efficacy of 65.2% was demonstrated by the transillumination technique. There was a significant difference ($p<0.05$) in numbers of encysted larvae recovered at necropsy between Treated and Control Groups horses by both techniques.

Table 3.7: Means of cyathostome larvae recovered from the intestinal mucosa of control and treated horses by mucosal digestion or transillumination.

Method	Control		Treatment		Efficacy		*p* value
	AM	GM	AM	GM	AM%	GM%	
Mucosal digestion	61720	60610	22213	22091	64.0	63.55	0.001
Transillumination	56673	55344	19717	19588	65.2	64.6	0.001

AM = arithmetic mean; GM = geometric mean

3.3.3.2.4 Peritoneal and arterial parasite stages

Table 3.8 and Table 3.9 show the number of migrating stages of *S. vulgaris* recovered from the cranial mesenteric artery including the nodules on the intestinal wall and *S. edentatus* recovered from sub-peritoneal sites together with a statistical evaluation of efficacy of AOB 698.

In this study, migrating stages of *S. vulgaris* were present in the cranial mesenteric artery of all horses except one Control Group horse. The inflammatory lesions found in the cranial mesenteric artery provided visual evidence of verminous arteritis at this location for both the Control and Treated Groups horses. Nodules were found in the walls of the caecum and ventral colon, some of which contained migrating stages of *S. vulgaris* in both groups of horses. From 35 to 109 (arithmetic mean 51) of these nematodes were recovered from the cranial mesenteric arteries and nodules from the caecum and v. colon of the Control Group horses, and 2-50 (arithmetic mean 15) were recovered from the Treated Group horses.

Migrating stages of *S. edentatus* were recovered from retro-peritoneal sites in the abdominal wall just beneath the parietal peritoneum of all Control Group horses. From 3 to 33 (arithmetic mean 13) nematodes were recovered from the sub-peritoneal sites of Control Group horses. In Treated Group horses these larvae were absent and even degenerated larvae were not seen.

Table 3.8: Numbers of migrating *Strongylus* spp. recovered from arterial dissections and sub-peritoneal sites of the Control Group and the Treated Group horses.

Horse No.	Organ	*Strongylus vulgaris*	Organ	*Strongylus edentatus*
Control				
B13	CMA	78	Sub-peritoneal sites	3
Y10	CMA	35	Sub-peritoneal sites	50
A4	CMA	37	Sub-peritoneal sites	7
A6	CMA	46	Sub-peritoneal sites	11
A2	CMA	0	Sub-peritoneal sites	2
Y2	CMA	109	Sub-peritoneal sites	3
Treated				
A5	CMA	3	Sub-peritoneal sites	0
Y7	CMA	14	Sub-peritoneal sites	0
Y1	CMA	27	Sub-peritoneal sites	0
Y8	CMA	33	Sub-peritoneal sites	0
A3	CMA	11	Sub-peritoneal sites	0
A1	CMA	0	Sub-peritoneal sites	0

CMA = cranial mesenteric artery including the nodular *Strongylus*.

Therefore, AOB 698 was only 70.6% effective in killing *S. vulgaris* in the mesenteric artery, but this reduction was significant (p=0.02). In contrast, AOB 698 was highly effective (100%; p<0.001) against *S. edentatus* in sub-peritoneal sites.

Table 3.9: Mean numbers of *Strongylus* spp. larvae recovered from arterial dissections and peritoneal sites

Organ	Parasites	Control		Treated		Efficacy		*p* value
		AM	GM	AM	GM	AM%	GM%	
Mesenteric artery	*Strongylus vulgaris*	51	28	15	9	70.6	67.9	0.020
Sub-peritoneal sites	*Strongylus edentatus*	13	6	0	0	100	100	0.001

AM = arithmetic mean; GM = geometric mean

3.4 Discussion

The test product AOB 698 anthelmintic was formulated with the same combination of actives of abamectin and oxibendazole but the dose of bithionol was increased to 7mg/kg from 5mg/kg than in AOB 198 which was used in the Dose Confirmation Study.

This product used an apple flavor to hide the bitterness of AOB 698 and increase the acceptance by the horse. However, during administration it was very difficult to assess the acceptance of this drug, because these were unbroken feral horses not used to being handled.

Loose faeces were observed from many of the study horses, before and after the time of treatment. This could be related mainly to the fresh (and wet) pasture available through this period, but a laxative effect from the treatment may have contributed. It is noted that a benign laxative effect has previously been reported for some horses following treatment with bithionol, usually at higher dosages (30mg/kg or greater) (Fukui, 1960; Akbaev, 1979) and was also seen in the DCS.

At 20 days after treatment, faecal egg counts were zero for all treated horses. Thus the efficacy in reduction of strongylid egg counts was 100% despite there being a small burden of adult cyathostomes which were apparently not producing many, if any eggs.

The tested product was highly effective in eliminating the common adult gastrointestinal parasites of horses including *S. edentatus, S. vulgaris* and *T. axei.* It was also highly effective against gastric stages of *G. intestinalis.* These results were essentially the same as those of the DCS results and were as expected.

The formulation was highly effective against adult luminal cyathostomes. The efficacy against *Cyathostomum* spp., *Cylicocyclus* spp., *Cylicostephanus* spp. ranged from 98.0 to 99.1%. However, it was less effective against luminal larval stages of cyathostomes where the efficacy ranged from 55.8% to 74.6%. The presence of more larvae and adults in this study compared to the DCS is likely to be related to the emergence of encysted cyathostome larvae from the mucosa between the time of treatment and necropsy at Day 20. As activity against mucosal larvae is limited, it may be assumed that fourth-stage larvae will have progressively re-established in the lumen of the gastrointestinal tract as soon as effective levels of

drugs were no longer present. The efficacy achieved against luminal larvae and adults in the DCS was ≥98.7% and ≥99.9% respectively which is likely to be a fairer measure of efficacy against these stages. There are many references (see Section 1.2) which show that other avermectins including ivermectin, and oxibendazole are 99-100% and 94.2-100% effective respectively against luminal stages of adult and larval cyathostomes.

Although an insufficient number of fourth stage *Oxyuris equi* larvae were recovered from control horses to properly evaluate efficacy of AOB 698, the result is consistent with 100% efficacy against *O. equi* larvae. The earlier assessment of AOB 198 in the DCS also supported 100% efficacy against *O. equi* larvae. As previous studies support high efficacy for oxibendazole, and other avermectins including ivermectin against larval stages of *O. equi,* good control of larvae and adults should be expected (Klei *et al.* 1993; French *et al.*, 1994; Cobra *et al.*, 1995; Monahan *et al.*, 1996; Costa *et al.*, 1998).

For *G. intestinalis*, good efficacy was demonstrated for gastric stages which is consistent with the findings in the DCS with AOB 198. Activity against these stages, as well as for other species of *Gasterophilus*, was expected according to reported activities of the related anthelmintic, ivermectin (Britt and Preston, 1985; Tolliver *et al.,* 1993; Klei *et al.* 1993; French *et al.*, 1994; Cobra *et al.,* 1995; Costa *et al.,* 1998). Only one *Gasterophilus nasalis* larvae was found in one control horses but none in any of the treated horses, therefore no conclusions can be made about the efficacy of AOB 698 against this species because of the very low burden seen. Similarly examinations were not made for larvae in tongue and cheek tissues. So no conclusions can be made about the efficacy of AOB 698 against these stages.

Migrating stages of *S. vulgaris* were recovered from the cranial mesenteric artery in both group of horses, and all showed visual evidence of verminous arteritis at this location. In contrast all control horses contained migrating stages of *S. edentatus* in the area immediately beneath the parietal peritoneum of the abdominal wall, but none were found in treated horses. Ivermectin at 0.2mg/kg has been shown to be highly effective against all migrating *Strongylus* spp. (Herd, 1992 and Slocombe *et al.*, 1982) but oxibendazole at 10mg/kg has not been shown to be effective against these migrating stages of *Strongylus* spp. (Kates *et al.*, 1975; Lyons *et al.*, 1981). The observations in this experiment showed that this combination was highly effective against migrating stages of *S. edentatus* achieving an efficacy of 100% but

was less effective against migrating stages of *S. vulgaris* in the mesenteric artery and its branches where it only achieved an efficacy of 70.6%. These results contrast with the efficacy of AOB 198 during the Dose Confirmation Study which showed that it had no efficacy against migrating stages of *S. vulgaris* and *S. edentatus.* This discrepancy is likely to be due to the longer time interval between treatment and slaughter, which has probably allowed dead *S. edentatus* migrating stages larvae to be removed from the retro-peritoneal fat which did not occur in the DCS. The result from *S. vulgaris* migrating stages in the cranial mesenteric artery is likely to be a true reflection of the efficacy of AOB 698. This is surprising given the high efficacy of the related avermectins against these stages and indicates there are some differences in efficacy between ivermectin and abamectin in horses. Abamectin (0.2mg/kg) would not appear to be very effective in killing migrating stages of *S. vulgaris.*

Lower numbers of encysted larvae were recovered from treated horses compared with control horses, and the difference was statistically significant ($p<0.01$). As assessed by mucosal digestion, an efficacy of 64% was obtained against encysted mucosal cyathostomes at 20 days after treatment. The experiment recorded that the efficacy against non-luminal cyathostome nematodes is remarkably consistent between the DCS (at ~65 %) and this study, at 64 %. As discussed previously this is similar to the efficacy of 77% achieved using ivermectin (0.2mg/kg) reported by Love *et al.*, (1995) and higher than other studies using ivermectin (0.2mg/kg) (Herd, 1992; Eysker *et al.* 1992; Klei *et al.*, 1993 and Xiao *et al.*, 1994). Oxibendazole was unlikely to contribute much to killing these stages as a previous report had shown poor efficacy against mucosal stages of cyathostomes (Kates *et al.*, 1975). It would appear that AOB 698 is possibly slightly more effective in killing mucosal stages of cyathostomes than ivermectin (0.2mg/kg).

As in the DCS the numbers of developing mucosal stages were different between the two techniques, probably because the mucosa contained very small larval stages which cannot be seen by transillumination, thus higher numbers are detected by digestion. This is similar to findings previously described by Murphy and Love (1997).

Efficacy was 100% against *Anoplocephala perfoliata* using 7mg/kg bithionol and is an improvement on the 88.2% efficacy obtained with the dose of 5 mg/kg bithionol in the Dose Confirmation Study. This result is supported by the few Japanese

unpublished reports which showed high efficacy (100%) against *Anoplocephala* spp using 7mg/kg of bithionol.

In summary, the results of this study and the Dose Confirmation Study show that the combination of abamectin (0.2mg/kg), oxibendazole (10mg/kg) and bithionol (7mg/kg) anthelmintic paste as used was highly effective against luminal stages of adult *Strongylus vulgaris, Strongylus edentatus,* cyathostomes, *Trichostrongylus axei* and *Anoplocephala perfoliata* and larval stages of retro-peritoneal migrating stages of *S. edentatus,* and gastric stages of *Gasterophilus* spp. larvae, but less effective against mucosal stages of cyathostomes and arterial stages of *S. vulgaris*. In case of *Triodontophorus* spp. and *Oxyuris equi* larvae, the efficacy was difficult to assess in the PES but the DCS show that this formulation was highly effective against them.

CHAPTER FOUR

Taxonomy

4.1 Introduction

Strongylidae are common parasites of horses and are a worldwide threat to the health and well-being of horses. The family Strongylidae have a well developed buccal capsule, a mouth collar with two leaf-crowns and a typical strongyloid copulatory bursa. They can be separated into two subfamilies: 1) Strongylinae and 2) Cyathostominae. The subfamily Strongylinae is separated again into four genera: *Strongylus, Oesophagodontus, Triodontophorus* and *Craterostomum*. This four genera system is accepted by most taxonomists. Nematodes in the genera *Strongylus*, *Triodontophorus* and *Oesophagodontus* have been reported in New Zealand (Tetley, 1948).

The subfamily Cyathostominae consists of eight genera and more than 41 species and these are the most difficult to identify for inexperienced persons. The following characteristics are important for identification; the mouth collar, cephalic papillae, internal leaf-crowns (ILC), external leaf crowns (ELC), extra-chitinous supports of the ELC, buccal capsule shape and size and presence of a oesophageal funnel. The 8 genera of Cyathostominae of horses are *Cyathostomum* (Molin, 1861), *Cylicocyclus* (Ihle,1922), *Cylicodontophorus* (Ihle, 1922), *Cylicostephanus* (Ihle, 1922), *Poteriostomum* (Quiel, 1919), *Gyalocephalus* (Looss, 1900), *Caballonema* (Abuladze, 1937) and *Cylindropharynx* (Leiper, 1911).

The aim of this experiment was to identify which nematodes were present in New Zealand horses as represented by horses in the two efficacy studies. In this chapter the author discusses and identifies those parasites which were found during the Dose Confirmation Study (Chapter 2) and the Principal Efficacy Study experiments (Chapter 3).

4.2 Materials and methods

Nematodes from three control horses from each of the Dose Confirmation Study (Chapter 2) and the Principal Efficacy Study (Chapter 3) were used for species identification. The horses were killed during autumn (May) in the DCS trials whereas the PES horses were killed in summer (December). Twenty cyathostome nematodes were identified to species level from each organ in each horse. From the Principal

Efficacy Study the 3 treated horses with the largest cyathostome burdens were also used for species identification and from caecum, ventral colon and dorsal colon of these horses at least 20 cyathostome parasites were identified to species level.

Each specimen was studied in a temporary wet mount. They were mounted and cleared in lactophenol and examined using an interference contrast microscope (Olympus, BH 2).

Calculation and statistics: Proportion was calculated with the number of species in each part divided by the total number of parasites examined. Statistical analysis was done with the Rank Sum test using the Mann-Whitney U statistic for comparison between the species present in the different parts of the large intestine and between the prevalence of the parasites in two trials.

4.3 Identification

The taxonomy of the species of Cyathostominae has been revised (Lichtenfels and Klei, 1988). However, since these changes have no direct bearing on the result of this study, for identification the names of the species as used here are from Lichtenfels (1975) and Popova (1958, English translation, 1965).

Specimens were identified from the descriptions given by Popova (1958, English translation, 1965) and Lichtenfels (1975). The identifying characteristics summarised below are based on these descriptions. For a few species there were some differences in identifying features from the published descriptions and these are noted.

4.3.1 Genus: *Strongylus*

These are large stout nematodes. The buccal capsule is globular, deeper than wide and the greatest diameter is near the middle.

4.3.1.1 Species: *Strongylus vulgaris* (Fig. 4.1a,b)

Ten specimens were identified as *S. vulgaris*. These were typical of the species as described. They were 1.5 to 2.5 cm long. Identifying features included: prominent rounded teeth present in the buccal cavity with lobes of the teeth high and smooth, extending almost one-half the depth of the buccal cavity; the ILC about one-fourth as long as the ELC.

4.3.1.2 Species: *Strongylus edentatus* (Fig. 4.2a,b)

Fourteen specimens were identified as *S. edentatus*. These were typical of the species as described. They were 2.0 to 4.5 cm long; buccal capsule globular in shape; leaf crown present; teeth absent in the buccal cavity.

4.3.2 Genus: *Triodontophorus*

These are medium sized nematodes. The buccal capsule is subglobular and three large teeth are present in the oesophageal funnel.

4.3.2.1 Species: *Triodontophorus serratus* (Fig 4.3a,b)

Seven specimens were identified. Identifying features included: mouth collar appearing in optical section as an inflated round tube in a ring around the mouth; female tail long; vulva separated from anus by approximately 1.5-3mm.

4.3.2.2 Species: *Triodontophorus tenuicollis* (Fig 4.4a,b)

Five specimens were identified. Identifying features included: mouth collar flattened; female tail short; vulva separated from anus by less than 1mm; dorsal lobe of bursa short.

4.3.2.3 Species: *Triodontophorus minor* (Fig 4.5a,b)

Three specimens were identified. Identifying features included: mouth collar flattened; dorsal lobe of bursa long; submedian papillae long, narrow and pointed.

4.3.3 Genus: *Cyathostomum*

The general features of *Cyathostomum* spp. are: cylindrical or ring-shaped buccal capsule which is not greatly elongated and not more than 1.5 times deeper than wide; anterior end of the oesophagus not greatly dilated; elements of internal leaf crown shorter; usually narrower and more numerous than elements of external leaf crown; lateral papillae usually not prominent; base of ILC quite posterior to anterior margin of buccal capsule; extra-chitinous supports of ELC present; buccal collar usually not depressed; buccal cavity of uniform diameter or wider anteriorly.

4.3.3.1 Species: *Cyathostomum coronatum* (Fig 4.6a,b)

There were 118 specimens identified as *C. coronatum*. The identifying features included: extra-chitinous supports of the ELC prominent and the ILC inserted in an even line around the buccal cavity; buccal cavity as deep as broad, walls thick and bent inwards near the middle; the ELC contains about 20 elongated petals. The nematodes identified in this study agreed with these descriptions.

4.3.3.2 Species: *Cyathostomum labiatum* (Fig 4.7a,b)

There were 36 specimens identified as *C. labiatum*. The identifying features included: the extra-chitinous supports of the ELC are spindle-shaped and prominent; the mouth collar is notched to form 4 distinct lips; the ELC contains 19 elements and the ILC 40-42 short elements; the buccal cavity much broader than deep with relatively straight walls; the excretory pore is near the junction of the middle and posterior thirds of the oesophagus. The nematodes identified as *C. labiatum* generally had most of these characteristics. However, some of the specimens in this study were different from the Popova (1958, English translation, 1965) and Lichtenfels (1975) description, they had the ELC which contained only 10 – 12 visible elements and the ILC contained only 14 – 16 visible elements; had a very small dorsal gutter and buccal capsules with very thick walls, but were still considered to be *Cyathostomum labiatum*.

4.3.3.3 Species: *Cyathostomum catinatum* (Fig 4.8a,b)

There were 27 specimens identified as *C. catinatum*. The identifying features included: extra-chitinous supports of the ELC not prominent; walls of buccal capsule thickened posteriorly; the ILC inserted more anteriorly on the lateral sides of the buccal capsule than on the dorsal and ventral sides, but not in a sinuous line; the ELC contains 21 elements. The nematodes identified as *C. catinatum* in this study generally agreed with these descriptions. However, in some of the specimens the ELC contained only 12 –14 visible elements; extra-chitinous supports were very short and in line with the walls of the buccal capsule.

4.3.4 Genus: *Cylicocyclus*

The general features of the genus are: the anterior end of oesophagus not dilated; buccal capsule not greatly elongated and not more than 1.5 times deeper than wide; elements of ILC shorter, usually narrower and more numerous than elements of ELC;

posterior margin of buccal capsule has a ring-like hoop-shaped thickening, lateral papillae usually large, broad and hom-like.

4.3.4.1 Species: *Cylicocyclus nassatus* (Fig 4.9a,b)

There were 186 specimens identified as *C. nassatus*. The identifying features included: buccal capsule not extremely shallow or delicate; both lateral papillae and ELC prominent, extending beyond the mouth collar; dorsal gutter present extending for one-half of the depth of the buccal cavity; submedian papillae long, extending beyond the mouth collar; the ELC consists of 20 elements; buccal capsule usually with internal shelf like cuticular projection. The nematodes identified as *C. nassatus* generally showed most of these characteristics. Other characteristics features observed and utilized in this study were: that the lateral papillae were round and quite distinct; and the walls of the buccal capsule were thin and relatively straight.

4.3.4.2 Species: *Cylicocyclus insigne* (Fig 4.10a,b)

There were 18 specimens identified as *C. insigne*. The identifying features included: buccal capsule not extremely shallow or delicate; dorsal gutter absent; lateral papillae not extremely long; excretory pore and cervical papillae near oesophago-intestinal junction; the ELC elements narrow, number about 38 in males and 44 in females; the ILC elements much shorter than the ELC elements and of uniform length. The nematodes identified as *Cylicocyclus insigne* in this study generally showed these characteristics.

4.3.4.3 Species: *Cylicocyclus leptostomus* (Fig 4.11a,b)

There were 12 specimens identified as *C. leptostomus*. The identifying features included: buccal capsule not extremely shallow or delicate; dorsal gutter absent; lateral papillae not extremely long; excretory pore and cervical papillae anterior to oesophago-intestinal junction; oesophageal funnel small; oesophagus long with posterior half enlarged and pyriform; oesophago-intestinal valve elongated; buccal cavity small; the ELC contains 20–24 elements; elements of the ELC almost as long as the buccal capsule is deep. The nematodes identified as *Cylicocyclus leptostomus* in this study generally showed these characteristics.

4.3.4.4 Species: *Cylicocyclus radiatus* (Fig 4.12a,b)

There were 30 specimens were identified as *C. radiatus.* The identifying features included: buccal capsule not extremely shallow or delicate; dorsal gutter absent; lateral papillae not extremely long; excretory pore and cervical papillae anterior to oesophago-intestinal junction; oesophageal funnel small; oesophagus long with posterior half enlarged and pyriform; oesophago-intestinal valve not elongated; buccal cavity large; the ELC contains 22-24 elements; elements of the ELC about one-third as long as buccal capsule is deep; ILC consists of small quadrangular elements. The nematodes identified as *Cylicocyclus radiatus* in this study generally agreed with these descriptions with the exception that the ILC elements were dome-shaped rather than quadrangular.

4.3.5 Genus: *Cylicostephanus*

The general features of the genus are: anterior end of oesophagus not dilated; buccal capsule not greatly elongated and not more than 1.5 times deeper than wide; mouth collar depressed; elements of ILC shorter, usually narrower and more numerous than elements of ELC; extra-chitinous supports of ELC absent; buccal cavity usually narrower anteriorly.

4.3.5.1 Species: *Cylicostephanus poculatus* (Fig 4.13a,b)

There were 41 specimens identified as *C. poculatus.* The identifying features included: buccal capsule much deeper than broad in lateral view; walls of buccal capsule much thicker posteriorly than anteriorly; ELC composed of about 36 elements. The nematodes identified a *Cylicostephanus poculatus* in this study generally showed these characteristics.

4.3.5.2 Species: *Cylicostephanus minutus* (Fig 4.14a,b)

There were 10 specimens identified as *C. minutus.* The identifying features included: buccal capsule much deeper than broad in lateral view; walls of buccal capsule nearly uniform thickness anteriorly and posteriorly; the ELC composed of about 8 elements with triangular and submedian papillae notched at a point one-half distance between the tips and the buccal collar; excretory pore and cervical papillae on one plane and near to the cephalic end. It was also noted that the excretory pore is located about three quarters of the distance down the oesophagus; around the base of the buccal capsule contains 3 plate-like teeth around the base.

4.3.5.3 Species: *Cylicostephanus calicatus* (Fig 4.15a,b)

There were 81 specimens identified as *C. calicatus.* The identifying features included: buccal capsule much deeper than broad in lateral view; walls of buccal capsule nearly uniform thickness anteriorly and posteriorly; the ELC composed of about 12-18 elements; submedian papillae notched near tips and cervical papillae near the excretory pore. The nematodes identified as *Cylicostephanus calicatus* in this study generally agreed with these characteristics with the exception that no cervical papillae were evident.

4.3.5.4 Species: *Cylicostephanus longibursatus* (Fig 4.16a,b)

There were 29 specimens identified as *C. longibursatus.* The identifying features included: buccal capsule nearly as broad or broader than deep in the lateral view; walls of the buccal capsule with slight compound curve slightly thicker anteriorly; elements of the ELC more than twice as long as broad; dorsal gutter extends halfway or less toward the base of the ILC and button like; elements of the ELC and the ILC in 1:1 ratio; dorsal ray of male bursa extremely long; female tail straight and teeth in oesophageal funnel not prominent. The nematodes identified as *Cylicostephanus longibursatus* in this study generally showed these characteristics.

4.3.5.5 Species: *Cylicostephanus goldi* (Fig 4.17a,b)

There were 56 specimens identified as *C. goldi.* The identifying features included: buccal capsule nearly as broad or broader than deep in the lateral view; walls of the buccal capsule with slight compound curve slightly thicker anteriorly; elements of the ELC more than twice as long as broad; dorsal gutter extends halfway or less toward base of the ILC and button like ; elements of the ILC almost twice as numerous as elements of the ELC; dorsal ray of male bursa not unusually long; female tail bent and prominent teeth in oesophageal funnel. The nematodes identified in this study as *C. goldi.* In addition, in the present study the specimens had a tear-shaped structure on the top of the buccal capsule lying with the ELC which should not be confused with the extra-chitinous support.

