Studies on the abomasal parasites of cattle in subtropical and temperate climates

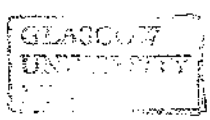
by

Stella Josefina Maciel, D.V.M. (Paraguay)

A Thesis submitted for the degree of Doctor of Philosophy in the Faculty of Veterinary Medicine of the University of Glasgow.

Department of Veterinary Parasitology

October, 1984.



ACKNOWLEDGEMENTS

Firstly, I would like to record my thanks and indebtedness to Professor J. Armour, under whose supervision these studies were conducted, and wish to express my deepest gratitude for his guidance and valuable advice, support, patience and understanding throughout the past three years.

I am indebted to Professor G.M. Urquhart, in whose Department the work was carried out, for his advice and encouragement throughout the study.

I would particularly like to thank Dr. A.M. Dunn for his help in the identification of the species.

I am especially grateful to Mr. K. Bairden for his friendly and skillful technical help, who never failing assistance was greatly appreciated.

I would like to extend my thanks to Dr. G. Gettinby in the Mathematics Department for his help.

For their able and friendly assistance in the laboratory, I would like to thank Mr. S. Brown, Mr. C. Hartley and Miss J. Henderson. I am also grateful to Mr. P. Tiebosch and Mrs. C. Connelly for their enthusiastic cooperation. I would also like to thank Mr. A. May and Mr. A. Finnie in the Photography Department for the illustrations which appear in this thesis.

My thanks is also due to Mr. Murphy for his able assistance in the post-mortem. For typing the manuscript so efficiently I wish to express my gratitude to Mrs. J. Nybo and Mrs. E. Millar.

I would also like to thank the members of the Department of Veterinary Parasitology and others for the constant encouragement

received.

I am most grateful to the Overseas Development Ministry who has provided the financial support for this research.

I would particularly like to thank Professor Eduardo Ruiz Almada, Dean of the Faculty of Veterinary Medicine of Asuncion, Paraguay, who allowed me to complete the study.

I am also indebted to all my colleagues and friends for their moral support.

Finally, I wish to dedicate this thesis to my mother who did so much with her constant encouragement and support.

October, 1984.

SUMMARY

In this thesis the literature pertaining to abomasal parasitism of cattle is reviewed in relation to temperate, subtropical and tropical climates. This review is followed by the experimental sections in which factors affecting the ecology and epidemiology of abomasal parasitism are investigated in Paraguay and Scotland.

In the ecology sections the free-living development of a bovine trichostrongyle Haemonchus contortus suited to tropical or subtropical climates and that of a bovine trichostrongyle Ostertagia ostertagi more suited to a temperate climate were studied under theoretically optimal conditions for each parasite. In the sub-tropics namely, Paraguay, development of H. contortus from egg to infective third stage larvae occurred throughout the year but was apparently most successful during the winter and spring. The numbers of larvae on the pasture at these times, although sufficient to cause significant infections in permanent tracer cattle under commercial farming conditions, were very low in terms of the actual yield of larvae from the numbers of eggs deposited in the faeces, never exceeding one per cent. Survival of the larval stages was closely linked to that of the intact faecal pat and mortality occurred rapidly once the faeces disintegrated.

By contrast, survival of O. ostertagi in the temperate climate of West Scotland was not so closely linked to survival of the intact faecal pat and infective larval stages were capable of surviving in considerable numbers for several months

beyond pat disintegration. Some of these larvae survived on the herbage and others in the soil, possibly associated with terrestrial transport hosts such as earthworms. Yields of O. ostertagi larvae from eggs deposited were also low, but not as markedly so as those of H. contortus and in two months of the year, namely, June and July, the percentage yield exceeded ten per cent.

The ecology of another Ostertagia species, O. leptospicularis was studied for the first time. Generally, the results were similar to those of O. ostertagi and yields of infective larvae from eggs were again low except for the months of June and July. A major difference occurred in the ability of the free-living stages of the two species to survive the winter with only relatively low numbers of O. leptospicularis being present in the following spring.

Confirmation that O. leptospicularis and O. ostertagi had similar requirements of temperature for their development but differed in those required for survival were obtained under laboratory experimental conditions. When inocula containing ten per cent of O. leptospicularis and 90 per cent of O. ostertagi were administered to parasite-naive calves and passaged five more times through other calves the more fecund O. leptospicularis become the dominant species present in the calves, reaching a level in excess of 80 per cent in the sixth calf. When the larval progeny of the Ostertagia worm burdens in that calf were stored at 4°C for a period of several months to simulate winter conditions, the O. leptospicularis larvae succumbed completely. It was proposed that the lack of fitness

for survival at cold temperatures of O. leptospicularis explained why this very pathogenic species was only involved sporadically in outbreaks of bovine ostertagiasis. Where such outbreaks occurred the source of infection is likely to result from deer, the natural host of this parasite, having grazed and contaminated the fields prior to susceptible cattle.

The abomasal worm burdens of the permanent tracer calves examined in Paraguay were a mixture of species, namely H. contortus, H. similis and T. axei. There was some evidence that the proportions of these present altered seasonally and gave rise to clinical outbreaks of parasitic gastritis in the late winter and spring. The T. axei burdens appeared to make a major contribution to these outbreaks and although several thousand Haemonchus spp. were also present the cattle were only moderately anaemic. While the seasonal nature of the worm burdens may reflect the differing ecological requirements of the free-living stages there was some indication that the interactions of the one species on the other, possibly involving cross immunity, may also have been involved. In view of the interesting results obtained with the two Ostertagia species, experimental studies are recommended to evaluate the interactions of the two Haemonchus spp. and T. axei.

Since recognition of larval stages at species level is a pre-requisite to such a programme a computer analysis comparing data on larval morphology and measurements from O. ostertagi and O. leptospicularis as a model was undertaken. Statistically different results were obtained which were unfortunately

difficult to recognise visually.

CONTENTS

SUMMARY

A LITERATURE REVIEW OF THE GASTROINTESTINAL NEMATODES PRESENT IN CATTLE IN SOME TEMPERATE, SUBTROPICAL TROPICAL ZONES OF THE WORLD 1

INTRODUCTION 2

Temperate zone 3

Subtropical and tropical zones 15

Subtropical 16

Tropical 19

Summary 27

MATERIALS AND METHODS 29

EXPERIMENTAL ANIMALS 29

PARASITOLOGICAL TECHNIQUES 29

Faeces 29

Experimental plots 31

Worm burdens 31

Herbage 33

Larval measurement 34

Statistical methods 35

Soil 35

INTRODUCTION TO THE EXPERIMENTAL STUDIES 36

HAEMONCHUS SECTIONS (A & B) 39

SECTION A 39

A STUDY ON THE ECOLOGY OF THE FREE-LIVING STAGES OF HAEMONCHUS CONTORTUS IN PARAGUAY 39

INTRODUCTION 40

The genus Haemonchus 40

Life cycle of Haemonchus contortus 41

The development of eggs to infective third stage larvae 42

The longevity of infective larvae on pasture 45

The availability of infective larvae 46

EXPERIMENTAL DESIGN 48

MATERIALS AND METHODS 48

Animals 48

Preparation of experimental grass plots 48

Faecal pats 49

Climatic observation 49

Parasitological data 49

RESULTS 50

Climatic observations 50

Parasitological data 50

Faecal pats 50

Recovery of Haemonchus contortus eggs and larvae from faeces 51

Migration and survival of infective larvae on grass 51

DISCUSSION 53

FIGURES AND TABLES

SECTION B 58

A STUDY OF ABOMASAL WORM BURDENS IN TWO BEEF CATTLE HERDS IN PARAGUAY 58

INTRODUCTION 59

EXPERIMENTAL DESIGN 61

Location of study 61

MATERIALS AND METHODS 63

Animals 63

Clinical 64

Metereological data 64

Parasitological data 64

Post-mortem examination 65

RESULTS 65

Clinical data 65

Metereological data 66

Faecal egg counts 67

Post-mortem worm burdens 67

Ratio of *Haemonchus contortus* and *Haemonchus similis* 69

DISCUSSION 69

FIGURES AND TABLES

OSTERTAGIA SECTIONS (C, D, E & F) 80

GENERAL INTRODUCTION 81

The genus *Ostertagia* 81

SECTION C 84

THE DIFFERENTIATION OF OSTERTAGIA OSTERTAGI AND OSTERTAGIA LEPTOSPICULARIS FREE-LIVING LARVAL STAGES 84

INTRODUCTION 85

MATERIALS AND METHODS 85

Animals 85

Original of larvae 85

Culture of larvae 86

Larval measurement 86

Statistics 87

RESULTS 87

DISCUSSION 89

FIGURES AND TABLES

SECTION D 92

THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF FREE-LIVING STAGES OF OSTERTAGIA OSTERTAGI AND OSTERTAGIA LEPTOSPICULARIS UNDER LABORATORY CONDITIONS 92

INTRODUCTION 93

EXPERIMENTAL DESIGN 94

MATERIALS AND METHODS 94

Animals 94

Origin of Larvae 94

RESULTS 95

DISCUSSION 96

FIGURES AND TABLES

SECTION E 98

A STUDY ON THE ECOLOGY OF THE FREE-LIVING STAGES OF OSTERTAGIA OSTERTAGI AND OSTERTAGIA LEPTOSPICULARIS IN WEST SCOTLAND 98

INTRODUCTION 99

Development and survival of larvae in the faecal pat 99

Migration from the faecal pat 104

Migration through soil 105

EXPERIMENTAL DESIGN 106

MATERIALS AND METHODS 106

Animals 106

Original of larvae 106

Experimental grass plots 106

Parasitological 107

Sample collection 107

RESULTS 108

Faeces deposited in spring 109

Faeces deposited in summer (June-August) 111

Faeces deposited in autumn (September-November) 113

Faeces deposited in winter (December-March) 115

DISCUSSION 116

FIGURES AND TABLES

SECTION F 123

THE EFFECT OF SERIAL EXPERIMENTAL PASSAGE IN CALVES ON THE RELATIVE PROPORTIONS OF OSTERTAGIA OSTERTAGI AND OSTERTAGIA LEPTOSPICULARIS IN A POPULATION OF MIXED OSTERTAGIA SPP. 123

INTRODUCTION 124

EXPERIMENTAL DESIGN 126

MATERIALS AND METHODS 126

Animals 126

Clinical 127

Parasitological observations 127

Larval inocula 127

Origin of larvae 127

Post-mortem 127

RESULTS 128

Worm burdens 128

DISCUSSION 130

FIGURES AND TABLES

GENERAL DISCUSSION 135

REFERENCES 146

LIST OF PLATES

Plate 1 Faecal collection system showing harness and bag.

Plate 2 Outdoor ecology plots.

Plate 3 Computer/digitiser system for larval measurement.

Plate 4 Male bursae of *H. contortus* and *H. similis*.

Plate 5 Vulvar region of *Haemonchus* spp female (ex Pirity) showing eggs.

Plate6 Sheath prolongations of *O. ostertagi* and *O. leptospicularis* third stage larvae.

Plate 7 Male bursae of *O. ostertagi*, *O. leptospicularis*, *S. lyrata* and *S. kolchida*.

LIST OF TABLES

Table A1 Mean fortnightly maximum and minimum temperatures (^{o}C) plus rainfall data at Asuncion.

Table A2 Survival (in weeks) and weight of cattle faeces (g) deposited on plots.

Table A3 Total number of Haemonchus contortus eggs in faecal pats (computed by weight of faeces x eggs per g).

Table A4 Infective larvae of Haemonchus contortus on grass plots (L_3/kdh) after deposition of cattle faeces.

Table A5 Weight of grass (g) collected at fortnightly sampling after deposition of faeces.

Table A6 Total number of Haemonchus contortus L_3 recovered from grass samples collected at a radius of 0.25 cms around faeces.

Table A7 Haemonchus contortus infective larvae on herbage expressed as a percentage of eggs deposited in the faeces.

Table B1 Necropsy.

Table B2 Mean Haemonchus contortus faecal egg counts at Pirity.

Table B3 Mean Haemonchus contortus faecal egg counts at Barrerito.

Table B4 a-f Abomasal worm burdens from cattle at Pirity.

Table B5 a-f Abomasal worm burdens from cattle at Barrerito.

Table B6 Ratio of Haemonchus contortus : H. similis at Pirity.

Table B7 Ratio of *Haemonchus contortus* : *H. similis* at Barrerito.

Table C1 Summary of oesophageal measurements lengths (microns) from 10 *Ostertagia ostertagi* and *O. leptospicularis* 1st/2nd stage larvae on days 2-9 and results of analysis of variance.

Table C2 Summary of intestinal measurements lengths (microns) from *Ostertagia ostertagi* and *O. leptospicularis* 1st/2nd stage larvae on days 1-9 and results of analysis of variance.

Table C3 Summary of anus to tail measurements lengths (microns) from *Ostertagia ostertagi* and *O. leptospicularis* 1st/2nd stage larvae on days 2-9 and results of analysis of variance.

Table C4 Summary of measurements lengths (microns) from 100 *Ostertagia ostertagi* and *O. leptospicularis* 3rd stage larvae.

Table D1 Percentage recovery of larvae from daily faecal cultures at 15°C.

Table D2 Percentage recovery of larvae from daily faecal cultures at 21°.

Table E1 Mean fortnightly maximum and minimum temperatures (°C) plus rainfall at Glasgow.

Table E2 Survival (in weeks) and weight of cattle faeces (g) deposited on *Ostertagia ostertagi* plots.

Table E3 Survival (in weeks) and weight of cattle faeces (g) deposited in *Ostertagia leptospicularis* plots.

Table E4 Weight of grass (g) collected from Ostertagia ostertagi plots (in weeks).

Table E5 Weight of grass (g) collected from Ostertagia leptospicularis plots (in weeks).

Table E6 Percentage of eggs, embryonated eggs, $L_1 + L_2$ and L_3 of Ostertagia ostertagi surviving in faecal pats (in weeks).

Table E7 Percentage of eggs, embryonated eggs, $L_1 + L_2$ and L_3 of Ostertagia leptospicularis surviving in faecal pats (in weeks).

Table E8 Infective larvae of Ostertagia ostertagi on grass plots (L_3/kdh) after deposition of cattle faeces.

Table E9 Infective larvae of Ostertagia leptospicularis on grass plots (L_3/kdh) after deposition of cattle faeces (in weeks).

Table E10 Total number of Ostertagia ostertagi L_3 recovered from grass samples collected at a radius of 0.25 cms around faeces (in weeks).

Table E11 Total number of Ostertagia leptospicularis L_3 recovered from grass samples collected at a radius of 0.25 cms around faeces (in weeks).

Table E12 Ostertagia ostertagi infective larvae on herbage expressed as a percentage of eggs deposited in the faeces (in weeks).

Table E13 Ostertagia leptospicularis infective larvae on herbage expressed as a percentage of eggs deposited in the faeces (in weeks).

Table E14 Numbers of Ostertagia ostertagi L_3 recovered from replicate soil cores (in weeks).

Table E15 Numbers of Ostertagia leptospicularis L_3 recovered from replicate soil cores (in weeks).

Table E16 The mean percentage of Ostertagia spp deposited from June through to September which become L_3 and the period of their survival.

Table F1 Experimental design.

Table F2 Experimental design.

Table F3 Total Ostertagia worm burdens at necropsy 24 days post-infection.

Table F4 Percentage of Ostertagia type species establishment at necropsy (100 males examined).

LIST OF FIGURES

Figure A1 *Haemonchus* ecology experiment at Asuncion: Climatic data, numbers of eggs in faeces, L_3 kdh.

Figure A2 Bioclimatograph for *Haemonchus contortus* based on climatic data at Asuncion 1979/80.

Figure B1 Climatic data plus *H. contortus* and *T. axei* worm burdens from calves at Pirity.

Figure B2 Climatic data pluc *H. contortus* and *T. axei* worm burdens from calves at Barrerito.

Figure B3 *H. contortus* L_4 burdens from calves at Pirity and Barrerito.

Figure C1 Frequency distributions of oesophageal and sheath prolongation lengths of *O. ostertagi* and *O. leptospicularis* third stage larvae.

Figure D1 Percentage larval recoveries of *O. ostertagi* and *O. leptospicularis* cultured at 15°C.

Figure D2 Percentage larval recoveries of *O. ostertagi* and *O. leptospicularis* cultured at 21°C.

Figure E1 *Ostertagia* ecology experiment at Glasgow: Climatic data plus *O. ostertagi* and *O. leptospicularis* L_3/kdh following summer contamination of plots.

A LITERATURE REVIEW OF THE GASTROINTESTINAL NEMATODES PRESENT IN CATTLE IN SOME TEMPERATE, SUBTROPICAL AND TROPICAL ZONES OF THE WORLD

INTRODUCTION

Parasitic gastroenteritis (PGE) in cattle is a worldwide problem which in the last few decades has received an increasing amount of scientific attention. This is largely due to a growing awareness of the serious economic losses which can result from even moderate burdens of gastrointestinal parasites.

PGE is a disease seen mainly in young animals during their first year of life, although animals up to 2 years may be affected. Clinical symptons of PGE are rare among adult stock and generally only appear if the herbage larval infestation is high and/or the level of nutrition poor.

The most important nematodes associated with PGE in cattle are:

(a) from the abomasum: Haemonchus contortus, H. placei, H. similis,; Ostertagia species including: O.ostertagi, O. leptospicularis, Skrjabinagia (Ostertagia) lyrata, S. kolchida and Trichostrongylus axei.

(b) from the small intestine: T. vitrinus, T. colubriformis, Cooperia punctata, C pectinata, C. oncophora; Nematodirus helvetianus, Bunostomum phlebotomum, Toxocara vitulorum and Strongyloides papillosus.

(c)from the large intestine: Oesophagostomum radiatum.

It is generally considered that the abomasal parasites are more pathogenic than those from the intestine, but since most infestations comprise a combination of genera at any one time, their individual, as opposed to collective pathogenic effect on the host can be difficult to assess.

The basic life cycles of most genera causing PGE in cattle are similar, being direct and consisting of external and internal phases of development. Thus, by its nature the successful completion of such a life cycle is very largely dependent on climate. It follows that the parasite fauna in similar geographical regions will be alike.

Three distinct macro-climate types are recognised. These are: Temperate, subtropical and tropical and the distribution and prevalence of gastrointestinal nematodes within examples of each is discussed below.

Temperate Zone

A temperate climate generally has a mild moist spring, followed by a warmer drier summer period, with a wet autumn which precedes the onset of the coldest part of the annual cycle, i.e. winter.

In areas of the Northern Hemisphere with a temperate climate the normal grazing period for animals is from late April to October i.e. spring through autumn, the animals being housed during the winter; by contrast in the Southern Hemisphere cattle are usually grazed all year round. Throughout the temperate climatic zones O. ostertagi, T. axei and C. oncophora are the main species contributing to PGE among cattle. While these three species share a number of features which account for their similar distribution O. ostertagi is undoubtedly the most important as can be seen from the large numbers of disease outbreaks reported from all temperate regions of the world, which are attributed to its presence in high numbers, (Armour 1970; Brunsdon and Adam 1975; Herd 1980).

As the seasonal incidence of parasitic nematodes is closely related to the ecology of their free-living stages, climate plays an important role particularly in the development and survival of these external stages, (Levine 1963). During unfavourable conditions larval development may not take place, can be delayed for several weeks, or the larvae which develop may not survive for long. When favourable, development can be rapid and survival for many months is possible, Rose (1961), Williams and Bilkovich (1971, 1973) Gibson and Everett (1976), Young and Anderson (1981). When lower temperatures prevail, egg hatching and larval development may cease, although the survival of larvae already present on herbage or in faecal pats may be enhanced, (Levine and Andersen, 1973). Once ingested, the establishment of a parasite is affected by such factors as the host's age (Herlich, 1960; Viljoen, 1969; Gordon, 1973), sex (Dobson, 1964; Bawden, 1969b), nutritional status (Bawden, 1969a), state of resistance (Donald, Dineen, Turner and Wagland, 1964), presence of other parasites in the preferred site (Reinecke, 1974) or the host specificity of the parasite (Southcott and Barger, 1975).

A rapid development within the host coupled with a rapid development on pasture leads to a short generation interval (Donald and Waller, 1973) whereas delayed development on pasture or arrested development in the host may result in only a single generation per annum (Connan 1971; Waller and Thomas, 1975).

The seasonal availability to grazing calves of infective larvae has been studied by several workers in different parts of the world. Thus, in the Northern Hemisphere, observations made

by Anderson, Armour, Jennings, Ritchie and Urquhart (1969) have shown that over a 12 month period in the West of Scotland, the populations of O. ostertagi and C. oncophora in calves grazed from May to October were highest in September and October, that is at the end of the summer grazing period.

In England, Michel (1967) and Rose (1962) found that eggs of O. ostertagi and C. oncophora deposited on the herbage in April, May and June appeared as infective third stage larvae in mid-July; thereafter the period taken for eggs to hatch and develop to third stage larvae (L_3) lengthened and by September little or no development took place. This led them to the conclusion that, at least in Britain, only one generation of O. ostertagi per annum occurred with a second generation possible only in some of the warmer areas. Some dissension with the general concept of a limited number of annual parasitic generations was shown by LeJambre and Ratcliffe (1971) who claimed that several generations of H. contortus occur annually in the State of New York, USA.

Similar epidemiological studies have been carried out in Europe by Burger, Eckert, Wetzel and Michel (1966) in West Germany, in The Netherlands by Kloosterman (1971), in Poland by Malczewski (1970), in France by Raynaud, Laudren and Jolivet (1971), in Sweden by Nilsson and Sorelius (1973), in Denmark by Henriksen, Jørgensen, Nansen Sejrsen and Klausen (1975), in Switzerland by Eisenegger and Eckert (1975) and in Norway by Tharaldsen (1976).

Studies carried out in North America by Malczewski, Westcott, Spratling and Gorham (1975), Yazwinski and Gibbs

(1975), Williams and Knox (1976), in Canada by Smith (1974) and in winter rainfall areas of Australia by Anderson (1971) and Smeal, Robinson and Fraser (1980) have all demonstrated the seasonability of infection with only a limited number of annual cycles.

In Britain, outbreaks of parasitic gastroenteritis have been described by Martin, Thomas and Urquhart (1957), Reid, Armour, Jennings, Kirkpatrick and Urquhart (1967), Taylor and Cawthorne (1972), Taylor, Cawthorne, Kenny and Regan (1973), Cawley and Lewis (1976), Selman, Reid, Armour and Jennings (1976). Detailed field studies of suspected outbreaks of PGE dominated by O. ostertagi were carried out in South West Scotland and Northern England by Anderson, Armour, Jarrett, Jennings, Ritchie and Urquhart (1965b). From a detailed history of each outbreak, clinically affected and in some cases non-affected cattle were obtained for post mortem examinations. From the results of this study it was apparent that the clinical disease occurred in two main forms. The first, named Type I ostertagiasis, was seen in young cattle during the summer principally between July and October, and the other, Type II, occurred in late winter or spring i.e. March to May, usually in cattle housed after their first grazing season. Working in France, Raynaud (1978) classified ostertagiasis in a similar manner i.e. Type I in calves during their first year at pasture, and Type II in animals after their first year at grass. He also described a variant of Type II which he called "oedematous ostertagiasis" in young animals after their first or second

winter i.e. where abomasal folds and sometimes the submucosa, are noticeably oedematous, being filled with a watery fluid. Nodules were found on the mucosal surface.

Since this original description of two distinct clinical types of ostertagiasis, field and laboratory observations have been carried out on the epidemiology of the two disease forms in Britain by Michel (1966), Anderson, Armour, Jennings, Ritchie and Urquhart (1965-1969), Armour, Jennings and Urquhart (1969), Michel (1969), Michel, Lancaster and Hong (1970, 1972, 1976), Chiejina (1978), Pott, Jones and Cornwell (1978), Armour, Bairden, Duncan, Jennings and Parkins (1979), Bairden, Parkins, Armour (1979), Armour, Al Saqur, Bairden, Duncan and Urquhart (1980).

The epidemiological patterns of PGE in Western Europe are essentially similar to those in Britain with very little variation in the incidence of species. Norwegian studies have shown that heavy clinical outbreaks of parasitism occur in young calves in their first grazing period as a result of overwintered larvae of O. ostertagi and C. oncophora with N. helvetianus also being present. Helle (1973). In Sweden, O. ostertagi and C. oncophora are the only gastrointestinal nematodes of economic importance in cattle. These two species are distributed all over the country and they almost always occur together in mixed infections. Nilsson (1973) Olsson (1977) and Nilsson (1981) reported ostertagiasis due to overwintered larvae. Infections with trichostrongylids are prevalent in the Danish cattle population, the most frequently occurring species being O. ostertagi and C. oncophora, but H. contortus, T. axei,

N. helvetianus and Oe. radiatum can also be found (Jørgensen, Nansen, Henriksen, 1973).

In The Netherlands, Ostertagia spp are the most numerous trichostrongylids accompanied by the less important T. axei. Cooperia spp generally occur in small numbers while Haemonchus, Bunostomum and Oesophagostomum spp are seldom seen (Swierstra, 1973). Borgsteede and Van der Burg (1981) reported O. ostertagi (99.2%) and T. axei (75%) in the abomasa of dairy cows. In 1981, the same authors, on the basis of faecal egg counts and cultures from adult dairy cows, reported O. ostertagi as the most prevalent species (87.9%) with other genera being present in lesser numbers - H. contortus (7.7%), Trichostrongylus spp. (47.7%), Bunostomum spp (13.1%), Oesophagostomum spp (9.4%), C. oncophora (8.7%), C. punctata (30.5%).

Trichostrongylid infections are very common in cattle in Switzerland. Severe outbreaks of ostertagiasis were observed in calves near Zurich by Eckert (1973) and when helminth-free tracer calves grazed the same pastures nine species of parasites were recovered at post mortem, but O. ostertagi, C. oncophora and N. helvetianus were the most prevalent (Eisenegger and Eckert, 1975). The same parasites were mentioned by Eckert, Perl and Inderbitzin (1981).

In France, parasitic gastroenteritis is widespread in Normandy and the wet pastures of the central and eastern parts of the country, Euzeby (1973). The main parasites concerned are: O. ostertagi and C. oncophora in cattle (Raynaud, Nage, Le Stand and Jones, 1981). Outbreaks of bovine ostertagiasis have been

reported by Raynaud and Bouchet (1976). In West Germany, O. ostertagi, Trichostrongylus spp and Cooperia spp are the predominant species in cattle as reported by Burger (1973), Bernhard, Barth and Lamina (1978) and Barth, Bernhard and Lamina (1981). Other species include O. lyrata, O. leptospicularis, T. axei, Haemonchus spp and Oesophagostomum radiatum.

Although PGE has little significance in the cattle population in Hungary, parasites such as Ostertagia, Haemonchus, Trichostrongylus, Nematodirus and Cooperia can be found, yet outbreaks of the disease seldom occur (Varga, 1973).

Patýk (1960) in Poland examined about 78 cattle in an abattoir at Wroclaw and reported the following species : Oe. radiatum, C. punctata, B. phlebotomum, Ostertagia lyrata and T. discolor. The most prevalent were O. ostertagi, C. oncophora and H. contortus. Later, Malczewski (1970) found that O. ostertagi, T. axei and H. contortus were the most important parasites of cattle.

Pecheur and Pouplard (1974) in Belgium in a survey made on fifty calves from four areas in the south of Belgium and examined at an abbatoir, reported O. ostertagi, C. oncophora and N. helvetianus as the dominant species, these findings being similar to those in other European countries.

In a survey of gastrointestinal parasites made in Austria by Hinaidy, Gutierres and Supperer (1972) in which fifty-five gastrointestinal tracts were examined the following species were recovered: O. ostertagi (80%), T. axei (66%), O. leptospicularis (20%), S. kolchida (18%), H. contortus (14%), S. lyrata (10%),

Spiculoteragia bohmi (4.1%), Rinadia matthevossiani (2%), Trichostrongylus longispicularis (2%), C. oncophora (4%), C. surnabada (18.1%), C. punctata (12.7%), N. helvetianus (10.9%), B. phlebotomum (7.2%), Oe. radiatum (29%), Oe. venulosum (18.1%), Chabertia ovina (10%) and Trichuris discolor (1.8%). S. bohmi and R. mathevossiani are reported as new records from cattle. Gutierres (1972) described the same parasites from bovines.

In Italy, Gallo (1962) reported outbreaks of trichostrongylosis causing mortality among young cattle in South Eastern Sicily. O. ostertagi was mainly responsible, but T. axei was also present. Balbo (1973) recorded O. ostertagi (81%), H. placei (61%), T. axei (25%), C. oncophora (27%) and Oe. radiatum (41%). at necropsy of 44 cattle. Genchi, Preti and Celano (1979) also found Ostertagia spp, Haemonchus spp and Trichostrongylus spp at necropsy. Genchi and Forner (1980) reported O. ostertagi (91.30%), O. lyrata (2.18%) C. punctata (6.52%), in the abomasa of 45 cattle. Vigliani (1981) described Ostertagia spp, Trichostrongylusspp, Haemonchus spp, Cooperia spp and Oesophagostomum spp as the most frequent parasites of calves.

In Spain, Valle Suarez, Rojo Vasquez, Diez Banios (1979) reported the following species in decreasing order of frequency: O. ostertagi, C. oncophora, N. helvetianus, O. lyrata, T. axei, H. contortus, O. trifurcata, T. vitrinus and C. punctata at necropsy of 61 cattle in Leon, Spain.

In Canada, McGregor and Kingscote (1957) studying the incidence of parasitism in an Ontario herd found that animals

under one year were the most susceptible with 64.5% having helminth infestations, followed by 35.9% for animals of one to two years of age, while only 16% of the adult cattle were infected. Smith and Archibald (1968) studying the effect of age in the develoment of gastrointestinal parasitism pointed out that animals which had little or no exposure to parasitism are much more susceptible to the effects of parasites than those with a previous exposure, irrespective of age. Later, Smith (1970) compared the development of O. ostertagi, C. oncophora and N. helvetianus in 15 month old parasite-naive yearlings and parasite naive three month old calves and demonstrated that yearlings have considerably greater resistance to gastro-intestinal parasitism than calves. Similar studies were carried out by Frechette and Gibbs (1971) in Quebec and showed that fewer adults than yearlings and fewer yearlings than calves were infected, the percentage of the three groups infected being respectively, 14%, 21% and 32%. From faecal cultures it appears that O. ostertagi was the most prevalent species (50%) followed by Cooperia spp (32%) and Bunostomum spp (11%). Smith and Perreault (1972) recorded an outbreak of ostertagiasis in yearlings and adult cattle in New Brunswick, Slocombe (1974) in Ontario, Canada, showed that 56, 20 and 17 animals respectively contained Ostertagia, Haemonchus and Trichostrongylus spp and 30 were negative for all nematodes. The predominant nematode was O. ostertagi, but other species present included O. trifurcata, S. lyrata and O. circumcincta.

While ostertagiasis is widely known as a common disease

entity in cattle throughout North America and is very likely a cause of major production deficiency due to subclinical parasitism, it is most common in the south central and south eastern parts of the USA, particularly in those states bordering the Gulf of Mexico (Williams, 1983) and this is reported in the subtropical section. Clinical ostertagiasis has been reported in the temperate areas by Bailey and Herlich (1953) in Georgia, Becklund (1962), Worley and Sharman (1966) in Montana and Herd (1980) in Ohio. A number of studies have been made on the incidence and epidemiology of gastrointestinal nematodes of cattle in the Northern U.S.A. Two sources of information were taken into consideration: (a) worm counts at necropsy and (b) serial examination of faecal egg counts and identification of cultured larvae from faeces.

Thus, by means of worm counts and parasite identification at necropsy, Malczewski, Westcott, Spratling and Gorham (1975) found that Ostertagia spp was the most common nematode in Washington State; Randall and Gibbs, (1977) working in Maine, found an incidence of 85.4% for Ostertagia spp (O. circumcincta, O. lyrata, O. ostertagi) and 81.2% for Cooperia spp (C. mcmasteri, C. oncophora, C. punctata, C. pectinata) in nematodes recovered at necropsy of 48 animals. Other species present included: H. placei, B. phlebotomum, T. axei and N. helvetianus.

In their survey of Wisconsin dairy calves, Gutierres, Todd and Crowley (1979) recorded Ostertagia spp (O. ostertagi and O. lyrata) as the most prevalent genera (80%), Cooperia spp (C. oncophora, C. punctata, C. mcmasteri) and Trichostrongylus

spp (T. axei, T. colubriformis and T. longispicularis) were found in 46.6%, H. placei in 42% and Oe. radiatum in 28.8% of calves. By means of faecal egg counts and identification of infective larvae cultured from faeces of calves, Leland, Caley and Ridley (1973) in Kansas, found an incidence of 73% Cooperia, 14% Trichostrongylus spp and 3% Haemonchus with Oesophagostomum and Nematodirus spp being present in less than 1%. Yazwinski and Gibbs (1975) working in Maine, examined the faeces of 250 animals and found that 97% were positive for trichostrongylid eggs (Ostertagia, Haemonchus, Cooperia, Trichostrongylus). Cox and Todd (1962) recorded a 78% incidence of parasitism in Wisconsin dairy herds and Zimmerman and Hubbard (1961) an incidence of 50% in Iowa herds.

In New Zealand, Brunsdon (1964) studied the incidence of gastrointestinal nematodes in cattle and found that the most important parasites were Ostertagia spp, T. axei and Cooperia spp. Similar findings in dairy calves were reported by Brunsdon (1968; 1969; 1972) who concluded that O. ostertagi was the most important parasite. Outbreaks of ostertagiasis have been reported in animals of all ages and from several parts of the country by Wedderburn (1970), Bisset (1980) and Chalmers(1980).

In Southern Australia, throughout the temperate climatic zone, O. ostertagi, T. axei and C. oncophora are the main species contributing to PGE among cattle according to Smeal, Hotson, Mylrea, Jackson, Campbell and Kirton (1977), Bowen (1979), Donald, Alexen, Morley, Waller and Donnelly (1979) and Smeal,

Fraser and Robinson (1980). Of the other cattle nematodes Oe. radiatum and Trichostrongylus spp occurred in low numbers and Oe. venulosum was seen occasionally (Smeal et al, 1977; Anderson, Donald and Waller, 1983). Ostertagia spp are the predominant and most pathogenic parasites of the tablelands of New South Wales according to Hotson (1967), Smeal et al (1977), Bowen (1979), Smeal, Nichols, Webb, Hotson, Dougherty and Harding (1981). Peterson (1957) working in Western Australia, also reported O. ostertagi, and C. oncophora as the most important parasites in the region.

In temperate Argentina, epidemiological studies carried out by Rosa, Lukovic and Niek (1971) on the basis of faecal egg counts and larval cultures, reported O. ostertagi, T. axei, H. placei, T. colubriformis, C. oncophora and other Cooperia spp Strongyloides papillosus and Oesophagostomum spp in calves in the district of Tres Arroyos, Cnel. Pringles and Cnel. Dorrego, Province of Buenos Aires. Using the same method, Rosa, Niek, Lukovic, Vidal - Monje and Hofer (1977) recorded H. placei, H. contortus, O. ostertagi, Ostertagia spp, T. axei, T. colubriformis, C. oncophora, Cooperia spp, Nematodirus spp, B. phlebotomum and Oe. radiatum in calves in Concepcion del Uruguay, Province of Entre Rios.

Entrocasso and Steffan (1980) found Ostertagia spp (57.4%), Trichostrongylus spp (34.2%), Cooperia spp (6.4%), Haemonchus spp (0.8%) and Oesophagostomum and Nematodirus spp. (0.1%) at necropsy of calves in Cnel Pringles, Province of Buenos Aires.

Epidemiological studies were carried out in temperate Chile by Sievers (1978) in which 41 calves were grazed on rotational

pastures; faecal egg counts and pasture larval populations were monitored during the different seasons of the year. In summer (February) the pasture infestation was low at 99 L_3 per square metre (L/m^2), increased abruptly to 9.200 L/m^2 at the beginning of the autumn rains (May) and by the end of autumn (June) a maximum of 59,600 L/m^2 was reached. Numbers then decreased during winter (July-August) to 4,600 L/m^2 and in spring coinciding with grass growth (October) the larval infestation of the pasture had decreased to 170 L/m^2. From this result it was concluded that calves which ingested the autumn infestation were likely to develop PGE.

Subtropical and Tropical Zones

Subtropical and tropical climates are generally characterised by very hot humid summers, a relatively warm wet autumn followed by mild winter conditions. Spring is similar to summer conditions in temperate areas being warm and dry. In the more arid tropics helminth infections are considered less important due to prolonged periods of drought plus the type of vegetation present and the cattle management practised (Barger, Bremmer and Waller, 1983). Examples of these climates are found in North Western Australia, parts of Southern U.S.A., areas of Africa and South America and, unlike the majority of the temperate areas, cattle in the tropics graze throughout the year.

The prevalence of helminth parasitism in cattle in the more humid tropical and subtropical areas is largely determined by rainfall. The prevailing temperatures of these regions are generally high enough to permit hatching and development of eggs

to infective larvae all the year round, although development may be temporarily curtailed under very hot or very cold conditions (Durie 1961, Roberts, O'Sullivan and Riek 1952, Bryan, 1980). Larvae can develop to the infective stage in one week in summer or in two to three weeks in winter. Fortunately for the animals, this rapid and continuous rate of development is balanced by a high mortality rate so that few larvae survive on pasture for more than eight weeks. Haemonchus spp, Cooperia spp and Oesophagostomum spp are the main genera responsible for helminth disease in these areas.

Subtropical

In Alabama, U.S.A., Porter (1942) examining the gastrointestinal tracts of 84 cattle reported an incidence of 91% for C. punctata, 83% for H. contortus and 74% for O. ostertagi, 62% for B. phlebotomum, 59% for Oe. radiatum, 48% for H. similis, 47% for T. axei, 44% for Trichuris spp (T. ovis and T. discolor), 32% for C. pectinata, 21% for S. papillosus and 15% for N. helvetianus. Becklund (1959) reported Haemonchus spp in 82%, C. punctata in 82%, T. axei in 72%, O. ostertagi in 72%, Oe. radiatum 69%, C. pectinata 52%, H. similis 41%, Setaria cervi 34%, T. colubriformis 7% and C. spatulata 7% at necropsy of cattle in Georgia. Becklund (1961) later recorded H. contortus, O. ostertagi, T. axei, C. pectinata , C. punctata and Oe. radiatum from calves in Florida.

In South Africa, Reinecke (1960) in a survey based on faecal egg counts of calves in the North Western Cape, a semi arid region, reported H. placei, C. pectinata, B. phlebotomum and Oe. radiatum as the most common parasites and Hobbs (1961)

working in the Natal Coastal area found the same range of parasites, including Trichostrongylus spp in late winter.

A survey in which tracer calves were slaughtered at regular intervals in order to ascertain the incidence of parasitism during different months of the year was carried out by Horak and Louw (1978) on the Transvaal Highveld. In their experiment in which the calves were grazed for two months on an irrigated pasture and then slaughtered, Haemonchus spp were the most abundant nematodes, the largest number being recovered from May to August, Trichostrongylus spp (T. axei and T. colubriformis) were recovered in low numbers, while the percentage of Cooperia spp (C. punctata and C. pectinata) increased from February to June; O. circumcincta, N. spathiger and B. phlebotomum were also recovered. Shroder (1979), also using tracer calves found that Cooperia was the predominant species with a peak infestation in November, H. placei, B. phlebotomum and Oe. radiatum were also present, the worm burdens being higher during the rainy season in the Northern Transvaal Highveld.

Epidemiological studies carried out in Salta, North Argentina, which has a subtropical climate with summer rainfall and a single dry period in the winter, by Le Riche, Kunhe and Dwinger (1982) by means of worm counts at post mortem showed a prevalence of Oesophagostomum (90%), Haemonchus spp (89%), Cooperia spp (74%), T. axei (67%), Ostertagia spp (50%), Trichuris spp (43%), Dictyocaulus spp (34%), and Bunostomum spp (14%) in calves. Bustos and Herrera (1973) also working in Salta in dairy herds discovered a prevalence of Haemonchus spp,

Ostertagia spp and Cooperia spp in calves based on faecal egg counts and larval culture. Also using faecal egg counts and culture techniques Rosa, Niec, Lukovic, Dindart and Barberan (1973) found a prevalence of Cooperia spp in cattle followed by Oesophagostomum spp, Haemonchus spp, Ostertagia spp and Trichostrongylus spp in Corrientes.

In another survey in Corrientes, in which 4843 faecal samples pooled by age, sex and race were examined, Lombardero, Moriena and Schiffo (1976) reported the following: Calves of six to twelve months of age were most frequently parasitised (84%), followed by three-year old bulls (62.8%), steers (49.7%) heifers (42%). There was no appreciable difference between Zebu and European crossbred animals. Larval cultures were correlated with the host type and monthly changes in climate. Haemonchus spp (35%) were the most common, occurring throughout the year, followed by Cooperia spp (20%), and Ostertagia spp (13.1%) the latter showing a marked decrease during winter. Trichostrongylus, Bunostomum and Oesophagostomum spp rarely appear above 12%.

In Paraguay, Allen, Ruiz, Massi and Vergara, (1965) recorded Haemonchus spp, B. phlebotomum and Oe. radiatum as the most frequently occurring species in cattle and mention Trichostrongylus spp, Ostertagia spp and Cooperia spp as also being present at necropsy.

In Brazil in the subtropical area, several surveys have been carried out in different states. Thus, in Guaira, Sao Paulo, by means of worm counts and parasite identification at necropsy, Nogueira, Costa, Machado and Kasai (1976) listed the

following parasites in order of prevalence. C. punctata, C. pectinata, H. similis, H. contortus, T. axei, Oe. radiatum and B. phlebotomum. In Jabotical, Sao Paulo, Costa, Campos, Kasai, Paulillo and Costa (1978) found C. punctata, C. pectinata, H. contortus, H. similis, T. axei, B. phlebotomum, Oe. radiatum, S. papillosus, T. discolor, the first five species being predominant. H. contortus, H. similis, T. axei, B. phlebotomum, C. punctata, C. pectinata, Oe. radiatum and T. discolor were seen by Costa, Noguiera and Costa (1978) in calves born in Guaira, Sao Paulo. While in Barretos, Sao Paulo, Machado, Starke, Girio, Samara and Berigo (1979) reported C. punctata (81.4%), C. pectinata (34.2%), H. similis (63.2%), H. contortus (7.9%), T. axei (31.6%), T. colubriformis (18.4%), Oe. radiatum (63.2%), B. phlebotomum (5.3%) in cattle. Using faecal egg counts and larval culture data Fenerich, Groceta, Moraes and Marques (1979) in Vale do Ribeira, Sao Paulo, found that Haemonchus and Trichostrongylus were most abundant in autumn and Cooperia during spring. Other genera present were Strongyloides, Bunostomum, Trichuris, Dictyocaulus and Oesophagostomum.

Tropical

In Australia, in the more tropical areas a marked incidence of rain occurs in summer. In Queensland, seasonal trends of faecal egg counts have been examined by Roberts, O'Sullivan and Riek (1952), Riek, Roberts and O'Sullivan (1953) and Winks (1968). The species of helminth larvae encountered at culture of these faecal samples included H. contortus, T. axei, O. ostertagi, B. phlebotomum, C. punctata, C. pectinata,

Nematodirus spp and Oe. radiatum. The majority of the outbreaks occur during the drier and cooler months of the year, namely July to October (winter - early spring), possibly because the nutritional value of the pasture is much reduced after the summer and autumn rains. Helminthiasis may occasionally occur in late spring or early summer but such outbreaks are comparatively rare and are usually associated with very heavy pasture infestation (Roberts et al (1952). Infections with the temperate zone species, O. ostertagi, T. axei and C. oncophora occasionally reach pathogenic levels in the coolest southeast of Queensland, but are of little significance elsewhere in the region (Anderson, Donald and Waller, 1983). Henderson and Kelly (1978) working in the East Kimberley and Victoria River districts of Northern Australia reported H. placei and Cooperia spp as the most important species in young cattle. Only small numbers of B. phlebotomum, N. spathiger and Oe. radiatum were present in the gastrointestinal tract of weaner and adult cattle which were slaughtered bi-monthly over a two year period.

In a survey carried out by El-Moukdad (1979) in Syria in which 34 abomasa and small intestines were examined for the presence of parasites, he recorded the following nematodes: O. ostertagi (61.8%), C. oncophora (61.8%), C. punctata (47.1%), T. axei (41.2%), C. surnabada (38.2%), B. phlebotomum (20.6%), H. contortus (17.6%), T. vitrinus (11.8%), S. lyrata (5.9%), T. colubriformis, T. longispicularis and Toxocara vitulorum each 2.9%.

Savir, Neuman and Neuman (1964) working in Israel reported the death of 10 beef cattle, the cause of death being attributed to

O. ostertagi and T. axei. In a survey in which the alimentary tract of 100 cattle were examined to establish the incidence of parasitism Eslami and Fakhrzadegan (1972) in Iran found the following parasites: Gongylonema pulchrum, Haemonchus spp (H. contortus or H. placei), O. ostertagi, T. axei, Marshallagia marshalli, C. oncophora, B. phlebotomum, S. papillosus, N. fillicolis, T. ovis, Ch. ovina, Oe. venulosum, Oe. radiatum and Setaria cervi. Haemonchus spp were quite rare, and on the whole, the level of parasitism was low.

In a survey in which egg counts and larval cultures were performed in order to establish the incidence of nematodes in Japan, Kudo, Hatta, Kishi, Ito, Taniguchi and Sano (1974) indicated the presence of C. oncophora, C. punctata, O. ostertagi, Mecistocirrus digitatus, T. axei, Oe. radiatum, N. helvetianus, Ch. ovina, B. phlebotomum and S. papillosus. The first four species were the most common.

