Incidence, clinical appraisal and treatment of haemonchosis in small ruminants of resource-poor areas in South Africa

by

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When asked if he was never discouraged by the little fruit his efforts seemed to yield, the Master told the story of a snail that started to climb a cherry tree one cold, windy day in late spring.

The sparrows on a neighbouring tree had a good laugh at his expense. Then one flew over and said, "*Hey, blockhead, don't you know there are no cherries on this tree?*"

The little fellow did not stop as he replied, "*Well, there will be when I get there*."

Anthony de Mello, 1992

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I also wish to thank my family for their support and Sr Pat McGregor for caring for my cats while I was away on field trips.

Declaration

The study was conducted within the ambit of a Food and Agriculture Organization of the United Nations Technical Co-operation Project (FAO TCP SAF/8821). As such, in establishing study sites I received assistance from Dr J.A. van Wyk. With the planning and execution of field trips and collection of samples, I was assisted by Mr S. Molebiemang and Mr W. Motswatswe (Kraaipan, North-West Province), by Dr G. Msiza and Dr W. Rabolao (Rust de Winter, Gauteng Province), and by Mr J.F. de Villiers, Ms B. Letty and Mr S. Madiba (Impendle, KwaZulu-Natal). In the laboratory, I was extensively assisted with the preparation of nematode and trematode faecal egg counts (FECs), haematocrits and cultures for nematode third-stage larvae and with the reading of nematode FECs and haematocrits by Mr D. Chipana, Mr W. Shima, Mr L. Tshikhudo, Ms O. Nefolovhodwe and Mr F. Masubelle, and on occasion by Ms E. van Wijk and Mr D. Booyse. Ms L. Michael and Mr J. van Rensburg assisted with the reading of the trematode FECs and the identification of the nematode third-stage larvae, respectively, during the beginning of the project and on occasion thereafter. Mr M. Boshoff and Ms E. van Wijk undertook field trips in my absence. Dr J. Hansen provided valuable advice during the planning, execution and writing-up phases of the project. Prof H. Groeneveld was consulted for statistical advice until June 2000 when Mr J. Grimbeek provided this service. Dr M. van der Linde was responsible for the data handling in SAS and for the writing of programmes for the execution of the analyses.

Notwithstanding the inputs of the abovementioned people, whose particular assistance was critical to the execution of the study, and the assistance of those people thanked in the Acknowledgements, this study is original work of the candidate and has not been submitted in any form to another University.

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Abstract

A novel clinical assay for the assessment and subsequent treatment of *Haemonchus* infection in sheep to slow down the development of anthelmintic resistance – the FAMACHA© system – has been developed, tested and validated in South Africa. The system is based on a colour chart with five colour categories depicting varying degrees of anaemia that are compared with the colour of the conjunctival mucous membranes of sheep. The animal is then scored from severely anaemic (pale) through anaemic to non-anaemic (red) and those animals considered in danger of succumbing to the effects of haemonchosis are treated. This method was tested in the present study in goats and sheep farmed under resource-poor conditions in South Africa.

The diversity and predominance of nematode genera in goats and sheep at Rust de Winter, Gauteng Province, in goats at Impendle, KwaZulu-Natal Province, and in goats and sheep at Kraaipan, North-West Province, were determined by means of a longitudinal study of the nematode faecal egg counts (FECs) and differential third-stage larvae. The animals were bled for haematocrit determination, scored for pallor of ocular mucous membranes using the FAMACHA© method, and body condition scored. A longitudinal study of the pooled trematode FECs was conducted at the same time.

Lower haematocrit values were registered for the goats during periods of heavier *Haemonchus* infection, which periods occurred from December/January to March for Rust de Winter; from December to March/April for Impendle; and from November/December to February or April for Kraaipan. For the sheep, the periods of heavier *Haemonchus* infection occurred from October to March at Rust de Winter and from September/October to February or April at Kraaipan. There was agreement too between the lower haematocrits and paler mucous membranes scored according to the FAMACHA© method.

Analyses in goats performed during the summers of 1998/1999 and 1999/2000 show a test sensitivity of 76% and 85%, respectively, meaning that the system may be used to identify correctly 76% to 85% of those animals in need of treatment with an anthelmintic. However, the test specificity remains low at 52% to 55%. This means that a large proportion of those animals that would not require treatment would in fact be treated. On the other hand, when the use of the FAMACHA© system is compared with conventional dosing practices where all the animals are treated, using the FAMACHA© system would result in a large proportion of the animals being left untreated. The untreated animals are then able to deposit the eggs of anthelmintic-susceptible worms on the pasture, while the treated ones should pass very few ova, given an effective anthelmintic. This maintains a reservoir of susceptible larvae *in refugia*, and should slow down the development of anthelmintic resistance.

The use of the FAMACHA© system may be recommended as part of an integrated approach to worm control in the resource-poor areas studied and may have wide application in the tropics and subtropics of sub-Saharan Africa and elsewhere.

Seasonal variations in body condition were evident in the goats at Impendle with the animals showing lower body condition scores (BCS) from June to September. The sheep at Kraaipan showed lower BCS from July to December. The small ruminants at Rust de Winter did not show clear seasonal variations, although the goats at Rust de Winter showed lower BCS from mid-July to early December and the sheep from August to mid-February. Although body condition was maintained by the goats at Kraaipan, the scores remained low overall. The BCS for Rust de Winter where the animals were grazed on a private farm were generally higher than those of the other sites, where communal grazing is practised.

The amphistome FECs followed a seasonal pattern, with an increase in the counts during the warmer months of the year (September to April). The study seems to indicate a different pattern of infection in goats raised under resource-poor conditions in South Africa from that on commercial farms, where outbreaks of clinical paramphistomosis occur during autumn and winter.

Keywords: Anthelmintic resistance; Body condition scores; Clinical assay; Eye colour chart; Faecal nematode and trematode egg counts; FAMACHA©; Goats; Haematocrit; *Haemonchus* spp.; Sheep

Opsomming

'n Nuwe kliniese toets — die FAMACHA©-sisteem — vir die ondersoek en daaropvolgende behandeling van *Haemonchus*-besmetting in skape is in Suid-Afrika ontwikkel, getoets en geldig verklaar om die ontwikkeling van wurmmiddelweerstand te vertraag. Die sisteem is gebaseer op 'n kleurkaart met vyf kleur kategorieë wat uiteenlopende grade van bloedarmoede uitbeeld en met die kleur van die oogslymvliese van skape vergelyk word. Die dier word as ernstig bloedarmoedig (bleek) tot bloedarmoedig tot nie-bloedarmoedig (rooi) aangewys en in dié gevalle waar dit verwag word dat die diere aan hemonchose sal vrek, word behandeling toegepas. Dié metode is in hierdie ondersoek getoets in bokke en skape waarmee daar onder hulpbron-beperkte omstandighede in Suid-Afrika geboer word.

Die verskeidenheid en belang van nematodegenera in bokke en skape by Rust de Winter, Gauteng Provinsie, in bokke by Impendle, KwaZulu-Natal Provinsie, en in bokke en skape by Kraaipan, Noordwes Provinsie, is bepaal deur middel van 'n longitudinale studie van miseiertellings (METs) van nematodes en onderskeiding van derde-stadium larfies. Die diere is gebloei om die hematokrit te bepaal, die kleur van hulle oogslymvliese is aangewys deur middel van die FAMACHA©-metode, en die diere se kondisie is bepaal. 'n Longitudinale studie van die gepoelde METs van trematodes is terselfdertyd uitgevoer.

Laer hematokritwaardes van bokke is gedurende die periode van swaarder *Haemonchus*-besmetting aangeteken. Dié periodes was van Desember/Januarie tot Maart by Rust de Winter; van Desember tot Maart/April by Impendle; en van November/Desember tot Februarie of April by

Kraaipan. In skape was die periodes van swaarder *Haemonchus*-besmetting van Oktober tot Maart by Rust de Winter en van September/Oktober tot Februarie of April by Kraaipan. Die periodes van bleker oogslymvliese wat aangeteken is volgens die FAMACHA©-metode het ook met die laer hematokritte ooreengestem.

Verwerkings wat vir die data van die bokke vir die somers van 1998/1999 en 1999/2000 gedoen is, het onderskeidelik ‘n toetssensitiwiteit van 76% en 85% getoon, wat beteken dat die sisteem gebruik mag word om 76% tot 85% van dié diere wat behandeling met ‘n wurmmiddel nodig het, korrek te identifiseer. Die toetsspesifisiteit het egter laag gebly en was 52% tot 55%. Dit beteken dat ‘n groot deel van dié diere wat nie behandeling nodig het nie, wel behandel sal word. Wanneer die FAMACHA©-sisteem egter met die gewone doseerpraktyke vergelyk word, sal die gebruik van die FAMACHA©-sisteem tot volg hê dat ‘n groot gedeelte van die diere nie behandel sal word nie. Die onbehandelde diere kan daarna die eiers van wurmmiddelvatbare wurms op die weiding uitskei, terwyl die behandelde diere baie min eiers sal uitskei, mits die wurmmiddel effektief is. Dit handhaaf ‘n reserwe van vatbare larfies *in refugia*, en behoort die ontwikkeling van wurmmiddelweerstand te vertraag.

Die gebruik van die FAMACHA©-sisteem mag as deel van ‘n geïntegreerde benadering tot wurmbeheer in die hulpbron-beperkte gebiede wat bestudeer is, aanbeveel word en mag wye toepassing in die tropiese en subtropiese gebiede van Afrika besuide die Sahara en elders hê.

Seisoenale variasies in liggaamskondisie is vir die bokke by Impendle aangeteken en die diere het laer liggaamskondisietellings (LKTs) van Junie tot September getoon. Die skape van Kraaipan het laer LKTs van Julie tot Desember getoon. Die klein herkouers van Rust de Winter het nie duidelike seisoenale variasies gewys nie, alhoewel die bokke by Rust de Winter laer LKTs van middel Julie tot vroeg in Desember en die skape van Augustus tot middel-Februarie getoon het. Alhoewel die bokke by Kraaipan liggaamskondisie behou het, het die tellings oor die algemeen laag gebly. Die LKTs vir Rust

de Winter waar die diere op ‘n privaat plaas gewei het, was oor die algemeen hoër as dié van die ander plekke, waar die weiding gemeenskaplik gebruik word.

Die amfistoom METs het ‘n seisoenale patroon gevolg, met ‘n vermeerdering in die tellings gedurende die warm maande van die jaar (September tot April). Dit skyn dat die studie ‘n verskillende patroon van infeksie aandui in die bokke waarmee daar onder hulpbron-beperkte omstandighede geboer word, in teenstelling met die situasie op kommersiële plase waar uitbreke van kliniese paramfistomose gedurende die herfs en winter voorkom.

Sleutelwoorde: Bokke; FAMACHA©; *Haemonchus* spp.; Hematokrit; Kliniese toets; Liggaamskondisietellings; Miseiertellings van nematodes en trematodes; Oog kleurkaart; Skape; Wurmmiddelweerstand

Introduction

Small ruminants play an important role in the local economies of many resource-poor communities in South Africa. However, little is known about the prevalence and effects of diseases in such animals in South Africa. The importance of internal parasites as causes of mortality and poor production is, however, recognized in commercial sheep farming in this country. In the summer rainfall area, haemonchosis is the most important helminth infection in sheep. As such it is necessary to examine its role in the resource-poor farming sector with the aim of developing worm management strategies for these areas.

With the worldwide emergence of anthelmintic resistance in sheep and goats, the testing and application of worm control strategies which reduce the use of anthelmintics is critical. One such strategy, the FAMACHA© system, was developed for use in sheep. The method is based on the principle that anaemic animals may be identified by examining the colour of the conjunctival ocular mucous membranes and these animals may be treated with an anthelmintic. A large proportion of the flock is left untreated and the number of treatments administered is considerably reduced.

Aims

The aims of the present study were thus :

- to conduct a longitudinal study of the helminth infections of goats and sheep raised by resource-poor farmers in the summer rainfall area of South Africa;
- to determine the effect of worm infection on the eye colour, haematocrit and body condition of the small ruminants; and
- to apply and evaluate the use of the FAMACHA© system in goats.

This dissertation consists of eight chapters which have been written following the format of *Veterinary Parasitology*. Chapter 1 gives the background to the study and includes a literature review. Chapter 2 describes the general materials and methods used. Chapters 3, 4, 5 and 6 describe the results for the goats studied while Chapter 7 gives the results for the sheep.

Chapter 3 discusses the sensitivity and specificity of the FAMACHA© assay in goats. It also gives the results of faecal egg count reduction (FECR) tests carried out in this species.

Chapter 4 gives the proportional nematode faecal egg counts (FECs) of the various strongyle worm genera found in the goats. This chapter also describes the results of the microhaematocrit determinations and provides a summary of the eye colour scores.

Chapter 5 describes the body condition scores (BCS) for the goats in relation to their FECs.

The results of the trematode FECs for the goats are given in Chapter 6.

Chapter 7 records the strongyle FECs, the microhaematocrit results, the FAMACHA© scores, the BCS and the trematode FECs for the sheep. The results of the FECR test conducted in the sheep at Kraaipan are also reported here.

Chapter 8 concludes the dissertation and provides suggestions for the application of the FAMACHA© system elsewhere in Africa.

Chapter 1

Background

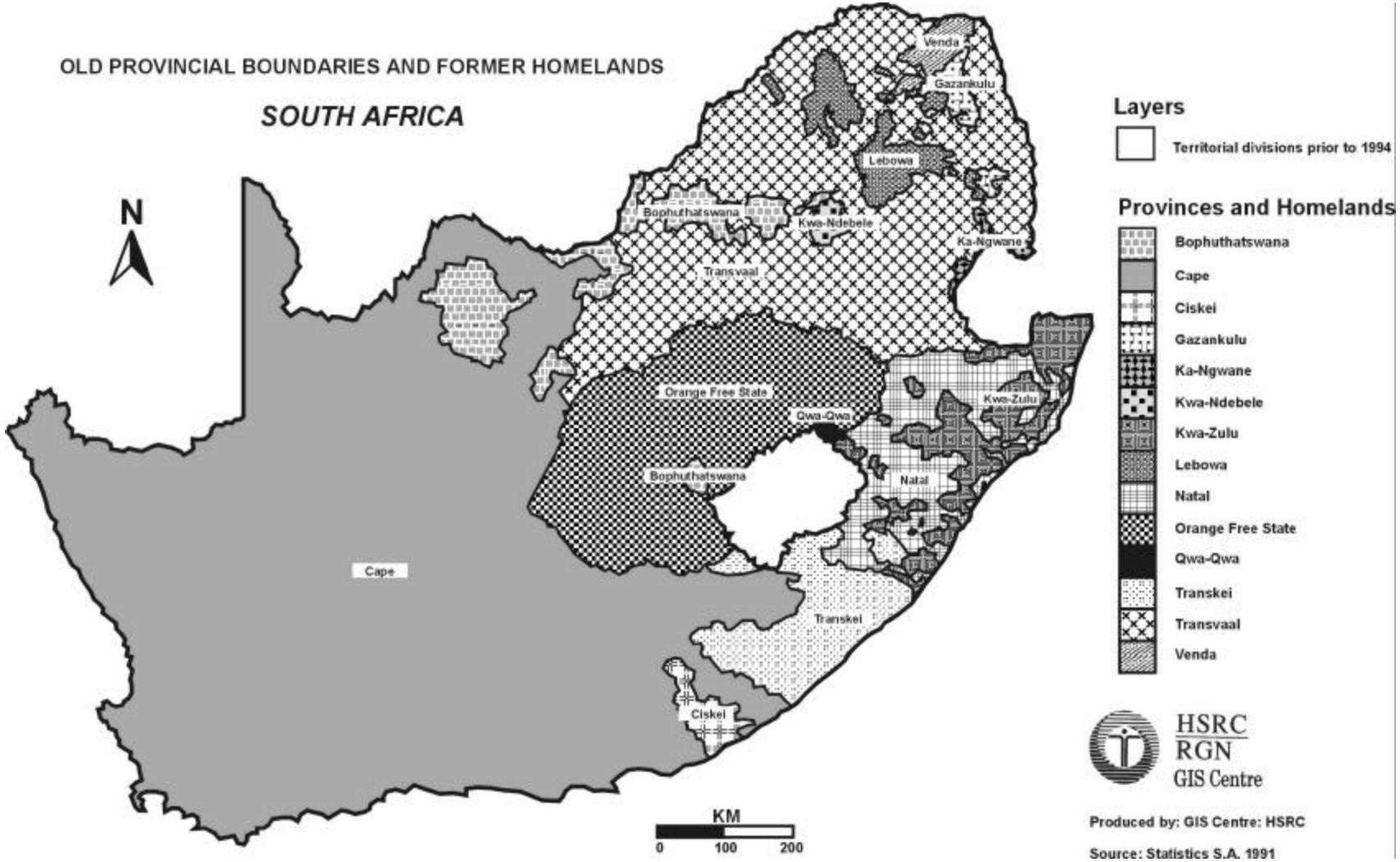
The small ruminant industry of South Africa comprises 36 million units of which 29 million are sheep and six-and-a-half million are goats (Anon, 1999).

Small ruminants, and livestock in general, serve as a stabilising factor in farming especially on marginal lands. Crop farming is so much more dependent on weather and climatic conditions. A maize crop may fail, for example, because of lack of rain whereas sheep and especially goats will be able to withstand a temporary drought. Small ruminants are important for the resource-poor farmer as they serve as an economic reserve. The sale of a few cull animals provides a solution to cash flow problems (own observations, 1998). In addition, small ruminants serve as sacrificial animals for celebrations and other traditional purposes and as a source of food in times of need.

A resource-poor community may be said to be characterised by:

- "At least 60% of the community living below the poverty data line;
- Poor access to facilities, information (literacy, education), infrastructure, finance, agricultural inputs and support services (e.g. access to markets);
- No security of land tenure; …
- Skewed demographics (higher proportions of women, aged, young);
- History of dependency on state-provided services (e.g. dipping);
- Low livestock production and reproduction;
- Needs include better accessibility and affordability of information, products and services" (Krecek et al., 1999). In many cases, resource-poor areas correspond to the so-called homelands of the previous political dispensation (Fig. 1.1).

Fig. 1.1


OLD PROVINCIAL BOUNDARIES AND FORMER HOMELANDS
SOUTH AFRICA
N
Venda
Gazankulu
Lebowa
Bophuthatswana
Kwa-Ndebele
Ka-Ngwane
Transvaal
Orange Free State
Kwa-Zulu
Qwa-Qwa
Bophuthatswana
Natal
Cape
Transkei
Ciskei
KM
0
100
200
Layers
Territorial divisions prior to 1994
Provinces and Homelands
Bophuthatswana
Cape
Ciskei
Gazankulu
Ka-Ngwane
Kwa-Ndebele
Kwa-Zulu
Lebowa
Natal
Orange Free State
Qwa-Qwa
Transkei
Transvaal
Venda
HSRC
RGN
GIS Centre
Produced by: GIS Centre: HSRC
Source: Statistics S.A. 1991

Agricultural development involves increasing the overall productivity and sustainability of the farming system (Norman, 1993). Improved production and utilisation of sustainable technology is necessary to assist resource-poor farming communities to contribute to the nation's food security and to their own social and economic advancement (Connor et al., 1994). Resource-poor farmers have expressed concern that their goats "don't multiply" (B.A. Letty, personal communication, 2001), a statement which conceals poor reproductive performance and low productivity as well as poor herd management and lack of understanding of disease. At the same time, they have also expressed the desire to keep their animals healthy in order to offset the expenses of, for example, hiring land (own observation, 1999).

The importance of gastro-intestinal parasites as major constraints to small ruminant health and production is well recognised world-wide and in Africa. Studies demonstrating this include, for example, those listed in Table 1.1. Gastrointestinal nematodes cause losses owing to mortalities, reduced liveweight gains, poor reproductive performance and condemnation of meat at abattoirs.

Echoing this fact, in September 1993 at a workshop held at the premises of the Foundation for Research Development (now National Research Foundation), interim priorities were set pending finalisation of the proposed new veterinary science research programme (Connor et al., 1994). One such priority was the control of internal parasites.