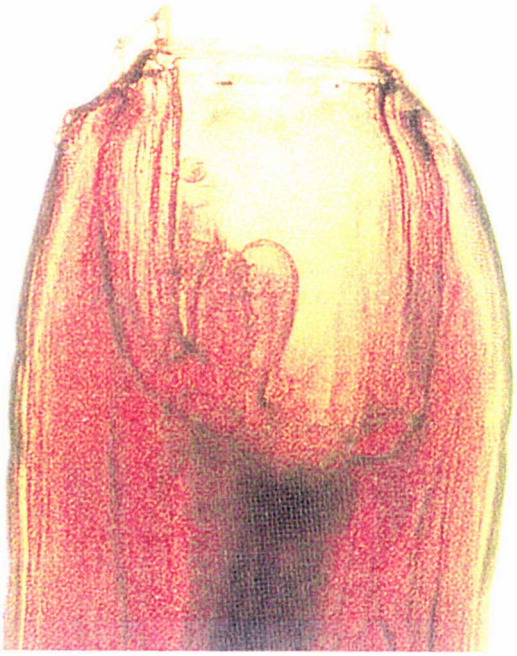


Figure No. 4.1a *Strongylus vulgaris* × 21

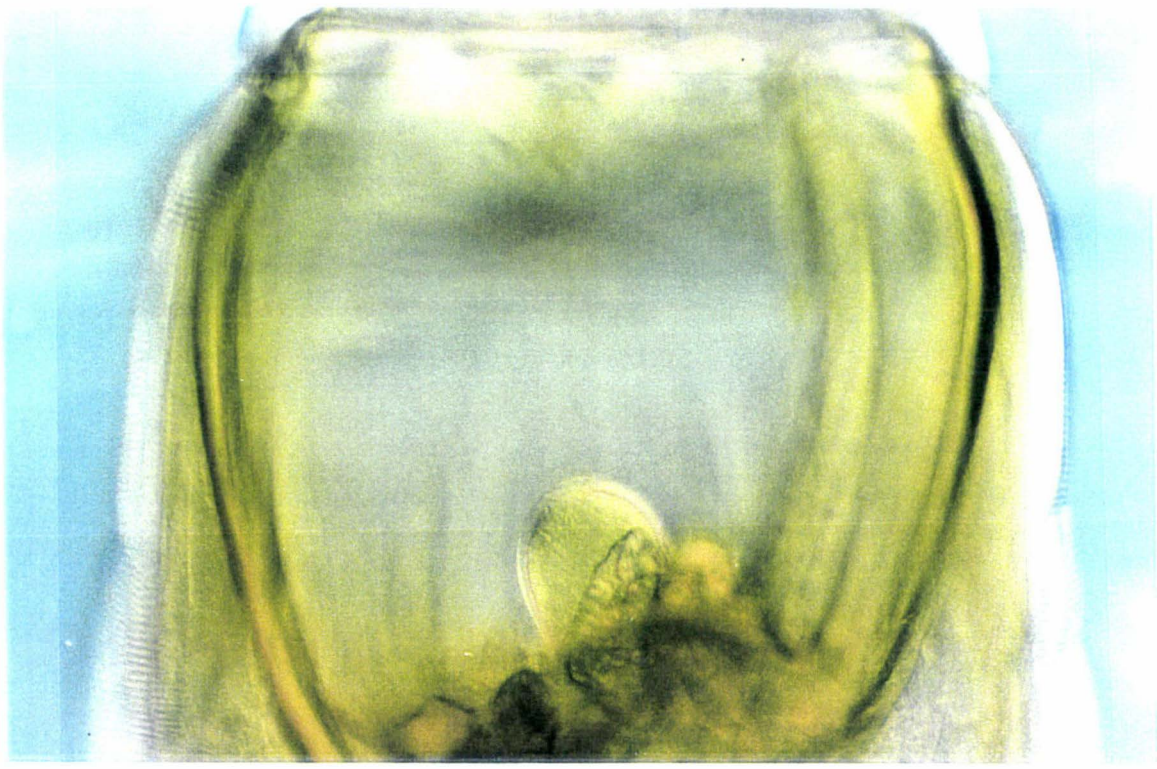


Figure No. 4.1b *Strongylus vulgaris* × 52

Figure No. 4.2a *Strongylus edentatus* × 21

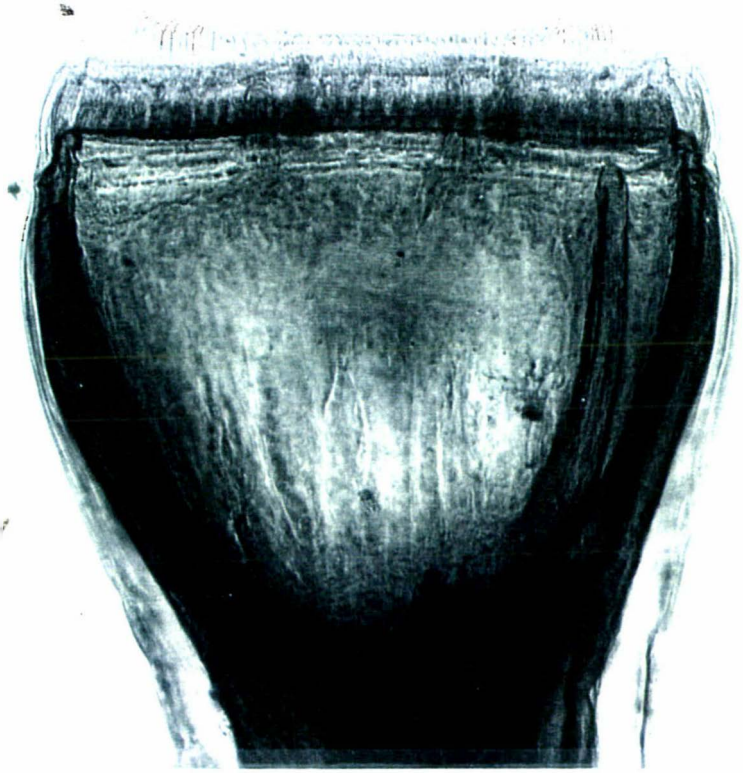


Figure No. 4.2b *Strongylus edentatus* × 21

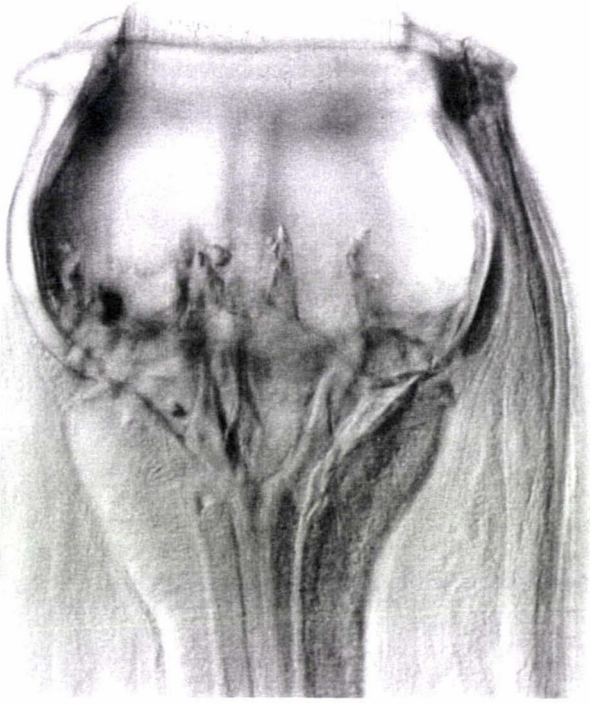


Figure No. 4.3a *Triodontophorus serratus* ×104

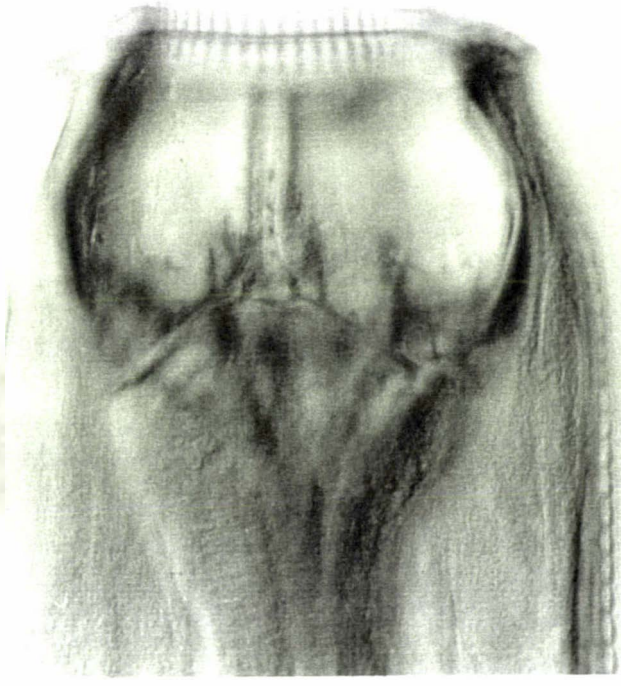


Figure No. 4.3b *Triodontophorus serratus* ×104

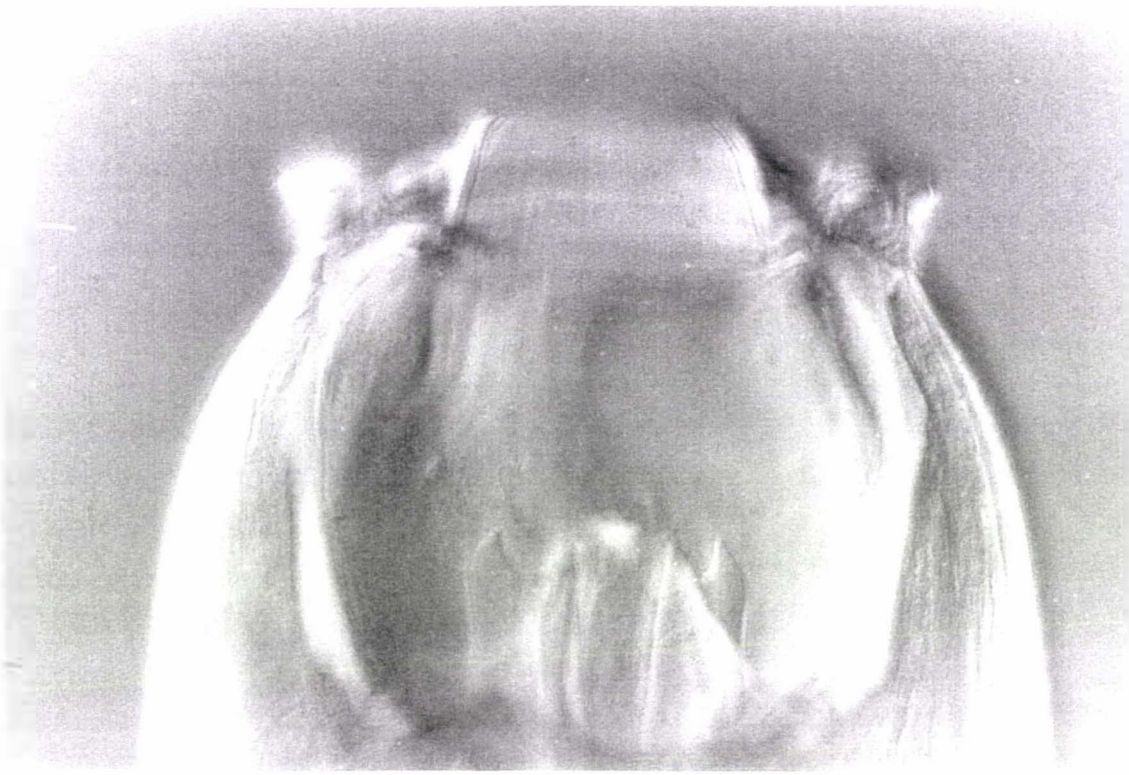


Figure No. 4.4a *Triodontophorus tenuicollis* ×104

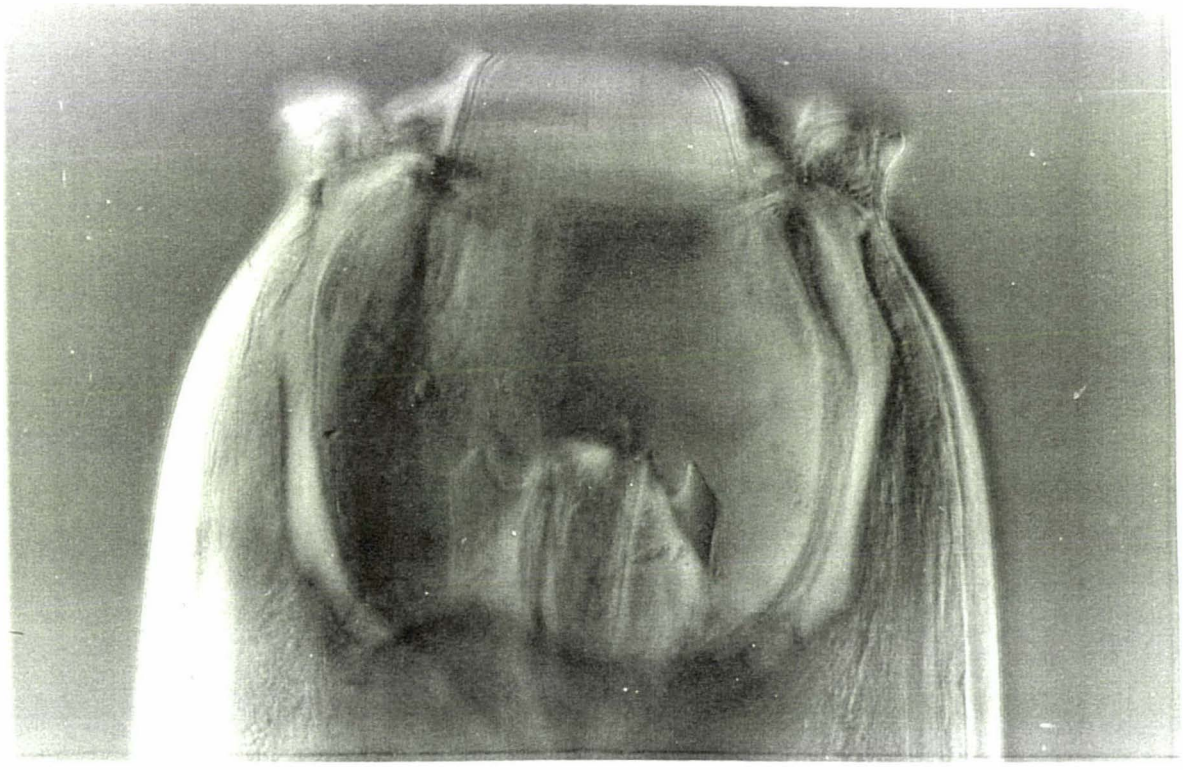


Figure No. 4.4b *Triodontophorus tenuicollis* ×104

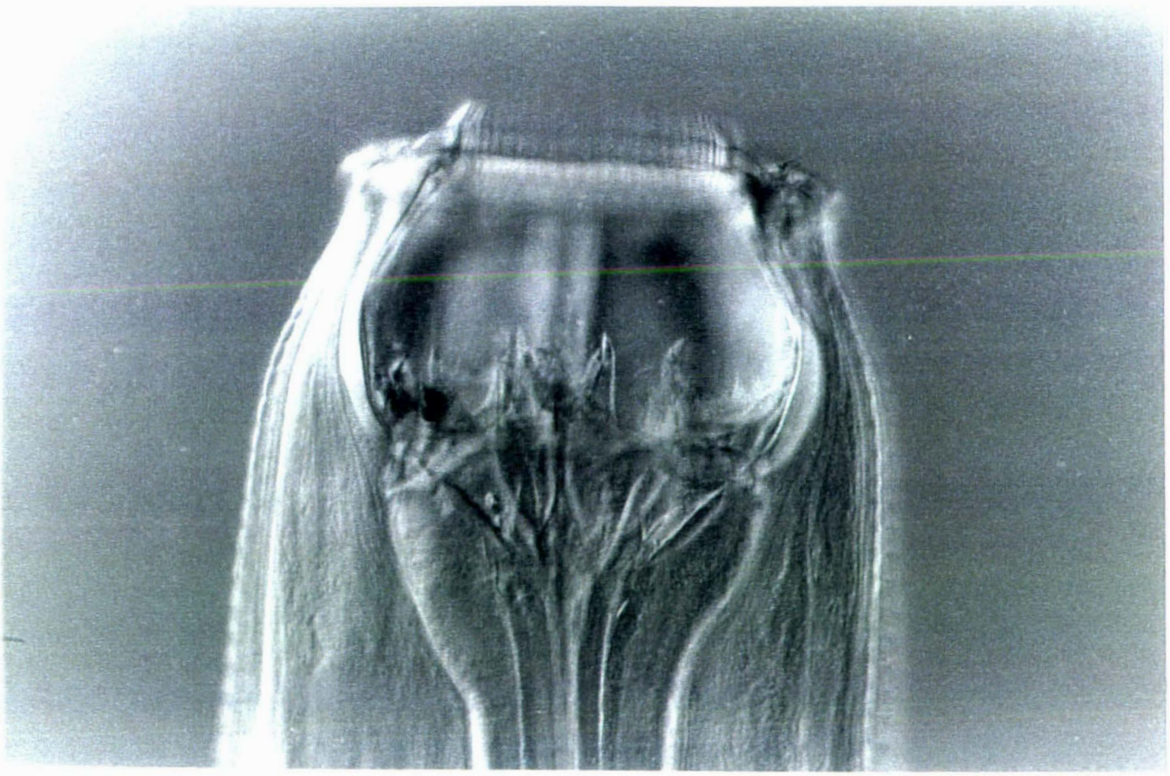


Figure No. 4.5a *Triodontophorus minor* ×104

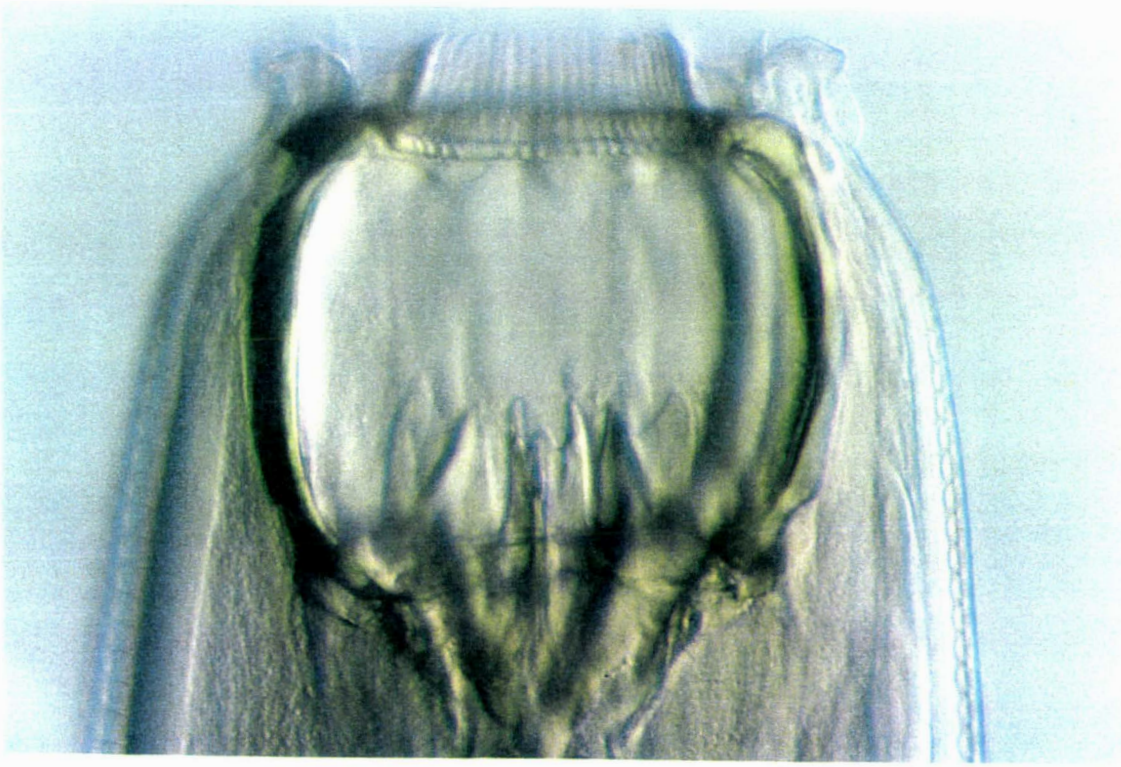


Figure No. 4.5b *Triodontophorus minor* ×104

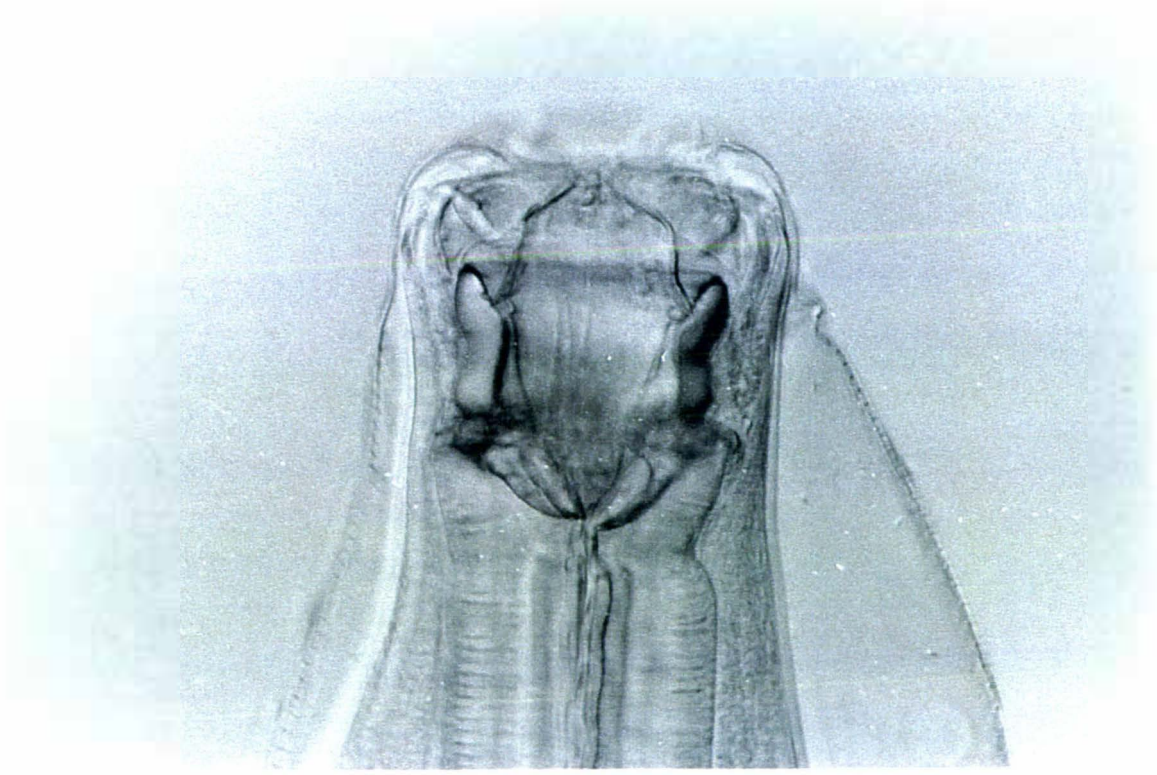


Figure No. 4.6a *Cyathostomum coronatum* × 208

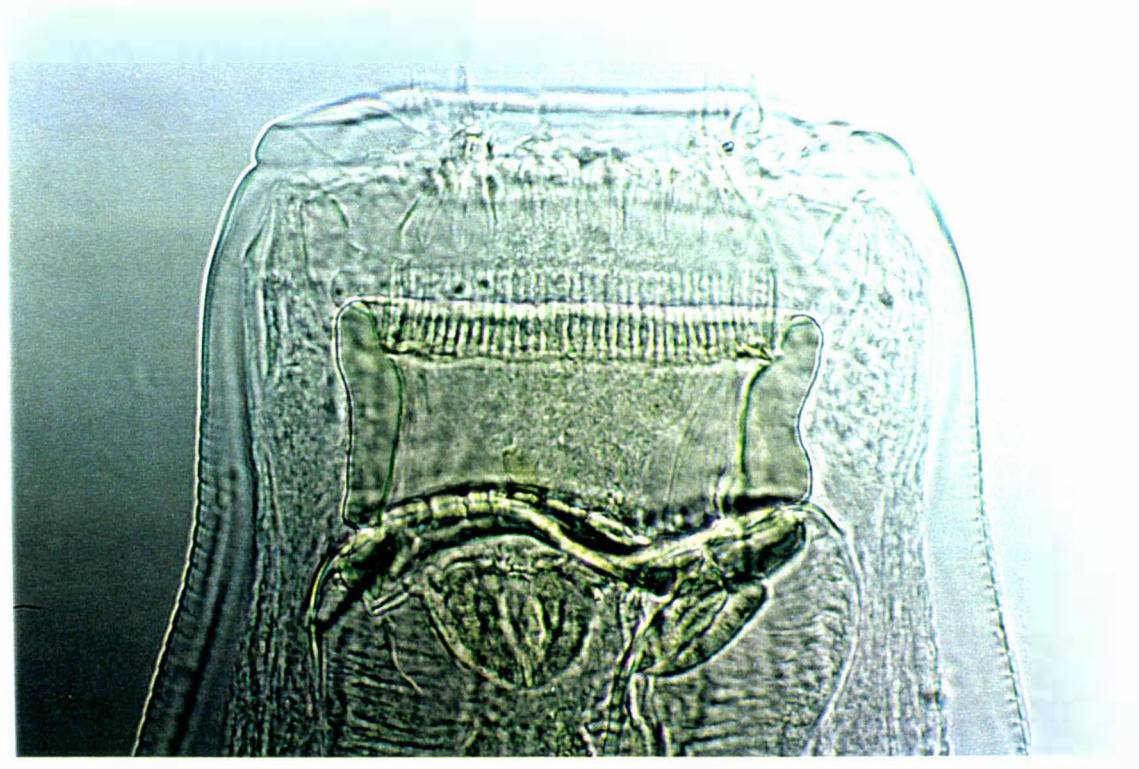


Figure No. 4.6b *Cyathostomum coronatum* × 416

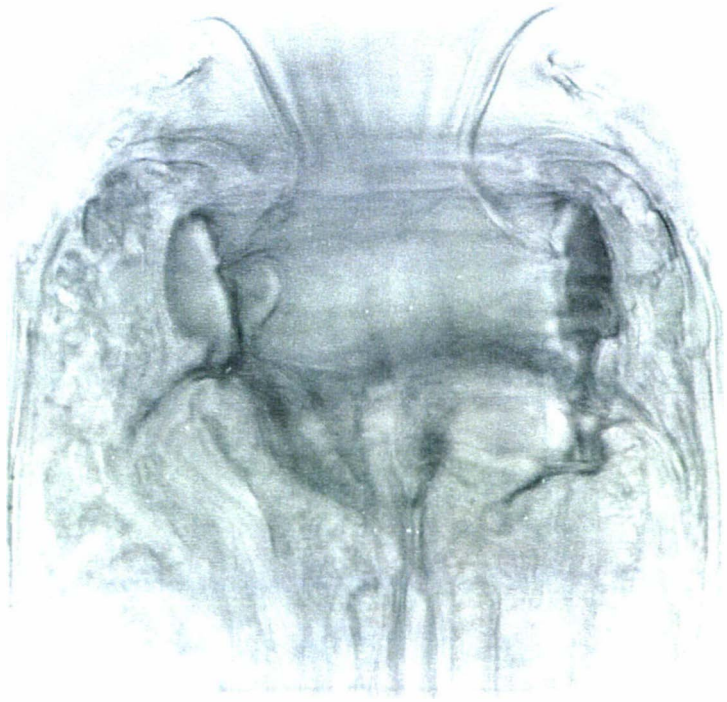
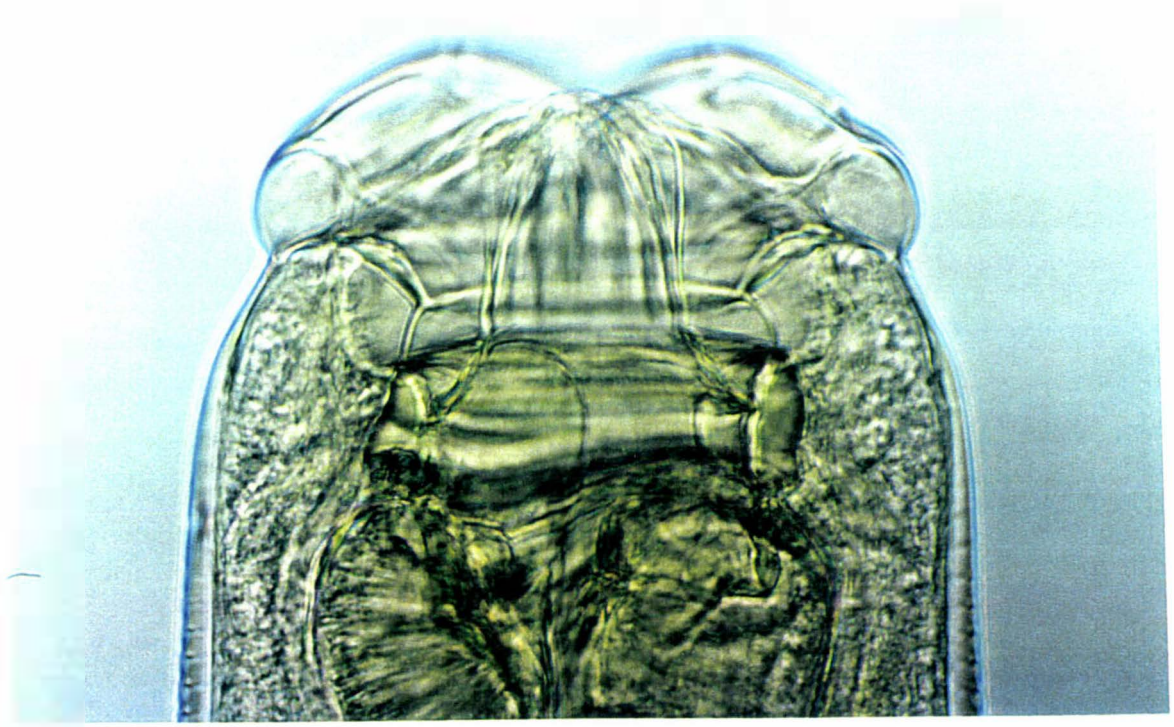


Figure No. 4.7a *Cyathostomum labiatum* ×416



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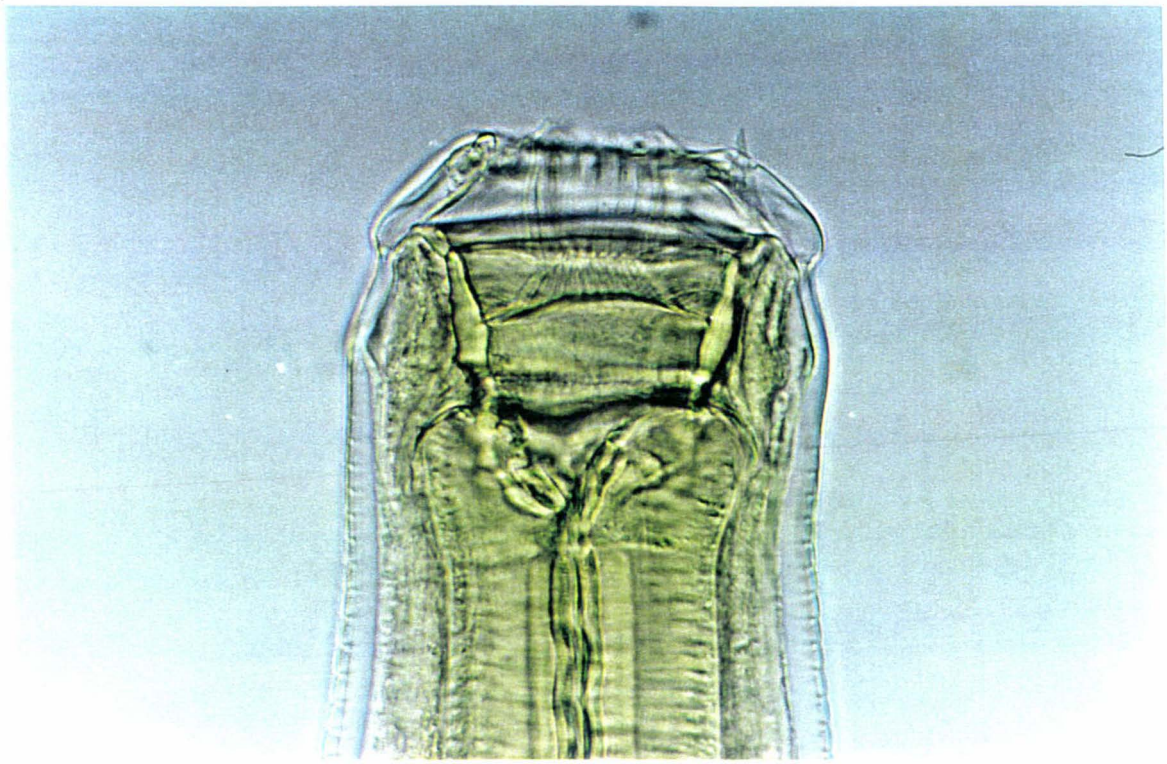