Similar studies carried out in Central Taiwan by Wang (1979) revealed H. placei (14%), O. ostertagi (5.9%), T. axei (5.8%), S. papillosus (2.7%), Capillaria bovis (0.6%), B. phlebotomum (0.3%), Trichuris spp (4.9%), Oe. radiatum (8.8%) based on larvae cultured from faeces of 708 dairy cattle.

In tropical areas of Brazil, several studies have been made on the incidence of parasites by means of worm counts at post mortem. Thus, in Minas Gerais, Costa, Freitas and Guimaraes (1970) described Haemonchus spp, Cooperia spp, B. phlebotomum, Oe. radiatum and T.discolor in cattle. Costa, Costa, Guimaraes and Freitas (1971) working in Minas Gerais, reported Cooperia spp

in all of the 77 calves necropsied and more than 75% were infected with Haemonchus spp, B. phlebotomum, Oe. radiatum and T. discolor. Grisi and Nuernberg (1971) found an incidence of B. phlebotomum in 82.4% and C. pectinata in 17.6%, H. similis in 76.1%, H. contortus in 23.9%, Oe. radiatum in 26.1%, B. phlebotomum in 23%, T. discolor in 7.6% and T. axei in 6.1% in 65 cattle carcasses in Matto Grosso. Costa, Freitas, Costa and Guimaraes (1973) recorded a 100% prevalence of Cooperia spp (C. punctata, C. pectinata, C. oncophora) in 59 calves in Calciolandia, Minas Gerais, with Haemonchus spp in 88.13% (H. contortus and H. similis), Oe. radiatum in 79.66%, B. phlebotomum in 79.66%, T. discolor in 83.05%, T. axei in 61.02% and Trichostrongylus spp in 39.66%.

Guimaraes, Freitas, Costa and Costa (1975) necropsied 145 calves in Minas Gerais and reported Cooperia spp and Oe. radiatum in calves aged 10 - 16 months while numbers of Haemonchus spp and Trichostrongylus spp increased up to 10 - 12 months of age and S. papillosus reached a peak at 5 - 6 months. The gradual increase in the numbers of helminths up to 15 - 16 months was mainly due to Cooperia spp and Oe. radiatum. Barboza and Costa (1977) found a prevalence of H. contortus and C. punctata (60%) at necropsy of 20 calves from the state of Ceara, but H. similis, T. axei, C. pectinata and Oe. radiatum were also present. At necropsy of 12 calves, Barboza and Costa (1979) in Fortaleza listed H. contortus (91.66%), C. punctata (66.66%), T. axei (33.33%), Oe. radiatum (25%), H. similis (8.33%) and C. pectinata (8.33%). In eight calves necropsied in Rio Grande do Norte, Maciel and Lima (1980) recorded the following parasites

- H. contortus, H. similis, T. axei, T. colubriformis, C. punctata, C. pectinata, Cooperia spp, S. papillosus, B. phlebotomum, Oe. radiatum, Trichuris spp in Rio Grande do Norte. Oliveira and Oliveira (1982) described a prevalence of C. punctata and Oe. radiatum in 100%, T. ovis in 79%, Haemonchus spp 92.9%, Trichostrongylus spp in 29%, B. phlebotomum in 22% and S. papillosus in 7.2%, at necropsy of 16 calves in Bahia. Duarte, Gomez, Sant'Anna (1982) mentioned the recovery of 71.4% H. contortus, 11.4% T. axei, 22.9% B. phlebotomum, 11.4% C. pectinata, 31.4% S. papillosus , 60% T. discolor, 29% Trichuris spp plus 31.4% Oe. radiatum at autopsy of 35 calves in Cantagalo, Rio de Janeiro. Barboza Cardozo, Nonato Girao and Costa Murta (1979) recorded a 71% incidence of infection of young cattle and 46% of adults with Haemonchus, Cooperia, Bunostomum, Strongyloides and Oesophagostomum by means of faecal egg counts and larval cultures in Fortaleza, Ceara.

Costa, Costa, Silva, Carvalho, Castro and Galesco (1979) found the following helminths in fourteen, 2 - 14 month old calves from Uruana County, Goias, in decreasing order of intensity of infection: C. punctata, H. contortus, H. similis, Oe. radiatum, B. phlebotomum, T. axei, S. papillosus, T. discolor, C. pectinata and T. colubriformis. The authors do not mention how the identification was made.

A list of nematodes which infect cattle was made by Chavez (1953) in Peru. In this, the commonest parasites in cattle include: H. contortus, O. ostertagi, T. axei, N. spathiger, C. punctata, Oe. radiatum, B. phlebotomum and C. oncophora.

In Colombia, Mussman, Rave, Norman, Mullenax and Rincon (1967) discovered Trichostrongylus, Haemonchus, Ostertagia and Cooperia spp to be present in 71%, Bunostomum spp in 21%, Oesophagostomum spp in 20% and Strongyloides spp in 7% of 85 cattle at necropsy.

Vassiliades (1978) made a list of the most important helminths present in cattle in Senegal from samples taken from different abbatoirs at Senegal and Dakar respectively. He mentioned Haemonchus spp, Trichostrongylus spp, Cooperia spp, Oesophagostomum spp, and Strongyloides spp as the most important helminths present in mixed infections in cattle.

Epidemiological studies carried out by Sauvage, Brown, Parkinson, Rossiter and McGovern (1974) in Uganda by means of faecal egg counts and larval identification recorded Haemonchus spp, B. phlebotomum and Oe. radiatum as the most prevalent parasites. They also reported that calves under one year old had higher e.p.g.'s.

Lee, Armour and Ross (1960) in an investigation on the seasonal availability of infective larvae to cattle in the savannah grasslands of North Nigeria found that the highest infestation with Haemonchus spp, Cooperia spp, Oesophagostomum spp and Bunostomum spp occurred during the wet season which extended from May to September, the results being obtained by periodically slaughtering calves and counting and differentiating their worm burdens.

Hart (1964) studied the incidence of nematodes during the dry season in Northern Nigeria by slaughtering groups of four calves, in December, two months after the dry season started and

in March, at the end of the dry season. Heavier worm-burdens of Haemonchus spp, T. axei and Oe. radiatum occurred at the end of the dry season probably due to the maturation of arrested immature stages. Fabiyi, Oluyede and Negedu (1979) reported an outbreak of parasitic gastroenteritis four months after the onset of the dry season; H. placei, C. punctata and C. pectinata being the predominant species present in two of the animals which were necropsied. They assumed that the outbreak must be due to the maturation of inhibited larvae, because infective larvae are usually absent from pasture during the dry season.

Donald (1964) in Fiji, studied the incidence of gastro-intestinal parasitism over a 3 year period by means of faecal egg counts. He recorded the presence of C. punctata, C. pectinata, B. phlebotomum, H. placei, H. similis, Oe. radiatum, Trichostrongylus spp, M. digitatus and Tox. vitulorum and found that protection of calves from infection until they are 6 months of age did not confer any advantage on the acquisition of pathogenic infection, only delayed it. The parasite burdens as estimated by egg counts were more related to the age of the host than to the season.

Epidemiological studies were carried out by Owen and Talbot (1983) in Papua, New Guinea in which 3 or 4 tracer calves were introduced every 6 weeks and allowed to graze for the same period together with permanent weaner calves. After 6 weeks of grazing the tracer calves plus 3 or 4 weaner calves were slaughtered and their worm burdens counted in order to establish the availability and species of helminths present throughout the year. From the

result of this trial the following species of nematodes were identified: H. placei, C. punctata, B. phlebotomum, T. longispicularis, Oe. radiatum, S. papillosus, T. discolor and Capillaria spp. The two most prevalent worm genera throughout the trial period both in tracer and weaner calves were Haemonchus and Cooperia. Highest worm burdens were recovered during the wet season which extended from October to April thus indicating the close relationship between the level of pasture contamination and rainfall. During the drier months larval availability on pasture as indicated by the lower worm counts was relatively low.

In a survey of the incidence of gastrointestinal helminths of cattle in the Phillipines, Dumag (1972) by means of identification of eggs in the faeces of 345 cattle indicated the following percentage of infection: Cooperia spp 32.1, Haemonchus spp 27.9, Oesophagostomum spp 21.7, Trichostrongylus spp 16.9, B. phlebotomum 14.7, M. digitatus 10.6, T. ovis 3.5, S. papillosus 1.8 and Tox. vitulorum 1.3, while Tongson and Caspe (1975) working in two provinces, Oriental Mindoro and Palawan and utilising the same method reported Cooperia as the most common parasite in both provinces (80.5 and 76.5% respectively), Haemonchus was present in 91.1% of cattle in Palawan but only in 22% in Oriental Mindoro. Bunostomum was present in 78.5% at Oriental Mindoro and 18.5% of those in Palawan.

Manuel (1979) in a review of gastrointestinal helminthiasis listed M. digitatus, Haemonchus spp (H. contortus and H. similis), Trichostrongylus spp (T. axei and T. colubriformis), Bunostomum spp (B. phlebotomum and B. trigonocephalum), Cooperia spp (C. pectinata and C. punctata) and Oesophagostom spp

(Oe. colombianum, Oe. radiatum), Tox. vitulorum and S. papillosus as the most common species encountered in the field. Among the abomasal worms, M. digitatus and Haemonchus spp are the most dangerous parasites of cattle in the Phillipines.

Apart from these studies described above, there are a vast number of papers from the Middle East countries and from Asia but the general pattern of species prevalence is the same and clearly dictated by climate and host species present. One could also present data on species occurring in other ruminants such as goats and buffaloes but this would make the review excessively wordy.

Summary

From the above literature search it is clear that the most prevalent gastrointestinal nematodes of cattle in the temperate zones are the Ostertagia group particularly O. ostertagi, with lower numbers of O. leptospicularis, Skrjabinagia lyrata and T. axei all occurring in the abomasum and C. oncophora in the small intestine. Occasionally, N. helvetianus, S. papillosus and Oe. radiatum cause problems.

Clearly, the important parasite species in the sub-tropics and tropics are different from those in the more temperate zones. In the tropics Haemonchus spp is very important. Two Cooperia spp, C. punctata and C. pectinata are also very prevalent but it should be pointed out that these are different and more pathogenic species to C. oncophora which predominates in the temperate areas.

In the sub-tropics Haemonchus spp (H. contortus/H. placei

and H. similis), T. axei and the Ostertagia group are the main problems in the abomasum. In the small intestine two Cooperia spp (C. pectinata and C. punctata) are prevalent. B. phlebotomum in the small intestine and Oe. radiatum in the large intestine are quite common while many young calves harbour Tox. vitulorum.

However, in each zone the abomasal parasites seem to dominate with Ostertagia spp pre-eminent in the temperate zone and parts of the sub-tropics and Haemonchus spp in the tropics with T. axei occupying an intermediate position.

From the above review it is also clear that in many tropical areas a tremendous amount of effort has gone into identifying the species of nematodes present and much less into studying the seasonal prevalence of these infections and the ecology of the free-living larval stages. This is in contrast to the studies in the temperate zones.

MATERIALS AND METHODS

Standard parasitological methods were practiced throughout these studies and are detailed in the following chapter. Where necessary modifications/improvements in technique were introduced and in some instances new procedures designed.

EXPERIMENTAL ANIMALS

Indoor reared, parasite-free male calves were used throughout the experimental procedures. Weaned animals of approximately four months of age and 70 kilograms bodyweight were found to be the optimal size for handling and harnessing. Dairy animals, mainly Ayrshire or Friesian breeds were used.

PARASITOLOGICAL TECHNIQUES

Faeces

Faecal examination to detect trichostrongyle eggs was carried out using the quantitative McMaster flotation technique devised by Gordon and Whitlock (1939).

In the McMaster method three grams of faeces were homogenised with 42 ml of tap water. The resultant suspension was then passed through a 250 micron aperture sieve (Endecotts Test Sieves Ltd., Croydon) which retained the larger particles of debris. After thorough mixing a 15 ml aliquot of the filtrate was centrifuged in a flat bottomed glass tube for two minutes at 2000 rpm. The supernatant which contained the very fine debris was discarded and the remaining faecal pellet broken up by rotary agitation (Whirlmixer, Griffin and George Ltd., Britain). The tube was then filled to its former level with saturated sodium chloride solution, inverted several times and sufficient

of the suspension to fill both chambers quickly transferred to a McMaster slide (Gelman Hawksley Ltd., England). The number of eggs/larvae under both etched squares were then counted and the result multiplied by 50 to give the number of eggs per gram of faeces.

When a sample proved negative by this technique a further step was taken. The tube was filled to the top with saturated sodium chloride solution until a positive meniscus was obtained. A 22x22 mm square coverglass was placed on top, allowed to remain in contact with the solution for five minutes and then carefully removed and placed on a microslide. Each egg/larva present was counted and the total recorded as eggs or larvae/gram of faeces. In the indoor experiments, rectal samples were collected whilst faecal pat material was used in the outdoor ecology study.

Faecal cultures, both as a source of infective material and for larval morphology studies, were set up using a mixture of faeces and vermiculite (horticultural vermiculite No. 2 size). This was incubated in screw topped jars at 24°C for 10 - 14 days. The larvae were harvested by adding warm water to the jar, allowing to stand for three hours and then subjecting the suspension to the Baermann technique. To minimise the possibility of contamination between species the faeces of one animal only was processed on any one day and all equipment thoroughly sterilised.

A bag and harness system, shown in Plate 1, was employed to collect faeces from donor animals, separate bags and harnesses being kept for the different parasite species.

For the ecology studies, faecal pats were artifically

Plate 1 Faecal collection system showing harness and bag.

produced by collecting the total faecal output of 1 - 2 donor calves over a period of seven days. This was bulked, thoroughly mixed and aliquots of 400 - 500 grams transferred to separate polythene bags. During the collection period each day's faeces was stored at 4°C to prevent premature development of eggs. Prior to deposition, McMaster counts were performed on random samples to determine the mean number of eggs per gram and so provide an estimate of the numbers of eggs per faecal pat. Since the egg output of individual donor animals varied considerably it was decided to standardise the weight of the faecal mass rather than attempt to have a uniform number of eggs deposited each time.

Experimental plots

For the U.K. studies two hectare plots of a paddock ungrazed for two to three years were fenced off and marked out in 0.5 metre squares. Plastic lawn edging was then sunk to a depth of 5 cms to form the pattern shown in Plate 2. At regular intervals the grass over the entire area was clipped in order to maintain an approximate herbage length of 10 cms.

In Paraguay an area of 150 sq. metres was delineated into 12 strips each having a width of 1 metre. Longitudinal division was achieved using bricks sunk to a depth of 10 cms but no transverse sections were introduced. All other techniques regarding maintenance of herbage length etc. were similar to those of the U.K. project.

Worm Burdens

At necropsy, abomasal worm burdens were established using

Plate 2. Outdoor ecology plots.

the technique of Ritchie, Anderson, Armour, Jarrett, Jennings and Urquhart 1966. The basic procedure was as follows:

The calves were killed using a captive bolt pistol and immediately bled out. On opening the abdominal cavity the alimentary tract from rumen to large intestine was removed. As soon as possible after death the abomasum was tied off at the pyloric/duodenal sphincter area to prevent loss of parasites.

The abomasum was opened along its greater curvature and washed carefully under slow running water. The washings were then made up to four litres and duplicate samples of 200 ml removed and formalised. In addition the remainder of the contents were passed through a coarse mesh sieve (250 micron aperture) and the material retained on the screen formalised and stored.

After scraping off the abomasal mucosa it was digested in a pepsin/hydrochloric acid mixture for six hours at 42°C. The digested material was made up to four litres with water and again 200 ml samples taken for examination.

Prior to microscopy, iodine was put into the sample and on transferring the 4 ml aliquot to a lined petri plate for counting, sodium thiosulphate solution was added. The parasites retained the stain while the background cleared thus facilitating counting. For parasite speciation adult worms from the unstained duplicate pot were used.

From the 200 ml sub sample ten x 4 ml aliquots were examined using the x 12 or x 25 objective of a Wild M5 stereoscopic microscope (Leitz) and the parasites present classified as adult male and female, developing larval stages (DL_4) and early fourth

stage larvae (EL_4). The percentage species establishment was assessed by mounting 100 male worms in Berlese's fluid from each animal necropsied. Although male trichostrongylid parasites can be readily identified to species level it is more difficult to so classify females.

Herbage

All herbage within the 0.5 m square was clipped using lawn shears and placed in plastic bags for transport to the laboratory, where it was weighed and immersed in 12 litres of warm water to which a few drops of non-ionic detergent (Lissapol, ICI Ltd.) had been added. After a minimum soaking of six hours the herbage was transferred to a fresh 12 litres of water and again allowed to soak. Six hours later the grass was removed, wrung out and placed on trays to dry. The washings were passed through a 37 micron aperture sieve and the material retained Baermannised. This latter step, ie. sieving, was not practised in the Paraguay experiments.

A slight modification to the traditional Baermann technique, as practised in this department, was adopted. Instead of passing the larval suspension through a double milk filter (Maxa Filters Ltd.) and then placing this larval side up on a metal supporting sieve, the washings were passed through a coarse filter paper (Whatmans No.1) using a Buchner apparatus. A single milk filter was placed on top and the whole inverted and placed directly on the water surface of the Baermann apparatus. This was found to be self supporting when just enough water was used to enable the outer rim of the milk filter to fold up to allow the stronger

paper to rest on the funnel slope.

There were two main advantages to this modification. Firstly it eliminated the loss of larvae associated with the use of only milk filters in the Buchner and secondly it greatly reduced the possibility of cross contamination between samples via the supporting meshes since the material used is disposable.

Larval Measurement

Linear measurement of larvae or parts thereof is one of the main criteria used in their identification and has been achieved in many ways. For example compass dividers can be employed or a calibrated map measurer with a small rotating wheel which is run over the larval trace can be employed. Both of the latter techniques are useful for estimating the length of reasonably long straight lines but tend to be too coarse for small distances such as oesophageal or prolongation of sheath measurements. In order to achieve a more accurate estimation of these it was decided to adopt a more sophisticated approach. By courtesy of Mr. Gordon of the Glasgow University Geography Department a computerised system with a programme capable of such fine work tolerances was used. The basic set-up included a microcomputer (Commodore, Pet) linked to a digitiser and printer and is illustrated in Plate 3. The lines to be measured were traced on plain white paper using a microscope drawing tube (Leitz Ltd.) at a known magnification. These traces were followed using a digitiser which can effectively measure very small straight lines. In essence the trace was thus divided into a number of straight sections, their measurements recorded and summated at the end of the digitising process to arrive at the final figure.

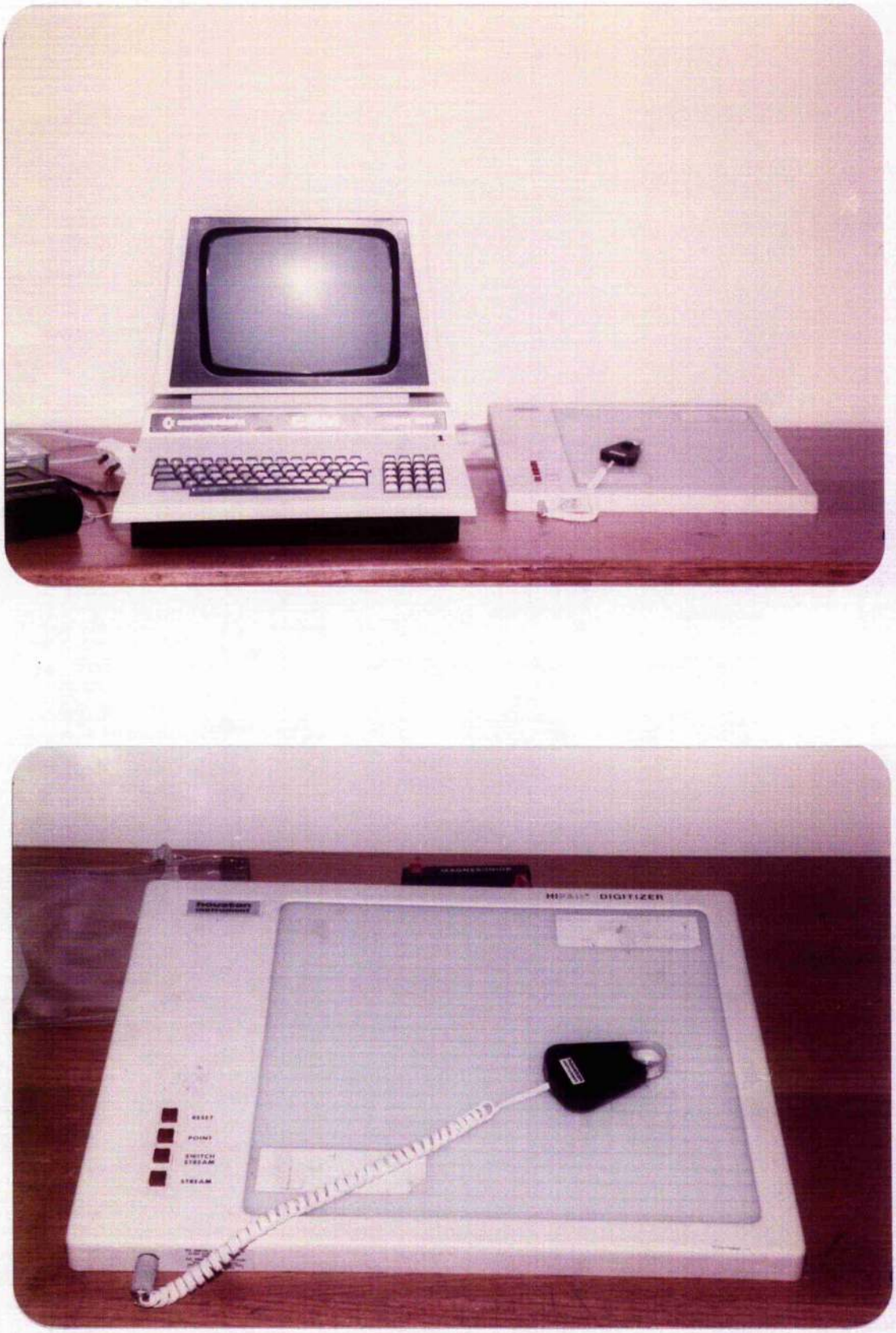


Plate 3. Computer/digitiser system for larval measurement.

Statistical Methods

Analysis of L_1 and L_2 stages was undertaken using a two way analysis of variance with factors of day of development and species. This enabled tests of significance for species differences and species X day of development interactions to be undertaken. In the event of significant interactions the pattern of mean lengths of each species on each day were examined.

Analysis of the L_3 stages i.e. oesophagus, intestine, anus to tail and sheath prolongation measurements were undertaken using the two sample t-test p values of 0.05 and less being considered significant.

Soil

In the outdoor ecology studies In Section E, after removal of the herbage component a single soil core was taken from the area directly under the faecal pat at each time of sampling. This was treated as described by Bairden (1980) except that the core was not divided into layers. A process of soaking followed by agitation and sedimentation was employed to remove most of the larger particles of soil and stones etc. The remaining suspension was then passed through a 37 micron sieve and the material retained on this filter Baermannised. As with the herbage 10 ml were drawn off from the Baermann funnel after a minimum of six hours and the larvae in one ml (i.e. 1/10th) counted and identified. When this 1/10 sub sample proved negative the remaining 9 ml were examined.

INTRODUCTION TO THE EXPERIMENTAL STUDIES

In order to control and so prevent economic loss the seasonal prevalence of the parasite species involved and the factors affecting that prevalence require to be studied. As mentioned in the literature review, many of the studies in the tropical countries have concentrated on identifying the species available, rather than looking at their seasonal prevalence and the ecology of the larval stages.

Two techniques are generally employed for estimating the prevalence of parasitism. In the first, the populations of free-living larval stages on the pasture are estimated by collecting and processing herbage so as to analyse the numbers of larvae present in a fixed quantity, usually a kilogram, of dried herbage. This technique has been widely used in studies on larval ecology in temperate zones but much less so in the tropics. This may be because of the type of extensive terrain and the grasses present in the tropics which do not lend themselves to this type of analysis but there is no reason that the larval ecology cannot be studied on plots of specially prepared ground as advocated by Armour (1982). Furthermore, the technique is not costly to undertake. The main difficulty may arise in the preparation of donor calves with monospecific infections.

The second technique widely used is the use of indoor-reared parasite-naive tracer calves. These are costly to rear and may not be representative since they often fail to graze alongside the main herd because of the discrepancy in rearing methods and

in some instances, age. However, this technique has the advantage that slaughter of these calves provides not only an estimate of the larval species present on the pasture but their stadial distribution in the host and this is important in areas where seasonal arrested larval development occurs (Armour, 1982). An alternative to using parasite-naive tracer calves is simply to withdraw cattle for slaughter at regular intervals over a year and analyse their worm burdens. These permanent tracers have the advantage that they are representative of the herd or flock but the worm count data lacks precision in relation to seasonal prevalence because of natural loss of some of the worm population (Armour, 1982).

In the first part of this thesis, Sections A and B, the ecology of the free-living stages of Haemonchus contortus, primarily a tropical parasite is studied in a subtropical environment, namely, Paraguay and the seasonal prevalence of H. contortus and other abomasal parasites are also studied in Paraguay using permanent tracer cattle. The effect of age of the cattle and the influence of parasite species interaction are also investigated and analysed.

In the second part of the thesis (Sections C, D, E and F) the factors affecting the development and survival in a temperate climate of two Ostertagia species, namely O. ostertagia and O. leptospicularis are studied under experimental and field conditions and this data compared with that of H. contortus in Paraguay. The interaction of these two species following their simultaneous inoculation into calves is also studied.

Tables and Figures pertaining to each section are placed at

the end of the text of each section and coded with the section letters and appropriate number.

HAEMONCHUS SECTIONS (A & B)

SECTION A

A STUDY ON THE ECOLOGY OF THE FREE-LIVING STAGES OF HAEMONCHUS CONTORTUS IN PARAGUAY

INTRODUCTION

The Genus Haemonchus

Though the first mention of Haemonchus is probably that of Johann Christian Fabricius in 1790, it was only recognisably described by Karl Asmund Rudolphi who, in 1803, recorded an abomasal parasite of sheep which he named Strongylus contortus. The genus Strongylus gradually accumulated very large numbers of different species and became so unwieldy that, towards the end of the last century, many taxonomists made new groups of species, among them being Cobb who, in 1898, erected the genus Haemonchus to accommodate Rudolphi's S. contortus.

More than twenty species of Haemonchus have been described, but only nine are now recognised as valid in the recent and probably definitive revision by Gibbons (1979) who, on a basis of synonymy, has not only eliminated, among several others, the previously widely accepted species H. placei, but also the six indeterminate sub-species of H. contortus proposed by Das and Whitlock (1960), Rao and Rahman (1967), Martinez Gomez (1968) and Sukhapesna (1974).

The genus Haemonchus probably has its origins in Africa, which is its chief endemic area, and where all nine species are found. Six of these are predominantly parasitic in wild ruminants, and the only three recorded outside Africa have domestic ruminants as their principal host, H. contortus occurring mainly in sheep, cattle and goats, H. similis in cattle and water buffalo, and H. longistipes in camels (Gibbons 1979). It has been suggested that these species have spread with their

host from their original African habitats, H. contortus world-wide, H. similis to South America, the southern states of North America and Asia, and H. longistipes to Southern Europe and Asia (though it has not yet been recorded in the camels of Australia). In its geographical dispersion H. similis appears to have retained some specificity within the host genus Bos, being commoner in Zebu than in the humpless european breeds in South America, in contrast to H. contortus which infects B. taurus and B. indicus indiscriminately.

Life Cycle of Haemonchus contortus

The life cycle of H. contortus is direct (Ransom, 1906) and consists of two parts: the pre-parasitic and the parasitic phases. The pre-parasitic phase takes place outside the host and starts with the development and hatching of the fertilised egg to the first larval stage. This larva progresses by two moults to the third or infective stage. The time taken for this pre-parasitic phase of the cycle to reach its conclusion is variable depending on external factors such as temperature and humidity, but, under optimal conditions this may be only four days. The female parasite can lay 5000 - 10,000 eggs per day and has one of the highest biotic potentials of parasitic nematodes.

The environmental conditions necessary for the successful embryonation of H. contortus eggs and their development through the first and second larval stages to the infective third stage have been studied by many workers under both field and laboratory conditions. Unfortunately, there is some disparity in their findings due perhaps to the cursory nature of some of the investigations conducted under a wide range of climatic

conditions. It is also possible that some of the inconsistencies depend on the existence of strains of H. contortus which have different optimal conditions for their pre-parasitic development. Since the survival of infective larvae is not necessarily a reliable indication of infectivity, the validity of some worker's conclusions based entirely on larval motility may also be somewhat suspect when applied to epidemiological studies.

For infective larvae to be available to grazing animals three conditions must be fulfilled:-

Firstly, the egg must have hatched and developed successfully to the infective larval stage.

Secondly, the infective larva must survive until the time of ingestion and thirdly, it must be in a suitable position on the herbage to be available to grazing ruminants. It is apparent, therefore, that under field conditions the bionomics of larval infectivity is a complex study and it is proposed to review the relevant literature under the three headings described above.

The development of eggs to infective third stage larvae

Much of the experimental work on Haemonchus spp has been carried out on the embryonic and larval development of species recovered from sheep, a lot of it utilising H. contortus.

Ransom (1906) working in the USA showed that some eggs of H. contortus in faecal cultures at 16°C to 20°C hatched within two days while others in the same culture did not hatch until a week had elapsed. In 1907 he reported that at 35°C infective larvae appeared in the faecal cultures in three to four days, at 21°C in six to 14 days and at an average of 10°C, three to four

weeks were necessary for the eggs to hatch and the larvae to develop to the infective stage. He found that the eggs showed some resistance to the adverse effects of drying or freezing when the temperature was below 4°C, the eggs remaining dormant. In this condition they retained their viability for two or three months, afterwards hatching out when the weather became warmer.

Veglia (1915) in South Africa found that at constant temperatures ranging from 26°C to 35°C the majority of eggs hatched within 14 to 24 hours. He considered 35°C to be within the normal temperature range for larval development and in faecal culture at that temperature, migration of the third stage larvae up the side of the culture jar began on the fourth day. Between 22°C and 33°C, temperatures which he regarded "as well defined optimal limits" larvae could grow without showing any marked variations. Development to the third stage required six days at 18°C, eight days at 15°C and 18 to 21 days at 8°C.

Dinaburg (1944) studied the effect of climate on the development of ruminant nematode larvae, working with H. contortus from sheep at Beltsville, Maryland, USA. He found that H. contortus eggs failed to develop into infective larvae when the monthly mean maximum temperature was below 18.3°C regardless of rainfall, but when the monthly mean maximum temperatures were betwwen 18.9°C to 28.9°C the numbers of larvae recovered from the field varied with the amount of rainfall.

Rather similar findings were reported by Dinnik and Dinnik (1958) from the Highlands of Kenya. A diurnal fluctuation in temperature (high day temperature and low night temperature) between a mean minimum of 12.2°C and a mean maximum of 23.3°C,

with at least one inch of rainfall evenly distributed within their ten day observation period was found to be the most favourable for larval development.

However, in experiments carried out at various constant temperatures under laboratory conditions in Scotland, Silverman and Campbell (1959) reported that hatching of eggs occurred at temperatures of 9°C with a maximum survival of eggs occurring at 11°C to 14°C. Of the eggs which failed to develop completely, most died in the pre-morula or morula stage, while eggs which reached the gastrula or tadpole stage survived longer, for up to four months at 7.2°C.

Although both Veglia (1915) and Barberian and Mizelle (1957) also observed that developed eggs were relatively more resistant to the effects of desiccation than freshly passed ones, the general consensus of opinion is that desiccation is unfavourable to larval development. Indeed, Rose (1963) concluded that the effects of desiccation are such that few if any, H. contortus eggs in dehydrated faecal pellets develop into infective larvae.

A conclusion somewhat at variance with those quoted above has been expressed by Silverman and Campbell (1959) who reported that well developed eggs containing larvae survived at room temperature for six weeks in dried faecal pellets.

Finally the advantage of the retention of the L_2 sheath by the larval L_3 was demonstrated by Ellenby (1968) who showed that the ensheathed larvae were relatively more resistant to the desiccating effect of a 43% relative humidity than the exsheathed stages.

The Longevity of Infective Larvae on Pasture

Despite the numerous reports on this important aspect of H. contortus epidemiology it is difficult to draw precise conclusions. This is partly due to the different conclusions reached by various workers and partly to the impossibility of extrapolating these observations to the daily fluctuations in temperature and relative humidity encountered in the field.

Under laboratory conditions an optimum temperature for longevity has been reported to range from 11°C to 21°C (Silverman and Campbell, 1959; Rose, 1963), while Gibson and Everett (1976) and Shorb (1943, 1944) under field conditions found a similar optimal temperature range. The subject has been recently reviewed by Levine (1963) and Todd, Levine and Whiteside (1970). The former, on the basis of his own work concluded that H. contortus third stage larvae survived for 8 1/2 months in faecal pellets at room temperature (the actual temperature was not defined) and in water at 4°C to 5°C for up to 22 months. Only in the first of these experiments was the infectivity of the surviving larvae confirmed by "in vivo" experiments. Todd and colleagues adopted a rather different approach to studying the survival of L_3. They suspended Haemonchus L_3 in tap, distilled and triple distilled water and then subjected the larvae to desiccation by evaporating them in a variety of relative humidities and temperatures. Larvae subjected to this moisture stress survived best at a relative humidity of 75% and a temperature of 20°C, remaining motile for 80 days. The longest surviving larvae were those suspended in the triple distilled water a result which the authors ascribed to the absence of

mineral salts.

In experiments in which infective larvae were exposed outdoors on grass plots during the four different seasons of the year, Dinaburg (1944) reported a maximum survival of infective larvae for 70 days in the spring, the mean maximum temperature at that time being 23.3°C in Beltsville, Maryland.

Under field conditions, the longevity of infective larvae on pasture has been found to show considerable variation, probably due to the different climatic conditions to which the larvae were exposed. Reports range from up to 38 days by Levine, Todd and Boatman (1974) in Illinois, USA and up to 22 months, Boughton and Hardy (1936) in Texas, USA.

In temperate Southern England, Rose (1964) found that only a very few larvae survived for a year while Braga and Honer (1982) reported a maximum survival of 18 weeks in tropical Rio de Janeiro, Brazil.

The Availability of Infective Larvae

It is generally assumed that a high relative humidity and high temperatures aid the movement of infective larvae out of faeces and towards the tips of herbage, thus facilitating their ingestion. For H. contortus larvae this has been demonstrated by Rees (1950) and Silangwa and Todd (1964) who concluded that a high relative humidity of 93% and temperatures of around 25°C to 27°C produced a maximal vertical migration of infective larvae, Rose (1963) reported that many larvae migrated laterally to around 5 cms from the faecal pellet and a few larvae were recovered up to 15 cms from the soil surface.

Skinner and Todd (1980) studied the lateral migration of infective larvae of H. contortus in tall grass (20 cms) and short grass (10 cms). Generally, over 90% of the larvae were found within 10 cms of the faeces and the number decreased as the distance from the faeces increased. Migration in short grass was 60% more than in tall grass. The pasture in the studies consisted mainly of blue grass (Poa pratensis) but other species of grasses were also present.

Clearly most of the work on the ecology of H. contortus larvae has been done with a strain from sheep and utilising faeces from infected sheep or infective larvae. Based on these data, Gordon (1953) concluded that provided rainfall exceeded 50 mm per month and mean monthly maximum temperatures were greater than 18.3°C, conditions were optimal for development and transmission. Levine (1963) stated that optimal conditions for the parasite are when monthly rainfall exceeds 50 mm and the mean monthly temperatures range from 15°C to 37°C. Later, Levine, Todd and Boatman (1974) studying the development and survival of H. contortus in Illinois, USA, found that the mean monthly maximum temperatures were a better guide and that a temperature range of 20°C to 33°C was optimal.

In tropical and subtropical areas, haemonchosis, due to the combination of the high biotic potential of the female parasite and favourable temperatures for the development of the pre-parasitic stages, poses a major threat to the economic production of meat and wool. This certainly applies to Paraguay where the parasite is recognised as an important pathogen but little is known about the ecology of the larvae or the seasonal variation

in worm burdens. Furthermore, there have been very few studies world-wide using a cattle strain of H. contortus. In this section the ecology of the free-living stages of H. contortus was studied over a twelve month period, using a strain isolated from cattle, the major ruminant species on Paraguayan farms.

EXPERIMENTAL DESIGN

Faecal pats containing known numbers of H. contortus eggs were deposited monthly on larval free pasture. Regularly, samples of faeces and herbage were removed and examined for the presence of helminth eggs and larvae. Rainfall and temperature data were also recorded.

MATERIALS AND METHODS

Animals

At intervals of three months, single helminth-naive donor Friesian calves aged approximately 10 weeks, were surgically implanted with approximately 600 male and female H. contortus isolated at necropsy of a naturally infected calf. Cortisone (1 ml) was administered daily to maintain the faecal egg counts at around 500 to 600 e.p.g.

Preparation of Experimental Grass Plots.

0.5 hectares of grassland free from trichostrongyle L_3 and consisting mainly of Paspalum notatum and situated with the grounds of the Faculty of Veterinary Science, Asuncion were utilised.

An animal-proof fence was constructed around a plot of 150 square metres which was then sub-divided into 12 strips each of 12 x 1 metre and each segregated by a wall of single bricks sunk

to a level of 10.0 cms into the ground and projecting 40.0 cms above ground level to prevent lateral migration of L_3.

Faecal Pats.

Freshly collected faeces from the donor calves containing 500 to 600 H. contortus epg were placed on one of the grass strips at monthly intervals during a period of one year (14th November to 14 October, 1980). Within each strip, 24 faecal deposits, each weighing approximately 500 g, i.e. equivalent to a faecal pat from a yearling calf, were placed at intervals of 0.5 m.

Climatic Observations.

Daily rainfall and weekly maximum and minimum temperatures were obtained from a local metereological station and the temperatures recorded in a shelter 120 cms above ground level.

Parasitological Data

Faeces. On day 1 i.e. when the faeces were placed on the herbage and thereafter at fortnightly intervals, one of the faecal deposits was removed, weighed and examined by the flotation technique for the presence of eggs and larvae. The presence of unembryonated and embryonated eggs was noted but only the total number of eggs present was counted.

Herbage. Coincident with the removal of the faecal mass, the grass within an area of 0.25 m radius around the faeces was clipped to ground level, removed, weighed and analysed for the presence of infective larvae (Parfitt, 1955) which were expressed as L_3 per kilogram of dried herbage (L_3/kdh). These observations continued until both faeces and herbage were negative for three

successive samplings, i.e. for six weeks. Throughout the experiment the grass height was maintained at no more than 15.0 cms.

RESULTS

Climatic Observations

To coincide with the fortnightly sampling of faeces and pasture the rainfall and mean maximum and minimum temperatures are presented on a fortnightly basis in Figure A1 and Table A1. The data is presented over a 16 month period, to cover that immediately prior to the plots being contaminated with faeces and the final disappearance of eggs and larvae. Rain occurred in every fortnight except July 1980 and the mean fortnightly minimum temperature ranged from 10°C to 23 °C and the mean fortnightly maximum temperature from 20°C to 34.6°C.

Parasitological Data

Data pertaining to the faeces deposited each month and including the weight of faeces and grass, the number of eggs remaining in the faeces and the L_3 per kdh are given in Tables A2 A7.

Faecal Pats

With regular rainfall, thick crusts did not form on the surface of the faeces. However, despite the presence of only a thin crust, persistence of the faecal mass was good over the first two months and during this period as can be seen from Table A2, the "apparent" weight loss, albeit complicated by the added contribution of rain water was less than 25%; thereafter a steady and more rapid disintegration occurred.

Recovery of H. contortus eggs and larvae from faeces

Eggs and larvae were recovered as long as faeces persisted. Embryonated eggs were abundant within two weeks of faecal deposition. However, many of these apparently failed to hatch and prior to the final disintegration of the faeces the eggs present were mostly embryonated. Although larvae were recovered from all of the faeces sampled the numbers were very low indicating that migration onto pasture or mortality must have occurred rapidly. The total numbers of eggs (embryonated and unembryonated) present in the faeces at the time of deposition and those samples removed at fortnightly intervals are also shown in Figure A1 and in Table A3.

From this result it is clear that over 50% of the total eggs deposited remained within the faeces after ten weeks, irrespective of season, and the numbers only declined as the rate of faecal disintegration increased.

Migration and Survival of Infective Larvae on Grass

Migration of L_3 occurred throughout the year. Every month, considerable numbers of H. contortus L_3 were present on herbage within two weeks of faecal deposition. Since few L_3 were recovered from within the faeces this tends to confirm that rapid migration occurred after embryonation and hatching.

The numbers of L3 recovered at each fortnight and expressed as L_3/kdh are also given in Figure A1 and Table A4 while the weight of the grass samples are given in TableA5. Infective larvae were recovered for at least 14 weeks after deposition and in some cases for up to 20 weeks. Larval recovery followed very

closely that of the survival of faeces and L_3 disappeared rapidly after the final disintegration of the faeces.

Most L_3 were recovered from plots within two to four weeks after deposition of faeces and the maximum numbers occurred during May through to October 1980 i.e. late autumn, winter and spring. During this period and particularly in August high numbers of L_3 continued to be present for up to 12 weeks after faecal deposition.

Between May and October the mean fortnightly minimum temperatures ranged from 10°C to 18.4°C and the mean fortnightly maximum between 20°C to 27°C. It is of course possible that during the other months of the year when temperatures were higher (November-April) that just as many L_3 developed and migrated to the pasture but succumbed rapidly within the 14 day period between the first and second samplings. Certainly from the egg count data shown in Table 3 there was a constant loss of eggs throughout the year suggesting that development of larvae and their transmission from the faecal pat was occurring throughout the year.

The weight of the grass samples (Table A5) ranged from a maximum of 240 g in the summer (December) to a minimum of 54 g in mid-winter (June to August).

DISCUSSION

Paraquay is situated at 57° W and 25° S and the climate is subtropical with non seasonal rainfall and seasons which are differentiated mainly on temperatures. Occasionally, prolonged periods of drought or flooding occur although this varies locally from region to region.

In the current study rain occurred during every month in Asuncion where the larval ecology plots were situated.

The cooler months of the year were apparently the most suitable for larval development, migration and survival. Thus, maximal numbers of L_3/kdh occurred on the plots from May through to October when the mean fortnightly maximum temperatures were between 20°C and 27°C and the mean fortnightly minimum temperatures were between 10°C and 18.4°C (Figure A1). These temperatures are rather lower than those proposed as optimal by Dinaburg (1944) and Levine et al (1974). However, in the present experiment temperatures were recorded in a weather shelter and Levine et al (1974) used ground temperatures to calculate the mean monthly maximum. The temperatures in the shelter used to record temperatures, are likely to be less extreme than those at the soil surface and therefore in any comparison a few degrees should be added to the present figures. Nevertheless, and allowing for this correction the maximum numbers of L_3 were recovered from the pastures when the mean monthly maximum temperatures were in the range of 23.5°C to 27°C and when they rose above 30°C as in December and January far fewer L_3 were recovered. However, as has been suggested in the results

section, it is possible that since the faeces and grass were not examined between days one and 14, eggs might have hatched quickly at the higher temperatures and the resultant larvae then suffered a heavy mortality, particularly at the labile L_1 and L_2 stages. Examination of the data on egg numbers present in the faecal material certainly shows a similar rate of disappearance of eggs from the dung pat (Table A3) throughout the year suggesting that hatching and rapid mortality had indeed taken place in some months.

It is also possible that a few L_3 migrated further than 25 cm from the faeces but the number is likely to be very low since it is well established that most are found within 10 cm (Skinner and Todd 1980).

In order to help visualise the effects of temperature and precipitation on the development of eggs to the infective stage, Gordon (1948) introduced the use of bioclimatographs. These are graphs in which the total precipitation is plotted against the mean temperature for the month and the resultant points are joined by a closed curve.

Following Dinaburg's temperature line of 18.3°C, Gordon selected mean monthly maximum temperatures for his bioclimatographs. On the latter he superimposed lines indicating the limits of climatic conditions most favourable for the free-living stages of a particular parasite in different localities.

Subsequently, Gordon (1953), Forsyth (1953) and Pullar (1953) considered that a total monthly rainfall of 50 mm or more together with a mean monthly maximum temperature above 18.3°C provided optimum conditions for the transmission of H. contortus

L_3 of sheep.

During the course of this study higher numbers of larvae were recovered from plots contaminated from May to October (autumn through spring) even though the conditions for transmission based on Gordon's data were also favourable during the whole year, with the exception of July as shown in the bioclimatograph in Figure A2.

The high number of larvae recovered in July could be due to the fact that even if the rainfall was minimal during that month (3 mm) there was still sufficient moisture in the soil and herbage from the rainfall in the previous month (75 mm). As the mean maximum temperature at that time was only 23.5°C and the overall mean only 17.05°C, evaporation would not be so great and the soil moisture therefore maintained for a longer time. The lower number of L_3 during summer in the present trial could have been due to a rapid mortality of the larvae as suggested above.

Another possible explanation for the differences in temperature response of the current results and those of Gordon (1953) and Levine et al (1974) is that the strains of H. contortus used were physiologically different, Gordon's and Levine's originating from sheep and a cattle strain being used in this trial. Certainly, physiological differences have been noted by Le Jambre and Whitlock (1976) for strains of H. contortus in New York State, USA and Southwest England.

In this experiment the Haemonchus L_3 persisted on the pastures for 4 to 5 months which is much longer than the maximum of 80 days recorded by Levine et al (1974) in Illinois, USA.

However, Levine and his colleagues worked with sheep pellets containing H. contortus eggs whereas cattle faeces were used in the present study and the results emphasise the greater protection afforded to eggs and larvae by the bovine faecal pat and its potential as a reservoir of infection.