Barrow (1964), Rossiter (1964), Horak et al. (1976), Horak and Louw (1977), Horak (1978) and Biggs and Anthonissen (1982) have investigated the epidemiology of gastrointestinal helminths in sheep raised under commercial farming conditions within the summer rainfall area of South Africa (Fig. 1.2). Boomker et al. (1994) and Kusina et al. (1999) have studied the parasites of goats kept under resource-poor conditions in South Africa and Zimbabwe, respectively. The studies have indicated *Haemonchus* to be the most important parasite of small ruminants within the summer rainfall area of South Africa. Summer in the Southern Hemisphere may be defined to occur from December to

Table 1.1

Studies demonstrating the importance of gastro-intestinal parasites as major constraints to small ruminant health and production in Africa

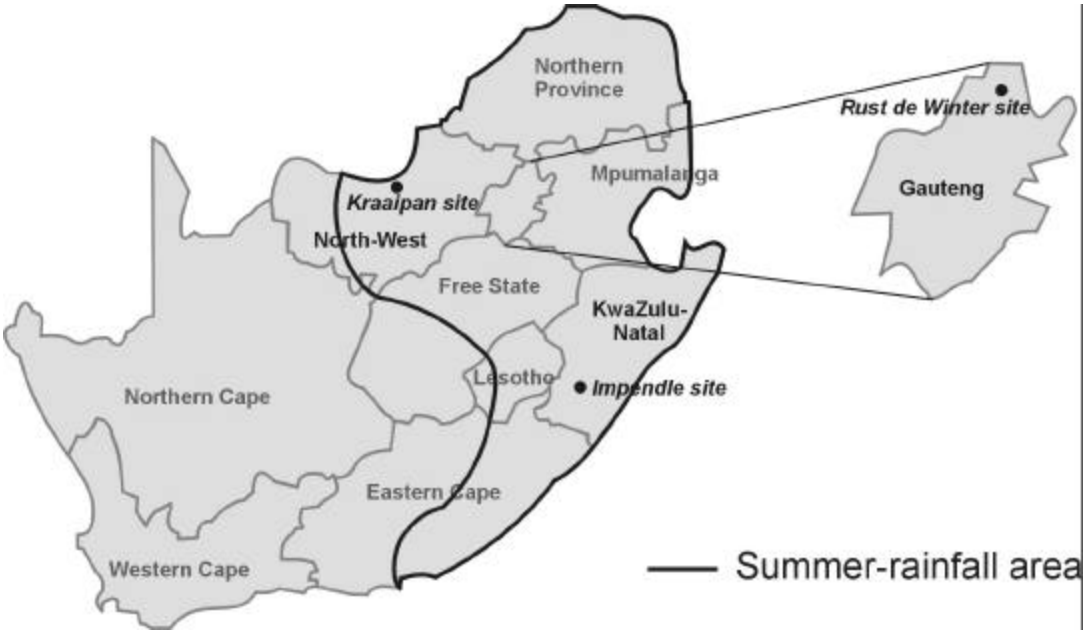
Country	Reference
Botswana	Carmichael, 1972
Cameroon (Bamenda)	Ndamukong et al., 1987
Ethiopia (Asmara)	Tseggai, 1981
Ethiopia (Debre Berhan)	Bekele et al., 1992
Ethiopia (highlands)	Tembely et al., 1996
Kenya	Peeler and Wanyangu, 1998
Kenya (Lolgorien Area, Narok District)	Muenstermann and Tome, 1989
Kenya (Machanga Embu)	Okeyo et al., 1996
Kenya (Nairobi—Limuru—Ruiru area)	Lutu, 1983
Kenya (Naivasha)	Allonby and Urquhart, 1975
Morocco (Middle Atlas, Gharb and Rhamna)	Dakkak and Ouhelli, 1988
Namibia (Kalahari region)	Biggs and Anthonissen, 1982
Nigeria (eastern)	Fakae, 1990
Sierra Leone (Freetown)	Asanji, 1988
Sierra Leone (Freetown)	Asanji and Williams, 1987
Sudan	Yagoup et al., 1981
Tanzania (northern)	Njau, 1987
Tanzania (southern)	Connor et al., 1990
Tropical regions e.g. northern Nigeria	Fabiyi, 1987
West Africa (sub-Saharan)	Zinsstag, 1998
Zimbabwe (Marandellas)	Grant, 1981
Zimbabwe (Mashonaland East Province)	Kusina et al., 1999

February; autumn from March to May; winter from June to August; and spring from September to November (Climatic Information Office, South African Weather Bureau).

Haemonchus occurs in highest numbers from January to April or May, but may be an important species even until July. From February to April, *Haemonchus* occurs increasingly as hypobiotic fourth-stage

larvae which allows the parasites to overwinter. When the mean maximum temperature and the monthly rainfall rise above 18°C and 50 mm respectively (Allonby and Urquhart, 1975), conditions are once again favourable for the development of the parasite on pasture. *Trichostrongylus* and *Teladorsagia* prefer the cooler months of the year. *Oesophagostomum* may occur all year round in moderate numbers (Rossiter, 1964), although conditions on pasture are optimal for infective larvae during late summer and autumn (Reinecke, 1983).

Fig. 1.2 : Study sites for sampling



With the emergence of the highly effective broadspectrum anthelmintics over the past three decades, anthelmintics have become the mainstay of worm control in the small ruminant industry. Anthelmintics are often registered for use in goats at the same dosage rate as for sheep (Hennessy et al., 1993a). However, various studies have subsequently indicated differences in the metabolism of the remedies in goats when compared with sheep. As such, the use of sheep dosage rates in goats has been suggested as a reason for the reduced efficacy of the drugs in this species. Oxfendazole has been shown to have a significantly lower systemic availability in goats than in sheep (Hennessy et al.,

1993a). A dosage rate of 10 mg kg^{-1} (double the sheep dose rate) is recommended in goats. This is administered as one single dose (5 mg kg^{-1}) followed by two half doses 12 and 24 hours later (Sangster et al., 1991). Closantel has a similar systemic availability in sheep and goats (Hennessy et al., 1993c), but the elimination rate has been shown to be two to three-fold greater in goats than in sheep. This means that the residual action against establishment of infection with gastro-intestinal nematodes is reduced in goats. The reason for the differences in oxfendazole and closantel metabolism in goats than those in sheep is thought to be a result of an enhanced metabolism of the drugs in the liver in goats. Albendazole has also been shown to have a lower systemic availability in goats than in sheep (Hennessy et al., 1993b), but in contrast with oxfendazole and closantel, albendazole is thought to be sequestered to a greater extent in the liver of goats than that of sheep. An increase in the dose rate from 4.75 mg kg^{-1} for sheep to 7.5 mg kg^{-1} in goats has been suggested. A higher dose rate for levamisole of 12 mg kg^{-1} has been suggested for goats compared with the 7.5 mg kg^{-1} for sheep since levamisole is metabolised more rapidly in goats than it is in sheep (Coles et al., 1989). Ivermectin should probably be used at one-and-a-half times the sheep dose rate (0.2 mg kg^{-1}) in goats (Coles, 1997).

The use of the sheep dosage rate in goats has also been proposed as a reason for the development of resistance of gastro-intestinal parasites to anthelmintics (for example, Bjørn et al., 1991), which has become a world-wide phenomenon and is considered to be increasing at an alarming rate. Reports of anthelmintic resistance in goats in Africa have been relatively scant (Table 1.2), although this is probably more a case of insufficient studies having been done than a case of resistance not being present. All of the five goat studies cited in Table 1.2 found resistance in goats kept on research, university or commercial farms where frequent treatments with anthelmintics were given. This differs somewhat from the resource-poor farming set-up where treatments are less intensive or not given at all. Nevertheless, resistance may be present on these farms through the introduction of animals as breeding stock from neighbouring commercial or experimental farms for improving animal production (Maingi et al., 1998; Mwamachi et al., 1995). Resource-poor small ruminant farming in South Africa appears

Table 1.2

Reports of anthelmintic resistance from goats in Africa

Country	Anthelmintic	Breed	Reference	Resistant worm genera
Cameroon	Fenbendazole Thiabendazole	-	Ndamukong et al. (1992)	Strongyles
Kenya	Thiabendazole	East African, Galla, Toggenburg/East African crossbreeds	Njanja et al. (1987)	*H. contortus*
Kenya	Fenbendazole Ivermectin Levamisole	Galla, Small East African	Mwamachi et al. (1995)	*H. contortus* *Trichostrongylus* spp. *Oesophagostomum* spp.
Tanzania	Thiophanate	-	Ngomuo et al. (1990)	*H. contortus*
Tanzania	Albendazole Fenbendazole Thiabendazole	Black Head Persian lambs[a]	Bjørn et al. (1991)	*H. contortus*
Zimbabwe	Thiabendazole	-	Chavunduka (1970)	Strongyles

[a]Anthelmintic efficacy was not tested in the goats, but these were grazed together with the sheep.

to be in the same situation the present commercial farming sector was in about 25 years ago when the first report of anthelmintic resistance in South Africa was published (Berger, 1975). However, even in resource-poor farming areas there appear to be levels of resistance. Van Wyk et al. (1999) reported anthelmintic resistance in sheep on an experimental farm in Lebowa where there was resistance to two compounds from different anthelmintic groups. Varying degrees of resistance also occurred even in four of the five sheep flocks they tested in 1993 on the communal pastures in this region. It is thus essential that plans be made now to prevent the development of the same extent of resistance on the resource-poor farms as exists in the commercial farming sector of South Africa today.

The FAMACHA© system

Waller (1993) cites a number of approaches that may hold promise for the sustainable control of nematodes, including the better use of existing drugs, helminth vaccines, breeding for host resistance, nematode growth regulators and biological control. Included under the better use of drugs are such strategies as specific, epidemiologically based treatments, pasture spelling, alternation of anthelmintic chemical groups, and alternate grazing between species and between age classes within species. Waller and Larsen (1996) stated that non-chemotherapeutic control options needed to be researched and field evaluated as a matter of urgency. In their search for a solution for the threatening problem of resistance of *Haemonchus* to anthelmintics in sheep, Malan and Van Wyk (1992) succeeded in identifying those sheep which were in danger of succumbing to *Haemonchus* infection unless dewormed, through the clinical observation of the colour of the ocular mucous membranes of the sheep. In sheep on irrigated pastures in Badplaas in Mpumalanga Province (an ideal climate for *Haemonchus* infection), they further succeeded in reducing the use of anthelmintics by approximately 90% in these sheep. The corresponding reduction in selection for resistant worms was still greater, as a result of the fact that only those individual animals that were considered in danger of dying were treated with an anthelmintic. There is for the first time a cheap, effective method for identifying the individual animals in a flock that are unable to withstand the worm challenge. This agrees with findings by Barger (1985) which indicated that important nematodes of sheep are overdispersed with more than half of the worms found in less than half of the hosts. Those animals that are not treated still shed susceptible worm eggs on to the pasture. Bath et al. (1996) called the concept the FAMACHA© system after its originator, i.e. Francois "FAffa" MAlan CHArt. They proposed that it be implemented in practice as they found it cheap to apply and easily taught, even to illiterate people. "The technique is very easy and reasonably reliable once learned under guidance of a competent instructor." (Bath et al., 1997.) Testing the FAMACHA© system under other farming conditions was proposed and is needed

before this method is validated as an additional tool for integrated worm management. It is described in greater detail in section 2.2 and Fig. 2.2 below.

Chapter 2

General materials and methods

2.1. Study sites, animals and sampling

Three study sites were selected within the summer-rainfall area of South Africa: one near Rust de Winter, Gauteng Province, one in Impendle, KwaZulu-Natal Province, and one in Kraaipan, North-West Province (Fig. 1.2). A summary of the trial periods and frequencies of visits, breeds of animals, sample sizes, and anthelmintics used is given in Table 2.1.1 The grazing practices, vegetation types, winter supplements and long-term average rainfall for each study site are given in Table 2.1.2. During the day, the goats at Rust de Winter browsed on the natural vegetation while the farmer at Site 2, Impendle, allowed the animals to graze communal pasture surrounding his homestead. At Site 1, Impendle, and at Kraaipan the animals were grazed on communal pasture tended by a shepherd. All the animals were penned in kraals at night. The sheep at Rust de Winter initially grazed with the goats, but from May 1999 they were grazing separately in an enclosed paddock of fallow land and were also penned separately at night.

Climatic data were supplied by the South African Weather Bureau from the weather station nearest to the study sites (Table A1.1) and are given in Figs. A1.1-A1.3.

During visits in November 1999 to January 2000, all the animals present were aged by examining their incisors and sexed. The profiles for the herds on those days are given in Table 2.2.

At Rust de Winter, all the weaner and adult goats and sheep present at each visit were sampled/scored. For the two farmers (Sites 1 and 2) at Impendle, a representative sample of the weaner and adult goats was selected based on the first animals brought into the crush at the first visit, and when available the same goats were sampled/scored throughout the trial period. Unfortunately, the initial sample sets for each of the two sites at Impendle had diminished in

Table 2.1.1

Study sites : summary of trial periods and frequencies of visits; breeds of animals; sample sizes; and anthelmintics used

Study site	Trial period	Frequency of visits	Breed	Approximate numbers of animals present at each visit	Mean number of animals sampled/ scored (range)	Anthelmintic used (dosage)[b]
Rust de Winter (25°16'52"S; 28°38'51"E)	Sep 1998 - Apr 2000	Fortnightly	Boer goat crossbreed	18-47	31 (18-47)	Levamisole ($7.5\ mg\ kg^{-1}$)[c]
			Dorper sheep crossbreed	3-7	5 (3-7)	Ditto
Site 1, Impendle (29°34'39"S; 29°47'7"E)	Nov 1998 - Apr 2000	Monthly	Indigenous Zulu goat crossbreed	±60	18 (9-22)	Ivermectin ($0.2\ mg\ kg^{-1}$)[d]
Site 2, Impendle (29°35'4"S; 29°47'15"E)	Ditto	Ditto	Ditto	±35	19 (14-24)	Ditto
Kraaipan (26°19'16"S; 25°16'44"E)	Oct 1998 - Apr 2000	Monthly[a]	Indigenous goat crossbreed	±60	16 (10-23)	Levamisole ($7.5\ mg\ kg^{-1}$)[c]
			Indigenous fat-tailed sheep crossbreed	±60	14 (5-22)	Ditto

[a]Excepting for two visits over three months at start of trial.
[b]All anthelmintics were administered *per os*.
[c]Predominantly Tramisol™ liquid (Hoechst Roussel Vet, now Intervet); on a few occasions initially, Levisol™ liquid (Bayer).
[d]Ivomec™ tablets for sheep (Logos Agvet); used extra-labelly in goats: goats less than 25 kg in weight were given ½ tablet (5 mg) each and goats between 25 and 50 kg in weight were given 1 tablet (10 mg) each.

Table 2.1.2

Study sites : summary of grazing practices, vegetation types, winter supplementation and rainfall

Study site	Grazing	Vegetation[a]	Winter supplementation (1999)	Rainfall[b]
Rust de Winter	Private farm of 620 ha.	Mixed bushveld	Bone meal and salt lick	610 (Rust de Winter, 10)
Site 1, Impendle	Communal	Highland sourveld and Döhne sourveld	Poor quality *Eragrostis* spp. hay	993 (Donnybrook, 35)
Site 2, Impendle	Ditto	Ditto	Whole maize kernels - frequency and quantity not obtained	Ditto
Kraaipan	Communal	Sourish mixed bushveld	Bone meal and salt lick	539 (Mmabatho, 60)

[a]Acocks, 1975.
[b]Long-term average annual rainfall in mm (weather station, approximate kilometres in a direct line from study site). Source: South African Weather Bureau.

number by the end of the trial, owing to sales or slaughtering of animals and at least one death. At Kraaipan, a representative sample of the goat and sheep flocks was chosen in a similar manner as for Impendle, but the animal numbers also started to dwindle and for this reason every 10th animal brought into the crush in May 1999 was added to the sample group. This resulted in four goats and four sheep being added to the representative sample groups.

Animals were utilised for faecal egg count reduction (FECR) tests (see 2.2 and 2.4 below) towards the end of the trial. These animals had not been included in the sampling groups mentioned above, except for one of the goats and one of the sheep at Kraaipan. All the goats had no permanent incisors, except for three of the goats at Site 1, Impendle, two at Site 2, Impendle, and 10 at Kraaipan, which goats had two to four permanent incisors. Four of the sheep at Kraaipan had two to six permanent incisors while the rest of the sheep had deciduous incisors only. None of the animals had been treated with an anthelmintic effective against nematodes within 12 weeks of the start of the FECR tests at Rust de Winter and Kraaipan, and within 16 weeks at Impendle.

Table 2.2

Profile of total flocks by age and sex

Age in years[a] (no. of permanent incisors)	Up to ± 1.0 (0)	1.0 and < 1.5 (2)	± 2.0 (4)	± 2.5 (6)	2.5 (8)	3.0 (worn mouth) [b]	Totals
Rust de Winter - Goats - 15 Dec 99							
Female	17	3	2	2	15	0	39
Male	15	1	0	0	0	0	16
Wether	2	1	0	0	0	0	3
Totals	34	5	2	2	15	0	58
Rust de Winter - Sheep - 15 Dec 99							
Female	1	0	0	1	2	0	4
Male	0	0	1	0	0	0	1
Totals	1	0	1	1	2	0	5
Site 1, Impendle - Goats - 23 Nov 99							
Female	10	7	5	3	15	0	40
Male	8	0	0	1	1	0	10
Totals [c]	18	7	5	4	16	0	50
Site 2, Impendle - Goats - 23 Nov 99							
Female	10	2	3	2	13	1	31
Male	12	0	0	0	0	0	12
Totals [c]	22	2	3	2	13	1	43
Kraaipan - Goats - 4 Jan 00							
Female	21	7	1	7	20	4	60
Male	2	1	0	0	1	1	5
Weather	13	6	0	0	0	0	19
Totals	36	14	1	7	21	5	84
Kraaipan - Sheep - 4 Jan 00							
Female	20	4	1	5	25	0	55
Male	5	0	0	0	0	0	5
Weather	10	0	0	0	0	0	10
Totals	35	4	1	5	25	0	70

[a]Adapted from Kwantes, 1994.
[b]Probably ≥ 8.0 years old.
[c]One animal missed per site.

2.2. Parasitological diagnostic techniques

Faecal samples were collected at each visit from the animals at Rust de Winter and the representative sample sets at Impendle and Kraaipan (the "trial" animals). Additional samples were

collected from April 1999 at Kraaipan and from May 1999 at Impendle to ensure that there would be sufficient faeces for a good yield of third-stage nematode larvae (L_3) when cultures were made (see below). The faecal samples were processed for nematode faecal egg count (FEC), using a modified McMaster technique (Van Schalkwyk et al., 1995). This method is described in Appendix 2. Minor modifications to the method as well as the trade names of the equipment used are recorded in square brackets in this appendix. In brief, two grams of faeces were weighed off per animal. To this were added 58ml of sugar solution and the mixture homogenized using an electric mixer (IKA®-Labortechnik, Janke and Kunkel, N.T. Laboratory Supplies, Johannesburg). Two chambers of a McMaster slide (Eggs-Acto™ McMasters, E. Krecek, South Africa) were filled with the solution, and after allowing the slide to stand for at least two minutes, the nematode eggs in the chambers were counted under a compound microscope. The number of eggs counted per sample was multiplied by 100 to give the final result in eggs per gram of faeces (epg), according to the following formula :

$$\text{FEC (in epg)} = \frac{\text{number of eggs counted x volume of sample}}{\text{volume of McMaster chamber x number of chambers counted x mass of faeces in sample}}$$

$$\text{FEC (in epg)} = \frac{\text{number of eggs counted x 60}}{0{,}15 \text{ x } 2 \text{ x } 2}$$

$$\text{FEC (in epg)} = \text{number of eggs counted x } 100$$

Strongyloides, *Nematodirus* and *Trichuris* eggs were counted separately from the other nematode eggs, which are herein referred to as "strongyle" eggs (Order Strongylida - Molin, 1861).

Samples were screened for trematode eggs by means of the sedimentation method (Van Wyk et al., 1987, Appendix 2) which was modified for pooled samples as follows. Half a gram of faeces (1g for the sheep at Rust de Winter) was weighed off from each of 10 faecal samples (five faecal samples for the sheep at Rust de Winter) randomly selected from those collected at each visit to a site. The faeces were pooled and softened and/or homogenized with an electric mixer (IKA®-Labortechnik, Janke and Kunkel, N.T. Laboratory Supplies, Johannesburg) in water. The faeces

were then sieved through a 150µm sieve (United wire test sieve, Nigel, South Africa or equivalent) into a 38µm sieve (Labotec test sieve, Johannesburg or equivalent), using water sprayed from a nozzle at high pressure. The remaining sediment was washed into a two or three litre glass jar. This was filled with water and allowed to stand for at least 15 minutes. The supernatant was then decanted and the sediment washed by filling up the jar again. This process was repeated approximately three times until the resulting supernatant was clear. Thereafter the sediment was poured into a measuring cylinder, made up to 200ml with water and mixed well by blowing air through the suspension with a pipette. Twenty millilitres of this suspension were examined in a gridded perspex container (70mm x 70mm, E. Krecek, South Africa) under a stereomicroscope for trematode eggs.

The number of eggs per gram of faeces was calculated as follows :

$$\text{FEC (in epg)} = \frac{\text{Number of eggs present}}{\text{Mass of faeces}}$$

$$= \frac{\text{Number of eggs counted x 10}}{10 \text{ x } ½}$$

$$= \text{Number of eggs counted x 2}$$

Faeces remaining after the FECs had been processed were cultured for third-stage nematode larvae (L_3). The method described in Van Wyk et al. (1987) for the collection of large numbers of larvae was used (Appendix 2). In brief, the faeces were mixed with vermiculite and a small amount of water, and lightly compacted in large fruit jars (approximately one litre). The faeces were placed in an incubation room at a temperature of approximately 25°C until November 1999 when a new room was used and the temperature then adopted was approximately 26°C. L_3 were harvested into small jars (approximately 100ml) by flushing the sides of the jar and the surface of the faeces with water. After allowing the larvae to settle to the bottom of the jar for at least 15 minutes (but often considerably longer) a sample(s) of larvae was (were) drawn up from the bottom of the sample with a Pasteur pipette (Liquipettes™, Elkay, Ireland or equivalent), placed on a slide(s), stained with a dilute iodine solution and where possible at least 50 L_3 were identified under a compound

microscope. The keys of Van Wyk et al. (1997a, Appendix 2) and Dunn (1978) were used. Where few larvae were recovered from a culture, the larvae from a sample were allowed to settle in a test tube. No attempt was made to differentiate *Teladorsagia* spp. from *Trichostrongylus* spp. The proportions of the strongyle L_3 were used to estimate the proportional FECs of the various strongyle worm genera.

The animals were bled from the jugular vein into evacuated ethylene diamine tetra-acetic acid (EDTA) tubes (Vacutainer Systems^{TM}, Becton Dickinson, France). Two heparinised microhaematocrit tubes (Marienfeld, Germany, or equivalent) were filled with blood per sample and centrifuged (Kubota 3100, N.T. Laboratory Supplies, Johannesburg or Hermle Z230 HA, Germany) for seven minutes at 12000 revolutions per minute. The haematocrits were read for each capillary tube and the mean of the two readings used in the analyses.