Figure No. 4.8a *Cyathostomum catinatum* ×416

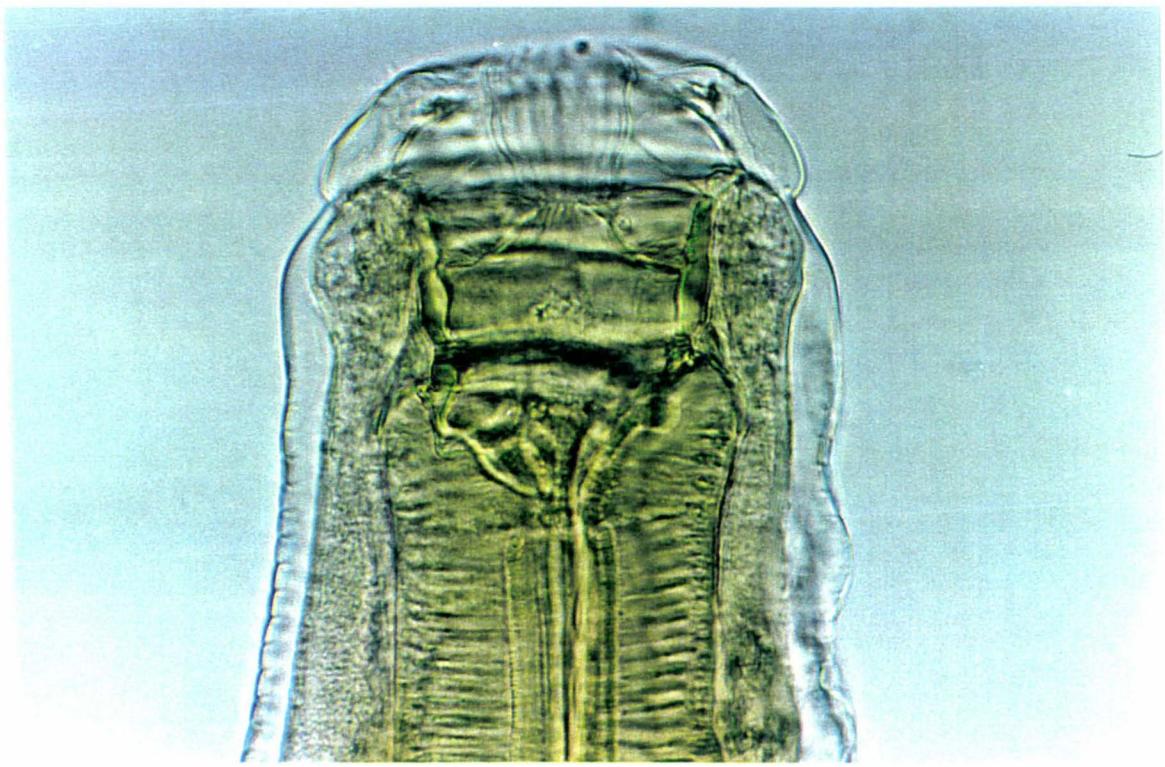


Figure No. 4.8b *Cyathostomum catinatum* ×416

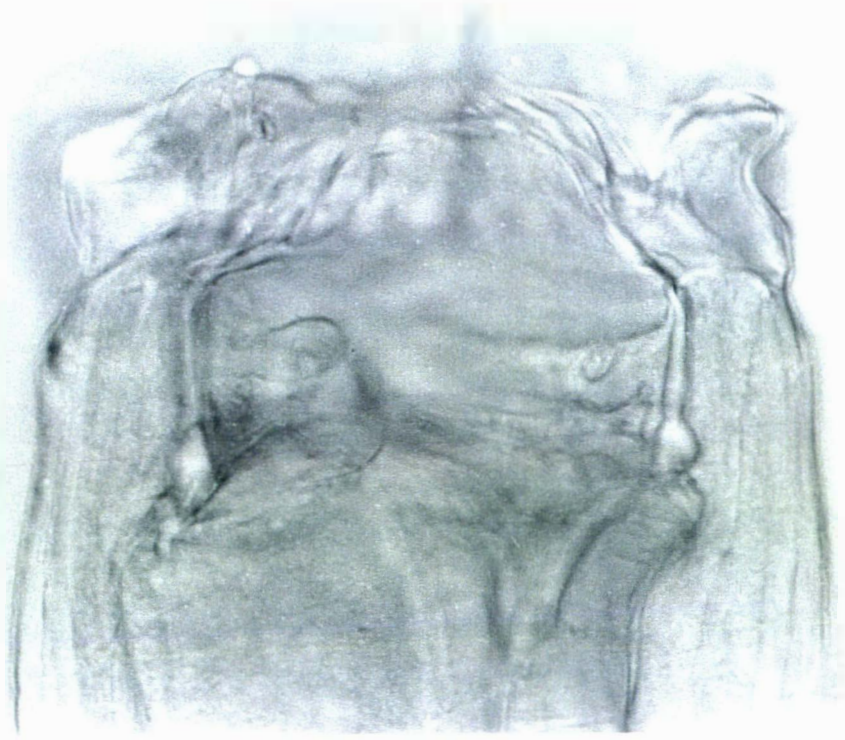


Figure No. 4.9a *Cylicocyclus nassatus* × 416

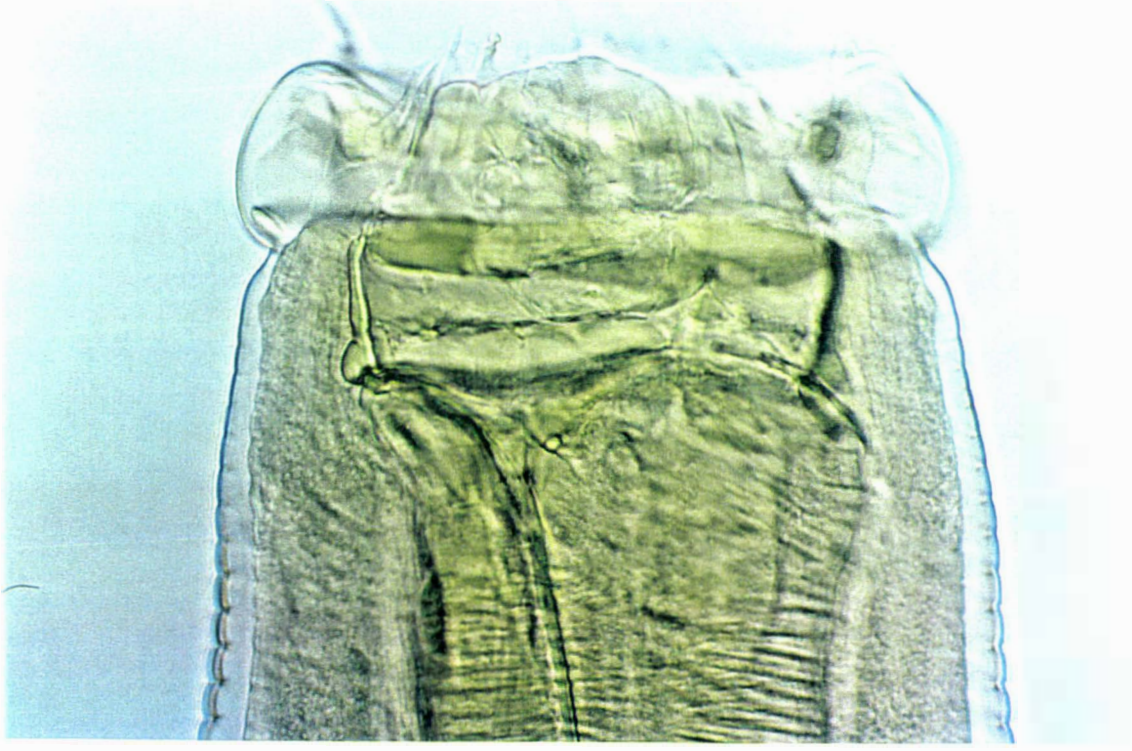


Figure No. 4.9b *Cylicocyclus nassatus* × 416

Figure No. 4.10a *Cylicocyclus insigne* × 416

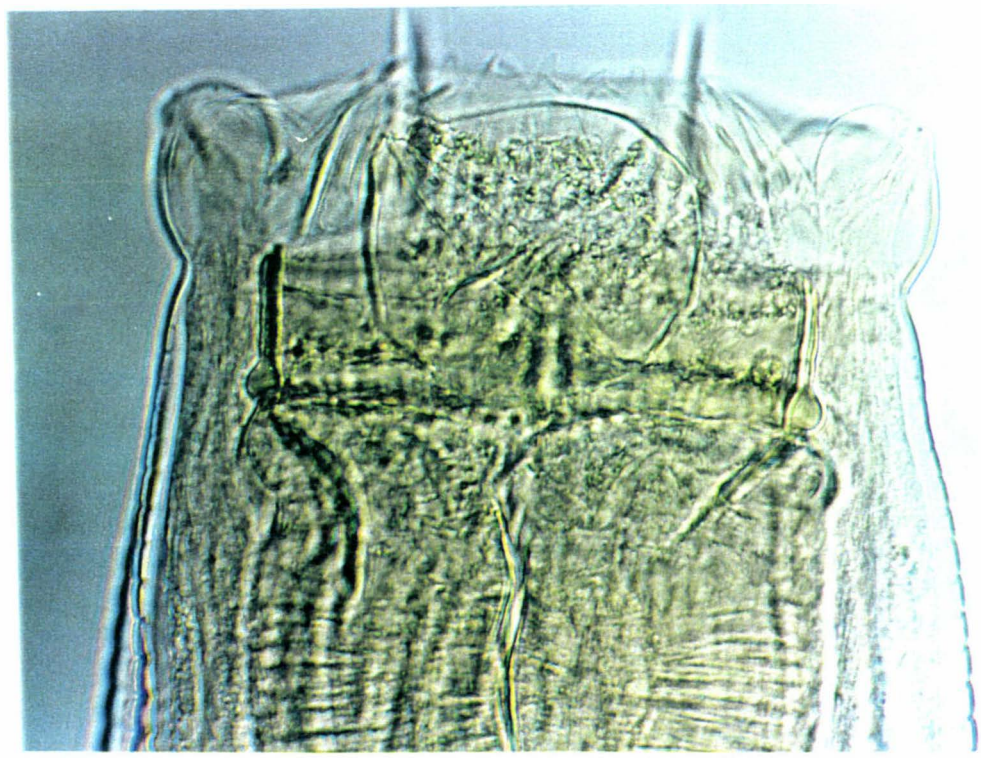


Figure No. 4.10b *Cylicocyclus insigne* × 416

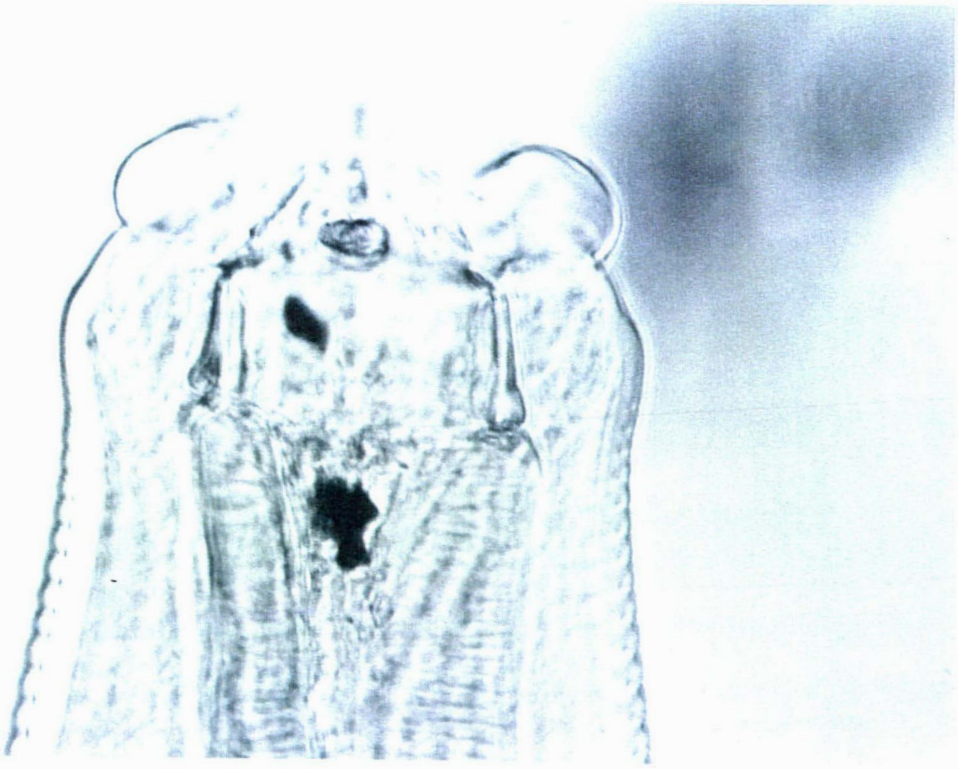


Figure No. 4.11a *Cylicocyclus leptostomus* × 416

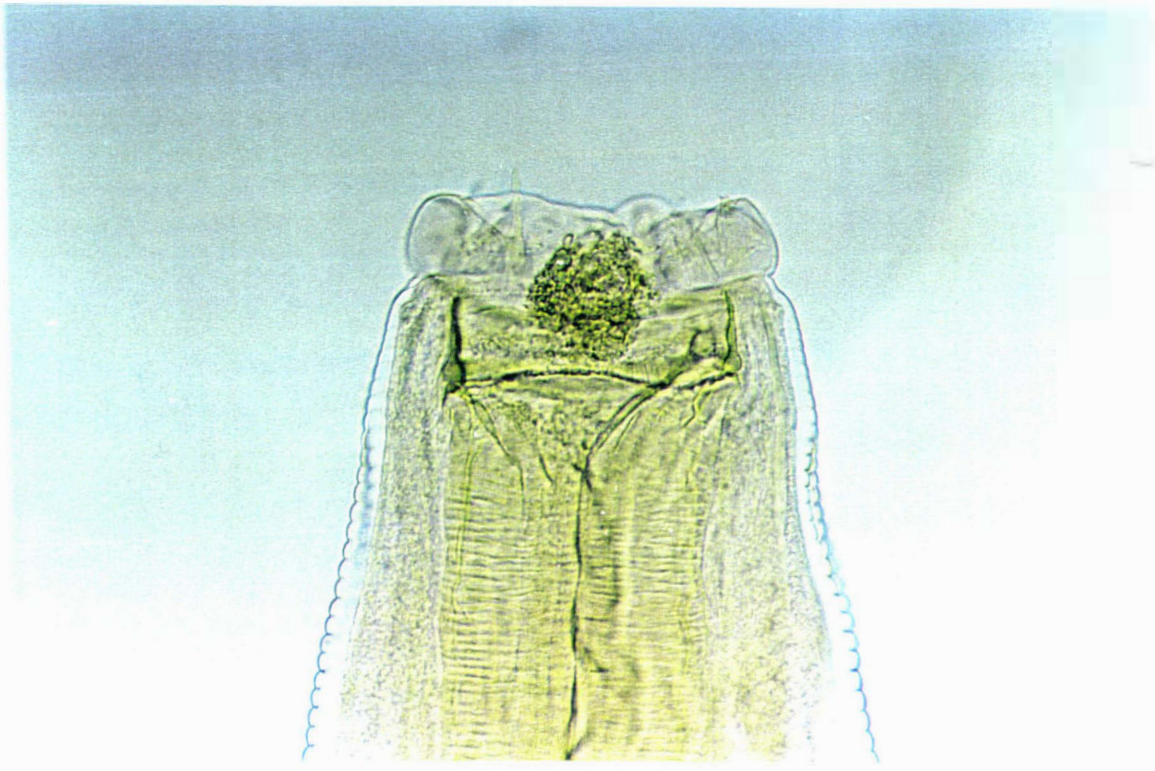


Figure No. 4.11b *Cylicocyclus leptostomus* × 416

Figure No. 4.12a *Cylicocyclus radiatus* × 416

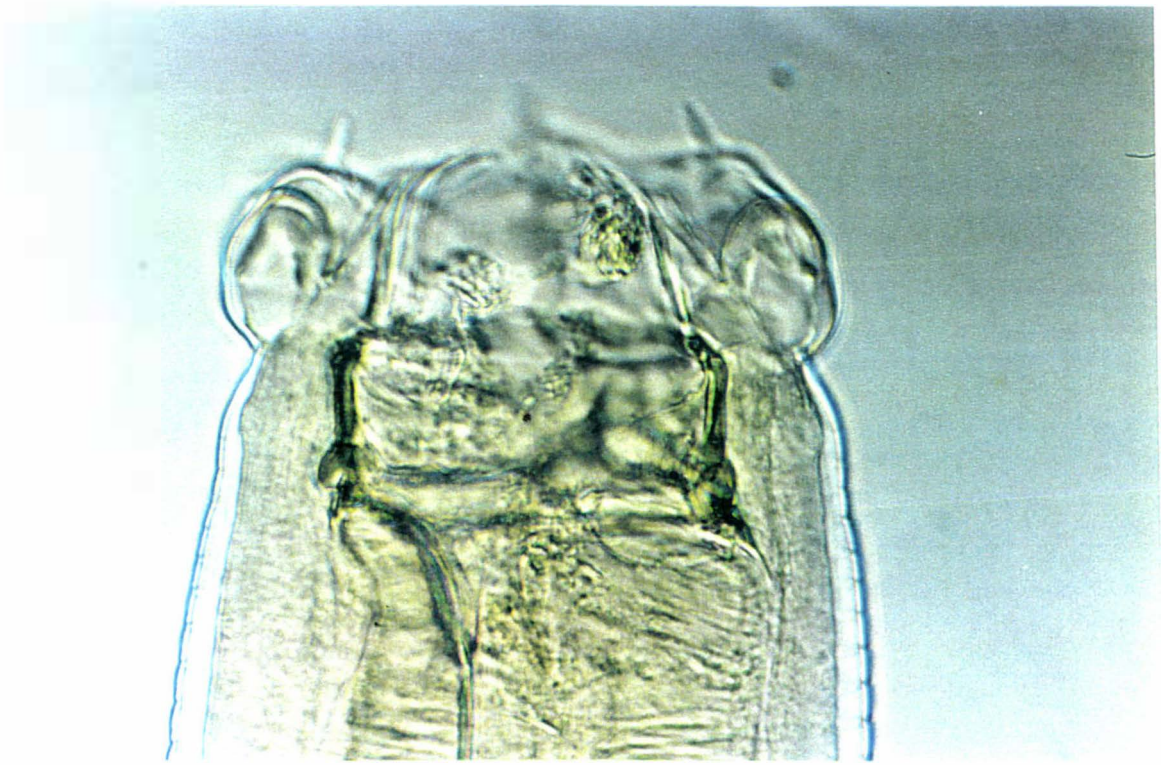


Figure No. 4.12b *Cylicocyclus radiatus* × 416

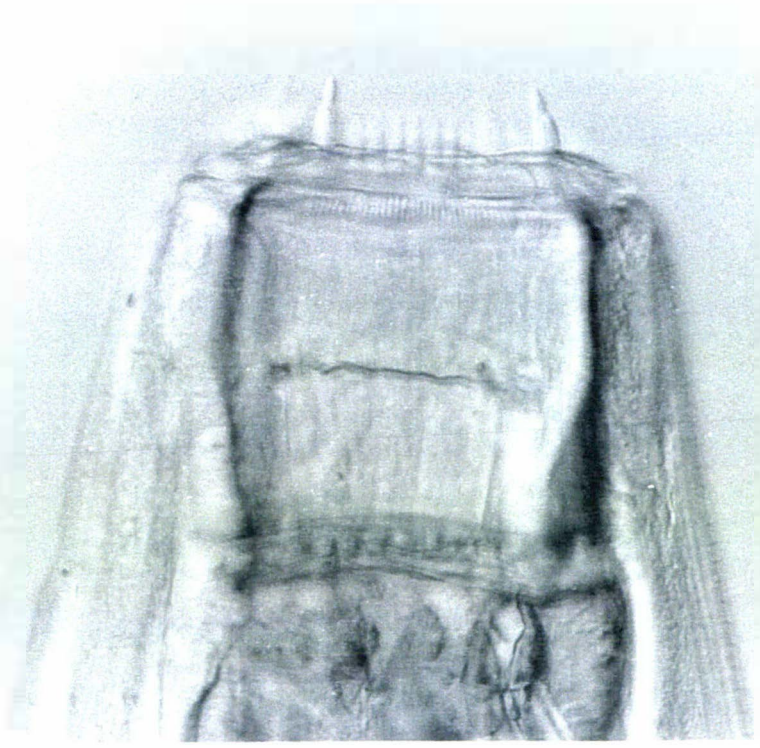


Figure No. 4.13a *Cylicostephanus poculatus* ×416

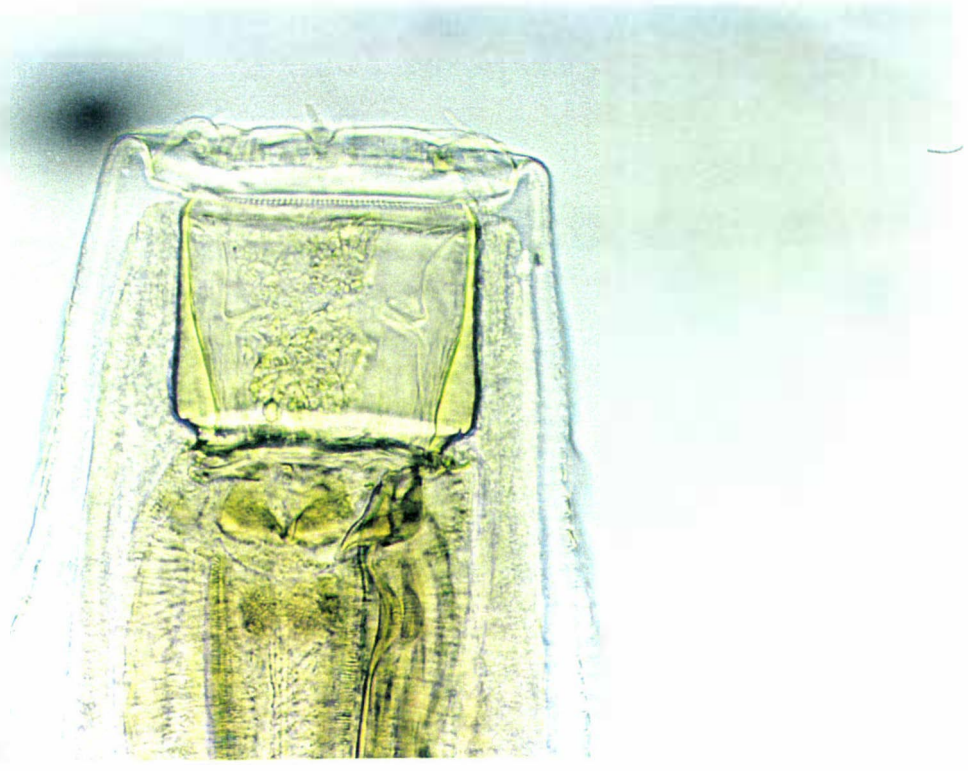


Figure No. 4.13b *Cylicostephanus poculatus* ×416

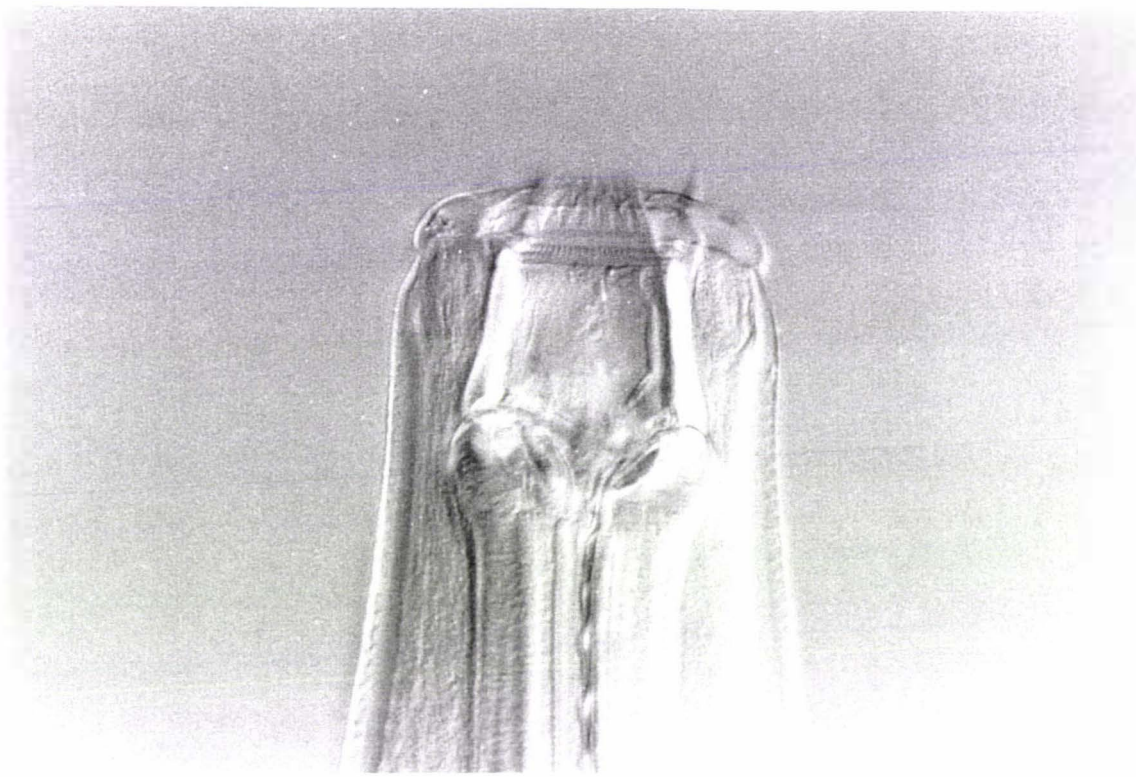


Figure No. 4.14a *Cylicostephanus minutus* × 416

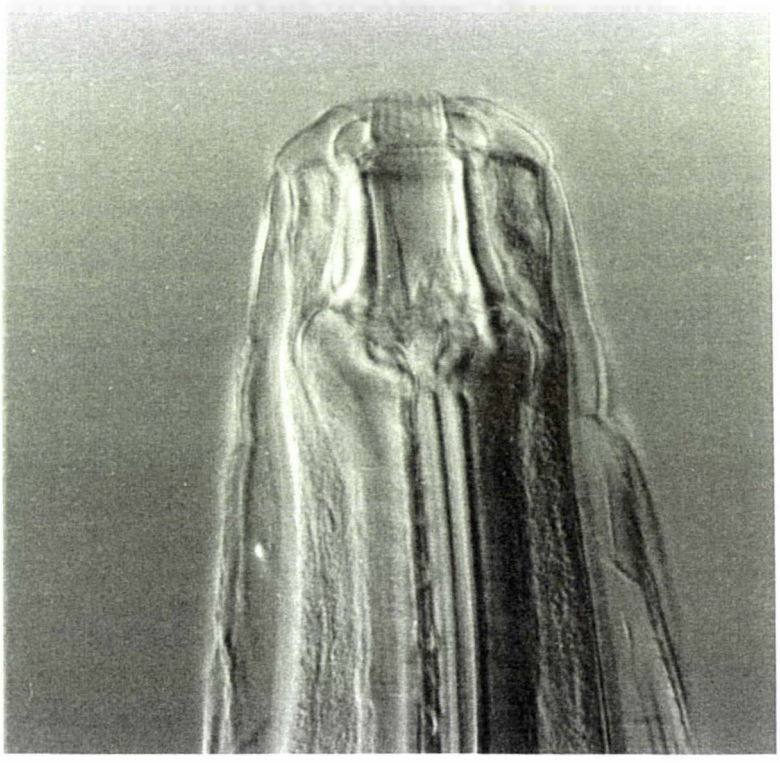


Figure No. 4.14b *Cylicostephanus minutus* × 416

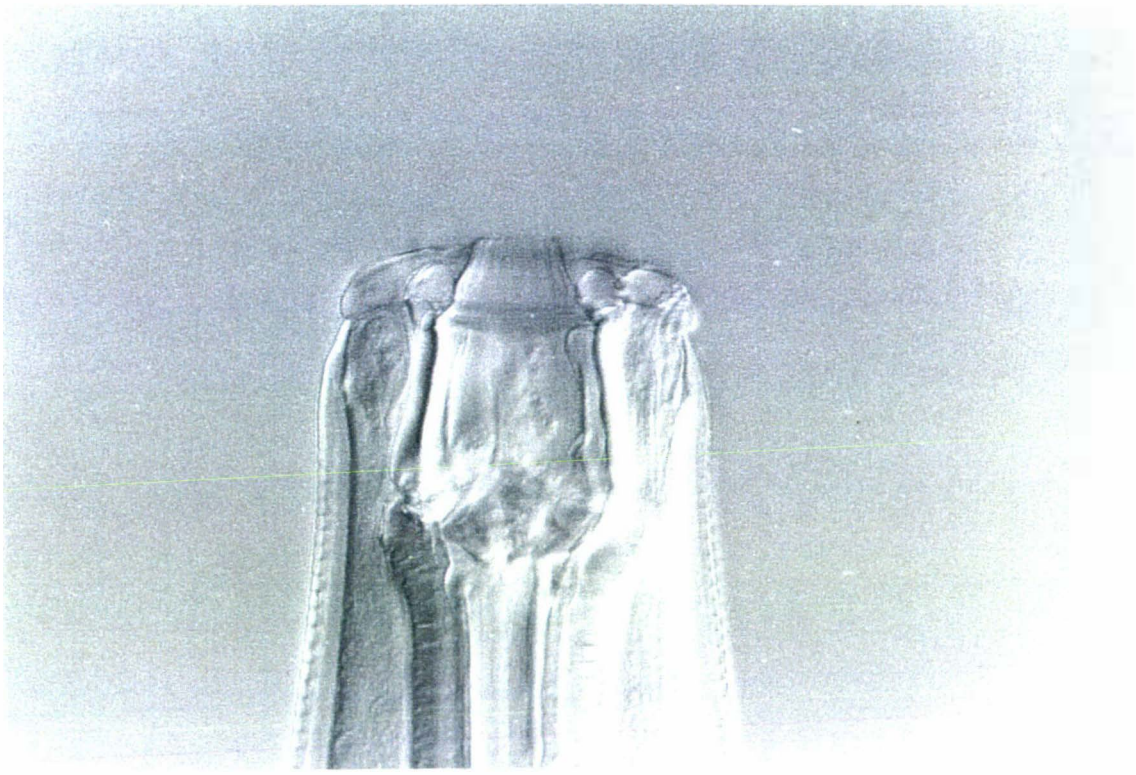
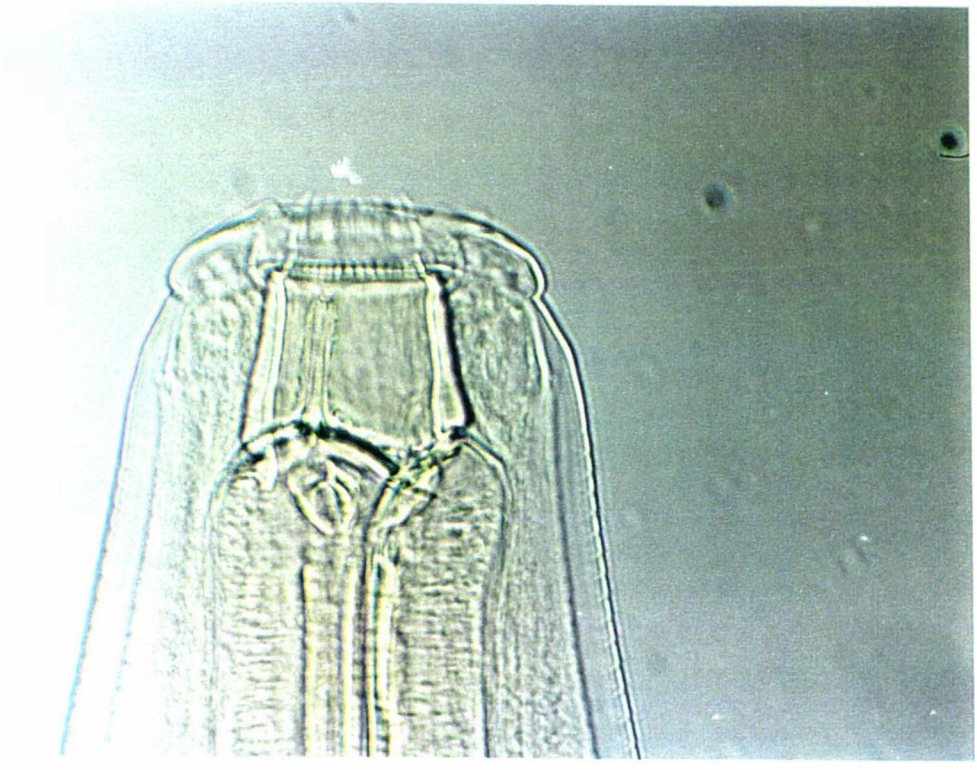


Figure No. 4.15a *Cylicostephanus calicatus* × 416



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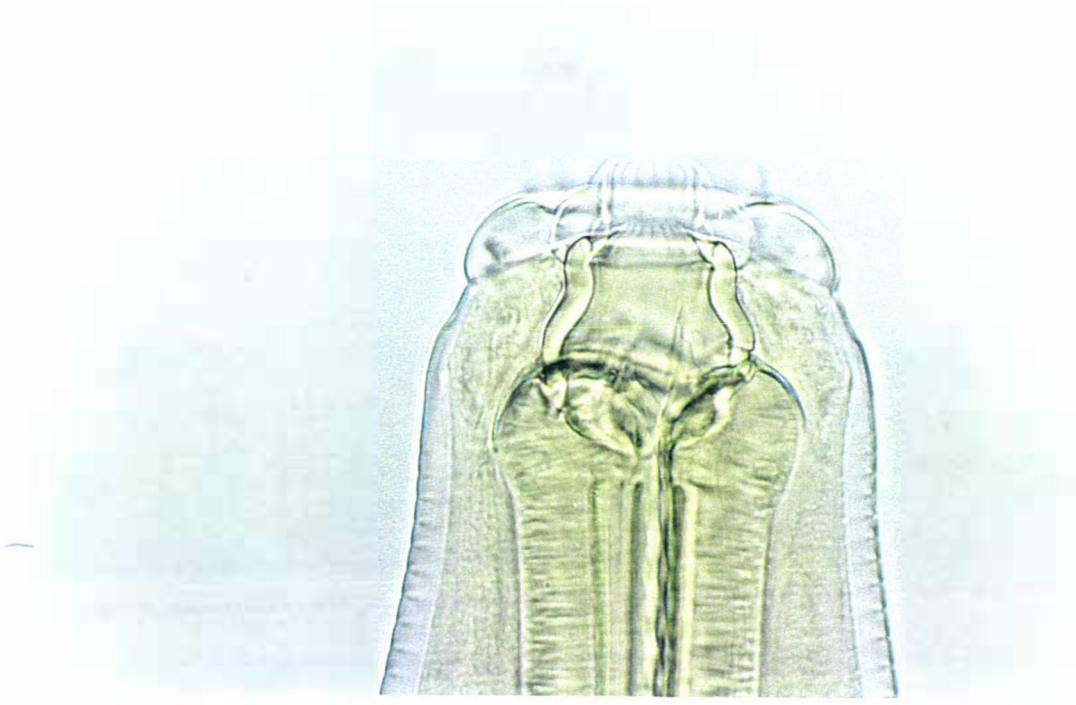

Figure No. 4.16a *Cylicostephanus longibursatus* × 208

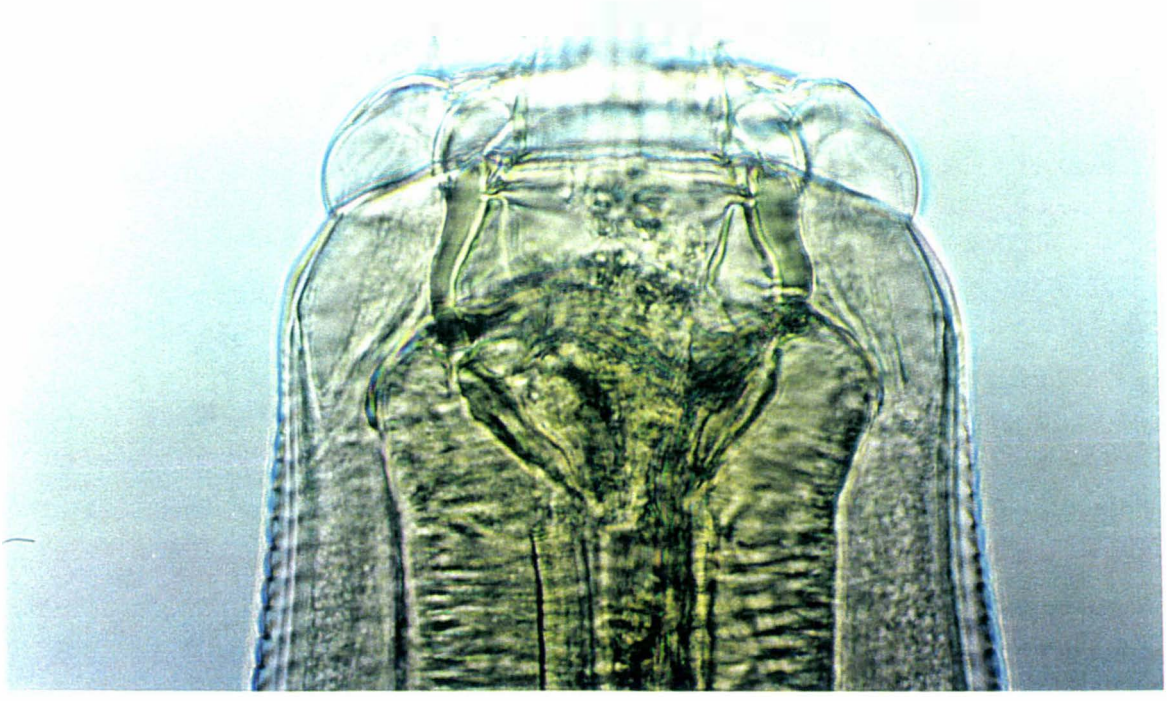

Figure No. 4.16b *Cylicostephanus longibursatus* × 416

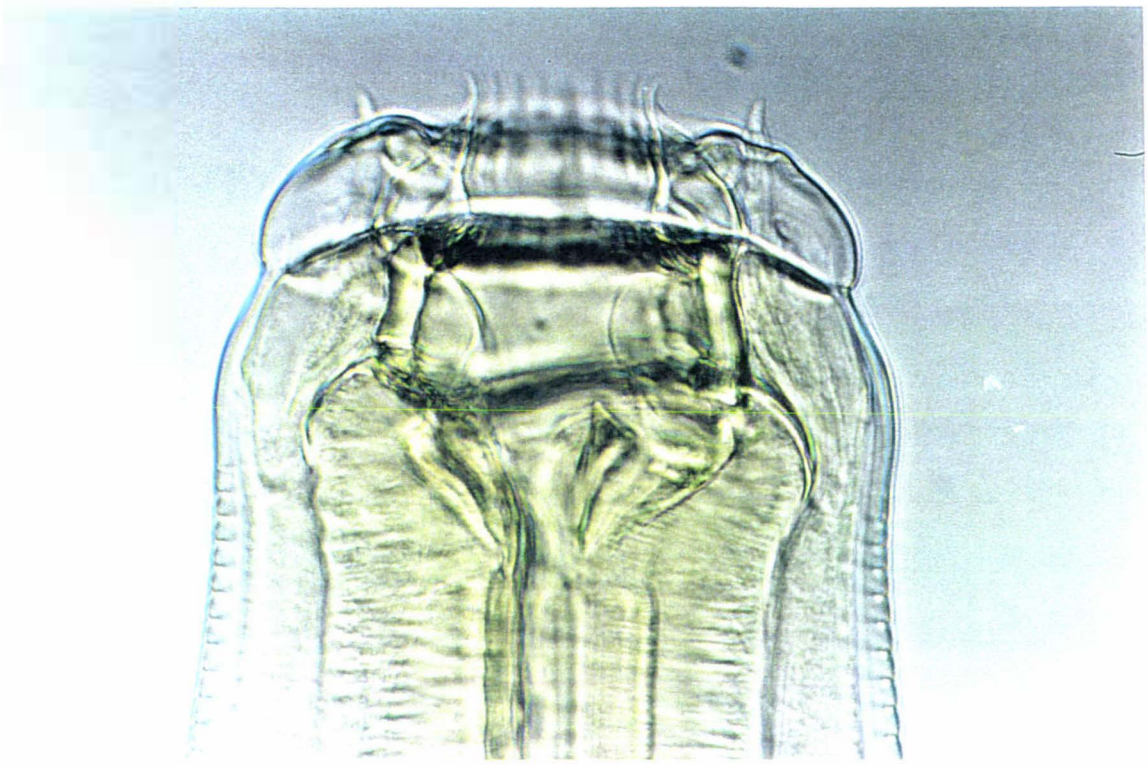

Figure No. 4.17a *Cylicostephanus goldi* × 416

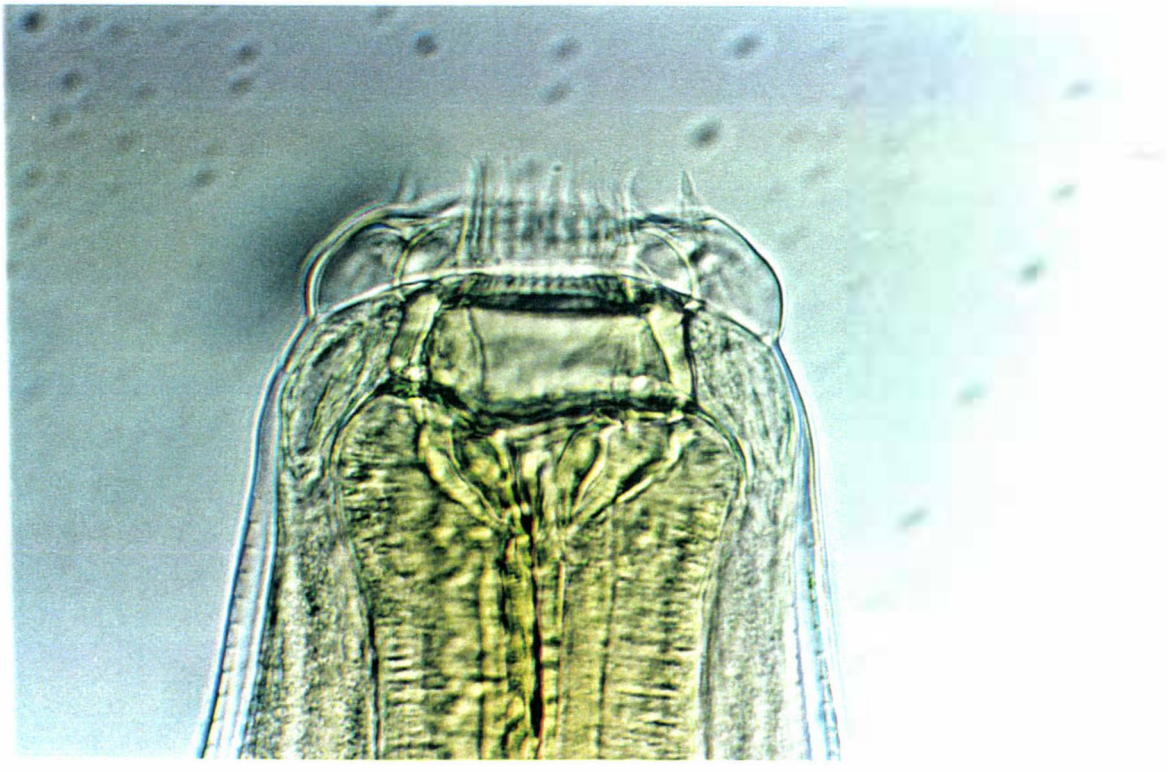

Figure No. 4.17b *Cylicostephanus goldi* × 416

4.4 Summary of species identified

In this study, twelve Cyathostome species were found. They were *Cyathostomum coronatum, Cyathostomum labiatum, Cyathostomum catinatum, Cylicocyclus nassatus, Cylicocyclus leptostomus, Cylicocyclus radiatus, Cylicocyclus insigne, Cylicostephanus poculatus, Cylicostephanus minutus, Cylicostephanus calicatus, Cylicostephanus longibursatus* and *Cylicostephanus goldi* (Appendix V). All these species were found in all sections of the large intestine including the caecum, the large colon and the small colon. There was no apparent difference in species composition between the two trials.

Figures 4.18a and 18b shows the mean proportions of each cyathostome species in different parts of the large intestine from the control horses in both trials (see also Appendix V). Figure 4.19 shows the mean proportion of each cyathostome species of the two trials combined. The species with the highest mean proportion was *C. nassatus* with 0.27. The next highest was *C. coronatum* with 0.18, then *C. calicatus* with 0.14 and then several species with quite similar proportions. Some species showed a definite site preference. The ventral and dorsal colon were the preferred site for *C. catinatum, C. poculatus, C. nassatus, C. leptostomus, C. insigne, C. minutus, C. longibursatus* and *C. goldi* while *C. coronatum, C. radiatus, C. labiatum* and *C. calicatus* preferred the caecum.

Figure 4.20 shows the total cyathostome worm burdens by species (calculated from total cyathostome counts) in the two different trials (see also Appendix V). The most prevalent species were *C. nassatus* which ranged from 4850 to 8350 (mean 6558) in the DCS and 9424 to 19159 (mean 13208) in the PES, *C. coronatum* which ranged from 2839 to 5919 (mean 4224) in the DCS and 4936 to 12234 (mean 8310) in the PES and *C. calicatus* which ranged from 2960 to 3177 (mean 3049) in the DCS and4416 to 9233 (mean 6287) in the PES. The mean numbers of the remaining nine species ranged from 529 to 2331 in the DCS and 216 to 4388 in the PES. Figure 4.20 also shows that the prevalence and intensity of infection with *C. nassatus, C. coronatum, C. calicatus, C. poculatus, C. catinatum, C. labiatum, C. catinatum, C. labiatum* and *C. goldi* were significantly different ($p<0.05$) between the DCS and PES horses.

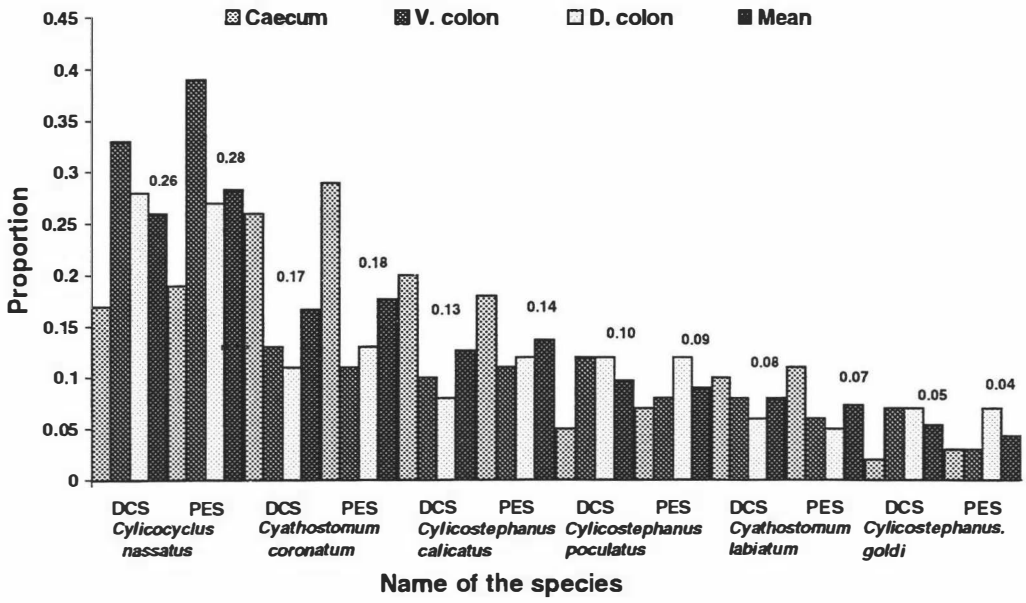

Figure 4.18a: The mean proportion by organ of cyathostome species in control horses of the DCS and PES trials (n = horses per trial)

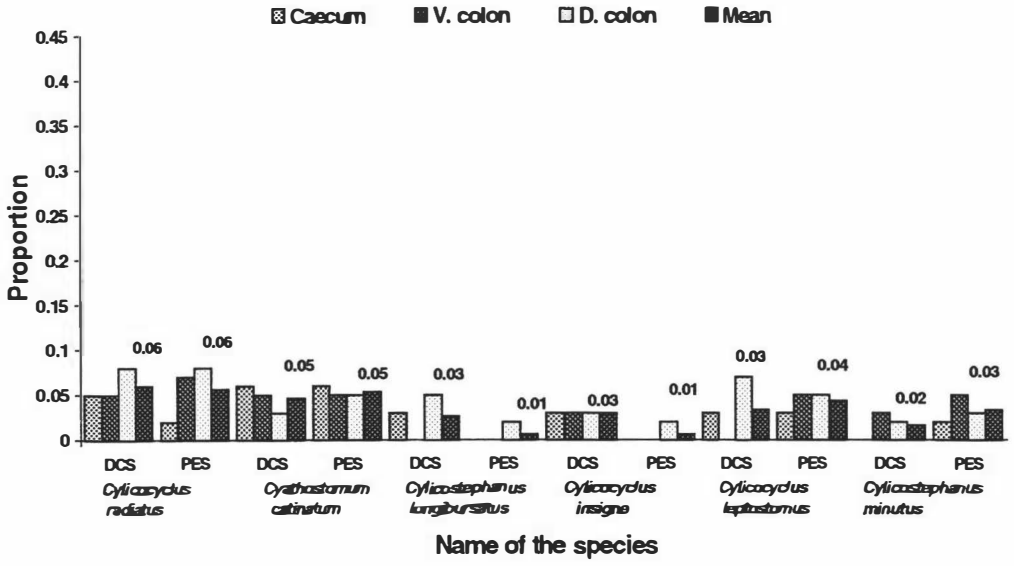

Figure 4.18b: The mean proportion by organ of cyathostome species in control horses of the DCS and PES trials (n = horses per trial)

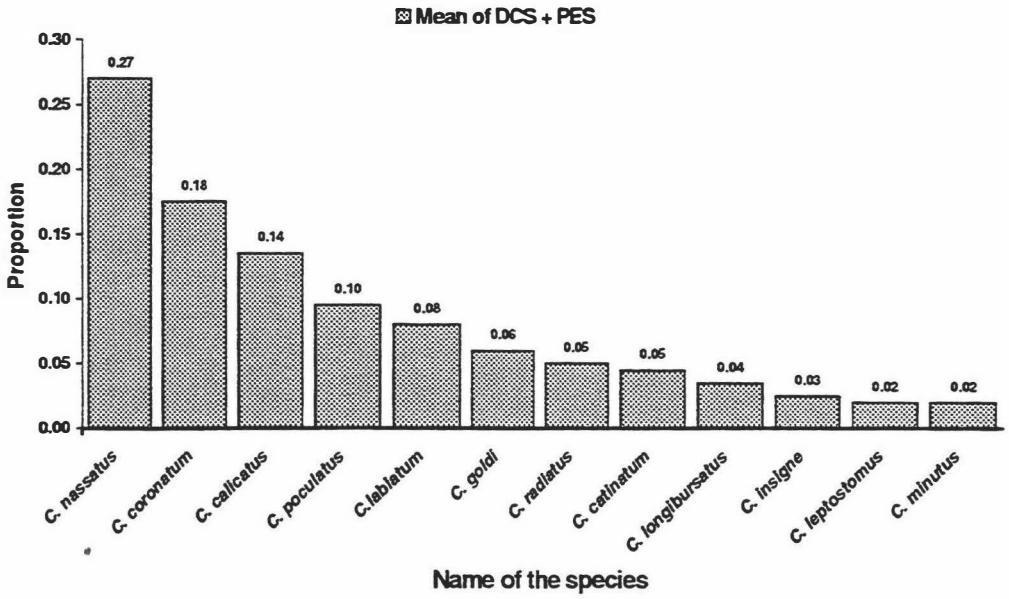

Figure 4.19: Mean proportion of cyathostome species in control horses of the DCS and PES trials (n = 3 horses per trial)

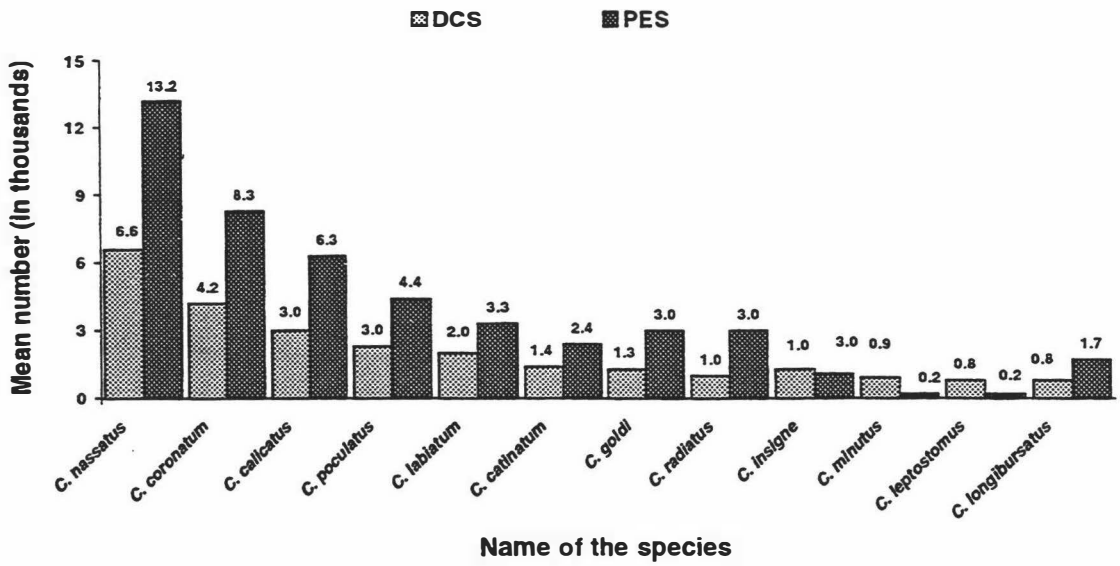

Figure 4.20: Total cyathostome worm counts in both the DCS and PES trials (n = 3 control horses per trial)

Figure 4.21 shows the identified parasites in the 3 treated horses of the PES trial. By 20 days post treatment the horses contained seven cyathostome species including *C. nassatus, C. coronatum, C. goldi, C. calicatus, C. radiatus, C. longibursatus* and *C. catinatum* and were found in the caecum and large colon. The mean proportions of these species *C. nassatus, C. coronatum, C. goldi, C. calicatus, C. radiatus, C. longibursatus* and *C. catinatum* are 0.32, 0.23, 0.16, 0.11, 0.10, 0.06 and 0.03 respectively. These are similar proportions to these found in the untreated horses in

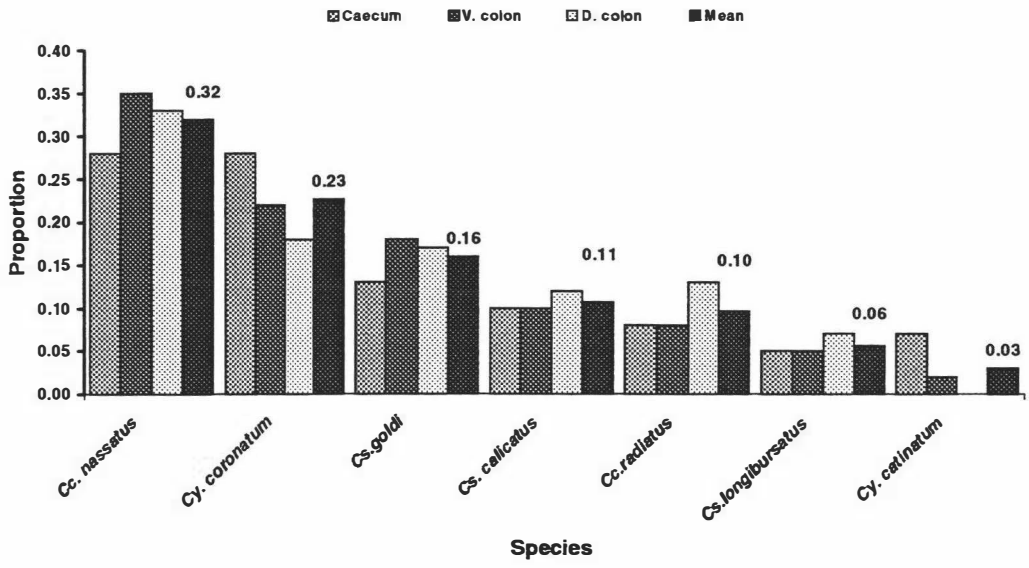

Figure 4.21: The mean proportion by organ of cyathostome species in treated horses of the PES trial (n = 3 horses)

4.5 Discussion

The species mentioned in above paragraph 4.2.1 to 4.2.5 have been grouped in genera according to Lichtenfels (1975) and the species were identified according to Popova (1958, English translation, 1965) and Lichtenfels (1975). Although some characteristics were not exactly as described, most were similar to those described by Popova (1958, English translation, 1965) and Lichtenfels (1975) and were reasonably easy to identify. In this study it was observed that at least two species of *Strongylus*, three species of *Triodontophorus* and twelve species of the subfamily Cyathostominae are present in New Zealand. The three most common genera of cyathostomes were *Cyathostomum, Cylicocyclus* and *Cylicostephanus*. Two species of *Triodontophorus* and eight species of cyathostome have been previously reported in New Zealand (Tetley, 1948; McKenna, 1997). However, another *Triodontophorus*

species (*T. minor*) and four cyathostome species (*C. labiatum, C. leptostomus, C. radiatus* and *C. poculatus*) were identified in these studies and are first records for these species in New Zealand.