It may be argued that computing the herbage larval data as L_3/kdh is a biased method of presentation since the size of the grass samples varied throughout the year (Table A5). However, since grazing animals have to ingest or would try to ingest a minimum quantity of dry matter per day, L_3/kdh is probably as representative a method of analysing the larval data as any other available. However, for the sake of comparison the larval data is presented in two other ways. In the first, the total L_3 recoveries from each grass sample are presented in Table A6 and in the second the numbers of L_3 recovered, divided by the number of eggs deposited in the faeces, are expressed as a percentage in Table A7.

From the results in both Tables A6 and A7 it is clear that the same seasonal trend is present as in Table A5. Thus the highest total numbers of L_3 present in the grass samples collected (Table A6) was 1675 in June with higher numbers generally being recovered from May to October.

Persistence of L_3 was greatest in August. The percentage of eggs which yielded L_3 was also highest in June (0.56) with relatively high yields also being recorded from May to October. Overall the yields of L_3 from the eggs were extremely low and did not exceed one per cent on any occasion. However, since there was no sampling between days one and 14 the original yields

could have been greater, but if so the mortality in the first 14 days must have been very high.

The fact that the L_3 of H. contortus were present on the pasture for as long as the faecal pats could be identified is a most important finding. Since the L_3 disappeared within 8 weeks after the final disintegration of the faeces, which persisted for between 14 and 20 weeks, pastures could be considered clear of larval infection by 28 weeks though this might be longer in areas of shade or in drought conditions.

It is interesting to note that in Queensland, Australia, at latitude 10°S and longitude 140°W, Durie (1961) found that cattle faecal pats persisted for between five and eight months, the extended survival periods being associated with periods of drought and that once the L_3 reached the herbage they survived for a maximum of 8 weeks during cool conditions, which is similar to the results of the current study.

The significance of the present findings is that higher numbers of L_3 are present on the pastures in the winter and early spring months in Paraguay and constitute a major hazard to cattle at this time. The effect of the parasite may also be exacerbated by the sub-optimal nutrition induced by the poorly grown pasture in winter and early spring as can be seen from the lower weights of the grass samples at this time.

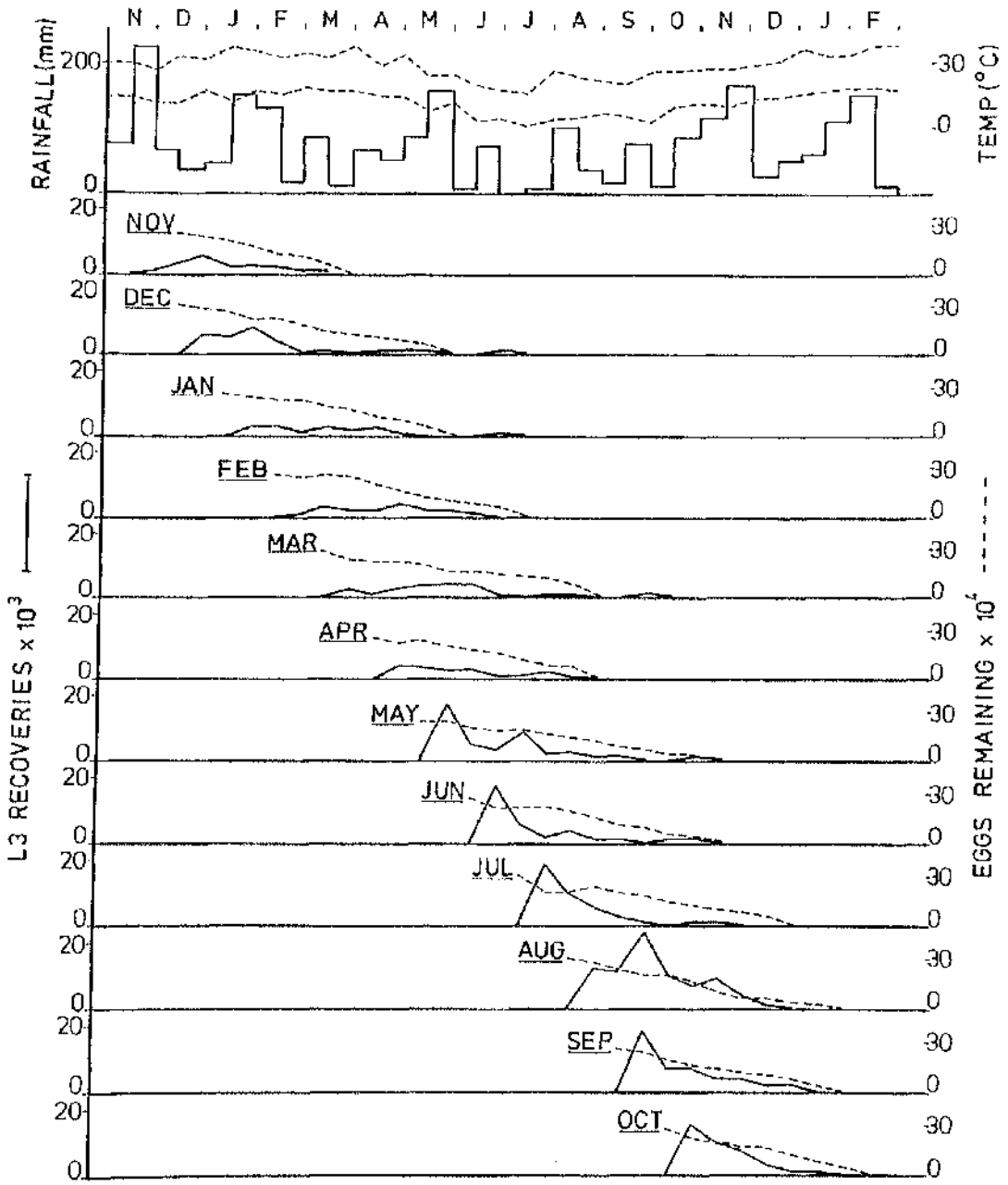


Figure A1. Haemonchus ecology experiment at Ascuncion: climatic data, numbers of eggs in faeces plus L3/kdh.

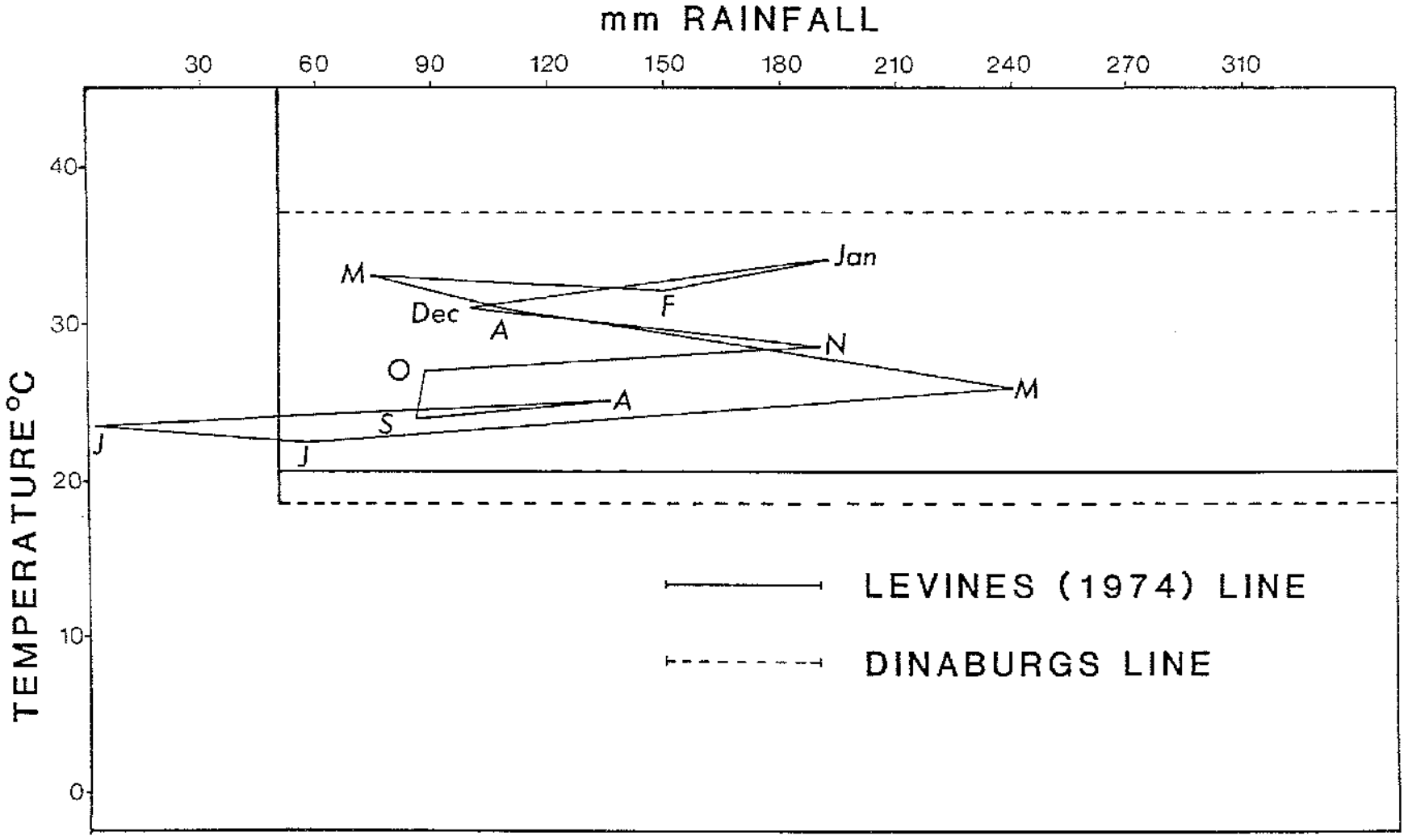


Figure A2. Bioclimatograph for Haemonchus contortus based on climatic data at Ascuncion 1980

TABLE A 1.

MEAN FORTNIGHTLY MAXIMUM AND MINIMUM TEMPERATURES (°C)

PLUS RAINFALL DATA AT ASUNCION

	MONTH	TEMPERATURE				RAINFALL	
		MAXIMUM		MINIMUM			
		1st - 15th	16th - 30th	1st - 15th	16th - 30th	1st - 15th	16th - 30th
1979	NOVEMBER	–	28	–	17	–	189
	DECEMBER	32	31	22	21	64	36
1980	JANUARY	35	34	18	21	38	152
	FEBRUARY	32	33	20	22	132	16
	MARCH	32	35	21	21	66	10
	APRIL	29	32	19	19	64	52
	MAY	26	26	18	16	85	154
	JUNE	23	24	12	11	6	69
	JULY	20	27	10	11	–	3
	AUGUST	25	24	13	14	101	34
	SEPTEMBER	24	24	12	10	13	73
	OCTOBER	27	27	15	15	88	20
	NOVEMBER	29	28	16	17	121	157
	DECEMBER	32	31	21	20	23	41
1981	JANUARY	34	33	21	20	52	108
	FEBRUARY	31	33	21	23	148	11

TABLE A 2. SURVIVAL (IN WEEKS) AND WEIGHT OF CATTLE FAECES (g) DEPOSITED ON PLOTS

MONTH FAECES DEPOSITED	2	4	6	8	10	12	14	16	18	20 weeks
NOV.	450	401	370	325	280	204	169	98	-	-
DEC.	468	422	391	296	221	198	176	152	94	-
JAN.	444	407	386	320	278	219	176	115	-	-
FEB.	430	452	439	392	314	266	202	173	121	- -
MARCH	418	399	401	366	318	289	255	197	139	96
APRIL	431	466	407	372	314	261	189	111	-	-
MAY	473	414	376	351	303	269	233	191	150	88
JUNE	379	396	405	362	288	241	193	117	69	-
JULY	342	414	391	344	299	238	198	-	-	-
AUGUST	451	396	315	285	239	176	128	94	71	58
SEPT.	423	379	315	287	241	198	147	96	-	-
OCT.	404	366	301	269	188	141	88	-	-	-

TABLE A 3. TOTAL NUMBER OF *Haemonchus contortus* EGGS IN FAECAL PATS

(computed by weight of faeces x eggs per g.)

MONTH FAECES DEPOSITED	Weeks							Weeks			
		2	4	6	8	10	12	14	16	18	20
NOV. 1979	300,000	270,000	240,600	222,000	196,000	168,000	122,400	101,400	58,200	-	-
DEC.	300,000	280,800	253,200	234,600	223,800	177,600	132,600	118,800	105,600	93,000	56,400
JAN. 1980	275,000	244,200	223,850	212,300	176,000	152,900	120,450	96,800	63,250	-	-
FEBR.	250,000	215,000	226,000	219,500	196,000	157,000	133,000	101,000	86,500	60,500	-
MARCH	275,000	229,900	219,450	220,450	201,300	174,900	158,950	140,250	108,350	76,450	52,800
APRIL	250,000	215,500	223,000	203,500	186,000	157,000	130,500	94,500	55,500	-	-
MAY	250,000	236,500	207,000	188,000	175,500	151,500	134,500	116,500	95,500	75,000	44,000
JUNE	275,000	208,450	217,800	222,750	199,100	158,400	132,550	106,150	64,350	37,950	-
JULY	300,000	205,200	248,400	234,600	206,400	179,400	142,800	118,800	105,600	93,600	55,900
AUG.	300,000	270,600	237,600	189,000	171,000	143,400	105,600	76,800	56,400	42,600	34,800
SEPT.	250,000	211,500	189,500	157,500	143,500	120,500	99,000	73,500	48,000	-	-
OCT.	275,000	222,200	201,300	165,550	147,950	103,400	77,550	48,400	-	-	-

TABLE A 4. INFECTIVE LARVAE OF *Haemonchus contortus* ON GRASS PLOTS (L3/Kdh)

AFTER DEPOSITION OF CATTLE FAECES

MONTH FAECES DEPOSITED	SURVIVAL OF FAECAL PATS	TOTAL *H.contortus* EGGS IN FAECES DEPOSITED	Weeks 2	4	6	8	10	12	14	16	18	20
NOV.	4 months	300,000	417	3,661	5,000	2,180	1,860	1,892	427	549	-	-
DEC.	5 months	300,000	5,468	5,107	7,173	5,309	625	1,271	537	769	666	657
JAN.	4 months	300,000	3,231	3,409	937	2,400	1,785	2,307	1,086	378	-	-
FEB.	4 months	275,000	1,027	2,862	2,430	2,573	3,571	2,102	1,458	450	229	-
MARCH	5 months	275,000	2,966	909	1,694	3,504	4,014	3,911	694	-	641	526
APRIL	4 months	250,000	4,772	3,571	2,777	3,125	531	675	1,898	357	-	-
MAY	6 months	250,000	11,666	5,451	3,629	8,035	1,623	2,058	1,119	1,293	-	-
JUNE	5 months	300,000	17,091	6,164	1,785	3,370	1,250	925	-	201	490	-
JULY	5 months	300,000	17,916	8,505	5,555	2,666	1,209	271	555	257	-	-
AUG.	5 months	300,000	11,785	11,475	30,092	10,040	8,669	8,478	3,125	524	233	203
SEPT.	5 months	250,000	17,672	6,720	7,051	4,375	4,050	1,679	1,520	233	-	-
OCT.	4 months	250,000	8,636	9,579	7,172	2,743	438	994	211	-	-	-

TABLE A5. WEIGHT OF GRASS (g) COLLECTED AT FORTNIGHTLY SAMPLING AFTER DEPOSITION OF FAECES

Time of faecal applic.	Weeks									
	2	4	6	8	10	12	14	16	18	20
NOV.1979	180	145	225	172	121	196	234	184	-	-
DEC.	96	93	115	113	240	177	93	65	75	76
JAN.1980	147	154	240	125	84	65	69	132	118	-
FEB.	219	131	72	78	70	107	120	167	109	-
MARCH	59	55	59	107	112	147	72	57	78	95
APRIL	110	126	126	144	94	74	79	70	-	-
MAY	90	133	62	56	77	85	67	58	-	-
JUNE	98	73	70	89	60	54	-	124	102	-
JULY	60	97	54	75	62	92	90	97	-	-
AUGUST	70	61	54	122	124	115	104	143	107	123
SEPT.	58	93	117	120	117	134	148	107	-	-
OCT.	110	107	122	164	171	176	118	-	-	-

TABLE A6. TOTAL NUMBER OF *H.contortus* L_3 RECOVERED FROM GRASS SAMPLES COLLECTED AT A RADIUS OF 0.25 CMS AROUND FAECES

Time of faecal applic.	Weeks									
	2	4	6	8	10	12	14	16	18	20
NOV.1979	75	525	1125	374	225	351	99	100	-	-
DEC.	605	475	825	510	150	225	50	50	50	50
JAN.1980	475	525	225	300	150	150	75	50	-	-
FEB.	225	375	175	175	250	225	175	75	25	-
MARCH	175	50	100	375	450	575	50	-	50	50
APRIL	525	450	350	450	50	50	150	25	-	-
MAY	1050	725	225	450	125	175	75	75	-	-
JUNE	1675	450	125	300	75	50	-	25	50	-
JULY	1075	825	300	200	75	25	50	25	-	-
AUGUST	825	700	1625	1225	1075	975	325	75	25	25
SEPT.	1022	625	825	525	475	225	225	25	-	-
OCT.	950	1025	825	450	75	175	25	-	-	-

TABLE A7. *H.contortus* INFECTIVE LARVAE ON HERBAGE EXPRESSED AS A PERCENTAGE OF EGGS DEPOSITED IN THE FAECES

Time of faecal applic.	Weeks									
	2	4	6	8	10	12	14	16	18	20
NOV.1979	0.03	0.2	0.4	0.12	0.1	0.12	0.03	0.03	-	-
DEC.	0.20	0.16	0.3	0.2	0.05	0.1	0.02	0.02	0.02	0.02
JAN.1980	0.2	0.2	0.1	0.1	0.05	0.05	0.025	0.02	-	-
FEB.	0.1	0.13	0.1	0.1	0.1	0.1	0.1	0.02	0.01	-
MARCH	0.1	0.01	0.03	0.13	0.16	0.21	0.01	-	0.01	-
APRIL	0.21	0.2	0.14	0.2	0.02	0.02	0.1	0.01	-	-
MAY	0.42	0.3	0.1	0.2	0.05	0.1	0.03	0.03	-	0.02
JUNE	0.56	0.15	0.04	0.1	0.025	0.16	-	0.01	0.16	-
JULY	0.34	0.3	0.1	0.1	0.03	0.01	0.02	0.01	-	-
AUGUST	0.3	0.23	0.54	0.41	0.4	0.32	0.11	0.025	0.01	0.01
SEPT.	0.41	0.25	0.33	0.21	0.2	0.1	0.1	0.01	-	-
OCT.	0.4	0.41	0.35	0.2	0.03	0.1	0.01	-	-	-

SECTION B

A STUDY OF ABOMASAL WORM BURDENS IN TWO BEEF CATTLE HERDS IN PARAGUAY

INTRODUCTION

The seasonal prevalence of gastrointestinal nematode infections of cattle has been studied in some depth in Western Europe, particularly in Great Britain and Australasia. In the sub-tropics there have been fewer comprehensive studies although there are several current investigations in the Southern USA (Williams, 1983) and in Brazil (Beck, Melo, Bianchin and Suarez, 1979; Melo, Bianchin, Ribeiro and Beck, 1980) and in several other countries as indicated in the literature review.

Apart from the rather dated technique of monitoring infection by the use of faecal egg counts, followed in some instances by faecal larval cultures to facilitate genus identification, the assessment of seasonal prevalence has largely rested on the use of tracer animals or pasture larval counts. The former technique, first used in sheep by Tetley (1959) and in calves by Durie (1961) involves the introduction of successive groups of parasite-naive animals or "tracers" to pastures known to be contaminated with eggs or larvae of gastrointestinal nematodes, for short periods of around 14 days. At the end of each test period the animals are removed, housed for 7 to 10 days and then slaughtered. The worm burdens at slaughter are used as an index of the level of infection during the period grazed and the burdens found in successive batches of tracers plotted to indicate the seasonal variation in availability of larvae on the pasture.

This technique in which parasite-naive tracers are grazed with permanent stock has been successfully used in many temperate

countries (reviewed by Armour and Ogbourne, 1982) but less often in tropical areas, although it has been used in Papua, New Guinea by Owen and Talbot (1983).

Although costly in terms of calves, the tracer technique has the main advantage that the stadial distribution of parasite stages can be monitored in relation to season. Without the use of this technique the seasonal nature of arrested larval development would not have been first identified by Anderson et al (1965 b) working in the temperate climate of Western Scotland.

However, Winks and Bremner (1978) have suggested that arrested larval development of nematodes is of comparatively little importance in the sub-tropics and tropics although the results of Hart (1964) working in Nigeria and Williams and his colleagues in Louisiana (1983) would appear to contradict that statement.

Perhaps the biggest drawback to the use of the tracer technique in tropical areas is that most farms are involved in beef production under systems of extensive management. When indoor-reared tracer calves, strange to the pasture and to the other cattle, are introduced to the grazing area they tend to graze as a small group away from the main herd and do not necessarily provide a true reflection of the larval uptake by the herd. Also, tracer calves do not yield data on the acquisition of immunity by the herd.

Likewise, the sampling of extensive pastures for the presence of larvae is not particularly helpful due to the relatively small areas sampled and the lack of information on

immunity or arrested larval development.

Because of the above difficulties an alternative method had to be sought to investigate gastrointestinal parasitism on Paraguayan estancias. It was decided to allow calves to graze naturally with their dams until weaning and thereafter as a separate group of weaners. At regular intervals up to two years old, groups of animals were removed and their worm burdens analysed to provide an indication of the seasonal worm populations. It was considered that despite certain qualifications on the use of these "permanent tracers" the advantages outweighed the disadvantages and these are discussed in more detail later. This technique was also used during contemporaneous studies by Henderson and Kelly (1978) in Northern Australia.

This section reports on a two-year study of abomasal worm burdens in young cattle at two estancias in Paraguay.

EXPERIMENTAL DESIGN

Location of Study

Two estancias were used, both situated in the south of Paraguay. The two farms selected for this study varied in several aspects.

Estancia Pirity, situated 280 km south of the capital is a private farm and is considered to be representative of the usual farming system. It had 5,000 cattle on 19,000 hectares. As it is an extensive property, the paddocks are usually bigger, approximately 400 to 500 hectares and the pastures are mainly natural unimproved grass of the same species as in Barrerito.

The mean annual rainfall over the past 10 years was 1,400 mm and the mean weekly temperatures usually ranged from a minimum of 10°C in the winter to a mean maximum of 35°C in summer. At this estancia flooding was common after heavy rains. The stocking rate was one cow per two hectares. Calving is also seasonal, usually in spring. The cattle were entirely Zebu breeds. Even though the calves graze with their dams until weaning, the amount of milk available to young calves is often well below the optimum, so that they had to depend largely on pasture grazing for their nutritional requirements.

Estancia Barrerito situated 170 km south from the capital Asuncion, is an experimental farm and belongs to the Ministry of Agriculture and Livestock. The management system is well above the average of the private farms as it serves as an experimental station. It had 6,000 cattle on 7,000 hectares. The area dedicated to the livestock is divided into several paddocks of about 200 hectares. The mean annual rainfall over the past 10 years was 1,500 mm and temperatures ranged from a mean weekly minimum of 11°C in winter to a mean weekly maximum of 35°C in summer. The pastures were mainly of natural grasses (Cynodon dactylon, Setaria spp, Andropogon lateralis, Panicum spp) though there are some paddocks of improved Colonial grass. The stocking rate was one cow or equivalent per hectare. The cattle population consisted of both Zebu and European breeds. Calving is geared to occur annually in August and September to coincide with the increased growth of grass in spring. Calves usually run with their dams until weaned.

Following the customary routine, the calves grazed with their dams from birth in September until the following March when they were weaned and grazed separately at the stocking rates employed on each estancia.

At regular intervals, from weaning in March 1979 until December 1980, four calves, born in September 1978 at each estancia were housed and slaughtered within 48 hours. These calves were selected at random, from the 36 untreated calves, hereafter designated "permanent tracers" and the dates chosen for their slaughter were weaning and thereafter at intervals of two to three months until the animals were over two years old. It was hoped that this design would provide information on the species and stadial structure of the nematode worm populations from weaning until puberty. To detect any differences due to age or acquired immunity groups of four calves from the 1979 crop were removed and slaughtered at the same time as the survivors of the 1978 crop during late 1979 and 1980. The dates of slaughter are shown in Table B 1.

MATERIALS AND METHODS

Animals

At each estancia 36 newly-born calves were purchased, branded and ear-tagged within one month of birth in September 1978. At the same time 12 cows and another 20 calves belonging to the estancia were also identified. In September 1979 a further 18 recently born calves were purchased, branded and ear-tagged and 12 more cows also identified. The cattle at Pirity

were entirely Zebu breeds whereas those at Barrerito were crossed with varying European breeds.

All of the animals received vaccinations against foot and mouth disease at four monthly intervals but unlike their cohorts the purchased calves received no anthelmintic treatment at these times.

Clinical

Both estancias were visited at intervals of approximately 14 days, although occasional visits could not be made due to the roads being impassable following heavy rain.

At each visit the calves were weighed and examined clinically. The bodyweight of the 20 identified calves belonging to the estancia were also recorded to provide information on growth rates.

Metereological Data

Daily rainfall figures and maximum and minimum temperatures were collected at both estancias. At Pirity they were collected from a metereological station situated 10 kms away from the farm and at Barrerito they were collected on the farm. Temperatures were recorded at 120 cms above ground level in a Stevenson screen.

Parasitological Data

Faeces. Faecal samples were collected from the permanent tracers, the 20 identified estancia calves and 12 cows and examined for the presence of nematode eggs or larvae as described previously by the flotation technique or the modified McMaster method, Gordon & Whitlock 1939 and the numbers present expressed as eggs per gram (epg).

The faeces from the permanent calves were cultured by the method of Roberts and O'Sullivan (1950) and the larvae harvested and identified as described by Keith (1953). Depending on the percentage of different larval species present in the larval cultures, the epg were corrected.

Post-Mortem Examination

At necropsy the lungs were removed and searched for the presence of Dictyocaulus viviparus. The gastrointestinal tract was then removed and processed as described by Ritchie et al (1966). Any worms present in the washings or mucosal digest were counted and identified by microscopic examination. To establish the proportions of H. contortus and H. similis 100 male worms were examined microscopically. In calves which had few worms, all the male worms obtainable were examined.

RESULTS

Only the results pertaining to the abomasal species are presented in full as the detailed clinical data and those from the small and large intestines are being presented elsewhere by a collaborator.

Clinical Data

The growth rates of the 20 calves weighed regularly were slow, averaging 0.32 kg/day at Pirity and 0.33 kg/day at Barrerito. However, the calves did not show any obvious clinical signs of parasitism until July 1980 when severe diarrhoea occurred at Pirity in the 1978 born calves which by then were adult animals of 22 months old. Diarrhoea was present in several of the untreated permanent tracers as well as in the rest of the

weaned herd which had been treated with anthelmintics three times annually. Since several calves had died in the herd anthelmintic treatment was instituted except for the permanent tracers.

Meteorological Data

The rainfall and the mean minimum and maximum temperatures at each estancia are expressed fortnightly in Figures B1 and B2. The rainfall in 1979 was typical for southern Paraguay occurring non-seasonally through the year. At Pirity, the typical mean maximum temperature was recorded in January (mid-summer) with 37°C and the lowest in June (mid-winter) was 8°C. The highest mean fortnightly maximum temperature was recorded at Barrerito in February (late summer) with 37°C and the lowest in June (mid-winter) with 9°C. In 1980 the temperatures were similar, at Pirity the highest temperature was recorded in January with 33°C and the lowest in July with 9°C. However, the rainfall picture in 1980 was atypical as a severe drought occurred at Pirity. The drought commenced in winter and extended over six months through spring and summer(June-December). At Barrerito the drought occurred on two occasions, during autumn for three months (March to May) and spring through summer (September to December).

Parasitological Data

At each estancia the following species of nematodes were identified from the abomasa of the calves: Haemonchus contortus and H. similis, Ostertagia ostertagi, O. leptospicularis, Skrjabinagia lyrata and Trichostrongylus axei.

As mentioned previously the data from the lungs and intestine will be presented elsewhere, but it is worth mentioning that apart from Haemonchus spp, Cooperia spp were the most

prevalent species found.

Faecal Egg Counts

The Haemonchus spp faecal egg counts at Pirity and Barrerito are shown in Tables B2 and B3 respectively. These counts were obtained by applying the percentage yield of Haemonchus L_3 from faecal culture, to the total trichostrongyle faecal egg counts. For young cattle harbouring Haemonchus spp the counts are extremely low. At Pirity, in the 1978 born-calves they remained at or below 120 epg before weaning and only increased to above that level in August and thereafter ranged from 70 to 200 epg until September 1980 when they fell to zero. In the 1979 born-calves the counts were generally higher and reached 340 epg prior to weaning. The levels remained above 100 epg until September when they fell to very low levels during the drought.

At Barrerito the trends were similar with the pre-weaning epg's being higher in the 1979 born-calves (up to 400) again as at Pirity they fell to very low levels in September 1980.

Since T. axei larvae were not recovered from the faecal cultures it was not possible to identify any faecal epg counts attributable to this parasite.

Post-Mortem Worm Burdens

The mean total burdens of Haemonchus spp and T. axei which were the abomasal species commonly present at Pirity and Barrerito are shown in histogram form in Figures B1 and B2 respectively and individual burdens in Tables B4 a to f and B5 a to f respectively. The Haemonchus spp consisted of H. contortus and H. similis and the percentages of each species

at each sampling are detailed in Table B6. Very low numbers of "Ostertagia type" worms were recovered and these are also shown in Tables B4 and B5.

At Pirity a seasonal pattern of infection was common to each of the above genera. In the 1978 born-calves, the mean total adult burdens were relatively low until March 1980 when they exceeded 2,000 for Haemonchus spp and 3,000 for T. axei and individual calves had burdens of 4,300 Haemonchus spp and 7,100 T. axei. During the following winter an increase occurred in the worm burdens of the two genera and the adult Haemonchus spp burdens reached 5,350 (range 4,600 - 5,900) and that of T. axei 20,475 (range 5,100 - 51,100). Subsequently, by late December and after six months' drought, only 500 Haemonchus spp were present in one calf and none in the others, T. axei were present in two calves, 3,600 in the one and 25,300 in the other.

The same general pattern was seen in the 1979 born-calves at Pirity except that the increase in adult worm burdens in July 1980 was of a lower magnitude than in the older calves. At the end of the drought very high T. axei burdens were present with a mean of 43,950 in the two calves, one harbouring 20,000 and the other 67,900.

At Barrerito the adult worm burdens of the 1978 born-calves were generally low and less than those at Pirity, and did not show a marked seasonal pattern. In July 1980, when an increase in worm burdens was recorded at Pirity the mean Haemonchus spp burden at Barrerito was only 1,075 (range 0 - 3,100) and that of T. axei 1,175 (range 0 - 3,600). In late December, after the second drought at Barrerito, Haemonchus spp worm burdens were

also lower although one calf had a Haemonchus spp burden of 4,800. Some increase of T. axei burdens occurred in December although again this was much lower than at Pirity.

At each necropsy, fourth larval stages of Haemonchus spp were usually present and the numbers of these are shown in Figure B3. There was no indication of a significant accumulation of arrested Haemonchus spp L_4 at any season in either estancia.

Ratio of H. contortus and H. similis

From the morphology of the spicules present (Plate 4) the ratio of each species present at each estancia was established.

At Pirity in the 1978 born-calves the H. contortus - H. similis ratio varied from 1 : 0.37 to 1 : 1.66 in 1979 and from 1 : 1 to 1 : 3.40 in 1980. In the 1979 born-calves H. similis was more dominant with H. contortus being present only in July 1980 when the H. contortus - H. similis ratio was 1 : 6.7.

At Barrerito, in the 1978 born-calves the ratio varied from 1 : 0.8 to 1 : 3.5 in the 1979 necropsies while in the 1980 necropsies H. similis was the only species present. In the 1979 born-calves necropsied in 1980 H. similis was the dominant species n 1980 with H. contortus being present only in March, when the H. contortus-H. similis ratio was 1 : 4.6.

DISCUSSION

As mentioned previously, the climate in Southern Paraguay is subtropical with non-seasonal rainfall and seasons which are differentiated mainly on temperature. Occasionally, prolonged periods of drought or flooding occur although this varies locally

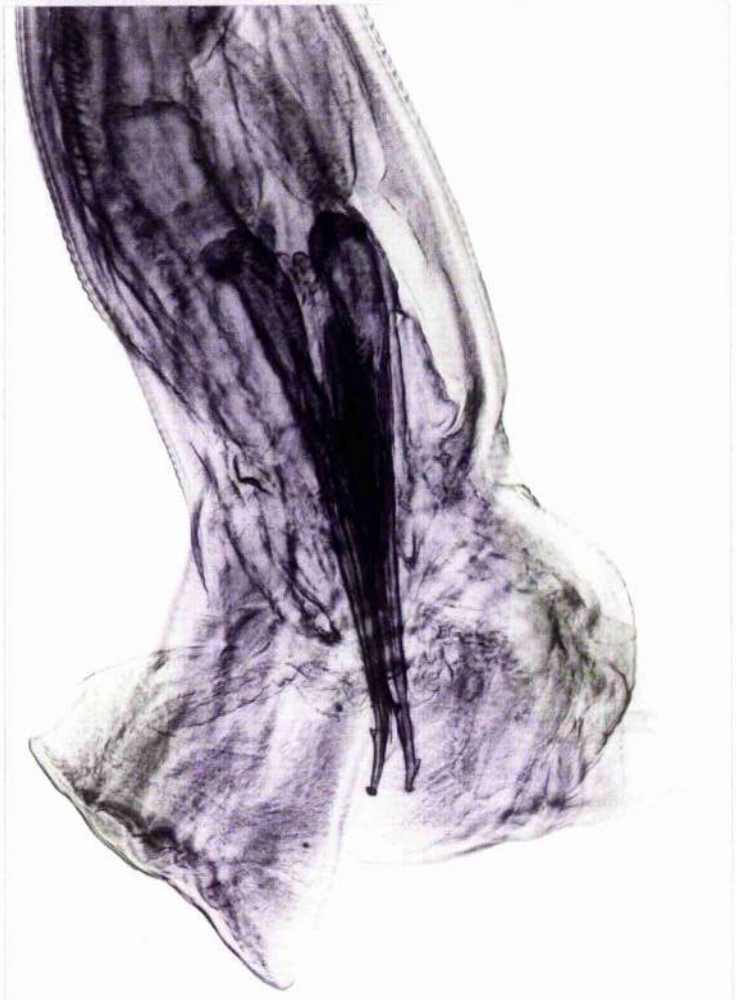
H. contortus (Tail of male)

This specimen features only the spicules in detail. The identifying character in this view is the presence of barbs at dissimilar distances from the spicule tips, that on the left being at about 1/18th of the spicule length, and the right at about 1/9th.

H. similis (Tail of male)

In this specimen also, only the spicules are clearly visible. Differentiation from H. contortus is easily made, the barbs lying at about 1/5th and 1/6th of the spicule length.

H. contortus



H. similis

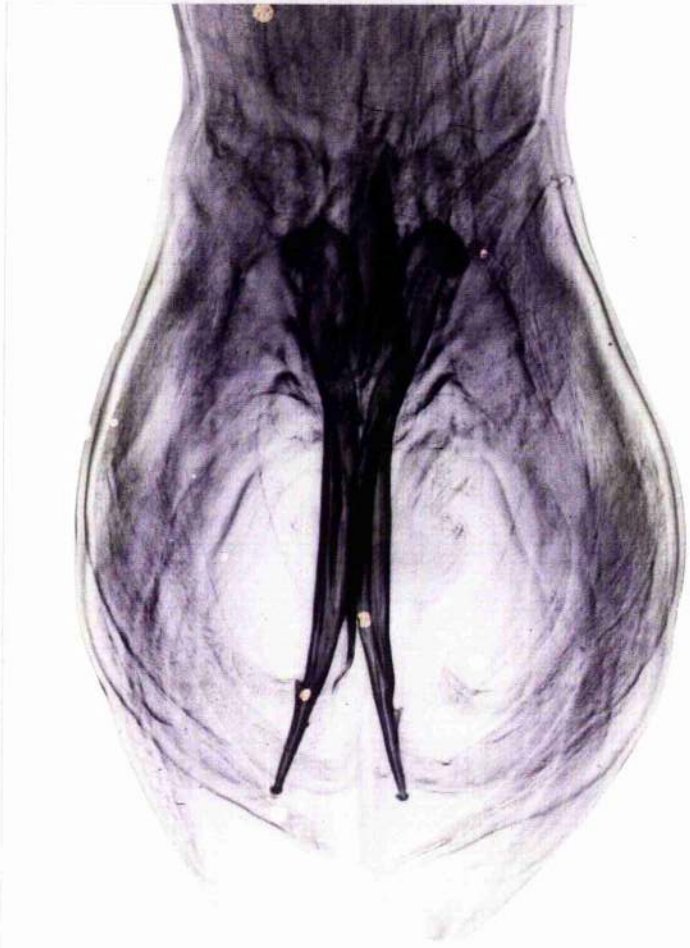


Plate 4. Male bursae of H. contortus and H. similis.

from region to region.

In the current investigation and in contrast to the larval ecology study at Asuncion approximately 170 km to the north, rain did not occur in every month. At Barrerito, there were two periods of drought, the first for three months in March, April and May of 1980 and the second from September to December 1980. At Pirity a prolonged period of drought occurred from June through to December 1980.

From the worm burdens in Tables B3 a to f and B4 a to f it is apparent that the climate at both estancias is suitable for Haemonchus spp, generally regarded as a tropical parasite and T. axei, very much a subtropical parasite; the climate was clearly too warm for Ostertagia spp, which are traditionally temperate creatures.

At Pirity, the availability of Haemonchus spp and T. axei L_3 increased from March through to July 1980, i.e. autumn and winter. Thus the maximum total worm burdens of Haemonchus spp and T. axei occurred in the 1978 born-calves necropsied in July 1980 i.e. when they were 22 months old; coincidentally a marked increase occurred in the worm burdens of the 1979 born-calves necropsied in July 1980 and clinical parasitic gastritis occurred. In the 1979 born-calves these totals were exceeded in the December 1980 necropsies which followed six months drought; T. axei was the dominant species present to the almost total exclusion of Haemonchus spp which had been present in considerable numbers at the July necropsies.

Whether this indicates that the eggs and larvae of T. axei

resist drought conditions better than Haemonchus spp, or whether it reflects a poorer immune response by the cattle to this species is not known.

Ecological studies on T. axei and the closely related T. colubriformis have been confined to experiments using sheep faeces and the protective effect of the bovine faecal pat has not been investigated. Waller and Donald (1970) found that fully embryonated eggs of T. colubriformis resisted desiccation quite well but Mirzayans (1969) and Callinan (1978) found that the optimal temperatures for development were 20°C to 27°C and above 27°C mortality was high. Even allowing for the greater protective effect of the bovine faecal pat some mortality of T. axei eggs and larvae was likely once the mean maximum temperatures increased to above 30°C in December 1980 and during the latter part of the drought.

A possible explanation for the increase in the T. axei burdens of the calves is as follows: a loss of both Haemonchus spp and T. axei could have occurred as part of the recognised turnover of nematode populations in cattle (Michel 1970). The Haemonchus spp were then not replaced because of the greater susceptibility to drought of their free-living stages possibly accentuated by the development of some immunity to these species. In contrast, eggs and L_3 of T. axei survived the early drought better so some reinfection of the cattle took place.

At Barrerito the pattern was different with only relatively low mean burdens throughout. However individual Haemonchus spp burdens often exceeded 1,500 (Table B 5) a level generally expected to give clinical disease yet the calves remained

clinically healthy. The explanation for this is not known but it may be associated with the better grazing at Barrerito, and the good nutritional status of the animals may have helped them to compensate for any blood loss due to the Haemonchus spp worms present throughout in both the 1978 and 1979 born-calves.

The apparent differences between the results obtained at Pirity and Barrerito may also be a reflection of the different weather patterns. Thus, the autumn drought from March to May at Barrerito could have prevented the development of eggs and therefore the availability of L_3 which occurred at Pirity where there was adequate rainfall at this time.

Climatic difference might also account for the differences in worm burdens in July and December 1980 when the drought was more severe at Pirity. Thus, despite the lower stocking rate at Pirity the poorer quality of the grazing in the early drought would have forced the animals to congregate around watering points or in shaded areas where herbage was more prevalent and so contamination from eggs was increased in those areas and eventually the numbers of infective L_3. Under drought conditions grazing is even less selective and local overstocking becomes even more prominent and eventually the animals are restricted in their grazing to the vicinity of a few waterholes, thus increasing the chances of the infective larvae being ingested.

An important factor which determines the host response to parasitism is the plane of nutrition. Undoubtedly, the quantity and the quality of the pasture are much reduced during the winter and even more so with the added complication of drought. If the

pattern of infection shown in this trial is typical, outbreaks of PGE should occur during winter and early spring when the pastures have little growth. According to Taylor (1934), Gordon (1948,1950) and Roberts et al (1952) this has a very important influence on increasing the numbers of larvae that gain access to the host. In such conditions the animals are forced to graze closer to the ground and to spend more time in grazing.

Another possible explanation for the reduction in Haemonchus spp burdens during the drought is that the presence of high T. axei numbers in the abomasum has interferred with their establishment and this has been shown under field conditions by Muller (1968) and experimentally by Reinecke (1974) in South Africa. The latter author attributed this to the unfavourable conditions created for Haemonchus spp in the abomasum, by the elevation of pH which occurs in heavy T. axei infestations (Ross, Purcell and Todd, 1969). This theory could explain the differences between the worm burdens of the calves at Pirity and Barrerito since the T. axei populations at the latter estancia were lower and may not have reached the level required to cause the elevation of pH necessary to affect establishment of Haemonchus spp.

The validity of using worm burdens of untreated permanently grazed calves as an index of seasonal variation in worm populations can be questioned. However, as mentioned in the introduction, in extensive systems such as those used in the beef ranches in Paraguay the use of parasite-naive tracer calves such as are used in temperate regions or intensive systems in the sub-tropics is not practical, and at the time this study was planned

information on the use of anthelmintic-treated permanent calves as tracers was lacking.

Since then, Horak (1978) and Schroder (1978) in South Africa, Graig (1979) in the USA and Le Riche, Kuhne and Dwinger (1982) in Argentina have used permanently grazed calves, given prior anthelmintic treatment, as tracers to monitor the seasonal availability of infective trichostrongyle L_3. The first three of these studies achieved very good results with this type of tracer but Le Riche et al (1982) were less successful, probably because their tracers were housed for long periods after treatment and they failed to adapt quickly to grazing.

Our current results indicate that immunity is acquired very slowly to the main genera of trichostrongyles present in Paraguayan cattle as shown by the high burdens in the calves necropsied in July 1980 when they were 22 month-old, so that in future Paraguayan studies anthelmintic treated permanent tracers could be used without the fear of an acquired immunity significantly preventing the acquisition of abomasal worms.

Despite the criticism that the worm burdens in permanent calves may simply reflect a steady accumulation of these burdens over a prolonged period, this is unlikely, for two reasons. First, Michel (1970) has demonstrated that in many trichostrongyle infections of cattle the worm burdens do not simply accumulate but turn-over in a regular fashion and that the rate of turn-over is related to larval intake.

Secondly, the very marked increase in the worm burdens present in July 1980 suggest that a real increase in the numbers

of larvae available at that time had occurred.

Confirmation that the cooler months of the year, June to August are the most suitable for larval development, migration and survival in Paraguay, provided there is adequate rainfall, comes from the larval ecology studies with H. contortus described in Section A.

The results of this study in terms of abomasal worm species present and their seasonal prevalence are rather similar to those in Southeast Queensland, Australia and some parts of Brazil (Roberts et al 1952; Rick et al 1953; Beck et al 1979; Melo et al 1980). These workers found that infection was present all year round and that Haemonchus spp increased in winter and T. axei in late winter and early spring. Even in drought conditions infections with these species persisted and these infections were ascribed to congregation of stock in wet areas where pasture was still available.

Working in Northwest Argentina, Le Riche et al (1982) also found the same range of abomasal species but the seasonal prevalence differed in that maximum numbers occurred in autumn followed by a marked drop in infection in the winter. The authors ascribed this drop to the development of immunity based on the fact that tracer calves picked up low numbers of trichostrongyles and moderate numbers of Dictyocaulus viviparus. However, in view of the results in the current experiment in which animals failed to develop a good immunity to Haemonchus spp especially H. similis the explanation for Le Riche and his colleague's results may be a reflection of the dry weather in the winters of Northwest Argentina. In the absence of rain all

trichostrongyle larvae would be retained with the faecal pats and not become readily available to the cattle; in contrast lungworm larvae would reach the pasture via the spores of the fungus Pilobolus (Robinson 1962, Jorgensen 1982).

There are several other interesting parasitological findings from the study. The first is the relatively low faecal egg counts of the cattle infected with Haemonchus spp which are known to be high egg producers. This low faecal egg count occurred despite the presence of eggs in the adult females examined (Plate 5). Possibly the low epg could be a reflection of the host diet or nutritional plane. Thus, the majority of the highest epg's were recorded prior to weaning when the calves were dependent solely on a milk diet; after weaning when the calves were dependent on a herbage diet the epg fell to moderately low levels which persisted with the exception of minor elevations. There are several examples of nematode faecal egg counts becoming elevated when the diet was changed and/or the nutritional plane improved (Brunsdon, 1964; Herbert et al, 1969; Whitlock and Georgi, 1976). It is possible that the reverse i.e. a drop in epg's could happen when young cattle are dependent on the meagre post-weaning diet available under extensive grazing conditions although it should be pointed out that Le Riche et al (1982) found much higher Haemonchus spp epg under similar conditions, despite the worm burdens being lower than in the present trial. It is of course possible that the egg counts were being held in check by a developing acquired immunity which was only partially effective and it is known that reduced egg production by worms is

Plate 5. Vulvar region of Haemonchus species female (ex Pirity) showing eggs.

often the first indication of a developing host immunity to gastrointestinal parasites (Urquhart, Jarrett and Mulligan 1962).