The efficacies of the anthelmintics used in the trial were assessed by means of the FECR test (Coles et al., 1992; Presidente, 1985; Van Schalkwyk et al., 1995), which uses the reduction in FECs following anthelmintic treatment as an indication of anthelmintic efficacy. In each FECR test, the faeces remaining after the FECs had been done were cultured as follows for L_3 recovery: for the initial date per test all the faeces were pooled together (pre-treatment culture), while the faeces for the second date were pooled separately per group (post-treatment cultures). The proportions of L_3 were applied to the strongyle egg counts to estimate the relative contribution of each genus (Coles et al, 1992; Presidente, 1985).

Table 2.3 gives the mean FECs of the animals at the visit dates immediately prior to the dates on which the anthelmintic treatments for the FECR tests were carried out. The animals were ranked according to these FECs from lowest to highest. The animals were then divided into groups of two or three, depending on whether one or two anthelmintics were being tested. Each individual within each group was then randomly assigned to a treatment or control group (Table 2.3) with the help of a table of random numbers. The initial and post-treatment dates of the FECR tests and the sizes of the groups included in the tests are also recorded in Table 2.3.

Table 2.3

Faecal egg count reduction tests : details of groups

Location	Mean FEC (interval)[a]	Treatment date of FECR test (interval)[b]	Anthelmintic (dosage)[c]
Goats			
Rust de Winter	2171 (14)	24 Feb 2000 (13)	Levamisole (7.5 mg kg^{-1})
Site 1, Impendle	3775 (28)	14 Mar 2000 (15)	Ivermectin (0.2 mg kg^{-1})
Site 2, Impendle	3147 (28)	14 Mar 2000 (15)	Ivermectin (0.2 mg kg^{-1})
Kraaipan	778 (28)	29 Feb 2000 (10)	Levamisole (7.5 mg kg^{-1}) Rafoxanide (7.5 mg kg^{-1})
Sheep			
Kraaipan	8230 (28)	29 Feb 2000 (10)	Levamisole (7.5 mg kg^{-1}) Rafoxanide (7.5 mg kg^{-1})

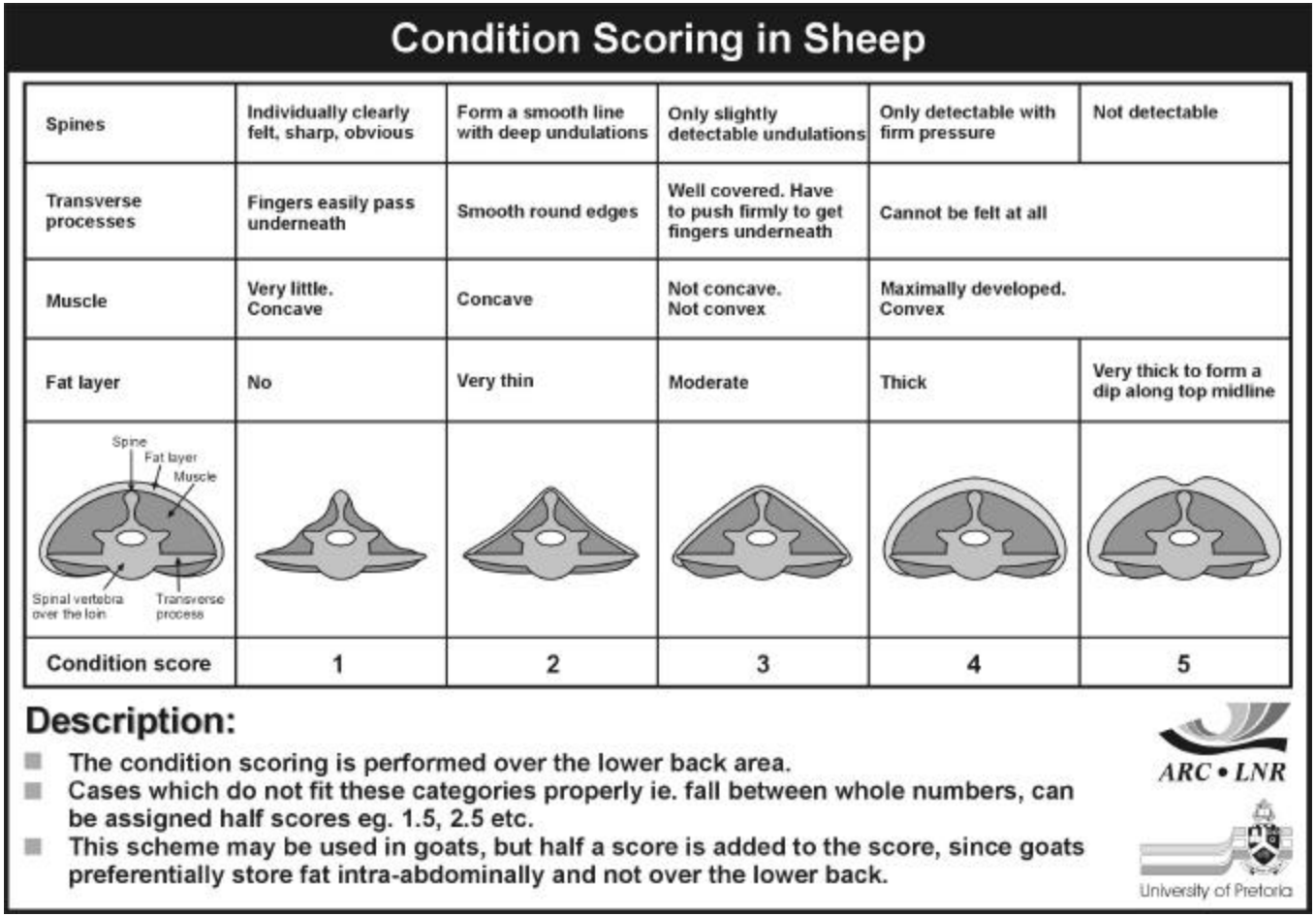
[a]Mean strongyle faecal egg counts in eggs per gram of faeces at last visit before FECR test (interval in days between last visit and FECR test).
[b]Treatment date of FECR test (interval in days between pre- and post-treatment collection of faecal samples).
[c]All anthelmintics were administered *per os*.

The animals were body condition scored according to the chart depicted in Fig. 2.1 which method agrees with that of Williams (1990).

2.3. Scoring for level of anaemia

At the scheduled visits, the author or one of the assistants on the project scored each animal for level of anaemia using the FAMACHA© colour card (Fig. 2.2). The author ensured that each assistant for whom scores were recorded had been adequately trained in the method. Excepting for the few visits that the author could not undertake, the scoring was always performed under his direct supervision. Occasionally, monitoring was done in-between scheduled visits by the farmer at Site 1, Impendle, and by the animal health technicians (AHTs) assisting with the project at Kraaipan. However, these scores were not included in any of the analyses discussed in this dissertation. Only the animals that were considered to be pale, i.e. colour chart categories four and

Fig. 2.1 : Condition scoring in sheep



five, were treated with an anthelmintic. At times, animals scored as category three were erroneously treated by the AHTs at Kraaipan and the farmer at Site 2, Impendle, initially misunderstood the aim of the trial and treated all his goats sometime between 24 November and 22 December 1998. Between 23 November and 21 December 1999, 10 goats were apparently treated by the shepherd of the farmer at Site 1, Impendle, but the animal identifications were not recorded.

To promote farmer co-operation, animals that showed signs indicative of *Oestrus ovis* infection (profuse mucous nasal discharge and difficulty in breathing through the nose) or infection with cestodes were at times also treated with an appropriate remedy. With respect to the trial animals, only one goat (at Kraaipan) was treated with rafoxanide [Nasalcur™, Hoechst Roussel Vet (now Intervet), 7.5 mg kg^{-1}] for *Oestrus ovis* infection during the trial. One to two sheep were treated on four occasions at Rust de Winter, and one to eight were treated on twelve occasions at Kraaipan. Twenty-two of the goats at Site 1, Impendle, and 25 of the goats at Site 2 were treated with niclosamide [Ex-a-lint™, Hoechst Roussel Vet (now Intervet), 50 mg kg^{-1}] during January
1999 for cestodes.

2.4. Statistical analyses

The data were entered in SAS (SAS Institute Inc., Cary, NC, USA) and this software was used throughout the analysis of the data. Sensitivity, specificity, predictive value of a negative and predictive value of a positive were calculated for the FAMACHA© clinical assay in goats. Smith (1995) defines sensitivity as the proportion of infected or diseased individuals with a positive test, or in the case of the FAMACHA© clinical assay, the proportion of anaemic animals correctly identified as anaemic. Test specificity is defined as the proportion of disease-free individuals that test negative, or the proportion of non-anaemic animals that are categorised as such. In the case of the FAMACHA© method, predictive value of a negative is the probability that an animal is not anaemic when the test result is negative for anaemia and vice versa for the predictive value of a

Fig. 2.2 : FAMACHA© anaemia guide (colours are not a completely accurate reflection of the original card)

positive. The sensitivity and specificity were tested statistically by means of Fisher's Exact Test for a two-by-two contingency table.

Two data sets for 1998/1999 and 1999/2000, respectively, were created from the FAMACHA© scores and haematocrit values obtained from the trial goats. Two-way frequency tables of haematocrit by FAMACHA© were drawn up, with FAMACHA© values four and five (or three, four and five) considered positive for anaemic animals and FAMACHA© values one, two and three (or one and two) considered negative test results, respectively (Table 2.4). Haematocrit was used as the gold standard by which anaemia was measured and two cut-off values for anaemia were assigned (less than 18% and less than 19%, respectively). In establishing the properties of a test, cut-off values are assigned to define the level of a test result that is needed to make or reject a diagnosis, in this case a diagnosis of anaemia (Smith, 1995).

The author chose to maximise the sensitivity and specificity of the FAMACHA© method in goats when the average of the sensitivity and specificity attains its highest value. This was calculated by the following equation for the present data:

$$(\text{sensitivity} + \text{specificity}) / 2 \quad \ldots (1)$$

In order to determine the percentage of animals treated in each case (FAMACHA© cut-off of four as opposed to FAMACHA© cut-off of three), the following calculation was also applied to the data:

$$(\text{true positive} + \text{false positive}) / \text{total number of animals} \times 100 \quad \ldots (2)$$

With respect to the FECR tests, anthelmintic efficacy was calculated by two methods: that of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992) and that of Presidente (1985). In the WAAVP method, the arithmetic mean of the treatment and control groups at 10 to 14 days after treatment are utilised to calculate the percentage reduction of FECs and the upper and lower 95% confidence intervals. Resistance is determined to be present

Table 2.4

Two-way frequency table of haematocrit by FAMACHA© with haematocrit cut-off of 18% (or 19%) and FAMACHA© scores 4 and 5 (or 3, 4 and 5) considered positive test results

		Anaemia	
		Present Ht[a] <18% (Ht <19%)	Absent Ht ≥18% (Ht ≥19%)
FAMACHA© score	Positive 4,5 (3,4,5)	True positive (TP)	False positive (FP)
	Negative 1,2,3 (1,2)	False negative (FN)	True negative (TN)

[a]Haematocrit.

Sensitivity = TP/(TP+FN) x 100
Specificity = TN/(FP+TN) x 100
Predictive value of a negative = TN/(FN+TN) x 100
Predictive value of a positive = TP/(TP+FP) x 100

if the percentage reduction is less than 95% and the lower confidence interval is less than 90%. If only one of the conditions is met, resistance is only suspected. In the method of Presidente (1985), the geometric or arithmetic means of FECs both on the day of treatment and 10 to 14 days thereafter are used in the calculation of the percentage reduction. Geometric means were used in the current calculations. Resistance has been said to occur when the percentage reduction is less than 80% in goats (Kettle et al., 1983). Reduced efficacy of anthelmintics in goats may be the result of a faster metabolism of the drugs in this species. Hence the lower value is used for goats when no difference between the dose for sheep and goats is indicated. The WAAVP method is considered to be a more conservative measure of anthelmintic efficacy (Coles et al., 1992). Focus has been placed on this method to allow for comparison between data of different authors, but the method of Presidente was included for completeness' sake.

Chapter 3

Testing for clinical anaemia caused by *Haemonchus* spp. in goats farmed under resource-poor conditions in South Africa using an eye colour chart developed for sheep *

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Abstract

A novel clinical assay for the assessment and subsequent treatment of *Haemonchus* infection in sheep to slow down the development of anthelmintic resistance – the FAMACHA© system – has been developed, tested and validated in South Africa. The system is based on a colour chart with five colour categories depicting varying degrees of anaemia that are compared with the colour of the mucous membranes of the eyes of sheep. The animal is then scored from severely anaemic

* *With the exception of Fisher's Exact Test, the current draft is that published in Veterinary Parasitology 99 (2001) 1-14.*

(pale) through anaemic to non-anaemic (red) and those animals considered in danger of succumbing to the effects of haemonchosis are treated. This method was tested in goats farmed under resource-poor conditions in South Africa. Analyses in goats performed during the summers of 1998/1999 and 1999/2000 show a test sensitivity of 76% and 85%, respectively, meaning that the system may be used to identify correctly 76% to 85% of those animals in need of treatment with an anthelmintic. However, the test specificity remains low at 52% to 55%. This means that a large proportion of those animals that would not require treatment would in fact be treated. However, when the use of the FAMACHA© system is compared with conventional dosing practices where all the animals are treated, using the FAMACHA© system would result in a large proportion of the animals being left untreated. The untreated animals are then able to deposit the eggs of anthelmintic-susceptible worms on the pasture, while the treated ones should pass very few ova, given an effective anthelmintic. This maintains a reservoir of susceptible larvae *in refugia*, and should slow down the development of anthelmintic resistance. The validation of the FAMACHA© system for goats for use by resource-poor farmers, which this paper describes, may have wide application in the tropics and subtropics of sub-Saharan Africa and elsewhere.

Keywords: Anthelmintic resistance; Clinical assay; Eye colour chart; FAMACHA©; Goat; *Haemonchus* spp.

3.1. Introduction

Haemonchosis is ranked among the most important diseases of small ruminants in the summer-rainfall area of South Africa. Anthelmintics are currently almost inexpendable in the control of the disease. However, recent surveys indicate that resistance to anthelmintics has reached high levels on commercial sheep farms in South Africa, and already occurs in the resource-poor farming sector (Van Wyk et al., 1999). As a means to reduce the number of anthelmintic treatments

given to sheep within the summer-rainfall area and hence the selection pressure for the development of anthelmintic resistance, a colour chart was developed to depict five categories within a range of ovine haematocrit values from healthy (red, "A") to severely anaemic (pale, "E") (Van Wyk et al., 1997b). The chart is compared with the ocular mucous membranes of sheep to assess the need for treatment with anthelmintics and those animals considered to be in danger of dying are selectively treated (categories "D" and "E"). The method presupposes that the anaemia is caused by *Haemonchus* infection. In the latest version of the FAMACHA© chart (Bath, 2000), the letters, "A" to "E", have been substituted by the numbers, one to five, and hence this latter convention has been used in this paper.

The broad objective of the present study was to apply and evaluate the FAMACHA© system in goats in resource-poor farming areas as a tool for anthelmintic resistance management and integrated parasite control. Within this broad objective, three specific aims were identified as follows: to use the FAMACHA© system to determine which animals require dosing with anthelmintics to prevent mortalities; to use the FAMACHA© system to ensure more effective dosing to slow down the development of anthelmintic resistance; and to investigate the efficacy of the anthelmintics used in the small ruminants following a year of anthelmintic treatment according to the FAMACHA© clinical assay.

3.2. Materials and methods

3.2.1. Study sites, animals, sampling, diagnostic techniques and scoring for level of anaemia

The study sites and materials and methods are discussed more fully in Vatta et al. (2000). In short, three study sites were selected within the summer-rainfall area of South Africa: Rust de Winter, Gauteng, Impendle, KwaZulu-Natal, and Kraaipan, North-West Province. A summary of the trial periods and frequencies of visits, sample sizes, breeds of goats, vegetation types and grazing practices, and anthelmintics used for each study site is given in Table 3.1. At Rust de

Table 3.1

Study sites : summary of trial periods and frequencies of visits; sample sizes; breeds of animals; vegetation types and grazing practices; and anthelmintics used

Study site	Trial period	Frequency of visits	Approximate numbers of animals present at each visit	Mean number of animals sampled/ scored (range)	Breed	Grazing	Vegetation[b]	Anthelmintic used (dosage)[c]
Rust de Winter	Sep 1998 - Apr 2000	Fortnightly	18-47	31 (18-47)	Boer goat crossbreed	Private	Mixed bushveld	Levamisole (7.5 mg kg^{-1})[d]
Site 1, Impendle	Nov 1998 - Apr 2000	Monthly	±60	18 (9-22)	Indigenous Zulu crossbreed	Communal	Highland sourveld and Döhne sourveld	Ivermectin (0.2 mg kg^{-1})[e]
Site 2, Impendle	Nov 1998 - Apr 2000	Monthly	±35	19 (14-24)				
Kraaipan	Oct 1998 - Apr 2000	Monthly[a]	±60	16 (10-23)	Indigenous crossbreed	Communal	Sourish mixed bushveld	Levamisole (7.5 mg kg^{-1})[d]

[a]Excepting for two visits over three months at start of trial.
[b]Acocks, 1975.
[c]All anthelmintics were administered *per os*.
[d]Predominantly Tramisol™ liquid (Hoechst Roussel Vet, now Intervet); on a few occasions initially, Levisol™ liquid (Bayer).
[e]Ivomec™ tablets for sheep (Logos Agvet); used extra-labelly in goats: goats less than 25 kg in weight were given ½ tablet (5 mg) each and goats between 25 and 50 kg in weight were given 1 tablet (10 mg) each.

Winter, all the weaner and adult goats present at each visit were sampled. For the two farmers (Sites 1 and 2) at Impendle, a representative sample of the weaner and adult animals was selected based on the first animals brought into the crush at the first visit, and when available the same goats were sampled/scored throughout the trial period. Similarly for Kraaipan, a representative sample of the herd was taken, but the animal numbers started to dwindle and for this reason every 10^{th} goat brought into the crush in May 1999 was added to the sample group. Faecal samples were collected at each visit from the goats and were processed for faecal nematode egg count, following the method of Van Schalkwyk et al. (1995) and at a sensitivity of 100 eggs per gram of faeces (epg), and for identification of third-stage nematode larvae (L_3), using the keys of Van Wyk et al. (1997a) and Dunn (1978). The goats were also bled and their haematocrits were determined by using the microhaematocrit method.

At the scheduled visits, one of the authors (A.F. Vatta) or one of the assistants on the project scored each animal for level of anaemia using the FAMACHA© card. The first author ensured that each assistant for whom scores were recorded had been adequately trained in the method. Excepting for the few visits that the first author could not undertake, the scoring was always performed under his direct supervision. Occasionally, monitoring was done in-between scheduled visits by the farmer at Site 1, Impendle, and by the animal health technicians (AHTs) assisting with the project at Kraaipan. However, these scores were not included in any of the analyses discussed below. Only the animals that were considered to be pale, i.e. categories four and five, were treated with an anthelmintic. At times, animals scored as category three were erroneously treated by the AHTs at Kraaipan and the farmer at Site 2, Impendle, initially misunderstood the aim of the trial and treated all his goats sometime between 24 November and 22 December 1998. On 7 January 1999, one of the goats at Kraaipan showed signs of *Oestrus ovis* infection, indicated by a mucoid nasal discharge. The animal showed difficulty in breathing through the nose and was treated with rafoxanide [Nasalcur™, Hoechst Roussel Vet (now Intervet), 7.5 mg kg^{-1}]. In January 1999, 22 of

Table 3.2.

Two-way frequency table of haematocrit by FAMACHA© with haematocrit cut-off of 18% (or 19%) and FAMACHA© scores 4 and 5 (or 3, 4 and 5) considered positive test results

		Anaemia	
		Present Ht[a] <18% (Ht <19%)	Absent Ht ≥18% (Ht ≥19%)
FAMACHA© score	Positive 4,5 (3,4,5)	True positive (TP)	False positive (FP)
	Negative 1,2,3 (1,2)	False negative (FN)	True negative (TN)

[a]Haematocrit.

Sensitivity = TP/(TP+FN) x 100 Predictive value of a negative = TN/(FN+TN) x 100

Specificity = TN/(FP+TN) x 100 Predictive value of a positive = TP/(TP+FP) x 100

the goats at Site 1, Impendle, and 25 of the goats at Site 2 were treated with niclosamide [Ex-a-lint™, Hoechst Roussel Vet (now Intervet), 50 mg kg^{-1}] for cestodes.

All the animals utilised for the faecal egg count reduction (FECR) tests (see 3.2.3 below) had not been included in the sampling groups mentioned above, except for one of the goats at Kraaipan. All the goats had no permanent incisors, except for three of the goats at Site 1, Impendle, two at Site 2, Impendle, and 10 at Kraaipan, which goats had two to four permanent incisors. None of the animals had been treated with an anthelmintic effective against nematodes within 12 weeks of the start of the FECR tests at Rust de Winter and Kraaipan, and within 16 weeks at Impendle.