In these studies, the common species of cyathostomes were *Cyathostomum coronatum, Cyathostomum labiatum, Cyathostomum catinatum, Cylicocyclus nassatus, Cylicocyclus leptostomus, Cylicocyclus radiatus, Cylicocyclus insigne, Cylicostephanus poculatus, Cylicostephanus minutus, Cylicostephanus calicatus, Cylicostephanus longibursatus* and *Cylicostephanus goldi*, which is similar to other studies in Australia and USA (Bucknell *et al.*, 1995; Mfitilodze and Hutchinson, 1990; Uhlinger, 1991).

All cyathostome species were found in all locations throughout the large intestine in the caecum, ventral, dorsal and small colon. The caecum, ventral colon and dorsal colon were the preferred sites for the cyathostome species. However some do show a preference for a particular site. In this study, *C. catinatum, C. poculatus, C. nassatus, C. leptostomus, C. insigne, C. minutus, C. longibursatus* and *C. goldi* preferred the ventral and dorsal colon on the other hand *C. coronatum, C. radiatus, C. labiatum* and *C. calicatus* preferred the caecum. These are similar to the preferances noted in previous reports (Foster and Pedro Ortiz, 1937; Ogbourne, 1976; Reinemeyer *et al.*, 1988; Bucknell *et al.*, 1995)

This experiment indicates that there was no seasonal variation of the species composition between autumn and summer when the two trials were conducted which was similar to other results as described by Dunsmore and Jue Sue, (1985). These studies also showed that the intensity of infection of the 12 species of cyathostomes for each of these species were higher in summer than autumn. This may be due to a number of factors including better nutrition available to the horses in the autumn possibly contributing to a more effective immune response.

This study also showed that *C. nassatus, C. coronatum, C. calicatus, C. catinatum, C. labiatum,* and *C. goldi* were present in treated horses during the PES trial period. It may be related to the emergence of encysted cyathostome larvae from the submucosa but implies that efficacy of AOB 698 against mucosal stages was not species dependent but was equally ineffective against all of them.

CHAPTER FIVE

Larval Development Assay Optimization

5.1 Introduction

The evolution of the larval development assay (LDA) has been described in Coles *et al.*, (1988), Taylor, (1990), Hubert and Kerboeuf, (1992) Gill *et al.*, (1995). However at the time this work was undertaken, there had been few reports of this technique being used with horse nematodes. In order to conduct a survey of anthelmintic resistance it was necessary to optimize the assay for use with horse nematodes. The aim in this study was to take the method described by Gopal *et al.*, (1999) which is a modification of that described by *Gill et al.*, (1995) and further modify this for use with horses. Use of the unmodified procedure with horses resulted in poor development of cyathostome eggs.

5.2 Materials and methods

5.2.1 Nematode egg recovery technique

Fresh faeces were collected from infected horses. The nematode egg recovery procedure was as described by Hubert and Kerboeuf (1992) and as described in Appendix II and was not varied. In brief about 100g of faeces were made into a slurry with water, sieved through a 1.0 mm aperture sieve, then through a 100µm aperture sieve with the eggs being collected on a 20µm sieve. The eggs were further concentrated by centrifugation in 20% magnesium sulphate (density 1.10) at 1500g for 5 minutes. Eggs were then recollected by passing the supematant through a 20µm sieve, then washed thoroughly with water to remove the magnesium sulphate and recovered from the sieve in water. The number of eggs/ml was estimated on the basis of 7 counts of 20µl and the concentration was adjusted to approximately 80egg/60µl of egg suspension.

5.2.2 Larval development assay

The basis for the assay was the descriptions by Gill *et al.*, (1995) and Gopal *et al.*, (1999) for sheep nematodes.

In brief, the larval development assay was carried out in 96 microtitre plates. Two µl of anthelmintic diluted in DMSO was placed in each well with only DMSO being added to control wells. Two replicates of different concentrations of anthelmintics

were normally run in each plate. Then 100µl of 2% agar was poured into each well and allowed to cool. Then 100µl of egg suspension containing nutritive medium and approximately 80eggs were layered on top of the agar matrix. The plates were incubated for 7 days at 25°C. The numbers of eggs, first, second and third stage larvae in each well were counted after staining with Lugol's iodine solution.

5.2.3 Data analysis

Evaluation was based on the number of L_3's of cyathostomine nematodes. The dominant type of larvae present were those of nematodes in the sub-family Cyathostominae. A dose response curve could be determined for these after correcting for the number of L_3's that developed in control wells. It was not possible to correct for the variation in eggs distributed to each well as these were naturally infected horses with mixed infections. The proportion was calculated as follows:

$$\text{Proportion} = \frac{\text{Number of } L_3\text{'s in test well}}{\text{Number of eggs plus different stages of larvae in each well.}}$$

The adjusted proportion was fitted into a sigmoid curve against $\log_{10}$ transformation of anthelmintic concentrations using the software, Slide Write Version 3.0, Advanced Graphics Software Inc.). LD_{50} values were then calculated with this same software. An example of the resulting sigmoid curve is shown in Fig 5.1.

5.3.1 Optimization Experiment 1

5.3.1.1 Aim: To investigate the development success of equine strongylid eggs using a standard sheep procedure as described by Gopal *et al.*, (1999).

5.3.1.1 Materials and methods

Fresh faeces were collected from infected horses. The assay was carried out as described above (Section 5.2). Each variation was replicated 3 times. The final components added to each well were: 100µl agar + 2µl DMSO solution + 60µl egg solution + 20µl Yeast-Earle's solution + 20µl *E. coli* +1.2µl Amphotericine B.

5.3.1.2 Results

Results were extremely variable. Most of the eggs did not hatch and of those that hatched most died soon after and degenerated with very few developing to third stage larvae.

5.3.1.3 Conclusion

Decided to investigated the proportion and the amount of Yeast-Earle's balanced salt and *E. coli* solutions.

5.3.2 Optimization Experiment 2

5.3.2.1 Aim: To vary the concentrations of ingredients in the nutritive media to attempt to improve development success.

5.3.2.2 Materials and methods

Fresh faeces were collected from infected horses. The assay was carried out as described above with the following variations. No anthelmintic was added at this stage. Each variation was replicated 3 times. The following trials were done:

Series A: 100µl agar + 60µl egg solution.
Series B: 100µl agar + 2µl DMSO solution + 60µl egg solution.
Series C: 100µl agar + 2µl DMSO solution + 60µl egg solution + 20µl *E. coli*.
Series D: 100µl agar + 2µl DMSO solution + 60µl egg solution + 20µl Yeast-Earle's solution + 20µl *E. coli*.
Series E: 100µl agar + 2µl DMSO solution + 60µl egg solution + 20µl Yeast-Earle's solution + 20µl *E. coli* +1.2µl Amphotericine B.
Series D: 100µl agar + 2µl DMSO solution + 60µl egg solution + 20µl Yeast-Earle's solution +1.2µl Amphotericine B.
Incubated at 25^{0}C for 7 days.

5.3.2.3 Results

The results were extremely variable and not satisfactory as shown in Table 5.1

Table 5.1: Development rate of each stage using LDA test.

Wells Series	Mean % of each stage				
	Eggs	L1	L2	L3	Degenerated larval stages
Series A	15.4	5.1	33.3	19.2	26.9
Series B	13.0	10.4	32.5	15.6	28.6
Series C	8.9	12.7	35.4	40.5	2.5
Series D	10.3	11.5	21.8	9.0	47.4
Series E	11.8	9.2	28.9	44.7	5.3
Series F	13.2	18.4	27.6	5.8	35.0

5.3.2.3 Conclusion

The results from this experiment indicated none of the combinations were satisfactory. Further experiments investigating the nutrient medium were required.

5.3.3 Optimization Experiment 3

5.3.3.1 Aim: The aim of this assay is to further investigate the amount of Yeast-Earle's balanced salt solution and *E. coli* was used.

5.3.3.2 Materials and methods: Fresh faeces were collected from infected horses. The assay was carried out as described above with the following variations. No anthelmintic or DMSO was added at this stage. Each variation was replicated 3 times.

A: 100µl agar + 60µl egg solution + 10µl Yeast and Earle's solution + 10µl *E. coli.*

B: 100µl agar + 60µl egg solution + 10µl Yeast and Earle's solution + 10µl *E. coli* + 20µl water + 1.2µl Amphotericine B.

C: 100µl agar + 60µl egg solution + 20µl Yeast and Earle's solution + 10µl *E. coli* + 10µl water + 1.2µl Amphotericine B.

D. 100µl agar + 60µl egg solution + 20µl Yeast and Earle's solution + 20µl *E. coli* + 1.2µl Amphotericine B.

E. 100µl agar + 60µl egg solution + 10µl Yeast and Earle's solution + 20µl *E. coli* + 1.2µl Amphotericine B +10µl water.

F. 100µl agar + 60µl egg solution + 10µl Yeast and Earle's solution + 20µl *E. coli* + 2.4µl Amphotericine B +10µl water.

G. 200µl agar + 60µl egg solution + 20µl Yeast and Earle's solution + 20µl *E. coli* + 1.2µl Amphotericine B.

Incubated at 25°C for 7 days.

5.3.3.3 Results

The results are shown in Table 5.2. Results were extremely variable between different series (A-G). In Series B, E and F the development rate of L_3 was more than in the others and Series E had the highest development of these three series. The development rate of the eggs and larvae were high in all wells of Series E.

Table 5.2: Development rate of each stage using LDA test.

Wells Series	**Mean % of each stage**				
	Eggs	**L1**	**L2**	**L3**	**Degenerated larval stages**
Series A	4.2	4.2	33.8	57.7	0
Series B	3.8	5.1	18.2	74.0	0
Series C	13	9.1	36.4	23.4	15.6
Series D	10.5	14.5	38.2	18.4	18.4
Series E	2.7	2.7	16.2	78.4	0
Series F	2.7	5.3	32.0	60.0	0
Series G	10.3	2.6	48.7	33.3	7.7

5.3.3.4 Conclusion

Series E showed the most promise but the developmental success was still not satisfactory. Further modifications were needed. No anthelmintic drug was added during this experiment, only control wells were started.

5.3.4 Optimization Experiment 4

5.3.4.1 Aim: Optimization Experiment 3 showed that the variation Series E resulted in the highest development rate. Further variations were studied using smaller incremental changes from this in the concentration of *E. coli* and amphotericine B.

5.3.4.2 Materials and methods

Fresh faeces were collected from infected horses. The assay was carried out as described above in section 5.2 with the following variations. No anthelmintic was added at this stage. Each variation was replicated 3 times.

A: 100µl agar + 60µl egg solution + 10µl Yeast and Earle's solution + 20µl *E. coli* + 1.2µl Amphotericine B +10µl water.

B: 100µl agar + 60µl egg solution + 10µl Yeast and Earle's solution + 15µl *E. coli* + 1.5µl Amphotericine B +15µl water.

C. 100µl agar + 60µl egg solution + 10µl Yeast and Earle's solution + 25µl *E. coli* + 1.2µl Amphotericine B +5µl water.

D. 100µl agar + 60µl egg solution + 10µl Yeast and Earle's solution + 20µl *E. coli* + 1.5µl Amphotericine B + 8.5µl water.

Incubated at 25^{0}C for 7 days.

5.3.4.3 Results

The results are shown in Table 5.3. After reducing the volume of the yeast solution, increasing the amount of amphotericine B and using same volume of *E. coli* as used by Gopal *et al.*, (1999), the development rate of eggs and larvae were high, especially in Series-D.

Table 5.3: Development rate of each stage using LDA test.

Wells Series	Mean % of each stage				
	Eggs	**L1**	**L2**	**L3**	**Degenerated larval stages**
Series A	3.9	2.6	20.8	72.7	0
Series B	6.5	3.9	27.3	62.3	0
Series C	5.2	10.4	31.2	53.2	0
Series D	2.6	2.6	5.3	89.5	0

5.3.4.4 Conclusion

This experiment suggested that variation of *E. coli* from that used with sheep nematode, did not show any improvement to the development rate. On the other hand this experiment showed variation of amphotericine B did appear to improve the development rate. Therefore, it was decided to use Series-D as the most appropriate nutritive media combination for use with horse strongylid nematodes. Still no anthelmintic drug was added during this experiment, only control wells without DMSO were run.

5.3.5 Optimization Experiment 5

5.3.5.1 Aim: To investigate if the addition of DMSO, which will be used as a diluent for anthelmintics, affects larval development.

5.3.5.2 Materials and methods:

The same component of nutrient media as described for Optimization Experiment 4 in series D were used, except this time 2µl DMSO were added to half of the wells.

No anthelmintic drug was added during this experiment, only control wells were run.

5.3.5.3 Results

This time very good development was achieved. The development rate of this plate was 85.1-93.6% for L_3, 3.3-9.5% for L_2, 0-1.3% for L_1 and 1.6-4% for eggs. There was no effect on development success by adding 2µl DMSO.

5.3.5.4 Conclusion

DMSO (2µl) had no influence on development rate. Decided to be continue with this volume.

5.3.6 Optimization Experiment 6

5.3.6.1 Aim: To investigated the influence of incubation period on development success.

5.3.6.2 Materials and methods

The component of the nutrient media were used as described for Optimization Experiment 5.

No anthelmintic drug was added during this experiment, only control wells were run.

Incubated at 25^0 C for 7 days.

5.3.6.3 Results

The results in this experiment show that the highest percentage success of development rate to L_3 was after an incubation period of at least 7 days (Table 5.4).

Table 5.4: Development rate of each stage using LDA test.

Incubation Time	Mean % of each stage				
Days	Eggs	L_1	L_2	L_3	Degenerated larval stages
3	2.8	43.5	53.7	0	0
5	2.4	20.3	68.4	8.9	0
7	2.6	1.3	6.4	89.7	0
10	3.6	1.8	5.4	89.3	0

5.3.6.4 Conclusion

This experiment indicated that 7 days was the best period for incubation. There was no advantage in continuing to 10 days.

5.3.7 Optimization Experiment 7

5.3.7.1 Aim: To determine appropriate anthelmintic concentrations for ivermectin, ivermectin aglycone and thiabendazole.

5.3.7.2 Materials and methods

5.3.7.2.1 Anthelmintics

The same concentrations as described by Gopal *et al.*, (1999) for sheep nematodes were used. Each concentration was run in duplicate. The LDA procedure was as used in Optimization Exp. 6 but with anthelmintics included in the DMSO at varying concentrations.

In brief the anthelmintics were diluted in DMSO and the concentrations were adjusted from 0.00073 μg/ml to 24μg/ml for ivermectin, from 0.0008μg/ml to 28.3 μg/ml for ivermectin aglycone and from 0.0078μg/ml to 258 μg/ml for thiabendazole. Fresh

eggs were collected from infected horses. LDA's were prepared and analysed as described in Experiment 5 and in addition different concentration of anthelmintics were added. This time 2µl anthelmintic drug in DMSO were added in each test well and 2µl DMSO in each control wells of microtitre plates.

5.3.7.3 Result

The results for ivermectin, ivermectin aglycone and thiabendazole were shown in shown in Table 5.4, Table 5.5 and Table 5.6. The coefficient of determination (r^2) was 0.987, 0.997 and 0.999 for thiabendazole, ivermectin and ivermectin aglycone respectively. Good dose response curves could generated for each anthelmintic with the dilutions chosen.

Table 5.5: Development rate of each stages in different concentrations of ivermectin and in control wells.

Control wells	Egg	L_1	L_2	L_3	Total	Prop
	5	3	4	70	82	0.85
	4	1	3	67	75	0.89
	2	3	4	66	75	0.88
	3	1	5	67	76	0.88
	4	2	5	69	80	0.86
Concentrations						
0.0007	3	2	4	69	78	0.88
0.0014	5	2	3	68	78	0.87
0.0029	4	1	5	66	76	0.87
0.0058	3	3	4	66	76	0.87
0.0117	4	2	15	56	77	0.72
0.0235	6	4	24	44	78	0.56
0.047	3	6	47	21	77	0.27
0.094	4	16	48	6	74	0.08
0.187	6	9	55	7	77	0.10
0.375	5	10	55	3	73	0.04
0.75	6	4	66	0	76	0
1.5	5	7	66	0	78	0
3	7	3	68	0	78	0
6	2	8	66	0	76	0
12	6	7	64	0	77	0
24	8	12	56	0	76	0

Table 5.6: Development rate of each stages in different concentrations of ivermectin aglycone and in control wells.

Control wells	Egg	L_1	L_2	L_3	Total	Prop
	2	1	2	74	79	0.94
	3	2	0	77	82	0.94
	1	4	2	71	78	0.91
Concentrations						
0.0008	2	4	5	66	77	0.86
0.0017	4	7	5	64	80	0.80
0.0034	3	10	9	53	75	0.71
0.0069	2	15	25	36	78	0.46
0.0138	2	18	38	20	78	0.26
0.0277	2	23	45	8	78	0.10
0.0553	2	28	46	2	78	0.03
0.1106	4	30	42	1	77	0.01
0.2212	2	32	46	0	80	0.00
0.4425	3	28	50	0	81	0.00
0.885	2	27	51	0	80	0.00
1.77	2	34	43	0	79	0.00
3.54	1	31	47	0	79	0.00
7.07	2	38	40	0	80	0.00
14.15	2	36	41	0	79	0.00
28.3	2	47	28	0	77	0.00

Table 5.7: Development rate of each stages in different concentrations of thiabendazole and in control wells.

Control wells	Egg	L_1	L_2	L_3	Total	Prop
	2	3	1	78	84	0.93
	3	1	0	76	80	0.95
	1	3	2	70	76	0.92
	3	1	2	72	78	0.92
Concentrations						
0.0039	3	2	4	69	78	0.88
0.0078	2	2	8	77	89	0.87
0.0157	2	3	7	67	79	0.85
0.0316	4	2	9	76	91	0.84
0.0632	3	6	13	59	81	0.73
0.1264	4	6	17	52	79	0.66
0.252	4	14	17	51	86	0.59
0.504	7	15	23	39	84	0.46
1.008	12	6	31	35	84	0.42
2.016	14	4	30	32	80	0.40
4.032	14	31	27	13	85	0.15
8.064	13	38	22	6	79	0.08
16.125	11	41	30	0	82	0.00
32.25	14	48	18	0	80	0.00
64.5	17	55	7	0	79	0.00
129	11	51	18	0	80	0.00

5.3.7.4 Conclusion

Good dose response curves could be obtained against horse strongylid nematodes using the same drug concentrations as used for sheep.

5.4 Discussion

The series of optimization experiments has shown that only a minor variation to the protocol used for sheep was required to optimize this assay for use with horse nematodes.

In particular a reduction in the proportion of Yeast-Earle's solution was required. The optimum protocol was in Optimization Experiment 7 and is described in detail in Appendix II. Other authors have found that the concentration of Yeast-Earle's is critical to achieve larval development in an LDA (Coles *et al.* 1988; Hubert and Kerboeuf, 1992). Interestingly horse strongylid nematodes appear to require a lower concentration of Yeast-Earle's than sheep. However, the standard sheep protocol of Gopal *et al.* (1999), was apparently used in the LDA by Ihler and Bjorn (1996), and Young *et al.*, (1999) with horses and apparently achieved good development rates.

Amphotericine B has been used by some but not others in the LDA for sheep. The concentration determined to be necessary in these experiments is higher than that used for sheep (Lacey *et al.* 1990; Gill *et al.*, 1995; Hubert and Kerboeuf, 1992 and Gopal *et al.*, 1999) and presumably for horse by (Ihler and Bjorn 1996 and Young *et al.*, 1999).

Coles *et al.*, (1988) and Hubert and Kerboeuf, (1992) determined that *E. coli* was necessary for the development of infective strongylid larvae in a LDA. Similarly these studies have shown that *E coli* is necessary for the development of infective larvae of horse strongylid nematodes.

Only one temperature (25^0C) was examined in these series of experiment. Given that good results were achieved no comparison with different temperatures were made.

These studies also showed that the optimal period for incubation is 7 days for the development of infective larvae which is similar to other authors (Coles *et al.*, 1988; Hubert and Kerboeuf, 1992 and Gill *et al.*, 1995) when incubated at 25^0C.

In summary, the observation from this series of experiments suggested that the larval development assay for horse strongylid nematodes is carried out in the following

way: 1) use of 2µl of test drugs in DMSO, 2) use of 100µl of 2% agar matrix, 3) use of 96 well microtitre plates, 4)laying about 80 eggs per well in total volume of 100µl (60µl egg solution + 10µl earle's-yeast solution + 20µl *E. coli* + 1.6µl amphotericine B + 8.4µl water), 5) optimum temperature is 25^{0}C, 6) incubation period 7 days.

CHAPTER SIX

Survey of Anthelmintic Susceptibility using a Larval Development Assay

6.1 Introduction

The prevalence of anthelmintic resistance continues to increase in different geographical areas, both in the number of species affected and the range of drugs involved. The larval development assay (LDA) is an *in vitro* assay which has proved suitable for detection of resistance to benzimidazoles, levamisole and ivermectin (Lacey *et al.*, 1990; Taylor,1990; Gill *et al.*, 1995; Amarante *et al.*, 1997; Gopal *et al.*, 1999). With increasing prevalence of resistance to benzimidazoles and the appearance of resistance in other drug classes, e.g. in the pyrantel group (Chapman *et al.*, 1996), it has become essential to know the anthelmintic resistance status of the area and even of the individual farm, when designing a worm control programme.

Although resistance to benzimidazole anthelmintics is considered common in horse strongylid nematodes in New Zealand, there is only one published report (Hope and Kemp, 1980). Resistance to ivermectin by horse nematodes has not been reported in New Zealand or elsewhere. In this experiment use is made of the LDA to survey anthelmintic susceptibility in horse nematodes in New Zealand.

6.2 Materials and Methods

The LDA was essentially run as described in Optimization Experiment 7, Chapter 5 (Section 5.4.7).

6.2.1 Animals

6.2.1.1 Kaimanawa feral horses

LDAs were made from 22 Kaimanawa feral horses which were used to set-up the base-line for non-resistant strains of nematodes for the three anthelmintics used: ivermectin aglycone, ivermectin and thiabendazole. These horses are feral horses which had never been drenched with anthelmintics, were free ranging and it was expected that all sorts of parasites were present.

Faeces were collected from 6 Kaimanawa horses in 1997 (K1-K6) when they were

on a holding farm after capture from their feral range 7 months previously. A further 5 samples (K7-K11) were collected after the horses had been transferred from this holding farm to the property where the efficacy studies were conducted. Another 11 samples (K12-K22) were collected when horses were mustered on their feral range in 1999.

6.2.1.2 Farm horses

A survey was made of 47 "farm horses" which included thoroughbred horses, slaughtered horses and exported horses. The faeces were collected from several geographical locations around New Zealand including Canterbury, Manawatu, Hawke's Bay and Auckland.

Faecal samples were collected per rectum by veterinarians and submitted to the laboratory by courier. On arrival faecal egg counts were estimated by a modified McMaster technique (Appendix I). For inclusion in the survey a minimum of 200 epg and 100g faeces was required. Veterinarians could submit several samples from the one farm and from 2 farms more than one horse was included in the survey.

6.2.2 Anthelmintics

Three anthelmintics were used: thiabendazole (99.3% powder, kindly donated by MSD AgVet, Australia) Ivermectin (0.08%, Ivomec oral drench for sheep, cattle and goats – MSD AgVet., New Zealand Ltd) and Ivermectin aglycone (99.2% powder, kindly donated by Virbac Laboratories, NZ Ltd.). All anthelmintics were diluted in DMSO and 2 µl of these dilutions were added to each well before adding the agar. The concentrations in DMSO before being added to the agar were adjusted from 0.0078µg/ml to 258µg/ml for thiabendazole, from 0.00073µg/ml to 24µg/ml for Ivermectin and from 0.0008µg/ml to 28.3µg/ml for ivermectin aglycone. The final concentration in agar are shown in Appendix II. Each concentration of anthelmintics and control wells were run in duplicate.

6.2.3 Nematode egg recovery technique

The Egg Recovery Technique was as described in the previous chapter (Chapter 5; Section 5.2.1) and detailed in Appendix II. Briefly, eggs were recovered by differential sieving and floatation in $MgSO_4$ as described by Hubert and Kerboeuf (1992).

6.2.4 Larval development assay

The LDA was as described in previous chapter (Chapter 5; Section 5.2.2) and detailed in Appendix II. In each well of 96 microtitre plates 2µl of anthelmintic in DMSO or just 2µl of DMSO for controls were added and overlaid with 100µl of 2% agar matrix. Over this eggs in nutritive medium were laid and the plate was then incubated at 25^{0}C for 7 days. The nutritive media was as finally used as in Section 5.4.7 and composed of 10µl Yeast-Earle's solution + 20µl *E. coli* + 1.6µl amphotericine B + 8.4µl water. The numbers of eggs, first, second and third stage larvae in each well were counted after staining with Lugol's iodine solution.

6.2.5 Data analysis

The numbers of eggs, first, second and third stage larvae in each well were counted, but evaluation was based on the number of L_3's as they were the only ones where it is possible to classify the different genera of a mixed infection.

The Cyathostomes were the dominant parasites and the only one for which a dose response curve could be determined. It was not possible to correct for the variation in eggs distributed to each well as these were naturally infected horses with mixed infections. A dose response curve could be determined for these after correcting for the number of L_3's that developed in control wells. The proportion was calculated by the number of cyathostomes L_3's in test wells divided by the numbers of cyathostome L_3's in control wells and was calculated as follows:

$$\text{Proportion} = \frac{\text{Number of cyathostome } L_3\text{'s in test well}}{\text{Mean number of cyathostome } L_3\text{'s in control well.}}$$

The proportion was fitted into a sigmoid curve as shown in the example in Fig 6.1 after $\log_{10}$ transformation of values for the different anthelmintic concentrations. The curve was fitted using the software package Slidewrite Version 3.0, Advanced Graphic Software Inc.

The concentration at which only 50% of eggs developed to L_3 was estimated from the dose response curve.

The mean and standard deviation (SD) for the LD_{50} values from the Kaimanawa horses were estimated for each anthelmintic. The LD_{50} values of mean + 2×SD of Kaimanawa horses was calculated and used as the cutoff for the upper limit of normal.

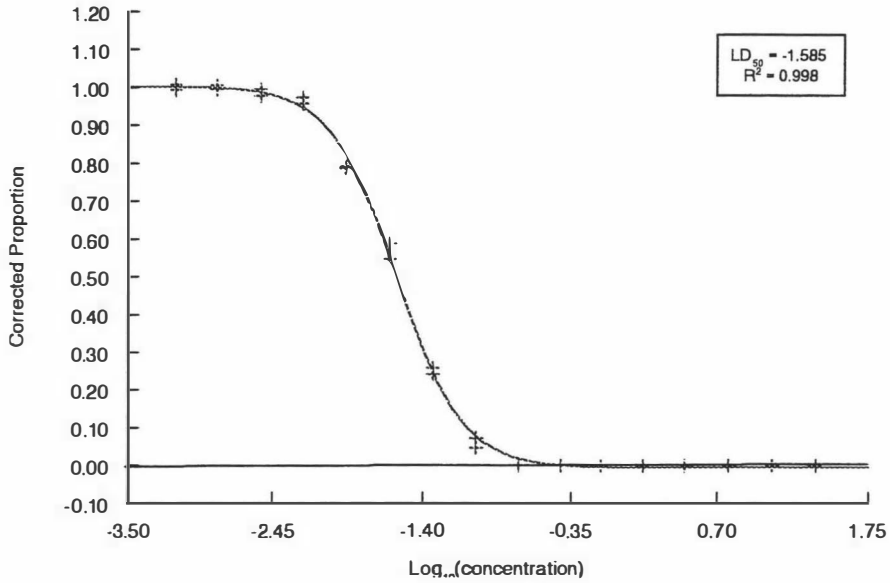

Figure 6.1: A typical dose response curve relating to the proportion of developed L_3 to logarithm concentration of ivermectin.

Resistance factors were calculated for each drug by dividing the LD_{50} value from the farm horse against the mean LD_{50} value from the Kaimanawa horses.

$$RF = \frac{LD_{50} \text{ value from the farm horse}}{\text{Mean of } LD_{50} \text{ value from the Kaimanawa horses}}$$

The coefficient of determination for fitting the sigmoid curve (r^2) was determined for each dose response curve. Any assay with a r^2 below value 0.95 was rejected and not included in the survey.

6.3 Results

The mean coefficient of determination (r^2) was 0.99, 0.989 and 0.98 for IVM, IVM aglycone and thiabendazole respectively. In general all these respective curves showed good fits with the data and little variation between anthelmintics. There were six assays (one IVM , two IVM aglycone and three thiabendazole) in which the r^2 was below <0.95 and the results from these assays were not included. The LD_{50} values from the *in vitro* larval development assays with ivermectin, ivermectin aglycone and thiabendazole were shown in Figure 6.2 to 6.4 and the raw data are shown in Appendix VI. All figures show the frequency of LD_{50} values in different farm horses and Kaimanawa horses. The LD_{50} values of ivermectin aglycone ranged from 0.089ng/ml to 8.44ng/ml of agar and 0.068ng/ml to 2.759ng/ml of agar, ivermectin from 0.112ng/ml to 1.869ng/ml of agar and 0.132ng/ml to 0.615ng/ml of agar and thiabendazole from 1.143ng/ml to 19.767ng/ml of agar and 0.559ng/ml to 8.307ng/ml of agar in the farm horses and feral horses respectively. For ivermectin aglycone (Fig. 6.2), nematodes from 6 of 47 horses (12% horses) gave an LD_{50} value above the nominal cutoff value with resistance factors ranging from 2.9 to

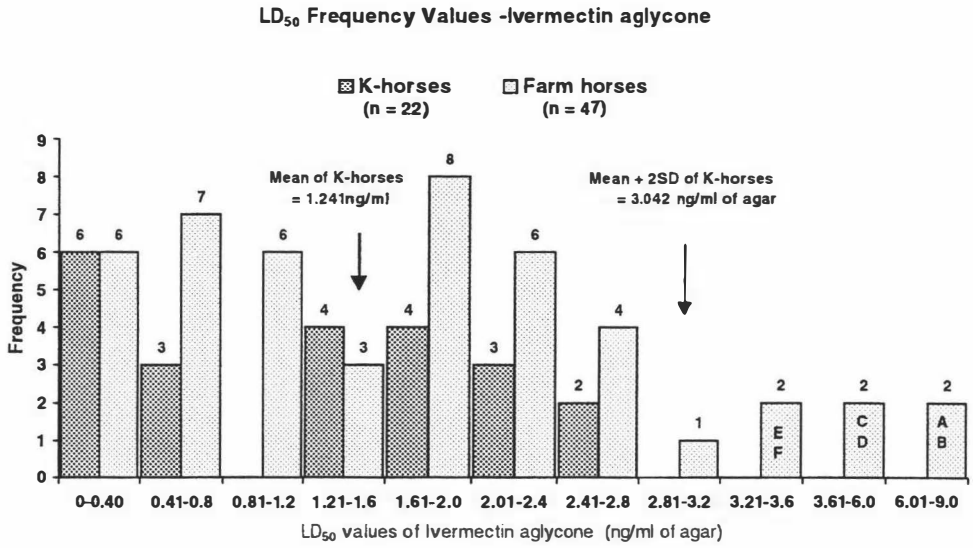

Figure No. 6.2: LD_{50} values of Ivermectin aglycone in ng/ml of agar.
Note: A,B,C,D,E and F are individual horses with high LD_{50} values

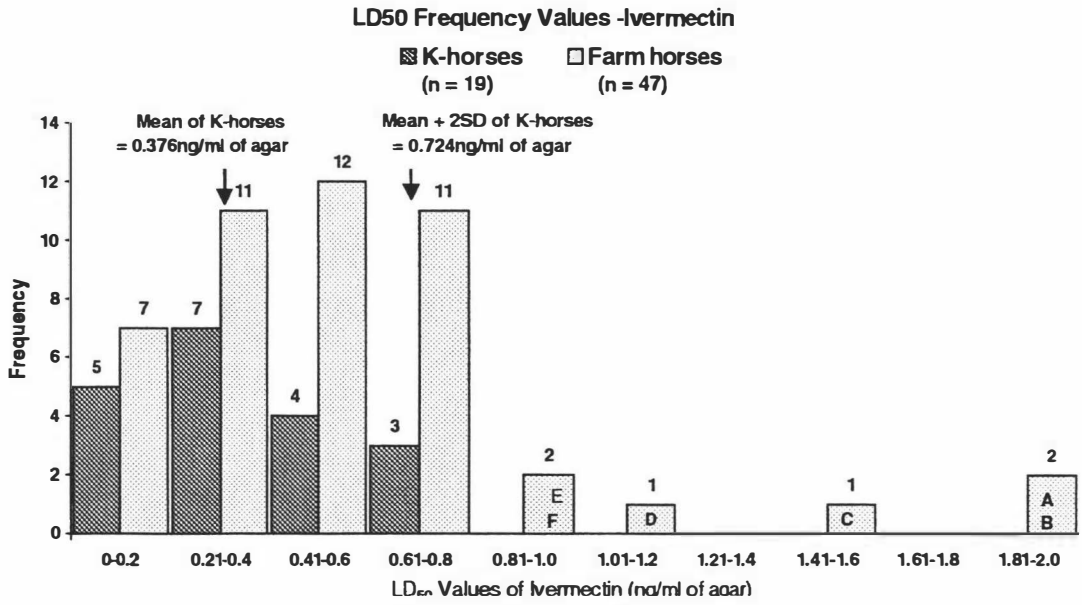

Figure No. 6.3: LD_{50} Values of Ivermectin in ng/ml of agar.
Note: A,B,C,D,E and F are individual horses with high LD_{50} values.