The second is the apparent low level of arrested larval development or hypobiosis. Hypobiosis is now widely recognised as a form of adaptation whereby the parasite arrests at a particular stage in the host when environmental conditions are adverse for its free-living development, e.g. in winter in Europe or in the dry season in parts of Africa (Armour, 1980). In Paraguay, where conditions for larval development are usually suitable all year round then it appears that there is no great need for the parasite to select for a high degree of hypobiosis.

Thirdly, the interesting interaction between H. contortus and H. similis. From the data on Table B6 and Table B7 it is possible to observe a difference in the rate of establishment of both species during the two years. Thus, in the calves born in 1978 at both estancias, it can be seen from Table B6 and based on the ratio of the two species that the percentage establishment of H. contortus was greater than H. similis, while in the 1979 born-calves at both estancias H. similis appeared to be the dominant species present, particularly at Barrerito. It is interesting to note that the weather pattern in 1979 was typical for Southern Paraguay with rainfall distributed all year round. On the contrary, in 1980 a severe drought occurred, in two instances at Barrerito, the first from April to May and the second from September to December, while at Pirity the drought commenced in June and extended to December and it was during this latter period that the dominance of H. similis became established

although the actual numbers present were low. Even though it is assumed that there is no difference between the two species related to the requirements of the free-living or parasitic stages, it could be suggested from the results that the infective larvae of H. similis resist drought conditions better than H. contortus and this may account for their dominance apart from the fact that H. similis is naturally a cattle parasite. An alternative explanation is that cattle acquire an immunity to H. contortus more rapidly than to H. similis. Thus, the dominance of H. similis was greater at Barrerito where it reached virtually 100% in 1980 whereas at Pirity some H. contortus were still present. Perhaps the prolonged drought and poor nutrition at Pirity could have lowered the resistance of the cattle allowing more H. contortus to develop. Interestingly, there was no indication that H. similis was more prevalent in the Zebu cattle at Pirity but in fact occurred more frequently in the cross Zebu animals at Barrerito.

In summary the results of these field investigations show that in both herds the bulk of infections were acquired post-weaning, which occurred during autumn at the end of March and when the calves were six months old. At one estancia a marked increase in worm burdens, which consisted mainly of H. contortus, H. similis and T. axei occurred in the winter and spring of one year and led to clinical parasitic gastroenteritis.

In the other estancia, there was no clear increase in burdens in that year probably related to an autumn drought in this estancia. There was no indication that young cattle up to

two years-old had acquired a significant immunity to T. axei and Haemonchus spp (especially H. similis). Only low numbers of arrested larvae were present throughout.

Finally, it would have been interesting to have continued this study beyond the drought and examine whether Haemonchus spp infections had persisted in the faecal pats or perhaps in the soil. Unfortunately, funding did not permit such studies.

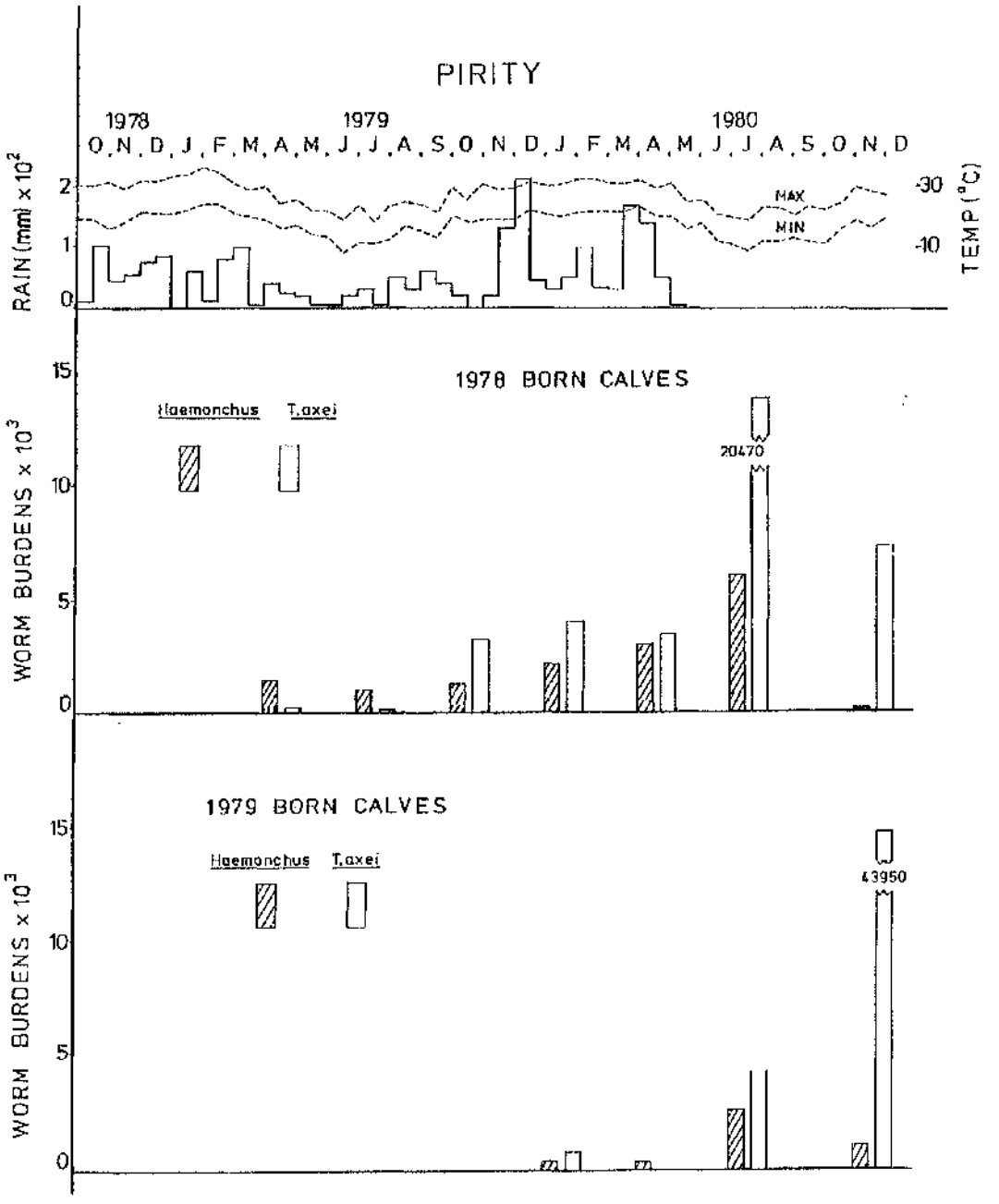


Figure B1. Climatic data plus H.contortus and T.axei worm burdens from calves at Pirity.

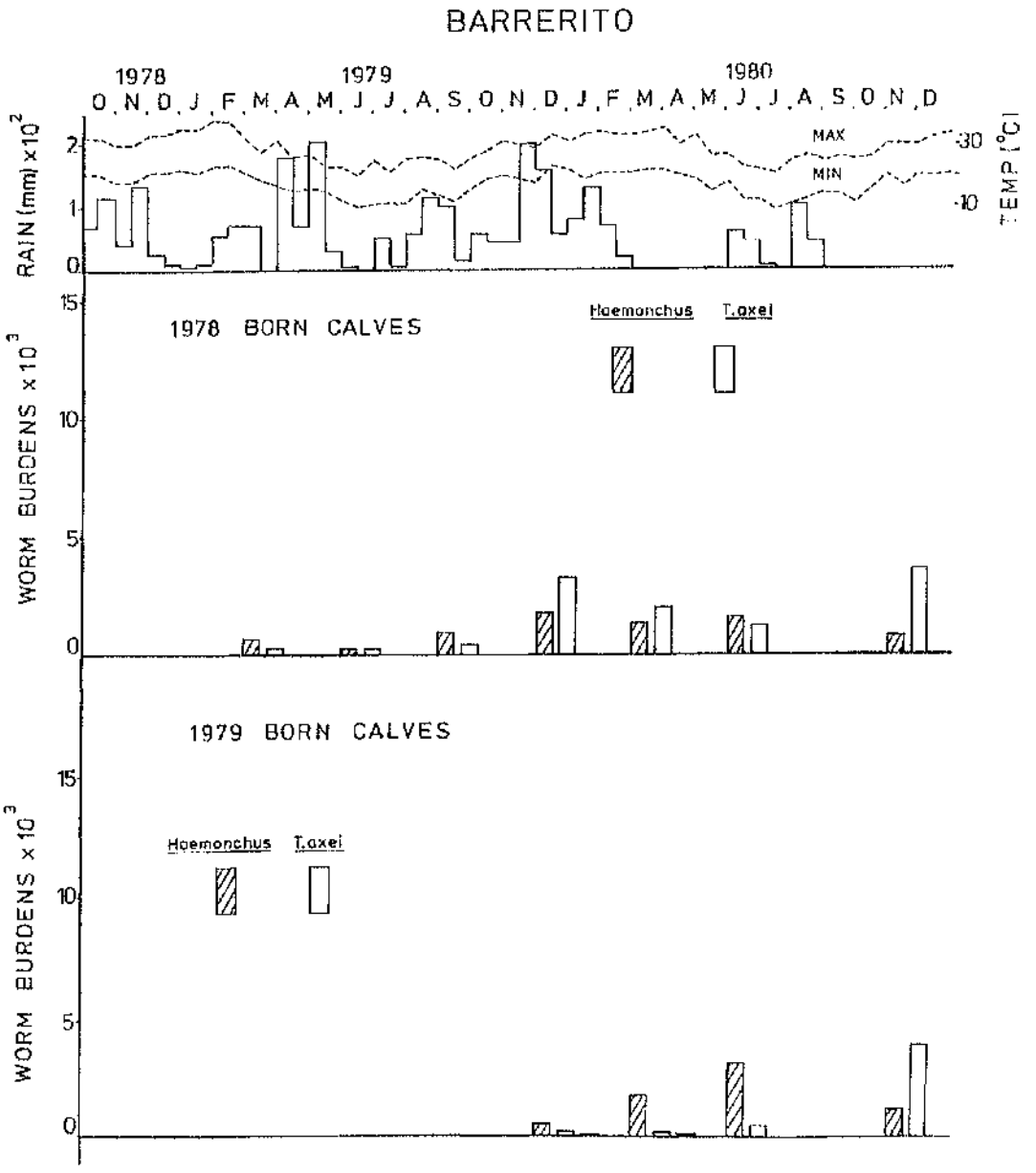


Figure B2. Climatic data plus H. contortus and T. axei worm burdens from calves at Barrerito.

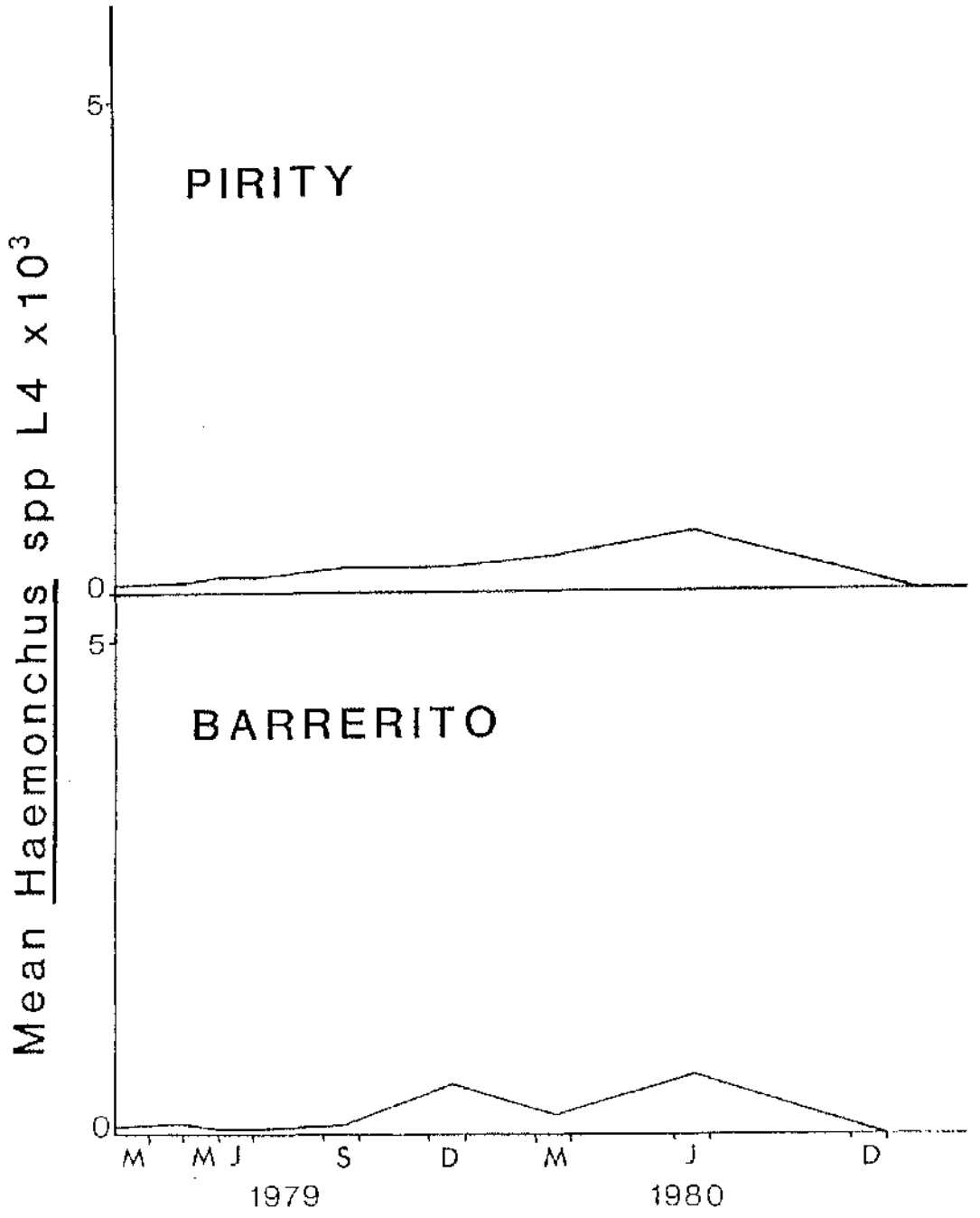


Figure B3. H. contortus L4 burdens form calves at Pirity and Barrerito.

TABLE B 1.

NECROPSY DATES

Season	1978 born-calves	1979 born-calves
Autumn	March 1979 (weaning)	-
Autumn	May	-
Winter	July	-
Spring	September	-
Summer	December	December 1979
Autumn	March 1980	March (weaning) 1980
Winter	July	July
Summer	December	December

TABLE B 2.

MEAN Haemonchus contortus FAECAL EGG COUNTS AT PIRITY

DATE	1978 CALVES	1979 CALVES
11.12.78	95	
28.12.78	100	
9.1.79	120	
26.1.79	80	
6.2.79	75	
24.2.79	75	
9.3.79	15	
30.3.79	25	
25.4.79	40	
9.5.79	75	
3.5.79	60	
26.6.79	85	
9.7.79	80	
30.7.79	75	
23.8.79	170	
21.9.79	210	
5.10.79	135	125
25.10.79	85	210
16.11.79	180	190
12.12.79	130	340
28.12.79	200	110
10.1.80	70	100
7.2.80	75	185
7.3.80	150	180
10.4.80	145	180
2.5.80	150	110
13.6.80	85	100
7.7.80	100	100
15.7.80	125	50
11.8.80	70	45
5.9.80	0	0
8.10.80	0	0
4.12.80	0	

TABLE B 3.

MEAN Haemonchus contortus FAECAL EGG COUNTS AT BARRERITO

DATE	1978 CALVES	1979 CALVES
29.11.78	200	
12.12.78	160	
27.12.78	100	
10.1.79	125	
23.1.79	95	
6.2.79	40	
20.2.79	60	
9.3.79	85	
28.3.79	40	
25.4.79	35	
9.5.79	10	
23.5.79	10	
28.6.79	10	
5.7.79	10	
25.7.79	15	
21.8.79	10	
19.9.79	0	
2.10.79	30	
22.10.79	25	175
14.11.79	50	400
17.12.79	60	310
26.12.79	65	320
12.1.80	70	380
5.2.80	40	210
5.3.80	40	90
25.3.80	25	45
13.6.80	85	110
7.7.80	100	100
15.7.80	125	100
11.8.80	70	50
5.9.80	0	45
7.10.80	0	0
21.11.80	0	0
3.12.80	0	0

TABLE B 4 a.

ABOMASAL WORM BURDENS FROM CATTLE AT PIRITY

DATE	CALF NO.	HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
		A	L_4	A	L_4
March 1979	6	400	100	200	-
	15	700	100	-	-
	31	100	-	-	-
	33	600	100	-	-
	TOTAL	1,800	300	200	-
	MEAN	450	75	50	-
Percentage of H.contortus		60%			
Percentage of H.similis		40%			
May 1979	2	400	-	-	-
	13	200	100	-	-
	19	1,300	300	600	100
	29	1,500	100	200	-
	TOTAL	3,400	500	800	100
	MEAN	850	125	200	25
Percentage of H.contortus		55.5%			
Percentage of H.similis		44.4%			
	O.ostertagi	200			

TABLE B 4 b.

DATE	CALF NO.	HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
		A	L_4	A	L_4
June 1979	3	700	100	-	-
	11	2,000	400	400	-
	34	700	-	-	-
	36	200	-	-	-
	TOTAL	3,600	500	400	-
	MEAN	900	125	100	-
Percentage of H.contortus		72.7%			
Percentage of H.similis		27.3%			
September 1979	8	800	400	2,600	400
	18	2,500	400	4,800	800
	19	400	-	400	-
	30	700	200	3,500	500
	TOTAL:	4,400	1,000	11,300	1,700
	MEAN	1,100	250	2,825	425
Percentage of H.contortus		37.5%			
Percentage of H.similis		62.5%			
	O.ostertagi	500			

TABLE B 4 c.

DATE	CALF NO.		HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
			A	L_4	A	L_4
December 79	16		200	100	700	100
Old calf	17		5,700	600	5,200	900
	26		300	-	2,900	200
	32		1,500	200	4,700	900
		TOTAL	7,700	900	13,500	2,100
		MEAN	1,925	225	3,375	525
Percentage of H.contortus			57.14%			
Percentage of H.similis			42.86%			
December 79	5		500	100	700	-
New calf	6		-	-	500	100
	7		100	-	100	100
	9		1,200	-	2,000	-
	14		300	-	200	-
		TOTAL	2,100	100	3,500	200
		MEAN	420	20	700	40
Percentage of H.similis			100%			

TABLE B 4 d.

DATE	CALF NO.	HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
		A	L_4	A	L_4
March 1980	5	3,400	800	7,100	400
Old calf	9	2,900	100	2,400	100
	23	4,300	300	2,900	-
	27	100	-	500	200
	TOTAL	10,700	1,200	12,900	700
	MEAN	2,675	300	3,225	175
Percentage of	H.contortus	22.73%			
Percentage of	H.similis	77.27%			
	O.leptospicularis	200			
	O.ostertagi	500			
March 1980	1	400	-	-	-
New calf	4	100	100	-	-
	11	500	-	100	100
	12	200	100	-	-
	TOTAL	1,200	200	100	100
	MEAN	300	50	25	25
Percentage of	H.similis	100%			

TABLE B 4 e.

DATE	CALF NO.	HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
		A	L_4	A	L_4
July 1980	4	4,600	1,200	5,100	700
Old calf	10*	5,900	600	51,100	1,600
	20	5,300	–	7,300	900
	22	5,600	600	18,500	2,000
	TOTAL	21,400	2,400	81,900	5,200
	MEAN	5,350	600	20,475	1,300
Percentage of	H.contortus	59.26%			
Percentage of	H.similis	40.74%			
	O.ostertagi	2,300			
	S.lyrata	100			
	S.Kolchida	100			
	* No. 10	Died in extremis			
July 1980	2	1,800	300	22,000	200
New calf	3	1,700	600	4,300	500
	15	300	400	1,400	200
	16	4,700	200	7,300	1,300
	TOTAL	8,500	1,500	15,000	2,200
	MEAN	2,125	375	3,750	550
Percentage of	H.contortus	12.90%			
Percentage of	H.similis	87.1%			

TABLE B 4 f.

DATE	CALF NO.		HAEMONCHUS SPP.		TRICHOSTRONGYLUS SPP.	
			A	L_4	A	L_4
Old calf	7		-	-	3,600	500
December 80	12		-	-	-	-
	25		500	-	25,300	1,200
	35		-	-	-	-
		TOTAL	500	-	28,900	1,700
		MEAN	125	-	7,225	425
Percentage of H.contortus			50%			
Percentage of H. similis			50%			
New calf	10		800	300	67,900	7,500
December 80	13		700	100	20,000	800
		TOTAL	1,500	400	87,900	8,300
		MEAN	750	200	43,950	4,150
Percentage of H.similis			100%			
		Ostertagia spp.	200			

TABLE B 5 a.

ABOMASAL WORM BURDENS FROM CATTLE AT BARRERITO

DATE	CALF NO.	HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
		A	L_4	A	L_4
March 1979	3	400	100	100	-
	11	400	-	900	-
	25	-	100	100	-
	28	500	100	-	-
	TOTAL	1,300	300	1,100	-
	MEAN	325	75	275	-
Percentage of H.contortus		50%			
Percentage of H.similis		50%			
May 1979	12	300	-	100	-
	15	500	-	-	-
	29	800	200	300	-
	33	1,900	200	100	-
	TOTAL	3,500	400	500	-
	MEAN	875	100	125	-
Percentage of H.contortus		55.5%			
Percentage of H.similis		44.4%			

TABLE B 5 b.

DATE	CALF NO.	HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
		A	L_4	A	L_4
June 1979	6	400	–	–	–
	18	500	200	400	–
	22	–	–	–	–
	23	–	–	–	–
	TOTAL	900	200	400	–
	MEAN	225	50	100	–
Percentage of H.similis		100%			
	O.ostertagi	400			
September 79	2	900	100	900	–
	9	600	100	300	–
	24	800	–	300	–
	32	600	200	300	–
	TOTAL	2,900	400	1,800	–
Percentage of H.contortus		54.54%			
Percentage of H.similis		45.46%			

TABLE B 5 c.

DATE	CALF NO.		HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
			A	L_4	A	L_4
December 1979	5		2,400	400	3,200	-
*Old calf	7		1,500	800	6,400	300
	26		-	-	-	-
	31		800	700	3,300	-
		TOTAL	4,700	1,900	12,900	300
		MEAN	1,175	475	3,225	75
Percentage of H.contortus			22%			
Percentage of H.similis			78%			
	Ostertagia spp.		100			
December 1979	155		500	-	100	-
**New calf	281		900	-	600	-
	415		800	300	400	-
	432		-	-	-	-
	444		-	-	-	-
		TOTAL	2,200	300	1,100	-
		MEAN	440	60	200	-
Percentage of H.similis			100%			

* Old calves - Born September 1978

** New calves - Born September 1979

TABLE B 5 d.

DATE	CALF NO.		HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
			A	L_4	A	L_4
March 1980	13		600	100	400	-
Old calf	16		3,300	100	1,900	-
	21		300	-	-	-
	34		300	500	5,900	300
		TOTAL	4,500	700	8,200	300
		MEAN	1,125	175	2,050	75
Percentage of	H.similis		100%			
	O.ostertagi		400			
March 1980	87		800	200	-	-
New calf	218		1,600	200	-	-
	462		1,700	-	-	-
	635		2,100	300	200	-
		TOTAL	6,200	700	200	-
		MEAN	1,550	175	50	-
Percentage of	H.contortus		17.75%			
Percentage of	H.similis		82.35%			
	S.lyrata		100			

TABLE B 5 e.

DATE	CALF NO.		HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
			A	L_4	A	L_4
July 1980	10		-	-	300	-
Old calf	19		3,100	2,300	3,600	-
	30		1,000	-	800	-
	35		200	-	-	-
		TOTAL	4,300	2,300	4,700	-
		MEAN	1,075	575	1,175	-
Percentage of	H.similis		100%			
	O.ostertagi		1,700			
	O.leptospicularis		200			
July 1980	49		2,700	300	600	-
New calf	212		200	-	-	-
	250		3,900	2,800	1,200	-
	425		1,800	-	500	-
		TOTAL	8,600	3,100	2,300	-
		MEAN	2,150	775	575	-

TABLE B 5 f.

DATE	CALF NO.		HAEMONCHUS SPP.		TRICHOSTRONGYLUS AXEI	
			A	L_4	A	L_4
December 80	4		-	-	300	500
Old calf	8		-	-	1,700	200
	17		4,800	300	11,500	500
	20		-	-	-	-
	27		-	-	8,400	-
	36		300	-	400	-
		TOTAL	5,100	300	22,300	1,200
		MEAN	850	50	3,716	200
Percentage of H.similis			100%			
December 1980	63		1,500	300	10,500	500
New calf	232		-	-	-	-
	280		1,600	-	2,100	-
	320		600	300	2,200	200
		TOTAL	3,700	600	14,800	700
		MEAN	925	150	3,700	175
Percentage of H.similis			100%			

TABLE B 6.

RATIO H.contortus : H.similis AT PIRITY

Month	1978 born calves		1979 born calves	
	H.C.	H.S.	H.C.	H.S.
MARCH 1979	1	0.66	-	-
MAY	1	0.80	-	-
JUNE	1	0.37	-	-
SEPTEMBER	1	1.66	-	-
DECEMBER	1	0.75	0	∞
MARCH 1980	1	3.39	0	∞
JULY	1	0.68	1	6.75
DECEMBER	1	1	0	∞

TABLE B 7.

RATIO H.contortus - H.similis AT BARRERITO

Month	1978 born calves			1979 born calves		
	H.C.	:	H.S.	H.C.	:	H.S.
MARCH 1979	1	:	1	-	:	-
MAY	1	:	0.8	-	:	-
JUNE	0	:	∞	-	:	-
SEPTEMBER	1	:	0.83	-	:	-
DECEMBER	1	:	3.54	0	:	∞
MARCH 1980	0	:	∞	1	:	4.6
JULY	0	:	∞	-	:	-
DECEMBER	0	:	∞	0	:	∞

OSTERTAGIA SECTIONS (C,D,E & F)

GENERAL INTRODUCTION

As mentioned in the literature review most outbreaks of bovine parasite gastroenteritis (PGE) in temperate regions involve the abomasal nematode O. ostertagi and there has been a great deal of work on the pathogenesis, epidemiology and control of ostertagiasis which is reviewed by Armour (1980).

Although Ostertagia is by no means the only genus to be regularly encountered at post-mortem of cattle, others such as Trichostrongylus, Cooperia and Nematodirus being frequently present, it is widely recognised that Ostertagia is by far the most pathogenic of the genera responsible for bovine PGE.

The genus Ostertagia

As was stated earlier (p.40) the genus Strongylus created nearly two centuries ago, eventually contained a great many species which had little in common except the presence of a copulatory bursa in the male. The genus Haemonchus was erected in 1898 by Cobb to accomodate Strongylus contortus, and in 1907 Ransom proposed the genus Ostertagia, in which he placed Strongylus ostertagi of Stiles (1892) as the type and, at that time, the sole species. As had happened with Strongylus, Ransom's new genus had the same defect of having insufficient criteria to limit its members to closely related species, and within a few years it contained species as diverse as O. rubidus, O. marshalli and O. mentulatus, later to be given their own generic status as Hyostrongylus, Marshallagia and Camelostrongylus respectively. After many reallocations the group of genera related to Ostertagia was finally placed in a new

subfamily, the Ostertagiinae by Lopez-Neyra in 1947.

In the subfamily are contained the four species relevant to this work, two members of the redefined genus Ostertagia O. ostertagi and O. leptospicularis, and two of the newer genus Skrjabinagia S. lyrata and S. kolchida.

It is not proposed to discuss the relationship in this work, but it should, perhaps, be remarked that these four species are the basis of the interspecific polymorphism hypothesis of Lancaster, Hong and Michel (1983) and Drozdz (1974), O. ostertagi being "paired" with S. lyrata and O. leptospicularis with S. kolchida. The data presented here may, nevertheless, provide additional basic information which may be useful to those involved in this interesting, though still speculative, field of taxonomy.

Concurrent infection with two or more parasitic genera in the same organ is common but with the very low prevalence of bovine haemonchosis in temperate regions the only other abomasal genus found in association with Ostertagia spp is Trichostrongylus, in the form of T axei. Intergenera competition between parasites whether for space (Reinecke, 1974) or nutrients (Holmes, 1961, 1962) has been reported and more recently interspecies interactions have been studied e.g. Oesophagostomum venulosum and Oe. columbianum (Dash, 1981) and O. ostertagi and O. leptospicularis (Al Saqur, Armour, Bairden, Dunn, Jennings and Murray, 1982). In the first section of this thesis there was also an indication that changes in the relative populations of H. contortus and H. similis could be due to interaction between these species or their response to climate or

host influence.

In the case of O. ostertagi and O. leptospicularis the interaction appeared to assume economic importance in that the presence of the latter parasite, even in relatively low numbers, increased the pathogenicity of O. ostertagi (Al Saqur et al, 1982).

These interesting interactions between O. ostertagi and O. leptospicularis and the H. contortus and H. similis data reported earlier prompted a series of experiments in an attempt to answer some of the questions which have arisen. In particular, it was decided to study under both laboratory and field conditions whether there were subtle differences in the ecology of the free-living stages of O. ostertagi and O. leptospicularis which might account for the apparent high frequency of the former parasite and the occasional appearance of the latter in large numbers. The interaction of the two parasites was also investigated by adding low numbers of O. leptospicularis L_3 to a high number of O. ostertagi L_3 and subjecting the mixed larvae to serial passage in calves. Since a pre-requisite to any epidemiological studies in the field would be the differentiation of the infective larvae of the two species this task was undertaken first of all.

SECTION C

THE DIFFERENTIATION OF OSTERTAGIA OSTERTAGI AND O. LEPTOSPICULARIS FREE-LIVING LARVAL STAGES

INTRODUCTION

Studies on the epidemiology of gastrointestinal nematode infections are generally carried out in two principal ways. In the first, the worm burdens of tracer calves are used as an index of the infective larval populations on the herbage. Since most epidemiological studies run for a minimum of 18 to 24 months this is expensive in terms of calves. In the second and more widely used, at least in temperate areas, herbage samples are collected, soaked in water and detergent and the residue analysed for the presence of larvae. The major drawback of this inexpensive technique is that morphological identification of larvae can usually only be made to genus level. For differentiation of larvae to species level it is necessary to measure, the total length of larvae or certain parts of the larvae, the distance between the tail of the third stage larvae and the tip of the L_2 sheath being one criterion which is frequently used.

In this chapter an attempt is made to establish criteria for the differentiation of the L_1, L_2 and L_3 free-living stages of O. ostertagi (O.O.) and O. leptospicularis (O.L.).

MATERIALS AND METHODS

Animals

Two parasite-naive Friesian calves, aged four months were each infected with 100,000 Ostertagi L_3, one calf receiving O. O. and one O. L.

Origin of Larvae

The O. O. culture was derived from a field strain containing 97% O. O. and 3% S. lyrata. It was isolated from a farm near Glasgow and passaged six times. It is the same isolate as that

used by Al Saqur et al (1982) and coded GB3 by these authors; with extra passages it was coded GB6 at the onset of the current experiment.

The O. L. culture was isolated from a mixed culture of O. O. and O. L. described by Al Saqur et al (1982) as strain GC3 and also isolated locally in the Glasgow area. It also contained low numbers of S. lyrata and S. kolchida. To purify the O. L. it was passaged twice through sheep and at necropsy of the second sheep yielded 100% O. L. male worms on morphological identification.

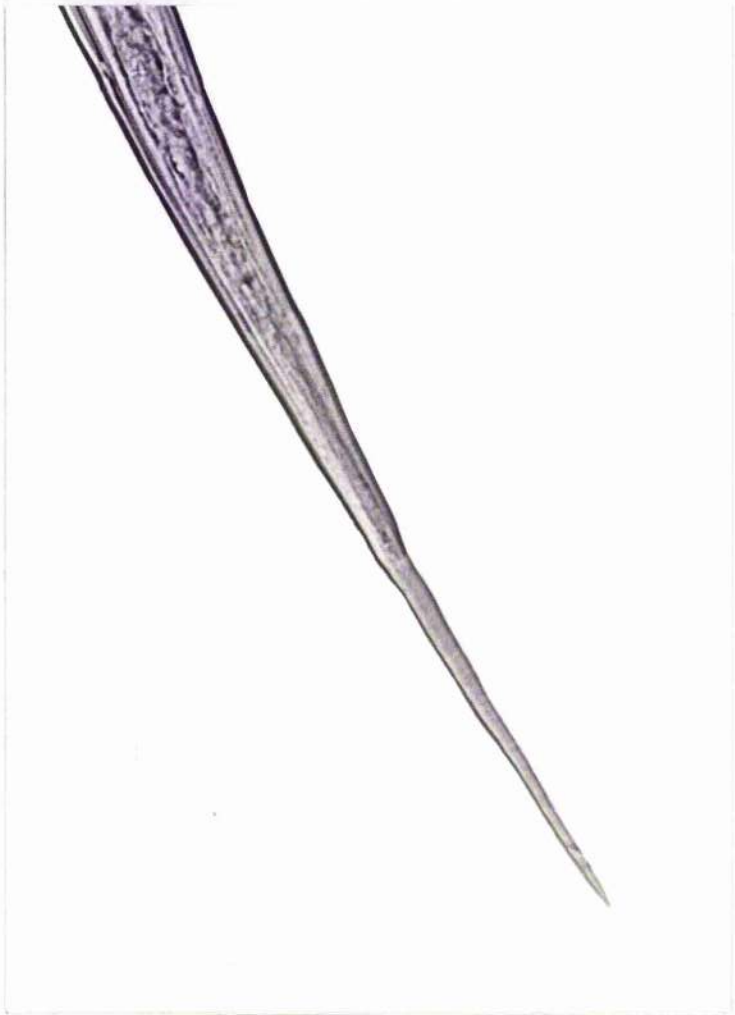
Culture of Larvae

Twenty-one days after infection the faeces of each calf were mixed with vermiculite, placed in 26 jars and cultured for 13 days at 21°C as detailed in the General Materials and Methods. On each day following incubation larvae were harvested from one culture jar, killed and subjected to measurement.

Larval Measurement

One hundred larvae, killed with 10% formalin were traced daily from day 2 to day 13. One hundred larval traces were made each day; those from day 2 through to day 9 were on L_1 and L_2 and from day 10 to day 13 on L_3. The traces, initially drawn using a Leitz drawing tube attached to a Leitz M20 compound microscope at a magnitude x 10, were measured using a digitiser (Plate 6) linked to a Commodore PET computer and printer as detailed in the General Materials and Methods. The more traditional approach to larval measurement using a calibrated eyepiece micrometer was also employed and using this method the sheath prolongations of 100 O. O. and 100 O. L. third stage

O.ostertagi



O. leptospicularis

Plate 6. Sheath prolongations of O.ostertagi and O.leptospicularis third stage larvae.

larvae were measured on day 13.

In addition to measuring sheath prolongations in L_3, the oesophageal length (buccal capsule to end of oesophagus), the intestinal length (end of oesophagus to anus) and the tail length (anus to tip of tail) were marked on the traces and measured.

The distance was calculated in microns and the results analysed using a VAXC Mini Tab computer programme (Penn.State University, 1982) at Strathclyde University Mathematics Department.

Statistics

A two sample T test was used to analyse the data from L_3 of both species while the interaction between the early L_1/L_2 stages was checked using a two way analysis of variance.

RESULTS

Larvae were first recovered on day 2 and the results are presented from day 2 through to day 13. For the pre-infective stages (L_1/L_2) comparative measurements of the three criteria measured through days 2 to 9 are summarised in Tables C 1, C 2 and C 3 which also include the mean and standard deviation values. Figure 1 compares the frequency distributions of the parameters measured for both Ostertagia spp at the L_3 stage and the mean and standard deviation values are given in Table C4.

To take account of data collected on different days, analysis for species' differences was undertaken using an analysis of variance in which the data were blocked according to day of development. Table C 1 shows the results of the analysis of oesophageal lengths and indicates a significant interaction between species and day of development. This means that the

development pattern of length over days 2 to 9 is not the same for the different species. The means and standard deviations for each species on each day are also shown in Table C 1. Examination of the means indicate the nature of the interaction. On day 2 the mean O.O. length is 38.16 u less than the mean of the O. L. lengths. However, by day 6 this difference drops to 5.11 u. Thereafter the mean lengths diverge again and on day 9 the mean O. O. length is 32.20 u less than those of the O. L. lengths.

Similar analyses were undertaken for intestinal and anus-tail lengths. The results are shown in Tables C 2 and C 3 and in both cases significant interactions are indicated. In the case of intestinal lengths, shown in Table C 2, the interaction pattern is very irregular. On days 2, 4, 8 and 9 the O. L. have the higher means whereas on days 3, 5, 6 and 7 the O. O. have the higher means. Finally (Table C 3) for anus-tail length, the means are similar initially but from day 5 until day 8 the O. O. mean lengths are consistently higher than those of the O. L. mean lengths. By day 9 the O. L. larvae had increased to a level 5 u longer than the O. O.

It can be seen from Figure C1 that only the results pertaining to the oesophageal length and anus to tail are presented in histogram form. This is because of the wide scatter of measurements of the intestinal lengths and their lack of significance.

From the results shown in Table C 4 and Figure C 1 it is clear that the oesophageal length and sheath prolongation of the

two species were significantly different. Thus, the mean oesophageal length of O. O. was only 125.50 u and that of O. L. was 140.40 u, a difference which was significant at a level of $P < 0.001$. The mean sheath prolongation was 59.21 for O. O. and 52.02 for O. L a difference of 7.19 which was again highly significant ($P < 0.001$); the differences in sheath prolongation are illustrated in Plate 6. There were no significant differences in the measurements of intestine, anus-tail and total lengths.

DISCUSSION

The object of this experiment was to compare measurements of free-living larvae derived from relatively pure cultures of O. O. and O. L. in order to obtain accurate data which would facilitate the differentiation of these species. For this purpose a computerised method of measurement and analysis was used. From the results of measurements of the L_1 and L_2 stages there are significant differences in the development rate of the oesophagus and intestine in each species. The anus to tail lengths were also significantly different. The anus-tail measurements of O. O. were consistently greater than those of O. L. and this is consistent with the differences in sheath prolongation length noted in the L_3. At least some of these differences may be related to varying times of moulting from L_1 to L_2 although no clear indication of the time of ecdysis was obtained. However, from a practical viewpoint the data on the L_1 and L_2 are rather academic since it is usual to compare the morphology of trichostrongyle L_3 as a means of species recognition rather than L_1 and L_2.

From analysis of the data on L_3, two of the parameters measured namely the oesophageal length and the sheath prolongation yielded data on lengths which were statistically different. The oesophagus of the L_3 of O. O. was shorter by a mean of 14.09 u and the sheath prolongations were longer by a mean of 7.19 u. Shorter measurements than these were consistently obtained using visual measurements. However, despite the fact that the visual measurements were always less than those obtained by the computerised system the trend was the same and the differences were almost identical suggesting that simple measurements could detect the differences in the two species. However, although these measurements are mathematically different it is questionable whether they are great enough to be recognised simply by visual examination without recourse to measurement. There is also considerable overlap which would necessitate measuring at least 100 larvae.

There have been no other similar studies with O. L. to compare the data with but O. O. larvae have been measured by several authors. In most instances those have been visual measurements of larvae from undefined strains and the only comparable measurements are total larval length and sheath prolongation.

For example, Keith (1953) found that the total length of O. O. ranged from 842 - 917 u compared with a mean of 783 (range 641 - 1025 u) in the present observations. This simply highlights the marked differences which can occur between larvae of apparently the same morphological species from different

laboratories and emphasises the need to specify origin and passage of strains of larvae. In Keith's analysis the range of sheath prolongation was 50 to 71 u against 45 to 80 u in the current trial. In another publication (Ministry of Agriculture, 1971) the total length of O. O. is said to range from 780 - 980 u which is closer to the result of the present trial but still much higher overall. From the results of the current experiment it would not be possible to differentiate O. O. and O. L. infective larvae on total lengths.

Generally, these results indicate that the differentiation of O. O. and O. L. free-living larvae at field laboratory level would be very difficult. Also, the considerable range of measurements obtained for the total length of O. O. and the differences to the results of other laboratories emphasises the difficulty for field investigators in differentiating L_3 trichostrongyle species without clear morphological characteristics. It would also help if laboratories standardised the techniques for producing, storing and measuring larvae to obviate the effects of shrinkage and optical delusion.

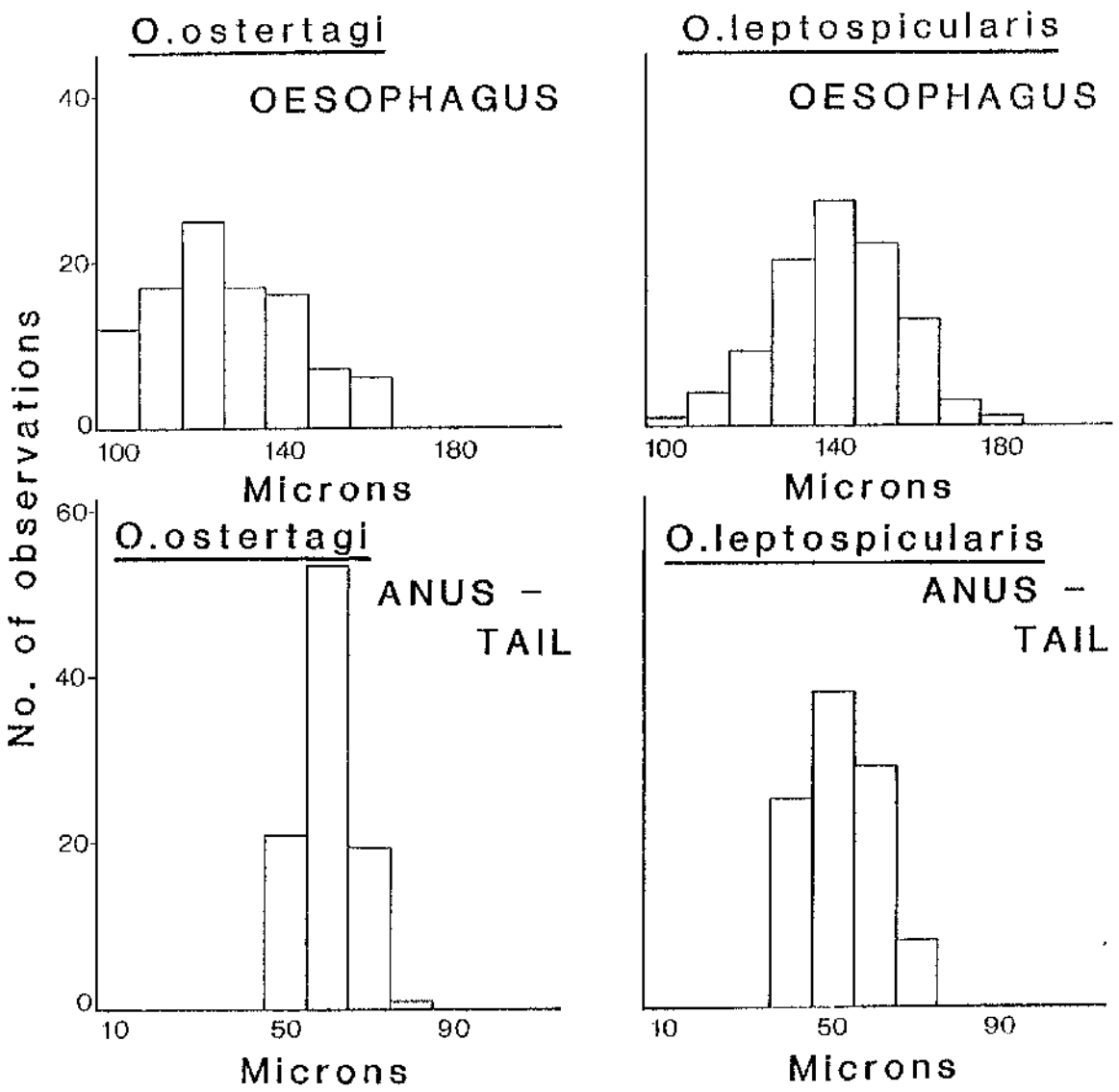


Figure C1. Frequency distributions of oesophageal and anus to tail lengths of O.ostertagi and O.leptospicularis third stage larvae.

TABLE C 1.

SUMMARY OF OESOPHAGEAL MEASUREMENT LENGTHS (MICRONS) FROM 10 O.ostertagi AND O.leptospicularis 1ST/2ND STAGE LARVAE ON DAYS 2 - 9 AND RESULTS OF ANALYSIS OF VARIANCE

		DAY							
		2	3	4	5	6	7	8	9
O.o.	Mean	74.09	79.80	87.26	108.80	115.47	122.87	107.27	121.47
	sd	18.22	14.16	8.18	9.34	14.80	15.50	18.17	12.55
O.l.	Mean	112.25	104.94	112.76	118.00	120.58	147.25	143.89	153.67
	sd	22.74	16.94	11.16	16.00	15.77	16.00	13.11	23.88

Analysis of variance

Source	DF	SS	MS
Days	7	449.18	64.17
Species	1	240.76	240.76
Day x speceis interaction	7	50.27	7.18
Error	144	365.84	2.54
Total	159	1106.05	

$F = 2.82$ Significant $p < 0.01$

TABLE C 2.