3.2.2. Evaluation of the FAMACHA© clinical assay

Two data sets for 1998/1999 and 1999/2000, respectively, were created from all the FAMACHA© scores and haematocrit values obtained from the goats at Rust de Winter and the representative sample sets at Impendle and Kraaipan. Two-way frequency tables of haematocrit by FAMACHA© were drawn up, with FAMACHA© values four and five (or three, four and five) considered positive for anaemic animals and FAMACHA© values one, two and three (or one and two) considered negative test results, respectively (Table 3.2). Haematocrit was used as the gold

standard by which anaemia was measured and two cut-off values for anaemia were assigned (less than 18% and less than 19%, respectively). In establishing the properties of a test, cut-off values are assigned to define the level of a test result that is needed to make or reject a diagnosis, in this case a diagnosis of anaemia (Smith, 1995). Sensitivity, specificity, predictive value of a negative and predictive value of a positive were calculated for the data. Smith (1995) defines sensitivity as the proportion of infected or diseased individuals with a positive test, or in the case of the FAMACHA© clinical assay, the proportion of anaemic animals correctly identified as anaemic. Test specificity is defined as the proportion of disease-free individuals that test negative, or the proportion of non-anaemic animals that are categorised as such. In the case of the FAMACHA© method, predictive value of a negative is the probability that an animal is not anaemic when the test result is negative for anaemia and vice versa for the predictive value of a positive. The sensitivity and specificity were tested statistically by means of Fisher's Exact Test for a two-by-two contingency table. The authors chose to maximise the sensitivity and specificity of the FAMACHA© method in goats when the average of the sensitivity and specificity attains its highest value. This was calculated by the following equation for the present data:

(sensitivity + specificity)/2 … (1)

In order to determine the percentage of animals treated in each case (FAMACHA© cut-off of four as opposed to FAMACHA© cut-off of three), the following calculation was also applied to the data:

(true positive + false positive)/ total number of animals x 100 … (2)

3.2.3. Faecal egg count reduction tests

The efficacies of the anthelmintics used in the trial were assessed by means of the FECR test (Coles et al., 1992; Presidente, 1985; Van Schalkwyk et al., 1995), which uses the reduction in egg counts following anthelmintic treatment as an indication of anthelmintic efficacy. In each FECR test, the faeces remaining after the egg counts had been done were cultured as follows for L_3

recovery: for the initial date per test all the faeces were pooled together (pre-treatment culture), while the faeces for the second date were pooled separately per group (post-treatment cultures). The proportions of L_3 were applied to the strongyle egg counts to estimate the relative contribution of each genus (Coles et al., 1992; Presidente, 1985). In the World Association for the Advancement of Veterinary Parasitology (WAAVP) method for the detection of anthelmintic resistance (Coles et al., 1992), the arithmetic mean of the treatment and control groups at 10 to 14 days after treatment are utilised to calculate the percentage reduction of faecal egg counts and the upper and lower 95% confidence intervals. Resistance is determined to be present if the percentage reduction is less than 95% and the lower confidence interval is less than 90%. If only one of the conditions is met, resistance is only suspected. In the method of Presidente (1985), the geometric or arithmetic means of faecal egg counts both on the day of treatment and 10 to 14 days thereafter are used in the calculation of the percentage reduction. Geometric means were used in the current calculations. Resistance has been said to occur when the percentage reduction is less than 80% in goats (Kettle et al., 1983). Reduced efficacy of anthelmintics in goats may be the result of a faster metabolism of the drugs in this species. Hence the lower value is used for goats when no difference between the dose for sheep and goats is indicated. The WAAVP method is considered to be a more conservative measure of anthelmintic efficacy (Coles et al., 1992). Focus has been placed on this method to allow for comparison between data of different authors, but the method of Presidente was included for completeness' sake.

Table 3.3 gives the mean faecal egg counts of the animals at the visit dates immediately prior to the dates on which the anthelmintic treatments for the FECR tests were carried out. The animals were ranked according to these faecal egg counts from lowest to highest. The animals were then divided into groups of two or three, depending on whether one or two anthelmintics were being tested. Each individual within each group was then randomly assigned to a treatment or control group (Table 3.3), with the help of a table of random numbers. The initial and post-treatment dates of the FECR tests and the sizes of the groups included in the tests are also recorded in Table 3.3.

Table 3.3

Faecal egg count reduction tests : details of groups and results

Location	Mean FEC (interval)[a]	Treatment date of FECR test (interval)[b]	Anthelmintic (dosage)[c]	Control group			Treatment group			FEC reduction (W.A. A.V.P.[f])	95% CI[g] (W.A. A.V.P.)	FEC reduction (Presidente[h])
				Mean FEC[d]		*n*	Mean FEC		*n*			
				Pre[e]	Post[e]		Pre	Post				
Rust de Winter	2171 (14)	24 Feb 2000 (13)	Levamisole (7.5 mg kg^{-1})	1791	2438	8	1133	340	10	86%	0%, 98%	99%
Site 1, Impendle	3775 (28)	14 Mar 2000 (15)	Ivermectin (0.2 mg kg^{-1})	5909	4211	5	6824	33	8	99%	97%, 100%	100%
Site 2, Impendle	3147 (28)	14 Mar 2000 (15)	Ivermectin (0.2 mg kg^{-1})	4074	4857	7	3222	13	8	100%	98%, 100%	100%
Kraaipan	778 (28)	29 Feb 2000 (10)	Levamisole (7.5 mg kg^{-1})	-[i]	573	9	-	4	11	99%	98%, 100%	-
			Rafoxanide (7.5 mg kg^{-1})	-	573	9	-	12	11	98%	93%, 99%	-

[a]Mean faecal strongyle egg counts in eggs per gram of faeces at last visit before FECR test (interval in days between last visit and FECR test).
[b]Treatment date of faecal egg count reduction test (interval in days between pre- and post-treatment collection of faecal samples).
[c]All anthelmintics were administered *per os*.
[d]Mean faecal *Haemonchus* egg counts in eggs per gram of faeces.
[e]Pre: Pre-treatment; Post: Post-treatment.
[f]Coles et al., 1992.
[g]Confidence intervals.
[h]Presidente, 1985.
[i]No larval culture results available and hence proportions of *Haemonchus* eggs in mean FEC could not be determined.

Table 3.4.1

Comparison of results for application of the FAMACHA© system in goats during 1998/1999 and 1999/2000

	Sensitivity (%)		Specificity (%)		Predictive value (%)			
					Negative		Positive	
	98/99	99/00	98/99	99/00	98/99	99/00	98/99	99/00
FAMACHA© values 4 and 5 considered positive test results								
Ht cut-off <18%[a]	31.1	35.6	91.2	90.2	95.6	94.9	17.7	21.3
Ht cut-off <19%	23.0	28.4	91.3	90.4	91.9	91.6	21.5	25.3
FAMACHA© values 3, 4 and 5 considered positive test results								
Ht cut-off <18%	80.0	86.7	54.3	50.7	97.8	98.1	9.6	11.6
Ht cut-off <19%	75.7	85.1	55.3	52.0	95.6	96.8	14.9	17.0

[a] Haematocrit cut-off value used.

3.3. Results

3.3.1. Evaluation of the FAMACHA© clinical assay

Periods of heavier worm infection during which time *Haemonchus* was the predominant species occurred from December/January to March/April at Rust de Winter; from December to March/April at Impendle; and from November/December to February/March at Kraaipan (Chapter 4). For this reason, the data used to draw up the two-way frequency tables included paired values of haematocrit and FAMACHA© score from November 1998 to April 1999 and from November 1999 to April 2000. A total number of 787 pairs of haematocrit and FAMACHA© values were included for 1998/1999 and 648 for 1999/2000. The sensitivities, specificities and predictive values for the two different levels of positive FAMACHA© scores (four and five, and three, four and five, respectively), for the two different haematocrit cut-off values for anaemia (less than 18% and less than 19%, respectively) and for the two summer seasons (1998/1999 and 1999/2000, respectively) are given in Table 3.4.1. The results for the application of equations (1) and (2) and for Fisher's Exact Test are recorded in Table 3.4.2.

Table 3.4.2

Comparison of results for application of the FAMACHA© system in goats during 1998/1999 and 1999/2000 (continued)

	(Sensitivity + specificity)/2[a]		Goats treated (%)[b]		P values for Fisher's Exact Test	
	98/99	99/00	98/99	99/00	98/99	99/00
FAMACHA© values 4 and 5 considered positive test results						
Ht cut-off <18% [a]	61.2	62.9	10.0	11.6	4.5×10^{-5}	9.6×10^{-6}
Ht cut-off <19%	57.2	59.4	10.0	11.6	7.1×10^{-4}	5.3×10^{-5}
FAMACHA© values 3, 4 and 5 considered positive test results						
Ht cut-off <18%	67.2	68.7	47.6	51.9	8.6×10^{-6}	6.1×10^{-7}
Ht cut-off <19%	65.5	68.6	47.6	51.9	3.2×10^{-7}	3.8×10^{-9}

[a]Equation 1 (see text).
[b]Equation 2 (see text).

3.3.2. Faecal egg count reduction tests

Strongyloides spp., *Trichuris* spp. and other nematode eggs were detected in the faecal egg counts and *Haemonchus* spp., *Oesophagostomum* spp., *Teladorsagia/Trichostrongylus* spp. and *Strongyloides* spp. were recorded in the faecal cultures. When *Strongyloides* was excluded from the differential counts of the L_3 cultured from the faeces collected on the day of treatment, *Haemonchus* spp. predominated in each case (Table 3.5). In the cultures made for the second visit, *Haemonchus* spp. was the most prevalent genus in the controls. For these reasons, analyses of efficacy were applied only for *Haemonchus* spp. The calculation of anthelmintic efficacy according to the methods of Coles et al. (1992) (WAAVP method) and Presidente (1985) are given in Table 3.3. The results indicate that resistance was not found for any of the groups tested, except for the goats

at Rust de Winter where resistance to levamisole was detected. This is indicated in the WAAVP method by the fact that the percentage reduction is less than 95% and the lower confidence interval is less than 90%. Resistance was not detected by the method of Presidente.

Table 3.5

Percentage of *Haemonchus* spp. in larval cultures made from faecal samples taken pre- and post-treatment for faecal egg count reduction tests

Location	Anthelmintic	Pre-treatment percentage (n^a)	Post-treatment percentage (n^a)	
			Control group	Treatment group
Rust de Winter	Levamisole	75% (28)	100% (3)	0
Site 1, Impendle	Ivermectin	92% (97)	94% (100)	88% (99)
Site 2, Impendle	Ivermectin	98% (100)	100% (96)	100% (19)
Kraaipan	Levamisole	-[b]	82% (99)	2% (45)
	Rafoxanide	-[b]	82% (99)	6% (32)

[a]Number of larvae counted - *Strongyloides* spp. was initially included in the differential larval counts, but has been excluded here.
[b]No larval culture results available

3.4. Discussion

3.4.1. Evaluation of the FAMACHA© clinical assay

A cut-off haematocrit value for anaemia of less than 18% was assigned when the two-way frequency table was drawn initially. The reason behind assigning this cut-off value was that the range of haematocrit values subjectively determined for sheep for categories four and five were 13-17% and <13%, respectively (Table 3.6). For the period 1998/1999, the sensitivity of the FAMACHA© system to identify animals that are anaemic (i.e. with a haematocrit less than 18%) when only those animals falling into FAMACHA© categories four and five are treated was poor at 31,1%. The specificity of the method was, however, good at 91,2%. It was then hypothesized that considering FAMACHA© categories three, four and five as anaemic may render a better sensitivity. The sensitivity at a

Table 3.6

Relationship between FAMACHA© score and haematocrit range for sheep

FAMACHA© score	Approximate haematocrit[a]	Haematocrit range[b]	Recommendation with regard to dosing
1	35 %	≥28%	Do not dose
2	25 %	23-27%	Do not dose
3	20 %	18-22%	Dose if uncertain
4	15 %	13-17%	Dose
5	10 %	≤12%	Dose

[a]Van Wyk et al., 1997b.
[b]Van Wyk, 2000.

haematocrit cut-off of less than 18% increased from 31,1% to 80,0%. However, the specificity decreased from 91,2% to 54,3%.

Schalm's Veterinary Hematology gives a normal haematocrit range for goats as 19-38% (Jain, 1986). Using a cut-off value of 19% and where FAMACHA© categories four and five are considered anaemic, the sensitivity and specificity for the 1998/1999 data are 23,0% and 91,3%, respectively (Table 3.4.1). These values change to 75,7% and 55,3%, respectively, where categories three, four and five are considered anaemic. One notices that as the sensitivity increases, so the specificity decreases when one changes from a FAMACHA© cut-off of four to a cut-off of three. This is expected for any diagnostic test (Smith, 1995). Where FAMACHA© categories four and five are considered anaemic, Fisher's Exact Test shows that the sensitivity of the FAMACHA© system to detect animals with a haematocrit less than 19% is highly significant but not as significant as with a haematocrit less than 18%. This may indicate that the subjectively assigned range for categories four and five (less than 18%, Table 3.6) may be accurate. However, the sensitivities for a FAMACHA© cut-off of four do not compare favourably with those for a cut-off of three. Moreover, for the latter sensitivities, Fisher's Test also gives more highly significant P values.

On a day-to-day basis, one is more concerned with the sensitivity of the FAMACHA© chart since the consequences of not treating an anaemic animal (possible mortality) are more severe than treating an animal that did not actually require treatment. A greater sensitivity is preferred as one does not wish to miss animals that are anaemic. If the assumption is made that the most important cause of the anaemia is haemonchosis (Chapter 4), then by extension, one does not want to miss animals that are suffering from haemonchosis. In considering the first specific objective of the study, to use the FAMACHA© system to determine which animals require dosing with anthelmintics to prevent mortalities, one can say that the FAMACHA© clinical assay may be used with a sensitivity of between 76% and 85%, provided that animals in categories three, four and five are treated and one wishes to identify animals with haematocrits less than 19%. Using this grouping of animals into anaemic (categories three, four and five) and non-anaemic (categories one and two), the sensitivity and specificity are maximized (equation 1, Table 3.4.2). This applied to the data both for 1998/1999 and 1999/2000. It should be borne in mind that the FAMACHA© assay is a clinical estimate of anaemia and that these results reflect the scores of only a few workers including the first author and those trained by him. However, until work by other authors reveals otherwise, the cut-off for anaemia should be taken as FAMACHA© category three in the goat.

The results for 1999/2000 are similar to those of 1998/1999, although better results for the calculation for sensitivity were obtained overall for 1999/2000 than for 1998/1999. The specificity of the method decreased marginally in 1999/2000. The improvement in sensitivity of the FAMACHA© method during the second summer season may be an indication of greater proficiency in its use.

Resource-poor farming in South Africa has been neglected in terms of agricultural extension, i.e. those activities aimed at providing farmers with the results of research, innovative technology and other information that may assist them to improve their agricultural production. These aspects are currently being addressed, however. Recommendations that resource-poor farmers are receiving as a result reflect the convention of commercial agriculture, i.e. to treat all the small ruminants for worms every

Table 3.7

Anthelmintic treatment programme supplied to farmer at Rust de Winter

Month	Deworming aimed at
January	Nasal bot (*Oestrus ovis*), wireworm (*Haemonchus* spp.)
February	Nodular worm (*Oesophagostomum* spp.), wireworm
March	Wireworm, nasal bot, liver fluke (*Fasciola* spp.)
May	Nasal bot, liver fluke
July	Bankrupt worm (*Trichostrongylus* spp.), nasal bot
September	Wireworm, nasal bot, liver fluke
October	Wireworm, nasal bot
December	Wireworm, nasal bot
Lambs[a]	Milk tapeworm (*Moniezia* spp.)

[a]A recommendation was made that lambs be treated for milk tapeworm (*Moniezia* spp.), but no time of year was supplied.

time that it is deemed necessary for any individual animal in the flock or herd (personal observation, 1998/1999). An example of an anthelmintic treatment programme given to one of the farmers before the current trial was implemented is shown in Table 3.7. Horak et al. (1976) recommended four to five strategic anthelmintic treatments for roundworms per year. However, to achieve maximum production in lambs during their first year, these authors recommended that regular, short-interval treatment be applied, presumably even at four-weekly intervals, which was the regimen tested in the study. However, the emergence of anthelmintic resistance and investigations into its subsequent causes have shown that high frequency of anthelmintic treatment of all the animals in a herd within or close to the prepatent period of the worm in question increases the selection pressure for the development of anthelmintic resistance (Jackson, 1993). In a sense, then, the fact that extension to resource-poor farmers was not as effective as to the commercial sector may be a blessing in disguise since the

extension may now take anthelmintic resistance into account. It is critical that resource-poor farmers do not follow the same path of those commercial farmers who, with their frequent drenching of all the animals in a flock, have in many cases selected for severe anthelmintic resistance (Van Wyk et al., 1999). The question that arises, then, is whether the application of the FAMACHA© system represents a potential improvement on the recommendation to treat the whole herd at any one time, irrespective of whether an animal requires treatment or not (second objective of the study). Based on the fact that only between 47,6% and 51,9% of the animals are treated when goats categorised as three, four or five are dewormed (equation 2, Table 3.4.2), the authors believe that the use of the FAMACHA© method would reduce the selection pressure for anthelmintic resistance because a large proportion of the animals would be left untreated (Besier, 1997). For the resource-poor farmer wanting to control haemonchosis in goats, the use of the FAMACHA© system represents an attractive tool to be employed in implementing integrated internal parasite control on the farm.

3.4.2. Faecal egg count reduction tests

Coles et al. (1989) showed that levamisole at 7.92 mg kg^{-1} was only 83% effective against immature *Haemonchus contortus* in goats. At a dosage of 11,8 mg kg^{-1}, immature worms were still present in five of the seven goats treated. Coles et al. (1989) speculated that the reason for the poor efficacy is due to a faster metabolism of levamisole in goats than in sheep, in which species the drug is considered highly effective at 7.5 mg kg^{-1} (Coles, 1986). In the present study, the dosage recommended by the manufacturer (7.5 mg kg^{-1}) was used at all times, and no difference in dosage was indicated between sheep and goats. In a goat herd in Eastern Virginia, USA, Zajac and Gipson (2000) initially found that levamisole showed an efficacy of 0.75% at a dosage of 11.8 mg kg^{-1}. Use was discontinued and seven months later, efficacy appeared to have improved and was then 74%. The following year, the efficacy of levamisole was found to be 97% and use of the drug was reinstated. When the drug was again tested a year later, efficacy was found to be 73%. Grimshaw et al. (1996) have shown that

treatment with levamisole at 7.5 mg kg^{-1} was only 88% effective against the immature stages of a levamisole-susceptible, benzimidazole-resistant strain of *H. contortus* in lambs. It is conceivable that immature nematodes could develop into adults, the eggs of which are detected when samples are analysed more than 10 days after treatment. In the first two tests of Zajac and Gipson, samples were collected 5 days post-treatment. These authors speculated, then, that the efficacy of levamisole might have been underestimated during the first two tests. For the same reason, the efficacy of levamisole at Rust de Winter may have been underestimated since samples were collected 14 days after treatment. However, Martin et al. (1985) proposed that levamisole causes a temporary suppression in egg laying in *Ostertagia* spp., which egg production may resume only 10 days after treatment. If samples are taken before this time, the efficacy of the anthelmintic may be overestimated. It is the authors' opinion that levamisole resistance is present in the goats at Rust de Winter. Although the origin of the goats was not confirmed, it was understood that the animals were brought on to the farm in 1989 when the farmer settled there. During the trial period, the farmer also moved some of the goats between the farm and the farmer's actual place of residence, a township where communal grazing of animals is practised. It is possible that the animals were infected with resistant worm strains on the communal grazing, or more likely, on commercial farms before being bought at local auctions by the resource-poor farmer. Since a FECR test was not carried out at the start of the trial, it is not possible to determine whether the resistance status of the flock changed over the trial period. As such, it is difficult to speculate on the role that the treatment of animals according to the FAMACHA© system may or may not have played in the development of the resistance. An advantage of the clinical assay is the fact that a large proportion of the animals are left untreated and are able to contaminate the pasture with the eggs of, in particular, anthelmintic-susceptible worms. This practice contributes to the maintenance of a large proportion of anthelmintic-susceptible infective larvae on the pasture which is able to dilute the larvae of any resistant strains and in that way delay the emergence of anthelmintic resistance (Jackson, 1993). This would

suggest that the application of the FAMACHA© clinical assay might not have played a large role in the development of the resistance.

The FAMACHA© assay represents a potential revolution in internal parasite management and its validation for goats for use by resource-poor farmers, which this paper describes, is of particular relevance for South Africa and indeed for the tropics and sub-tropics of sub-Saharan Africa and elsewhere. In many of these areas, haemonchosis represents a major disease constraint on increasing production in small ruminants. The principle on which the FAMACHA© method is based, namely the treatment of only those animals that are susceptible to the disease, provides a method by which tremendous savings in the use of anthelmintics can be realised. Where appropriate, the method should be taught as part of an integrated approach to worm control within participatory rural extension programmes. Further testing of the FAMACHA© clinical assay should also be pursued in other goat farming systems.

Chapter 4

Incidence of *Haemonchus* spp. and effect on haematocrit and eye colour in goats farmed under resource-poor conditions in South Africa*

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Abstract

The diversity and predominance of nematode genera in goats of resource-poor farmers at Rust de Winter, Gauteng Province, Impendle, KwaZulu-Natal Province, and Kraaipan, North-West Province, South Africa, was determined by means of a longitudinal study of the nematode faecal

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egg counts and differential third-stage nematode larvae. The animals were bled for haematocrit determination and scored for pallor of ocular mucous membranes using the FAMACHA© method, an assay for clinical evaluation of anaemia caused by *Haemonchus* spp. Animals considered to be in danger of dying from anaemia caused by haemonchosis were selectively treated with an anthelmintic.

Lower haematocrit values were registered during periods of heavier *Haemonchus* infection, which periods occurred from December/January to March for Rust de Winter; from December to March/April for Impendle; and from November/December to February or April for Kraaipan. There was agreement too between the lower haematocrits and paler mucous membranes scored according to the FAMACHA© method. The use of this system may be recommended as part of an integrated approach to worm control in goats kept in the resource-poor areas studied.

Keywords: Eye colour chart; FAMACHA ©; Goats; Haematocrit; *Haemonchus* spp.

4.1. Introduction

Small ruminants play an important socio-economic role within traditional farming systems in many developing countries, including South Africa. However, in this country, little is known about the role of diseases within these animals, including helminthosis. Boomker and Horak have studied worms in goats raised under commercial farming conditions (Boomker et al., 1989; Horak et al., 1991) and Boomker in indigenous goats (Boomker et al., 1994). The epidemiology of helminths in sheep raised within the summer-rainfall area of South Africa, where the present study was conducted, has also been well-described (Horak, 1978; Horak and Louw, 1977). Nevertheless, little is known about the epidemiology of internal parasites and their effects on the production of small ruminants raised under resource-poor conditions.