6.8 (2.9, 2.9, 3.9, 3.9, 6.4 & 6.8). For ivermectin (Fig. 6.3), 6 of 47 horses (12% horses) had nematodes which gave an LD_{50} value above the nominal cutoff with resistance factors ranging from 2.2 to 5.3 (2.2, 2.4, 2.8, 3.0, 4.2, & 5.3). For thiabendazole (Fig. 6.4), nematodes from 20 of 47 horses (42.5% horses) gave LD_{50} values above the nominal cutoff value with resistance factors ranging from 2.6 to 4.8. Nematodes from the 6 horses with LD_{50} values for ivermectin aglycone above the cutoff also had LD_{50} values for thiabendazole above the cutoff. These 6

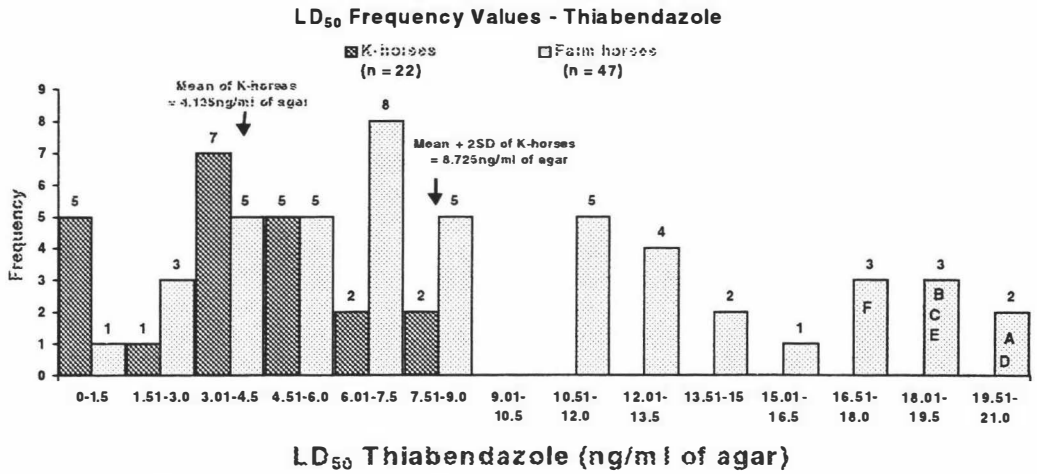

Figure No. 6.4: LD_{50} values of thiabendazole in ng/ml of agar.
Note: A,B,C,D,E and F are individual horses with high LD_{50} values

horses (A,B,C,D,E and F) that had high LD_{50} values with ivermectin aglycone, ivermectin and thiabendazole are shown in Figure 6.2, 6.3 and 6.4 and also in.Appendix VI. There were 3 horses (D, E and F) from the same farm in Hastings and 2 horses (B and C) from the same farm in Takanini. As can be seen, the horses from the same farm had similar LD_{50} values for each anthelmintic

6.4 Discussion

This study indicated that the larval development assay can used to detect increased tolerance to ivermectin, ivermectin aglycone and thiabendazole in cyathostomes. The coefficient of determination (r^2) ranged from 0.95 to 0.999, which indicated good dose response curves with the data and very few data sets were rejected because of a poor dose response curve. As cyathostomes were almost the only larvae seen in the LDA of farm horses, no information on nematodes in the subfamily Strongylinae was obtained.

This study was based on the method used by Amarante *et al.*,(1997) and Gopal *et al.*, (1999) in sheep. There are only two published reports where the LDA has been used in horses. Ihler and Bjorn (1996) and Young *et al.*, (1999), both were used the LDA for detection of anthelmintic resistance and successfully detected anthelmintic resistance to benzimidazole in horses but neither detected resistance to ivermectin. Young *et al.*, (1999) detected a resistant sub-population in both domestic horses and in a population of feral horses but these was no evidence of such a sub-population in the current study.

Two different analogues were used to investigate increased tolerance to the avermectins. With ivermectin aglycone six horses from three separate properties had high LD_{50} values and the resistance factors ranged up to 6.8 which is strongly suggestive of an increased tolerance by cyathostomes to the avermectins. With ivermectin six of the same six horses had high LD_{50} values and the resistance factors ranged up to 5.3 which is also strongly suggestive of an increased tolerance by cyathostome to the avermectins. However, the finding of increased tolerance by some nematodes does not necessarily mean they are not being killed in horses treated with a therapeutic dose of an avermectin.

There would appear to be only a small advantage in using ivermectin aglycone rather than ivermectin in the LDA to investigate tolerance to the avermectins. Since ivermectin is readily available and ivermectin aglycone is not. It would appear that ivermectin would be as suitable for use in field investigations. Further studies with other analogues may find one which result in better discrimination. For example, with an ivermectin-resistant *Haemonchus contortus* isolate Gill *et al*, (1995) found the resistance ratios of three different analogues (IVM, AVM B1 and AVM B2) were different being 5,10 and 11 respectively.

The thiabendazole data from the LDA results indicates that approximately 43% of the farm horses contained cyathostome nematodes which have an increased tolerance to BZs by comparison with the Kaimanawa feral horses. This result was supported by Ihler and Bjorn (1996) and Young *et al.*, (1999). Surprisingly, there is only one documented case of resistance to benzimidazoles in cyathostomes in New Zealand (Hope and Kemp, 1980). However, this data is consistent with the general understanding that there is widespread resistance in cyathostomes to BZs in New Zealand as has been found elsewhere around the world (Bauer *et al.*, 1986; Nilsson *et al.*, 1989; Boersema *et al.*, 1991; Bjorn *et al.*, 1991; Ihler, 1995 and Craven *et al.*, 1998).

Similar LD_{50} values were obtained for horses from the same property for all 3 actives suggesting reasonable repeatability of the assay.

These results do not give a true estimate of the prevalence of anthelmintic tolerance in cyathostomes as further work is required to validate the assay but these observations suggest that this problem is widespread. In New Zealand 12 cyathostome species were identified (See Chapter 4) and it is possible that different

species show different tolerance to avermectins or thiabendazole. It is theoretically possible that the proportion of different species in a horse may vary and this could

influence the LD_{50} value rather than represent resistance *per se.* This may account for the resistant sub-population identified by Young *et al.*, (1999) in both feral and domestic horses.

Of concern is the observation that the six horses which showed increased tolerance to avermectins also have very high tolerances to the benzimidazole which could be suggestive of multiple resistance to both these action families.

These observations suggest that increased tolerance to avermectins and benzimidazoles is widespread in farm horses in New Zealand and it is likely that the problem will be of increasing importance in the future. However, further work is required to validate the use of the LDA and allow a better interpretation of the resistance factors calculated here.

CHAPTER SEVEN

7.1 General Discussion

The optimization of the larval development assay (LDA) for horses worked well after a slight modification from the standard sheep methodology used previously (Gill *et al.*, 1995; Gopal *et al.* 1999). Interestingly there are two reports apparently using the sheep methodology successfully in horses (Ihler, 1995; Young *et al.*, 1999). However, raw data were not presented with either to indicate developmental success of control wells so it is not clear if they experienced the same problems as seen with the sheep methodology in this study.

The benzimidazole results in the survey generally align with the perception that there is wide spread BZ resistance in horses in New Zealand. The size of the resistance ratio average 4.8 is not that high compared to the levels seen in ruminants (Gill *et al.*, 1995) but further refinements to the cut off levels may result in a higher resistance ratio. Regardless, the usefulness of the LDA to detect BZ resistance still needs to be confirmed by some means, preferably a slaughter study.

The avermectin results in this survey suggest an increased tolerance by cyathostomes on three properties of the forty four properties from which samples were obtained. However, this increased tolerance of cyathostomes by some nematodes does not necessarily mean they are not being killed in horse nematodes treated with a therapeutic dose of ivermectin. Given that there are at least twelve species of cyathostome present in New Zealand, there may be different tolerances to avermectins expressed by different species and more tolerant species may be over represented on these three properties. Nevertheless, these results are suspicious and given the widespread use of ivermectin for 20 years it would be expected that resistance will be beginning emerge. This study also showed that there is little advantage in using ivermectin aglycone over ivermectin in the LDA. However, use of other analogues should also be investigated as they may result in a larger separation between resistant and susceptible nematodes.

In the two efficacy studies the conditions and timings used were slightly different, especially with respect to the interval between treatment and slaughter. This has implications for counting mucosal stages of cyathostomes and larvae of migratory species where dead larvae may take time to disappear. It also has implications for counting luminal stages of cyathostomes, which can emerge in the interval between

treatment and slaughter. When the two results are combined it is possible to say that abamectin (0.2mg/kg) combined with oxibendazole (10mg/kg) and bithionol achieved an efficacy of more than 95% required for a control claim against adult luminal stages of *S. vulgaris, S. edentatus,* cyathostomes and *Gasterophilus* spp. larvae. It was necessary to use 7mg/kg bithionol to achieve high efficacy against *A. perfoliata* as 5mg/kg had only 86% efficacy which is too low to warrant a control claim. These results are similar to those by Takahashi, K (unpublished report) in Japan. This suggests that the presence of oxibendazole, which might have been expected to have some efficacy against *Anoplocephala*, has not had an additive effect to that of bithionol.

When ivermectin paste is used alone at 0.2mg/kg, it was highly effective (>97% efficacy) against luminal *Strongylus* spp., *Triodontophorus* spp., cyathostomes, *Habronema* spp., *Oxyuris equi* and *Gasterophilus* spp. (Klei *et al.*, 1993; Xiao *et al.*, 1994; French *et al.*, 1994; Cobra *et al.*, 1995; Monahan *et al.*, 1996; Costa *et al.*, 1998). Moreover Slocombe *et al.*, (1982) and Herd, (1992) observed that it was also effective against migrating larval stages of *S. vulgaris* and *S. edentatus*. Although similar efficacy was achieved against *S. edentatus* larvae in this study, the efficacy against migratory *S. vulgaris* was poor. This indicates that ivermectin and abamectin are not identical in their range of efficacy. The activity against the mucosal stages of cyathostomes was remarkably consistent in the two studies (~65%) which is similar to one studies with ivermectin (Love *et al.*, 1995) and greater than other studies (Eysker *et al.*, 1992; Klei *et al.*, 1993; Xiao *et al*. 1994).

Similarly when oxibendazole was used alone, it (10mg/kg) was highly effective (>95%) against luminal stages of *Strongylus* spp., *Triodontophorus* spp., cyathostome, *T. axei*, and *O. equi* (Kates *et al.*, 1975; Nawalinski and Theodorides, 1976; Nawalinski and Theodorides, 1977; Lyons *et al.*, 1981; Theodorides *et al.*, 1982; Drudge *et al.*, 1984; Stromberg *et al.,* 1985; Tolliver *et al.*, 1993) but was less effective (62-90%) against luminal larval stages of cyathostomes but had no efficacy against migrating stages of *Strongylus* spp. In this study oxibendazole combined with abamectin was expected to have high efficacy against *Strongylus* spp., *Triodontophorus* spp., cyathostomes, *T. axei*, and *O. equi,* reflecting the high efficacy achieved by both components alone.

The advantage of the combination anthelmintic is that efficacy against nematodes resistant to either should be achieved. It is also suggested that combination of

anthelmintics can slow development of resistance (Rolfe and Dawson, 1994; Anderson *et al.*, 1988). In the current situation, where BZ resistance in cyathostome is considered to be common this is mainly aimed at delaying development of resistance to avermectins.

In New Zealand, three species of *Strongylus*, two species of *Triodontophorus* and eight species of cyathostome have been previously reported (Tetley,1948; McKenna, 1997). In this study one additional species of *Triodontophorus* and four additional species of cyathostome were identified. The presence of these species was expected given they are found in horses in similar climatic regions around the world. The fact they were not recognised previously reflects the limited research and identification of horse parasites in New Zealand. The timing of these two efficacy studies allowed a comparison of worm burdens in summer and winter. It was found there was no difference in species present on their relative intensity of infection suggesting no seasonality of occurrence. However, these worms are probably long-lived so further investigations are required to confirm this. The distribution of cyathostomes throughout the large intestine was as previously described (Mfitilodze and Hutchinson, 1990; Reinemeyer *et al.*, 1988) with some species showing a site preference but others not.

APPENDIX I

A1. Standard Operating Procedures

Contents

Based on SOP's Utilised by Dr.T.R. Klei, Principle Investigator, Equine Parasitology Laboratory, Department Of Veterinary Science, Louisiana Agricultural Experiment Station, Louisiana State.

These SOP's are modified specifically to suit this study in some respects, from preliminary work.

A1.1 Egg Counting Procedure : Modified McMaster Technique

Equipment

McMaster counting slide – Olympic Equine: grid size 1cm^2, volume under each grid 0.15ml.
Small stainless bowl – size 9.2 x 3.5cm, volume 100ml.

Microscope (Olympus).

Pasteur pipette.

Domestic tea strainer 1mm aperture, 6cm diameter.

Electronic strainer (Delta Range^R USA) 0.1g.

Domestic plastic tea spoon.

Universal bottle, volume 28ml.

Saturated NaCl specific gravity 1.2.

Procedure

1. Weigh 2 g of faeces into the sieve within the bowl.

2. Mix with 28 ml of saturated NaCl in the bowl.

3. Pass through the coarse sieve (tea strainer) to remove the large particulate matter.

4. Mix the filtrate and with a Pasteur pipette and fill each of 2 chambers of a standard McMaster slide.

5. Count the eggs that appear under the grid lines in the two chambers at 40X magnification and multiply by 50 to get egg per gram.

This counting system relies on 2g of faeces displacing 2ml volume, which together with 28ml of saline totals 30mls. The volume under each of the 2 grid lines is 0.15ml for a total of 0.3ml, which represents an aliquot of 0.01. However, each egg count represents 50 eggs/g as there are 2g of faeces.

Faecal Flotation Technique

Mix 2g of faeces with 28ml saturated NaCl and pass through a coarse sieve as above. Pour the filtrate into a universal jar, top up with the flotation medium to a shallow meniscus and place a coverslip on top. Remove the cover after 15 minutes and place on a slide and then scan at 100X magnification. Count all eggs seen. The number of eggs divided by 2 represents the number of eggs/g.

A1.2 Necropsy procedures for recovery of endoparasites

Equipment

Gut scissors

Knife

Petri dish

Plastic bucket (10L to 50L) capacity marked at litre

Polythene rubbish bin (5kg – 50kg) capacity

2L preserving jar

Ladles 100 to 500ml capacity plastic beakers

2000ml plastic marked beaker

Wooden stirrer-flat in section 5cm x 1.0cm x 40-50cm long

1.25mm aperture sieve

104µm aperture sieve

53µm aperture sieve

String

Hanging scales ranged from 0.0 to 22kg (0.5kg interval)

50ml and 100ml sample container

Petridish with scored lines

A stereo microscope

10% formalin

Pepsin A powder (BDH) contains lactose as diluent: activity about 1 Anson unit per g (calculated as at 35.5^{0}C) (1:3000NF).

Conc. Hydrochloric acid (BDH).

Procedure

1. Throughout the necropsy, record all observations by a scribe.

2. Open the abdomen.

3. Remove the gastro-intestinal tract in its entirely together with the mesentery and the aorta, approximately 10cm anterior to and 10 cm posterior to the entrance to the cranial mesenteric artery.

4. Trim the retro-peritoneal fat from the hanging carcass on both sides of the abdomen and place in a labelled plastic bag.

5. Remove the cranial mesenteric artery together with the mesenteric arteries by trimming the mesentery at the level of the serosa. Place these in a labelled plastic bag.

6. Ligate the GI tract at the eosophagus-stomach junction, the pylorus, the ileocaecal junction, the junction of the caecum and ventral colon, the junction of the dorsal and ventral colon, the junction of the dorsal and small colon. Separate each of these segments and place in a labelled plastic bag.

7. Store all of these organs at -20^{0}C.

A1.3 Preparation and examination of the contents of the stomach

1. Thaw and open the stomach. Then wash under a forceful stream of water and collect the contents in a bucket.

2. Allow the contents to settle, then remove the supernatant fluid by decantation to an area just above the settled solid material.

3. Thoroughly resuspend the mixture and remove 2 X 5% aliquot using the ladle to fill a beaker to the required volume.

4. Examine one 5% aliquot under a dissecting microscope (10X) and remove, identify, and count the parasites found. Keep the other 5% aliquot as a reserve.

5 Sieve the remaining 90% and the reserve 5% aliquot through a 104µm aperture mesh and examine visually for large parasites including *Gasterophilus* spp. larvae. Collect all *Gasterophilus* spp. larvae and preserve in 10% formalin.

6 Under a dissecting microscope at 10X, determine the species and larval stage of *Gasterophilus* spp. larvae.

7 Total number of parasites = number of parasites present in aliquot X by reciprocal factor.

8 Store any parasites found in 10% formaldehyde, identify bottles as to horse and drug trial.

A1.4 Preparation and examination of the contents of the small intestine

1. Thaw and open the duodenum, jejunum and ileum, wash under a forceful stream of water and collect all the contents in a bucket.

1 Allow the contents to settle, then remove the supernatant fluid by decantation to an area just above the settled solid material.

2 Thoroughly resuspend the mixture and remove 2 X 5% aliquots using the ladle to fill a beaker to the required volume.

3 Examine one 5% aliquot under a dissecting microscope (7X to 10X) and remove, identify, and count all parasites found. Keep the other 5% aliquots as a reserve.

4 Wash the remaining 90% and the reserve 5% aliquot thoroughly through a 104µm aperture sieve and examine for *Parascaris equorum* and *Anoplocephala* spp.

5 Total number of parasites = number of parasites present in aliquot X reciprocal factor.

6 Store any parasites found in 10% formaldehyde, in bottles identified as to horse and drug trial.

A1.5 Preparation of the contents of the Caecum

1 Thaw and open the caecum.

2 Wash the surface under a forceful stream of water and collect all the contents in a 50 L bucket. Remove any attached strongylid nematodes or *Anoplocephala* to 10% formaldehyde solution. Take any desired tissue samples.

3 Bring the contents of the bucket up to a multiple of 10 L with water. While

thoroughly stirring with a heavy paddle, remove 2 X 5% aliquots of the mixture using the 500ml plastic beaker as a ladle to fill the 2L beaker to the required volume.

4 Place the aliquots into a 2L labelled preserving jar with screw cap and preserve with 10% by volume of formaldehyde solution. Keep one 5% aliquot as a reserve.

5 Sieve the remaining 90% and the reserve 5% aliquot of the intestinal contents through a 1.25mm aperture mesh.

6 Store any parasites found in 10% formaldehyde.

7 Weigh the caecum and spread out on a table.

8 Take 6-11 pieces approximately 12cm × 12cm representing about 5-10% of the total caecal weight. Space these evenly throughout the length of the caecum.

9 Place these in a labelled plastic bag and store in the freezer at -20^{0}C.

A1.6 Preparation of the contents of the Ventral Colon

1 Thaw and open the ventral colon.

2 Wash the surface under a forceful stream of water and collect all the contents in to a 50L bucket. Remove any attached strongylid nematodes to 10% formaldehyde solution and take any desired tissue samples.

3 Bring the contents of the bucket up to a multiple of 10L with water. While thoroughly stirring using a heavy paddle, remove 2 X 5% aliquot of the mixture using the 500ml plastic beaker as a ladle to fill the 2L beaker to the required volume.

4 Place the aliquots into a 2L labelled preserving jar with screw cap and preserve these aliquots with 10% by volume of formaldehyde solution. Keep one 5% aliquot keep as a reserve.

5 Sieve the remaining 90% and the reserve 5% aliquot of the intestinal contents through a 1.25mm aperture mesh.

6 Store any parasites found in 10% formaldehyde.

7 Weigh the ventral colon and spread out on a table.

8 Take 7-14 pieces approximately 12cm × 12cm representing about 5-10% of the total ventral colon weight. Space these evenly throughout the length of the ventral colon.

9 Place these in a plastic bag, labelled properly and store in the freezer at -20^{0}C.

A1.7 Preparation of the contents of the Dorsal Colon

1 Thaw and open the dorsal colon.

2 Wash the surface under a forceful stream of water and collect all the contents in a 50L bucket. Remove any attached strongylid nematodes and *Oxyuris equi* to 10% formaldehyde solution. Take any desired tissue samples.

3 Bring the contents of the bucket up to a multiple of 10L with water. While thoroughly stirring using a heavy paddle, remove 2 X 5% aliquot of the mixture using the 500ml plastic beaker as a ladle to fill the 2L beaker to the required volume.

4 Place the aliquots into a 2L labelled preserving jar with screw cap and preserve these aliquots with 10% by volume of formaldehyde solution. Keep one 5% aliquot as a reserve.

5 Sieve the remaining 90% and the reserve 5% aliquot of the intestinal contents through a 1.25mm aperture mesh.

6 Store any parasites found in 10% formaldehyde.

7 Weigh the dorsal colon mucosa and spread out on a table.

8 Take 5 - 9 pieces approximately 12cm × 12cm representing about 5-10% of the total dorsal colon weight. Space these evenly throughout the length of the dorsal colon.

9 Place these in a plastic bag, labelled properly and store in the freezer at -20^{0}C.

A1.8 Preparation of the contents of the Small Colon

1 Thaw and open the small colon.

2 Wash the surface under a forceful stream of water and collect all the contents in

a 50L bucket. Remove any attached strongylid nematodes and *Oxyuris equi* larvae to 10% formaldehyde solution. Take any desired tissue samples.

3 Bring the contents of the bucket up to a multiple of 10L with water. While thoroughly stirring using a heavy paddle, remove 2X 5% aliquot of the mixture using the 500ml plastic beaker as a ladle to fill the 2L beaker to the required volume.

4 Place the aliquots into a 2L labelled preserving jar with screw cap and preserve these aliquots with 10% by volume of formaldehyde solution. Keep one 5% aliquot as a reserve.

5 Sieve the remaining 90% and the reserve 5% aliquot of the intestinal contents through a 1.25mm aperture mesh.

6 Store any parasites found in 10% formaldehyde.

A1.9 Examination of the 5% aliquots of the caecum and colon

1. Select one 5% aliquot from each organ from each horse and carefully decant the supernatant formaldehyde solution, taking care not to decant any "loose" solids. The "bottoms" are stirred and the jar is filled with water and set aside to settle again.

2. Repeat 1. three or four times, until the odour of formaldehyde is greatly reduced.

3. Fill the jar with water and sieve through a 53µm aperture mesh and collect the material on the sieve in a 1.5L beaker with water.

4. Examine all of this material visually, under strong light, in glass dishes 140 mm in diameter, against a dark background and count any parasites seen.

5. Examine only one 5% aliquot from each organ from each horse at a time, collect at least 50 parasites into one container for species identification, label and store in 10% formalin.

6. Save the second 5% aliquot from the same organ of each horse as a reserve.

A1.10 Enumeration and identification of parasites

A1.10a. Large Parasite:

1 Count the large parasites after sieving the whole of the intestinal contents (including both 5% aliquots).

2 Identify to species by using a stereo microscope in a petri dish, with the worm suspended in water.

3 This is the TOTAL NUMBER of large parasites. Large parasites included *Strongylus* spp., *Triodontophorus* spp., *Gasterophilus* spp., *Oxyuris equi* and *Anoplocephala* spp.

A1.10b. Small Parasites

1 Place 2 or 3 small parasites in lactophenol on a microscope slide and place a cover slip on top. Examine each parasite under 40 or 100X magnification.

2 Identify at least 50 stored small parasites (*Cyathostomum, Cylicocyclus* and *Cylicostephanus*) to genus level from the 5% aliquot according to Lichtenfels, J.R., 1975. Helminths of Domestic Equids, Proc. Helminth. Soc. Wash. 42, special issue. and Strongyloides of Animals and Man/ Trichonematidae, Popova, T.I., volume VII.

3. The numbers of small parasites present in the Aliquot X the reciprocal of the fraction examined = TOTAL NUMBER in caecum and large intestine.

A1.11 Procedure for the quantitative recovery of *Strongylus vulgaris* from the mesenteric vasculature

1. Thaw and carefully dissect the arteries by opening longitudinally with scissors.

2. Remove the grossly visible larvae with forceps and identify with a stereo microscope.

3. Scrape the internal surfaces of all these vessels with a scalpel blade and examine the resulting material for parasites under a stereomicroscope.

4. Save any recovered parasites in 10% formalin in appropriately labelled pottles.

A1.12 Procedure for the quantitative recovery of *Strongylus edentatus* from the retro-peritoneal fat

1 Thaw and carefully examine the retro-peritoneal fat with scissors and forceps in the laboratory.

2 Remove the grossly visible parasites with forceps and identify with a stereo microscope.

3 Save any recovered parasites in 10% formalin in appropriately label pottles.

A1.13 Enumeration of mucosal cyathostomes

1 Perform each of these steps perform separately for each section of large intestine.

A1.13.a Transillumination

1. Stretch each piece of the caecum, ventral colon, and dorsal colon over a 24 cm^2 circular opening cut out of a thin plastic pipe and tack down.

2. View the stretched pieces at 10 - 15X with a stereo microscope with a fibre optic light source underneath.

3. Count all encysted larvae in each piece and record.

4. Excise each circular piece, weigh and record larvae count as larvae/gm tissue.

A1.13.b Digestion

1. Use the same pieces of mucosa that was counted by transillumination. Scrape with a glass slide and then cut into small pieces.

2. Digest the combined material in pepsin (BDH)/HCl (BDH) solution (digestion fluid: 10gm pepsin added with 15ml conc. HCl in 1 litre distilled water).

3. Add 200ml digestion fluid to approximately 30gm mucosal tissue and incubate at 35-40^{o}C.

4. Shake the samples frequently and place in a heated shaker water bath.

5. Continue digestion until no obvious mucosal surface or large pieces of tissues remain visible. This is for a minimum of 2 hours and a maximum of 6 hours.

6. Wash the digestion mixtures through a 53µm apertures sieve and examine the retained material under the dissecting microscope.

7. Count the larvae under the dissecting microscope at 20X.

8. Calculate the total number of larvae by taking the number of larvae present in the combined mucosal samples, and multiply by the appropriate multiplication factor to give the total number of parasites in each organ.

APPENDIX II

A2. The Larval Development Assay

A2.1 Equipment

Electronic balance (Delta Range^{R} USA) 0.1g

Measuring cylinders

Beakers – volume 100 ml to 3000 ml

Plastic test tubes (Falcon) – volume 50 ml.

Eppendrop 5µl, 20µl, 200µl and 1000µl pipettes.

Multi dose pipette (Multi-pipette Plus).

Grooved microscope slides.

Cover slips.

96-well (Nunclon^{MT}) round flat-bottomed microtitre plates – volume of each well 300µl.

Rectangular shaped Plastic container as a desiccator (to incubate plates).

Incubator.

Microwave (Panasonic).

Squeeze bottles – volume 250 ml.

Sieves – 1mm aperture diameter 12cm, 20µm aperture diameter 10.2cm, 40µm aperture diameter 4.2cm, 60µm aperture diameter 7.5cm and 100µm aperture diameter 10.2cm.

Microscope (Olympus)

Centrifuge (ILEC Centra-8 International Equipment Company).

Laboratories multiple counter (Clay Adams)

A2.2 Chemicals

Anthelmintics

Ivermectin (0.08%) (Ivomec oral drench for sheep and goats) – MSD AgVet, New Zealand Ltd.)

0.08% Ivermectin drench = 0.8g/litre i.e. 0.8mg/ml i.e. 800µg/ml.

Therefore 1ml of 800µg/ml + 33.3ml Dimethyl sulphoxide (DMSO) gives 24µg/ml stock solution. 2µl of 24µg/ml in 100µl of agar = 2×24/100 = 0.48µg/ml.

The following dilutions were used for ivermectin:

1) Stock solution 24µg/ml in 100 agar =0.48µg/ml.
2) 1 ml of 24µg/ml + 1ml DMSO = 12µg/ml = 0.24µg/ml in agar.
3) 1 ml of 12µg/ml + 1ml DMSO = 6µg/ml = 0.12µg/ml in agar.
4) 1 ml of 6µg/ml + 1ml DMSO = 3µg/ml = 0.06µg/ml in agar.
5) 1 ml of 3µg/ml + 1ml DMSO = 1.5µg/ml = 0.03µg/ml in agar.
6) 1 ml of 1.5µg/ml + 1ml DMSO = 0.75µg/ml = 0.015µg/ml in agar.
7) 1 ml of 0.75µg/ml + 1ml DMSO = 0.375µg/ml = 0.0075µg/ml in agar.
8) 1 ml of 0.375µg/ml + 1ml DMSO = 0.1875µg/ml = 0.0038µg/ml in agar.
9) 1 ml of 0.1875µg/ml + 1ml DMSO = 0.09375µg/ml = 0.0019µg/ml in agar.
10) 1 ml of 0.09375µg/ml + 1ml DMSO = 0.046875µg/ml = 0.00094µg/ml in agar.
11) 1 ml of 0.046875µg/ml + 1ml DMSO = 0.02343µg/ml = 0.00047µg/ml in agar.
12) 1 ml of 0.02343µg/ml + 1ml DMSO = 0.01172µg/ml = 0.000234µg/ml in agar.
13) 1 ml of 0.01172µg/ml + 1ml DMSO = 0.005859µg/ml = 0.000117µg/ml in agar.
14) 1 ml of 0.005859µg/ml + 1ml DMSO = 0.002929µg/ml = 0.00006µg/ml in agar.
15) 1 ml of 0.002929µg/ml + 1ml DMSO = 0.001465µg/ml = 0.00003µg/ml in agar.
16) 1 ml of 0.001465µg/ml + 1ml DMSO = 0.0007325µg/ml = 0.000015µg/ml in agar.

Ivermectin aglycone

Ivermectin aglycone (99.2% powder, kindly donated by Virbac Laboratories Ltd.).

Molecular weight of ivermectin aglycone = 566.73

1 molar = 566.73g in 1000 ml i.e. 566.73×10^6µg in 1000 ml

i.e. 566.73×10^3µg in 1ml

i.e. 1µM = 0.567µg/ml

i.e. 50µM = 0.567 × 50 = 28.35µg/ml

Therefore, 1ml contain = 28.35µg

i.e. 50 ml contain = 1.4mg

Therefore, 1.4mg powder diluted in 50ml DMSO = 28.35µg/ml.

i.e. 2 µg/ml in 100µg of agar = 0.567µg/ml

Then series of 1:2 dilution were made as follows:

1) Stock solution 28.3µg/ml = 0.567µg/ml in agar

2) 1ml of 28.3µg/ml + 1ml DMSO = 14.15µg/ml = 0.283µg/ml in agar.

3) 1ml of 14.15µg/ml + 1ml DMSO = 7.07µg/ml = 0.1415µg/ml in agar.

4) 1ml of 7.07µg/ml + 1ml DMSO = 3.54µg/ml = 0.0707µg/ml in agar.

5) 1ml of 3.54µg/ml + 1ml DMSO = 1.77µg/ml = 0.0354µg/ml in agar.

6) 1ml of 1.77µg/ml + 1ml DMSO = 0. 885µg/ml = 0.0177µg/ml in agar.

7) 1ml of 0. 885µg/ml + 1ml DMSO = 0. 4425µg/ml = 0.00885µg/ml in agar.

8) 1ml of 0. 4425µg/ml + 1ml DMSO = 0. 2212µg/ml = 0.00442µg/ml in agar.

9) 1ml of 0. 2212µg/ml + 1ml DMSO = 0. 1106µg/ml = 0.00221µg/ml in agar.

10) 1ml of 0. 1106µg/ml + 1ml DMSO = 0.0553µg/ml = 0.001105µg/ml in agar.

11) 1ml of 0.0553µg/ml + 1ml DMSO = 0.0277µg/ml = 0.00055µg/ml in agar.

12) 1ml of 0.0277µg/ml + 1ml DMSO = 0.0138µg/ml = 0.000276µg/ml in agar.

13) 1ml of 0.0138µg/ml + 1ml DMSO = 0.0069µg/ml = 0.000138µg/ml in agar.

14) 1ml of 0.0069µg/ml + 1ml DMSO = 0.00345µg/ml = 0.000069µg/ml in agar.

15) 1ml of 0.00345µg/ml + 1ml DMSO = 0.00172µg/ml = 0.000034µg/ml in agar.

16) 1ml of 0.00172µg/ml + 1ml DMSO = 0.0008µg/ml = 0.000017µg/ml in agar.

Thiabendazole:

Thiabendazole (99.3% powder, Kindly donated by MSd AgVet, Australia).

30 mg of thiabendazole powder was added in 115.5 ml of DMSO or 12.9mg powder were dissolved in 50ml DMSO. Then it was stirred and shaken properly until the powder was dissolved. This gave a stock solution of 258µg/ml of thiabendazole.

i.e. 2µl of 258 µg/ml in 100µl of agar = 258µg/ml.

Then series of 1:2 dilution were made as follows:

1) 2µl of 258 µg/ml in 100µl of agar = 258µg/ml.

2) 1 ml of 258 µg/ml + 1ml of DMSO = 129µg/ml = 1.29µg/ml in agar.

3) 1 ml of 129 µg/ml + 1ml of DMSO = 64.5µg/ml = 0.645µg/ml in agar.

4) 1 ml of 64.5 µg/ml + 1ml of DMSO = 32.25µg/ml = 0.3225µg/ml in agar.

5) 1 ml of 32.25 µg/ml + 1ml of DMSO = 16.125µg/ml = 0.1612µg/ml in agar.

6) 1 ml of 16.125 µg/ml + 1ml of DMSO = 8.0625µg/ml = 0.0806µg/ml in agar.

7) 1 ml of 8.0625 µg/ml + 1ml of DMSO = 4.03125µg/ml = 0.0403µg/ml in agar.

8) 1 ml of 4.03125 µg/ml + 1ml of DMSO = 2.015625µg/ml = 0.02016µg/ml in agar.

9) 1 ml of 2.01562 µg/ml + 1ml of DMSO = 1.0078125µg/ml = 0.01008µg/ml in agar.

10) 1 ml of 1.0078 µg/ml + 1ml of DMSO = 0.503906µg/ml = 0.00504µg/ml in agar.

11) 1 ml of 0.5039 µg/ml + 1ml of DMSO = 0.25195µg/ml = 0.00252µg/ml in agar.

12) 1 ml of 0.25195µg/ml + 1ml of DMSO = 0.125877µg/ml = 0.00126µg/ml in agar.

13) 1 ml of 0.12587 µg/ml + 1ml of DMSO = 0.06299µg/ml = 0.00063µg/ml in agar.

14) 1 ml of 0.06299 µg/ml + 1ml of DMSO = 0.03149µg/ml = 0.00031µg/ml in agar.

15) 1 ml of 0.03149µg/ml + 1ml of DMSO = 0.01575µg/ml = 0.000155µg/ml in agar.

16) 1 ml of 0.0157µg/ml + 1ml of DMSO = 0.00787µg/ml = 0.000077µg/ml in agar.

Nutritive media

1g Yeast Extract (Y-1000 Sigma) was added in 90 ml of 0.85% saline solution (Oxoid). The yeast solution was stored in 9ml bottles in the freezer. At the time of assay 1ml of Earle's Balanced Salt solution (E7510, Sigma) was added to every bottle 9ml of Yeast Extract and this combination was referred to as the nutritive media.

***E. coli* suspension**

15mg of lyophilised *E. coli* (Strain W (ATCC) 9637, Sigma) were added to 100 ml of distilled water. These was dispensed into 3ml Bijou bottles and then sterilized by autoclave.

Amphotericin B solution

25 mg of Amphotericine B (A-9525, Sigma) was dissolved in 100 ml of distilled water. This was dispensed into 1ml aliquots in ample and stored in the freezer until use.

2% Agar matrix

2g agar (Bacto-agar; Y-1000 Sigma) were dissolved in 100ml of distilled water by heating in a microwave for about 2-3 minutes and kept warm on a heating plate during the application of agar to the plates.

20% Magnesium Sulphate solution

100g of magnesium sulphate were dissolved in 500 ml of water. This gives a specific gravity of 1.1.

Lugol's iodine solution

5% iodine and 10% potassium iodide in water.

Dimethyl sulphoxide (DMSO) (Sigma New Zealand Ltd.)

A2.3 Procedure

A2.3.1 Nematode eggs recovery

Nematode egg recovery was as described by (Hubert and Kerboeuf, 1992; Amarante *et al.*, 1997 and Gopal *et al.*, 1999).

1) Before recovering the nematode eggs, faecal egg counts were carried out to know the epg of this samples. A minimum requirement to conduct the assay was 200 epg, and amount of 100g of faeces available. This ensured there were enough eggs for preparing plates. For this experiment approximately 80 eggs in each well of the 96 wells microtitre plate was used.

2) The faeces were washed through a 1mm-mesh screen into a 3L beaker. Then the faeces were washed through a 100μm aperture sieve into a 2L beaker. The faecal suspension was then washed on a 20μm aperture sieve which retained the eggs. The eggs were then washed off the screen into a 250ml beaker with a jet of water from a squeeze bottle.

3) The egg suspension was transferred to several 50ml plastic test tubes which were then filled to the 50ml mark level with water. These were centrifuged at 1500g for 10 minutes to concentrate the eggs. The supernatant was discarded and magnesium sulfate (density 1.10) was added in the tubes up to the 50ml mark. This was then mixed by shaking and then centrifuged for another 5 minutes at 1500g.

4) The supernatant was then washed through a 60μm aperture sieve inside a 20μm sieve with a forceful stream of tap water. The eggs were retained on the 20μm aperture sieve. The eggs were then washed off the sieve into another test tube with a jet of water from a squeeze bottle.

5) Care must be taken, as a long exposure to $MgSO_4$ damages the eggs, it is important to complete this step quickly (Hubert and Kerboeuf, 1984).

6) The egg suspension was then transferred into a graduated 500 ml glass tube and allowed to settle for one hour. The supernatant was then removed to reduce the volume to about 50ml.

7) The egg suspension was then transferred to a 50 ml graduated test tube and again allowed to settle for one hour. The supematant was removed to reduce the volume and the concentration of the egg suspension was estimated in eight 20µl samples and counted at 100x with a compound microscope.

8) The final volume of the eggs suspension was adjusted to approximately 1.333eggs/ml (80 eggs/60µl of suspension).

A2.3.2 Preparation of LDA-plates

1) This test was carried out in 96 well microtitre plates largely as described by Gill *et al.* (1994) with two replicates of each anthelmintic dilution.