SUMMARY OF INTESTINAL MEASUREMENT LENGTHS (MICRONS) FROM 10 *O.ostertagi* AND *O.leptospicularis* 1ST/2ND STAGE LARVAE ON DAYS 2 – 9 AND RESULTS OF ANALYSIS OF VARIANCE

		DAY							
		2	3	4	5	6	7	8	9
O.c.	Mean	176.64	215.62	285.85	373.76	444.19	511.36	365.02	419.39
	sd	30.40	19.45	36.80	47.39	38.06	38.51	90.92	81.29
O.l.	Mean	221.79	206.98	294.76	298.55	361.68	474.04	490.80	428.81
	sd	56.62	43.42	32.75	32.47	49.28	46.50	85.46	41.51

Analysis of variance

Source	DF	SS	MS
Day	7	19,952.2	2,850.3
Species	1	1.3	1.3
Day x species interaction	7	1,433.1	204.7
Error	144	3,916.2	27.2
Total	159	25,302.8	

$F = 7.52$ Significant $p < 0.01$

TABLE C 3

SUMMARY OF ANUS TO TAIL MEASUREMENT LENGTHS (MICRONS) FROM 10 O.ostertagi AND O.leptospicularis 1ST/2ND STAGE LARVAE ON DAYS 2 - 9 AND RESULTS OF ANALYSIS OF VARIANCE

		DAY							
		2	3	4	5	6	7	8	9
O.o.	Mean	59.94	107.90	91.34	125.43	144.50	143.47	122.18	125.64
	sd	12.16	15.65	29.67	6.89	14.67	6.79	19.79	9.46
O.l.	Mean	60.56	95.68	105.05	110.80	114.76	136.24	116.50	130.43
	sd	4.57	34.07	10.02	21.14	28.33	31.90	22.45	22.09

Analysis of variance

Source	DF	SS	MS
Days	7	838.42	119.77
Species	1	29.02	29.02
Day x species interaction	7	70.81	10.12
Error	137	619.98	4.52
Total	159	1,558.24	

$F = 2.23$ Significant $p < 0.05$

TABLE C 4.

SUMMARY OF MEASUREMENT LENGTHS (MICRONS) FROM

100 O.ostertagi AND O.leptospicularis 3RD STAGE LARVAE

		O.o.	O.l.	t-value	Significance
Oesophagus	Mean	125.50	140.40	5.69*	$p < 0.001$
	sd	21.60	14.70		
Intestine	Mean	479.40	476.80	0.22	NS
	sd	97.50	69.70		
Anus-tail	Mean	117.10	117.20	0.06	NS
	sd	14.60	27.70		
Sheath prolongation	Mean	59.21	52.02	-6.81**	$p < 0.001$
(Digitiser system)	sd	6.59	8.24		
Sheath prolongation	Mean	40.57	32.80	10.81	$p < 0.001$
(Visual measurement)	sd	4.54	5.57		
Total length	Mean	783.03	785.93	1.067	NS
	sd	90.02	80.67		

* Positive t-value indicates oesophageal lengths of O.o. are significantly shorter than those of O.l.

** Negative t-value indicates sheath prolongations of O.o. are significantly longer than those of O.l.

SECTION D

THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF FREE-LIVING STAGES OF OSTERTAGIA OSTERTAGI AND O. LEPTOSPICULARIS UNDER LABORATORY CONDITIONS

INTRODUCTION

Al Saqur *et al* (1982a) demonstrated that the faecal egg counts (epg) of calves infected with *O. leptospicularis* (O. L.) either in pure culture or mixed with *O. ostertagi* (O. O.) were markedly higher than those of pure or almost pure O. O. infections. Under field conditions a similar elevation of the epg's of calves given a mixed infection of O. O. and O. L. compared to pure O. O. infection was observed (Al Saqur *et al*, 1982b).

From the results of these latter observations one would have expected that high numbers of O. L. would become established in grazing calves yet Al Saqur *et al* (1982) found that only four per cent of the total *Ostertagia* worm population in calves necropsied in late autumn at the end of the grazing season were O. L. the majority being O. O. This may simply reflect that calves acquire an immunity to O. L. more rapidly, hence the relative accumulation of O. O. worms. However, Since at the time it was not possible to differentiate the free-living larvae of O. O. and O. L. from the pasture samples it was impossible to estimate levels of challenge and so confirm whether fewer O. L. had established; hence the experiment described in Section C.

Another explanation could be that the free-living stages of the two species have different temperature and humidity requirements for their development and survival and so it was decided to first study their development under different constant temperatures in the laboratory and then under the varying temperatures and humidities which occur in the field . This

experiment reports the results of the laboratory studies on the effect of two temperatures on the rate of larval development and the yield of O. O. and O. L. from faecal culture.

EXPERIMENTAL DESIGN

Faeces, collected from the monospecific donor calves, were incubated at two temperatures (15°C and 21°C). Replicate samples were examined daily from day 2-25 and the rate of development and larval yield from a known number of eggs studied.

MATERIALS AND METHODS

Animals

Two parasite-naive male Friesian calves aged four months were each infected with 100,000 Ostertagia L_3, one calf receiving O. O. and one O. L.

Origin of Larvae

Ostertagia eggs of both species were obtained from a single passage of the O. O. strain (coded GB3 by Al Saqur et al 1982) and since passaged three times (now coded GB6) and the O. L. strain (coded GC3 by Al Saqur et al 1982) through two parasite-naive calves (now coded GC5).

Faeces were collected from the donor calves using a harness and bag system as described in the General Materials and Methods.

As with the normal culture procedure the faeces were mixed with vermiculite until a firm consistency was obtained. Approximately the same volume of faeces was placed in each container (the exact faecal weight was then determined) and the jar lids loosely screwed on.

A sufficient number of individual samples to enable

replicate examinations at two temperatures, at 24 hourly intervals, over a period of 25 days were prepared. The jars were then stored in two incubators, one at 15°C and the other at 21°C.

RESULTS

The larval recoveries expressed as a percentage of the eggs cultured are shown in Figure D1 for the 15°C culture. Tables D1 and D2 detail the yields numerically.

Larvae were recovered from most cultures 24 hours post-incubation with the exception of O. L. at 21°C when 72 hours elapsed before larvae were obtained from one replicate.

At both temperatures the recoveries of early first and second stage larvae were relatively low with individual peaks of 20% and 15.6% being recorded for O. O. and O. L. on days 6 and 8 respectively.

At 15°C O. O. larvae reached maturity, i.e. to the infective third stage in 10 days while O. L. required only nine days to reach this point, mean recoveries being 1.2% and 10.4% respectively.

After days 9 to 10 larval recoveries of L_3 were greater with individual maximum yields of 31.5% O. O. and 57% O. L. being obtained at 15°C on days 22 and 15 respectively. However, at the higher temperature of 21°C both species attained the infective L_3 stage simultaneously on day nine, with means of 6.9% of O. O. and 8.4% of O. L. eggs having successfully developed. At 21°C an individual maximum recovery of 43% for O. O. was recorded on day 17 with the highest single recovery of O. L. again being 57% on day 23.

There appeared to be a greater fluctuation of results from

both species at 21°C and it was also noticed that from day 20 onwards a progressively higher number of dead larvae were present.

DISCUSSION

The object of this experiment was to investigate the effects of two temperatures on the development of O. O. and O. L. in an attempt to explain the relative dominance of O. O. in grazing calves (Al Saqur et al, 1982b).

The temperatures of 21°C and 15°C were chosen to simulate the mean maximum temperatures in mid-summer and in early autumn respectively in West Scotland. It had been observed that O. L. established in reasonably high numbers in late spring and summer but the numbers of this species were much reduced by the autumn (Al Saqur et al, 1982b) possibly due to unfavourable conditions for its development.

The results in yields of larvae are interesting in that much lower numbers of L_1 and L_2 were recovered at both temperatures and with both species. This is probably due to the sluggish nature of the L_1, L_2 compared to the L_3 and the recovery technique which relies heavily on the motility of the larvae. The harvesting of larvae without baermannising (the use of sieves), was chosen to minimise total larval loss but may have biased the yields of L_1 and L_2 compared to the more motile L_3.

The yield of L_3 of both species was very satisfactory reaching over 50% in the case of O. L. at the highest temperature of 21°C. Egg to L_3 production by this species was also generally greater at 15°C. Clearly, the apparent drop in the numbers of

O. L. established in grazing calves in the autumn could not be attributed to the effect of lower mean maximum temperatures. However, it may be that the survival of O. L. larvae is affected by the lower minimum temperatures found in autumn and this aspect is investigated in the field studies in the next section.

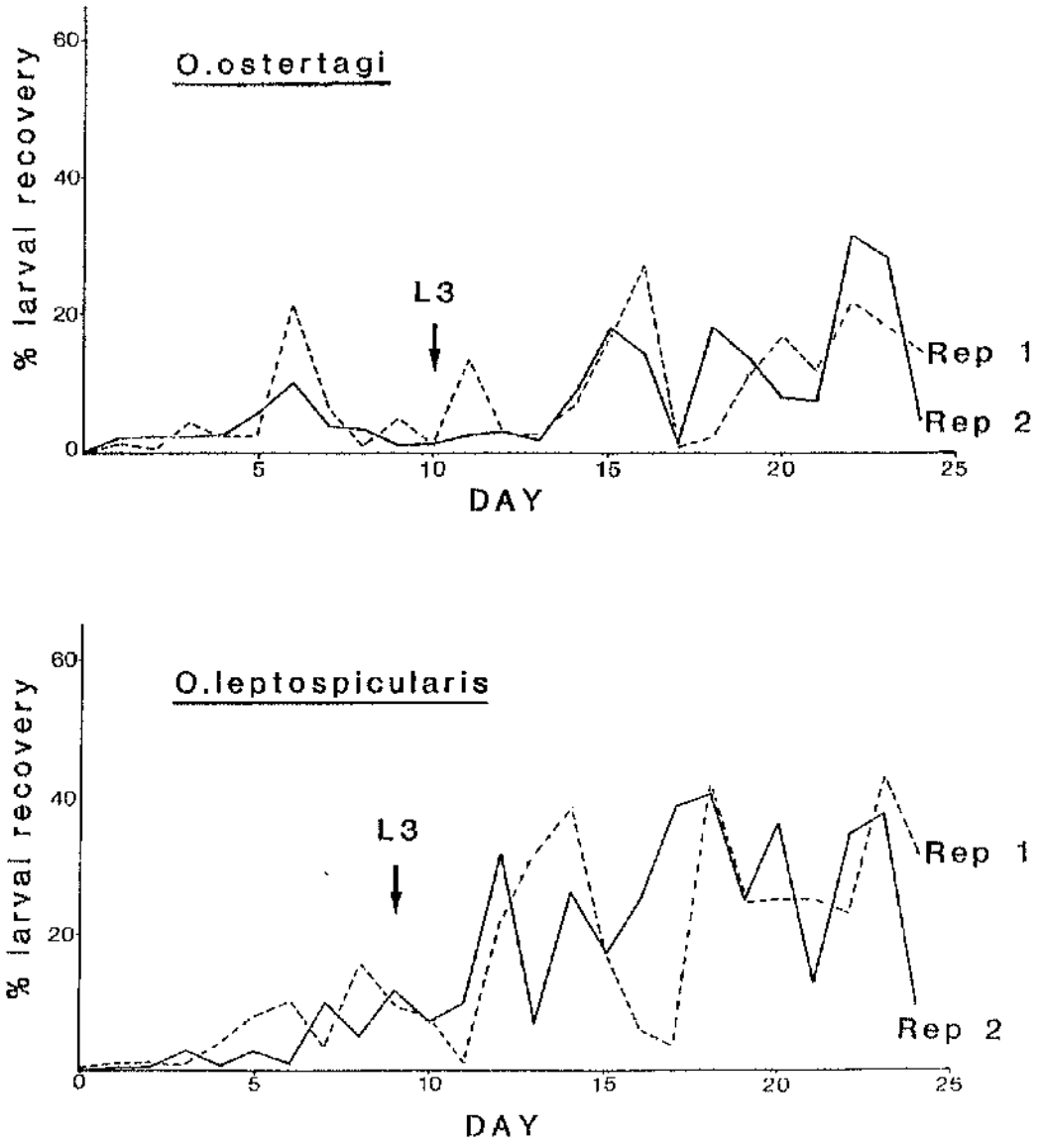


Figure D1. % larval recoveries of O.ostertagi and O.leptospicularis from faeces cultured at 15°C.

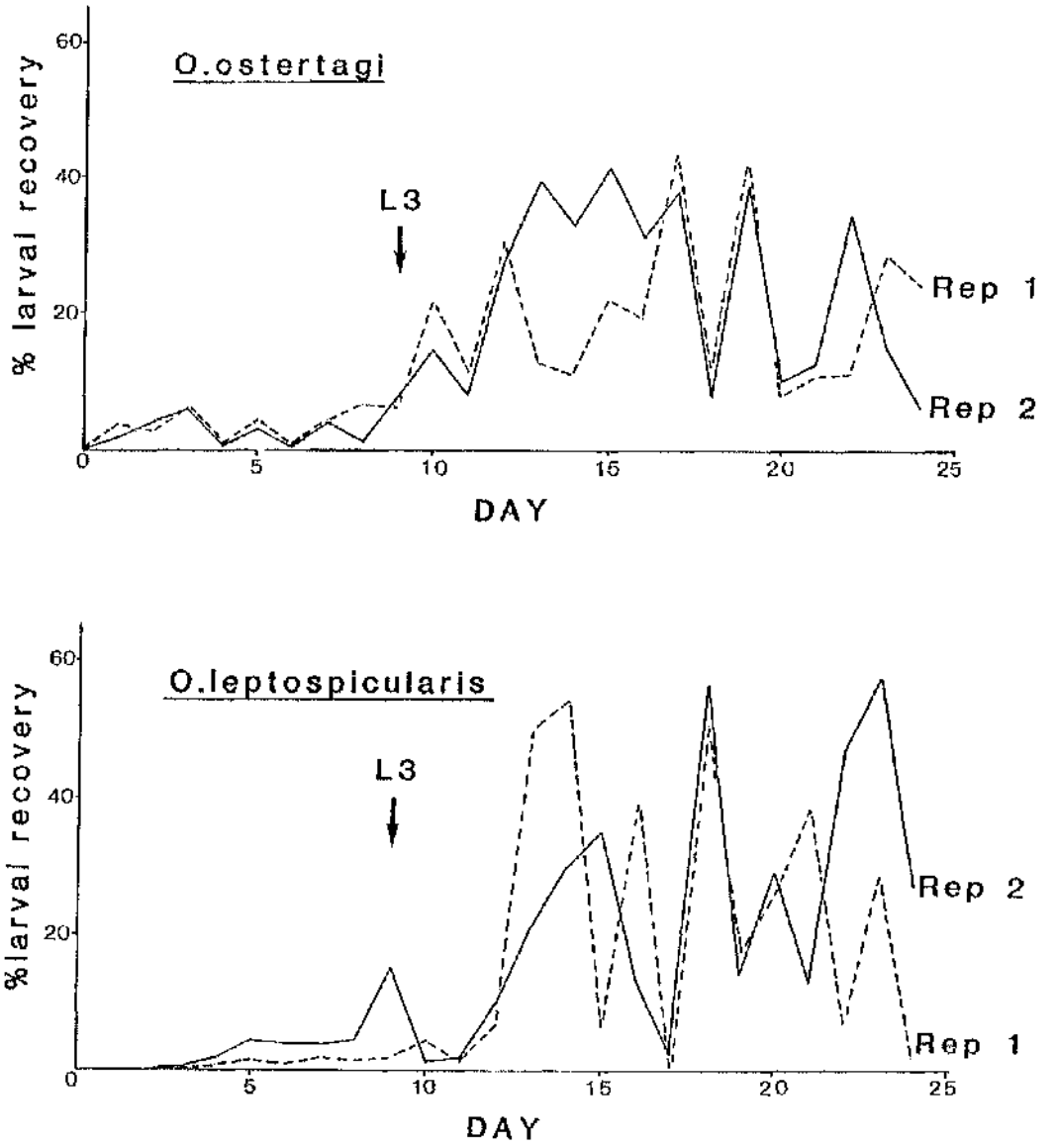


Figure D2. % larval recoveries of O.ostertagi and O.leptospicularis from faeces cultured at 21°C.

TABLE D 1. % RECOVERY OF LARVAE FROM DAILY FAECAL CULTURES AT 15° C

		O.ostertagi			O.leptospicularis		
	Day	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean
	0	0	0	0	0	0	0
	1	2.09	1.54	1.81	0.12	0.33	0.23
	2	2.15	0.27	1.21	0.33	0.97	0.65
	3	1.97	4.02	2.99	2.84	0.87	1.86
	4	2.29	2.27	2.28	0.77	4.09	2.43
	5	5.46	2.23	3.85	2.89	7.71	5.30
	6	9.83	20.84	15.34	1.02	9.75	5.39
	7	3.42	6.35	4.89	9.80	3.06	6.43
	8	3.13	0.85	1.99	4.73	15.68	10.21
	9	0.83	4.65	2.74	L_3 11.36	9.34	10.35
L_3	10	1.00	1.31	1.16	7.12	7.74	7.43
	11	2.21	12.60	7.41	9.86	1.02	5.44
	12	2.77	2.44	2.61	31.40	21.73	26.57
	13	1.58	2.34	1.96	6.81	32.13	19.47
	14	8.27	6.60	7.44	26.00	38.54	32.27
	15	17.67	15.49	16.58	17.11	57.13	37.12
	16	14.09	26.62	20.36	25.18	5.85	15.52
	17	0.97	0.79	0.88	38.42	3.53	20.98
	18	17.60	1.97	9.79	40.23	41.23	40.73
	19	13.20	11.30	12.25	24.91	24.81	24.86
	20	7.29	16.80	12.05	36.07	25.30	30.69
	21	6.90	11.74	9.32	12.57	24.59	18.58
	22	31.50	21.27	26.39	34.43	23.09	28.76
	23	28.06	18.04	23.05	37.72	42.51	40.12
	24	4.45	14.29	9.37	9.60	31.69	20.65

TABLE D 2. % RECOVERY OF LARVAE FROM DAILY FAECAL CULTURES AT 21°C

	O.ostertagi			O.leptospicularis		
Day	Replicate 1	Replicate 2	Mean	Replicate 1	Replicate 2	Mean
0	0	0	0	0	0	0
1	2.12	3.92	3.02	0	0	0
2	4.19	2.87	3.53	0	0.07	0.04
3	6.00	5.94	5.97	0.22	0.02	0.12
4	1.08	1.63	1.36	2.00	0.79	1.40
5	2.96	4.40	3.68	4.43	1.53	2.98
6	0.65	0.83	0.74	3.88	0.91	1.94
7	3.98	4.30	4.14	4.05	2.03	3.04
8	1.27	6.76	4.02	4.55	1.65	3.10
L_3 9	7.39	6.37	6.88	L_3 14.63	1.98	8.41
10	14.39	21.40	17.90	1.40	4.53	2.97
11	7.92	11.32	9.62	2.00	1.69	1.85
12	27.11	30.20	28.66	19.83	6.93	13.38
13	39.13	11.53	25.33	20.93	49.49	35.21
14	32.89	10.96	21.93	29.74	53.86	41.80
15	41.23	21.42	31.33	35.17	6.19	20.68
16	30.99	19.02	25.01	13.88	39.33	26.61
17	37.65	43.13	40.39	2.67	0.79	1.73
18	7.82	10.89	9.36	56.31	50.47	53.39
19	38.56	36.83	37.70	14.35	16.96	15.66
20	10.56	8.33	9.45	28.95	25.78	27.37
21	12.56	10.79	11.68	13.20	37.92	25.56
22	34.03	11.23	22.63	47.26	6.96	27.11
23	15.48	28.31	21.90	56.96	28.47	42.72
24	6.53	24.09	15.31	27.21	2.10	14.66

SECTION E

A STUDY ON THE ECOLOGY OF THE FREE-LIVING STAGES OF OSTERTAGIA OSTERTAGI AND O. LEPTOSPICULARIS IN WEST SCOTLAND

INTRODUCTION

It is generally accepted that eggs of O. ostertagi (O. O.) passed in the faeces are in the two to eight cell stage and in warm weather they hatch in a day or two releasing motile first stage larvae (L_1) which feed on bacteria in the faeces and then moult to second stage larvae (L_2). These also feed on bacteria and then moult to the infective third stage (L_3), which retain the sheath of the L_2 (Dunn, 1969).

Two basic requirements of this process are oxygen and adequate moisture, and the time taken to reach the infective stage depends largely upon temperature. These conditions occur in the faecal pat and the L_3 then migrate under moist conditions onto the herbage. Since the L_3 retains the outer sheath of the L_2, this is the most resistant of the free-living stages. While the life cycle of O. leptospicularis (O. L.) is probably similar there have been no definitive studies in this species.

Previous studies on the larval ecology of O. O. can be conveniently divided into development and survival in the faecal pat, migration from the faecal pat and finally survival on pasture.

Development and survival of larvae in the faecal pat

Rose (1961, 1962) carried out an intensive study on the bionomics of O. O. under natural conditions in the South of England. In 1961 he found that the faecal pat was an ideal environment for the development of infective larvae. Although the pat dried out, it did so relatively slowly and while a dry crust, a quarter of an inch thick had formed over the surface of

the faeces by the fourth or fifth day, it took almost a month for the whole pat to dry out. The minimum and maximum time taken for eggs to develop into third stage larvae was 11 and 19 days respectively during April and May and six to 14 days in June and July. Larval development in the faecal pat took place all year round except during the winter and eggs which had remained viable resumed development in the spring with the rise in temperature. In 1962 he made a comparison between the development and viability of the free-living stages in faeces spread over small plots and in faeces deposited as pats. Some of the plots were watered artificially. Faeces were again found to be an ideal environment for larval development. However, infective larvae were not recovered from faeces spread over the grass plots which were not watered, nor were any found on the herbage or in the upper 0.5 inch of soil. The faeces dried out within four to five days and this was not enough time for the eggs to develop to the infective stage. Other findings were that third stage larvae could survive on the herbage for up to two years, large numbers survived eight months and some larvae could withstand winter conditons.

Vincente (1961) in Spain, studied the development of eggs of O. ostertagi deposited out of doors during the autumn (October, November and December) when temperatures of -3°C to 14.5°C were noted. Infective larvae were found 45 to 60 days after deposition of faeces and migration of larvae onto the herbage occurred prior to the 35th day. All larvae found on the herbage in January were infective.

Goldberg (1970) studying development, migration and survival

of infective larvae of O. ostertagi in faecal pats deposited during the summer months at Betsville, Maryland, found infective larvae on herbage two weeks after deposition of faeces, their numbers reaching a peak in four weeks with the larvae surviving for 24 weeks.

Williams and Bilkovich (1971, 1973) studied the development and survival of infective larvae of O. ostertagi under Louisiana weather conditions. In their study they compared the total number of larvae recorded from faeces and herbage on plots contaminated with compact pats, or scattered in small portions and as pats or watery faeces. The percentage recovery of larvae was higher on plots with disseminated faeces than from plots with faecal pats. During the coolest months of the year, i.e. December to February, development of larvae to the infective stage was often delayed for between two and six weeks after faeces was placed on pasture. From June to September (summer), infective larvae were first recovered six to 14 days after faeces was placed on pasture. During these months development to the infective stage was quick, but mortality in the first two to six weeks of exposure was high. Larval recovery was generally low from herbage or faeces collected from plots throughout the summer months (May - September). However, from July to October more larvae were recovered from scattered faeces than from faecal pats but the difference was not significant. The longest survival of larvae were observed on plots contaminated in October, November and December (autumn - early winter), from which larvae were recovered for eight months post faecal deposition. Length of

survival was also lowest during the summer months; the effect of form of faeces on survival of infective stages being negligible. Optimal temperature conditions for development of larvae to the infective stage were considered to be in the range of 13°C to 23°C mean monthly mean air temperature with 7.5 cm to 17.5 cm of total monthly precipitation requirement. Optimum mean monthly temperature conditions for survival of infective larvae were considered to be between 8°C to 23°C and 7.5 cm to 17.5 cm total monthly rainfall. These conditions occurred from October to April.

It is interesting to point out that in Rose's comparative studies with spread faeces and pats (1962) conducted during spring and summer (April to July) he did not recover any larvae from spread faeces which were not artificially watered. He indicated that the faecal pat was more protective of developing eggs and larvae regardless of prevailing weather. He also considered that spreading of freshly deposited faeces on pasture in spring and summer may either limit or facilitate development of infective larvae, the precise effect depending on what climatic conditions subsequently prevail. In the work of Williams et al, they showed the dissemination of faeces was advantageous to development during the coolest months and of less significance during the rest of the year. They also reported that during spring and summer months scattered faeces or faecal pats dried out at approximately similar rates. Neither form of faeces appeared to be more protective to developing eggs and larvae than the other.

Persson (1974) studied the survival of eggs and infective larvae of O. ostertagi for two years under outdoor conditions in Sweden. He reported that in plots contaminated with faeces during January - Feburary only a few infective larvae were found in the following spring and summer. In plots contaminated in March and April infective larvae were recovered at the beginning of May, while in plots contaminated in June and July viable larvae were found on the herbage after 14 days. Plots contaminated in August - October gave rise to larvae during late autumn and the following spring. No larvae developed until the next spring in faeces deposited in November - December. The herbage infestation remained high for 10 to 12 months and maximum survival time was 19 months on plots contaminated in May. The results of this study indicate that under the climatic conditions in Stockholm, Sweden infective larvae of O. ostertagi are present in all seasons of the year.

Young and Anderson (1981) studied the ecology of the free-living stages of O. ostertagi in a winter rainfall region of Australia. In field experiments in Victoria, faecal pats were deposited on pasture plots at intervals of about six weeks from June 1976 until 1977. They also compared the development of larvae on irrigated plots during the summer months. Dung pats deposited during the winter and spring were not irrigated. Development of larvae was delayed from three to 12 days in summer and for 34 to 68 days in winter. The rate of development was closely related to the temperature in the soil and dung pats and the mortality rate of pre-infective stages increased with increasing temperature and decreasing moisture levels. Infective

larvae were present in abundance on herbage and in soil between six and 10 weeks after deposition providing that the moisture content of this was high. Mortality of the larvae was low during the winter and early spring but increased rapidly in mid to late spring irrespective of the time of deposition of dung pats. Mortality rates of infective larvae in dung pats deposited in either spring or summer were low and a large proportion of these larvae were capable of moving into herbage and soil after the autumn rains. Irrigation during summer did not provide ideal conditions for the development of infective larvae from eggs, but hastened larval migration from dung pats.

Migration from the faecal pat

Migration of the third stage larvae onto the herbage appears to depend upon the condition of the faecal pats which is, in turn, influenced by the climatic conditions.

Rose (1961) showed that the larvae of O. ostertagi tended to congregate in and just beneath the hard crust of the faecal pat, even when deeper parts contained more moisture. Larval migration to the pasture was a gradual process and occurred only when this crust was softened by rain. Some larvae migrated on to the herbage soon after becoming infective and the majority had left the pat by the end of four months. It seems that dry conditions can be regarded as unfavourable to migration from faeces, whereas wet conditions are favourable. At all times the faecal pat served as a reservoir of infection for several months.

Lateral migration was limited, the majority of larvae were found at 5 cm from the faecal pat and only few were recovered 10

to 15 cm away. Vertical migration was also limited, the majority of larvae were found less then 5 cm above the soil surface. A migration of 13 cm from the faecal pat was reported by Goldberg (1970). In their studies of lateral distribution of larvae to herbage, Williams and Bilkovich (1971) indicated that most larvae were recovered at a distance 2.5 cm to 15 cm from faecal pats and in the vertical distribution, upper herbage (8 cm to 16 cm from soil surface) consistently yielded greater numbers of larvae than lower herbage (soil surface to 8 cm).

Migration through soil

Migration of infective third stage larvae of O. ostertagi through soil has been reported by many workers. Thus, Rose (1961) reported the migration of larvae to the upper 0.5 inch of soil.

In the studies of Persson (1974) in which eggs and infective larvae were buried under the soil, he found that infective third stage larvae could migrate upwards from a level 20 cm below the soil surface although the greatest numbers were recovered from those buried at 10 cm. Survival in the soil was for up to one year.

Fincher and Stewart (1979) in Georgia carried out experiments in which faeces containing eggs of O. ostertagi were buried in the soil. They reported an upward vertical migration of 12.5 cm. Under laboratory conditions, the larvae can migrate up to 15 cm.

Bairden (1980) showed that infective L_3 of bovine Ostertagia spp could persist in the soil to a depth of 10 cm throughout the year, while Al Saqur et al (1982) suggested in

similar studies that Ostertagia spp L_3 would migrate downwards during the winter and upwards during the spring and summer. In their studies they found infective larvae to a depth of 15 cm.

EXPERIMENTAL DESIGN

Two series of experiments were carried out in order to compare the ecology of the free living stages of O.O. and O.L., emphasis being placed on the rate of development and survival of the two species in faeces, herbage and soil.

Faecal pats were deposited on clean pasture at monthly intervals and regular examination of replicate herbage and faecal samples carried out to determine epg. and larval counts.

MATERIALS AND METHODS

Animals

To obtain fresh Ostertagia species eggs for the outdoor ecology study it was necessary to infect two donor animals each month.

Two parasite naive Ayrshire male calves aged four months were infected each month with 100,000 O. O and O. L., each derived from the larval culture of the previous month.

Origin of larvae

Initially the strain used was the same as for the laboratory experiment (i.e. O.O. GB6 and O.L. GC5) but thereafter fresh larvae from each donor calf was used to infect the next pair. Thus, by the end of the twelve month deposition period the isolates used had been passaged a further 12 times.

Experimental grass plots

Each month, from April 1983 to March 1984, replicate samples

of fresh faeces containing eggs of O. O. or O. L. were placed on each plot at a distance of 50 cm from the centre of each pat. In total 48 replicates for each species were set up for each month to enable sampling to take place for at least one year.

Parasitological

Faeces Due to considerable variation in the establishment of both O. O. and O. L. in the culture calves, there was a wide scatter in their faecal egg counts. It was decided that standardisation of faecal pat size was more important than standardisation of the number of eggs per faecal deposit since there was a large variation in the latter.

Faeces were carefully mixed by kneading and weighed out into 400 g. masses which were shaped into pats approximately 15 cms. in diameter and 5 cms. high. This provided approximately equal numbers of eggs in each species replicates. Faecal egg counts were performed on 3 replicates before they were deposited on pasture.

Sample collection

Samples of faeces and herbage were collected from plots at 7 to 15 days after faeces were deposited and at fortnightly intervals thereafter except in the winter months (December to March) when they were collected monthly. On each occasion two faecal deposits for each species were removed, weighed and analysed for the presence of eggs and larvae. Stages of development were classified according to Silverman and Campbell (1959) into unembryonated eggs, embryonated eggs, $L_1 + L_2$ and L_3. The technique used here of examining samples from a total faecal

pat is the most practical for work with bovine faeces. However, the accuracy is less than can be obtained in studies using sheep faeces where individual pellets can be removed and examined e.g. as was used by Levine, Todd and Boatman (1974) in their studies on H. contortus. All herbage within a square of 50 cm. surrounding each dung pat was clipped at ground level. Soil cores were removed to a depth of 5 cm. from the area directly under the faecal pat. The grass and soil was then processed as detailed in the General Materials and Methods.

RESULTS

The weather pattern in 1983 and up to June 1984 was normal for the West of Scotland with rain occurring in most months of the year. Monthly temperatures ranged from as mean maximum of 4.2 to 21.8 °C and a mean minimum of -10°C to 9°C. In July and August 1983 there was a spell of very dry weather extending over a three week period. Following heavy rain in September and October, November was drier than usual. In 1984 the spring months of April and May were exceedingly dry. The monthly data is summarised in Table E1 and Fig. E1.

The main criteria studied were the duration of the faecal pats, the development and longevity of eggs and free-living larval stages (in faeces, soil and herbage) over a one year period.

The survival of the faecal pats and their weights are given in Tables E2 for O. O. and E3 for O. L. The weights of the grass samples collected from the O. O. and O. L. plots are given in Tables E4 and E5 respectively.

Tables E6 and E7 present the data on the survival and

development of eggs, embryonated eggs, $L_1 + L_2$ and L_3 in the faecal pats of O. O. and O. L. For ease of presentation these are given as percentages of the original population of eggs.

The data pertaining to the presence and survival of L_3 following their migration from the faecal mass is presented in three different ways. In the first, they are given in the traditional manner being expressed as L_3/kdh. These are tables E8 and E9 for O. O. and O. L. respectively.

In the second the total numbers of O. O. and O. L. recovered from each sample are shown in Tables E10 and E11 while in the third the total numbers of L_3 recovered divided by the total number of eggs deposited are expressed as a percentage in Tables E12 and E13.

Finally, the total numbers of L_3 recovered from the soil are given in Tables E14 and E15 for O. O. and O. L.

Since there were marked seasonal differences in the results these have been grouped seasonally and are reported in the order of spring (April-May), summer (June-July-August), autumn (September-October-November) and winter (December-January-February-March). To illustrate the seasonal pattern the data on herbage numbers of L_3/kdh from faeces deposited in summer are given in Figure E1 together with the climatic data.

Faeces deposited in spring

The faecal pats containing each species and deposited in April survived fairly intact for about 12 weeks i.e. until July. Thereafter they disintegrated slowly and most had disappeared by 36 weeks (November)

Eggs in faecal pats deposited in April developed very slowly for both species even though some of the eggs reached the embryonated stage by the first week. It was not until after two weeks that L_1 and L_2 of O. O. were recovered from the faeces and L_3 from the herbage at the same time. L_1 and L_2 of O. L. appeared also at two weeks. Temperatures during this time were very low, ranging from a minimum of 1.3 to a maximum of 10.3°C. The percentage yields of L_3 from the eggs deposited were very low (Tables E8 and E9) for both species with a maximum recovery of 2% after 12 weeks (July) in the case of O. O. and 2.5% after 8 weeks (June) in the case of O. L. These maximum yields were associated with heavy rainfall over the previous two weeks (44 mm, Figure E1). Thereafter the percentage recovery of larvae decreased to very low levels for both species even though some larvae overwintered and survived for 12 months. Infective larvae were recovered from the faecal pats during eight weeks for O. O. and 8 weeks for O. L. No eggs or larvae were recovered from the faecal pats thereafter. Low numbers (a maximum of six) of infective larvae were recovered throughout from the soil samples in the O. O. plots two to 24 weeks after faecal deposition in April while for the O. L. April faeces, the maximum spring L_3 recovery of 27 was observed in week eight.

The faecal pats deposited in May remained intact for 8 weeks i.e. until July, thereafter they disintegrated gradually but persisted only until October i.e. for eight weeks less than the April pats. On plots contaminated in May with both species embryonated eggs appeared within a week; thereafter the L_1, L_2 and L_3 of O. L. appeared in the faeces on week 2 and the L_3 on

the herbage on week 8 although only 1.1% of the eggs deposited became L_3. The L_3 of O. O. first appeared in the faeces in 6 weeks and strangely on the herbage in week 2. The peak yield of O. O. was 2.2% in week 16 (September).

The survival of the O. O. larvae was much higher than the O. L. being 1.3% of the eggs deposited after 40 weeks compared to 0.06% for O. L. On the plots contaminated with O. O. in May, L_3 were found in soil on five occasions, the maximum recovery being 15 in week 16. O. L. faeces for this month produced very few soil L_3 throughout although one core was positive after 24 weeks.

Faeces deposited in summer (June-August)

Survival of faeces varied during the 3 months. There was little disintegration for the first 4 weeks. Thereafter the break-up of the pats occurred fairly quickly and the maximum survival was 24 weeks for the June pats. The August pats survived for only 16 weeks so all the faeces had disappeared by December.

In June, the embryonated eggs of both species and the L_1 and L_2 of O. L. appeared within a week and L_3 in the faeces within 2 weeks. The L_3 were also found on the herbage within 2 weeks.

Maximum yield of O. O. was 24.2% in week 16 (October) and of O. L. was 11.4% in week 12 (September). The yield of O. O. remained consistently higher than O. L. through the autumn e.g. in week 24, the percentage yield of O. O. was 5.8% and that of O. L. 0.5%. O. O. larvae survived the winter much better than those of O. L.

The highest numbers of larvae were recovered from soil in plots contaminated during the summer months. Thus, in the June O. O. plots, L_3 were recovered after one week with a peak figure of 320 being found in week 16. Thereafter numbers decreased to 100 in week 24 i.e. December, and were only positive on one more occasion. In the O. L. plots infective larvae were first recovered two weeks after faecal deposition with the highest number of 100 being seen in week eight. A decline in numbers followed but a relatively high figure of 70 were still present in the soil core taken in week 32, i.e. February.

In July the temperatures were much higher with a mean maximum of 21.8°C. This was reflected in a more rapid development with embryonated eggs and larval stages of O. O. appearing in the first and second week respectively. In the case of O. L. development was so rapid that embryonated eggs were never recovered and L_1 and L_2 were already present in week one.

O. O. infective larvae were on the herbage by week 2 and a maximum yield of 23% was recorded at 12 weeks (early October). O. L. infective larvae yielded 14.8% by week 8 and 15% in week 12. By week 20 (December) the yields of O. L. had fallen to 2.4% compared 7.3% for O. O. The larvae of the latter species survived the winter better and 15.4% of the eggs deposited were still present as L_3 at the end of the following June compared to 2% of O. L.

Week 2 was the first positive soil sampling for O.O. with a maximum of 290 in week 12 while O.L. were first found in week 4 with a peak of 180 at this time, L_3 of O.O. and O.L. persisted for 16 and 32 weeks respectively.

As in July, the temperatures in August were higher than the rest of the year with a mean maximum of 21.4%°C. Embryonated eggs of both species were present in the first week and $L_1 + L_2$ also in the first week. Infective larvae of O. O. were found in the faeces on week 4 and in 2 weeks for O. L.

Infective larvae of O. O. were found in week 1 on the herbage and similarly for O. L. A maximum yield of 3.5% of O. O. was recovered in week 12 (November) and 5% of O. L. in week 8 (October). Larvae of O. O. overwintered slightly better than O. L. (in week 40 in June, there were 3.8% of O. O. and 0.6% of O. L.).

Similar soil larval recovery trends were observed from the July and August plots of both species.

Weeks 8 (O. O.) and 4 (O. L.) were when maximum recoveries of L_3 were found being 290 and 70 respectively, O. O. and O. L. L_3 were still present 36 and 32 weeks after deposition i.e. in April and March.

Faeces deposited in autumn (September-November)

For the first two weeks there was little visible disintegration of faecal pats, thereafter breakdown was rapid with no faeces being left after week 16. Development of eggs to the embryonated stage in faeces deposited in September for O. O. took one week as did those of O. L. L_1 and L_2 of O. O were only recorded after four weeks with no L_3 of this species being recovered from faeces while in the O. L. pats L_1 and L_2 were found after two weeks but again no L_3 were ever recovered from the faeces. The lack of L_3 recovery from faeces indicates a

rapid translation to herbage which correlates well with the high rainfall (148 mm) in September.

L_3 of both species were initially recovered from herbage in week 1 with a maximum percentage of 0.14% at 8 weeks for O. O. and 0.7% at the same period for O. L. Subsequent recoveries of L_3 of both O. O. and O. L. were very low with less than 1% after 40 weeks. Embryonated eggs were first recovered from faeces in October after 2 weeks for O. O. and one week for O. L. L_1 and L_2 of both species were first recovered at week 4 and as in September no L_3 stages of either species were detected in faeces. In the plots contaminated with O. O. during September, L_3 were first recovered from soil after two weeks with the maximum recovery of 12 being found at this time. Thereafter a general decrease in recovery was observed although 1 L_3 was still present in week 24. Very few O. L. larvae were found in soil with the highest recovery of 2 being made in week 2. Two L_3 of O. L. were also found in week 12.

In October, herbage infective L_3 were first found in low numbers (0.02%) for both species in week 4 with extremely low maximal recoveries of 0.27% and 0.24% for O. O. and O. L. respectively in week 16. For the remainder of the sampling period L_3 recoveries from herbage were very low although they did persist until 36 weeks after faecal deposition. October faeces produced a maximum of ten O. O. infective larvae from soil in week 28, with the only other positive core being taken at eight weeks. The only O. L. larvae present in soil from the October faeces were seen four weeks after faecal deposition.

In the November faeces, embryonation was observed in week one for both species, L_1 and L_2 appeared in week 2 again for O. O.and O. L. and no L_3 were recovered from the faecal pats containing either. L_3 recoveries from herbage were again very low O. O. first appearing at 4 weeks (0.005%) and O. L. also requiring 4 weeks for successful translation (0.05%). The maximum recovery for O. O. was only 0.15% in week 20 while that of O. L. reached 0.1% in week 12. Low numbers (0.002%) of O. O. and O. L. were recovered 36 weeks after faecal deposition. In November soil cores taken in weeks 4 and 16 proved positive for O. O. L_3 thereafter they remained negative. O. L. faeces produced only one positive core i.e. in week 16.

Throughout autumn, temperatures were low with a mean minimum of 3.8°C in November and a mean maximum of 15.2% in September.

Faeces deposited in winter (December-March)

Due to adverse weather conditions sampling through the winter period was restricted to monthly intervals and thereafter this practice was continued. In addition no data was recorded for the month of January due to heavy snow cover.

Duration of faeces was short with intact pats being recovered only up to 4 weeks after deposition. By April (week 16) they had virtually disappeared. In faeces put out in December embryonated eggs of O. O. and O. L. were first detected in week 8. L_1 and L_2 of O. O. were recovered from faeces in week 8 with no early larval stages being found in the O. L. pats. At no time were L_3 of either species recovered from faeces. Minimal L_3 recoveries from herbage were observed for both species first in week 8 for O. L. and also week 8 for O. O. Extremely low

peaks of 0.04% at week 12 for O. O. and 0.01% for O. L. in week 8. Again very low numbers of L_3 persisted throughout the sampling period until 24 weeks for O. O. and 24 weeks for O. L.

In February, embryonation was first recorded in week 4 for eggs of both species with L_1 and L_2 of only O. O. being recovered (week 8). No L_3 were recovered from faeces. Percentage recoveries from herbage of 0.002% at week 4 for O. O. and 0.001% at week 4 for O. L. were first recorded. Persistence of L_3 was of a 24 week duration for O. O. (0.01%) while O. L. L_3 were found up to 24 weeks after faecal deposition (0.01%).

O. O. and O. L. embryonated eggs were first detected from March faeces in week 4 with L_1 and L_2 larval stages of O. O. also being present at this time. L_1 and L_2 stages of O. L. were consistently absent from faeces as were the L_3 of both species. L_3 of both O. O. and O. L. were present on herbage from weeks 4 with 0.002% O. O. and 0.005% O. L. being recorded. An O. O. maximum recovery of 0.1% was recorded in week 16. Infective larvae of both species persisted on herbage for 16 weeks with recoveries of 0.1% (O. O.) and 0.02% (O. L.). No soil L_3 were recovered during the winter sampling period i.e. December, February and March.

DISCUSSION

Before discussing these results it is perhaps worth noting the rationale for the methods used in their presentation. Since the experimental design was chosen to compare the ecology of the free-living stages of two species, this necessarily imposed certain restrictions on sampling and presentation.

Thus, the larval populations on the herbage are discussed primarily in relation to the total yield of these larvae from the eggs deposited, expressed as a percentage. While this provides information on the production and survival of larvae it does not indicate the level of infection per unit area or herbage. However, for epidemiological reasons and comparison with other published work, larval data is also expressed as L_3/kdh, although this may be an artificial figure since the herbage sampled was only a maximum of 17 cm from the faeces.

It should also be pointed out that twelve consecutive passages of O.O. and O.L. were necessary to provide infected faeces and assuming two generations annually this would encompass six years under natural conditions and there may be an alteration in phenotype of the larvae at this time.

Nevertheless and in spite of the above strictures several interesting facts emerge from the results. First, there was little difference in the initial development times of eggs, and egg embryonation was rapid for both species deposited in all seasons, embryonated eggs being found after one week in most samples. It is interesting that in the case of autumn and winter faecal pats embryonated eggs persisted for eight weeks while those in spring and summer faeces had generally advanced to the L_1 and L_2 stage by four weeks. With the exception of the summer months the time required for egg hatching and development to the L_1 and L_2 stage took at least two weeks and thereafter these stages persisted until pat deterioration. The fate of pre-infective stages after pat disintegration is unknown and it is open to speculation as to whether they die or are able to

overwinter and so contribute to a new generation of L_3 on herbage. It is possible that earthworms while scavenging in faecal masses may ingest, transport and excrete these stages in soil as they have been shown to do for third stage larvae (Gronvold, 1979).

Secondly, there was a distinct seasonal variation in the yield of infective larval stages from the eggs deposited with marked increases in both replicates of each species occurring in the warmer summer months of June, July and August, particularly in June. Thus, the percentage yield of O.O. infective larvae from faeces deposited in June reached 24.2% in replicate A while for O.L. the highest yield of 11.4% was recorded in June again in replicate A. There was some indication that O. L. responded more quickly to the higher summer temperatures and that development of this parasite proceeded more rapidly during the first four weeks.

The data on O.O. agrees with the results of Rose (1961) working in a similar climate in Southern England. In his work the maximum yield of larvae was recorded from faeces in June. Rose employed total yield of larvae and where the present results are given in this manner the June deposited faeces also produced the highest total numbers of larvae on the pastures (Table E 10).