Indiscriminate and overuse of anthelmintics has led to the development of severe anthelmintic resistance on commercial sheep farms and resistance has been reported from sheep raised under resource-poor conditions in South Africa (Van Wyk et al., 1999), but had not been reported from goats in South Africa until now (Chapter 3). An emphasis is currently being placed on assisting resource-poor farmers to improve the production of their livestock and in this respect the management of worms is seen to be a researchable constraint. The testing of worm control strategies in small ruminants, appropriate to the resource-poor farmer and sustainable in terms of maintaining anthelmintic efficacy, is needed.

The aim of this study was to determine the diversity and predominance of nematode genera in small ruminants in three resource-poor farming study areas. Emphasis was placed on determining the incidence of *Haemonchus* spp., in relation to evaluating the effect of the level of worm infection on the haematocrit and to determine the suitability of the production system and management for the introduction of the FAMACHA© system. This assay uses the comparison of the colour of the ocular mucous membranes (eye colour) of a sheep to a colour chart for the classification of the animal into one of five colour categories reflecting the range of anaemia from "A" (healthy) to "E" (severely anaemic) (Van Wyk et al., 1997b). Only those animals that are considered to be in danger of dying from *Haemonchus* infection (categories "D" and "E") are selectively treated. In the latest version of the chart (Bath, 2000), the letters, "A" to "E", have been substituted by the numbers, one to five, and hence this latter convention has been used in this paper.

4.2. Materials and methods

4.2.1. Study sites, animals and sampling

Three study sites were selected within the summer-rainfall area of South Africa: one near Rust de Winter, Gauteng Province, one in Impendle, KwaZulu-Natal Province, and one in Kraaipan, North-West Province (Fig. 1.2). A summary of the trial periods and frequencies of visits, breeds of

animals, sample sizes, and anthelmintics used is given in Table 2.1.1 The grazing practices, vegetation types, winter supplements and rainfall for each study site are given in Table 2.1.2. During the day, the goats at Rust de Winter browsed on the natural vegetation while the farmer at Site 2, Impendle, allowed his animals to graze communal pasture surrounding his homestead. At Site 1, Impendle, and at Kraaipan, the animals were grazed on communal pasture tended by a shepherd. The animals at all sites were penned in kraals at night.

At Rust de Winter, all the weaner and adult goats present at each visit were sampled/scored. For the two farmers at Impendle, a representative sample of the weaner and adult goats was selected based on the first animals brought into the crush at the first visit, and when available the same goats were sampled/scored throughout the trial period. Unfortunately, the initial sample sets for each of the two sites at Impendle had diminished in number by the end of the trial, owing to sales or slaughtering of animals and at least one death. At Kraaipan, a representative sample of the goat flock was chosen in a similar manner as for Impendle, but the animal numbers also started to dwindle and for this reason every 10^{th} animal brought into the crush in May 1999 was added to the sample group. This resulted in four goats being added to the representative sample group.

4.2.2. Diagnostic techniques

Faecal samples were collected at each visit from the animals at Rust de Winter and the representative sample sets at Impendle and Kraaipan (the "trial" animals). Additional samples were collected from April 1999 at Kraaipan and from May 1999 at Impendle to ensure that there would be sufficient faeces for a good yield of third-stage nematode larvae (L_3) when cultures were made (see below). The faecal samples were processed for nematode faecal egg count (FEC), following the method of Van Schalkwyk et al. (1995) and at a sensitivity of 100 eggs per gram of faeces (epg). *Strongyloides*, *Nematodirus* and *Trichuris* eggs were counted separately from the other nematode eggs, which are herein referred to as "strongyle" eggs.

Samples were screened for *Fasciola* eggs by means of the sedimentation method (Van Wyk et al., 1987) performed on samples pooled from ten goats selected at random from each site at each visit.

Faeces remaining after the FECs had been processed were cultured for third-stage nematode larvae (L_3) at a temperature of approximately 25°C until November 1999 when a new room was used and the temperature then adopted was approximately 26°C. The L_3 were identified using the keys of Van Wyk et al. (1997a) and Dunn (1978). Where possible, at least 50 L_3 were identified per culture. No attempt was made to differentiate *Teladorsagia* spp. from *Trichostrongylus* spp. The proportions of the strongyle L_3 were used to estimate the proportional FECs of the various strongyle worm genera. (The L_3 results are recorded in Appendix 3.)

The animals were bled and their haematocrits were determined using the microhaematocrit method, they were scored for body condition (Vatta et al., 2000), and they were inspected for ticks.

4.2.3. Scoring for level of anaemia

This has been described in detail in Chapter 3. In brief, at the scheduled visits, the first author or one of the assistants on the project scored each animal for level of anaemia using the FAMACHA© card. Occasionally, monitoring was done in-between scheduled visits by the farmer at Site 1, Impendle, and by the animal health technicians (AHTs) assisting with the project at Kraaipan. Only the animals that were considered to be pale, i.e. categories four and five, were treated with an anthelmintic. At times, animals scored as category three were erroneously treated by the AHTs at Kraaipan and the farmer at Site 2, Impendle, initially misunderstood the aim of the trial and treated all his goats sometime between 24 November and 22 December 1998. Between 23 November and 21 December 1999, ten goats were apparently treated by the shepherd of the farmer at Site 1, Impendle, but the animal identifications were not recorded.

4.3. Results

4.3.1. General

Figs. 4.1-4.4 illustrate the FECs and mean haematocrits for the study sites. Complete results for the L_3 cultures were not obtained during October 1998, the beginning of November 1998 and late July 1999 owing to problems in the laboratory. In these cases, the averages of the proportions for *Haemonchus* spp. and for the other nematode genera for the visit dates immediately prior to and following the dates of missing data were applied to the FECs. These results are indicated by dotted lines in the figures. Since *Haemonchus* predominated in many of the cultures, the graphs were drawn to reflect the mean *Haemonchus* FECs and the mean total FECs for the other genera. Mean *Strongyloides* FECs were less than 200 epg for all sites and all visits, while *Trichuris* and *Nematodirus* FECs were negligible, maximum individual counts never exceeding 200 epg.

In summary, L_3 of *Haemonchus* spp., *Teladorsagia /Trichostrongylus* spp., *Oesophagostomum* spp. and *Strongyloides* spp. were identified in the faecal cultures from each of the trial sites. Periods of heavier worm infection during which *Haemonchus* was the predominant species occurred from December/January to March at Rust de Winter; from December to March/April at Impendle; and from November/December to February or April at Kraaipan. During these periods, the mean haematocrits dropped.

Treatment of goats from November to April (period of heavier *Haemonchus* infection) is summarised in Table 4.1. Both the percentage of animals treated (FAMACHA© values four and five) and the percentage of animals scored within the lower FAMACHA© categories (three, four and five) are given in Table 4.1. (Full details of the numbers of animals scored in each FAMACHA© category over time are recorded in Appendix 4.) Except for Site 1, Impendle, relatively more goats were treated during the *Haemonchus* seasons (November to April) than in-between (May to October).

Fig. 4.1 : Strongyle faecal egg counts and haematocrits from goats at Rust de Winter

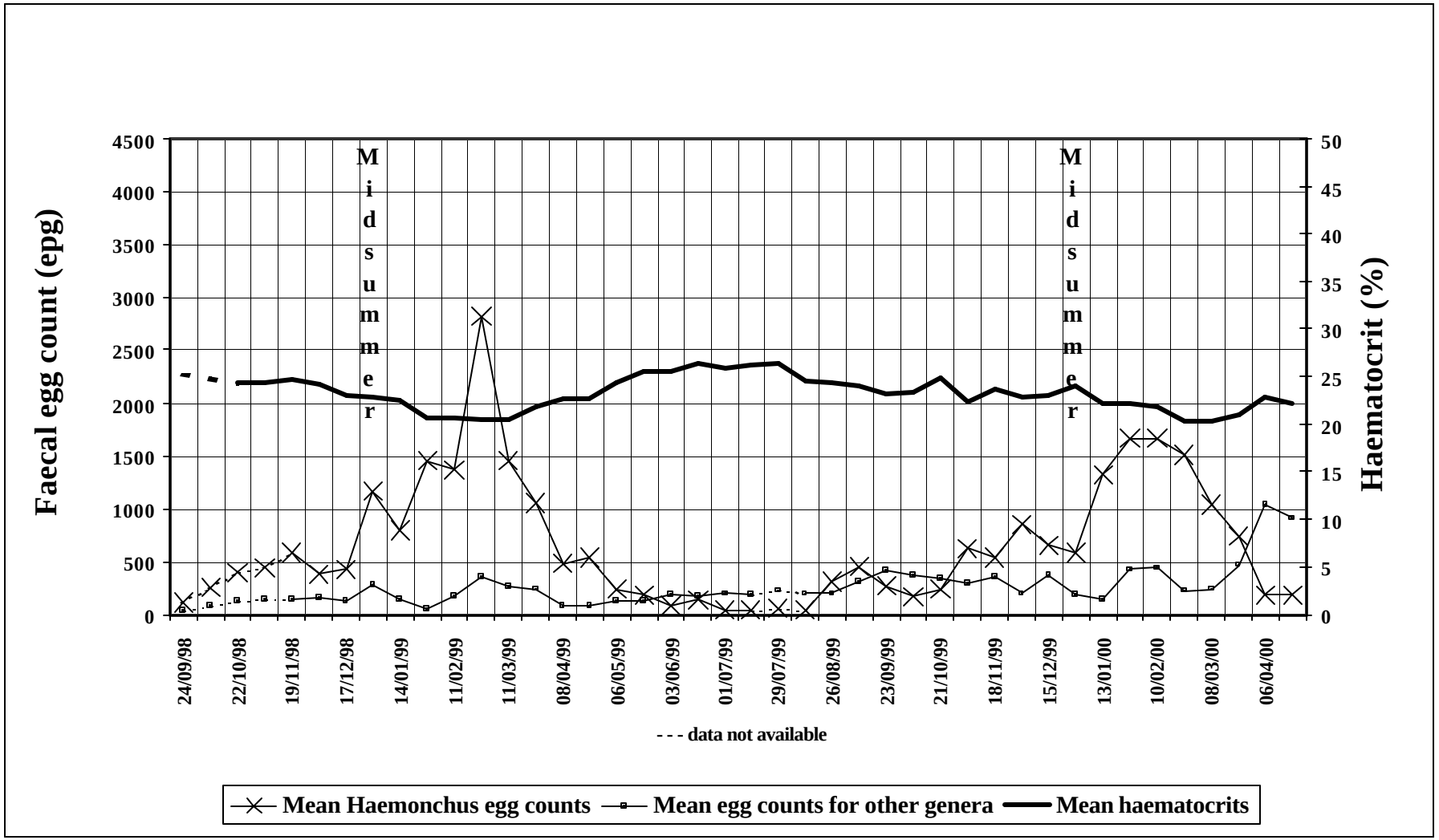

Fig. 4.2 : Strongyle faecal egg counts and haematocrits from goats at Site 1, Impendlle

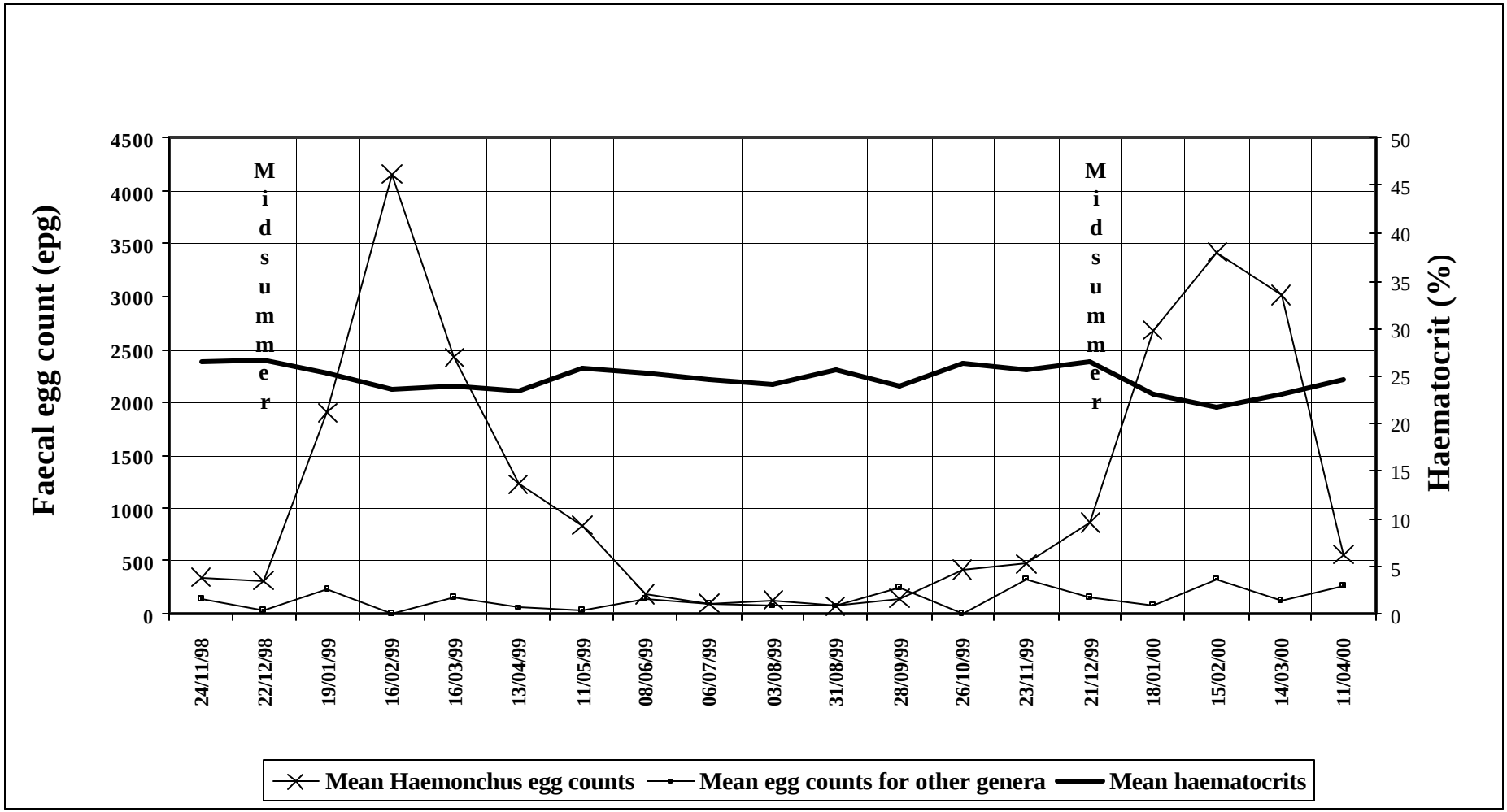

Fig. 4.3 : Strongyle faecal egg counts and haematocrits from goats at Site 2, Impendle

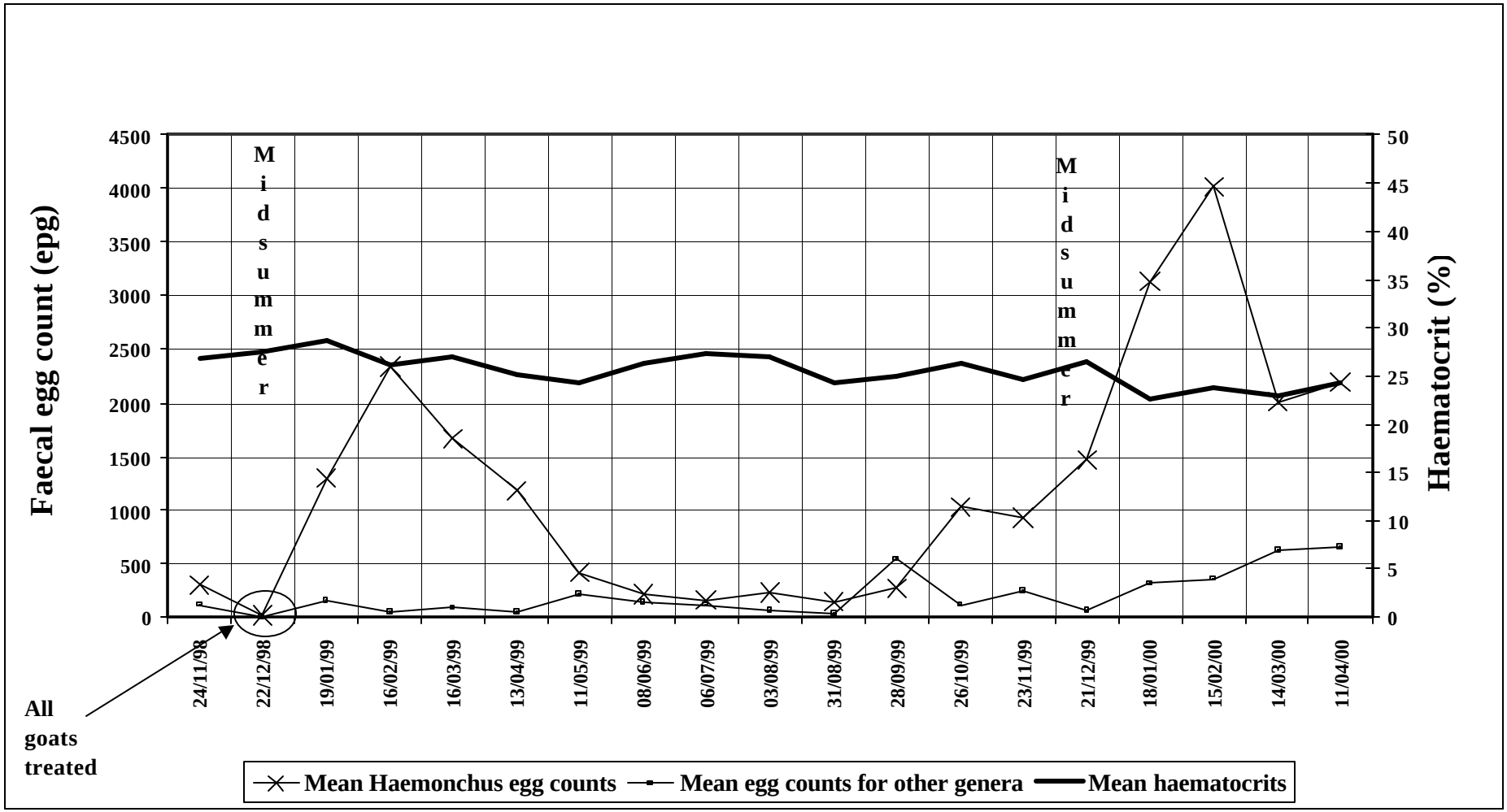

Fig. 4.4 : Strongyle faecal egg counts and haematocrits from goats at Kraaipan

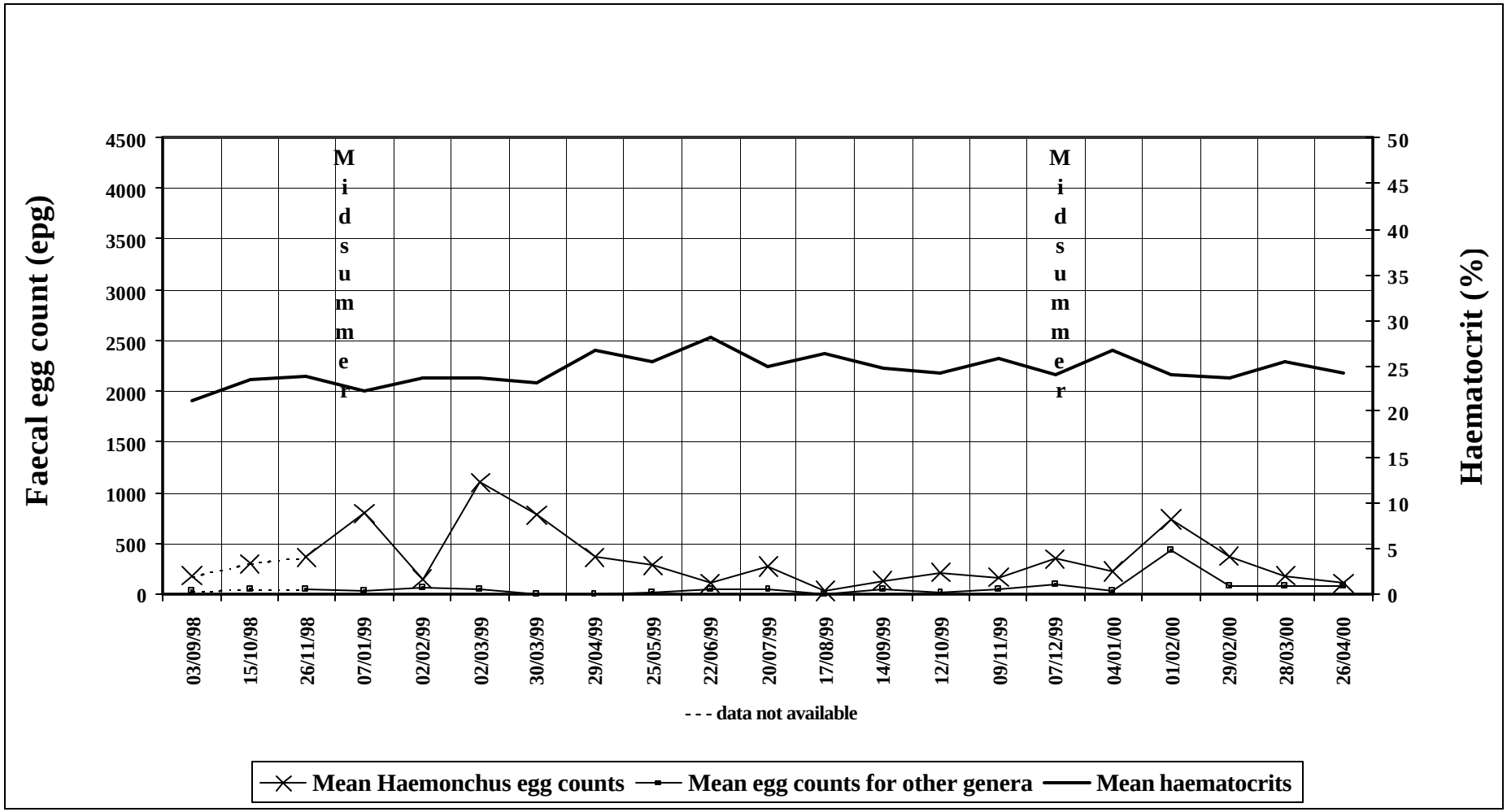

Table 4.1

Percentage of goats treated from November to April (*Haemonchus* season) and May to October

Location	Total examined	F© 4 and 5 treated (percentage)[a]	F© 3, 4 and 5 (percentage)[b]
Rust de Winter			
Nov 98 — Apr 99	434	9,4 %	47,2 %
Nov 99 — Apr 00	372	8,1 %	46,8 %
May — Oct 99	398	6,3 %	34,4 %
Site 1, Impendle			
Nov 98 — Apr 99	129	12,4 %	52,7 %
Nov 99 — Apr 00	83	19,3 %	55,4 %
May — Oct 99	141	14,9 %	58,2 %
Site 2, Impendle			
Nov 98 — Apr 99	134	8,2 %	48,5 %
Nov 99 — Apr 00	104	16,3 %	62,5 %
May — Oct 99	134	2,2 %	35,1 %
Kraaipan			
Nov 98 — Apr 99	121	11,6 %	43,0 %
Nov 99 — Apr 00	91	12,1 %	57,1 %
May — Oct 99	105	2,9 %	35,2 %

[a]FAMACHA© values 4 and 5 treated.
[b]FAMACHA© values 3, 4 and 5 treated (theoretical).