2) 2µl of diluted anthelmintic dilution was added to each well with sixteen different concentrations of ivermectin, ivermectin aglycone and thiabendazole used as previously described.

3) 2µl of dimethyl sulfoxide (DMSO) was added in each control well and eight wells were used as controls in each plates.

4) 100µl of hot 2% agar was added to each wells. At least half an hour was allowed for the agar to set and cool before proceeding.

A2.3.3 Preparation of cultures

1) 9 ml egg suspension was mixed with 3.0ml sterilized *E. coli* suspension plus 1.5ml nutritive medium (9ml Yeast Extract + 1ml Earle's Balanced Salt Solution) plus 240µl amphotericine-B plus1.5ml of water.

2) The solution was then mixed gently and 100µl of this mixture was layered on top of the agar matrix in each well of the microtitre plates.

3) The plates were then incubated at 25^{0}C for 7 days in a sealed plastic box which contains water to ensure a high humidity.

4) The liquid media containing larvae and eggs were transferred on a glass slide with a pipette.

5) The number of eggs, first, second and third stage larvae in each well were counted after staining with Lugol's iodine solution.

6) The proportion was calculated by the number of third stage cyathostome larvae in the test well divided by the number of third stage cyathostome larvae in control wells. This was calculated as follows:

$$\text{Proportion} = \frac{\text{Number of cyathostome } L_3\text{'s in each test well}}{\text{Mean number of cyathostome } L_3\text{'s in control well}}$$

8) LD_{50} values were calculated by using the software Slide Write Version 3.0.

APPENDIX III

A.3 Raw data from the Dose Confirmation Study with AOB 198

A3.1 Animal identification and allocation on the basis of faecal egg counts on Day - 25 and Day – 10.

Treated/ Control	Rank of pair	Neck band	Weight (Kg)	FEC* day -25 (epg)	FEC* day -10 (epg)	Average egg count
C	1	16	333	1250	450	850
T	1	17	336	700	900	800
C	2	23	290	1000	450	725
T	2	11	356	900	600	750
C	3	27	326	700	500	600
T	3	21	251	500	600	550
C	4	14	373	100	1000	550
T	4	4	345	550	450	500
C	5	18	380	500	400	450
T	5	2	336	NTR	450	450
C	6	20	276	400	400	400
T	6	24	338	350	300	350

*FEC = Faecal egg count; ranked by epg
Treatment: AOB198: Batch No. 97030/C; MFR: 23/04/98

A3.2 Treatment record of AOB 198 in the Dose Confirmation Study of each horses

Neck band	Weight Kg	FEC* day 0 (epg)	Pair	Calculated dose	Drenched dose
16	331	600	7	-	0
17	310	1600	7	15.5 g	18.2 g
23	282	600	8	-	0
11	340	800	8	17.0 g	17.7 g (spilt 2 g in yard) *
27	309	950	9	-	0
21	249	700	9	12.45 g	14.7 g (spilt 2 g in yard) *
14	360	800	10	-	0
4	340	500	10	17.0 g	20.7 g
18	349	NTR[▽]	11	-	0
2	371	700	11	18.55 g	20.72 g (spilt 2 g in yard) *
20	264	300	12	-	0
24	327	900	12	16.35 g	20.7 g (spilt 2 g in yard) *
			Average	1g/20kg	1.08g/20kg

Dosage: 2.5 grams per 50 kg b.w. ; *FEC = Faecal egg count; [▽]NTR = Not recorded
* = net dosage after allowing for estimated spillage.

Appendix III (Cont.)
Raw data from the Dose Confirmation Study with AOB 198

A3.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. 16

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	66	0	4644
Small intestine	97	0	0	0	0	0	0	0
Caecum	276	76	702	0	1040	0	0	0
Ventral colon	21	204	67	34	1600	0	0	0
Dorsal colon	0	0	2	9	3700	0	0	0
Small colon	0	1	0	0	420	0	0	0
CMA♦/IT. nodules	0	0	38	0	0	0	0	0
Subperitoneal sites	0	5	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Calculated total internal parasite numbers of horse no. 17

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	1	0	0	0	0	0	0	0
Caecum	0	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	0	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
CMA♦/IT. nodules	0	0	37	0	0	0	0	0
Subperitoneal sites	0	5	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Appendix III (Cont.)
Raw data from the Dose Confirmation Study with AOB 198

A3.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. 23

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	251	0	5049
Small intestine	36	0	0	0	0	0	0	0
Caecum	67	130	64	26	1460	0	0	0
Ventral colon	2	155	5	2	4460	3	0	0
Dorsal colon	0	0	0	0	700	0	3700	0
Small colon	0	0	0	0	280	0	40	0
CMA♦/IT. nodules	0	0	3	0	0	0	0	0
Subperitoneal sites	0	2	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Calculated total internal parasites of horse no. 11

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	5	0	117
Small intestine	44	0	0	0	0	0	0	0
Caecum	32	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	0	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
CMA♦/IT. nodules	0	0	19	0	0	0	0	0
Subperitoneal sites	0	2	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Appendix III (Cont.)
Raw data from the Dose Confirmation Study with AOB 198

A3.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. 27

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Trlodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi*	*Trichostrongylus axei*
Stomach	0	0	0	0	0	213	0	832
Small intestine	390	0	0	0	0	0	0	0
Caecum	375	89	407	73	1060	0	0	0
Ventral colon	7	135	18	14	13020	0	0	0
Dorsal colon	0	0	0	3	6120	2	210	0
Small colon	0	0	0	1	100	0	0	0
CMA♦/IT. nodules	0	0	28	0	0	0	0	0
Subperitoneal sites	0	18	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Calculated total internal parasite numbers of horse no. 21

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Trlodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi*	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	12	0	0	0	0	0	0	0
Caecum	1	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	0	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
CMA♦/IT. nodules	0	0	18	0	0	0	0	0
Subperitoneal sites	0	1	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Appendix III (Cont.)
Raw data from the Dose Confirmation Study with AOB 198

A.3.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. 14

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	312	0	12020
Small intestine	12	0	0	0	0	0	0	0
Caecum	258	344	503	25	2200	0	0	0
Ventral colon	1	178	5	13	14320	1	0	0
Dorsal colon	1	0	0	0	4880	0	0	0
Small colon	0	0	0	0	1740	0	0	0
CMA♦/IT. nodules	0	0	14	0	0	0	0	0
Subperitoneal sites	0	5	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Calculated total internal parasite numbers of horse no. 4

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	21	0	0	0	0	0	0	0
Caecum	9	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	0	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
CMA♦/IT. nodules	0	0	16	0	0	0	0	0
Subperitoneal sites	0	0	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Appendix III (Cont.)
Raw data from the Dose Confirmation Study with AOB 198

A3.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. 18

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi*	*Trichostrongylus axei*
Stomach	0	0	0	0	0	106	0	17554
Small intestine	16	0	0	0	24	2	0	0
Caecum	70	77	441	52	7740	2	0	0
Ventral colon	0	74	50	10	17140	1	0	0
Dorsal colon	0	0	0	8	7300	0	96	0
Small colon	0	0	1	1	2280	0	140	0
CMA♦/IT. nodules	0	0	52	0	0	0	0	0
Subperitoneal sites	0	1	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Calculated total internal parasites of horse no. 2

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	17	0	0	0	0	0	0	0
Caecum	32	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	100	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
CMA♦/IT. nodules	0	0	17	0	0	0	0	0
Subperitoneal sites	0	38	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Appendix III (Cont.)
Raw data from the Dose Confirmation Study with AOB 198

A3.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. 20

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	108	0	3568
Small intestine	45	0	0	0	0	1	0	0
Caecum	279	136	188	31	1580	0	0	0
Ventral colon	7	103	19	32	11580	1	0	0
Dorsal colon	0	3	0	0	6880	0	0	0
Small colon	0	0	0	0	6760	0	0	0
CMA♦/IT. nodules	0	0	32	0	0	0	0	0
Subperitoneal sites	0	3	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Calculated total internal parasite numbers of horse no. 24

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	26	0	0	0	0	0	0	0
Caecum	35	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	0	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
CMA♦/IT. nodules	0	0	12	0	0	0	0	0
Subperitoneal sites	0	1	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery; IT. Nodules = on the intestinal wall nodules

Appendix III (Cont.)

Raw data from Dose Confirmation Study with AOB 198

A.3.4 Raw data of intestinal content volumes and mucosal weights from each organ of each horses

Name of the Organ: Caecum

Horse No.	Total volume of contents (L)	5% volume of contents (ml)	Total mucosal weight (Kg)	Examined mucosal weight (g)	No. of pieces examined	% of examined mucosa
16	30	1500	3.2	173	8	5.4
17	10	500	2.7	142	6	5.3
18	20	1000	3.1	149	7	4.8
2	10	500	3.0	158	7	5.3
20	10	500	3.5	182	8	5.2
24	30	1500	4.1	214	11	5.2
14	20	1000	3.8	186	9	4.9
4	30	1500	4.2	198	10	4.7
27	20	1000	3.7	181	9	4.9
21	15	750	3.9	200	9	5.1
23	30	1500	4.0	205	9	5.1
11	20	1000	3.6	174	7	4.8

Name of the Organ: Ventral colon

Horse No.	Total volume of contents (L)	5% volume of contents (ml)	Total mucosal weight (Kg)	Examined mucosal weight (g)	No. of pieces examined	% of examined mucosa
16	30	1500	4.2	218	10	5.2
17	40	2000	4.4	206	10	4.7
18	30	1500	3.5	169	7	4.8
2	40	2000	4.6	222	11	4.8
20	30	1500	4.1	196	9	4.8
24	40	2000	5.2	252	12	4.8
14	40	2000	4.5	229	10	5.1
4	30	1500	4.8	246	11	5.1
27	30	1500	4.1	198	10	4.8
21	30	1500	4.9	253	11	5.2
23	30	1500	5.7	285	12	5.0
11	30	1500	4.8	250	11	5.2

Appendix III (Cont.)
Raw data from Dose Confirmation Study with AOB 198

A3.4 Raw data of intestinal content volumes and mucosal weights from each organ of each horse

Name of the Organ: Dorsal colon

Horse No.	Total volume of contents (L)	5% volume of contents (ml)	Total mucosal weight (Kg)	Examined mucosal weight (g)	No. of pieces examined	% of examined mucosa
16	30	1500	3.2	156	7	4.9
17	30	1500	3.5	165	8	4.7
18	20	1000	3.3	171	8	5.2
2	30	1500	3.8	204	9	5.4
20	40	2000	3.6	196	9	5.4
24	30	1500	3.5	182	8	5.2
14	40	2000	3.2	157	7	4.9
4	40	2000	3.4	162	8	4.8
27	30	1500	2.9	153	7	5.3
21	30	1500	3.3	170	8	5.2
23	30	1500	3.6	172	8	4.8
11	30	1500	3.4	183	8	5.4

Appendix III (Cont.)
Statistics

A3.5 DOSE CONFIRMATION STUDY: STATISTICAL ANALYSIS OF THE DIFFERENCE BETWEEN TREATED AND CONTROL GROUP BY RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR THE FOLLOWING PARAMETER:

All analysis were undertaken using the non-parametric Mann-Whitney test using STATISTIX version 4.1. Treatment is the Treated Group given AOB 198 on Day 0. Control is the Control Group which were not treated. All values were compared on the day of, or after slaughter. The probability value reported were all one-tailed as it was expected that the Control Group values would be higher than the Treated Group.

A3.5.1 Strongylid egg count (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT

	SAMPLE			
VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 6
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.2 *Strongylus vulgaris* (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT

	SAMPLE			
VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	54.000	6	33.000	9.0
TREATMENT	24.000	6	3.0000	4.0
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0076
TOTAL NUMBER OF VALUES THAT WERE TIED 7
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.3 *Strongylus edentatus* (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 6
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.4 *Triodontophorus* spp. (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	48.000	6	27.000	8.0
TREATMENT	30.000	6	9.0000	5.0
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0920
TOTAL NUMBER OF VALUES THAT WERE TIED 9
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.5 *Trichostrongylus axei* (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 6
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001

CASES INCLUDED 12 MISSING CASES 0

A3.5.6 *Anoplocephala perfoliata* (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 6
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.7 *Oxyuris equi* larvae (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	42.000	6	21.000	7.0
TREATMENT	36.000	6	15.000	6.0
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.4145
TOTAL NUMBER OF VALUES THAT WERE TIED 11
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.8 *Gasterophilus intestinalis* larvae (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 8

MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.9 Adult *Cyathostomum* spp. (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.10 Adult *Cylicocyclus* spp. (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.11 Adult *Cylicostephanus* spp. (Day 9)

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 0

MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.12 Larval stages of *Cyathostomum* spp. (Day 9).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT
SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	52.000	6	31.000	8.7
TREATMENT	26.000	6	5.0000	4.3
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0206
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.13 Larval stages of *Cylicocyclus* spp. (Day 9).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT
SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	53.000	6	32.000	8.8
TREATMENT	25.000	6	4.0000	4.2
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0130
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.14 Larval stages of *Cylicostephanus* spp. (Day 9).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT
SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	52.000	6	31.000	8.7
TREATMENT	26.000	6	5.0000	4.3
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0206
TOTAL NUMBER OF VALUES THAT WERE TIED 0

MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.15 Mucosal digestion (Day 9).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.16 Migrating Larvae of *Strongylus vulgaris* (Day 9).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	51.500	6	30.500	8.6
TREATMENT	26.500	6	5.5000	4.4
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0206
TOTAL NUMBER OF VALUES THAT WERE TIED 2
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.5.17 Migrating Larvae of *Strongylus edentatus* (Day 9).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME

THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 8
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A3.6 Statistical analysis by paired t-test for comparison of the numbers of mucosal stages of larvae between transillumination and digestion techniques on all horses for the following parameter:

A3.6.1 PAIRED T TEST FOR LOGDIGEST - LOGTRANS

NULL HYPOTHESIS: DIFFERENCE = 0
ALTERNATIVE HYP: DIFFERENCE <> 0
MEAN 0.1250
STD ERROR 0.0176
LO 95% CI 0.0864
UP 95% CI 0.1636
T 7.12
DF 11
P 0.0000

CASES INCLUDED 12 MISSING CASES 0

A3.7 Statistical analysis by ANOVA using General Linear Models Procedure in SAS for comparison of the numbers of mucosal stages of larvae in the caecum, ventral colon and dorsal colon. All analysis were undertaken with data transformed $\log_{10}(x+1)$.

This analysis was undertaken to compare the distribution of mucosal stages of cyathostome larvae between the caecum, ventral colon and dorsal colon. All data were transformed to $\log_{10}(x+1)$ because they are not normally distributed. Both the Duncan and Tukey's test were undertaken after the initial ANOVA. In this report "Duncan" = Duncan's Multiple Range Test; "Tukey" = Tukey's Studentized Range Test and "Logcount" = $\log_{10}(x+1)$ transformation of the value being tested.

A3.7.1 Comparison between caecum, ventral colon and dorsal colon using the transillumination techniques in Control Group of horses.

Analysis of larvae count data of the total data of control group (DCS)
General Linear Models Procedure

Class Level Information

Class	Levels	Values
LI	3	Dorsal colon Ventral colon caecum

Number of observations in data set = 18

Dependent Variable: LOGCOUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	29.53666317	14.76833159	12.88	0.0006
Error	15	17.20289021	1.14685935		
Corrected Total	17	46.73955338			

R-Square	C.V.	Root MSE	LOGCOUNT Mean
0.631941	12.15761	1.070915	8.808602

Source	DF	Type I SS	Mean Square	F Value	Pr > F
L1	2	29.53666317	14.76833159	12.88	0.0006

Means with the same letter are not significantly different ($p < 0.05$).

Duncan Grouping	Mean	N	L1
A	10.6120	6	Ventral Colon
B	8.0556	6	Caecum
B	7.7582	6	Dorsal colon

Means with the same letter are not significantly different ($p < 0.05$).

Tukey Grouping	Mean	N	L1
A	10.6120	6	Ventral Colon
B	8.0556	6	Caecum
B	7.7582	6	Dorsal colon

A3.7.2 Comparison between caecum, ventral colon and dorsal colon using the transillumination techniques in Treated Group of horses.

Analysis of larvae count data of the total data of control group (DCS)

General Linear Models Procedure

Class Level Information

Class	Levels	Values
LI	3	Dorsal colon Ventral colon caecum

Number of observations in data set = 18

Dependent Variable: LOGCOUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	24.05191944	12.02595972	7.52	0.0055
Error	15	23.97932495	1.59862166		
Corrected Total	17	48.03124439			

R-Square	C.V.	Root MSE	LOGCOUNT Mean
0.500756	16.00892	1.264366	7.897884

Source	DF	Type I SS	Mean Square	F Value	Pr > F
L1	2	24.05191944	12.02595972	7.52	0.0055

Duncan's Multiple Range Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 1.598622

Number of Means 2 3

Critical Range 1.556 1.631

Means with the same letter are not significantly different (p < 0.05).

Duncan Grouping	Mean	N	L1
A	9.4599	6	Ventral Colon
B	7.5344	6	Caecum
B	6.6993	6	Dorsal colon

Tukey's Studentized Range (HSD) Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 1.598622

Critical Value of Studentized Range = 3.673

Minimum Significant Difference = 1.8961

Means with the same letter are not significantly different (p < 0.05).

Tukey Grouping	Mean	N	COLON
A	9.4599	6	Ventral Colon
B	7.5344	6	Caecum
B	6.6993	6	Dorsal colon

A3.7.3 Comparison between caecum, ventral colon and dorsal colon using the digestion techniques in Control Group of horses.

Analysis of larvae count data

General Linear Models Procedure

Class Level Information

Class Levels Values

LI 3 Dorsal colon Ventral colon caecum

Number of observations in data set = 18

Dependent Variable: LOGCOUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	26.00704496	13.00352248	13.93	0.0004
Error	15	14.00296876	0.93353125		
Corrected Total	17	40.01001373			

R-Square	C.V.	Root MSE	LOGCOUNT Mean
0.650013	10.51488	0.966194	9.188828

Source	DF	Type I SS	Mean Square	F Value	Pr > F
L1	2	26.00704496	13.00352248	13.93	0.0004

Duncan's Multiple Range Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 0.933531

Number of Means 2 3

Critical Range 1.189 1.246

Means with the same letter are not significantly different (p < 0.05).

Duncan Grouping	Mean	N	L1
A	10.8873	6	Ventral Colon
B	8.4007	6	Caecum
B	8.2786	6	Dorsal colon

Tukey's Studentized Range (HSD) Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 0.933531

Critical Value of Studentized Range = 3.673

Minimum Significant Difference = 1.449

Means with the same letter are not significantly different (p < 0.05).

Tukey Grouping	Mean	N	L1
A	10.8873	6	Ventral Colon
B	8.4007	6	Caecum
B	8.2786	6	Dorsal colon

A3.7.4 Comparison between caecum, ventral colon and dorsal colon using the digestion techniques in Treated Group of horses.

Analysis of larvae count data of the total data of treatment group (DCS)

General Linear Models Procedure

Class Level Information

Class	Levels	Values
LI	3	Dorsal colon Ventral colon caecum

Number of observations in data set = 18

Dependent Variable: LOGCOUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	20.32930489	10.16465245	6.90	0.0075
Error	15	22.09372210	1.47291481		
Corrected Total	17	42.42302699			

R-Square	C.V.	Root MSE	LOGCOUNT Mean
0.479204	14.74330	1.213637	8.231786

Source	DF	Type I SS	Mean Square	F Value	Pr > F
L1	2	20.32930489	10.16465245	6.90	0.0075

Duncan's Multiple Range Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 1.472915

Number of Means 2 3

Critical Range 1.493 1.566

Means with the same letter are not significantly different (p < 0.05).

Duncan Grouping	Mean	N	L1
A	9.6817	6	Ventral Colon
B	7.8496	6	Caecum
B	7.1641	6	Dorsal colon

Tukey's Studentized Range (HSD) Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 1.472915

Critical Value of Studentized Range = 3.673

Minimum Significant Difference = 1.82

Means with the same letter are not significantly different (p < 0.05).

Tukey Grouping	Mean	N	L1
A	9.6817	6	Ventral Colon
B	7.8496	6	Caecum
B	7.1641	6	Dorsal colon

APPENDIX IV

A.4 Raw data from the Principal Efficacy Study with AOB 698

A4.1 Animal identification and allocation on the basis on faecal egg counts on Day –14 and Day -3

Treated /Control	Rank of pair	Neck tag	Weight kg	Day -14 FEC* (epg)	Day -3 FEC* (epg)	Average egg count
C	1	B13	395	700	800	750
T	1	A5	345	750	850	800
C	2	Y10	323	700	800	750
T	2	Y7	342	700	600	650
C	3	A4	321	650	550	600
T	3	Y1	303	500	600	550
C	4	A2	344	500	400	475
T	4	A3	394	350	450	400
C	5	A6	368	600	400	500
T	5	Y8	356	500	400	450
C	6	Y2	343	400	300	350
T	6	A1	341	450	350	400

FEC*= Faecal egg counts ; Treatment: AOB 698; Batch: 97030/H
Dosage: 2.5 grams per 50 kg b.w.

A4.2 Treatment Record of AOB 698 in the Principal Efficacy Study of each horses

Neck Tag	Weight kg	Pair C/T	FEC* Day 0 (epg)	Calculated Dose	Drenched Dose
B13	388	1	1000	0	0
A5	340	1	1000	17.0 g	18.5 g
Y10	325	2	550	0	0
Y7	334	2	750	16.7 g	17.5
A4	320	3	600	0	0
Y1	294	3	450	14.7 g	15.5
A2	332	4	350	0	0
A3	388	4	300	19.4 g	20.0
A6	367	5	650	0	0
Y8	353	5	600	17.65 g	18.0
Y2	339	6	300	0	0
A1	335	6	350	16.75 g	17.5
			Average	**1g/20 kg**	**1.04g/20 kg**

Dosage: 2.5 grams per 50 kg Body weight; *FEC = Faecal egg count

Appendix IV (Cont.)
Raw data from the Principal Efficacy Study with AOB 698

A4.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. B13

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	57	0	63620
Small intestine	0	0	0	0	0	0	0	1
Caecum	84	75	226	0	2780	1	0	0
Ventral colon	0	109	1	0	48940	2	0	0
Dorsal colon	0	4	0	9	440	0	0	0
Small colon	1	0	0	0	440	1	0	0
Aorta/CMA♦	0	0	78	0	0	0	0	0
Subperitoneal sites	0	3	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Calculated total internal parasite numbers of horse no. A5

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0	0	0
Caecum	0	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	8940	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
Aorta/CMA♦	0	0	3	0	0	0	0	0
Subperitoneal sites	0	0	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Appendix IV (Cont.)
Raw data from the Principal Efficacy Study with AOB 698

A4.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. Y10

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	110	0	8530
Small intestine	2	0	0	0	0	0	0	0
Caecum	126	73	66	6	4300	0	0	0
Ventral colon	0	107	0	12	62580	0	0	0
Dorsal colon	0	15	0	1	15680	0	2	0
Small colon	0	0	0	0	5100	0	0	0
Aorta/CMA♦	0	0	35	0	0	0	0	0
Subperitoneal sites	0	50	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Calculated total internal parasites of horse no. Y7

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi*	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0	0	0
Caecum	0	0	0	0	1560	0	0	0
Ventral colon	0	0	0	0	1920	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
Aorta/CMA♦	0	0	14	0	0	0	0	0
Subperitoneal sites	0	0	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Appendix IV (Cont.)
Raw data from the Principal Efficacy Study with AOB 698

A4.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. A4

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	107	0	8070
Small intestine	1	0	0	0	0	0	0	0
Caecum	83	77	59	0	1820	0	0	0
Ventral colon	0	54	2	0	25440	1	0	0
Dorsal colon	0	1	1	0	16860	0	0	0
Small colon	0	1	0	0	13180	1	0	0
Aorta/CMA♦	0	0	37	0	0	0	0	0
Subperitoneal sites	0	7	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Calculated total internal parasite numbers of horse no. Y1

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0	0	0
Caecum	0	0	0	0	420	0	0	0
Ventral colon	0	0	0	0	5680	0	0	0
Dorsal colon	0	0	0	0	80	0	0	0
Small colon	0	0	0	0	0	0	0	0
Aorta/CMA♦	0	0	27	0	0	0	0	0
Subperitoneal sites	0	0	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Appendix IV (Cont.)
Raw data from the Principal Efficacy Study with AOB 698

A4.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. A2

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	106	0	28930
Small intestine	0	0	0	0	0	0	0	0
Caecum	181	111	0	0	3060	0	0	0
Ventral colon	0	31	0	0	5400	3	0	0
Dorsal colon	0	1	0	2	1160	0	0	0
Small colon	0	1	0	0	80	1	0	0
Aorta/CMA♦	0	0	0	0	0	0	0	0
Subperitoneal sites	0	2	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Calculated total internal parasite numbers of horse no. A3

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0	0	0
Caecum	0	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	4260	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
Aorta/CMA♦	0	0	11	0	0	0	0	0
Subperitoneal sites	0	0	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Appendix IV (Cont.)
Raw data from the Principal Efficacy Study with AOB 698

A4.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. A6

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi*	*Trichostrongylus axei*
Stomach	0	0	0	0	0	157	0	25370
Small intestine	2	0	0	0	0	0	0	0
Caecum	198	58	66	0	2300	1	0	0
Ventral colon	0	156	4	0	82020	1	0	0
Dorsal colon	0	5	0	0	14560	2	0	0
Small colon	0	0	2	0	11420	0	0	0
Aorta/CMA♦	0	0	46	0	0	0	0	0
Subperitoneal sites	0	11	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Calculated total internal parasite numbers of horse no. Y8

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Triodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0	0	0
Caecum	0	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	0	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
Aorta/CMA♦	0	0	33	0	0	0	0	0
Subperitoneal sites	0	0	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Appendix IV (Cont.)
Raw data from the Principal Efficacy Study with AOB 698

A4.3 Total Parasite Counts

Calculated total internal parasite numbers of horse no. Y2

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Trlodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi* larvae	*Trichostrongylus axei*
Stomach	0	0	0	0	0	46	0	17610
Small intestine	0	0	0	0	0	1	0	0
Caecum	50	7	94	10	200	0	0	0
Ventral colon	8	20	25	10	30020	0	0	0
Dorsal colon	0	9	20	1	11040	2	0	0
Small colon	0	0	1	0	4300	0	0	0
Aorta/CMA♦	0	0	109	0	0	0	0	0
Subperitoneal sites	0	3	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Calculated total internal parasite numbers of horse no. A1

Organ name	*Anoplocephala perfoliata*	*Strongylus edentatus*	*Strongylus vulgaris*	*Trlodontophorus* spp.	Cyathostome species	*Gasterophilus intestinalis* 2nd and 3rd instar larvae	*Oxyuris equi*	*Trichostrongylus axei*
Stomach	0	0	0	0	0	0	0	0
Small intestine	0	0	0	0	0	0	0	0
Caecum	0	0	0	0	0	0	0	0
Ventral colon	0	0	0	0	9480	0	0	0
Dorsal colon	0	0	0	0	0	0	0	0
Small colon	0	0	0	0	0	0	0	0
Aorta/CMA♦	0	0	0	0	0	0	0	0
Subperitoneal sites	0	0	0	0	0	0	0	0

♦ CMA = Cranial mesenteric artery

Appendix IV (Cont.)

Raw data from the Principal Efficacy Study with AOB 698

A4.4 Raw data of intestinal content volumes and mucosal weights from each organ of each horses

Name of the Organ: Caecum

Horse No.	Total volume of contents (L)	5% volume of contents (ml)	Total mucosal weight (Kg)	Examined mucosal weight (g)	No. of pieces examined	% of examined mucosa
B13	20	1000	3.5	182	7	5.2
A5	20	1000	3.6	176	6	4.9
Y10	20	1000	3.8	179	8	4.7
Y7	20	1000	4.1	212	9	5.2
A4	10	500	4.2	227	9	5.4
Y1	20	1000	4.2	218	10	5.2
A2	10	500	3.7	193	9	5.2
A3	20	1000	4.0	207	9	5.2
A6	10	500	3.4	186	9	5.5
Y8	20	1000	3.7	194	9	5.2
Y2	10	500	4.0	211	9	5.3
A1	20	1000	3.8	197	9	5.2

Name of the Organ: Ventral colon

Horse No.	Total volume of contents (L)	5% volume of contents (ml)	Total mucosal weight (Kg)	Examined mucosal weight (g)	No. of pieces examined	% of examined mucosa
B13	30	1500	4.6	218	11	4.7
A5	30	1500	3.4	176	7	5.2
Y10	20	1000	4.5	213	11	4.7
Y7	30	1500	5.6	256	12	4.6
A4	30	1500	4.3	233	10	5.4
Y1	30	1500	4.9	258	11	5.3
A2	20	1000	3.5	184	8	5.3
A3	30	1500	4.3	226	10	5.3
A6	20	1000	4.0	214	10	5.4
Y8	40	2000	4.9	233	11	4.8
Y2	20	1000	6.7	328	14	4.9
A1	20	1000	4.6	237	11	5.2

Appendix IV (Cont.)

Raw data from the Principal Efficacy Study with AOB 698

A4.4 Raw data of intestinal content volumes and mucosal weights from each organ of each horse

Name of the Organ: Dorsal colon

Horse No.	Total volume of contents (L)	5% volume of contents (ml)	Total mucosal weight (Kg)	Examined mucosal weight (g)	No. of pieces examined	% of examined mucosa
B13	20	1000	3.0	137	6	4.6
A5	20	1000	2.6	134	6	5.2
Y10	20	1000	3.1	162	7	5.2
Y7	30	1500	3.0	145	6	4.8
A4	30	1500	3.1	153	7	4.9
Y1	20	1000	3.5	178	7	5.1
A2	20	1000	2.4	124	5	5.2
A3	20	1000	3.1	161	7	5.2
A6	20	1000	2.5	132	6	5.3
Y8	20	1000	3.9	206	9	5.3
Y2	20	1000	3.4	182	8	5.4
A1	20	1000	3.1	148	6	4.8

Appendix IV (Cont.)
Statistics

A4.5 PRINCIPAL EFFICACY STUDY: STATISTICAL ANALYSIS OF THE DIFFERENCE BETWEEN TREATED AND CONTROL GROUP BY RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR THE FOLLOWING PARAMETER:

All analysis were undertaken using the non-parametric Mann-Whitney test using STATISTIX version 4.1. Treatment is the Treated Group given AOB 698 on Day 0. Control is the Control Group which were not treated. All values were compared on the day of, or after slaughter. The probability value reported were all one-tailed as it was expected that the Control Group values would be higher than the Treated Group.

A4.5.1 Strongylid Egg Count (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT
SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 6
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.2 *Strongylus vulgaris* (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT
SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	54.000	6	33.000	9.0
TREATMENT	24.000	6	3.0000	4.0
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0076
TOTAL NUMBER OF VALUES THAT WERE TIED 7
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.3 *Strongylus edentatus* (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5

TOTAL 78.000 12

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 6
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.4 *Triodontophorus* spp. (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	48.000	6	27.000	8.0
TREATMENT	30.000	6	9.0000	5.0
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0920
TOTAL NUMBER OF VALUES THAT WERE TIED 9
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.5 *Trichostrongylus axei* (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 6
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.6 *Anoplocephala perfoliata* (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 6
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.7 *Oxyuris equi* larvae (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	42.000	6	21.000	7.0
TREATMENT	36.000	6	15.000	6.0
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.4145
TOTAL NUMBER OF VALUES THAT WERE TIED 11
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.8 *Gasterophilus intestinalis* larvae (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 8
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.9 Adult *Cyathostomum* spp. (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME

THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.10 Adult *Cylicocyclus* spp. (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.11 Adult *Cylicostephanus* spp. (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.12 Larval stages of *Cyathostomum* spp. (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	52.000	6	31.000	8.7
TREATMENT	26.000	6	5.0000	4.3
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0206

TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.13 Larval stages of *Cylicocyclus* spp. (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	53.000	6	32.000	8.8
TREATMENT	25.000	6	4.0000	4.2
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0130
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.14 Larval stages of *Cylicostephanus* spp. (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	52.000	6	31.000	8.7
TREATMENT	26.000	6	5.0000	4.3
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0206
TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.15 Mucosal digestion (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011

TOTAL NUMBER OF VALUES THAT WERE TIED 0
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.16 Migrating Larvae of *Strongylus vulgaris* (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	51.500	6	30.500	8.6
TREATMENT	26.500	6	5.5000	4.4
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0206
TOTAL NUMBER OF VALUES THAT WERE TIED 2
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001
CASES INCLUDED 12 MISSING CASES 0

A4.5.17 Migrating Larvae of *Strongylus edentatus* (Day 20).

RANK SUM TWO-SAMPLE (MANN-WHITNEY) TEST FOR CONTROL VS TREATMENT SAMPLE

VARIABLE	RANK SUM	SIZE	U STAT	MEAN RANK
CONTROL	57.000	6	36.000	9.5
TREATMENT	21.000	6	0.0000	3.5
TOTAL	78.000	12		

EXACT PROBABILITY OF A RESULT AS or MORE EXTREME
THAN THE OBSERVED RANKS (ONE-TAILED P-VALUE) 0.0011
TOTAL NUMBER OF VALUES THAT WERE TIED 8
MAXIMUM DIFFERENCE ALLOWED BETWEEN TIES 0.00001

CASES INCLUDED 12 MISSING CASES 0

A4.6 Statistical analysis by paired t-test for comparison of the numbers of mucosal stages of larvae between transillumination and digestion for the following parameter:

A4.6.1 PAIRED T TEST FOR LOGDIGEST - LOGTRANS

NULL HYPOTHESIS: DIFFERENCE = 0
ALTERNATIVE HYP: DIFFERENCE <> 0
MEAN 0.0467
STD ERROR 6.67E-03
LO 95% CI 0.0320
UP 95% CI 0.0613
T 7.00
DF 11
P 0.0000
CASES INCLUDED 12 MISSING CASES 0

A4.7 Statistical analysis by ANOVA using General Linear Models Procedure in SAS for comparison of the numbers of mucosal stages of larvae in the caecum, ventral colon and dorsal colon. All analysis were undertaken with data transformed $log_{10}(x)$

This analysis was undertaken to compare the distribution of mucosal stages of cyathostome larvae between the caecum, ventral colon and dorsal colon. All data were transformed to $log_{10}(x+1)$ because they are not normally distributed. Both the Duncan and Tukey's test were undertaken after the initial ANOVA. In this report "Duncan" = Duncan's Multiple Range Test; "Tukey" = Tukey's Studentized Range Test and "Logcount" = $Log_{10}(x+1)$ transformation of the value being tested.

A4.7.1 Comparison between caecum, ventral colon and dorsal colon using the transillumination techniques in Control Group of horses.

Analysis of larvae count data of the total data of treatment group
General Linear Models Procedure
Class Level Information
Class Levels Values
LI 3 Dorsal colon Ventral colon caecum

Number of observations in data set = 18

Dependent Variable: LOGCOUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	8.95014047	4.47507024	29.40	0.0001
Error	15	2.28310382	0.15220692		
Corrected Total	17	11.23324429			

R-Square	C.V.	Root MSE	LOGCOUNT Mean
0.796755	4.070636	0.390137	9.584179

Source	DF	Type I SS	Mean Square	F Value	Pr > F
L1	2	8.95014047	4.47507024	29.40	0.0001

Duncan's Multiple Range Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 0.152207

Number of Means 2 3

Critical Range .4801 .5033

Means with the same letter are not significantly different (p < 0.05).

Duncan Grouping	Mean	N	L1
A	10.3509	6	Ventral Colon
B	9.7530	6	Caecum
C	8.6486	6	Dorsal colon

Tukey's Studentized Range (HSD) Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 0.152207

Critical Value of Studentized Range = 3.673

Minimum Significant Difference = 0.5851

Means with the same letter are not significantly different (p < 0.05).

Tukey Grouping	Mean	N	L1
A	10.3509	6	Ventral Colon
B	9.7530	6	Caecum
C	8.6486	6	Dorsal colon

A4.7.2 Comparison between caecum, ventral colon and dorsal colon using the transillumination techniques in Treated Group of horses.

Analysis of larvae count data of the total data of treatment group

General Linear Models Procedure

Class Level Information

Class	Levels	Values
LI	3	Dorsal colon Ventral colon caecum

Number of observations in data set = 18

Dependent Variable: LOGCOUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	22.21974537	11.10987268	17.92	0.0001
Error	15	9.30146010	0.62009734		
Corrected Total	17	31.52120547			

R-Square	C.V.	Root MSE	LOGCOUNT Mean
0.704914	9.501429	0.787463	8.287833

Source	DF	Type I SS	Mean Square	F Value	Pr > F
L1	2	22.21974537	11.10987268	17.92	0.0001

Duncan's Multiple Range Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 0.620097

Number of Means 2 3

Critical Range 0.969 1.016

Means with the same letter are not significantly different (p < 0.05).

Duncan Grouping	Mean	N	L1
A	9.2319	6	Ventral Colon
A	8.9035	6	Caecum
B	6.7281	6	Dorsal colon

Tukey's Studentized Range (HSD) Test for variable: LOGCOUNT

Alpha = 0.05 df = 15 MSE = 0.620097

Critical Value of Studentized Range = 3.673

Minimum Significant Difference = 1.1809

Means with the same letter are not significantly different (p < 0.05).

Tukey Grouping	Mean	N	L1
A	9.2319	6	Ventral Colon
A	8.9035	6	Caecum
B	6.7281	6	Dorsal colon

A4.7.3 Comparison between caecum, ventral colon and dorsal colon using the digestion techniques in Control Group of horses.