However, it should be pointed out that the faeces containing O.O. and deposited in June contained twice the number of eggs at deposition compared to the July faecal samples. This highlights the benefit of calculating the percentage yield rather than the total yield of L_3/kdh. This is particularly important in

experiments involving Ostertagia spp where the faecal egg counts are so variable. Thus the view was also taken at the outset of the experiment that, because of the protective nature of the faecal pat to infective larvae, standardising the size of the faecal pat was more important than standardising the number of eggs present.

Thirdly, the yields of L_3 from eggs of both species deposited from June through August were higher than the rest of the year. Thus, the peak L_3 numbers of both species were reached in 12 to 16 weeks after deposition of the eggs and this means that larval numbers were maximum on the pasture from September through to November.

Fourthly, there was a variation in the rate of survival of the infective larvae of both species. Although there was some variation in the results of the replicate samples, the mean of the two replicates is presented in Table E16 to facilitate the reading of the discussion. Taking the June faecal deposit as an example it can be seen from Table E16 that the mean maximum percentage of L_3 present on the herbage following deposition of O.O. eggs in June was 23.21% and this was reached in October i.e. 16 weeks after deposition; this percentage yield had decreased to 5.1% by December i.e. week 24 and to very low levels over the remaining winter months. A similar fall was noted with O.L.; thus, the mean percentage yield of the July deposited eggs in October was 14.4% and this had fallen to 2,36% in December with less than 1% being recovered in February. In the spring months there was an increase in the larval numbers of both species and in the case of O.O. this reached over 12% in May and June, i.e.

week 44. The O.L. increase was much less marked and never exceeded 2.6%. The source of this larval increase in the spring is not definitely known but there are at least three possibilities; namely, that overwintered eggs had resumed development and then hatched; that L_3 had survived in the faecal pat or that they had survived in the soil. The first of these possibilities is unlikely since eggs could not be recovered from the June, July and August faecal depositions beyond a period of four weeks (Table E6 and E7). It is of course possible that eggs were washed free of the faecal debris but again this is unlikely to occur in large numbers from faecal pats in which the weights had not decreased significantly for at least 12 weeks (Table E 2). The second possibility depends on the survival of the faecal pat and as can be seen from Table E that faecal pats deposited in the summer had virtually disappeared by December.

The third possibility remains the most likely source of the larvae in view of the published work of Al Saqur et al (1982), in which an increase in pasture larval numbers of O. O. occurred at the same time as a fall in numbers of these larvae in the soil. However in the current experiment the results of the soil analyses shown in Tables E14 and E15 clearly demonstrate that although fairly high numbers of larvae were recovered from the soil core at a time when herbage larval populations were high and the faecal mass was still present, only negligible numbers were recovered in the following spring. It should be remembered however that the area of soil sampled was directly under the faecal pat and Al Saqur et al sampled randomly. If the larvae

which had migrated laterally from the faecal pats were then washed into the soil, it is unlikely that they would be recovered by the sampling procedure used in this experiment. The most likely reason for the increase in larval numbers in the spring is that larvae in the soil or root mat had migrated onto the herbage. Possibly, the fact that the spring of 1984 was abnormally dry compared to the year when Al Saqur et al (1982) carried out their work encouraged the larvae to migrate lower than the 5 cm sampled in this experiment.

From the results it therefore appears that the free-living stages of O.O. survive better than those of O.L. during the winter and this could be one explanation as to why O.L. appears to contribute to outbreaks of clinical ostertagiasis on only a few occasions (Bisset 1980, Al Saqur et al 1982). The optimal temperatures for the initial development of O.L. also appear to be rather higher than for O.O. It is probable that where O.L. is a problem in cattle there will have been contamination of the pastures by deer, the natural hosts of O.L.

Finally, it is interesting that, apart from the summer months (June - August), the yield of infective larvae from the eggs deposited is extremely low and never exceeded 2.5%. However, although the expression of data in this manner has its benefits it should be realised that these low yields can represent quite high values of L_3/kdh. For example the percentage yield of L_3 produced from the faeces of one replicate deposited in November was only 0.2% in the following April; if this data is expressed as L_3/kdh it is approximately 8,000. Since the average calf ingests 2.5 kg of dry matter per day this

would give a daily uptake of 20,000 L_3 which is sufficient to cause production loss. It could be argued that these L_3 were recovered within 25 cm of the faecal pat deposited and so within the so-called "ring of repugnance" for grazing animals. However, if the survival of faecal pats is studied (Table E2 and E3) it is clear that the November faeces survived for four months i.e. until March/April and therefore there would be no faecal presence to deter the animal from grazing the area contaminated by L_3.

Any future studies on this aspect should include estimates of larval uptake by calves grazing the experimental areas. The expression of data as L_3/kdh is useful for this purpose but the main method of presentation used here i.e. the larvae recovered expressed as a percentage of the eggs deposited is most useful for ecological comparisons.

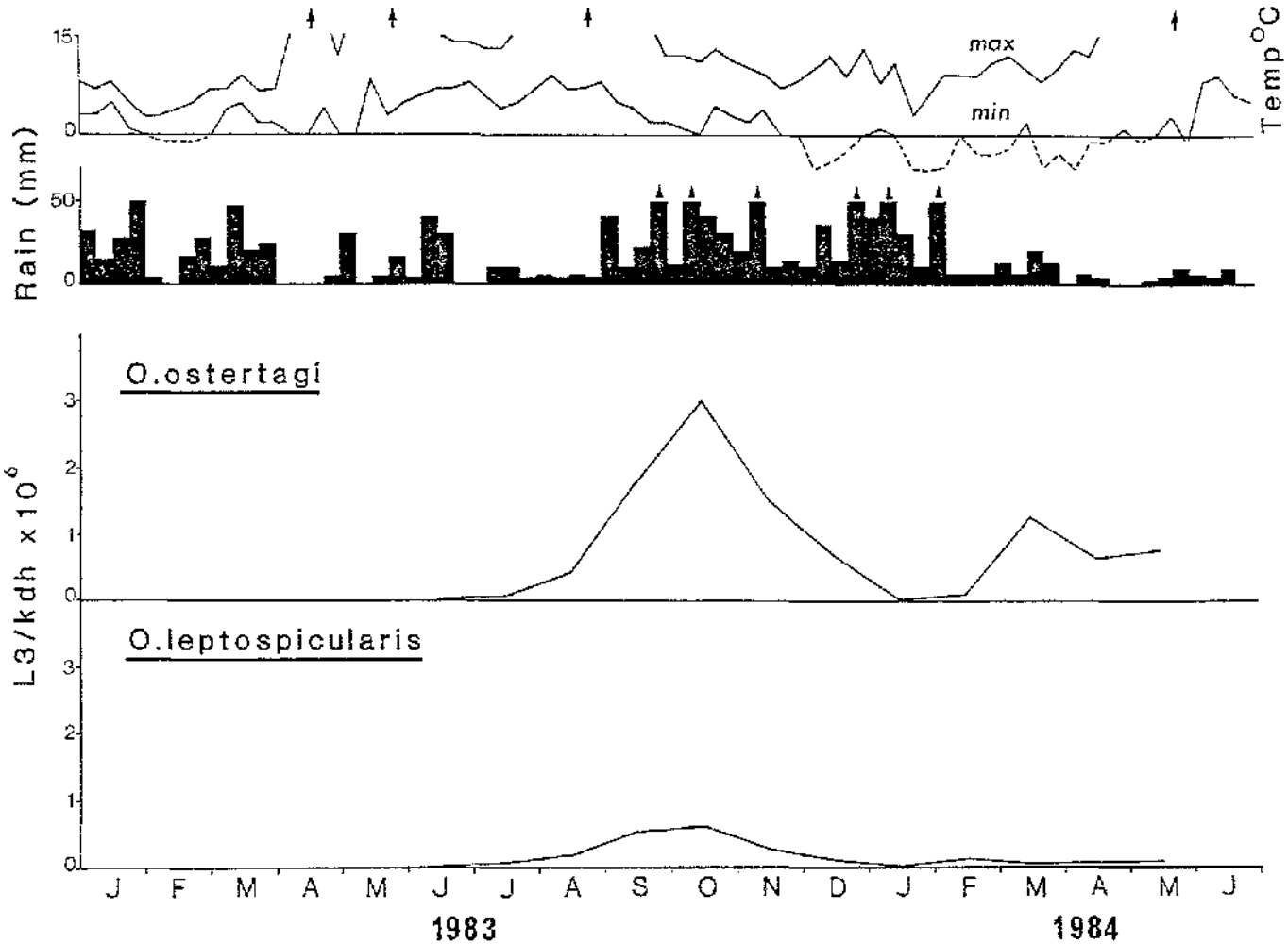


Figure E1. Ostertagia ecology experiment at Glasgow: climatic data plus O.ostertagi and O.leptospicularis L3/kdh following summer contamination of plots.

TABLE E 1.

MEAN FORTNIGHTLY MAXIMUM AND MINIMUM TEMPERATURES (°C)

PLUS RAINFALL DATA AT GLASGOW

	MONTH	TEMPERATURE				RAINFALL	
		MAXIMUM		MINIMUM			
		1st – 15th	16th – 30th	1st – 15th	16th – 30th	1st – 15th	16th – 30th
1983	JANUARY	7	5	2	2	48	77
	FEBRUARY	3	6	-1	0	4	37
	MARCH	8	7	4	2	58	45
	APRIL	15	15	0	2	0	5
	MAY	18	19	4	4	30	18
	JUNE	18	14	6	7	43	29
	JULY	13	17	5	6	9	15
	AUGUST	19	18	8	6	9	44
	SEPTEMBER	18	16	4	2	50	> 50
	OCTOBER	16	12	0	3	> 50	70
	NOVEMBER	9	7	3	0	> 50	24
	DECEMBER	11	11	-4	-1	47	87
	JANUARY	9	4	-2	-11	119	40
	FEBRUARY	9	10	-2	-3	90	11
	MARCH	11	9	1	-4	19	32
	APRIL	12	23	-3	1	6	4
	MAY	18	21	-1	1	1	14
	JUNE	23	19	8	5	9	13

TABLE E 2.

SURVIVAL (IN WEEKS) AND WEIGHT OF CATTLE FAECES (g) DEPOSITED ON *O.ostertagi* PLOTS

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	298	266	225	92	38	28	42	44	22	83	*	-	-
	B	320	295	179	112	20	26	38	59	38	76	*	-	-
MAY	A	279	266	155	71	75	97	83	36	45	*	-	-	-
	B	292	287	260	78	50	74	72	31	36	*	-	-	-
JUNE	A	199	277	122	176	120	111	33	49	*	-	-	-	-
	B	198	264	116	163	167	48	27	30	*	-	-	-	-
JULY	A	212	139	171	119	63	49	38	*	-	-	-	-	-
	B	233	150	201	116	60	41	36	*	-	-	-	-	-
AUGUST	A	274	327	278	82	30	45	*	-	-	-	-	-	-
	B	287	329	283	102	63	63	*	-	-	-	-	-	-
SEPTEMBER	A	342	325	122	43	18	*	-	-	-	-	-	-	-
	B	340	350	254	100	30	*	-	-	-	-	-	-	-

* No samples taken

TABLE E 2. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	393	324	273	133	*	20	-	-	-	-	-	-	-
	B	398	292	219	149	*	45	-	-	-	-	-	-	-
NOVEMBER	A	261	235	202	*	113	10	-	-	-	-	-	-	-
	B	235	222	198	*	151	27	-	-	-	-	-	-	-
DECEMBER	A	244	*	*	104	19	-	-	-	-	-	-	-	-
	B	232	*	*	145	28	-	-	-	-	-	-	-	-
FEBRUARY	A	*	*	231	210	75	190	-	-	-	-	-	-	-
	B	*	*	261	116	54	59	-	-	-	-	-	-	-
MARCH	A	*	*	303	105	35	-	-	-	-	-	-	-	-
	B	*	*	317	108	168	-	-	-	-	-	-	-	-

* No samples taken

TABLE E 3.

SURVIVAL (IN WEEKS) AND WEIGHT OF CATTLE FAECES (g) DEPOSITED ON *C.leptospicularis* PLOTS

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	312	268	225	75	22	20	34	45	42	18	*	-	-
	B	305	265	269	115	62	21	56	47	48	98	*	-	-
MAY	A	284	323	225	106	54	70	94	53	44	*	-	-	-
	B	298	305	253	128	74	92	85	58	67	*	-	-	-
JUNE	A	190	235	89	96	102	77	62	48	*	-	-	-	-
	B	197	241	109	102	144	109	72	55	*	-	-	-	-
JULY	A	337	306	204	146	67	96	69	*	-	-	-	-	-
	B	354	265	143	151	79	46	67	*	-	-	-	-	-
AUGUST	A	249	309	252	54	50	68	*	-	-	-	-	-	-
	B	258	306	273	71	98	92	*	-	-	-	-	-	-
SEPTEMBER	A	347	345	257	77	78	*	-	-	-	-	-	-	-
	B	332	222	208	122	43	*	-	-	-	-	-	-	-

TABLE E 3. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	349	308	189	109	*	27	-	-	-	-	-	-	-
	B	349	325	207	106	*	19	-	-	-	-	-	-	-
NOVEMBER	A	395	365	25	*	-	-	-	-	-	-	-	-	-
	B	370	378	239	*	29	-	-	-	-	-	-	-	-
DECEMBER	A	283	*	*	129	20	29	40	-	-	-	-	-	-
	B	286	*	*	110	3	49	-	-	-	-	-	-	-
FEBRUARY	A	*	*	187	128	-	10	-	-	-	-	-	-	-
	B	*	*	238	210	13	-	-	-	-	-	-	-	-
MARCH	A	*	*	137	30	51	-	-	-	-	-	-	-	-
	B	*	*	83	25	22	-	-	-	-	-	-	-	-

TABLE E 4.

WEIGHT OF GRASS (g) COLLECTED FROM *O.ostertagi* PLOTS (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	10	11	22	60	80	98	120	111	141	124	*	109	91
	B	12	9	23	62	73	93	90	112	101	119	*	98	94
MAY	A	32	31	54	101	119	134	128	126	146	*	128	131	116
	B	41	34	48	94	108	125	106	112	126	*	130	98	107
JUNE	A	46	46	59	75	79	80	89	120	*	82	78	86	96
	B	32	40	55	79	76	70	92	76	*	86	56	57	100
JULY	A	27	23	57	47	51	70	84	*	99	62	58	77	142
	B	38	31	53	43	53	71	87	*	115	60	83	90	107
AUGUST	A	20	9	13	25	40	52	*	34	44	50	52	117	30
	B	18	10	11	16	37	37	*	27	44	70	49	83	25
SEPTEMBER	A	9	11	4	15	15	*	12	46	61	58	121	32	63
	B	7	5	10	28	24	*	13	34	59	54	101	31	83

TABLE E 4. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	16	13	26	16	*	13	9	12	26	87	29	88	-
	B	36	27	26	21	*	19	6	28	29	41	32	117	-
NOVEMBER	A	5	8	8	*	3	11	9	24	65	38	116	-	-
	B	6	5	9	*	6	5	11	41	52	83	124	-	-
DECEMBER	A	4	*	*	4	3	10	19	38	38	-	-	-	-
	B	3	*	*	4	3	8	14	51	44	-	-	-	-
FEBRUARY	A	*	*	13	16	13	91	42	130	-	-	-	-	-
	B	*	*	13	6	32	24	40	169	-	-	-	-	-
MARCH	A	*	*	7	7	59	39	132	-	-	-	-	-	-
	B	*	*	29	14	13	43	111	-	-	-	-	-	-

TABLE E 5.

WEIGHT OF GRASS (g) COLLECTED FROM *O.leptospicularis* PLOTS (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	13	9	19	49	92	124	134	159	137	130	*	95	99
	B	13	12	18	54	77	105	134	145	161	134	*	76	119
MAY	A	19	43	50	75	105	168	157	139	135	*	110	91	–
	B	31	36	61	63	89	113	127	133	114	*	89	124	–
JUNE	A	40	28	48	59	83	91	110	97	*	82	112	83	113
	B	34	35	56	66	81	94	116	72	*	97	86	63	108
JULY	A	75	82	73	83	94	108	171	*	61	92	69	94	98
	B	55	69	71	84	85	147	162	*	45	79	109	89	102
AUGUST	A	41	18	14	27	37	52	*	43	29	29	37	70	38
	B	34	9	17	25	29	46	*	59	40	29	47	92	46
SEPTEMBER	A	8	9	11	19	29	*	10	9	22	33	87	54	106
	B	9	12	16	18	22	*	9	36	28	45	91	45	123

TABLE E 5. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	12	11	15	14	*	16	17	18	40	71	46	110	-
	B	20	26	19	23	*	16	36	22	54	90	42	119	-
NOVEMBER	A	10	8	5	*	12	12	18	36	37	142	-	-	-
	B	12	9	4	*	14	12	28	81	48	141	-	-	-
DECEMBER	A	5	*	*	5	11	14	22	19	62	-	-	-	-
	B	6	*	*	7	3	11	16	16	61	-	-	-	-
FEBRUARY	A	*	*	7	9	10	23	49	130	-	-	-	-	-
	B	*	*	10	15	31	74	23	110	-	-	-	-	-
MARCH	A	*	*	9	8	23	71	159	-	-	-	-	-	-
	B	*	*	14	15	42	58	128	-	-	-	-	-	-

TABLE E 6.

O. OSTERTAGI

PERCENTAGE OF EGGS, EMBRYONATED EGGS, $L_1 + L_2$ AND L_3 SURVIVING IN FAECAL PATS (IN WEEKS)

MONTH FAECES DEPOSITED		NO. OF EGGS DEPOSITED	Week 1				Week 2				Week 4			
			E	EE	L_1/L_2	L_3	E	EE	L_1/L_2	L_3	E	EE	L_1/L_2	L_3
APRIL	A	52,500	57.0	–	–	–	8.1	8.1	25.0	–	7.0	14.0	–	–
	B		20.0	5.1	–	–	9.0	18.5	9.0	–	11.0	11.0	51.0	–
MAY	A	52,500	62.0	8.5	–	–	17.0	–	–	–	10.0	–	–	–
	B		37.0	8.9	–	–	27.0	9.0	18.0	–	–	–	–	–
JUNE	A	408,100	0.8	8.0	–	–	–	–	26.0	7.0	–	–	2.5	26.3
	B		0.8	1.6	–	–	–	–	49.0	7.5	–	–	12.7	8.0
JULY	A	222,950	–	1.5	16.0	–	–	–	5.2	4.1	–	–	18.0	8.0
	B		3.4	3.4	5.2	–	–	–	8.0	3.4	–	–	45.0	15.0
AUGUST	A	526,400	6.5	6.5	14.0	–	10.3	6.2	35.0	–	8.0	3.5	2.0	5.3
	B		13.6	14.5	2.7	–	20.0	7.2	28.0	–	6.2	2.0	16.0	4.5
SEPTEMBER	A	426.400	59.6	6.6	–	–	55.0	8.8	–	–	12.0	4.0	3.3	–
	B		52.0	6.6	–	–	58.7	5.4	–	–	21.0	6.0	2.0	–
OCTOBER	A	213.200	74.0	–	–	–	53.0	15.0	–	–	29.0	8.4	8.4	–
	B		93.0	–	–	–	52.0	7.0	–	–	32.0	25.6	8.5	–
NOVEMBER	A	869,050	53.0	5.0	–	–	61.0	6.3	2.2	0.9	*	*	*	*
	B		42.1	9.0	–	–	45.0	4.7	0.8	0.4	*	*	*	*
DECEMBER	A	198,100	51.0	4.0	–	–	*	*	*	*	*	*	*	*
	B		50.7	–	–	–	*	*	*	*	*	*	*	*
FEBRUARY	A	435,550	*	*	*	*	*	*	*	*	23.0	34.0	–	–
	B		*	*	*	*	*	*	*	*	46.0	22.0	–	–
MARCH	A	431,550	*	*	*	*	*	*	*	*	23.0	34.0	–	–
	B		*	*	*	*	*	*	*	*	21.0	19.0	1.5	–

Table E6 (contd)

MONTH FAECES DEPOSITED		NO. OF EGGS DEPOSITED	Week 6				Week 8			
			E	EE	L_1/L_2	L_3	E	EE	L_1/L_2	L_3
APRIL	A	52,500	5.0	-	50.0	5.0	2.0	-	9.2	-
	B		7.0	-	7.0	17.4	-	-	-	5.5
MAY	A	52,500	-	-	83.0	3.0	-	-	33.0	-
	B		-	-	62.0	7.3	-	-	19.0	-
JUNE	A	408,100	-	-	5.0	12.5	-	-	7.3	11.3
	B		-	-	0.5	7.0	-	-	20.0	3.0
JULY	A	222,950	-	-	27.0	6.5	-	-	10.5	-
	B		-	-	46.0	4.7	-	-	6.0	-
AUGUST	A	526,400	0.3	-	1.2	-	-	-	0.7	0.13
	B		4.0	1.15	13.0	1.7	-	-	-	0.12
SEPTEMBER	A	426,400	-	-	0.7	-	1.1	0.3	3.0	-
	B		3.6	3.2	3.6	-	8.0	2.1	0.8	-
OCTOBER	A	213,200	10.4	4.0	53.0	-	0.5	0.15	1.2	-
	B		-	-	11.6	-	-	-	-	-
NOVEMBER	A	869,050	*	*	*	*	-	-	-	-
	B		*	*	*	*	-	-	-	-
DECEMBER	A	198,100	*	*	*	*	2.6	2.0	13.0	-
	B		*	*	*	*	12.1	4.8	4.8	-
FEBRUARY	A	445,550	*	*	*	*	1.6	5.0	15.0	-
	B		*	*	*	*	0.8	-	3.6	-
MARCH	A	431,550	*	*	*	*	1.6	5.0	15.0	-
	B		*	*	*	*	-	-	-	-

E = Eggs EE = Embryonated eggs L_1/L_2 = 1st and 2nd stage larvae

L_3 = Infective larvae

TABLE E 7.

O.LEPTOSPICULARIS

PERCENTAGE OF EGGS, EMBRYONATED EGGS, $L_1 + L_2$ AND L_3 SURVIVING IN FAECAL PATS (IN WEEKS)

MONTH FAECES DEPOSITED		NO. OF EGGS DEPOSITED	Week 1				Week 2				Week 4			
			E	EE	L_1/L_2	L_3	E	EE	L_1/L_2	L_3	E	EE	L_1/L_2	L_3
APRIL	A	122,500	21.0	8.4	-	-	-	7.3	3.6	-	-	3.04	46.0	3.0
	B		8.2	4.13	-	-	7.2	7.2	3.5	-	-	7.2	22.0	-
MAY	A	315,000	40.6	7.5	-	-	15.4	18.6	5.12	5.12	38.1	13.1	-	-
	B		33.0	14.2	-	-	30.6	8.0	6.4	-	6.6	2.6	10.7	2.6
JUNE	A	161,700	-	2.0	6.0	-	-	5.0	22.0	41.0	-	-	51.3	6.4
	B		-	2.0	2.0	-	-	-	62.0	19.8	-	-	24.7	6.7
JULY	A	150,000	4.0	-	7.4	-	-	-	88.3	3.3	-	-	87.6	16.0
	B		3.7	-	3.7	-	-	-	26.5	5.8	-	-	25.3	7.9
AUGUST	A	146,400	30.0	21.3	47.0	-	35.0	21.0	35.0	-	40.0	46.0	3.0	-
	B		6.0	6.0	26.4	-	21.0	7.0	66.0	7.0	34.0	12.0	-	-
SEPTEMBER	A	60,000	77.0	-	-	-	95.4	48.0	-	-	35.5	21.4	35.5	-
	B		46.0	18.3	-	-	24.0	12.0	9.6	-	11.5	5.6	46.2	-
OCTOBER	A	240,000	92.0	5.0	-	-	79.0	-	-	-	8.0	16.0	21.0	-
	B		75.0	7.0	-	-	90.0	11.0	-	-	7.0	8.6	17.2	-
NOVEMBER	A	506,400	80.0	9.0	-	-	74.0	11.0	6.0	-	-	*	*	*
	B		59.0	11.0	-	-	68.4	10.0	1.2	-	*	*	*	*
DECEMBER	A	717,500	81.4	1.3	-	*	*	*	*	*	*	*	*	*
	B		51.0	-	-	-	*	*	*	*	*	*	*	*
FEBRUARY	A	313,200	*	*	*	*	*	*	*	*	13.4	9.0	-	-
	B		*	*	*	*	*	*	*	*	38.0	15.0	-	-
MARCH	A	565,833	*	*	*	*	*	*	*	*	4.0	10.0	0.4	-
	B		*	*	*	*	*	*	*	*	3.1	6.0	-	-

TABLE E 7. (Cont'd)

MONTH FAECES DEPOSITED		NO. OF EGGS DEPOSITED	Week 6				Week 8			
			E	EE	L_1/L_2	L_3	E	EE	L_1/L_2	L_3
APRIL	A	122,500	-	-	18.0	1.5	-	-	5.0	4.0
	B		2.5	-	7.6	2.52	-	-	1.2	1.2
MAY	A	315,000	-	-	13.0	11.4	-	-	6.0	-
	B		-	-	2.3	2.3	-	-	17.3	-
JUNE	A	161,700	-	-	36.0	8.0	-	-	85.0	1.7
	B		-	-	22.8	18.0	-	-	52.3	1.4
JULY	A	150,000	-	-	33.0	5.0	-	-	33.6	-
	B		-	-	25.6	10.3	-	-	31.2	-
AUGUST	A	146,400	-	-	13.0	-	-	-	2.3	-
	B		-	-	3.0	0.6	-	-	-	-
SEPTEMBER	A	60,000	2.5	2.5	5.1	-	-	-	1.6	-
	B		11.0	5.3	72.0	-	4.0	-	5.0	-
OCTOBER	A	240,000	0.72	-	0.72	-	-	-	0.18	-
	B		-	1.5	3.0	-	-	-	0.12	-
NOVEMBER	A	506,400	*	*	*	-	-	-	0.16	-
	B		*	*	*	*	0.5	0.1	-	-
DECEMBER	A	717,500	*	*	*	*	10.4	13.1	-	-
	B		*	*	*	*	6.13	13.3	-	-
FEBRUARY	A	313,200	*	*	*	*	6.1	-	-	-
	B		*	*	*	*	5.5	2.2	-	-
MARCH	A	565,833	*	*	*	*	-	-	-	-
	B		*	*	*	*	-	-	-	-

E = Eggs EE = Embryonated eggs L_1/L_2 = 1st stage and 2nd stage larvae

L_3 = Infective larvae

TABLE E 8.

INFECTIVE LARVAE OF *O.ostertagi* ON GRASS PLOTS (L_3/kdh)

AFTER DEPOSITION OF CATTLE FAECES

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20
APRIL	A	-	181	1,818	7,083	13,043	1,317	1,250
	B	-	-	1,739	5,440	9,320	4,555	555
MAY	A	-	322	6,667	1,337	2,929	8,582	2,734
	B	-	-	2,500	3,085	3,318	3,187	8,920
JUNE	A	-	45,000	16,949	365,333	915,823	1,227,329	670,950
	B	-	35,250	15,091	418,354	796,053	1,291,428	697,283
JULY	A	-	4,348	64,912	728,421	744,118	621,276	194,048
	B	-	3,871	75,472	873,256	954,205	493,706	168,965
AUGUST	A	2,000	41,111	107,692	650,000	455,555	246,153	*
	B	5,555	12,000	518,181	971,875	428,000	281,081	*
SEPTEMBER	A	1,111	16,363	35,000	38,064	17,333	*	9,167
	B	1,428	4,000	16,000	21,403	4,583	*	13,077
OCTOBER	A	-	-	1,923	11,250	*	9,231	17,778
	B	-	-	2,307	7,143	*	31,053	43,333
NOVEMBER	A	-	-	2,500	*	60,000	83,636	147,778
	B	-	-	5,555	*	36,666	84,000	46,363
DECEMBER	A	-	*	*	5,000	26,666	2,000	1,579
	B	-	*	*	-	6,667	3,750	2,143
FEBRUARY	A	*	*	769	687	3,769	10,989	714
	B	*	*	-	1,692	343	47,083	500
MARCH	A	*	*	1,000	2,428	-	2,564	-
	B	*	*	172	1,500	-	7,209	-

TABLE E 8. (Cont'd)

MONTH FAECES DEPOSITED		24	28	32	36	40	44
APRIL	A	2,062	815	887	*	183	3,516
	B	1,822	1,633	756	*	714	2,553
MAY	A	2,648	11,027	*	2,656	3,053	3,793
	B	9,687	13,889	*	3,077	7,143	7,383
JUNE	A	148,333	*	8,536	456,410	363,953	279,167
	B	311,842	*	45,349	651,786	473,684	210,000
JULY	A	*	20,202	404,839	201,724	409,090	242,958
	B	*	13,043	163,333	113,253	247,778	201,869
AUGUST	A	38,235	450,000	78,000	261,538	171,795	666
	B	29,630	347,727	95,714	140,816	218,072	400
SEPTEMBER	A	2,174	6,557	13,793	3,884	312	–
	B	5,000	1,695	3,704	3,564	–	–
OCTOBER	A	4,167	3,846	1,149	–	–	–
	B	2,500	25,517	10,244	–	–	–
NOVEMBER	A	2,500	16,615	1,053	–	–	–
	B	2,195	12,500	482	80	–	–
DECEMBER	A	–	–	–	–	–	–
	B	784	–	–	–	–	–
FEBRUARY	A	154	–	–	–	–	–
	B	296	–	–	–	–	–
MARCH	A	–	–	–	–	–	–
	B	–	–	–	–	–	–

TABLE E 9.

INFECTIVE LARVAE OF *O.leptospicularis* ON GRASS PLOTS (L_3/kdh)

AFTER DEPOSITION OF CATTLE FAECES (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	-	-	1,368	56,938	1,793	927	746	1,666	73	153	*	2,105	60
	B	-	-	8,000	56,203	4,064	2,464	1,492	69	161	75	*	2,631	176
MAY	A	-	232	12,600	33,533	24,285	12,797	5,573	3,129	6,666	*	2,727	659	-
	B	322	278	16,229	55,079	20,000	18,141	16,614	5,150	9,649	*	16,853	1,693	-
JUNE	A	3,500	52,500	35,000	139,661	141,566	129,670	80,000	9,278	*	44,756	37,500	19,277	6,194
	B	3,529	38,873	34,285	108,796	227,777	134,574	68,103	972	*	37,319	32,558	14,285	5,833
JULY	A	-	16,585	126,027	213,855	239,893	104,629	19,883	*	24,590	8,695	40,579	37,553	22,959
	B	182	48,840	80,281	262,721	244,705	77,627	22,839	*	29,111	53,164	23,853	52,134	31,568
AUGUST	A	4,390	7,222	171,428	258,518	83,783	15,384	*	67,441	41,379	31,034	13,513	13,714	-
	B	-	13,333	222,857	249,019	131,034	106,521	*	57,627	15,000	24,137	16,170	7,934	-
SEPTEMBER	A	1,250	8,888	27,272	14,736	7,586	*	38,000	11,111	9,090	2,727	230	322	370
	B	-	1,666	14,375	23,513	7,727	*	14,444	2,777	3,571	4,000	109	110	-

TABLE E 9. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	-	-	8,000	7,142	*	4,375	17,647	27,777	2,250	985	-	182	-
	B	-	-	2,631	6,521	*	37,500	16,666	18,181	7,037	1,333	238	252	-
NOVEMBER	A	-	-	70,000	*	46,666	19,166	9,444	6,111	-	140	-	-	-
	B	-	-	112,500	*	29,285	66,833	23,214	5,802	-	71	-	-	-
DECEMBER	A	-	*	*	8,000	4,545	2,857	3,181	1,052	-	-	-	-	-
	B	-	*	*	11,428	3,333	2,727	3,125	1,875	-	-	-	-	-
FEBRUARY	A	*	*	571	222	8,000	14,782	612	461	-	-	-	-	-
	B	*	*	600	533	2,000	1,486	3,478	454	-	-	-	-	-
MARCH	A	*	*	1,889	-	84,782	1,690	-	-	-	-	-	-	-
	B	*	*	2,214	-	59,761	2,241	-	-	-	-	-	-	-

TABLE E 10.

TOTAL NUMBER OF *O.ostertagi* L_3 RECOVERED FROM GRASS SAMPLES

COLLECTED AT A RADIUS OF 0.25 CMS AROUND FAECES (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	-	2	40	425	1,050	130	150	230	115	110	*	40	44
	B	-	-	40	340	685	425	50	205	165	90	*	70	240
MARCH	A	-	10	360	135	350	1,150	350	335	1,610	*	340	400	440
	B	-	-	120	290	360	400	950	1,085	1,750	*	400	700	790
JUNE	A	-	2,070	1,000	27,400	72,350	98,800	60,050	17,800	*	700	35,600	31,300	26,800
	B	-	1,410	830	33,050	60,500	90,400	64,150	23,700	*	3,900	36,500	27,000	21,000
JULY	A	-	100	3,700	34,600	37,950	43,800	16,300	*	2,000	25,100	11,700	31,500	34,500
	B	-	120	4,000	37,550	51,050	35,300	14,700	*	1,500	9,800	9,400	22,300	21,600
AUGUST	A	40	370	1,400	16,250	18,450	12,800	*	1,300	19,800	3,900	13,600	20,100	20
	B	100	120	5,700	15,550	16,050	10,400	*	800	15,300	6,700	6,900	18,100	10
SEPTEMBER	A	10	180	140	590	260	*	110	100	400	800	470	10	-
	B	10	20	160	610	110	*	170	170	100	200	360	-	-

TABLE E 10. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	-	-	50	180	*	120	160	50	100	100	-	-	-
	B	-	-	60	150	*	590	260	70	740	420	-	-	-
NOVEMBER	A	-	-	20	*	180	920	1,330	60	1,080	40	-	-	-
	B	-	-	50	*	220	420	510	90	650	40	10	-	-
DECEMBER	A	-	*	*	20	80	20	30	-	-	-	-	-	-
	B	-	*	*	-	20	30	30	40	-	-	-	-	-
FEBRUARY	A	*	*	10	11	49	1,000	30	20	-	-	-	-	-
	B	*	*	-	11	11	1,130	20	50	-	-	-	-	-
MARCH	A	*	*	7	17	-	100	-	-	-	-	-	-	-
	B	*	*	5	21	-	310	-	-	-	-	-	-	-

TABLE E 11.

TOTAL NUMBER OF *O.leptospicularis* L_3 RECOVERED FROM GRASS SAMPLES COLLECTED AT A RADIUS OF 0.25 CMS AROUND FAECES (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	–	–	26	2,790	165	115	100	265	10	20	*	200	6
	B	–	–	144	3,035	315	260	200	10	26	10	*	200	21
MAY	A	–	10	630	2,515	2,550	2,150	875	435	900	*	300	60	–
	B	10	10	990	3,470	1,780	2,050	2,110	685	1,100	*	1,500	210	–
JUNE	A	140	1,470	1,680	8,240	11,750	11,800	8,800	900	*	3,670	4,200	1,600	700
	B	120	1,380	1,920	7,235	18,450	12,650	7,900	700	*	3,620	2,800	900	630
JULY	A	–	1,360	9,200	17,750	22,550	11,300	3,400	*	1,500	800	2,800	3,530	2,250
	B	10	3,370	5,700	22,200	20,800	11,450	3,700	*	1,310	4,200	2,600	4,640	3,220
AUGUST	A	180	130	2,400	7,250	3,100	800	*	2,900	1,200	900	500	960	–
	B	–	120	3,900	6,350	3,800	4,900	*	3,400	600	700	760	730	–
SEPTEMBER	A	40	80	300	280	220	*	380	100	200	90	20	20	–
	B	–	20	230	435	170	*	130	100	100	180	10	–	–

TABLE E 11. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	-	-	120	100	*	70	300	500	90	70	-	20	-
	B	-	-	50	150	*	600	600	400	380	120	10	30	-
NOVEMBER	A	-	-	350	*	560	230	170	220	-	20	-	-	-
	B	-	-	450	*	410	790	650	470	-	10	-	-	-
DECEMBER	A	-	*	*	40	50	40	70	20	-	-	-	-	-
	B	-	*	*	80	10	30	50	30	-	-	-	-	-
FEBRUARY	A	*	*	4	2	80	340	30	60	-	-	-	-	-
	B	*	*	6	8	62	110	80	50	-	-	-	-	-
MARCH	A	*	*	17	-	1,950	120	-	-	-	-	-	-	-
	B	*	*	31	-	2,510	130	-	-	-	-	-	-	-

TABLE E 12.

O.ostertagi INFECTIVE LARVAE ON HERBAGE EXPRESSED AS A PERCENTAGE OF EGGS DEPOSITED IN THE FAECES (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	-	0.003	0.076	0.8	2.0	0.24	0.28	0.43	0.22	0.21	*	0.04	0.6
	B	-	-	0.076	0.6	1.3	0.8	0.09	0.4	0.31	0.17	*	0.13	0.4
MAY	A	-	0.02	0.68	0.25	0.66	2.2	0.66	0.63	3.06	*	0.64	0.76	0.8
	B	-	-	0.22	0.55	0.68	0.76	1.8	2.06	3.33	*	0.76	1.3	1.5
JUNE	A	-	0.5	0.24	6.7	17.7	24.2	14.7	4.36	*	0.17	8.7	7.6	6.5
	B	-	0.34	0.20	8.09	14.8	22.15	15.7	5.8	*	0.95	8.9	6.6	5.1
JULY	A	-	0.04	1.65	15.5	17.0	19.6	7.3	*	0.89	11.25	5.2	14.12	15.4
	B	-	0.05	1.79	16.8	23.0	15.8	6.6	*	0.67	4.4	4.2	10.0	9.6
AUGUST	A	0.007	0.07	0.26	3.08	3.5	2.43	*	0.24	3.76	0.7	2.58	3.8	0.003
	B	0.02	0.02	1.1	2.95	3.04	2.0	*	0.15	2.9	1.3	1.3	3.4	0.001
SEPTEMBER	A	0.002	0.04	0.03	0.14	0.06	*	0.02	0.02	0.1	0.18	0.11	0.002	-
	B	0.002	0.004	0.03	0.14	0.02	*	0.03	0.03	0.02	0.04	0.08	-	-

TABLE E 12. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	-	-	0.02	0.08	*	0.05	0.07	0.02	0.04	0.04	-	-	-
	B	-	-	0.02	0.07	*	0.27	0.12	0.03	0.34	0.19	-	-	-
NOVEMBER	A	-	-	0.002	*	0.02	0.10	0.15	0.006	0.12	0.004	-	-	-
	B	-	-	0.005	*	0.02	0.04	0.05	0.01	0.07	0.004	0.001	-	-
DECEMBER	A	-	*	*	0.01	0.04	0.01	0.01	-	-	-	-	-	-
	B	-	*	*	-	0.01	0.01	0.01	0.02	-	-	-	-	-
FEBRUARY	A	*	*	0.002	0.002	0.01	0.23	0.006	0.006	-	-	-	-	-
	B	*	*	-	0.002	0.002	0.26	0.006	0.01	-	-	-	-	-
MARCH	A	*	*	0.002	0.005	-	0.03	-	-	-	-	-	-	-
	B	*	*	0.001	0.006	-	0.1	-	-	-	-	-	-	-

TABLE E 13

O.leptospicularis INFECTIVE LARVAE ON HERBAGE EXPRESSED AS A PERCENTAGE OF EGGS DEPOSITED IN THE FAECES (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
APRIL	A	-	-	0.02	2.3	0.13	0.1	0.1	0.22	0.008	0.016	*	0.16	0.0004
	B	-	-	0.12	2.5	0.26	0.21	0.16	0.008	0.02	0.008	*	0.16	0.02
MAY	A	-	0.003	0.2	0.8	0.8	0.7	0.3	0.14	0.28	*	0.09	0.02	-
	B	0.003	0.003	0.31	1.1	0.56	0.65	0.67	0.22	0.35	*	0.48	0.06	-
JUNE	A	0.08	0.9	1.04	5.09	7.26	7.29	5.44	0.55	*	2.26	2.6	0.93	0.4
	B	0.07	0.85	1.18	4.47	11.41	7.8	4.8	0.43	*	2.23	1.73	0.5	0.4
JULY	A	-	0.9	6.13	11.8	15.03	7.5	2.26	*	0.09	0.53	1.9	2.3	1.5
	B	0.006	2.24	3.8	14.8	13.8	7.6	2.46	*	0.87	2.8	1.7	3.1	2.1
AUGUST	A	0.12	0.08	1.63	5.0	2.1	0.54	*	2.0	0.8	0.6	0.34	0.6	-
	B	-	0.08	2.6	4.3	2.6	3.3	*	2.3	0.4	0.47	0.52	0.5	-
SEPTEMBER	A	0.01	0.13	0.5	0.46	0.36	*	0.63	0.16	0.32	0.15	0.03	0.03	-
	B	-	0.03	0.4	0.72	0.28	*	0.21	0.16	0.16	0.3	0.16	-	-

TABLE E 13. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36	40	44
OCTOBER	A	-	-	0.05	0.04	*	0.02	0.12	0.21	0.03	0.03	-	0.008	-
	B	-	-	0.02	0.06	*	0.25	0.25	0.16	0.16	0.05	0.004	0.01	-
NOVEMBER	A	-	-	0.07	*	0.11	0.04	0.03	0.04	-	0.003	-	-	-
	B	-	-	0.08	*	0.08	0.15	0.12	0.09	-	0.001	-	-	-
DECEMBER	A	-	*	*	0.005	0.006	0.005	0.009	0.002	-	-	-	-	-
	B	-	*	*	0.01	0.001	0.004	0.006	0.004	-	-	-	-	-
FEBRUARY	A	*	*	0.001	0.006	0.02	0.11	0.01	0.02	-	-	-	-	-
	B	*	*	0.001	0.002	0.02	0.03	0.02	0.01	-	-	-	-	-
MARCH	A	*	*	0.003	-	0.34	0.02	-	-	-	-	-	-	-
	B	*	*	0.005	-	0.44	0.02	-	-	-	-	-	-	-

TABLE E 14.

NUMBERS OF *O.ostertagi* L_3 RECOVERED FROM REPLICATE SOIL CORES (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36–40
APRIL	A	–	1	6	4	5	–	–	4	–	–	–
	B	–	2	3	2	2	3	4	–	–	–	–
MAY	A	–	–	1	3	2	15	–	–	–	–	–
	B	–	–	–	1	7	1	2	–	–	–	–
JUNE	A	10	40	130	70	310	270	–	–	–	–	–
	B	–	10	20	30	180	320	110	100	–	–	–
JULY	A	–	1	–	230	280	280	–	–	–	–	–
	B	–	10	260	170	290	20	–	–	–	–	–
AUGUST	A	–	–	30	290	120	–	–	–	10	20	–
	B	–	–	120	240	140	90	–	–	–	–	20

TABLE E 14. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36–40
SEPTEMBER	A	–	8	1	1	–	–	–	–	–	–	–
	B	–	12	3	–	–	–	–	1	–	–	–
OCTOBER	A	–	–	–	5	–	–	–	–	10	–	–
	B	–	–	–	7	–	–	–	–	–	–	–
NOVEMBER	A	–	–	3	–	–	2	–	–	–	–	–
	B	–	–	1	–	–	1	–	–	–	–	–
DECEMBER	A	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–
FEBRUARY	A	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–
MARCH	A	–	–	–	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–	–	–	–

TABLE E 15.

NUMBERS OF *O.leptospicularis* L_3 RECOVERED FROM REPLICATE SOIL CORES (IN WEEKS)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	36–40
APRIL	A	–	2	*	27	3	–	1	–	1	1	–
	B	–	–	–	26	2	1	–	–	–	–	–
MAY	A	–	1	2	1	–	3	–	–	–	–	–
	B	–	–	7	6	2	3	3	1	–	–	–
JUNE	A	–	30	–	100	90	70	–	–	–	–	1
	B	–	10	10	30	50	–	30	–	–	70	–
JULY	A	–	–	60	70	–	–	–	–	–	–	–
	B	–	–	180	70	30	10	–	–	–	20	–
AUGUST	A	–	1	70	50	50	10	–	–	1	–	–
	B	–	1	30	60	70	–	–	–	–	–	–

TABLE E 15. (Cont'd)

MONTH FAECES DEPOSITED		1	2	4	8	12	16	20	24	28	32	34 - 36
SEPTEMBER	A	-	-	2	1	2	-	-	-	-	-	-
	B	-	-	2	1	-	-	-	-	-	-	-
OCTOBER	A	-	-	1	-	-	-	-	-	-	-	-
	B	-	-	1	-	-	-	-	-	-	-	-
NOVEMBER	A	-	-	-	-	-	1	-	-	-	-	-
	B	-	-	-	-	-	1	-	-	-	-	-
DECEMBER	A	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-
FEBRUARY	A	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-
MARCH	A	-	-	-	1	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-

TABLE E 16.