Tick burdens were not deemed heavy enough to be important contributors to anaemia in any of the study areas. *Fasciola* eggs were found in faecal samples of goats at Rust de Winter and Site 2, Impendle, in low numbers (range: 2-4 epg, when positive) and incidence (Chapter 6). Periods of poorer body condition, as an indication of nutritional status, occurred from August to early December 1999 at Rust de Winter and from June to September 1999 at Impendle (Chapter 5). These periods did not clearly correspond to any periods of lower haematocrit. The goats at Kraaipan did not show any clear seasonal trend in body condition.

4.3.2. Specifics of study sites

At Rust de Winter, FECs started to rise during late August 1999 (Fig. 4.1). Mean FECs for the other worm genera reached a level of over 1000 epg in early April 2000.

At both sites at Impendle, the animals showed a distinct peak in *Haemonchus* FECs during February (Figs. 4.2 and 4.3). In addition to the above genera, L_3 resembling those of *Gaigeria* or *Bunostomum* spp. were also found in the faecal cultures from Site 2, Impendle. These L_3 occurred with a low frequency in November and December 1998 and in January, May, August and September 1999.

The mean FECs at Kraaipan remained below 1200 epg (Fig. 4.4) throughout the period of the investigation. The drops in haematocrit during the periods of heavier worm infection were less noticeable than those recorded for the sites discussed above.

4.4. Discussion

4.4.1. General

In common with the results of Boomker et al. (1994) in goats at Roedtan (in the Northern Province, approximately 80 km in a direct line from Rust de Winter), this study has demonstrated a seasonal distribution of *Haemonchus* spp. in the summer months. Similarly, Horak (1978) and Horak and Louw (1977) reported greatest numbers of *Haemonchus contortus* in sheep from January to May or June on dryland and irrigated pastures, respectively. Tick burdens, worm infections and inadequate nutrition were considered as differential diagnoses of anaemia. Of these, the most important of the possible causes of anaemia in the animals studied was *Haemonchus* spp. The high *Haemonchus* FECs during the summers account for the drops in haematocrit seen during these periods. These periods of lower haematocrit were reflected clinically by the fact that more animals were scored as having paler mucous membranes during the periods of heavier worm infection (November to April) than during the winter period (May to October). As discussed in Chapter 3, a sensitivity of 76% to 85% was established for the FAMACHA© method in goats provided categories three, four and five are treated. This means

that the system may be used to identify correctly 76% to 85% of those animals in need of treatment with an anthelmintic. Since this implies that some animals in need of treatment would be missed, the use of the FAMACHA© system in isolation to other worm management strategies cannot be recommended.

4.4.2. Specifics of study sites

An increase in worm infection in spring is known as a "spring rise" (Gordon, 1973) and is thought to arise from the resumption of development of larvae retarded in the fourth-stage during the cooler months of the year (Michel, 1974). Hence, the rise in FECs during late August 1999 at Rust de Winter may be attributed to this phenomenon, since no rain fell during June to August 1999 (South African Weather Bureau). While Horak and Louw (1977) and Horak (1978) showed retarded larval development in sheep in their studies, Boomker et al. (1994) suggest that arrested development is not significant in indigenous goats at Roedtan. On the other hand, lactating animals show a periparturient relaxation of resistance (PPRR) which also manifests as a rise in worm infection (Gordon, 1973). Since many of the female goats kidded in August and September 1999, the rise in FECs is perhaps also partially attributable to this phenomenon in the female animals. The mean haematocrits are seen to decrease concurrently with the increase in FECs, which would seem to agree with the findings by Dorny et al. (1995). These workers reported a significant drop in haematocrit and a significant rise in FECs (predominantly *Haemonchus contortus* infection) during the periparturient period in two sheep flocks and one of two goat herds kept under the traditional husbandry systems of peninsular Malaysia. In the second goat herd, the changes were similar, though not significant.

Fewer of the goats at Site 1, Impendle, were treated during the periods of heavier *Haemonchus* infection than during the other months. It is possible that the number of treatments given at scheduled visits was reduced because of treatment by the farmer or his shepherd in-between the scheduled visits. In order to confirm the identity of the L_3 resembling those of *Gaigeria* or *Bunostomum*, animals from

Site 2, Impendle, or an animal experimentally infected with L_3 recovered from faeces collected at the site would need to be necropsied for worm recovery.

At Kraaipan, nematodes assume greater importance only during the periods of higher FECs from November/December to February or April, when *Haemonchus* predominated. Treatment by AHTs of animals in-between scheduled visits may have reduced the number of treatments given at these visits.

4.5. Conclusion

There is agreement between the periods of heavier *Haemonchus* infection, lower haematocrits and paler mucous membranes scored according to the FAMACHA© method. As such the latter may be recommended as an appropriate intervention within the context of an integrated worm control programme for the areas studied.

Chapter 5

Effect of nematode burden as assessed by means of faecal egg counts on body condition in goats farmed under resource-poor conditions in South Africa

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Abstract

A longitudinal study was conducted of the nematode faecal egg counts (FECs) and body condition scores (BCS) of goats of resource-poor farmers at Rust de Winter, Gauteng Province, Impendle, KwaZulu-Natal Province, and Kraaipan, North-West Province, South Africa.

Periods of higher FECs occurred from December/January to March/April at Rust de Winter and at Impendle and from January to March at Kraaipan.

Seasonal variations in body condition were evident in the goats at Impendle with the animals showing lower BCS from June to September. The goats at Rust de Winter and at Kraaipan did not show clear seasonal variations, although the goats at Rust de Winter showed lower BCS from mid-July to early December. The BCS for Rust de Winter where the animals were grazed on a private farm were generally higher than those of the other sites, where communal grazing is practised.

Keywords: Body condition scores; Faecal nematode egg counts; Goats

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5.1. Introduction

Small ruminants play an important socio-economic role within traditional farming systems in many developing communities in Southern Africa. However, in many communal grazing areas, lack of sufficient and adequate pasture is one of the most important constraints to improving the production of ruminants, particularly during the dry season. Also, in these areas little is known about the effects of worms on the production of goats and the interaction between nutritional status and parasite burden.

The aim of the present study was to examine the relationship between worm burden as assessed by means of faecal egg counts on body condition in herds of goats owned by resource-poor farmers at three study sites within the summer rainfall area of South Africa. It forms part of a larger study in which the nematode faecal egg counts (FECs), haematocrits and ocular mucous membrane colour scores were also recorded. The present paper reports on the body condition scores (BCS) in relation to the FECs while the interactions between FECs, haematocrits and ocular mucous membrane colour scores have been discussed in Chapter 4.

5.2. Materials and methods

These have been extensively discussed in Chapters 2, 3 and 4. In brief, faecal samples were collected from the goats of a farmer near Rust de Winter, Gauteng Province, two farmers in Impendle, KwaZulu-Natal Province, and a farmer in Kraaipan, North-West Province, at fortnightly (Rust de Winter) to monthly intervals (other study sites) over a period of 18-20 months. The Rust de Winter site is a private farm, while the other farmers grazed their animals on communal pasture. At Rust de Winter, all the weaner and adult goats were sampled at each visit, while representative sample sets were identified at Impendle and Kraaipan. The goats were body condition scored according to the chart depicted in Fig. 2.1, which method agrees with that of Williams (1990). The faecal samples were analysed for FEC following the method of Van Schalkwyk et al. (1995).

5.3. Results

Figs. 5.1-5.4 depict the mean strongyle FECs and BCS over the study period. Calavas et al. (1998) indicate that the reproducibility of body condition scoring is poor, i.e. scoring performed by different operators on the same animals at the same time is poorly comparable between persons. Thus, only those BCS recorded by the first author are included.

Periods of higher FECs occurred from December/January to March/April at Rust de Winter and at Impendle and from January to March at Kraaipan. Results of identification of third-stage nematode larvae (L_3) cultured from the faeces remaining after FECs had been carried out indicated that for the most part of the trial *Haemonchus* was the predominant genus at all three study sites (Chapter 4, Appendix 2).

Fig. 5.1 : Strongyle faecal egg counts and body condition scores for goats at Rust de Winter

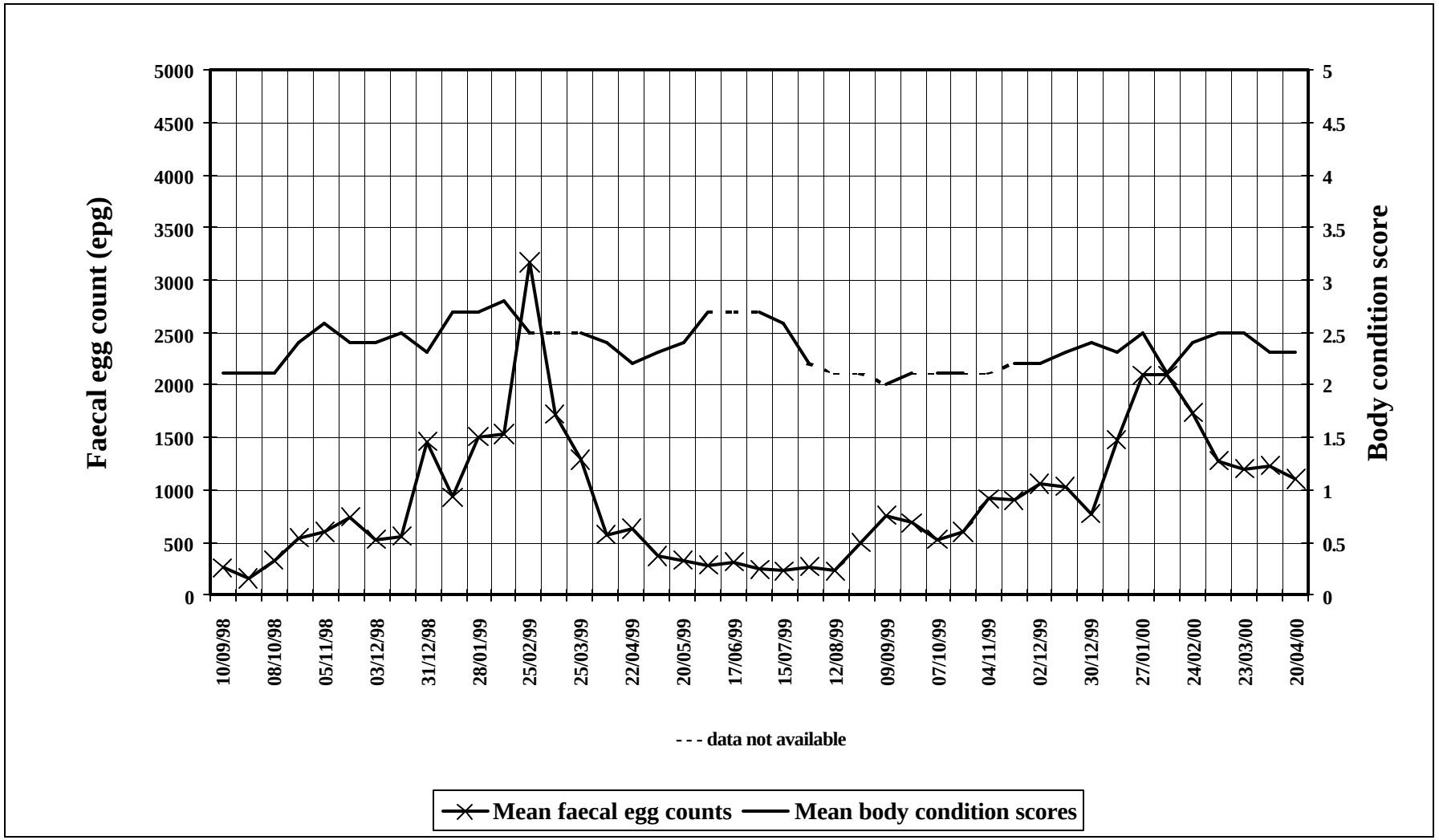


Fig. 5.2 : Strongyle faecal egg counts and body condition scores for goats at Site 1, Impendle

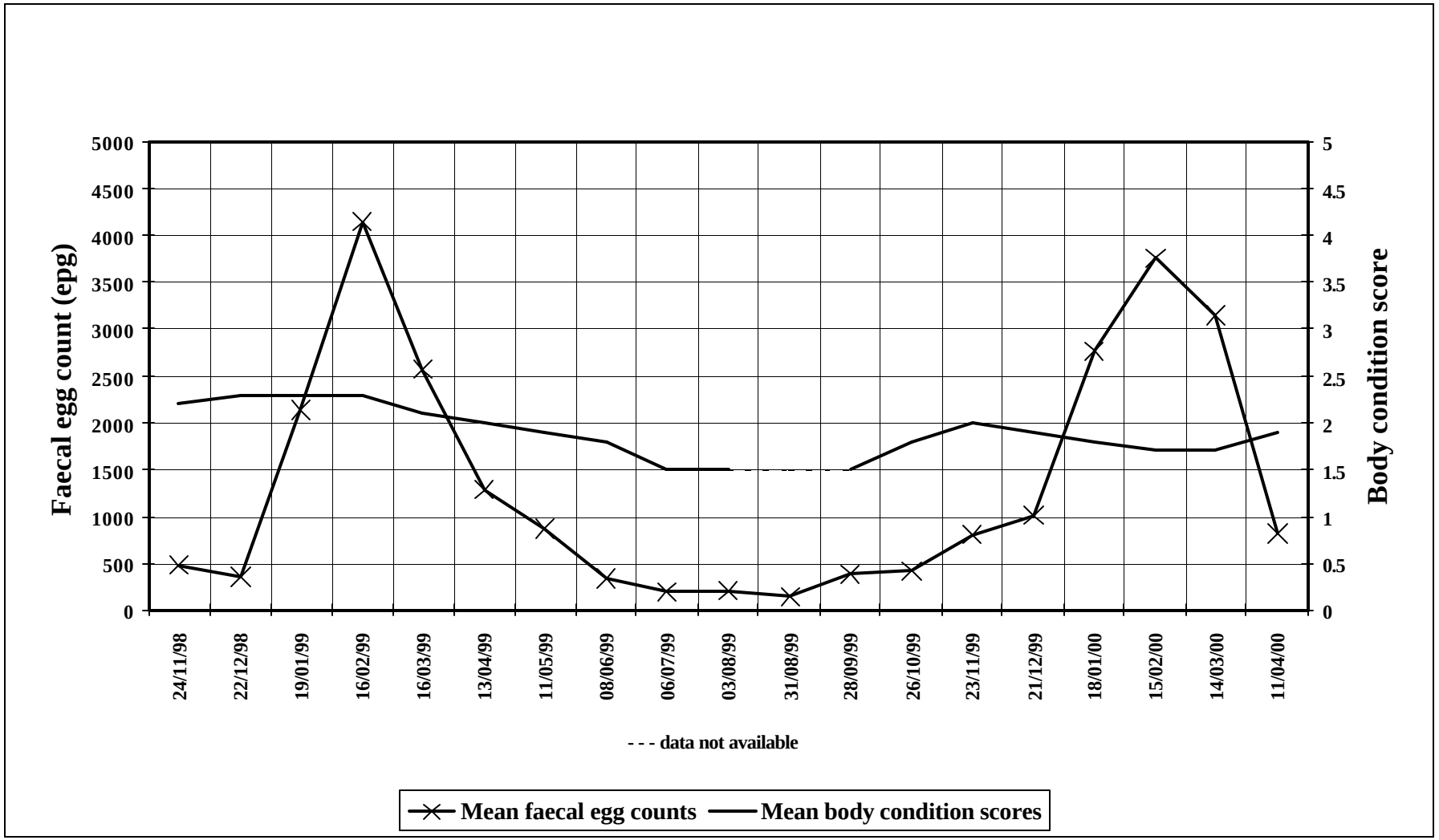


Fig. 5.3 : Strongyle faecal egg counts and body condition scores for goats at Site 2, Impendle

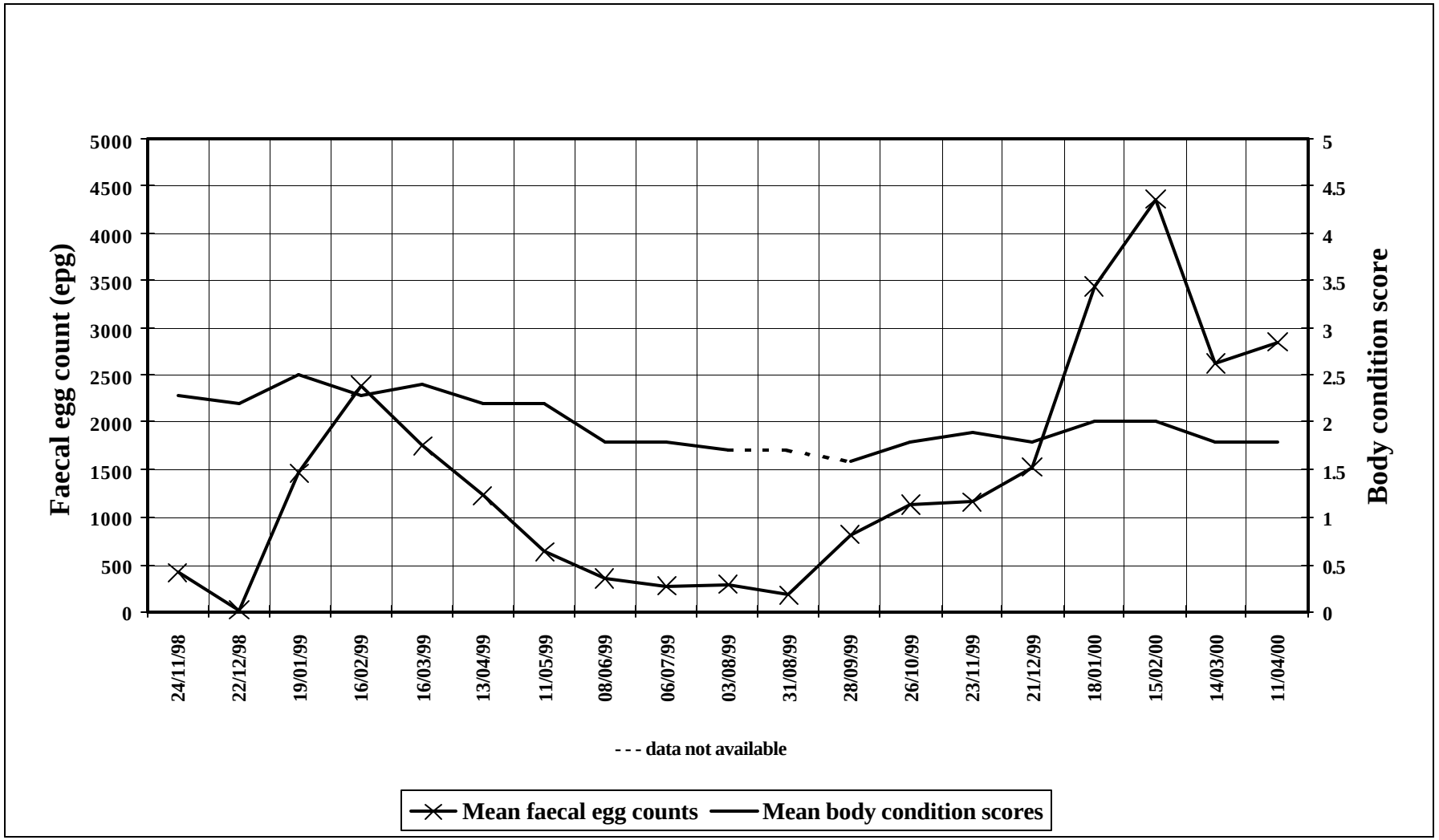
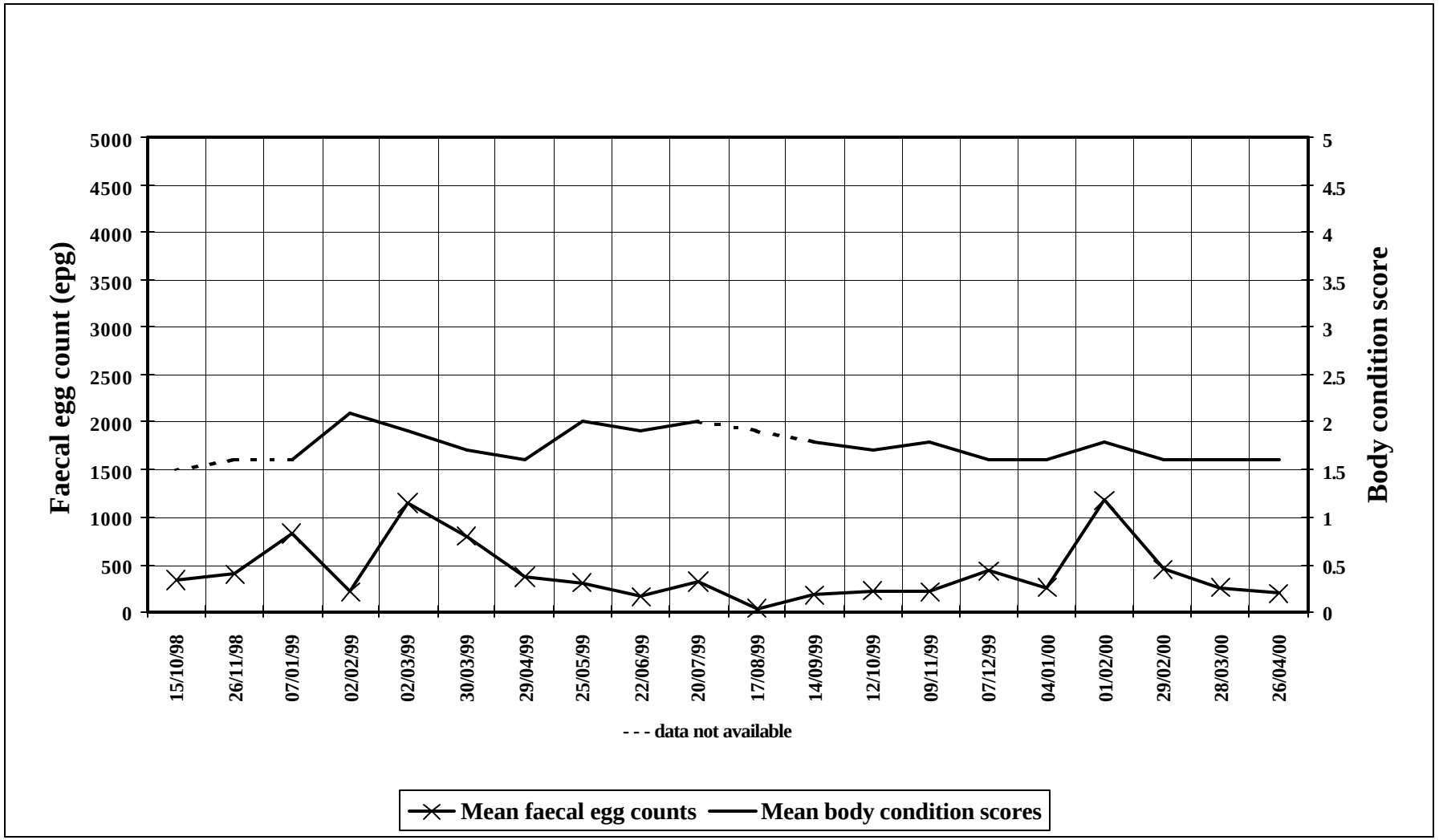