Analysis of larvae count data
General Linear Models Procedure
Class Level Information
Class Levels Values
LI 3 Dorsal colon Ventral colon caecum
Number of observations in data set = 18

Dependent Variable: LOGCOUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	8.89321922	4.44660961	33.15	0.0001
Error	15	2.01207297	0.13413820		
Corrected Total	17	10.90529219			

R-Square	C.V.	Root MSE	LOGCOUNT Mean
0.815496	3.783634	0.366249	9.679817

Source	DF	Type I SS	Mean Square	F Value	Pr > F
L1	2	8.89321922	4.44660961	33.15	0.0001

Duncan's Multiple Range Test for variable: LOGCOUNT
Alpha = 0.05 df = 15 MSE = 0.134138
Number of Means 2 3
Critical Range .4507 .4725

Means with the same letter are not significantly different (p < 0.05).

Duncan Grouping	Mean	N	L1
A	10.4382	6	Ventral Colon
B	9.8572	6	Caecum
C	8.7441	6	Dorsal colon

Tukey's Studentized Range (HSD) Test for variable: LOGCOUNT
Alpha = 0.05 df = 15 MSE = 0.134138
Critical Value of Studentized Range = 3.673
Minimum Significant Difference = 0.5492

Means with the same letter are not significantly different (p < 0.05).

Tukey Grouping	Mean	N	L1

A	10.4382	6	Ventral Colon
B	9.8572	6	Caecum
C	8.7441	6	Dorsal colon

A4.7.4 Comparison between caecum, ventral colon and dorsal colon using the digestion techniques in Treated Group of horses.

Analysis of larvae count data of the total data of treatment group (DCS)
General Linear Models Procedure
Class Level Information
Class Levels Values
LI 3 Dorsal colon Ventral colon caecum
Number of observations in data set = 18

Dependent Variable: LOGCOUNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	20.93987877	10.46993939	25.94	0.0001
Error	15	6.05422559	0.40361504		
Corrected Total	17	26.99410437			

R-Square	C.V.	Root MSE	LOGCOUNT Mean
0.775720	7.522028	0.635307	8.445955

Source	DF	Type I SS	Mean Square	F Value	Pr > F
L1	2	20.93987877	10.46993939	25.94	0.0001

Duncan's Multiple Range Test for variable: LOGCOUNT
Alpha = 0.05 df = 15 MSE = 0.403615
Number of Means 2 3
Critical Range .7818 .8195

Means with the same letter are not significantly different (p < 0.05).

Duncan Grouping	Mean	N	L1
A	9.3567	6	Ventral Colon
A	9.0503	6	Caecum
B	6.9309	6	Dorsal colon

Tukey's Studentized Range (HSD) Test for variable: LOGCOUNT
Alpha = 0.05 df = 15 MSE = 0.403615
Critical Value of Studentized Range = 3.673
Minimum Significant Difference = 0.9527

Means with the same letter are not significantly different ($p < 0.05$).

Tukey Grouping	Mean	N	L1
A	9.3567	6	Ventral Colon
A	9.0503	6	Caecum
B	6.9309	6	Dorsal colon

APPENDIX V

A5. Raw data of mean proportion of different species

A5.1 The mean proportion of different species in different organs for control horses from both the DCS and PES trials (n = 3 horses per trial) 20 (twenty) cyathostomes were examined from each organ

Species	Caecum	Ventral colon	Dorsal colon	Mean
Dose Confirmation Study				
Cyathostomum coronatum	0.26	0.13	0.11	0.17
Cyathostomum labiatum	0.10	0.08	0.06	0.08
Cyathostomum catinatum	0.02	0.07	0.07	0.05
Cylicocyclus nassatus	0.17	0.33	0.28	0.26
Cylicocyclus leptostomus	0.03	0.03	0.03	0.03
Cylicocyclus radiatus	0.06	0.05	0.03	0.05
Cylicocyclus insigne	0.00	0.03	0.02	0.02
Cylicostephanus poculatus	0.05	0.12	0.12	0.10
Cylicostephanus minutus	0.03	0.00	0.05	0.03
Cylicostephanus calicatus	0.20	0.10	0.08	0.13
Cylicostephanus longibursatus	0.03	0.00	0.07	0.03
Cylicostephanus goldi	0.05	0.05	0.08	0.06
Principal Efficacy Study				
Cyathostomum coronatum	0.29	0.11	0.13	0.18
Cyathostomum labiatum	0.11	0.06	0.05	0.07
Cyathostomum catinatum	0.03	0.03	0.07	0.04
Cylicocyclus nassatus	0.19	0.39	0.27	0.28
Cylicocyclus leptostomus	0.00	0.00	0.02	0.01
Cylicocyclus radiatus	0.06	0.05	0.05	0.05
Cylicocyclus insigne	0.02	0.05	0.03	0.03
Cylicostephanus poculatus	0.07	0.08	0.12	0.09
Cylicostephanus minutus	0.00	0.00	0.02	0.01
Cylicostephanus calicatus	0.18	0.11	0.12	0.14
Cylicostephanus longibursatus	0.03	0.05	0.05	0.04
Cylicostephanus goldi	0.02	0.07	0.08	0.06

Appendix V (Cont.)

Raw data of total worm burdens of different horses

A5.2 Total cyathostome worm counts for three control horses in both the DCS and PES trials (n = 3 horses per trial) 20 cyathostomes were examined from each organ

Species	H - 14	H - 18	H - 20	Mean
Dose Confirmation Study				
Cyathostomum coronatum	2839	5919	3914	4224
Cyathostomum labiatum	2501	2643	753	1966
Cyathostomum catinatum	0	2114	2032	1382
Cylicocyclus nassatus	4850	8350	6473	6558
Cylicocyclus leptostomus	1352	1057	0	803
Cylicocyclus radiatus	1690	0	1355	1015
Cylicocyclus insigne	0	1586	0	529
Cylicostephanus poculatus	1352	3382	2259	2331
Cylicostephanus minutus	0	2643	0	881
Cylicostephanus calicatus	3177	2960	3011	3049
Cylicostephanus longibursatus	1217	1057	0	758
Cylicostephanus goldi	1284	0	2559	1281
Principal Efficacy Study	**H – A4**	**H – Y10**	**H – Y2**	**Mean**
Cyathostomum coronatum	4936	12234	7761	8310
Cyathostomum labiatum	2468	4155	3326	3316
Cyathostomum catinatum	2598	4617	0	2405
Cylicocyclus nassatus	11041	19159	9424	13208
Cylicocyclus leptostomus	649	0	0	216
Cylicocyclus radiatus	3117	5771	0	2963
Cylicocyclus insigne	649	0	2772	1140
Cylicostephanus poculatus	649	8079	4435	4388
Cylicostephanus minutus	649	0	0	216
Cylicostephanus calicatus	4416	9233	5211	6287
Cylicostephanus longibursatus	5066	0	0	1689
Cylicostephanus goldi	3117	6002	0	3040

Appendix V (Cont.)

Raw data of mean proportion of different species

A5.3 The mean proportion of different species in different organs for treated horses from the PES trial (n = 3 horses). All available nematodes to a maximum of 20 cyathostomes were examined from each organ.

Species	Caecum	Ventral colon	Dorsal colon	Mean
Principal Efficacy Study				
Cyathostomum coronatum	0.28	0.22	0.18	0.23
Cyathostomum catinatum	0.07	0.02	0	0.03
Cylicocyclus nassatus	0.28	0.35	0.33	0.32
Cylicocyclus radiatus	0.08	0.08	0.13	0.10
Cylicostephanus calicatus	0.10	0.1	0.12	0.11
Cylicostephanus longibursatus	0.05	0.05	0.07	0.06
Cylicostephanus goldi	0.13	0.18	0.17	0.16

Appendix VI

A6 Raw data for larval development assay

A6.1 LD_{50} values and co-efficient of determination (r^2) of ivermectin (ng/ml of agar) in farm horse

Horse No.	LD_{50} value	Coefficient of determination	Horse No.	LD_{50} value	Coefficient of determination
FH1	0.292	0.994	FCa6	0.427	0.989
FH2	0.112	0.988	FCa7	0.595	0.997
FH3	0.514	0.997	FCa8	0.533	0.982
FH4♦	0.982	0.995	FTa1	0.730	0.994
FH5♦	1.059	0.996	FTa2	0.230	0.996
FH6♦	0.829	0.991	FTa3	0.620	0.992
FH7	0.217	0.987	FTa4♦	1.806	0.995
FH8	0.594	0.997	FTa5♦	1.476	0.996
FB1	0.198	0.994	FTa6	0.115	0.996
FB2	0.324	0.996	FTa7	0.283	0.997
FB3	0.642	0.994	FTa8	0.623	0.996
FB4	0.116	0.996	FTa9	0.150	0.997
FB5	0.733	0.977	FTa10	0.600	0.988
FB6	0.357	0.993	FTa11	0.296	0.994
FB7	0.775	0.993	FDy1	0.649	0.995
FP1	0.203	0.998	FE1	0.126	0.989
FP2	0.464	0.983	FE2	0.661	0.995
FP3	0.128	0.996	FE3	0.781	0.992
FP4	0.459	0.997	FE4	1.869	0.994
FCa1	0.560	0.998	FCh1	0.386	0.999
FCa2	0.463	0.994	FCh2	0.603	0.996
FCa3	0.542	0.997	FCh3	0.269	0.990
FCa4	0.637	0.994	FCh4	0.399	0.998
FCa5	0.434	0.995			

F = Farm; H = Hastings; B = Bombay; P = Palmerston North; Ca = Canterbury; Ta = Takanini; Dy = Drury; E = Exported horse; Ch = Christchurch;
♦ = from the same farm horses

Appendix VI (Cont.)

Raw date for larval development assay

A6.2 LD_{50} values and co-efficient of determination (r^2) of ivermectin aglycone (ng/ml of agar) in farm horses

Horse No.	LD_{50} value	Coefficient of determination	Horse No.	LD_{50} value	Coefficient of determination
FH1	0.744	0.985	FCa6	1.091	0.997
FH2	0.089	0.969	FCa7	1.954	0.978
FH3	1.323	0.999	FCa8	1.800	0.9996
FH4♦	3.538	0.991	FTa1	2.413	0.991
FH5♦	4.831	0.983	FTa2	0.498	0.985
FH6♦	3.543	0.991	FTa3	2.134	0.995
FH7	0.576	0.981	FTa4♦	7.940	0.992
FH8	2.059	0.984	FTa5♦	4.886	0.990
FB1	0.271	0.986	FTa6	0.216	0.998
FB2	0.976	0.995	FTa7	0.644	0.988
FB3	2.363	0.996	FTa8	2.141	0.991
FB4	0.102	0.992	FTa9	0.491	0.961
FB5	2.599	0.986	FTa10	1.952	0.991
FB6	1.026	0.997	FTa11	0.978	0.991
FB7	2.552	0.993	FDy1	2.491	0.998
FP1	0.626	0.993	FCh1	1.083	0.992
FP2	1.633	0.974	FCh2	1.679	0.997
FP3	0.132	0.999	FCh3	0.795	0.995
FP4	1.949	0.974	FCh4	1.177	0.997
FCa1	1.930	0.996	FE1	0.261	0.992
FCa2	1.243	0.982	FE2	2.265	0.990
FCa3	1.902	0.972	FE3	2.963	0.983
FCa4	2.094	0.992	FE4	8.440	0.991
FCa5	1.281	0.978			

F = Farm; H = Hastings; B = Bombay; P = Palmerston North; Ca = Canterbury; Ta = Takanini; Dy = Drury; E = Exported horse; Ch = Christchurch;
♦ = from the same farm horses

Appendix VI (Cont.)

Raw data for larval development assay

A6.3 LD_{50} values and co-efficient of determination (r^2) of thiabendazole (ng/ml of agar) in farm horses

Horse No.	LD_{50} value	Coefficient of determination	Horse No.	LD_{50} value	Coefficient of determination
FH1	5.842	0.984	FCa6	7.346	0.990
FH2	3.181	0.993	FCa7	10.593	0.995
FH3	7.513	0.994	FCa8	8.563	0.993
FH4♦	19.415	0.967	FTa1	13.853	0.980
FH5♦	19.707	0.989	FTa2	2.271	0.968
FH6♦	18.027	0.989	FTa3	12.934	0.960
FH7	4.619	0.953	FTa4♦	18.596	0.993
FH8	8.628	0.959	FTa5♦	17.593	0.994
FB1	3.579	0.977	FTa6	1.143	0.970
FB2	6.020	0.994	FTa7	3.398	0.971
FB3	16.540	0.994	FTa8	12.964	0.983
FB4	3.665	0.984	FTa9	6.849	0.962
FB5	14.636	0.993	FTa10	13.438	0.985
FB6	4.749	0.980	FTa11	6.679	0.971
FB7	15.579	0.994	FDy1	13.296	0.994
FP1	4.652	0.968	FE1	2.862	0.980
FP2	7.400	0.992	FE2	13.487	0.985
FP3	3.229	0.997	FE3	16.708	0.989
FP4	10.958	0.993	FE4	19.767	0.988
FCa1	7.651	0.984	FCh1	7.091	0.977
FCa2	11.390	0.995	FCh2	11.059	0.994
FCa3	8.467	0.986	FCh3	4.826	0.993
FCa4	11.818	0.969	FCh4	6.810	0.971
FCa5	7.077	0.983			

F = Farm; H = Hastings; B = Bombay; P = Palmerston North; Ca = Canterbury; Ta = Takanini; Dy = Drury; E = Exported horse; Ch = Christchurch;
♦ = from the same farm horses

Appendix VI (Cont.)

Raw data for larval development assay

A6.3 LD_{50} values and co-efficient of determination (r^2) of ivermectin aglycone, ivermectin and thiabendazole (ng/ml of agar) in Kaimanawa horse

Horse No.	LD_{50} value			Co-efficient of determination (r^2)		
	IVM aglycone	IVM	TBZ	IVM aglycone	IVM	TBZ
K1	0.126	0.181	0.927	0.991	0.981	0.988
K2	0.676	0.246	0.658	0.981	0.983	0.969
K3	1.487	0.463	0.717	0.989	0.994	0.975
K4	0.573	0.309	4.053	0.958	0.978	0.989
K5	0.109	0.132	0.980	0.996	0.996	0.992
K6	0.068	0.181	5.479	0.980	0.997	0.990
K7	0.139	0.234	1.132	0.997	0.994	0.950
K8	1.746	0.489	4.321	0.982	0.996	0.997
K9	0.144	0.198	0.559	0.999	0.991	0.968
K10	0.635	0.275	3.476	0.968	0.985	0.982
K11	1.444	0.324	3.172	0.990	0.986	0.990
K12	0.079	0.215	4.396	0.978	0.964	0.990
K13	2.487	0.626	6.652	0.988	0.991	0.993
K14	2.307	0.615	5.990	0.987	0.993	0.988
K15	2.350	0.585	7.878	0.996	0.992	0.998
K16	1.509	0.361	4.423	0.974	0.981	0.978
K17	1.324	0.488	5.703	0.950	0.996	0.985
K18	1.989	0.605	6.155	0.969	0.994	0.993
K19	2.759	0.608	8.307	0.980	0.993	0.997
K20	1.616		5.498	0.982		0.991
K21	2.045		2.512	0.993		0.993
K22	1.698		4.986	0.983		0.973

K = Kaimanawa; IVM = ivermectin; TBZ = thiabendazole; K1-K6 = faeces collected from feral horses in 1997; K7-K11 = faeces collected from the holding farm; K12-K22 = faeces collected from feral horses in 1999

Appendix VII

A7 Raw data by animals by anthelmintic

A7.1 Proportion of L_3 values of farm horses by concentrations of Ivermectin

Conc (µg/ml) IVM♦	F B1	F B2	F B3	F B4	F B5	F B6	F B7	F Ca1	F Ca2	F Ca3	F Ca4	F Ca5
0.0007	1.01	1.00	1.03	1.01	0.97	1.03	1.05	0.98	1.01	0.98	1.02	1.03
0.0007	0.99	1.01	1.04	0.97	0.98	1.02	1.02	1.00	0.98	1.00	1.03	1.02
0.0015	1.01	0.94	1.01	0.94	0.93	1.02	1.01	0.95	0.95	0.98	1.01	0.99
0.0015	0.98	0.92	1.03	0.97	1.00	0.97	1.02	0.97	1.02	1.02	1.02	1.03
0.0029	0.99	0.91	1.00	0.92	0.94	0.94	0.98	1.00	0.97	0.95	0.99	0.95
0.0029	0.98	0.98	0.97	0.91	0.96	0.91	1.01	0.98	0.98	0.94	0.96	0.97
0.0059	0.78	0.80	0.94	0.48	0.94	0.82	0.99	0.92	0.99	0.88	0.93	0.94
0.0059	0.74	0.78	0.96	0.45	0.92	0.90	0.95	0.95	1.01	0.91	0.95	0.97
0.0117	0.37	0.69	0.87	0.14	0.93	0.82	0.93	0.78	0.75	0.82	0.86	0.76
0.0117	0.34	0.66	0.94	0.16	0.94	0.79	0.95	0.80	0.78	0.79	0.93	0.80
0.0234	0.24	0.33	0.68	0.09	0.89	0.27	0.61	0.60	0.46	0.61	0.68	0.40
0.0234	0.20	0.30	0.67	0.07	0.87	0.29	0.60	0.62	0.51	0.58	0.66	0.42
0.0469	0.07	0.14	0.33	0.00	0.23	0.09	0.51	0.25	0.23	0.24	0.33	0.23
0.0469	0.11	0.17	0.26	0.00	0.25	0.11	0.48	0.28	0.19	0.23	0.26	0.21
0.0938	0.00	0.05	0.13	0.00	0.20	0.00	0.17	0.12	0.12	0.15	0.13	0.12
0.0938	0.00	0.03	0.09	0.00	0.22	0.00	0.13	0.09	0.11	0.12	0.09	0.15
0.1875	0.00	0.00	0.10	0.00	0.19	0.00	0.10	0.00	0.11	0.06	0.10	0.08
0.1875	0.00	0.00	0.12	0.00	0.18	0.00	0.12	0.03	0.09	0.08	0.11	0.07
0.375	0.00	0.00	0.09	0.00	0.15	0.00	0.04	0.00	0.04	0.00	0.09	0.03
0.375	0.00	0.00	0.07	0.00	0.12	0.00	0.03	0.00	0.03	0.00	0.07	0.04
0.75	0.00	0.00	0.09	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.09	0.00
0.75	0.00	0.00	0.07	0.00	0.07	0.00	0.00	0.00	0.01	0.00	0.07	0.01
1.5	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
1.5	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
3	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
3	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0C	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	70.5	66.2	69.0	69.2	73.2	65.7	68.6	65.0	74.6	66.0	69.6	74.6

IVM♦= ivermectin; F = farm; B = Bombay; Ca = Canterbury

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.1 Proportion of L_3 values of farm horses by concentrations of Ivermectin

Conc (µg/ml) IVM♦	F Ca6	F Ca7	F Ca8	F Ch1	F Ch2	F Ch3	F Ch4	F Dy1	F E1	F E2	F E3	F E4
0.0007	1.01	1.00	1.03	0.97	1.01	0.98	0.99	1.00	0.74	1.01	1.07	0.96
0.0007	1.03	1.01	1.00	0.95	0.97	0.96	0.97	1.03	0.71	1.03	1.03	0.98
0.0015	1.04	1.03	1.04	0.93	0.99	0.94	0.96	1.02	0.70	1.04	1.03	0.93
0.0015	1.05	0.98	1.03	0.97	1.01	0.98	0.92	1.05	0.66	1.01	1.01	0.95
0.0029	0.91	1.00	0.95	0.95	0.97	0.96	0.97	1.00	0.73	1.00	1.01	1.04
0.0029	0.90	0.98	0.98	0.93	0.93	0.94	0.91	1.02	0.67	1.01	0.98	0.92
0.0059	0.74	0.94	0.82	0.85	0.95	0.78	0.87	0.94	0.55	0.97	1.00	0.92
0.0059	0.73	1.00	0.81	0.87	0.92	0.74	0.89	0.99	0.50	0.99	0.95	1.03
0.0117	0.59	0.80	0.74	0.75	0.71	0.48	0.77	0.87	0.31	0.85	0.95	0.91
0.0117	0.56	0.83	0.75	0.72	0.71	0.50	0.74	0.99	0.29	0.87	0.91	0.89
0.0234	0.48	0.63	0.56	0.39	0.59	0.43	0.40	0.71	0.20	0.69	0.65	0.88
0.0234	0.48	0.66	0.53	0.41	0.57	0.39	0.42	0.66	0.15	0.72	0.69	0.92
0.0469	0.35	0.25	0.46	0.11	0.38	0.09	0.12	0.31	0.10	0.33	0.51	0.88
0.0469	0.37	0.32	0.42	0.15	0.37	0.07	0.15	0.27	0.07	0.28	0.50	0.91
0.0938	0.35	0.11	0.00	0.03	0.22	0.00	0.03	0.12	0.10	0.10	0.10	0.46
0.0938	0.34	0.08	0.00	0.05	0.18	0.00	0.05	0.10	0.07	0.13	0.06	0.49
0.1875	0.14	0.00	0.00	0.00	0.15	0.00	0.00	0.09	0.01	0.12	0.03	0.12
0.1875	0.13	0.05	0.00	0.00	0.11	0.00	0.00	0.10	0.03	0.13	0.00	0.15
0.375	0.06	0.00	0.00	0.00	0.09	0.00	0.00	0.06	0.00	0.07	0.00	0.04
0.375	0.03	0.00	0.00	0.00	0.05	0.00	0.00	0.06	0.00	0.04	0.00	0.05
0.75	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.03	0.00	0.00
0.75	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.04	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.01	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	71.2	65.2	69.2	61.0	54.6	54.0	59.5	67.8	68.6	67.0	68.2	73.8

IVM♦= ivermectin; F = farm; Ca = Canterbury; Ch = Christchurch; Dy = Drury; E = Exported horses.
This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.1 Proportion of L_3 value of farm horses by concentrations of Ivermectin

Conc (µg/ml) IVM*	F H1	F H2	F H3	F H4	F H5	F H6	F H7	F H8	F P1	F P2	F P3	F P4
0.0007	1.01	1.03	0.99	0.92	1.01	1.07	0.98	1.00	0.97	1.03	1.00	1.01
0.0007	1.00	0.98	0.98	0.93	1.00	1.06	0.99	1.06	1.03	0.99	0.94	1.02
0.0015	1.03	0.97	0.96	0.96	1.00	1.06	0.95	1.00	1.04	0.99	0.89	0.99
0.0015	1.01	1.03	0.99	0.97	0.99	1.04	1.00	1.04	0.99	1.03	0.93	1.02
0.0029	0.86	1.07	0.95	0.94	1.00	1.06	0.77	1.00	0.91	1.01	0.71	0.97
0.0029	0.91	1.01	0.99	0.97	1.00	1.01	0.80	0.90	0.97	0.95	0.70	0.96
0.0059	0.79	0.27	0.89	0.84	0.92	1.00	0.60	0.88	0.74	1.01	0.49	0.97
0.0059	0.77	0.25	0.91	0.81	1.00	0.92	0.59	0.83	0.81	0.97	0.52	0.96
0.0117	0.66	0.14	0.77	0.77	0.91	0.98	0.43	0.80	0.42	0.75	0.36	0.73
0.0117	0.65	0.16	0.75	0.74	0.92	0.94	0.45	0.77	0.44	0.70	0.37	0.77
0.0234	0.30	0.09	0.56	0.62	0.90	0.67	0.36	0.68	0.12	0.40	0.22	0.61
0.0234	0.28	0.07	0.60	0.66	0.88	0.70	0.33	0.64	0.15	0.37	0.20	0.58
0.0469	0.12	0.00	0.25	0.53	0.61	0.54	0.36	0.27	0.05	0.27	0.10	0.29
0.0469	0.10	0.00	0.24	0.54	0.62	0.55	0.30	0.31	0.03	0.33	0.05	0.34
0.0938	0.00	0.00	0.07	0.36	0.09	0.09	0.19	0.14	0.00	0.22	0.03	0.10
0.0938	0.00	0.00	0.04	0.35	0.10	0.10	0.21	0.15	0.00	0.18	0.03	0.06
0.1875	0.00	0.00	0.00	0.26	0.05	0.03	0.07	0.09	0.00	0.15	0.00	0.02
0.1875	0.00	0.00	0.00	0.24	0.06	0.01	0.07	0.06	0.00	0.16	0.00	0.05
0.375	0.00	0.00	0.00	0.12	0.03	0.00	0.00	0.01	0.00	0.11	0.00	0.00
0.375	0.00	0.00	0.00	0.15	0.04	0.00	0.00	0.00	0.00	0.13	0.00	0.00
0.75	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00
0.75	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	57.2	69.2	71.6	74.2	77.0	67.2	72.8	80.8	59.5	54.6	73.2	62.6

IVM*= ivermectin; F = farm; H = Hastings; P = Palmerston North

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.1 Proportion of L_3 value of farm horses by concentrations of Ivermectin

Conc (µg/ml) IVM♦	F Ta1	F Ta2	F Ta3	F Ta4	F Ta5	F Ta6	F Ta7	F Ta8	F Ta9	F Ta10	F Ta11
0.0007	0.87	1.02	0.95	1.00	0.96	0.99	1.00	1.02	0.97	0.99	0.81
0.0007	0.90	0.99	0.98	0.94	0.98	0.95	1.02	1.00	0.96	0.97	0.82
0.0015	0.88	1.00	1.01	1.00	0.93	0.90	1.02	1.00	0.94	1.02	0.87
0.0015	0.90	0.97	0.97	0.98	0.95	0.87	0.94	0.97	0.97	0.99	0.85
0.0029	0.86	0.95	0.92	0.96	1.04	0.64	0.96	0.97	0.76	1.05	0.77
0.0029	0.83	0.92	1.02	1.04	0.92	0.66	0.98	0.99	0.79	1.01	0.80
0.0059	0.78	0.79	0.78	1.01	0.92	0.48	0.80	0.97	0.54	0.98	0.68
0.0059	0.84	0.74	0.80	0.93	1.03	0.51	0.81	0.96	0.59	1.01	0.70
0.0117	0.87	0.46	0.72	0.90	0.91	0.35	0.63	0.83	0.39	0.94	0.64
0.0117	0.84	0.44	0.75	0.91	0.89	0.36	0.61	0.86	0.35	0.97	0.63
0.0234	0.82	0.30	0.61	0.83	0.88	0.21	0.26	0.65	0.20	0.49	0.30
0.0234	0.83	0.26	0.60	0.86	0.92	0.20	0.28	0.69	0.21	0.53	0.27
0.0469	0.21	0.09	0.37	0.77	0.75	0.09	0.07	0.31	0.10	0.40	0.10
0.0469	0.20	0.14	0.36	0.79	0.70	0.05	0.06	0.25	0.04	0.39	0.04
0.0938	0.09	0.00	0.24	0.52	0.37	0.03	0.00	0.09	0.00	0.04	0.00
0.0938	0.10	0.00	0.26	0.49	0.35	0.03	0.00	0.12	0.03	0.03	0.03
0.1875	0.04	0.00	0.17	0.15	0.12	0.00	0.00	0.10	0.00	0.00	0.00
0.1875	0.05	0.00	0.18	0.20	0.15	0.00	0.00	0.10	0.00	0.00	0.00
0.375	0.02	0.00	0.00	0.06	0.04	0.00	0.00	0.04	0.00	0.00	0.00
0.375	0.01	0.00	0.00	0.07	0.05	0.00	0.00	0.06	0.00	0.00	0.00
0.75	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	81.8	56.7	70.4	71.2	73.8	74.8	54.0	67.8	71.0	69.7	70.4

IVM♦= ivermectin; F = farm; T = Takanini.

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.1 Proportion of L_3 values of Kaimanawa horse by concentrations of Ivermectin

Conc (µg/ml) IVM*	H K1	H K2	H K3	H K4	H K5	H K6	H K7	H K8	H K9	H K10
0.0007	0.87	0.94	1.01	1.01	0.99	0.96	1.02	1.00	0.97	0.98
0.0007	1.05	0.99	0.98	1.05	1.01	0.99	1.01	1.04	0.95	0.99
0.0015	0.87	0.89	0.95	0.80	0.96	0.98	0.98	1.01	0.94	0.96
0.0015	0.93	0.93	1.02	0.82	1.02	1.01	0.99	1.04	0.97	0.99
0.0029	0.90	0.76	0.97	0.75	0.78	0.92	0.95	1.00	0.87	0.99
0.0029	0.93	0.77	0.98	0.76	0.81	0.87	0.92	1.01	0.86	0.98
0.0059	0.66	0.57	0.99	0.58	0.52	0.66	0.73	0.96	0.63	0.80
0.0059	0.51	0.63	1.01	0.64	0.50	0.64	0.75	0.97	0.65	0.77
0.0117	0.53	0.47	0.75	0.57	0.34	0.43	0.59	0.73	0.40	0.41
0.0117	0.48	0.50	0.78	0.60	0.35	0.42	0.56	0.67	0.46	0.43
0.0234	0.14	0.47	0.46	0.49	0.10	0.17	0.17	0.46	0.22	0.36
0.0234	0.16	0.46	0.51	0.50	0.13	0.13	0.13	0.49	0.23	0.40
0.0469	0.05	0.31	0.23	0.34	0.03	0.07	0.07	0.34	0.24	0.25
0.0469	0.07	0.30	0.19	0.37	0.01	0.04	0.04	0.31	0.23	0.23
0.0938	0.01	0.13	0.12	0.16	0.00	0.00	0.00	0.17	0.07	0.11
0.0938	0.03	0.10	0.11	0.20	0.00	0.00	0.00	0.15	0.08	0.08
0.1875	0.00	0.00	0.11	0.07	0.00	0.00	0.00	0.07	0.07	0.10
0.1875	0.00	0.00	0.09	0.04	0.00	0.00	0.00	0.08	0.07	0.07
0.375	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.06	0.00	0.00
0.375	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.04	0.00	0.00
0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	73.2	76.6	74.6	73.5	71.6	70.5	68.8	71.2	74.4	72.6

IVM*= ivermectin; F = farm; K = Kaimanawa.
This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.1 Proportion of L_3 values of Kaimanawa horse by concentrations of Ivermectin

Conc (µg/ml) IVM*	H K11	H K12	H K13	H K14	H K15	H K16	H K17	H K18	H K19
0.0007	0.98	1.05	1.03	0.99	1.01	1.01	1.02	1.02	1.01
0.0007	1.01	0.98	1.00	1.02	1.00	1.00	0.97	0.97	0.97
0.0015	0.99	1.00	0.97	0.97	0.97	0.98	1.02	1.02	1.04
0.0015	1.02	1.03	1.01	0.96	0.99	1.01	0.98	0.98	0.99
0.0029	0.96	0.95	0.96	1.00	1.03	1.03	1.00	1.00	1.01
0.0029	1.01	0.98	0.97	0.97	1.00	1.00	1.03	1.03	1.02
0.0059	0.81	0.49	0.94	0.94	0.90	0.78	0.94	0.94	0.91
0.0059	0.79	0.52	0.91	0.91	0.94	0.75	0.91	0.91	0.88
0.0117	0.48	0.40	0.79	0.78	0.77	0.50	0.77	0.77	0.75
0.0117	0.45	0.36	0.80	0.75	0.73	0.46	0.74	0.74	0.77
0.0234	0.42	0.39	0.74	0.72	0.68	0.48	0.48	0.68	0.69
0.0234	0.48	0.42	0.71	0.67	0.66	0.43	0.45	0.65	0.66
0.0469	0.27	0.30	0.26	0.28	0.24	0.30	0.32	0.32	0.28
0.0469	0.28	0.32	0.21	0.25	0.25	0.37	0.26	0.26	0.30
0.0938	0.10	0.09	0.13	0.15	0.15	0.21	0.17	0.17	0.14
0.0938	0.08	0.14	0.10	0.12	0.20	0.25	0.15	0.15	0.16
0.1875	0.11	0.12	0.07	0.04	0.06	0.07	0.09	0.09	0.11
0.1875	0.08	0.06	0.04	0.07	0.07	0.09	0.08	0.08	0.06
0.375	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.375	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	70.6	69.2	70.0	66.8	71.0	56.2	66.0	58.25	63.6

IVM*= ivermectin; F = farm; K = Kaimanawa.
This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.2 Proportion of L_3 value of farm horses by concentrations of Ivermectin aglycone

Conc (µg/ml) IVM ag♦	F B1	F B2	F B3	F B4	F B5	F B6	F B7	F Ca1	F Ca3	F Ca2	F Ca4	F Ca5
0.0008	0.96	1.01	0.98	0.99	1.03	1.03	1.01	1.02	0.92	1.01	1.01	0.93
0.0008	0.93	0.95	0.95	1.00	1.00	1.00	1.02	1.00	0.95	1.04	1.01	0.99
0.0017	1.00	0.99	0.96	0.78	0.99	1.02	1.02	1.03	0.92	1.00	1.01	0.90
0.0017	0.97	0.99	0.98	0.80	1.02	1.05	1.00	1.02	0.92	0.96	1.02	0.91
0.0034	0.84	0.97	1.00	0.58	0.99	1.00	0.98	0.99	0.91	0.92	0.97	0.90
0.0034	0.85	0.95	1.00	0.56	0.98	1.03	0.97	1.00	0.92	0.96	1.00	0.88
0.0069	0.78	0.95	0.96	0.48	1.08	0.95	0.86	1.03	0.83	0.90	1.03	0.77
0.0069	0.77	0.93	0.95	0.46	0.98	0.94	0.89	0.97	0.76	0.94	1.01	0.75
0.0138	0.42	0.75	0.95	0.27	0.96	0.88	0.85	1.00	0.82	0.76	0.95	0.78
0.0138	0.38	0.77	0.98	0.32	1.02	0.83	0.80	0.99	0.79	0.75	1.00	0.77
0.0277	0.32	0.73	0.96	0.10	1.04	0.78	0.72	0.87	0.69	0.65	0.96	0.61
0.0277	0.31	0.71	0.95	0.14	0.99	0.72	0.74	0.81	0.71	0.63	0.97	0.62
0.0553	0.16	0.44	0.65	0.03	0.90	0.48	0.71	0.70	0.63	0.54	0.95	0.58
0.0553	0.15	0.46	0.60	0.09	0.86	0.45	0.65	0.70	0.66	0.52	1.03	0.54
0.1106	0.09	0.26	0.44	0.00	0.62	0.25	0.49	0.42	0.57	0.51	0.41	0.52
0.1106	0.11	0.22	0.47	0.00	0.59	0.22	0.50	0.40	0.57	0.49	0.43	0.43
0.2212	0.04	0.15	0.37	0.00	0.25	0.11	0.44	0.28	0.03	0.24	0.17	0.20
0.2212	0.05	0.16	0.39	0.00	0.20	0.14	0.49	0.25	0.08	0.23	0.18	0.23
0.4425	0.03	0.11	0.21	0.00	0.12	0.05	0.30	0.13	0.03	0.00	0.07	0.00
0.4425	0.03	0.09	0.18	0.00	0.09	0.03	0.29	0.10	0.05	0.00	0.09	0.00
0.885	0.03	0.05	0.12	0.00	0.13	0.03	0.19	0.07	0.03	0.00	0.07	0.00
0.885	0.03	0.04	0.16	0.00	0.12	0.03	0.18	0.09	0.05	0.00	0.07	0.00
1.77	0.00	0.00	0.00	0.00	0.16	0.00	0.08	0.04	0.00	0.00	0.06	0.00
1.77	0.00	0.00	0.00	0.00	0.13	0.00	0.10	0.07	0.00	0.00	0.07	0.00
3.54	0.00	0.00	0.00	0.00	0.16	0.00	0.03	0.00	0.00	0.00	0.02	0.00
3.54	0.00	0.00	0.00	0.00	0.17	0.00	0.04	0.00	0.00	0.00	0.04	0.00
7.07	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.00	0.00	0.00	0.02	0.00
7.07	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.02	0.00
14.15	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.02	0.00
14.15	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.02	0.00
28.3	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Mean control	74.0	54.7	57.0	58.7	75.7	65.0	73.3	66.7	66.2	71.0	81.2	69.0

IVM ag♦ = ivermectin aglycone; F = farm; B = Bombay; Ca = Canterbury.