THE MEAN PERCENTAGE OF *Ostertagia* spp EGG DEPOSITED FROM JUNE THROUGH TO SEPTEMBER WHICH BECOME L_3 AND THE PERIOD OF THEIR SURVIVAL

DATE OF FAECAL DEPOSITION		Weeks after deposition												
		1	2	4	8	12	16	20	24	28	32	36	40	44
JUNE	0.0.	-	0.42	0.2	7.4	16.2	23.2	15.2	5.1	*	0.56	8.8	7.1	5.8
	0.L.	0.07	0.8	1.1	4.8	8.3	7.5	5.12	0.5	*	2.2	2.16	0.7	0.4
JULY	0.0.	-	0.04	1.7	16.15	20.0	17.7	7.0	*	0.78	7.8	4.7	12.0	12.5
	0.L.	0.03	1.6	5.0	13.3	14.4	7.5	2.36	*	0.5	1.6	1.8	2.7	1.8
AUGUST	0.0.	0.01	0.04	0.68	3.0	3.3	2.2	*	0.2	3.3	1.0	2.0	3.6	-
	0.L.	0.06	0.08	2.1	4.6	2.3	1.9	*	2.0	0.6	0.5	0.4	0.5	-
SEPTEMBER	0.0.	0.002	0.02	0.03	0.14	0.04	*	0.02	0.02	0.06	0.11	0.09	-	-
	0.L.	0.005	0.08	0.4	0.6	0.32	*	0.42	0.16	0.24	0.22	0.09	-	-
OCTOBER	0.0.	-	-	0.02	0.007	*	0.16	0.1	0.02	0.2	0.11	-	-	-
	0.L.	-	-	0.03	0.05	*	0.13	0.2	0.18	0.1	0.04	0.002	0.009	-
NOVEMBER	0.0.	-	-	0.003	*	0.02	0.07	0.1	0.008	0.09	0.004	0.0005	-	-
	0.L.	-	-	0.07	*	0.09	0.09	0.07	0.06	-	0.002	-	-	-

SECTION F

THE EFFECT OF SERIAL EXPERIMENTAL PASSAGE IN CALVES ON THE RELATIVE PROPORTIONS OF O. OSTERTAGI AND O. LEPTOSPICULARIS IN A POPULATION OF MIXED OSTERTAGIA SPP

INTRODUCTION

Recent studies by Bisset (1980) and Al Saqur et al (1982a, 1982b) have shown that O. leptospicularis (O. L.) can be responsible for outbreaks of bovine ostertagiasis sometimes as the dominant species present or mixed with the better known pathogen O. ostertagi (O.O.). In the most recent paper by Al Saqur, Armour, Bairden, Dunn, Jennings and Murray (1984) it was also demonstrated that when inocula of bovine Ostertagia spp which contained O. L. were given to calves, the percentage of worms established was markedly increased, compared with inocula devoid of O. L. The increased establishment of the mixed inocula was accompanied by an increased pathogenic effect and clinical signs.

Interactions between gastrointestinal nematode species leading to an enhanced establishment of these species had not been reported before the observations of Al Saqur and his colleagues although increased establishment of abomasal nematodes from different genera (Ostertagia and Trichostrongylus axei) have been recorded by Herlich (1959) and Ross, Purcell, Todd and Dow (1968). Kates and Turner (1953) also noted enhanced infectivity of Nematodirus spathiger and T. colubriformis when the larvae of these species were inoculated simultaneously.

By contrast, there have been two reports in which the interaction between abomasal nematodes from two different genera namely Haemonchus contortus and T. axei resulted in only a few Haemonchus becoming established in calves following experimental infections with the two genera (Reinecke, 1970); similar

observations were made in the field by Muller (1968). In Australia, Dash (1981) also reported a 50% decrease in the establishment of Oesophagostomum venulosum when larvae of both Oe. venulosum and Oe. columbianum were administered simultaneously to sheep.

In Section B of this thesis there was also an indication that the presence of H. similis might adversely affect the establishment of H. contortus.

In the studies of Al Saqur and his colleagues (1982a, 1982b) it was shown that O.L. was more fecund than O.O. and this fact together with the enhanced establishment of both species following their simultaneous inoculation under experimental conditions should have produced heavy contamination of pastures with O.L., when mixed larval infections were ingested under natural conditions; yet examination of the worm burdens of calves exposed to natural mixed infections of O.O. and O.L. at the commencement of grazing in the spring (May), had only a low proportion of O.L. in their Ostertagia burdens recovered at the end of the grazing season in September (Al Saqur et al, 1982b).

To try and elucidate the reasons for these fluctuations in prevalence of O. O. and O.L., apart from the ecological ones discussed in Sections D and E, the consequence of serial passage of an Ostertagia inoculum containing a known amount of O.O. and O.L. infective larvae was studied by assessing the numbers of worms established by each species.

The possible role of anthelmintics in selecting out one or other of the two species was also studied in a parallel experiment.

EXPERIMENTAL DESIGN

Two inocula, each containing approximately 90,000 infective larvae of O.O. and 10,000 infective larvae of O.L. were given to two calves. On day 14, one calf was given thiabendazole (Merck, Sharp & Dohme, Hoddesdon, Herts.) at 110 mg per kg bodyweight. From day 18 the faeces of each calf were collected for three days for larval culture. The calves were then necropsied on day 24. The infective larvae harvested from the faecal cultures were then inoculated, again at a dose of 100,000 L_3 into two more calves. This procedure was repeated on a total of six occasions as shown in the experimental design in Table F 2.

To control possible changes in the original larval populations used for the components of the mixed inocula, the original cultures of O.O. and O.L. infective larvae, hereafter referred to as monospecific cultures, were passaged and cultured on at least four occasions. This design is summarised in Table F 1.

Finally, because of the results obtained in the field ecology study and the results of the present experiment the larval progeny of the 6th passage of the mixed inoculum were stored for several months at 4°C to simulate winter conditions and inoculated into two calves.

MATERIALS AND METHODS

Animals

Indoor reared parasite-free male Friesian calves aged four months were used for all larval passages.

Clinical. The calves were examined daily.

Parasitological Observations

Faeces. Faecal egg counts were performed prior to inoculation of the larvae and daily from day 18 until necropsy. When Ostertagia eggs appeared in the faeces the latter were cultured at 21°C as described in the General Materials and Methods.

Larval inocula

Larval inocula were prepared by counting the number of larvae present in 40 x 0.025 ml aliquots. A minimum total number of 400 larvae were counted and to facilitate examination the larval dilution was so arranged that the number of L_3 per 0.025 ml aliquot did not exceed 30. Once the number of L_3 present in 1 ml was known the volume necessary to provide the required inoculum was pipetted out and made up to a volume of approximately 20 ml prior to dosing the calves. Throughout the counting procedure emphasis was placed on regular aggitation of the suspension to prevent clumping.

Origin of Larvae

A monospecific culture of O.L. obtained from previous studies by Al Saqur et al (1982) and since then subjected to experimental passage on two occasions and a monospecific laboratory strain of O.O. passaged four times were used for primary infections.

Post Mortem

At necropsy, the abomasum was opened and the contents and mucosae processed according to Ritchie et al (1966). Identification of individual Ostertagia spp were then made and a morphological examination of the spicules (Plate 7). Where

O. ostertagi (Tail of male)

The important morphological features are the slender, almost straight, spicules with truncate main and second branches. The third branch is delicate and somewhat hooked, and can be seen to the left of the flexible stem of the paddle shaped gubernaculum.

S. lyrata (Tail of male)

The spicules are massive, the main branch ending in a strong medially-directed point. A notable feature is the very stout second branch, which ends in a somewhat saucer-like expansion. The third branch, clearly visible in the left spicule, is very short and pointed. The "boss" or "eyelet" is about the middle of the spicule body.

O.ostertagi

S. lyrata

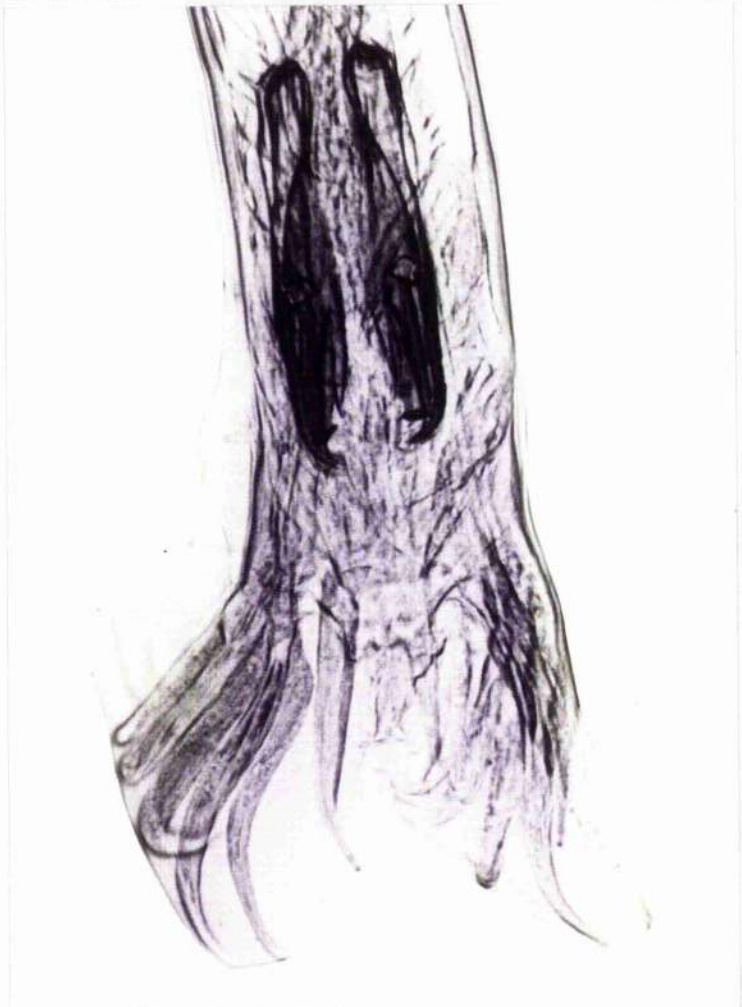


Plate 7. Male bursae of O.ostertagi and S. lyrata

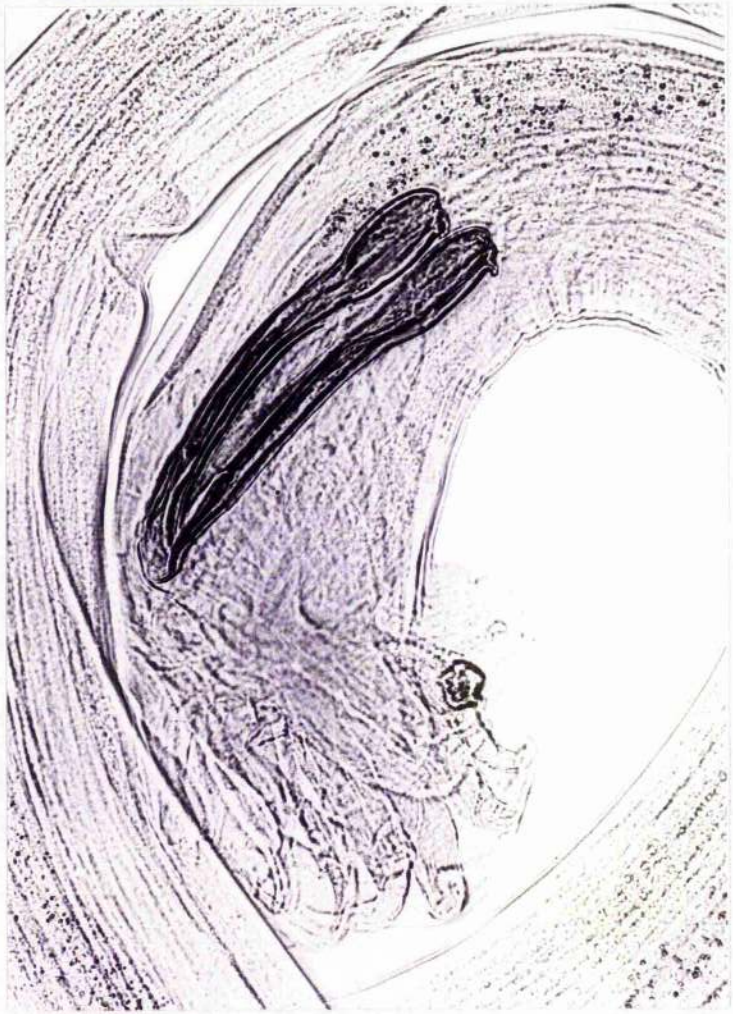
O. leptospicularis (Tail of male)

The features differentiating the spicules of this species from O.ostertagi are the pointed tip of the main branch, the "sickle" form of the third branch, which can be seen very faintly at the caudal end of the left spicule, and the spatulate form of the cephalic end.

S. kolchida (Tail of male)

Like S. lyrata, this species has stout spicules. It is differentiated from S. lyrata by the less massive form of the branches. The main branch ends in a pointed, medially-directed, "shoe-like" tip, while the second appears truncate when viewed, as in this specimen, from the dorsal aspect. The third branch is short and pointed, and is easily seen in the left-hand spicule. The "boss" is about the beginning of the distal third of the spicule body. The gubernaculum is "baton-shaped" and in this specimen has been dislodged from between the spicules to lie on the approximate location of the dorsal ray.

O. leptospicularis



S. kolchida

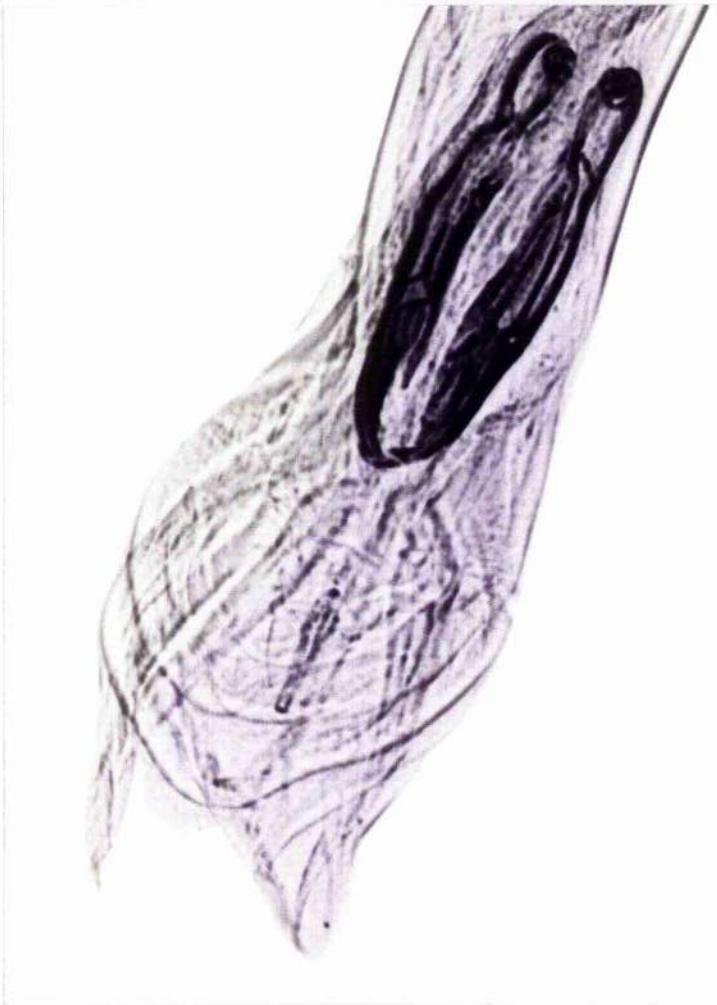


Plate 7 (contd). Male bursae of O. leptospicularis and S. kolchida.

measurements were required an eyepiece micrometer was used

RESULTS

Due to unforeseen circumstances there were two enforced changes to the original experimental design of the passages involving the monospecific cultures. The calves reared for the first and second passages suffered from an outbreak of "virus" pneumonia and it was decided that they were in such poor condition that they could not be infected. The first passage of the monospecific infective larvae therefore took place at the same time as the third passage of the mixed inocula.

Worm Burdens

Table F 3 details the worm populations of the calves following each passage while Table F 4 gives the percentage of each nematode species recovered. From these results it is clear that the effect of treatment on day 14 with thiabendazole had a minimal effect on the number of worms present at subsequent necropsy. Thus, the total Ostertagia burdens of the treated calves ranged from 8,300 to 41,200 compared with a range of 26,300 to 45,600 in the untreated animals. There was also no indication that the drug selectively removed more O.O. or O.L. This can be seen from Table F 4 in which the results show that the proportion of both O.O. and O.L. are virtually the same in the anthelmintic treated and untreated calves.

Overall worm populations were as might be expected from an infection dose of 100,000 L_3 except for one instance when a 63% establishment of O.L. was obtained. With the exception of the mixed untreated group (32.6%) the percentage establishments by

the time of passage 6 were markedly reduced with recoveries of only 14,7%; 12.6% and 8.3% being obtained.

Where monospecific control groups were present in passages 3 through 6, the percentage establishment of worms was higher in the calves given mixed inocula of larvae, on three out of the four untreated calves (P3, P5 and P6) compared to the O.O. controls and in only one of the calves (P6) compared to the O.L. controls.

In the worm burdens which accrued from the monospecific O.O. culture, S. lyrata was also recovered on three out of the four calves infected and ranged from 2 to 11% of the total burden; O.L. were recovered on two occasions at levels of 3 and 4%; O.O. was the only other species present.

From infections with the monospecific O.L. culture four Ostertagia spp were recovered; O.L. which ranged from 76 to 93% of the total; S. lyrata which ranged from 6 to 12% of the total; S. kolchida which ranged from 0 to 8% and the ovine species O. circumcincta which was present only in the first three passages at a level of 0 to 6%.

In the mixed inocula calves given no anthelmintic treatment four species were detected; O.O. ranged from 9 to 92%; O.L. from 0 to 88%; S. lyrata from 0 to 7% and S. kolchida from 0 to 2%.

In the calves given the mixed inocula plus anthelmintic treatment O.O. ranged from 13 to 91%; O.L. from 2 to 87%; S. lyrata from 0 to 6% and S. kolchida from 0 to 4%.

Typical spicules from each of these species are shown in Plate F 7.

Clearly, in the calves given the mixed inocula the percentage of O.L. present at necropsy increased through passages 1 to 5 at which point the untreated calf harboured 88% O.L. and the treated animal 87% of this species. After passage 6 a decrease in the numbers of O.L. was observed from both calves with 65 and 74% O.L. being recovered from the treated and untreated animals respectively.

Following storage at 4°C, for more than six months, of the L_3 cultured from the faeces of calves which received the sixth passage, the larvae inoculated into two calves yielded an almost pure population of O.O. with only 0.5% S. lyrata being recovered from 200 worms.

DISCUSSION

The object of this experiment was to examine the population interactions between two Ostertagia species O. ostertagi and O. leptospicularis which occurred when a mixed inoculum of these species was administered to calves and the larval progeny of this population then inoculated into other calves and the procedure repeated on six occasions. With this experimental design it was hoped to simulate a field situation over five or six generations of mixed Ostertagia spp.

For this purpose isolates of O.O. and O.L. thought to be virtually free of other species were used. However, it is clear from the results that the isolates were not pure. Thus the O.O. isolate contained low percentages of S. lyrata on three out of four occasions and O.L. at two necropsies. The O.L. isolate had an even greater variety of other Ostertagia spp, namely S. lyrata, S. kolchida and O. circumcincta mostly at less than

ten per cent of the total with O.L. being the dominant species.

These results are difficult to explain in view of current theories on Ostertagia populations in ruminants. Several authors have published on polymorphism in nematodes and in Ostertagia spp in particular. Polymorphism has been defined by Ford (1953) as "the occurrence together in the same habitat at the same time of two or more discontinuous forms of a species, the rarest of which is too frequent to be maintained merely by recurrent mutation".

The first indication that polymorphism occurs in Ostertagia spp and to be more specific the sub-family Ostertagiinae came from Copland (1965) who, whilst attempting to establish a pure culture of O. circumcincta in sheep by male selection with unknown origin females of mixed Ostertagia spp found males of O. circumcincta, O. trifurcata and Teladorsagia davtiani for several generations before achieving a pure culture. The author proposed that these findings suggested cross fertilisation between the species although he failed to find any hybrids.

Hybridisation has been reported under experimental conditions between two closely related Cooperia spp (C. oncophora and C. surnabada) by Isentein (1971b) in experiments in which the nematodes were given only a single choice of mate. Isenstein (1971a) also concluded that C. oncophora is polymorphic with C. surnabada as the alternative morph.

Polymorphism has also been demonstrated in H. contortus in relation to the frequency of smooth, knobbed or linguiform vulvar flaps (Daskalov, 1971). More recently, at Weybridge, Lancaster and Hong (1981) and Lancaster, Hong and Michel (1983) have

produced evidence that O. ostertagi is a polymorphic species and O. lyrata is the alternative morph (these authors prefer O. lyrata to S. lyrata). They also state that O. leptospicularis is polymorphic with S. kolchida as the alternative morph. In each of these relationships the dominant species is the one with slender male spicules while the other possesses stronger spicules (See Plate F 7).

If these theories on polymorphism are correct how can the mixture of species in the present experiment be explained? Taking the monospecific O.O. isolate first, the presence of O.L. without S. kolchida does not fit with the Weybridge theory. The presence of S. lyrata without O. ostertagi plus O. circumcincta in the monospecific O.L. isolate is even more confusing. The O. circumcincta is presumably a contaminant since this isolate was passaged through sheep and disappeared following three passages in calves. The presence of S. lyrata without O. ostertagi remains a mystery. One difference between the current study and the Weybridge work is that they examined 500 males from each necropsy whereas only 100 were examined here. However, the fact that S. lyrata the less common species was found at a level of 6 per cent after four passages and no O. ostertagi rather suggests that the differences are not due to the numbers of worms examined. Since great care was taken to avoid cross contamination of cultures the only possible conclusion is that the simple arrangement of pairs proposed by the Weybridge workers on O. ostertagi/O. lyrata and O. leptospicularis/S. kolchida requires several passages, apparently more than four, before the relationship is

standardised.

Even more difficult to explain are the results from serial passages of the mixed inocula. The first anomaly was the failure of thiabendazole to have any consistent effect on the numbers of worms established. Treatment at 110 mg per kg bodyweight was deliberately chosen to obtain an efficiency which was less than 80% (Armour, Jennings, Kirkpatrick, Malczweski and Urquhart, 1967) in order to have sufficient adult male worms for identification. The apparent very low efficiency against the 5th larval stages, which would be present at day 14, could be due to a variety of reasons; lack of efficiency against this particular stage or possibly resistance of this isolate to thiabendazole. The former seems the more likely since the adult stages of this isolate are known to be susceptible to thiabendaozle (Bairden, personal communication).

The steady increase in the proportion of O.L. found after four passages from 2 to 87% in the treated calves and 5 to 88% in the untreated ones is remarkable. It could possibly be ascribed to the higher egg production of O.L. also noted in Section D but why then is there a fall in the proportion of O.L. following the fifth passage.

Could there initially by a hybridisation of these species with these hybrids at first being more vigorous and fecund at the F 1 generation and then gradually becoming less fertile? This is unlikely as there was no morphological evidence of hybrid specimens at necropsy.

Could O.L. be an alternative morph of O.O. just as S. lyrata

and what was happening was a natural phenomenon, O.L. and O.O. alternating in dominance? This would not fit with the classical description of a morph (Ford, 1953) but might explain why O.L. is sometimes isolated as the major proportion of Ostertagia spp recovered from outbreaks of bovine ostertagiasis.

The other possibility is that the free-living larvae of O.L. are less fit for survival than those of O.O. in the temperatures at which larvae are usually stored i.e. 4°C to 6°C. In the first six passages the interval between larval harvest from culture and inoculation of calves was only a few days but to test the hypothesis that the O.L. larvae were less fit, the larval progeny of passage 6 were stored at 4°C for over six months. This prolonged storage produced a marked change in the proportion of Ostertagia spp in calves P 7A and P 7B. Thus the worm population was almost entirely O.O. with only one S. lyrata being found from the examination of 200 male worms. O. leptospicularis and S. kolchida had apparently disappeared following prolonged storage. This finding is rather similar to those in the larval ecology study in Section E where the free-living stages of O.L. survived the winter less well than those of O.O. and could explain the sporadic nature of its occurrence in bovine ostertagiasis possibly following contamination of pastures by deer.

How then does O.L. survive to infect deer each spring? From the evidence here they survive poorly outside the host and probably their survival from one season to another is as arrested 4th stage larvae in the deer. The worm populations recovered from calves P 7A and P 7B were all adult worms and storage of

Ostertagia larvae usually produces arrested 4th stage larvae on inoculation to calves. However, it has also been shown (Armour, Bruce, 1974) that after prolonged storage, in this experiment more than six months, the larvae prone to arrestment show an increased mortality and the survivors develop to the adult stage in the usual period of time.

Finally, it is worth noting that the higher establishment rate of inocula containing both O.L. and O.O. and noted by Al Saqur et al (1982a, 1982b) was not regularly observed here. This could possibly be due to the relative proportion of O.L. in the mixture or a change in the infectivity of both species following laboratory passage; Al Saqur and his colleagues used isolates which had been passaged a maximum of three times whereas in the current trial they had been passaged on at least six occasions.

GENERAL DISCUSSION

The studies reported in this thesis fall into two distinct sections.

In the first, the ecology of H. contortus free-living larval stages and the seasonal pattern of abomasal worm burdens in young cattle in a subtropical environment, namely, Paraguay, are investigated.

In the second, some aspects of the ecology of two Ostertagia spp, O. ostertagi and O. leptospicularis are studied in a temperate climate, namely West Scotland and the identification and development of these free-living stages examined under laboratory conditions. In addition, the interaction of these two species following serial passage in calves and laboratory culture

TABLE F 1.

EXPERIMENTAL DESIGN

Mixed inocula

	Calf	Day 0	D 14	D 18 – 21	D 22
P1	A	10,000 O.L. L_3 + 90,000 O.O. L_3	TBZ	Faecal culture	Necropsy
	B	10,000 O.L. L_3 + 90,000 O.O. L_3	NIL	Faecal culture	Necropsy
P2	C	100,000 Mixed L_3	TBZ	Faecal culture	Necropsy
	D	100,000 Mixed L_3	NIL	Faecal culture	Necropsy
P3 ↓ P4 ↓ P5 ↓ P6 ↓ P7	M	100,000 Mixed L_3	TBZ	Faecal culture	Necropsy
	N	100,000 Mixed L_3	NIL	Faecal culture	Necropsy

TABLE F 2.

EXPERIMENTAL DESIGN

Monospecific inocula

	Calf	Day 0	D 18 – 21	D 22
P1	O	100,000 O.L. L_3	Faecal culture	Necropsy
	P	100,000 O.O. L_3	Faecal culture	Necropsy
P2	Q	100,000 O.L. L_3		
	R	100,000 O.O. L_3		
P3				
P4	U	100,000 O.L. L_3	Faecal culture	Necropsy
	V	100,000 O.O. L_3	Faecal culture	Necropsy

TABLE F 3.

TOTAL *Ostertagia* WORM BURDENS AT NECROPSY

24 DAYS POST-INFECTION

Ostertagia spp. (% Establishment)

Passage	Monospecific infections		Mixed infections	
	O.O.	O.L.	O.O./O.L. (TBZ)	O.O./O.L. Untreated
P1	-	-	41,200 (41.2)	33,700 (33.7)
P2	-	-	14,100 (14.1)	45,600 (45.6)
P3	18,300 (18.3)	30,500 (30.5)	24,500 (24.5)	30,300 (30.3)
P4	34,700 (34.7)	44,800 (44.8)	12,400 (12.4)	29,000 (29.0)
P5	26,200 (26.2)	63,800 (63.8)	40,500 (40.5)	44,200 (44.2)
P6	14,700 (14.7)	12,600 (12.6)	8,300 (8.3)	32,600 (32.6)
P7A	-	-	-	30,300 (30.3)
P7B	-	-	-	26,300 (26.3)

TABLE F 4.

PERCENTAGE OF Ostertagia TYPE SPECIES ESTABLISHMENT

AT NECROPSY (100 males examined)

Monospecific O.ostertagi	Passage					
Species	P1	P2	P3	P4	P5	P6
O.O.	96	–	89	96	95	98
O.L.	0	–	0	4	3	0
S.L.	4	–	11	0	2	2
S.K.	0	–	0	0	0	0

Monospecific O.leptospicularis						
Species	P1	P2	P3	P4	P5	P6
O.O.	–	–	0	0	0	0
O.L.	–	–	76	81	93	93
S.L.	–	–	10	12	6	6
S.K.	–	–	8	5	0	1
O.C.	–	–	6	2	1	0

Mixed O.O./O.L. TBZ						
Species	P1	P2	P3	P4	P5	P6
O.O.	91	65	65	31	13	34
O.L.	2	28	33	66	87	65
S.L.	6	3	2	3	0	1
S.K.	1	4	0	0	0	0

Mixed O.O./O.L. Untreated							
Species	P1	P2	P3	P4	P5	P6	P7
O.O.	92	68	71	32	9	26	99.5
O.L.	5	24	26	67	88	74	0
S.L.	3	7	2	0	1	0	0.5
S.K.	0	1	1	1	2	0	0

O.O. = O.ostertagi O.L. = O.leptospicularis

S.L. = S.lyrata S.K. = S.kolchida

O.C. = O.circumcincta

were studied.

H. contortus in the sub-tropics of Paraguay and Ostertagia spp in the wet temperate climate of Scotland may seem poles apart, however, although the results indeed indicate that there are major differences in the larval ecology of the free-living stages, there are also some similarities.

Perhaps one of the most surprising features of the results has been the very low yield of infective larvae onto the herbage contaminated by faeces containing eggs from each of these parasitic genera. Larvae on herbage are usually presented as L_3/kdh and while this is useful for estimating the numbers of larvae available to grazing animals it does not tell us much about the proportion of eggs which actually produce an infective larva. In the case of H. contortus, the percentage yield of L_3 in the Paraguayan studies apparently never exceeded 0.58% of the eggs deposited; however, since the examinations of the faecal deposits and surrounding herbage were made at intervals of 14 days it is possible that on some occasions larvae produced within the 14 day period succumbed rapidly prior to the next sampling date. While acknowledging in retrospect, that it would have been beneficial to have made more frequent examinations, particularly within the first 14 days of faecal deposition, it does appear that a very low percentage of H. contortus eggs produced viable L_3 which remained on the herbage sufficiently long to facilitate the chances of ingestion by cattle grazing on the pasture.

In the studies on the two Ostertagia spp, the percentage yields were also very low except for the two summer months of June and July where individual faecal pats had egg to L_3 yields

of up to 24.2%. In the remaining months of the year the yield was always less than 5% and usually less than 3%.

From the present studies it is not clear why the crops of infective larvae were so low; in Haemonchus some embryonated eggs survived as long as the faecal pats and why more of these failed to hatch larvae in apparently ideal conditions is not known but it may be related to a low oxygen content in the older dung pats. In the Ostertagia study more eggs appeared to hatch but the mortality of the L_1/L_2 stages was apparently very high.

An interesting feature of the Ostertagia ecology was the increased crop of O. ostertagi larvae in the spring on plots contaminated in the previous summer and autumn. The source of these larvae remains unknown but this "spring rise" of larvae was also a feature of the two previous European studies on the ecology of O. ostertagi larvae, namely those of Rose (1962) in Southern England and Persson (1974) in Sweden. Whatever the source of these larvae they must have originated from eggs, larvae in the soil or terrestrial transport hosts such as earthworms since the faecal pats did not survive for sufficiently long to act as reservoirs. This was particularly true of pats deposited in the autumn when rainfall was very heavy and the formation of a crust on the faeces was probably prevented. In contrast, in Paraguay, the faecal pats persisted for several months and with embryonated eggs surviving in these pats some L_3 were constantly available to migrate onto the pasture. Presumably, the higher maximum temperatures in Paraguay allowed a rapid evaporation of moisture and some crust formation on the

surface of the faeces which helped to prolong survival of the faecal pats.

In the only other study on cattle nematodes in which percentage yield of L_3 from eggs was used in the data presentation, Young and Anderson (1981) working in Victoria, Australia, found that the maximum yield of O. ostertagi L_3 occurred from faeces deposited in winter and was 15.7%. In autumn the yields were 8.2% and 10.7% but during the rest of the year failed to exceed 4.2% and in summer and early autumn was always under 2%. These results were analogous to those of the current study since autumn temperatures in Victoria are similar to those of June and July in West Scotland.

It is interesting that in the Victorian study, O. ostertagi larval development was very rapid, taking only three to 12 days in summer temperatures of between 15°C and 30°C, not dissimilar to those found in Paraguay. However during this period the survival of the pre-infective L_1 and L_2 stages was very low but there was a prolonged survival in the dung pat of those larvae which had become L_3 with a few appearing on the herbage six to seven months later, in the following winter. Young and Anderson (1981) suggest that in the arid conditions of the Victorian summer the drying of the faecal pats progressively aereated the pats, so enabling eggs to develop to L_3 at a rate directly dependent on temperature. In contrast, Young, Nicholson, Tweedie and Shuh (1980) have reported that when the moisture content of the dung pat was high, the waterlogging effect reduced the amount of free oxygen and larval mortality, particularly of the L_1/L_2 was high and development of surviving larvae to L_3 was delayed.

Although the Australian workers were studying O. ostertagi these theories could also explain the poor yield of H. contortus under similar temperatures in Paraguay, in which, despite crust formation, the regular rainfall ensured that the moisture content of the faeces remained deleteriously high.

Desiccation is an important factor in larval and egg survival and repeated desiccation is especially important. Anderson and Levine (1968) working with sheep strains found that T. colubriformis infective larvae survived for 120 days at 20°C in water; with repeated daily desiccation they survived only 30 days. H. contortus survived much better following repeated desiccation, survival being as long as 80 days, at 20°C (Todd, Levine and Whiteside, 1970). However it is clear that repeated desiccation is much more detrimental than a single episode. Possibly T. axei and H. similis survive repeated desiccation better than the above species.

In Louisiana, another subtropical climate, Williams (1971, 1973) also found that O. ostertagi developed best in winter temperatures of 13°C to 23°C and that during the hot summer when the mean maximum temperatures reached 26.5°C to 34.8°C there was a rapid development of L_3 accompanied by a rapid mortality in the first two to six weeks.

In neither of the ecology studies was an attempt made to measure soil moisture. Soil moisture deficits have been used by workers in the USA (Anderson, Levine and Boatman, 1970; Levine et al, 1974) in Northern Ireland (Taylor 1975) as a guide to the conditions suitable for the development and survival of sheep

nematode larvae. In the Paraguayan study this data was not available and it was not included in the Scottish study since there appears to be some doubt as to whether the inclusion of such data is necessary. Thus, Anderson et al (1970) have shown that under the conditions in Urbana, Illinois, where precipitation usually occurs each week, the main factor controlling larval development and survival is temperature. Since rainfall occurred every week except on one occasion in Paraguay it was not considered necessary to try and obtain soil moisture values on site; the volume of data being handled in the Glasgow experiment also precluded the measurement of soil moisture and the fact that larval numbers remained high during the dry spell in July/August suggested that sufficient moisture was still present to maintain development, survival and movement of larvae.

However, it should be emphasised that despite the very low yields of infective larvae from the Haemonchus eggs and to a lesser extent the Ostertagia eggs the actual numbers of larvae present were capable of causing significant infections in grazing cattle. Thus in the Paraguayan study the L_3/kdh of H. contortus within four weeks of deposition was usually in excess of 2,000 throughout the year while the levels of O. ostertagi after the winter were at least 20,000 L_3/kdh. Since most faecal pats had disintegrated after the winter the latter larvae would be situated in areas which cattle would graze without the need to avoid faeces.

The results of the field study in Paraguay were interesting and confirmed that in the sub-tropics the winter and autumn are the periods when most trichostrongyle infections are acquired by

cattle. Apart from the seasonal pattern of infection observed in the permanent tracer calves, the influence of extreme weather conditions, in this case drought, on the grazing behaviour of the cattle and its effect on the magnitude of the worm burdens was most interesting and reinforced the observations of Durie (1961) on the role of the bovine faecal pat as a reservoir of larvae during a drought period.

However, from the field parasitologist's or veterinarian's point of view there were two disturbing results. The first of these were the very low epg's of the cattle, despite the fact that they were harbouring in some cases more than 5,000 Haemonchus spp and over 50,000 T. axei. There is no obvious reason for this discrepancy but it may be associated with the diet of the animals or a partial immunity which inhibited egg production. What is important is the fact that in South America the epg's of cattle are thought only to be significant if they exceed 500 (Cabral, personal communication). In this study the epg's were often 100 - 125, yet the cattle had pathogenic worm burdens.

The second disturbing feature was the inconsistent results obtained by the larval cultures. Haemonchus larvae were recovered regularly, but T. axei larvae were not, despite the animals having high burdens of this parasite. This may have been due to the temperatures at which the faecal cultures were stored (27°C) favouring the development of Haemonchus or it may reflect the naturally low epg output of T. axei. Whatever the reason it highlights the problems in differentiating trichostrongyle

infections in the field by the long established technique of faecal larval culture.

The presence of both H. contortus and H. similis in the abomasa of the cattle during 1979 and the dominance of the latter in 1980 was also very interesting. While this may reflect that H. similis free-living larvae are more resistant to the effects of desiccation and so survived the drought of 1980 there may be other reasons. For example, could the presence of H. similis interfere with the establishment of H. contortus or possibly affect egg production by the latter? This is an area which requires investigation and the egg production of H. contortus with or without the presence of H. simils should be investigated The morphology of H. contortus females is also worthy of a more detailed investigation to assess whether any morphological changes in the vulvar flap were present. Some authors (Michel, Lancaster and Hong, 1971) maintain that the absence of a vulvar flap in O. ostertagi is caused by a developing host immunity to the parasite and it may be that the same is true for H. contortus. This may be useful to know and would help in distinguishing whether the H. contortus acquired by other cattle in their second year were morphologically changed by a developing host immunity.

Another interesting and inexplicable feature of the field study was the ability of the cattle to cope with burdens of several thousand Haemonchus spp. without developing an anaemia or a loss of bodyweight. Although this aspect is being dealt with in detail in another thesis it is worthy of comment. Since the animals at Pirity were on a poor grazing and a diet low in

protein it seems remarkable that the cattle coped with the Haemonchus burdens without becoming markedly anaemic. The reason for this is not known but it may be related to a developing immunity, diet or some interaction with the other abomasal parasites present.

One aspect which might be worthy of further investigation is the relationship between the mineral status of the herds in Paraguay and their Haemonchus burdens. Workers in New Zealand and Ireland have shown a relationship between the Haemonchus burdens and the cobalt status of sheep. In cobalt deficient sheep supplemented with cobalt more H. contortus were established and the metabolism and pathogenic effect enhanced compared to deficient controls (Downey 1965, 1966). It is possible that if the Paraguayan cattle were deficient in cobalt this could have lowered the metabolism and pathogenic effect of established Haemonchus spp.

The differentiation of infective larvae from different species of the same genus has always been difficult although species occurring in different hosts can be identified e.g. O. circumcincta in sheep and O. ostertagi in cattle. However, during the field study in Paraguay the inability to distinguish larvae of H. contortus and H. similis prompted the experiment reported in Section C comparing O. ostertagi and O. leptospicularis. Using a computerised method to analyse the data it was hoped that populations of the two species of larvae could be readily separated. This was accomplished and significant differences in oesophageal length and sheath

prolongation of the L_3 were obtained. However, the differences were so small that they could not be readily discerned on visual examinations again highlighting the problems of identification of larvae at species level from faecal culture and herbage samples.

The final section of the thesis provided the answer to the problem of why O. leptospicularis occasionally causes problems in the field but O. ostertagi is the more common cause of bovine ostertagiasis. Several experimental passages in calves of a mixed larval inoculum, which initially contained 90 per cent of O. ostertagi and 10 per cent of O. leptospicularis, allowed the more fecund O. leptospicularis to gradually become dominant in the Ostertagia populations of the calves after four to five experimental passages. The indication from the field study on larval ecology that O. leptospicularis survived poorly under winter conditions prompted the decision to chill, for a prolonged period, the larvae produced following the sixth passage. Despite the majority of the worms in that passage being O. leptospicularis the chilling of the infective larval progeny for a few months, to simulate winter conditions, resulted in the disappearance of this species from the culture and this result offered an acceptable explanation as to why O. leptospicularis fails to become the dominant Ostertagia spp. in natural cattle populations of this genus. Similar studies in H. contortus and H. similis would be interesting and possibly provide information on why these parasites interact in the manner described in the field study.

Finally, mention should be made of the different Ostertagia

found in the larval strains used. If the dogma provided by recent papers on morphological variants of Ostertagia spp. is to be believed, the occurrence of S. lyrata without O. ostertagi is baffling. Perhaps it requires several laboratory passages of strains containing a variety of Ostertagia spp. before the proposed relationship between O. ostertagi and S. lyrata and O. leptospicularis and S. kolchida fall into place. There is also a need to study the hybridisation of these species under a variety of conditions although there was no gross morphological evidence of hybrids in the present study. A possible sequel of these studies would be an analysis of the size of the spicules belonging to the Ostertagia spp. to possibly identify hybrids. However, positive evidence of the existence of hybrids would require carefully managed experiments in which single males and females of the different species were placed in the abomasum and allowed to breed.

The entire field of nematology requires to be more careful in defining a parasite purely by its genus and species name. The number of laboratory passages should also be included and biochemical techniques possibly employed to identify tissue enzymes. In this way the identification of pathogenic and possibly more immunogenic strains could be made.

REFERENCES

Al Saqur, I., Armour, J., Bairden, K., Dunn, A.M., Jennings, F.W. and Murray, M. (1982a). Observations on the infectivity and pathogenicity of three isolates of Ostertagi spp. sensu lato in calves. Research in Veterinary Science 32, 106-112.

Al Saqur, I., Armour, J., Bairden, K., Dunn, A.M. and Jennings, F.W. (1982b). Field study on the epidemiology and pathogenicity of different isolates of bovine Ostertagia spp. Research in Veterinary Science 33, 313-318.

Al Saqur, I., Armour, J., Bairden, K., Dunn, A.M., Jennings, F.W. and Murray, M. (1984). Research in Veterinary Science (in press).

Allen, R.W., Ruiz, E., Pallares, R.M. and Vergara, G. (1966). New records of parasites of cattle in Paraguay. American Society of Parasitology, 41st Annual Meeting, San Juan, Puerto Rico, 31st October - 4th November, p. 33.

Andersen, F.L. and Levine, N. (1968). Effect of desiccation on survival of the free-living stages of Trichostrongylus colubriformis. Journal of Parasitology 54, 117-128.

Andersen, F.L., Levine, N.D. and Boatman, P.A. (1970). Survival of third-stage Trichostrongylus colubriformis larvae on pasture. Journal of Parasitology 56, 209-232.

Anderson, N. (1971). Ostertagiasis in beef cattle. Victoria Veterinary Proceedings 30, 36-38.

Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1965b). A field study of parasitic gastritis in cattle. Veterinary Record 77, 1196-1204.

Anderson, N., Armour, J., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1969). The sequential development of naturally occurring Ostertagiasis in calves. Research in Veterinary Science 10, 18-28.

Anderson, N., Donald, A.D. and Waller, P.J. (1983). Epidemiology and control of parasitic gastroenteritis of cattle in the temperate climate zone. In Epidemiology and Control of gastrointestinal parasites of cattle in Australia. Edited by: N. Anderson and P.J. Waller p. 47-61.

Armour, J. (1970). Bovine ostertagiasis: A review. Veterinary Record 86, 184-190.

Armour, J. (1980). The epidemiology of helminth disease in farm animals. Veterinary Parasitology 6, 7-46.

Armour, J. (1982). Nuclear techniques in the study of parasitic infections. IAEA, 367-377.

Armour, J., Al Saqur, I., Bairden, K., Duncan, J.L. and Urquhart, G.M. (1980). Parasitic bronchitis and ostertagiasis on aftermath grazing. Veterinary Record 106, 184-185.

Armour, J. and Bruce, R.G. (1974). The inhibition of development of Ostertagia ostertagi - a diapause phenomenon. Parasitology 69, 161-174.

Armour, J., Bairden, K., Duncan, J.L., Jennings, F.W. and Parkins, J.J. (1979). Observations on ostertagiasis in young cattle over two grazing seasons with special reference to plasma pepsinogen levels. Veterinary Record 105, 500-503.

Armour, J., Jennings, F.W., Kirkpatrick, K.S. Malczewski, A., Murray, M. and Urquhart, G.M. (1967). The use of thiabendazole in bovine ostertagiasis: Treatment of experimental Type I Disease. Veterinary Record, 510-514.

Armour, J., Jennings, F.W. and Urquhart, G.M. (1969). Inhibition of Ostertagi ostertagi at the early fourth larval stage. Research in Veterinary Science 10, 232-237.

Armour, J. and Ogbourne, G.P. (1982). Bovine ostertagiasis: A review and annotated bibliography. Commonwealth Agricultural Bureau, Farnham Royal, Bucks. Commonwealth Institute of Parasitology, No. 7.

Bailey, W.S. and Herlich, H. (1953). Epizootic. Auburn Veterinarian 105-110.

Bairden, K. (1980). Factors modifying the prevalence of bovine ostertagiasis with special reference to infective larvae in soil. MSc. Thesis, Glasgow University.

Bairden, K., Parkins, J.J. and Armour, J. (1979). Bovine ostertagiasis: A changing epidemiological pattern? Veterinary Record 105, 33-35.

Balbo, T. (1973). Investigation on the distribution of gastrointestinal nematodes in cattle from the province of Cuneo. Nuova Veterinaria 49, 307-314.

Barberian, J.F. and Mizelle, J.D. (1957). Development studies on Haemonchus contortus Rudolphi (1803). American Midland Naturalist 57, 421-439.

Barboza Cardoso, S., Nonato Girao, R., Santiago Girao, E. and Costa Murta, J.L.Da. (1979). Study of helminths in dairy cattle in the region of Fortaleza, Ceara. Revista do Centro de Ciencias Rurais 9, 387-390.

Barboza Cardoso, S. and Costa Murta, J.L. Da. (1980). Helmintos gastrintestinais parasitos de bovinos na area de influencia da facultade de Veterinaria Do Ceara. Anais do II Seminario Brasileiro de Parasitologia Veterinaria. Fortaleza-Ceara 298.

Barboza Cardoso, S. and Costa Murta, J.L.Da. (1980). Helmintos gastrintestinais parasitos de bovinos na micro-regiao homogenea de Fortaleza. Anais do II Seminario Brasileiro de Parasitologia Veterinaria. Fortaleza-Ceara 297.