Fig. 5.4 : Strongyle faecal egg counts and body condition scores for goats at Kraaipan



Mean BCS for the goats at Rust de Winter varied from 2.0 to 2.8 over the trial period (Fig. 5.1). From the middle of July 1999 the animals dropped in condition to a level which persisted until the scores increased again in late December 1999. However, the goats did not show clear seasonal variations in BCS.

Mean BCS for the goats at Site 1, Impendle, ranged between 1.5 and 2.3, and never rose above 2.6 (Fig. 5.2). Higher scores were seen during October to April while the BCS were lower during June to September. Mean BCS for the goats at Site 2, Impendle (Fig. 5.3) showed a similar pattern to that of Site 1 but the general trend is that the goats of Site 2 were in better condition than those of Site 1.

The BCS for the goats at Kraaipan varied between 1.5 and 2.1 (Fig. 5.4). In contrast with the other sites, body condition was maintained from June to December 1999.

5.4. Discussion

Although FECs are generally considered inaccurate indicators of worm burden, they are nevertheless often used for this purpose, particularly where necropsy for worm recovery is not feasible or practical, as in the present study. Gastrointestinal nematode infection is known to have detrimental effects on production in small ruminants, and body condition scores were used in the current study to monitor changes in fat reserves. Horak and Louw (1977) and Horak (1978) have shown that there may be a delay of several weeks between maximum contamination of pasture with *Haemonchus* eggs and maximum larval availability. In effect, maximum worm burdens may only occur during April and May (when FECs are lower) and these worms may consist predominantly of fourth-stage larvae. Nevertheless, the adult egg-laying worm population is that which is responsible for the mortality owing to anaemia in haemonchosis and it is this blood-sucking population that is of greatest concern. Hoste and Chartier (1993) have shown that a significant decrease in BCS occurred in goats experimentally infected with *Haemonchus contortus* and *Trichostrongylus colubriformis* compared with controls. This

decrease occurred concomitant to decreases in haematocrit and red blood cell count. As has been shown in Chapter 4, the greatest effects on the haematocrit occur during and, in some cases, slightly after the periods of heaviest egg output. One would expect, therefore, that the greatest effect on body condition would also occur during the times of heaviest egg laying. However, in the present study, trends towards a decrease in BCS are not evident during this time of heaviest egg-laying capacity.

Body condition is an indication of nutritional status, with poorer scores corresponding to poorer nutritional intake and/or greater metabolic need, the latter occurring, for example, during pregnancy and lactation. It is probable that the effects of worm burden are masked during the summer months because of sufficient browse being available. When the condition of the veld deteriorated in the dry winter months, poorer BCS were noted during mid-July to early December at Rust de Winter and during June to September at Impendle. Chronic infection with *Haemonchus* may however also have contributed to decreases in BCS (Allonby and Urquhart, 1975).

At Rust de Winter, many of the female goats kidded during the late winter/early spring of 1999 (August and September 1999), which would have placed additional strain on body fat reserves. Parturition and lactation would have contributed greatly to the drop in body condition during the period of August to early December 1999.

Two differences in management may explain the differences in BCS of the two farmers of Impendle. Firstly, it was observed that the farmer at Site 2 did supply some supplemental feed in the form of whole maize kernels, but the frequency and quantity supplied throughout the trial could not be obtained. The farmer at Site 1 also supplied some supplemental feeding in the winter in the form of poor quality *Eragrostis* spp. hay. However, supplementation with maize would be more nutritious than with poor quality hay. Secondly, the farmer at Site 1 employed a shepherd to take the animals to pasture on the hills surrounding the village. The other farmer, however, allowed the animals to graze pasture surrounding his homestead. The latter animals would, therefore, use less energy than the former

animals, which would expend more body reserves in walking to the pasture, and may have had relatively less time per day to graze.

At Kraaipan, the goats were able to utilise the dried leaves of shrubs during the winter period. This would have allowed them to maintain their body condition during the dry season better than the goats at Impendle where fewer shrubs were available.

The BCS for the goats at the other sites were in general lower than those of the goats at Rust de Winter, where mean condition scores did not drop below 2.0. This may result from the fact that there is less forage available at Impendle and Kraaipan where communal grazing is practised than at Rust de Winter where the goats are not overstocked.

The effect of worm burden on the body condition is not resolved by the current study, however the results do indicate that supplementation of the animals with additional sources of protein and energy is recommended, particularly during the times of lower body condition cited. Such supplementation would probably also assist the animals to overcome partially the detrimental effects of worms during the winter period. Supplementation of indigenous Tuli cattle in Zimbabwe with cottonseed meal between July and October (i.e. from the end of the dry period into the beginning of the rainy season) improved the liveweight gains of the animals above anthelmintic-treated and untreated controls in the presence of subclinical gastrointestinal nematode parasitism (Magaya et al., 2000).

Chapter 6

Trematode infection of goats farmed under resource-poor conditions in South Africa

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Abstract

A longitudinal study was conducted of the pooled trematode faecal egg counts (FECs) of samples collected from goats of resource-poor farmers at Rust de Winter, Gauteng Province, Impendle, KwaZulu-Natal Province, and Kraaipan, North-West Province. The amphistome FECs followed a seasonal pattern, with an increase in the counts during the warmer months of the year (September to April). The study seems to indicate a different pattern of infection in goats raised under resource-poor conditions in South Africa from that on commercial farms, where outbreaks of clinical paramphistomosis occur during autumn and winter.

Keywords: Amphistome; Faecal trematode egg counts; *Fasciola* spp.; Small ruminants

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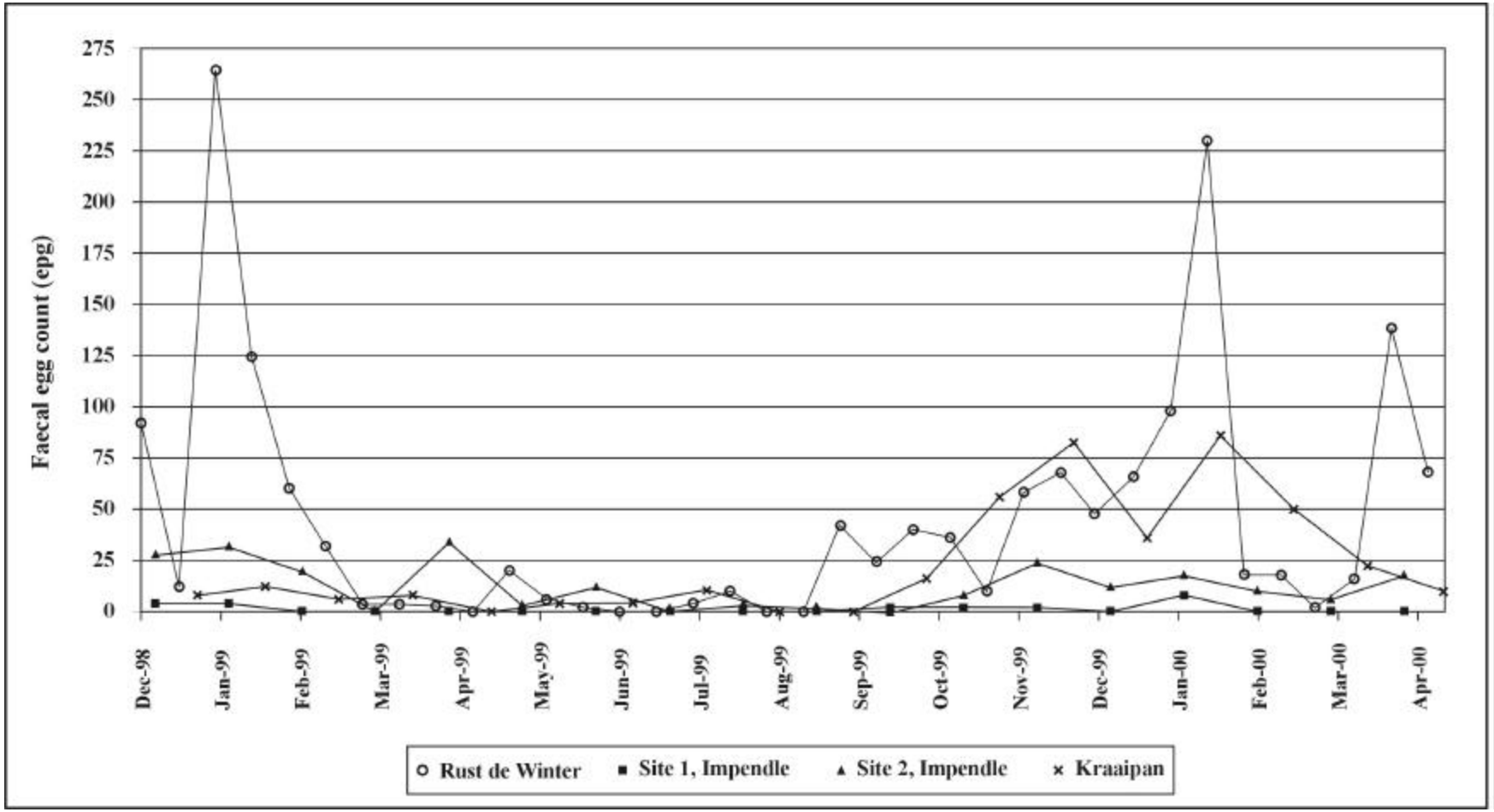
As part of a larger study to examine the nematode faecal egg counts (FECs), haematocrits, ocular mucous membrane (eye) colour scores and body condition scores (BCS) of goats owned by resource-poor farmers, a longitudinal study of the trematode FECs was conducted at the same time. Faecal samples were collected from September 1998 to April 2000 at three study sites within the summer rainfall area of South Africa at fortnightly (Rust de Winter, Gauteng Province) or monthly (Sites 1 and 2, Impendle, KwaZulu-Natal Province and Kraaipan, North-West Province) intervals. The results of the nematode FECs, haematocrits, BCS and eye colour scores for the goats are recorded in Chapters 4 and 5 while further details of the trial are recorded in Chapter 3.

Samples were screened for trematode eggs by means of the sedimentation method (Van Wyk et al., 1987) which was modified for pooled samples as follows. Half a gram of faeces (1g for the sheep at Rust de Winter) was weighed off from each of 10 faecal samples (five faecal samples for the sheep at Rust de Winter) randomly selected from those collected at each visit to a site. The faeces were pooled and softened and/or homogenized with an electric mixer (IKA® - Labortechnik, Janke and Kunkel, N.T. Laboratory Supplies, Johannesburg) in water. The faeces were then sieved through a 150µm sieve (United wire test sieve, Nigel, South Africa or equivalent) into a 38µm sieve (Labotec test sieve, Johannesburg, South Africa or equivalent), using water sprayed from a nozzle at high pressure. The remaining sediment was washed into a two or three litre glass jar. This was filled with water and allowed to stand for at least 15 minutes. The supernatant was then decanted and the sediment washed by filling up the jar again. This process was repeated approximately three times until the resulting supernatant was clear. Thereafter the sediment was poured into a measuring cylinder, made up to 200ml with water and mixed well by blowing air through the suspension with a pipette. Twenty millilitres of this suspension were examined in a perspex container (70mm x 70mm, E. Krecek, South Africa) under a stereomicroscope for trematode eggs.

Fig. 6.1 : Pooled amphistome faecal egg counts for goats at Rust de Winter, Sites 1 and 2, Impendle, and Kraaipan



The number of eggs per gram of faeces was calculated as follows :

$$\text{FEC (in epg)} = \frac{\text{Number of eggs present}}{\text{Mass of faeces}}$$

$$= \frac{\text{Number of eggs counted x 10}}{10 \text{ x } ½}$$

$$= \text{Number of eggs counted x 2}$$

The results of these analyses for the three study sites are recorded in Fig 6.1. The pooled amphistome FECs followed a seasonal pattern at all three study sites, with an increase in the counts during the warmer months of the year (September to April). This is especially evident for the goats at Rust de Winter. In contrast, the amphistome FECs for the goats at Impendle did not rise higher than 8 eggs per gram of faeces (epg) for Site 1 and 34 epg for Site 2. The infection levels at Kraaipan were also low during the first summer of the study but were higher from October 1999 to March 2000.

Fasciola eggs were recorded at levels of 2 and 4 epg in the goats at Rust de Winter and Site 2, Impendle, in August and January 1999, respectively. All other samples examined were negative for *Fasciola* eggs.

Reinecke (1983) reports that cattle and sheep on commercial farms are grazed on higher lying fallow lands in the summer-rainfall period. During this time, conditions on the lands are suitable for the survival of the intermediate snail hosts and they become heavily infected with *Calicophoron* (*Paramphistomum*) eggs. Prior to the winter, however, the snails migrate to areas around dams and marshes where they contaminate the surrounding vegetation with metacercariae. In the late summer, cattle and sheep are moved to the lower lying marshy areas to allow the fallow lands to be planted with wheat. Conditions on the pasture dry out and the animals seek out the better grazing surrounding the wetlands and become heavily infected with *Calicophoron* spp. Outbreaks of clinical paramphistomosis then occur in the autumn and winter.

Given that adult flukes start to pass eggs 69 days after goats have been infected with metacercariae (Horak, 1971), the animals in the present study were probably infected from July to February. Since it is the immature stages that are pathogenic (Horak and Clark, 1963), outbreaks of clinical amphistomosis may occur two to four weeks after infection (Horak, 1971), which in the present study would then have occurred during the late winter, spring and into summer. No signs of a copious, watery, foetid diarrhoea characteristic of amphistomosis (Horak and Clark, 1963) were noted in any of the study animals, however.

The present study seems to indicate a different pattern of infection in goats raised under resource-poor conditions in South Africa from that on commercial farms. Management practices differ markedly between the two farming systems, one difference being that the goats in the resource-poor areas have access to the same grazing throughout the year. This may allow for a more natural cycle of infection to develop in the resource-poor set-up than on the commercial farms where, as discussed above, during autumn and winter animals may be forced to graze camps in which water sources occur which are heavily infested with snails shedding metacercariae.

The low counts and incidence indicate that *Fasciola* spp. was not an important parasite in the animals in this study.

Chapter 7

Haemonchus spp. in sheep farmed under resource-poor conditions in South Africa - effect on haematocrit, eye colour and body condition

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Abstract

A longitudinal study was conducted of the differential faecal egg counts, haematocrits and body condition scores (BCS) of sheep of resource-poor farmers at Rust de Winter, Gauteng Province, and Kraaipan, North-West Province. The animals were scored for level of anaemia using the FAMACHA© method, an assay for the clinical evaluation of anaemia caused by *Haemonchus* spp. Animals considered to be in danger of dying from anaemia caused by haemonchosis were selectively treated with an anthelmintic.

Lower haematocrit values were registered during periods of heavier *Haemonchus* infection, which periods occurred from October to March for the sheep at Rust de Winter and from September/October to February or April for the sheep at Kraaipan.

Seasonal variations in body condition were clearly evident in the sheep at Kraaipan but not at Rust de Winter. The animals at Kraaipan showed lower BCS from July to December. The BCS for Rust de Winter where the animals were grazed on a private farm were generally higher than those of Kraaipan, where communal grazing is practised.

Although there was agreement between the higher *Haemonchus* egg counts, lower haematocrits and increased incidence of anaemic eye colour scores, very few animals were treated.

Keywords: Body condition; Eye colour; FAMACHA©; Haematocrit; *Haemonchus*; Sheep

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7.1. Introduction

Although worms have been studied in sheep raised under commercial farming conditions (Horak, 1978; Horak and Louw, 1977) and some work has been done in indigenous goats (Boomker et al., 1994), little is known of the effects of worms on production in sheep raised by resource-poor farmers. The objectives of the current study were the same as those described for the goats in Chapters 3-6. In brief, the aim was to evaluate the effect of *Haemonchus* infection on haematocrit, ocular mucous membrane (eye) colour and body condition score in sheep farmed under resource-poor conditions.

7.2. Materials and methods

7.2.1. Study sites, animals and sampling

Two study sites situated within the summer rainfall area of South Africa were selected: one near Rust de Winter, Gauteng Province, and one in Kraaipan, North-West Province. The sites correspond to those sites selected for the goats in Chapters 3 to 6. At Rust de Winter, all the weaner and adult sheep were sampled/scored at the visits. At Kraaipan, a representative sample of the sheep flock was selected based on the first animals brought into the crush at the first visit, and when available the same goats were sampled/scored throughout the trial period. Unfortunately, the initial sample set started to dwindle in number and for this reason every 10^{th} sheep brought into the crush in May 1999 was added to the sample group. This resulted in four sheep being added to the representative sample groups. A summary of the trial periods and frequencies of visits; breeds of animals; sample sizes; anthelmintics used; grazing practices; vegetation types; winter supplementation; and rainfall is given in Tables 2.1.1 and 2.1.2.

During the day, the sheep at Rust de Winter grazed on the natural vegetation, but from May 1999, they were grazing separately in an enclosed paddock of fallow land. At Kraaipan, the sheep were grazed on communal pasture tended by a shepherd. The animals at both sites were penned in kraals at night.

A faecal egg count reduction (FECR) test was carried out in the sheep at Kraaipan towards the end of the trial. Except for one sheep, the animals utilised for this purpose had not been included in the sampling group mentioned above. Four of the sheep included in the test had two to six permanent incisors while the rest had deciduous incisors only. None of the animals had been treated with an anthelmintic effective against nematodes within 12 weeks of the start of the FECR test.

7.2.2. Diagnostic techniques

Faecal samples were collected at each visit from the sheep at Rust de Winter and the representative sample set at Kraaipan (the "trial" animals). Additional samples were collected from April 1999 onwards at Kraaipan to ensure that there would be sufficient faeces for a good yield of third-stage nematode larvae (L_3) when cultures were made (see below). The faecal samples were processed for nematode faecal egg count (FEC), following the method of Van Schalkwyk et al. (1995) and at a sensitivity of 100 eggs per gram of faeces (epg). *Strongyloides*, *Nematodirus* and *Trichuris* eggs were counted separately from the other nematode eggs, which are herein referred to as "strongyle" eggs (Order Strongylida — Molin, 1861).

Samples were screened for trematode eggs using the following method. Half a gram of faeces (1g for the sheep at Rust de Winter) was weighed off from each of 10 faecal samples (five faecal samples for the sheep at Rust de Winter) randomly selected from those collected at each visit to a site. The faeces were pooled and the sedimentation method as described by Van Wyk et al. (1987) was modified for pooled samples as described in Chapter 6. Ten-percent aliquots were examined for trematode eggs.