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw Data by animals by anthelmintic

A7.2 Proportion of L_3 value of farm horses by concentrations of Ivermectin aglycone

Conc (µg/ml) IVM ag♦	F Ca6	F Ca7	F Ca8	F Ch1	F Ch2	F Ch3	F Ch4	F Dy1	F E1	F E2	F E3	F E4
0.0008	0.93	1.00	1.00	1.04	1.00	0.96	1.00	1.02	0.99	0.99	0.97	1.00
0.0008	0.96	1.01	1.02	1.05	1.01	1.00	1.03	1.00	0.96	1.01	0.99	0.99
0.0017	0.93	0.98	0.99	1.01	1.04	0.97	1.00	1.02	0.96	0.96	1.01	0.98
0.0017	0.95	1.01	1.00	1.02	0.98	1.00	1.01	0.98	0.99	1.00	1.03	0.94
0.0034	0.92	0.98	0.96	1.02	0.95	0.93	0.98	1.00	0.80	0.94	1.04	0.92
0.0034	0.99	1.00	0.98	1.04	0.97	0.94	1.06	1.00	0.83	0.96	0.99	0.91
0.0069	0.87	1.00	1.00	0.90	0.97	0.80	0.93	0.97	0.79	0.92	0.99	0.87
0.0069	0.90	0.94	0.95	0.89	0.94	0.79	0.96	1.05	0.76	0.90	1.01	0.85
0.0138	0.86	0.98	0.98	0.75	0.92	0.73	0.92	0.97	0.43	0.90	0.95	0.84
0.0138	0.83	0.95	0.99	0.75	0.91	0.71	0.89	1.00	0.42	0.92	1.01	0.87
0.0277	0.76	0.75	0.84	0.63	0.83	0.59	0.81	1.02	0.25	0.82	0.88	0.77
0.0277	0.70	0.70	0.81	0.64	0.77	0.57	0.74	0.98	0.28	0.80	0.91	0.79
0.0553	0.51	0.72	0.71	0.48	0.67	0.49	0.54	0.90	0.16	0.77	0.80	0.73
0.0553	0.47	0.70	0.68	0.52	0.64	0.46	0.49	0.89	0.15	0.79	0.78	0.75
0.1106	0.31	0.60	0.40	0.38	0.39	0.26	0.32	0.60	0.12	0.55	0.57	0.68
0.1106	0.26	0.59	0.37	0.34	0.37	0.28	0.28	0.62	0.09	0.59	0.59	0.69
0.2212	0.09	0.09	0.27	0.29	0.25	0.17	0.09	0.13	0.03	0.07	0.35	0.60
0.2212	0.06	0.06	0.22	0.31	0.22	0.14	0.06	0.11	0.03	0.08	0.34	0.58
0.4425	0.03	0.00	0.10	0.12	0.10	0.03	0.03	0.00	0.03	0.00	0.24	0.50
0.4425	0.04	0.00	0.09	0.08	0.12	0.05	0.05	0.00	0.01	0.00	0.22	0.49
0.885	0.03	0.00	0.06	0.00	0.06	0.02	0.03	0.00	0.01	0.00	0.16	0.47
0.885	0.01	0.00	0.07	0.00	0.07	0.00	0.02	0.00	0.03	0.00	0.12	0.45
1.77	0.00	0.00	0.03	0.00	0.06	0.00	0.00	0.00	0.01	0.00	0.11	0.24
1.77	0.00	0.00	0.06	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.08	0.23
3.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.23
3.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.20
7.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
7.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12
14.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
14.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
28.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	68.7	68.3	67.7	73.4	67.2	64.7	63.2	63.0	74.7	71.0	74.0	73.7

IVM ag♦ = ivermectin aglycone; F = farm; Ca = Canterbury; Ch = Christchurch; Dy = Drury; E = Exported.
This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw Data by animals by anthelmintic

A7.2 Proportion of L_3 value of farm horses by concentrations of Ivermectin aglycone

Conc (µg/ml) IVM ag♦	F H1	F H2	F H3	F H4	F H5	F H6	F H7	F H8	F P1	F P2	F P3	F P4
0.0008	0.99	0.95	1.00	0.97	1.00	0.99	1.06	0.98	1.09	0.99	0.89	1.01
0.0008	0.97	0.92	1.02	1.00	0.99	0.97	1.04	1.02	1.00	0.91	0.93	0.96
0.0017	0.97	0.70	0.99	0.97	1.00	0.94	1.00	1.03	1.05	1.10	0.86	0.93
0.0017	0.99	0.70	1.00	0.94	1.00	0.96	1.02	1.00	0.99	1.07	0.84	0.99
0.0034	0.96	0.56	1.00	0.97	0.99	0.94	0.94	0.96	0.96	0.91	0.71	1.04
0.0034	1.01	0.52	0.99	0.94	0.97	1.02	0.96	0.97	0.99	0.99	0.69	0.96
0.0069	0.97	0.47	0.99	0.91	0.95	0.93	0.98	0.95	0.87	1.05	0.48	1.03
0.0069	1.00	0.53	0.95	0.93	0.94	0.99	0.94	0.94	0.85	1.12	0.50	0.90
0.0138	0.96	0.21	0.93	0.93	0.95	0.97	0.91	0.92	0.74	0.99	0.27	1.06
0.0138	0.94	0.08	0.95	0.94	0.91	1.00	0.89	0.91	0.75	0.97	0.24	0.95
0.0277	0.52	0.02	0.90	0.88	0.92	1.02	0.43	0.89	0.61	0.78	0.13	0.74
0.0277	0.48	0.00	0.88	0.87	0.94	0.99	0.41	0.90	0.60	0.82	0.11	0.75
0.0553	0.43	0.00	0.60	0.84	0.91	0.88	0.26	0.92	0.32	0.86	0.03	0.66
0.0553	0.45	0.00	0.63	0.83	0.96	0.90	0.22	0.88	0.31	0.79	0.01	0.68
0.1106	0.06	0.00	0.23	0.67	0.90	0.65	0.20	0.39	0.08	0.30	0.01	0.62
0.1106	0.07	0.00	0.19	0.70	0.87	0.67	0.22	0.41	0.06	0.22	0.01	0.60
0.2212	0.00	0.00	0.05	0.46	0.42	0.47	0.15	0.19	0.00	0.10	0.00	0.09
0.2212	0.00	0.00	0.02	0.48	0.45	0.49	0.13	0.22	0.00	0.16	0.00	0.06
0.4425	0.00	0.00	0.00	0.03	0.31	0.03	0.11	0.19	0.00	0.14	0.00	0.00
0.4425	0.00	0.00	0.02	0.01	0.32	0.03	0.11	0.21	0.00	0.15	0.00	0.00
0.885	0.00	0.00	0.00	0.00	0.21	0.00	0.07	0.12	0.00	0.11	0.00	0.00
0.885	0.00	0.00	0.00	0.00	0.19	0.00	0.06	0.12	0.00	0.07	0.00	0.00
1.77	0.00	0.00	0.00	0.00	0.18	0.00	0.04	0.09	0.00	0.08	0.00	0.00
1.77	0.00	0.00	0.00	0.00	0.16	0.00	0.02	0.10	0.00	0.05	0.00	0.00
3.54	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.09	0.00	0.07	0.00	0.00
3.54	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.07	0.00	0.03	0.00	0.00
7.07	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.06	0.00	0.01	0.00	0.00
7.07	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.06	0.00	0.00	0.00	0.00
14.15	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.02	0.00	0.00	0.00	0.00
14.15	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.04	0.00	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	69.0	66.0	56.7	69.0	77.0	65.7	54.0	82.2	71.7	73.5	74.2	66.3

IVM ag♦ = ivermectin aglycone; F = farm; H = Hastings; P = Palmerston North.

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw Data by animals by anthelmintic

A7.2 Proportion of L_3 value of farm horses by concentrations of Ivermectin aglycone

Conc (µg/ml) IVM ag♦	F Ta1	F Ta2	F Ta3	F Ta4	F Ta5	F Ta6	F Ta7	F Ta8	F Ta9	F Ta10	F Ta11
0.0008	0.98	0.95	0.98	1.00	0.96	0.97	0.98	0.98	1.03	0.99	0.96
0.0008	1.00	1.01	1.00	0.99	1.00	1.01	0.99	1.01	1.04	0.97	0.98
0.0017	1.03	0.94	0.98	0.99	0.98	0.92	0.98	0.98	0.99	0.99	1.00
0.0017	1.08	0.95	0.97	0.97	0.90	0.94	1.02	0.98	1.00	0.96	0.94
0.0034	1.05	0.95	1.00	0.96	0.92	0.91	0.94	0.95	0.77	0.94	0.96
0.0034	0.98	0.94	1.03	0.95	0.89	0.87	0.95	0.94	0.79	0.93	0.93
0.0069	0.95	0.91	1.02	0.96	0.92	0.71	0.90	0.93	0.60	0.88	0.89
0.0069	0.93	0.77	1.00	0.97	0.89	0.74	0.91	0.97	0.59	0.93	0.81
0.0138	0.86	0.75	0.98	0.95	0.84	0.39	0.69	0.94	0.47	0.85	0.91
0.0138	0.93	0.57	0.97	0.93	0.85	0.37	0.66	0.91	0.47	0.87	0.87
0.0277	0.88	0.54	0.80	0.93	0.79	0.10	0.52	0.95	0.45	0.78	0.57
0.0277	0.85	0.34	0.83	0.95	0.77	0.08	0.50	0.93	0.48	0.82	0.59
0.0553	0.86	0.37	0.74	0.92	0.73	0.00	0.32	0.65	0.47	0.66	0.44
0.0553	0.83	0.33	0.69	0.89	0.74	0.02	0.36	0.72	0.45	0.61	0.46
0.1106	0.58	0.06	0.40	0.84	0.68	0.00	0.21	0.52	0.44	0.57	0.35
0.1106	0.56	0.09	0.48	0.86	0.65	0.00	0.24	0.57	0.45	0.55	0.33
0.2212	0.20	0.04	0.32	0.61	0.52	0.00	0.21	0.16	0.37	0.21	0.13
0.2212	0.17	0.01	0.34	0.55	0.47	0.00	0.22	0.18	0.36	0.18	0.15
0.4425	0.08	0.00	0.18	0.47	0.41	0.00	0.20	0.15	0.23	0.06	0.06
0.4425	0.07	0.00	0.14	0.42	0.33	0.00	0.19	0.16	0.25	0.09	0.04
0.885	0.03	0.00	0.05	0.32	0.24	0.00	0.11	0.08	0.07	0.04	0.02
0.885	0.03	0.00	0.02	0.29	0.30	0.00	0.12	0.10	0.05	0.03	0.00
1.77	0.00	0.00	0.00	0.20	0.22	0.00	0.07	0.07	0.03	0.00	0.00
1.77	0.00	0.00	0.02	0.22	0.21	0.00	0.05	0.07	0.03	0.00	0.00
3.54	0.00	0.00	0.00	0.17	0.19	0.00	0.05	0.04	0.01	0.00	0.00
3.54	0.00	0.00	0.00	0.12	0.22	0.00	0.04	0.04	0.03	0.00	0.00
7.07	0.00	0.00	0.00	0.06	0.08	0.00	0.03	0.03	0.01	0.00	0.00
7.07	0.00	0.00	0.00	0.03	0.03	0.00	0.03	0.03	0.01	0.00	0.00
14.15	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
14.15	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	59.0	70.5	65.0	68.7	63.2	59.5	75.7	73.5	73.3	66.7	54.0

IVM ag♦ = ivermectin aglycone; F = farm; Ta = Takanini.

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw Data by animals by anthelmintic

A7.2 Proportion of L_3 value of Kaimanawa horse by concentrations of Ivermectin aglycone

Conc (µg/ml) IVM ag♦	H K1	H K2	H K3	H K4	H K5	H K6	H K7	H K8	H K9	H K10	H K11
0.0008	0.88	0.99	0.94	0.81	0.86	0.90	1.05	1.03	0.93	0.94	1.01
0.0008	0.92	1.01	0.98	0.80	0.88	0.92	1.01	1.00	0.96	0.86	0.95
0.0017	0.94	1.03	0.99	0.61	0.78	0.67	0.99	1.01	0.89	0.76	0.93
0.0017	0.83	1.04	0.96	0.60	0.80	0.66	0.97	1.05	0.86	0.75	0.96
0.0034	0.74	0.99	0.93	0.56	0.68	0.47	0.79	0.90	0.75	0.72	0.95
0.0034	0.69	0.97	0.99	0.58	0.65	0.46	0.77	0.96	0.70	0.69	0.91
0.0069	0.42	0.75	0.82	0.54	0.43	0.42	0.47	0.93	0.51	0.64	0.93
0.0069	0.42	0.77	0.83	0.53	0.38	0.41	0.45	0.86	0.53	0.60	0.91
0.0138	0.35	0.61	0.80	0.52	0.26	0.20	0.30	0.67	0.30	0.61	0.88
0.0138	0.21	0.58	0.82	0.53	0.20	0.09	0.26	0.69	0.28	0.57	0.86
0.0277	0.09	0.55	0.77	0.49	0.15	0.03	0.15	0.63	0.15	0.53	0.71
0.0277	0.08	0.54	0.75	0.50	0.14	0.00	0.13	0.62	0.12	0.56	0.70
0.0553	0.00	0.38	0.66	0.47	0.09	0.00	0.04	0.53	0.05	0.51	0.50
0.0553	0.00	0.41	0.67	0.50	0.07	0.00	0.06	0.55	0.04	0.49	0.47
0.1106	0.00	0.36	0.37	0.30	0.03	0.00	0.00	0.49	0.01	0.21	0.47
0.1106	0.00	0.35	0.35	0.27	0.04	0.00	0.04	0.49	0.03	0.26	0.51
0.2212	0.00	0.30	0.08	0.15	0.00	0.00	0.00	0.45	0.00	0.06	0.22
0.2212	0.00	0.29	0.10	0.12	0.00	0.00	0.00	0.42	0.00	0.08	0.25
0.4425	0.00	0.06	0.02	0.07	0.00	0.00	0.00	0.35	0.00	0.00	0.13
0.4425	0.00	0.07	0.03	0.05	0.00	0.00	0.00	0.31	0.00	0.00	0.12
0.885	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.00	0.00	0.00
0.885	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00	0.00
1.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00
1.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00
3.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	66.2	69.0	62.5	73.7	74.0	76.3	46.6	71.2	74.25	72.0	60.2

IVM ag♦ = ivermectin aglycone; F = farm; K = Kaimanawa

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw Data by animals by anthelmintic

A7.2 Proportion of L_3 value of Kaimanawa horse by concentrations of Ivermectin aglycone

Conc (µg/ml) IVM ag♦	H K12	H K13	H K14	H K15	H K16	H K17	H K18	H K19	H K20	H K21	H K22
0.0008	0.93	1.01	1.00	1.01	0.95	1.02	1.01	1.01	0.99	0.97	1.00
0.0008	0.90	1.05	0.99	1.03	0.99	0.91	1.00	0.99	1.02	0.98	1.01
0.0017	0.67	0.85	0.98	1.01	0.90	0.71	0.98	1.02	0.99	0.94	1.07
0.0017	0.66	1.02	1.02	1.00	0.91	0.70	1.00	1.01	0.99	0.96	1.05
0.0034	0.53	0.98	0.96	1.00	0.89	0.67	0.98	0.94	0.97	0.93	1.03
0.0034	0.50	1.01	0.98	1.01	0.91	0.73	1.01	0.93	0.99	0.94	1.00
0.0069	0.45	0.96	0.93	0.97	0.89	0.71	1.03	0.96	0.96	0.89	0.98
0.0069	0.43	0.93	0.92	0.95	0.90	0.67	0.98	0.97	0.95	0.88	1.01
0.0138	0.21	0.95	0.92	0.94	0.87	0.70	1.00	0.94	0.92	0.86	0.93
0.0138	0.10	0.92	0.93	0.93	0.89	0.65	1.01	0.93	0.93	0.88	0.91
0.0277	0.03	0.76	0.83	0.75	0.86	0.64	0.79	0.69	0.73	0.76	0.66
0.0277	0.00	0.78	0.82	0.77	0.86	0.62	0.71	0.66	0.72	0.72	0.68
0.0553	0.00	0.62	0.79	0.64	0.46	0.67	0.43	0.55	0.45	0.53	0.47
0.0553	0.00	0.65	0.80	0.61	0.48	0.50	0.46	0.55	0.48	0.55	0.49
0.1106	0.00	0.48	0.56	0.51	0.35	0.47	0.45	0.52	0.35	0.51	0.45
0.1106	0.00	0.47	0.60	0.50	0.32	0.24	0.42	0.55	0.40	0.51	0.42
0.2212	0.00	0.38	0.07	0.36	0.30	0.21	0.43	0.47	0.32	0.37	0.37
0.2212	0.00	0.40	0.09	0.38	0.32	0.00	0.40	0.46	0.35	0.35	0.38
0.4425	0.00	0.30	0.00	0.26	0.26	0.00	0.30	0.39	0.30	0.29	0.26
0.4425	0.00	0.28	0.00	0.27	0.27	0.00	0.27	0.36	0.27	0.25	0.30
0.885	0.00	0.21	0.00	0.20	0.15	0.00	0.22	0.22	0.21	0.19	0.16
0.885	0.00	0.24	0.00	0.19	0.16	0.00	0.20	0.27	0.20	0.20	0.12
1.77	0.00	0.13	0.00	0.13	0.11	0.00	0.13	0.13	0.14	0.11	0.14
1.77	0.00	0.08	0.00	0.10	0.12	0.00	0.10	0.08	0.11	0.09	0.12
3.54	0.00	0.07	0.00	0.07	0.09	0.00	0.10	0.00	0.08	0.08	0.10
3.54	0.00	0.10	0.00	0.06	0.11	0.00	0.09	0.00	0.07	0.05	0.12
7.07	0.00	0.04	0.00	0.06	0.09	0.00	0.07	0.00	0.06	0.07	0.05
7.07	0.00	0.06	0.00	0.04	0.08	0.00	0.06	0.00	0.04	0.05	0.07
14.15	0.00	0.07	0.00	0.06	0.08	0.00	0.06	0.00	0.06	0.05	0.05
14.15	0.00	0.03	0.00	0.03	0.07	0.00	0.04	0.00	0.03	0.07	0.02
28.3	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
28.3	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	71.3	70.6	69.7	70.2	74.4	66.0	69.2	63.6	70.8	75.2	57.2

IVM ag♦ = ivermectin aglycone; F = farm; K = Kaimanawa

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw Data by animals by anthelmintic

A7.3 Proportion of L_3 value of farm horses by concentrations of Thiabendazole

Conc (µg/ml) TBZ	F B1	F B2	F B3	F B4	F B5	F B6	F B7	F Ca1	F Ca2	F Ca3	F Ca4	F Ca5
0.0039	0.98	0.98	0.98	0.92	1.02	1.05	0.91	0.97	1.01	1.00	1.00	0.99
0.0039	0.95	1.00	0.94	0.88	1.05	1.00	0.83	0.96	0.99	1.00	1.02	0.97
0.0078	0.99	0.95	0.98	0.91	1.00	1.08	0.82	0.92	0.95	1.02	0.98	0.95
0.0078	0.98	1.01	0.96	0.89	0.99	1.07	0.86	0.94	0.91	0.98	1.01	0.94
0.0157	0.93	1.00	0.96	0.89	0.90	1.02	0.79	0.84	0.93	0.96	0.97	0.95
0.0157	0.95	0.97	0.94	0.86	0.93	1.00	0.82	0.77	0.95	0.98	0.98	0.94
0.0316	0.69	0.98	0.93	0.67	0.85	1.04	0.80	0.77	0.94	0.98	0.96	0.81
0.0316	0.70	0.98	0.99	0.71	0.88	1.02	0.87	0.78	0.91	1.00	0.96	0.79
0.0632	0.68	0.97	0.89	0.58	0.82	0.83	0.88	0.77	0.90	0.78	0.94	0.70
0.0632	0.66	0.94	0.91	0.61	0.86	0.84	0.83	0.74	0.93	0.80	0.93	0.72
0.1264	0.50	0.74	0.86	0.53	0.83	0.61	0.76	0.76	0.95	0.71	1.00	0.75
0.1264	0.51	0.71	0.88	0.55	0.79	0.54	0.76	0.77	0.86	0.67	0.94	0.77
0.252	0.35	0.57	0.79	0.52	0.71	0.45	0.79	0.57	0.71	0.56	0.58	0.57
0.252	0.38	0.54	0.81	0.47	0.74	0.37	0.84	0.55	0.67	0.58	0.56	0.59
0.504	0.35	0.34	0.79	0.36	0.59	0.38	0.76	0.41	0.59	0.45	0.39	0.39
0.504	0.37	0.31	0.78	0.38	0.57	0.35	0.80	0.39	0.57	0.44	0.45	0.40
1.008	0.33	0.20	0.55	0.24	0.43	0.27	0.20	0.36	0.30	0.38	0.38	0.29
1.008	0.34	0.17	0.56	0.26	0.45	0.25	0.26	0.38	0.31	0.40	0.39	0.28
2.016	0.28	0.14	0.38	0.26	0.31	0.18	0.04	0.26	0.15	0.33	0.30	0.22
2.016	0.27	0.12	0.36	0.24	0.32	0.19	0.07	0.19	0.10	0.31	0.29	0.24
4.032	0.26	0.12	0.12	0.20	0.17	0.21	0.01	0.17	0.08	0.09	0.28	0.22
4.032	0.19	0.09	0.10	0.21	0.19	0.16	0.03	0.15	0.06	0.07	0.30	0.20
8.064	0.05	0.03	0.03	0.17	0.06	0.08	0.00	0.15	0.00	0.02	0.07	0.17
8.064	0.07	0.05	0.05	0.18	0.11	0.11	0.00	0.17	0.00	0.00	0.08	0.18
16.125	0.04	0.00	0.00	0.03	0.03	0.11	0.00	0.04	0.00	0.00	0.00	0.15
16.125	0.04	0.00	0.00	0.05	0.00	0.10	0.00	0.03	0.00	0.00	0.00	0.09
32.25	0.03	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	73.8	65.2	60.5	66.0	64.7	62.8	76.0	68.8	77.6	55.0	76.2	54.5

TBZ = Thiabendazole; F = Farm; B = Bombay; Ca = Canterbury.
This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.3 Proportion of L_3 value of farm horses by concentrations of Thiabendazole

Conc (µg/ml) TBZ	F Ca6	F Ca7	F Ca8	F Ch1	F Ch2	F Ch3	F Ch4	F Dy1	F E1	F E2	F E3	F E4
0.0039	1.04	0.99	0.95	1.09	1.00	0.99	1.01	0.93	0.98	1.03	0.92	1.04
0.0039	1.06	1.01	0.97	1.04	0.99	1.01	0.92	0.90	0.95	1.00	1.00	1.00
0.0078	1.01	0.98	0.99	1.07	1.01	1.02	0.92	0.85	1.01	1.05	1.00	1.02
0.0078	1.03	1.01	0.98	1.06	0.97	0.99	1.07	0.86	0.96	0.99	0.98	1.04
0.0157	0.93	0.99	1.01	1.07	0.97	1.01	1.03	0.85	0.89	0.86	0.95	1.02
0.0157	0.95	0.97	0.98	1.02	0.96	0.98	1.07	0.83	0.92	0.83	0.97	1.00
0.0316	0.82	0.97	0.97	0.99	0.94	0.98	1.01	0.79	0.89	0.72	1.00	0.96
0.0316	0.85	0.99	0.93	1.07	0.90	0.98	0.98	0.81	0.83	0.65	0.93	1.00
0.0632	0.76	0.98	0.82	0.74	0.87	0.81	0.95	0.78	0.69	0.62	0.86	1.00
0.0632	0.79	0.95	0.83	0.70	0.88	0.80	0.92	0.82	0.73	0.63	0.83	0.96
0.1264	0.71	0.78	0.75	0.75	0.83	0.61	0.94	0.77	0.44	0.40	0.80	0.98
0.1264	0.74	0.79	0.74	0.72	0.84	0.63	0.95	0.78	0.38	0.35	0.85	0.96
0.252	0.61	0.66	0.59	0.69	0.62	0.49	0.51	0.78	0.40	0.34	0.71	0.98
0.252	0.66	0.68	0.56	0.66	0.61	0.53	0.49	0.82	0.32	0.31	0.69	0.96
0.504	0.47	0.48	0.46	0.51	0.59	0.38	0.35	0.67	0.31	0.15	0.68	0.98
0.504	0.48	0.49	0.43	0.50	0.58	0.31	0.33	0.69	0.28	0.16	0.66	0.96
1.008	0.26	0.39	0.31	0.19	0.35	0.20	0.27	0.19	0.24	0.21	0.41	0.44
1.008	0.27	0.37	0.32	0.22	0.36	0.18	0.19	0.20	0.17	0.15	0.44	0.46
2.016	0.18	0.24	0.23	0.03	0.20	0.00	0.17	0.04	0.15	0.13	0.31	0.19
2.016	0.24	0.22	0.24	0.02	0.22	0.03	0.20	0.03	0.17	0.12	0.32	0.18
4.032	0.06	0.06	0.22	0.00	0.09	0.00	0.14	0.01	0.12	0.15	0.27	0.16
4.032	0.05	0.07	0.20	0.00	0.10	0.00	0.17	0.01	0.15	0.12	0.24	0.18
8.064	0.00	0.03	0.08	0.00	0.00	0.00	0.17	0.00	0.09	0.07	0.05	0.11
8.064	0.00	0.01	0.11	0.00	0.00	0.00	0.14	0.00	0.12	0.10	0.03	0.12
16.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.09	0.00	0.07
16.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.07	0.00	0.09
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	62.2	69.4	74.4	62.5	69.0	71.6	69.2	75.6	65.4	67.8	59.0	57.0

TBZ = Thiabendazole; F = Farm; Ca = Canterbury; Ch = Christchurch; Dy = Drury; E = Exported horse. This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.3 Proportion of L_3 value of farm horses by concentrations of Thiabendazole

Conc (µg/ml) TBZ	F H1	F H2	F H3	F H4	F H5	F H6	F H7	F H8	F P1	F P2	F P3	F P4
0.0039	0.97	1.01	1.04	0.99	1.03	1.00	1.03	1.02	1.02	0.94	1.00	0.99
0.0039	0.99	1.03	1.00	0.97	1.01	0.98	1.05	0.98	1.04	0.98	1.01	1.00
0.0078	0.96	0.97	1.02	1.00	1.04	0.95	1.03	1.00	0.99	0.98	0.97	1.02
0.0078	1.02	0.94	1.04	0.97	1.07	1.00	1.02	0.97	1.02	0.96	0.96	0.97
0.0157	0.97	0.84	1.02	1.01	1.03	1.00	1.03	1.01	1.00	0.95	0.98	1.04
0.0157	0.99	0.85	1.04	1.00	1.03	1.02	1.02	0.97	1.03	0.95	1.00	0.99
0.0316	0.96	0.73	0.99	0.88	0.95	0.97	1.05	0.90	0.79	0.94	0.97	0.99
0.0316	0.97	0.71	0.97	0.87	0.98	0.94	1.03	0.93	0.82	0.94	0.96	1.00
0.0632	0.88	0.64	0.95	0.75	0.91	0.89	1.03	0.88	0.81	0.90	0.87	0.95
0.0632	0.85	0.67	0.93	0.76	0.98	0.88	0.99	0.87	0.88	0.91	0.90	0.93
0.1264	0.67	0.50	0.92	0.74	1.00	0.86	0.54	0.81	0.81	0.70	0.62	0.75
0.1264	0.64	0.47	0.97	0.76	0.91	0.88	0.55	0.87	0.79	0.72	0.63	0.79
0.252	0.50	0.44	0.95	0.76	0.70	0.85	0.35	0.40	0.33	0.51	0.27	0.68
0.252	0.46	0.44	0.97	0.74	0.67	0.82	0.38	0.42	0.33	0.54	0.24	0.74
0.504	0.29	0.37	0.93	0.72	0.67	0.77	0.28	0.36	0.31	0.48	0.14	0.57
0.504	0.30	0.34	0.92	0.74	0.68	0.80	0.31	0.37	0.30	0.50	0.15	0.56
1.008	0.29	0.24	0.83	0.56	0.54	0.35	0.32	0.37	0.17	0.29	0.07	0.32
1.008	0.34	0.26	0.86	0.57	0.51	0.42	0.29	0.35	0.20	0.26	0.06	0.29
2.016	0.29	0.27	0.60	0.10	0.40	0.29	0.31	0.30	0.16	0.15	0.04	0.22
2.016	0.22	0.24	0.62	0.12	0.34	0.23	0.28	0.31	0.17	0.17	0.06	0.25
4.032	0.13	0.20	0.28	0.03	0.18	0.12	0.20	0.31	0.16	0.03	0.00	0.04
4.032	0.10	0.21	0.33	0.04	0.15	0.08	0.17	0.30	0.15	0.01	0.00	0.05
8.064	0.05	0.10	0.21	0.00	0.10	0.06	0.09	0.17	0.14	0.00	0.00	0.04
8.064	0.08	0.11	0.18	0.00	0.15	0.06	0.12	0.16	0.16	0.00	0.00	0.02
16.125	0.00	0.03	0.00	0.00	0.00	0.00	0.07	0.06	0.00	0.00	0.00	0.00
16.125	0.00	0.04	0.00	0.00	0.00	0.00	0.09	0.05	0.00	0.00	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	62.6	70.2	56.7	68.0	67.2	65.0	68.6	80.4	80.6	72.6	71.2	55.7

TBZ = Thiabendazole; F = Farm; H = Hastings; P = Palmerston North.
This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.3 Proportion of L_3 value of farm horses by concentrations of Thiabendazole

Conc (µg/ml) TBZ	F Ta1	F Ta2	F Ta3	F Ta4	F Ta5	F Ta6	F Ta7	F Ta8	F Ta9	F Ta10	F Ta11
0.0039	0.93	0.99	1.00	0.97	1.02	1.00	0.99	1.01	1.02	1.00	1.01
0.0039	0.98	1.02	1.03	0.96	1.03	1.02	0.97	0.98	1.00	1.02	0.96
0.0078	1.00	1.04	0.95	0.93	1.02	0.98	1.00	1.00	1.02	1.00	0.98
0.0078	1.04	1.00	1.04	0.97	0.99	1.00	0.99	1.00	0.99	0.98	0.92
0.0157	0.98	0.99	0.98	0.93	1.01	0.95	0.96	0.97	0.96	0.96	0.96
0.0157	0.91	0.96	0.92	0.97	0.98	0.97	0.94	0.98	0.97	1.00	0.98
0.0316	0.92	0.78	0.97	0.96	0.98	0.64	0.91	0.95	0.99	0.81	0.94
0.0316	1.03	0.77	0.95	0.98	0.96	0.58	0.88	0.93	0.94	0.80	0.92
0.0632	0.79	0.56	1.03	0.92	0.95	0.35	0.86	0.95	0.99	0.83	0.91
0.0632	0.80	0.54	0.98	0.91	0.96	0.38	0.87	0.93	0.93	0.81	0.92
0.1264	0.69	0.38	0.91	0.89	0.95	0.30	0.51	0.95	0.71	0.76	0.60
0.1264	0.70	0.34	0.88	0.92	0.93	0.33	0.45	0.92	0.63	0.74	0.64
0.252	0.68	0.33	0.53	0.87	0.82	0.27	0.32	0.89	0.40	0.70	0.52
0.252	0.69	0.30	0.52	0.84	0.83	0.23	0.38	0.87	0.41	0.69	0.41
0.504	0.51	0.33	0.40	0.89	0.67	0.23	0.28	0.74	0.37	0.56	0.33
0.504	0.53	0.36	0.46	0.85	0.69	0.17	0.23	0.72	0.32	0.57	0.38
1.008	0.46	0.33	0.45	0.41	0.55	0.12	0.26	0.17	0.34	0.33	0.35
1.008	0.47	0.32	0.43	0.43	0.51	0.14	0.22	0.15	0.31	0.35	0.38
2.016	0.43	0.10	0.36	0.00	0.17	0.11	0.20	0.18	0.28	0.33	0.30
2.016	0.43	0.08	0.39	0.00	0.16	0.09	0.22	0.14	0.31	0.31	0.35
4.032	0.19	0.10	0.32	0.00	0.03	0.11	0.16	0.15	0.27	0.28	0.09
4.032	0.18	0.07	0.29	0.00	0.06	0.12	0.17	0.17	0.24	0.28	0.06
8.064	0.07	0.08	0.03	0.00	0.04	0.09	0.12	0.15	0.19	0.24	0.03
8.064	0.08	0.10	0.00	0.00	0.01	0.11	0.14	0.14	0.15	0.22	0.03
16.125	0.00	0.01	0.01	0.00	0.00	0.00	0.10	0.00	0.04	0.11	0.00
16.125	0.00	0.03	0.00	0.00	0.00	0.00	0.09	0.00	0.09	0.13	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	73.8	72.8	69.2	76.2	68.6	66.0	69.0	65.2	67.8	54.0	70.5

TBZ = Thiabendazole; F = Farm; Ta = Takanini.
This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.3 Proportion of L_3 value of Kaimanawa horses by concentrations of Thiabendazole

Conc. (µg/ml) TBZ	H K1	H K2	H K3	H K4	H K5	H K6	H K7	H K8	H K9	H K10	H K11
0.0039	0.86	0.94	0.98	1.00	1.02	0.97	1.03	1.00	0.94	0.96	1.01
0.0039	1.08	1.01	0.94	1.03	1.01	1.00	1.02	1.03	0.90	0.97	0.98
0.0078	1.00	0.98	1.00	1.02	1.04	0.95	0.99	0.98	0.93	0.99	1.02
0.0078	0.98	0.89	1.01	1.00	0.98	0.97	1.00	0.98	0.92	0.94	0.97
0.0157	0.75	0.75	0.96	1.02	0.87	1.02	0.93	0.96	0.49	0.99	1.01
0.0157	0.80	0.80	0.89	0.98	0.88	0.98	0.96	0.93	0.53	0.97	0.99
0.0316	0.64	0.44	0.85	0.95	0.58	0.94	0.75	0.90	0.49	0.89	0.85
0.0316	0.62	0.40	0.87	0.94	0.59	0.91	0.72	0.88	0.47	0.90	0.88
0.0632	0.46	0.19	0.62	0.83	0.40	0.79	0.25	0.87	0.32	0.64	0.67
0.0632	0.42	0.30	0.59	0.86	0.37	0.76	0.29	0.86	0.35	0.58	0.61
0.1264	0.19	0.12	0.50	0.58	0.32	0.68	0.04	0.70	0.36	0.49	0.53
0.1264	0.15	0.16	0.48	0.57	0.30	0.70	0.03	0.67	0.22	0.50	0.51
0.252	0.07	0.04	0.50	0.38	0.12	0.59	0.00	0.45	0.22	0.40	0.36
0.252	0.04	0.01	0.46	0.42	0.17	0.56	0.03	0.41	0.25	0.44	0.40
0.504	0.00	0.00	0.44	0.35	0.06	0.35	0.00	0.24	0.17	0.36	0.30
0.504	0.01	0.00	0.41	0.40	0.03	0.41	0.00	0.27	0.19	0.33	0.33
1.008	0.00	0.00	0.25	0.09	0.00	0.12	0.00	0.10	0.13	0.26	0.20
1.008	0.00	0.00	0.30	0.08	0.00	0.08	0.00	0.07	0.11	0.29	0.26
2.016	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.10	0.08	0.09
2.016	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.11	0.11	0.10
4.032	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.07	0.04
4.032	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.08	0.07
8.064	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.064	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	72.2	74.4	56.2	65.0	65.6	66.0	71.8	71.2	70.4	72.0	70.4

TBZ = Thiabendazole; F = Farm; K = Kaimanawa

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

Appendix VII (Cont.)

Raw data by animals by anthelmintic

A7.3 Proportion of L_3 value of Kaimanawa horses by concentrations of Thiabendazole

Conc (µg/ml) TBZ	H K12	H K13	H K14	H K15	H K16	H K17	H K18	H K19	H K20	H K21	H K22
0.0039	1.04	1.03	0.99	1.02	1.03	1.04	1.01	0.98	1.01	1.06	1.00
0.0039	1.01	1.00	0.98	1.00	1.00	1.00	1.02	1.00	1.02	1.02	1.04
0.0078	1.02	0.99	0.96	1.03	1.05	1.06	1.04	1.01	0.98	1.04	1.02
0.0078	1.04	1.01	1.02	0.98	1.02	1.02	1.01	1.00	0.99	1.06	1.00
0.0157	1.01	0.99	1.01	0.99	0.97	0.97	0.95	0.98	0.95	0.94	1.02
0.0157	1.02	0.94	0.99	1.00	0.99	0.99	0.98	1.01	0.98	0.90	0.98
0.0316	0.86	0.87	0.93	0.93	1.00	1.00	0.91	1.00	0.90	0.80	0.95
0.0316	0.83	0.86	0.89	0.95	1.02	1.02	0.87	1.01	0.95	0.84	0.93
0.0632	0.79	0.85	0.91	0.91	0.78	0.92	0.87	1.03	0.92	0.61	0.98
0.0632	0.77	0.82	0.95	0.88	0.75	0.88	0.84	0.98	0.91	0.58	1.02
0.1264	0.71	0.76	0.76	0.76	0.47	0.57	0.73	0.81	0.63	0.51	0.51
0.1264	0.68	0.77	0.79	0.78	0.44	0.54	0.76	0.84	0.67	0.48	0.49
0.252	0.44	0.54	0.42	0.66	0.49	0.55	0.49	0.73	0.45	0.38	0.45
0.252	0.46	0.52	0.44	0.63	0.47	0.52	0.48	0.67	0.46	0.36	0.49
0.504	0.35	0.48	0.40	0.43	0.41	0.43	0.46	0.44	0.38	0.31	0.38
0.504	0.38	0.45	0.37	0.48	0.38	0.42	0.42	0.39	0.35	0.27	0.40
1.008	0.11	0.24	0.24	0.25	0.28	0.21	0.27	0.18	0.29	0.12	0.27
1.008	0.06	0.20	0.23	0.22	0.25	0.28	0.24	0.21	0.25	0.15	0.20
2.016	0.00	0.11	0.10	0.11	0.12	0.12	0.08	0.10	0.13	0.09	0.09
2.016	0.00	0.07	0.11	0.10	0.10	0.09	0.06	0.13	0.08	0.05	0.05
4.032	0.00	0.08	0.13	0.03	0.09	0.07	0.04	0.04	0.10	0.00	0.00
4.032	0.00	0.04	0.10	0.04	0.07	0.09	0.03	0.06	0.06	0.00	0.00
8.064	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8.064	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
64.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean control	63.6	71.0	70.6	72.8	67.8	57.8	71.4	71.2	71.4	58.6	55.0

TBZ = Thiabendazole; F = Farm; K = Kaimanawa

This proportion number as the number of cyathostome L_3 per well divided by the mean number of cyathostome L_3 in control well.

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