Barger, I.A., Bremner, K.C. and Waller, P.J. (1983). Factors influencing worm populations in cattle. In: The epidemiology and control of gastrointestinal parasites of cattle in Australia. Edited by N. Anderson and P.J. Waller, 35-45.

Barth, D., Bernhard, D. and Lamina, J. (1981). The prevalence of gastrointestinal nematodes in dairy cows. In Epidemiology and Control of Nematodiasis in cattle, 117-130. Editors P. Nansen, R.J. Jorgensen and E.J.L. Soulsby.

Bawden, R.J. (1969a). Relationship between Oesophagostomum colombianum infection and the nutritional status of sheep. Australian Journal of Agricultural Research 20, 601-606.

Bawden, R.J. (1969b). The establishment and survival of Oesophagostomum colombianum in male and female sheep given high and low protein diets. Australian Journal of Agricultural Research 20, 1151-1159.

Becklund, W.W. (1959). Worm parasites in cattle from South Georgia. Veterinary Medicine 54, 369-372.

Becklund, W.W. (1961). Helminth infections of healthy Florida cattle, with a note on Cooperia spatulata. Proceedings of the Helminthological Society of Washington 28, 183-184.

Becklund, W.W. (1962). Helminthiases in Georgia cattle. A clinical and economic study. American Journal of Veterinary Research 23, 510-515.

Beck, A.A.H., de Melo, H.J.H., Bianchin, I. and Suarez, M.R.J. (1979). Anais do I Seminario Nacional sobre parasitoses dos bovinos. Embrapa, Campo Grande, M.S.

Bernhard, D., Barth, D. and Lamina, J. (1978). Gastrointestinal nematodes in dairy cattle. Berliner und Munchener Tierarztliche Wochenschrift 91, 45-46.

Bisset, S.A. (1980). Species involved in Ostertagiasis in calves. New Zealand Veterinary Journal 28, 54.

Borgsteede, F.H.M. and van der Burg, W.P.J. (1981). Worm burdens in adult dairy cattle. In Epidemiology and Control of Nematodiasis in cattle, 131-141. Editors P.Nansen, R.J. Jorgensen and E.J.L. Soulsby.

Boughton, I.B. and Hardy, W.I. (1936). Stomach worms (Haemonchus contortus) of sheep and goats. Report of Texas Agriculture Experimental Station 49, 276-278.

Bowen, F.L. (1979). Nematode parasite control in weaner cattle on the Northern Tablelands of New South Wales. Australian Journal of Experimental Agriculture and Animal Science 19, 269-275.

Braga, R.M. and Honer, M.R. (1982). Sobrevivencia de larvas infectantes de nematodesos gastrintestinais de bovinos sob condicoes naturais do Rio de Janeiro. In Anais Do XVIII Congreso Brasileiro de Medicina Veterinaria, 18 - 23 Octubro, p. 211, Camboriu, S.C.

Brunsdon, R.V. (1964). The incidence of gastrointestinal nematodes in cattle in New Zealand. New Zealand Veterinary Journal 12, 135-139.

Brunsdon, R.V. (1968). Trichostrongyle worm infection in cattle: Ostertagiasis. Effect of a field outbreak on production, with a review of the disease syndromes, problems of diagnosis and treatment. New Zealand Veterinary Journal 18, 176-187.

Brunsdon, R.V. (1969). Trichostrongyle worm infection in cattle: Ostertagiasis and current infections in dairy calves: Seasonal patterns of occurrence, pathology and diagnosis. New Zealand Veterinary Journal 17, 161-172.

Brunsdon, R.V. (1972). Inhibited development of Ostertagia spp. and Cooperia spp. in naturally acquired infections in calves. New Zealand Veterinary Journal 20, 183-189.

Brunsdon, R.V. and Adam, J.L. (1975). Internal parasites and animal production. New Zealand Society of Animal Production Occasional Publication No. 4.

Bryan, R.P. (1980). Internal Parasites of livestock. CSIRO Division of Animal Health Annual Report 67.

Burger, H.J. (1973). Helminthological problems in Western Germany. In Helminth Diseases of Cattle, Sheep and Horses in Europe. Edited by Urquhart, G.M. and Armour, J. p.173-179.

Burger, H.J., Eckert, J., Wetzel, H. and Michael, S.A. (1966). Epizootiology of trichostrongylid infections of cattle in north-west Germany. Deutsche Tierarztliche Wochenschrift 73, (20) 503-508.

Bustos, J.A. De and Herrera, (1973). Preliminary findings on gastrointestinal parasitism in dairy calves in the Valle de Lerma (Salta). Argentina. Revista De Investigaciones Agropecuarias INTA 10, 1-8.

Callinan, A.P.L. (1978). The ecology of the free-living stages of Trichostrongylus axei. International Journal for Parasitology 8, 453-456.

Cawley, G.D. and Lewis, C.J. (1976). Type II ostertagiasis in adult cattle. Veterinary Record 344.

Chalmers, K. (1980). Ostertagiasis survey: summary and discussion. New Zealand Veterinary Journal 28, 27-34.

Chavez, C.E. (1963). Incidence and geographical distribution of parasitic diseases of economical importance in livestock of Peru.ForeignAgriculturalResearch Grant ProjectLima-Peru.

Chiejina, S.N. (1978). Field observations on the blood pepsinogen levels in clinically normal cows and calves and in diarrhoeic adult cattle. Veterinary Record 103, 278-281.

Cobb, N.A. (1898). Extract from Ministry Survey Report on the parasites of stock. Agricultural Gazette of New South Wales, 9, 296-321.

Connan, R.M. (1971). The seasonal incidence of inhibition of development in Haemonchus contortus. Research in Veterinary Science 12, 272-274.

Copland, J.W. (1965). Establishment of a pure infection of the nematode Ostertagia circumcincta in sheep. Nature 208, 1229-1230.

Costa, A.J., Campos, M.S., Kasai, N., Paulillo, A.C. and Costa, A.F. (1978). A preliminary study of gastrointestinal helminths of cattle in the municipality of Jabotical, Sao Paulo State, Brazil. Cientifica 6, 433-449.

Costa, H.M.A., Costa, J.O., Guimaraes, M.P. and Freitas, M.G. (1971). Helminths parasites of cattle from Ibia, Minas Gerais. Arquivos da Escola de Veterinaria de Universidade Federal de Minas Gerais 23, 221-228.

Costa, A.J., Costa, J.O., Silva, M.B., Carvalho, C., Pastor, J.C. and Galesco, H. (1979). Helminth parasites of calves from Uruana County, Goias, Brazil. Arquivos da Escola de Veterinaria da Universidade Federal de Minas Gerais 31, 33-36.

Costa, H.M.A., Freitas, M.G., Costa, J.D., and Guimaraes, M.P. (1973). Helminth parasites of cattle in the dairying area of Caldolandia, Brazil. Arquivos da Escola Veterinaria da Universidade Federal de Minas Gerais 25, 111-116.

Costa, H.M.A., Freitas, M.G. and Guimaraes, M.P. (1970). Helminth parasites of cattle from the area of Tres Caracoes-Brazil. Arquivos da Escola Veterinaria 22, 95-101.

Costa, A.J., Nogueir, C.Z. and Costa, J.O. (1978). The natural development of gastrointestinal helminthiases in calves born during the dry season in Guair, Sao Paulo State, Brazil. Arquivos Instituto do Biologico 45, 291-297.

Cox, D.D. and Todd, A.C. (1962). Survey of Gastro-intestinal parasitism in Wisconsin dairy cattle. Journal of the American Veterinary Medical Association 141 6, 706-709.

Das, K.M. and Whitlock, J.H. (1960). Subspeciation in Haemonchus contortus (Rudolphi, 1803) Nemata, Trichostrongyloidea. Cornell Veterinarian 50, 182-197.

Dash, K. M. (1981). Interaction between Oesophagostomum colombianum and Oesophagostomum venelosum in sheep. International Journal for Parasitology 11, 201-207.

Daskalov, P.B. (1971). Haemonchus contortus: genetically determined polymorphism in females. Experimental Parasitology 29, 351-366.

Dinaburg, A.G. (1944). The survival of the infective larvae of the common ruminant stomach worm, Haemonchus contortus on Outdoors Grass Plots. American Journal of Veterinary Research 32-37.

Dinaburg, A.G. (1944). Development and survival under outdoor conditions of eggs and larvae of the common ruminant stomach worm, Haemonchus contortus. Journal of Agricultural Research 69, 421-433.

Dinnik, J.A. and Dinnik, N.N. (1958). Observations on the development of Haemonchus contortus larvae under field conditions in the Kenya Highlands. Bulletin Epizootic Diseases of Africa 6, 11-21.

Dobson, C. (1964). Host endocrine interactions with nematode infections. I. Effects of sex, gondectomy and thyroidectomy on experimental infections in lambs. Experimental Parasitology 15, 200-212.

Donald, A.D. (1964). Nematode parasite populations in cattle in Fiji: a humid tropical environment. Parasitology 54, 273-287.

Donald, A.D. and Waller, P.J. (1973). Gastro-intestinal nematode parasite populations in ewes and lambs and the origin and time course of infective larval availability in pastures. International Journal for Parasitology 3, 219-233.

Donald, A.D., Axelsen, A., Morley, F.H.W., Waller, P.J. and Donnelly, J.R. (1979). Growth of cattle on Phalaris and Lucerne pastures II. Helminth parasite populations and effects of anthelmintic treatment. Veterinary Parasitology 5, 205-222.

Donald, A.D., Dineen, J.K., Turner, J.H. and Wagland, B.M. (1964). The dynamics of the host-parasite relationship. Parasitology 54, 527-544.

Downey, N.E. (1965). Some relationships between trichostrongylid infestation and cobalt status in lambs. Haemonchus contortus infestation. British Veterinary Journal 121, 362-370.

Downey, N.E. (1966). Some relationship between trichostrongylid infestation and cobalt status in lambs. II. Trichostrostrongylus axei infestation. British Veterinary Journal 122, 201-208.

Drozdz, J. (1974). The question of genetic isolation and of permanent coincidence of some species of the subfamily Ostertagiinae. Proceedings, Third International Congress of Parasitology, Munich 1, 477-478.

Duarte, M.J.De F., Gomez, P.A.C. and Sant'Anna, D.B. (1982). Prevalence and intensity of helminth infection in calves at Cantagalo, Rio de Janeiro, Brazil. Pesquisa Agropecuaria Brasileira 17, 1521-1524.

Dunn, A.M. (1969). Veterinary Helminthology. William Heineman Medical Books Ltd., London.

Durie, P.H. (1961). Parasitic gastroenteritis of cattle: The distribution and survival of infective strongyle larvae on pasture. Australian Journal of Agricultural Research 12, 1200-1211.

Eckert, J. (1973). Helminthiasis of cattle, sheep and horses in Switzerland. In Helminth Diseases of Cattle, Sheep and Horses in Europe. Edited by G.M. Urquhart and J. Armour, p. 167-173.

Eckert, J., Perl, R. and Inderbitzin, F. (1981). Significance of nematodiasis in cattle grazing on alpine pastures. In Epidemiology and Control of Nematodiasis in cattle, p. 177-191. Edited by P. Nansen, R.J. Jorgensen and E.J.L. Soulsby.

Eisenegger, H. and Eckert, J. (1975). Epizootiology and prophylaxis of dictyocauliasis and trichostrongylosis in cattle. Schweizer Archiv fur Tierheilkunde 117, 255-286.

El-Moukad, A.R. (1979). The helminth fauna of Syrian cattle. Angewandte Parasitologie 1, 11-16.

Ellenby, C. (1968). Desiccation survival of the infective larvae of Haemonchus contortus. Journal of Experimental Biology 49, 469-475.

Entrocasso, C. and Steffan, P. (1980). Programa de Control de parasitos gastrointestinales en sistemas reales de produccion de carne bovina. In III. Congreso Argentino de Ciencias Veterinarias. Memorias. 2 - 5 Noviembre p. 170-181. Buenos Aires.

Eslami, A.H. and Dakhrzadegan, F. (1972). Nematode parasites of the alimentary canal of cattle in Iran. Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux 25, 527-529.

Euzeby, J.A. (1973). Helminthic diseases of domestic ruminants and horses in France. In Helminth Diseases of Cattle, Sheep and Horses in Europe. Edited by G.M. Urquhart and J. Armour p.148-151.

Fabiyi, J.P., Oluyede, D.A. and Negedu, J.O. (1979). Late dry season outbreak of clinical haemonchosis and cooperiasis in cattle in Northern Nigeria. Veterinary Record 105, 399-400.

Fenerich, F.L. Grocetta, I., Moraes, W.F. and Marques, T. (1979). Occurrence of gastrointestinal helminths in cattle from one to 23 months of age in the Vale do Ribeiro region of Sao Paulo State, Brazil. Biologico, Brazil 45, 263-271.

Fincher, G.T. and Stewart, T.B. (1979). Vertical migration by nematode larvae of cattle parasites through soil. Proceedings of the Helminthological Society of Washington 46, 43-46.

Ford, E.B. (1953). The genetics of polymorphism in the Lepidoptera. Advances in Genetics 5, 43-87.

Forsyth, B.A. (1953). Epidemiology studies on helminthosis of sheep in southern New South Wales. Australian Veterinary Journal 29, 349-356.

Frechette, J.L. and Gibbs, H.C. (1971). Studies on the incidence of gastrointestinal helminths of cattle in Quebec. Canadian Veterinary Journal 12, 207-210.

Gallo, C. (1962). An outbreak of trichostrongylosis in young cattle on pasture in south-eastern Sicily. Veterinaria Italiana 13, 254-258.

Genchi, C. and Forner, F. (1980). Prevalence of abomasal nematodes in cattle in Udine Province, Italy. Rivista di Zootecnia e Veterinaria 2, 86-90.

Genchi, C., Preti, R. and Celano, A. (1979). Incidence of gastrointestinal strongyle infection in dairy cattle in Milan Province, Italy. Rivista di Zootecnia e Veterinaria 4, 234-238.

Gibbons, L.M (1979). Revision of the genus Haemonchus Cobb, 1898 (Nematoda: Trichostrongylidae). Systematic Parasitology 1, 3-24.

Gibson, T.E. and Everett, G. (1976). The ecology of the free-living stages of Haemonchus contortus. British Veterinary Journal 132, 50-59.

Goldberg, A. (1970). Development, migration and survival on pasture of gastrointestinal nematodes of cattle: Summer contamination. Proceedings of the Helminthological Society of Washington 37, 2, 166-169.

Gordon, H.McL. (1948). The epidemiology of parasitic diseases, with special reference to studies with nematode parasites of sheep. Australian Veterinary Journal 24, 17-45.

Gordon, H.McL. (1950). Some aspects of parasitic gastroenteritis of sheep. Australian Veterinary Journal 26, 14-28.

Gordon, H.McL. (1953). The epidemiology of helminthosis in sheep in winter rainfall regions of Australia. Australian Veterinary Journal 29, 337-348.

Gordon, H.McL. (1973). Epidemiology and control of gastrointestinal nematodoses of ruminants. Advances in Veterinary Science 17, 395-437.

Gordon, H. and Whitlock, H.V. (1939). A new technique for counting nematode eggs in sheep faeces. Journal of the Council for Scientific and Industrial Research, Australia, p. 12-50.

Graig, T. (1979). Seasonal transmission of bovine gastrointestinal nematodes in the Texas Gulf Coast. Journal of The American Veterinary Medical Association 174, 844-847.

Grisi, L. and Nuernberg, S. (1971). Incidence of gastrointestinal nematodes in cattle in the State of Mato Grosso. Pesquisa Agropecuaria Brasileira 6, 145-149.

Gronvold, J. (1979). On the possible role of earthworms in the transmission of Ostertagia ostertagi third stage larvae from faeces to soil. Journal of Parasitology 65, 831-832.

Guimaraes, M.P., Freitas, M.G.De., Costa, H.M.De and Costa, J.O. (1975). Parasitism by intestinal nematodes in calves of various ages. Arquivos da Escola de Veterinaria da Universidade Federal de Minas Gerais 27, 67-72.

Gutierres, V.C. (1972). The gastrointestinal parasites of cattle in Austria. Wiener Tierarztliche Monatsschrift 59, 341-342.

Gutierres, V.C., Todd, A.C. and Crowley, J.W. (1979). Natural populations of helminths in Wisconsin dairy cows. Veterinary Medicine/Small Animal Clinician 74, 369-374.

Hart, J.A. (1964). Observations on the dry season strongyle infestations of zebu cattle in Northern Nigeria. British Veterinary Journal 120, 87-94.

Helle, O. (1973). A survey of helminthiasis in cattle, sheep and horses in Norway. In Helminth Diseases of Cattle, Sheep and Horses in Europe. Edited by G.M. Urquhart and J. Armour, p 160-163.

Henderson, A.W.K. and Kelly, J.D. (1978). Helminth parasites of beef cattle in the East Kimberley and Victoria River Districts of Northern Australia. Tropical Animal Health and Production 10, 63-73.

Henriksen, S.A., Jorgensen, R.J., Nansen, P., Sejrsen, J.B.L. and Klausen, S. (1975). Gastrointestinal strongyles in cattle. I. Investigations on the infection in grazing calves. Danish Veterinary Tidestir 58, 441-444.

Herbert, I.V., Lean, I.J. and Nickson, E.W. (1969). Dietary factors and the production of Oesophagostomum spp. ova in breeding pigs. Veterinary Record 84, 569-570.

Herd, R. (1980). Animal health and public health aspects of bovine parasitism. Journal of the American Veterinary Medical Association 176, 737-743.

Herlich, H. (1959). Experimental infections of cattle with the stomach worms Ostertagi ostertagi and Trichostrongylus axei. Proceedings of The Helminthological Society of Washington 26, 97-102.

Herlich, H. (1960). Age resistance of cattle to nematodes of the gastrointestinal tract. Journal of Parasitology 46, 392-397.

Hinaidy, H.K., Gutierres, V.C. and Supperer, R. (1972). The gastrointestinal helminths of cattle in Austria. Zentralblatt fur Veterinarmedizin 19, 679-695.

Hobbs, N.B. (1961). Helminth research in South Africa. Seasonal incidence of nematode parasites in cattle in the Natal Coastal area. Journal of South Africa Veterinary Medical Association 23, 151-155.

Holmes, J.C. (1961). Effects of concurrent infections on Hymenolepis diminuta (Cestoda) and Moniliformis dubius (Acantocephala). I. General effects and comparison with crowding. Journal of Parasitology 47, 209-216.

Holmes, J.C. (1962). Effects of concurrent infections on Hymenolepis diminuta (Cestoda) and Moniliformis dubius (Acantocephala). III. Effects in Hamsters. Journal of Parasitology 48, 97-100.

Horak, I.G. and Louw, J.P. (1978). Parasites of domestic and wild animals in South Africa. VI. Helminths in calves on irrigated pastures on the Transvaal Highveld. Onderstepoort Journal of Veterinary Research 45, 23-28.

Hotson, I.K. (1967). Ostertagiasis in cattle. Australian Veterinary Journal 43, 383-387.

Isenstein, R.S. (1971a). Hybridisation of two species of nematodes parasitic in ruminants, Cooperia oncophora (Railliet 1898, Ransom 1907) and Cooperia pectinata (Ransom 1907). Journal of Parasitology 57, 320-326.

Isenstein, R.S. (1971b). The polymorphic relationship of Cooperia oncophora (Railliet 1898, Ransom 1907) to Cooperia surnabada (Antipin 1931) (nematoda: Trichostrongylidae). Journal of Parasitology 57, 316-319.

Jorgensen, R.J. (1982). Spread of infective Dictyocaulus viviparus larvae in pasture and to grazing cattle: Experimental evidence of the role of Pilobolus fungi. Veterinary Parasitology 10, 331-339.

Jorgensen, R.J., Nansen, P. and Henriksen, Sv.A. (1973). Helminthiasis in Denmark. In Helminth Diseases of Cattle, Sheep and Horses in Europe. Edited by G.M. Urquhart and J. Armour, p. 142-144.

Kates, K.C. and Turner, J.H. (1953). A comparison of the pathogenicity and course of infection of two nematodes of sheep, Nematodirus spathiger and Trichostrongylus colubriformis in pure and mixed infections. Proceedings of the Helminthological Society of Washington 20, 117-124.

Kloosterman, A. (1971). Observations on the epidemiology of trichostrongylosis of calves. Ph.D. Thesis, University of Wageningen, the Netherlands p. 114.

Keith, R.K. (1953). The differentiation of the infective larvae of some common nematode parasites of cattle. Australian Journal of Zoology 1, 223-235.

Kudo, T., Hatta, T., Kishi, K., Ito, S., Tanniguchi, R. and Sano, S. (1974). Studies on gastrointestinal nematodes in cattle. II. Surveys of worms in heifers on pasture in Hokkaido. Bulletin of the Hokkaido Prefectural Shintoku Animal Husbandry Experimental Station 6, 35-39.

Lancaster, M.B. and Hong, C. (1981). Polymorphism in nematodes. Systematic Parasitology 3, 29-31.

Lancaster, M.B., Hong, D. and Michel, J.F. (1983). Polymorphism in the Trichostrongylidae. Systematic Association Special Volume No. 22, 293-302.

Lee, R.P., Armour, J. and Ross, J.G. (1960). The seasonal variations of Strongyle infestations in Nigerian Zebu cattle. British Veterinary Journal 116, 34.

Leland, E.S., Caley, H.K. and Ridley, R.H. (1973). Incidence of Gastro-intestinal nematodes in Kansas cattle. American Journal of Veterinary Research 34, 581-585.

Le Jambre, L. and Ractliffe, L.H. (1971). Seasonal change in a balanced polymorphism in Haemonchus contortus populations. Parasitology 62, 151-155.

Le Jambre, L.F. and Whitlock, J.H. (1976). Changes in the hatch rate of Haemonchus contortus eggs between geographic regions. Parasitology 73, 223-238.

Le Riche, P.D., Kuhne, G.I. and Dwinger, R.H. (1982). An epidemiological study of helminthiasis in cattle in subtropical Argentina. Tropical Animal Health and Production 14, 207-215.

Levine, N. (1963). Weather, Climate and the Bionomics of Ruminant Nematode larvae. Advances in Veterinary Science 8, 215-261.

Levine, N.D. and Andersen, F. (1973). Development and survival of Trichostrongylus colubriformis on pasture. Journal of Parasitology 59, 147-165.

Levine, N., Todd, K. and Boatman, P.A. (1974). Development and Survival of Haemonchus contortus on Pasture. American Journal of Veterinary Research 35, 11, 1413-1422.

Lombardero, O.J., Moriena, R.A. and Schiffo, H.P. (1976). Epizootiology of verminous gastroenteritis of cattle in the northern area of the Province of Corrientes. Gaceta Veterinaria 38, 143-169.

Lopez-Neyra, C.R. (1947). Helmintos de los vertebrados Ibericos. Instituto Nacional de Parasitologia de Granada, 2, 413-802.

Machado, R.Z., Starke, W.A., Girio, R.J.S., Samara, S.I. and Berigo, P.R. (1979). Frequency and intensity of gastro-intestinal helminth infection in cattle at the Anglo slaughterhouse at Barretos, Sao Paulo State, Brazil. Cientifica 7, 103-109.

Maciel, F.C. and Lima, G.F.T. (1982). Helmintos gastrintestinais de bovinos na microregiao serido do Rio Grande do Norte. Anais do XVIII Congreso Brasileiro de Medicina Veterinaria, Camboriu 145.

Malczewski, A. (1970). Gastro-intestinal helminths of ruminants in Poland. III. Seasonal incidence of the stomach worms in calves, with consideration of the effect of the inhibition phenomenon on the spring rise phenomenon. Acta Parasitologica, Polonica 18, 417-434.

Malczewski, A., Wescott, R.B. Spratling, B.M. and Gorham, J.R. (1975). Internal Parasites of Washington Cattle. American Journal of Veterinary Research 36, 1671-1675.

Manuel, M.F. (1979). Parasitic diseases of ruminants in the Philippines. Philippine Journal of Veterinary Medicine 18, 1-15.

Martin, W.B., Thomas, B.A.C. and Urquhart, G.M. (1957) Chronic Diarrhoea in Housed Cattle Due to Atypical Parasitic Gastritis. Veterinary Record, 69, 736-739.

Martinez Gomez, F. (1968). Formula del proceso vulvar de Haemonchus contortus (Nematoda, Trichostrongylidae) en ovejas espanolas, con la descripcion de una posible subespecie, H. contortus hispanicus. Revista Iberica de Parasitologia 28, 473-477.

McGregor, J.K. and Kingscote, A.A. (1957). A survey of gastro-intestinal helminths of cattle in Ontario. Canadian Journal of Comparative Medicine 21 11, 370-373.

Melo, H.J.H., Bianchin, I., Ribeiro, H.S. and Beck, A.A.H. (1980). Anais do II Seminario Brasileiro de Parasitologia Veterinaria. Embrapa, Fortaleza, C.E.

Michel, J.F. (1967). The epidemiology and control of parasitic gastroenteritis in calves. In Proceedings of the 4th International Meeting of the World Association for Buiatrics, Zurich, 4-9 August, 1967, 1-16..

Michel, J.F. (1969). Observations on the epidemiology of parasitic gastroenteritis in calves. Journal of Helminthology 43, 111-113.

Michel, J.F. (1970). The regulations of populations of Ostertagia ostertagi in calves. Parasitology 61, 435-447.

Michel, J.F., Lancaster, M.B. and Hong, C. (1970). Field observations on the Epidemiology of Parasitic Gastro-enteritis in Calves. Research in Veterinary Science 11, 255-259.

Michel, J.F., Lancaster, M.B. and Hong, C. (1971). Host-induced effects on the size and body proportions of Ostertagia ostertagi (Stiles 1892) Ransom, 1907, a nematode parasite of cattle. Journal of Parasitology 57, 1185-1189.

Michel, J.F., Lancaster, M.B. and Hong, C. (1972). The epidemiology of gastrointestinal nematode infection in the single suckled calf. Veterinary Record 91, 301-306.

Michel, J.F., Lancaster, M.B. and Hong, C. (1976). Observations on the resumed development of arrested Ostertagia ostertagi in naturally infected yearling cattle. Journal of Comparative Pathology 86, 73-80.

Ministry of Agriculture, Fisheries and Food (1971). Technical Bulletin No. 18 Manual of Veterinary Parasitological Laboratory Techniques.

Mirzayans, A. (1969). The effect of temperature on the development of the eggs and larvae of Trichostrongylus axei. British Veterinary Journal 125, 37-38.

Muller, G.L. (1968). The epizootiology of Helminth infestation in sheep in the South Western Districts of the Cape. Onderstepoort Journal of Veterinary Research 35, 159-194.

Mussman, H.C., Rave, V.G., Norman, B., Mullenax, C.H. and Rincon, E. (1967). Informe clinicopatologico preliminar del ganado vacuno de los llanos orientales de Colombia. Rev.Fac.Med.Vet. y Zooteonia. Universidad Nacional de Colombia 30, 67-76.

Nilsson, O. (1973). Helminthological problems in Sweden. In Helminth Diseases of Cattle, Sheep and Horses in Europe. Edited by G.M. Urquhart and J. Armour p. 163-168

Nilsson, O. (1981). Winter ostertagiasis in Swedish cattle. In Epidemiology and Control of nematodiasis in cattle, p. 295-309. Edited by P. Nansen, R.J. Jorgensen and E.J.L. Soulsby.

Nilsson, O. and Sorelius, L. (1973). Trichostrongyle infections of cattle in Sweden. Nord.Veterinaer Med. 25, 65-78.

Nogueira, C.Z., Costa, A.J., Machado, R.Z. and Kasai, N. (1976). Natural development of gastrointestinal nematode infections of calves born during the rainy season in Guaira, Sao Paulo State, Brazil. Cientifica, Sao Paulo 4, 346-355.

Oakley, G.A. (1981). Survival of Dictyocaulus viviparus infection in earthworms. Research in Veterinary Science 30, 255-256.

Oliveira, N.L.H. and Oliveira, N.de C. (1982). Helmintos parasitas de bezerros procedentes da regiao fisiografica Piemonte da Diamantina, Bahia. Anais do XVIII Congreso Brasileiro de Medicina Veterinaria, Camboriu 135.

Olsson, G. (1977). Winter ostertagiasis in Sweden: A study using slaughter-house material. Svensk Veterinarktidning 29, 361-365.

Owen, I.L. and Talbot, N.T. (1983). Importance of gastrointestinal helminths in calves in Papua New Guinea. Tropical Animal Health and Production 15, 115-123.

Parfitt, J.W. (1955). Two techniques used for the detection and enumeration of the larvae Dictyocaulus viviparus in faeces and herbage. Laboratory Practice 4, 15-16.

Patik, S. (1960). Helminths in the alimentary tract of cattle in West Poland. Acta Parasitologica Polonica 8, 231-253.

Pecheur, H. and Pouplard, L. (1974). Gastrointestinal nematodes of cattle in South Belgium. Annales de Medicine Veterinaire 118, 519-523.

Peterson, J.E. (1957). Observations on parasitic gastroenteritis of cattle in Western Australia. Australian Veterinary Journal, 108-113.

Persson, L. (1974). Studies on the survival of eggs and infective larvae of Ostertagia ostertagi and Cooperia oncophora in herbage. Zentralblatt fur Veterinarmedizin B, 21, 787-798.

Persson, L. (1974). Studies on the bionomics of eggs and infective larvae of Ostertagia ostertagi and Cooperia oncophora in soil. Zentralblatt fur Veterinarmedizin B, 21, 318-328.

Porter, D.A. (1942). Incidence of gastrointestinal nematodes of cattle in the Southeastern United States. American Journal of Veterinary Research 30, 304-307.

Pott, J.M., Jones, R.M. and Cornwell, R.L. (1978). Observations on parasitic gastroenteritis and bronchitis in grazing calves: Untreated calves. International Journal for Parasitology 8, 331-339.

Pullar, E.M. (1953). The epidemiology of helminthosis in sheep in winter rainfall regions of Australia.

Randall, R.W. and Gibbs, H.C. (1977). Occurrence and seasonal behaviour of gastrointestinal nematodes infecting Maine dairy cattle. American Journal of Veterinary Research 38, 10, 1665-1668.

Ransom, B.H. (1906). The life history of the twisted wireworm Haemonchus contortus of sheep and other ruminants. Circular No. 39, Bulletin U.S.A. Department of Agriculture and Animal Industry, p. 7.

Ransom, B.H. (1907). Notes on parasitic nematodes, including descriptions of new genera and species, and observations on life histories. Circular No. 116, Bulletin, U.S. Department of Agriculture and Animal Industry.

Rao, N.S.K. and Rahman, S.A. (1967). The vulvar flap formula of Haemonchus contortus from local sheep. Mysore Journal of Agricultural Science 1, 168-175.

Raynaud, J.P. (1978). Facts and reflections on cattle ostertagiasis in France. In Facts and Reflections III. Editors F.H.M. Borgsteede, J. Armour and H. Jansen). Worshop on "Arrested development of nematodes in sheep and cattle". Central Veterinary Institute, Lelystad, The Netherlands, 18 - 19 May, 1978, p. 143-155.

Raynaud, J.P. and Bouchet, A. (1976). Results of post-mortem examinations and total worm counts on cattle with Ostertagia infection in France. In 9o Congres International sur les maladies du betail, 6 - 9 Septembre, 1976 Paris. Societe Francaise de Buiatrie p. 1083-1089.

Raynaud, J.P., Laudren, G. and Jolivet, G. (1971). Epidemiolcgical interpretation of bovine gastrointestinal nematode infections developing in tracer cattle at pasture. Annales de Recherches Veterinaires 5, 115-145.

Raynaud, J.P., Mage, C., Le Stang, J.P. and Jones, R.M. (1981). Recent results on epidemiology of nematode infections in beef and dairy cattle in France. In Epidemiology and Control of Nematodiasis in cattle, p. 193-214. Edited by P. Nansen, R.J. Jorgensen and E.J.L. Soulsby.

Rees, G. (1950). Observations on the vertical migrations of the third stage larva of Haemonchus contortus (Rud.) on experimental plots of Lolium perenne S24, in relation to metereological and micrometereological factors. Parasitology 40, 127-143.

Reid, J.F.S., Armour, J., Jennings, F.W., Kirkpatrick, K.S. and Urquhart, G.M. (1967). The fascioliasis/ostertagiasis complex in young cattle. Veterinary Record 80, 371-374.

Reinecke, R.K. (1960b). A field study of some nematode parasites of bovines in a semi-arid area, with special reference to their biology and possible methods of prophylaxis. Onderstepoort Journal of Veterinary Research 28, 365-464.

Reinecke, R.K. (1974). Studies on Haemonchus contortus. I. The influence of previous exposure to Trichostrongylus axei on infection with Haemonchus contortus. Onderstepoort Journal of Veterinary Research 41, 212-215.

Riek, R.F., Roberts, F.H.S. and O'Sullivan, P.J. (1953). Further observations on the epidemiology of parasitic gastroenteritis of cattle. Australian Veterinary Journal 29, 122-128.

Ritchie, J.S.D., Anderson, N., Armour, J., Jarrett, W.F.J., Jennings, F.W. and Urquhart, G.M. Experimental Ostertagia ostertagi infections in calves: Parasitology and Pathogenesis of single infection. American Journal of Veterinary Research 27, 659-667. (1966)

Roberts, F.H.S., O'Sullivan, P.J. and Riek, R. (1952). The epidemiology of parasitic gastroenteritis of cattle. Australian Journal of Agricultural Research 3, 187-226.

Robinson, J. (1962). Pilobolus spp. and the translation of the infective larvae of Dictyocaulus viviparus from faeces to pasture. Nature 193, 353-354.

Rosa, W.A., Lukovich, R. and Niec, R. (1971). Parasitismo gastrointestinal de los ovinos y bovinos en la zona sur de la provincia de Buenos Aires (Tres Arroyos, Cnel. Pringles y Cnel, Dorrego). Revista de Investigaciones Agropecuarias, INTA 8, 71-83.

Rosa, W.A.J., Niec, R., Lukovich, R., Dindart, J. and Barberan, R.(1973). Etiologia y variacion estacional del parasitismo gastrointestinal de los ovinos y bovinos en la zona de Mercedes (Provincia de Corrientes). Revista de Investigaciones Agropecuarias, INTA 10, 25-40.

Rosa, W.A., Niec, R., Lukovich, R., Marti Vidal, J., Monje, A.R. and Hofer, C. (1977). Evolucion del parasitismo gastrointestinal en bovinos en la zona de Concepcion del Uruguay, Pcia. de Entre Rios, Rep. Argentina. Revista de Investigaciones Agropecuarias, INTA 4, 1-14.

Rose, J.H. (1961). Some observations on the free-living stages of Ostertagia ostertagi, a stomach worm of cattle. Parasitology 51, 295-307.

Rose, J.H. (1962). Further observations on the free-living stages of Ostertagia ostertagi in cattle. Journal of Comparative Pathology 72, 11-18.

Rose, J.H. (1963). Observations on the free-living stages of the stomach worm Haemonchus contortus. Parasitology 53, 469-481.

Rose, J.H. (1964). Relationship between environment and the development and migration of the free-living stages of Haemonchus contortus. Journal of Comparative Pathology 74, 163-172.

Ross, J.C., Purcell, A., Todd, J.R. and Dow, C. (1968). Combined infections of calves with the nematode parasites Trichostrongylus axei and Ostertagia ostertagi. British Veterinary Journal 124, 299-304.

Ross, J.G., Purcell, D.A. and Todd, J.R. (1969). Experimental infections of calves with Trichostrongylus axei. Investigations using abomasal cannulae. Research in Veterinary Science 10, 46-56.

Rudolphi, K.D. (1803). Neue Beobacchtungen uber die Eingeweidewurmer. Archiv fur Zoologie und Zootomie 3, 1-31.

Savir, D., Neuman, M. and Neuman, F. (1964). Parasitic gastritis in a herd of beef cattle. Refuah vet. 21, 221-222.

Sauvage, J.P., Brown, J.R.H., Parkinson, J.G., Rossiter, P.B. and McGovern, P.T. (1974). Helminthiasis in cattle in the Ankole district of Uganda. British Veterinary Journal 130, 120-126.

Schroder, J. (1979). The seasonal incidence of helminth parasites of cattle in the Northern Transvaal Bushveld. Journal of the South African Veterinary Association 50, 23-27.

Selman, J.E., Reid, J.F.S., Armour, J. and Jennings, F.W. (1976). Type II Ostertagiasis in adult cattle. The Veterinary Record 99, 141-143.

Shorb, D.A. (1943). Survival on grass plots of eggs and pre-infective larvae of the common sheep stomach worm Haemonchus contortus. Journal of Parasitology 29, 284-289.

Shorb, D.A. (1944). Factors influencing embryonation and survival of eggs of the stomach worm Haemonchus contortus. Journal of Agricultural Research 69, 279-287.

Sievers, G. (1978). Epidemiology and trichostrongylosis of the calves in Southern Chile. In Fourth International Congress of Parasitology, 19 - 26 August, Warsaw, p. 168.

Silangwa, S.M. and Todd, A.C. (1964). Vertical migration of trichostrongylid larvae on grasses. Journal of Parasitology 50, 278-285.

Silverman, P. and Campbell, J.A. (1959). Studies on parasitic worms of sheep in Scotland. I. Embryonic and larval development of Haemonchus contortus at constant temperatures. Parasitology 47, 23-37.

Skinner, W. and Todd, K. (1980). Lateral migration of Haemonchus contortus larvae on pasture. American Journal of Veterinary Research 41, 395-398.

Slocombe, J.O.D. (1974). Abomasal nematodes in cattle in Ontario. Canadian Journal of Comparative Medicine 38, 18-21.

Smeal, M.G., Fraser, G.C. and Robinson, G.G. (1980). Seasonal changes in the structure of nematode populations of cattle in New South Wales in relation to inhibited larval development. Australian Veterinary Journal 56, 80-86.

Smeal, M.G., Robinson, G.G. and Fraser, G.C. (1980). Seasonal availability of nematode larvae on pasture grazed by cattle in New South Wales. Australian Veterinary Journal 56, 74-79.

Smeal, M.G., Hotson, I.K., Mylrea, P.J., Jackson, A.R., Campbell, N.J. and Kirton, H.C. (1977). Studies on nematode infections of beef cattle in New South Wales. Australian Veterinary Journal 53, 566-573.

Smeal, M.G., Nicholls, P.J., Webb, R.F., Hotson, I.K., Doughty, F.R. and Harding, W.B. (1981). The effect of anthelmintic treatments on growth of beef cattle in New South Wales. Australian Journal of Agricultural Research 32, 813-323.

Smith, H.J. (1970). On the development of gastrointestinal parasitism in bovine yearlings. Canadian Journal of Comparative Medicine 34, 303-307.

Smith, H.J. (1974). Inhibited development of Ostertagia ostertagi, Cooperia oncophora and Nematodirus helvetianus in parasite-free calves grazing fall pastures. American Journal of Veterinary Research 7, 935-937.

Smith, H.J. and Archibald, R. (1968). The effects of age and previous infection on the development of gastrointestinal parasitism in cattle. Canadian Journal of Comparative Medicine 32, 511-517.

Smith, H.J. and Perreault, J.P. (1972). A Type II Ostertagiasis outbreak in cattle in New Brunswick. Canadian Veterinary Journal 13, 114-117.

Southcott, W.H. and Barger, I.A. (1975). Control of nematode parasites by grazing management. II. Decontamination of sheep and cattle pastures by varying periods of grazing with the alternate host. International Journal for Parasitology 5, 45-48.

Stiles, C.W. (1892). A word in regard to the Filaridae found in the body cavity of horses and cattle: On the presence of Strongylus ostertagi (Ostertag, 1890) Stiles 1892 in America. Journal of Comparative Medicine and Veterinary Archives 13, 464-466.

Sukhapesna, D.V.M. (1974). A study of the morphological features of Kentucky isolates of Haemonchus spp. and the biological inter-relationships with special reference to drug resistance. Dissertation Abstracts International 35B, 4, 1978-1979.

Swierstra, D. (1973). Helminth diseases of The Netherlands. In Helminth Diseases of Cattle, Sheep and Horses in Europe. Edited by G.M. Urquhart and J. Armour, p. 157-160.

Taylor, E.L. (1934a). The epidemiology of parasitic gastritis in sheep. Journal of Agricultural Science, 192-208.

Taylor, E.L. (1938). Observations on the Bionomics of Strongyloid larvae in pastures. The Veterinary Record 50, 40, 1265-1272.

Taylor, S.M. and Cawthorne, J.G. (1972). Species of gastrointestinal nematodes of cattle in Northern Ireland. British Veterinary Journal 128, 311-315.

Taylor, S.M., Cawthorne, R.J.G., Kenny, J. and Regan, M. (1973). Acute parasitic gastroenteritis in calves in spring. Veterinary Record 93, 603-604.

Tetley, J.H. (1959). The availability of the infective stages of nematode parasites to sheep in early spring. Journal of Helminthology 33, 289-292.

Tharaldsen, J. (1976). The epidemiology of trichostrongylid infections in young cattle in Norway. Acta Veterinaria Scandinavia 17, 1-19.

Todd, K., Levine, N. and Whiteside, C. (1970). Moisture stress effects on survival of infective Haemonchus contortus larvae. Journal of Nematology 4, 330-333.

Tongson, M.S. and Caspe, C.E. (1975). Epidemiology of bovine parasitic gastroenteritis in the Philippines. VI. Incidence of bovine gastrointestinal nematodes in Oriental Mindoro and Palawan. Philippine Journal of Veterinary Medicine 14, 98-105.

Urquhart, G.M., Jarrett, W.F.H. and Mulligan, W. (1962). Helminth immunity. In Advances in Veterinary Science, Academic Press, New York and London 7, 87-129.

Varga, I. (1973). Epidemiological aspects of major helminth diseases of cattle, sheep and horses in Hungary. In Helminth Diseases in Europe. Edited by G.M. Urquhart and J. Armour, p. 152-155.

Valle Suarez, J.M. Del., Rojo Vazquez, F.A., Diez Banos, P. (1979). A study of the trichostrongyles of cattle in Leon. Hygia Pecoris 1, 82-107.

Vassiliades, G. (1978). Les affections parasitaires dues a des helminths chez les bovins du Senegal. Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux 31, 157-163.

Veglia, F. (1915). The anatomy and life history of the Haemonchus contortus. Third and Fourth Report of the Divsion of Veterinary Science and Animal Industry of South Africa, p. 347-500.

Vicente, S. (1961). Desarrollo embrionario de Ostertagia spp. y Trichostrongylus spp. en el ambiente externo. Revista Iberica de Parasitologia 21, 61-74.

Vigliani, E. (1981). Some remarks and control methods of gastrointestinal nematodiasis in cattle in Piedmont, Italy. In Epidemiology and Control of Nematodiasis in Cattle, 369-376. Edited by P. Jansen, R.J. Jorgensen and E.J.L. Soulsby.

Viljoen, J.H. (1969). Further studies on the epizootiology of nematode parasites of sheep in the Karoo. Onderstepoort Journal Veterinary Research 36, 233-264.

Waller, P.J. and Donald, A.D. (1970). The response to desiccation of eggs of Trichostrongylus colubriformis and Haemonchus contortus (Nematode: Trichostrongylidae). Parasitology 61, 195-204.

Waller, P.J. and Thomas, R.J. (1975) Field studies on inhibition of Haemonchus contortus in sheep. Parasitology 71, 285-291.

Wang, J.S. (1979). A study on the endoparasitic species in dairy cattle in central Taiwan. Journal of the Chinese Society of Veterinary Science 5, 115-123.

Wedderburn, J.F. (1970). Ostertagiasis in adult cattle: A clinical report of an outbreak in the field. New Zealand Veterinary Journal 18, 168-170.

Whitlock, J.H. and Georgi, J.R. (1976). Biological controls in mixed trichostrongyle infections. Parasitology 72, 207-224.

Whorley, D.E. and Sharman, G.A.M. (1966). Gastritis associated with Ostertagia bisonis in Montana range cattle. Journal of the American Veterinary Medical Association 149, 1291-1294.

Williams, J.C. (1983). Ecology and Control of Gastrointestinal Nematodes of Beef Cattle. Veterinary Clinics of North American Large Animal Practice 5, 183-205.

Williams, J.C. and Bilkovich, F.R. (1971). Development and survival of infective larvae of the cattle nematode Ostertagia ostertagi. Journal of Parasitology 57, 327-338.

Williams, C. and Bilkovich , F.F. (1973). Distribution of Ostertagia ostertagi infective larvae on pasture herbage. American Journal of Veterinary Research 34, 10, 1337-1344.

Williams, J.C. and Knox, J.W. (1976). Effect of nematode parasite infection on the performance of stocker cattle at high stocking rates on Coastal Bermudagrass pastures. American Journal of Veterinary Research 37, 453-464.

Winks, R. (1968). Epidemiology of helminth infestation of beef cattle in Central Queensland. Australian Veterinary Journal 44, 367-372.

Winks, R. and Bremner, K.C. (1978). Internal parasites of livestock. CSIRO Division of Animal Health Annual Report, p. 66.

Yazwinski, T.A. and Gibbs, H.C. (1975). Survey of helminth infections in Maine dairy cattle. American Journal Veterinary Research 36, 11, 1677-1682.

Young, R.R. and Anderson, N. (1981). The ecology of the free-living stages of Ostertagia ostertagi in a winter rainfall region. Autralian Journal of Agricultural Research 32, 371-388.

Young, R.R., Nicholson, R.M., Tweedie, R.I. and Shuh, H.J. (1980). Quantitative modelling and prediction of development times of the free-living stages of Ostertagi ostertagi under controlled and field conditions. Parasitology 81, 493-505.

Zimmermann, W.J. and Hubbard, E.D. (1961). Gastrointestinal parasitism in Iowa cattle. Journal of the American Veterinary Medical Association 139, 5, 555-559.