Faeces remaining after the FECs had been completed were cultured for third-stage nematode larvae (L_3) at a temperature of approximately 25°C until November 1999 when a new room was used and the temperature then adopted was approximately 26°C. Where possible, at least 50 L_3 were identified per culture, using the keys of Van Wyk et al. (1997a) and Dunn (1978). No attempt was made to differentiate *Teladorsagia* larvae from those of *Trichostrongylus* spp. The proportional FECs of the various strongyle worm genera were calculated using the proportions of the strongyle L_3.

The animals were bled and their haematocrits were determined using the microhaematocrit method. The strongyle FECs and haematocrits of the sheep (this chapter) and goats (Chapter 4) at Kraaipan were compared statistically. The details of this analysis are, however, recorded in Appendix 5.

The efficacies of the anthelmintics used in the sheep at Kraaipan were assessed by means of the FECR test using the method of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Coles et al., 1992), which uses the reduction in FECs following anthelmintic treatment as an indication of anthelmintic efficacy. The arithmetic mean of the treatment and control groups at 10 to 14 days after treatment are utilised to calculate the percentage reduction of FECs and the upper and lower 95% confidence intervals. If the percentage reduction is less than 95% and the lower confidence interval is less than 90% resistance is determined to be present. Resistance is only suspected if only one of the conditions is met. The faeces remaining after the FECs had been done were pooled separately per group and cultured for L_3 recovery (post-treatment cultures). The proportions of L_3 were applied to the strongyle FECs to estimate the relative contribution of each genus (Coles et al., 1992; Presidente, 1985). However, since *Haemonchus* was the predominant strongyle genus in the post-treatment culture for the control animals (82%, $n = 79$), the calculations were performed only for the *Haemonchus* egg counts.

Table 7.1 gives the mean FECs of the animals at the visit dates immediately prior to the dates on which the anthelmintic treatments for the FECR tests were carried out. The animals were ranked according to these FECs from lowest to highest. The animals were then divided into groups of three. Each individual within each group was then randomly assigned to a treatment or control group (Table 7.1), with the help of a table of random numbers. The treatment and post-treatment dates of the FECR tests and the sizes of the groups included in the tests are also recorded in Table 7.1.

The sheep were body condition scored on a scale of one (emaciated) to five (obese) and half scores were assigned where appropriate (Fig. 2.1).

7.2.3. Scoring for level of anaemia

At the scheduled visits, the author or one of the assistants on the project scored each animal

Table 7.1

Faecal egg count reduction test in sheep : details of groups and results

Location	Mean FEC (interval)[a]	Treatment date of FECR test (interval)[b]	Anthelmintic (oral dosage)	Control group		Treatment group		FEC reduction	95% Confidence Interval
				Mean Post-treatment FEC[c]	n	Mean Post-treatment FEC[c]	n		
Kraaipan	8230 (28)	29 Feb 2000 (10)	Levamisole (7.5 mg kg^{-1})	7844	6	0	8	100	Undefined
			Rafoxanide (7.5 mg kg^{-1})	7844	6	0	7	100	Undefined

[a]Mean faecal strongyle egg counts in eggs per gram of faeces at last visit before treatment date of FECR test (interval in days between last visit and FECR test).
[b]Treatment date of faecal egg count reduction test (interval in days between treatment date and date of post-treatment collection of faecal samples).
[c]Mean faecal *Haemonchus* egg counts in eggs per gram of faeces.

for level of anaemia using the FAMACHA© card (Van Wyk et al., 1997b). The author ensured that each assistant for whom scores were recorded had been adequately trained in the method. Excepting for the few visits that the author could not undertake, the scoring was always performed under his direct supervision. Occasionally, monitoring was done in-between scheduled visits by the animal health technicians (AHTs) assisting with the project at Kraaipan. However, these scores were not included in any of the analyses discussed in this paper. Only the animals that were considered to be pale, i.e. categories four and five, were treated with an anthelmintic. At times, animals scored as category three were erroneously treated by the AHTs at Kraaipan.

To promote farmer co-operation, animals that showed signs indicative of *Oestrus ovis* infection (profuse mucous nasal discharge and difficulty in breathing through the nose) were at times also treated with rafoxanide [Nasalcur™, Hoechst Roussel Vet (now Intervet), 7.5 mg kg^{-1}]. With respect to the trial animals, one to two sheep were treated on four occasions at Rust de Winter, and one to eight were treated on 12 occasions at Kraaipan.

7.3. Results

Figs. 7.1 and 7.2 depict the FECs, mean haematocrits and mean BCS for the two study sites. Third-stage larvae of *Haemonchus* spp., *Teladorsagia*/*Trichostrongylus* spp., *Oesophagostomum* spp. and *Strongyloides* spp. were identified in the faecal cultures from both trial sites (Appendix 3). However, since *Haemonchus* predominated in many of the cultures, the graphs of the FECs were drawn to reflect the mean *Haemonchus* FECs and the mean total FECs for the other strongyle genera. Maximum individual *Strongyloides* FECs never exceeded 200 epg and *Nematodirus* and *Trichuris* eggs were not found. Complete results for the L_3 cultures were not obtained on numerous occasions for the sheep at Rust de Winter mainly because of difficulties in obtaining sufficient faeces from the animals for FEC and culture. L_3 culture results were incomplete for the sheep at

Fig. 7.1 : Strongyle faecal egg counts, haematocrits and body condition scores from sheep at Rust de Winter

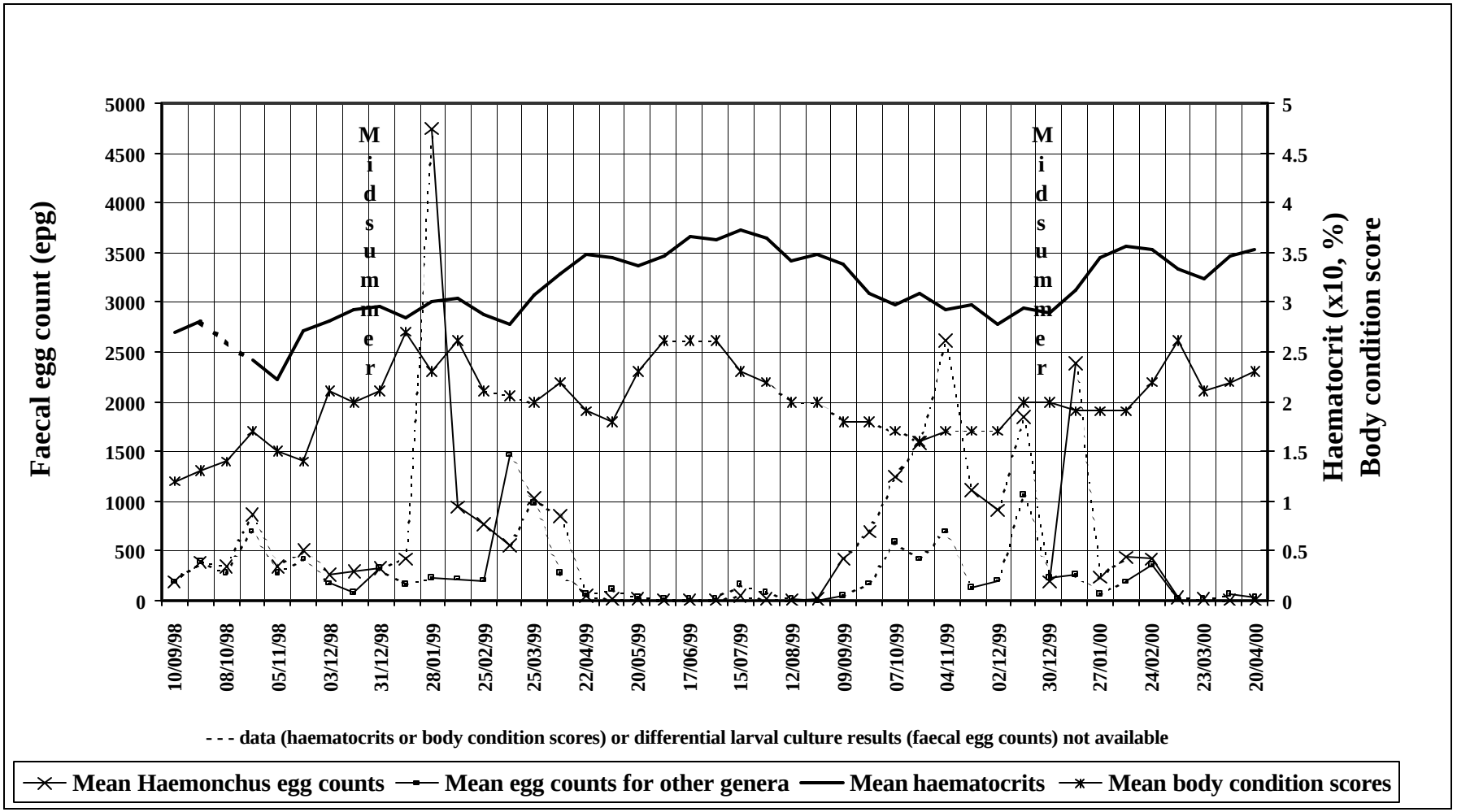
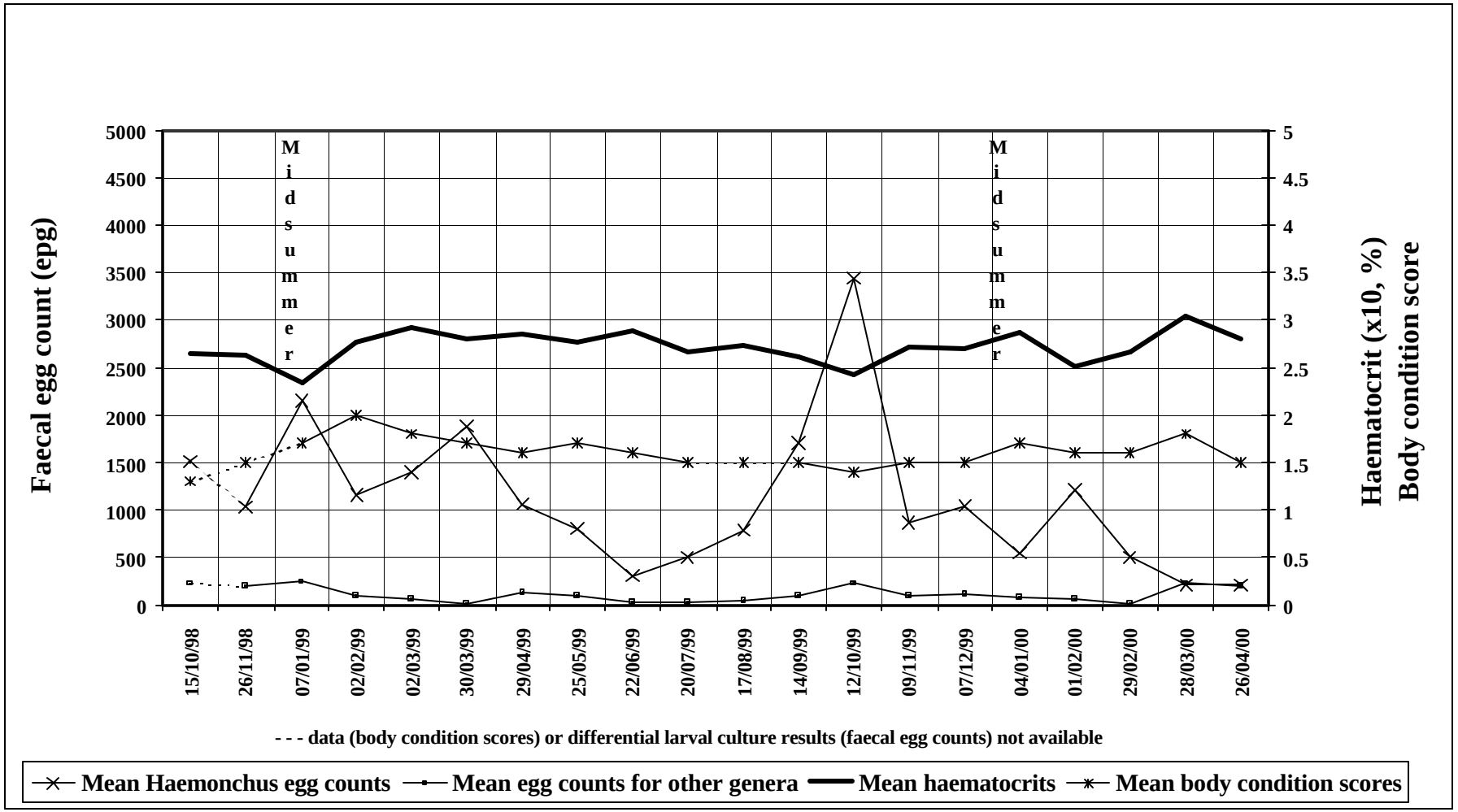


Fig. 7.2 : Strongyle faecal egg counts, haematocrits and body condition scores from sheep at Kraaipan



Kraaipan during October 1998 owing to problems in the laboratory. In these cases, the averages of the proportions for *Haemonchus* spp. and for the other nematode genera for the visit dates immediately prior to and following the dates of missing data were used to estimate the proportional FECs. These results are indicated by dotted lines in Figs 7.1 and 7.2.

A period of higher *Haemonchus* egg counts occurred from October to March at Rust de Winter (Fig. 7.1) and from September/October to February or April at Kraaipan (Fig. 7.2). The mean haematocrits were lower during the periods of heavier worm infection at Rust de Winter and during January and October 1999 and February 2000 at Kraaipan.

The pooled trematode FECs followed a seasonal pattern of amphistome infection in the sheep at Rust de Winter and Kraaipan, with an increase in the counts during the summer months of December/January to March/April. FECs were low, however, with a maximum count of 40 epg recorded in February 1999 at Rust de Winter and 54 epg at Kraaipan. A count of 30 epg was recorded during October 1999 at Kraaipan. All sheep samples examined were negative for *Fasciola* eggs.

Calavas et al. (1998) indicate that body condition scoring performed by different operators on the same animals at the same time is poorly comparable between persons. Thus only those scores recorded by the first author are depicted in Figs. 7.1 and 7.2. The BCS at Rust de Winter ranged between 1.2 and 2.7, but from the beginning of December 1998 onwards, the scores did not fall below 1.6. Although no clear seasonal pattern is evident in the BCS of the sheep at Rust de Winter (Fig. 7.1), the BCS were lower during August 1999 to mid-February 2000. The BCS at Kraaipan were higher during the summer months but lower during July to December 1999 (Fig. 7.2). Overall the BCS at Kraaipan remained poor, however, with scores ranging from 1.3 to 2.0.

Very few sheep were treated at Rust de Winter and Kraaipan during the trial (Table 7.2). Theoretically, considering FAMACHA© category three as anaemic in addition to categories four and five did not increase the total scores for anaemic sheep by a substantial degree. Although more animals were scored in FAMACHA© categories four and five (and three, four and five) during

Table 7.2

Percentage of sheep treated from October to March (*Haemonchus* season) and April to September

Location	Total examined	F© 4 and 5 treated[a]	F© 3, 4 and 5[b]
Rust de Winter			
Oct 98 - Mar 99	74	6,8%	21,6%
Oct 99 - Mar 00	65	1,5%	20,0%
Apr - Sep 99	65	0	7,7%
Kraaipan			
Oct 98 - Mar 99	130	0,8%	9,2%
Oct 99 - Mar 00	86	1,2%	17,4%
Apr - Sep 99	110	0	5,5%

[a]FAMACHA© values 4 and 5 treated.
[b]FAMACHA© values 3, 4 and 5 treated (theoretical).

October to March than during April to September, on most occasions the sheep were scored as non-anaemic, i.e. in categories one and two.

Resistance was not detected in the sheep at Kraaipan by the FECR test (Table 7.1).

7.4. Discussion

The high *Haemonchus* FECs during the summers account for the drops in haematocrit seen during these months. Trematodes do not appear to be important parasites in the sheep studied, although confinement of the sheep at Rust de Winter to the enclosed paddock of fallow land from May 1999 onwards would have limited exposure of the sheep to trematode infection.

The sheep at Rust de Winter had been bought not long before the start of the trial, and the poorer condition scores at the beginning of the trial probably reflect a poorer nutritional status at purchase, which improved when the animals started to graze. Rainfall measured at Rust de Winter was below average for June to November 1999 (South African Weather Bureau) and the condition of the grass in the fallow camp where the sheep were confined deteriorated markedly in the winter period as did the

communal grazing at Kraaipan. The sheep were not fed sufficient additional feed to maintain their body condition. At least two of the ewes at Rust de Winter lambed in the spring/summer of 1999/2000 while at the visit to Kraaipan in September 1999 it was observed that many of the ewes had lambed recently. Late pregnancy/parturition/lactation would have placed additional strain on body fat reserves. Higher egg counts were recorded in the Rust de Winter and Kraaipan sheep during October 1999 to January 2000, and in October 1999, respectively. Lack of sufficient grazing, the stresses of the periparturient period and the worms responsible for the higher egg counts probably all contributed to the lower BCS seen during August to mid-February and July to December at Rust de Winter and Kraaipan, respectively.

There is agreement between the higher *Haemonchus* egg counts, lower haematocrits and increased incidence of anaemic eye colour scores. Consequently, the application of the FAMACHA© system is suitable for the two study sites. Under supervision of adequately trained personnel, the FAMACHA© system has been shown to be an appropriate method of worm control in sheep within the context of an integrated worm control approach on commercial farms (Anonymous, 2000). Nevertheless, the numbers of sheep used in the current study were small and wider application of the FAMACHA© system under resource-poor conditions in sheep should be further investigated.

Chapter 8

Conclusion

This study has adapted the FAMACHA© clinical assay for use in goats of resource-poor farmers in the summer-rainfall area of South Africa. *Haemonchus* spp., the parasite targeted by the FAMACHA© system, was shown to be an important worm in these animals.

The FAMACHA© assay may be used with a sensitivity of between 76% and 85% in the goat, provided that animals in categories three, four and five are treated and one wishes to identify goats with haematocrits less than 19%. This means that between 76% to 85% of the animals that are anaemic are identified as such.

Based on the fact that only between 47,6% and 51,9% of the animals are treated when goats categorised as three, four or five are dewormed, the use of the FAMACHA© method would reduce the selection pressure for anthelmintic resistance because a large proportion of the animals would be left untreated. This assists in maintaining on the pasture a large proportion of anthelmintic-susceptible infective larvae derived from eggs passed in the faeces of untreated animals. The susceptible larvae dilute the larvae of any resistant strains and in that way delay the emergence of anthelmintic resistance (Jackson, 1993).

Given that the sensitivity of the FAMACHA© method in the goat is less than 100%, it is obvious that some animals in need of treatment would be missed. As such, the use of the FAMACHA© system alone in the absence of other worm managemental strategies cannot be recommended, but only within the context of an integrated worm control programme.

The recommendation that the FAMACHA© clinical assay be used in the goats studied is based on the fact that *Haemonchus* spp. was shown to have a seasonal distribution in the summer months. The worm was found to be the most important of the possible causes of anaemia in the animals studied. These periods of lower haematocrit were reflected clinically by the fact that more

Table 8.1

Countries in Africa in which *Haemonchus* spp. has been reported as a parasite of major importance in small ruminants and in which the possibility of introducing the FAMACHA© system exists (the list is incomplete)

Country	Period of importance of *Haemonchus* infection	Host species	Reference
Cameroon (Bamenda)	Most of year, particularly wet season (mid-March to November)	Sheep and goats	Ndamukong et al., 1987
Kenya (Naivasha)	August to October and June and July (also March and April when pasture condition is poor)	Sheep	Allonby and Urquhart, 1975
Kenya (Nairobi-Limuru-Ruiru)	Following rainfall (September to November and March to June), but pattern inconsistent in this study	Goats	Lutu, 1984
Nigeria (derived savannah of eastern Nigeria)	April to October (wet season, cropping season — animals confined under traditional husbandry system)	Sheep and goats	Fakae, 1990
Nigeria (northern)	May to October (wet season), particularly early wet season	Sheep and goats	Fabiyi, 1987
Sierra Leone (Freetown)	November to June (dry season)	Sheep and goats	Asanji and Williams, 1987
Sierra Leone (Freetown)	October to January (dry season)	Sheep and goats	Asanji, 1988
Tanzania (northern)	March to August (wet season)	Sheep and goats	Njau, 1987
Tanzania (southern)	December to April (wet season)	Goats	Connor et al., 1990
Zimbabwe (Marandellas)	November to March (warm, wet season)	Sheep	Grant, 1981
Zimbabwe (Mashonaland East)	October to May (wet season)	Goats	Kusina et al., 1999

goats were scored as having paler mucous membranes during the periods of heavier worm infection (November to April) than during the intervening winter period.

The numbers of sheep used in the current study were small and wider application of the FAMACHA© system under resource-poor conditions in sheep should be further investigated.

The FAMACHA© system may be further relevant for the tropics and sub-tropics of sub-Saharan Africa and elsewhere. In many of these areas (Table 8.1), haemonchosis represents a major disease constraint on increasing production in small ruminants. Where appropriate, the method should be taught as part of an integrated approach to worm control within participatory agricultural extension programmes. Further testing of the FAMACHA© clinical assay should also be pursued under these other epidemiological and farming conditions.

Appendix 1

Climatic data for study sites

Table A1.1

Weather stations from which climatic data for study sites was obtained

Study site	Rainfall	Temperature
Rust de Winter	Rust de Winter dam (10) [a]	Soshanguve (70)
Impendle	Donnybrook (35)	Emerald Dale (40)
Kraaipan	Kraaipan (study period)	Mafikeng (60)
	Mmabatho (30-year average) (60)	

[a]Approximate kilometres in a direct line from study site.

Fig. A1.1 : Climatic data for Rust de Winter

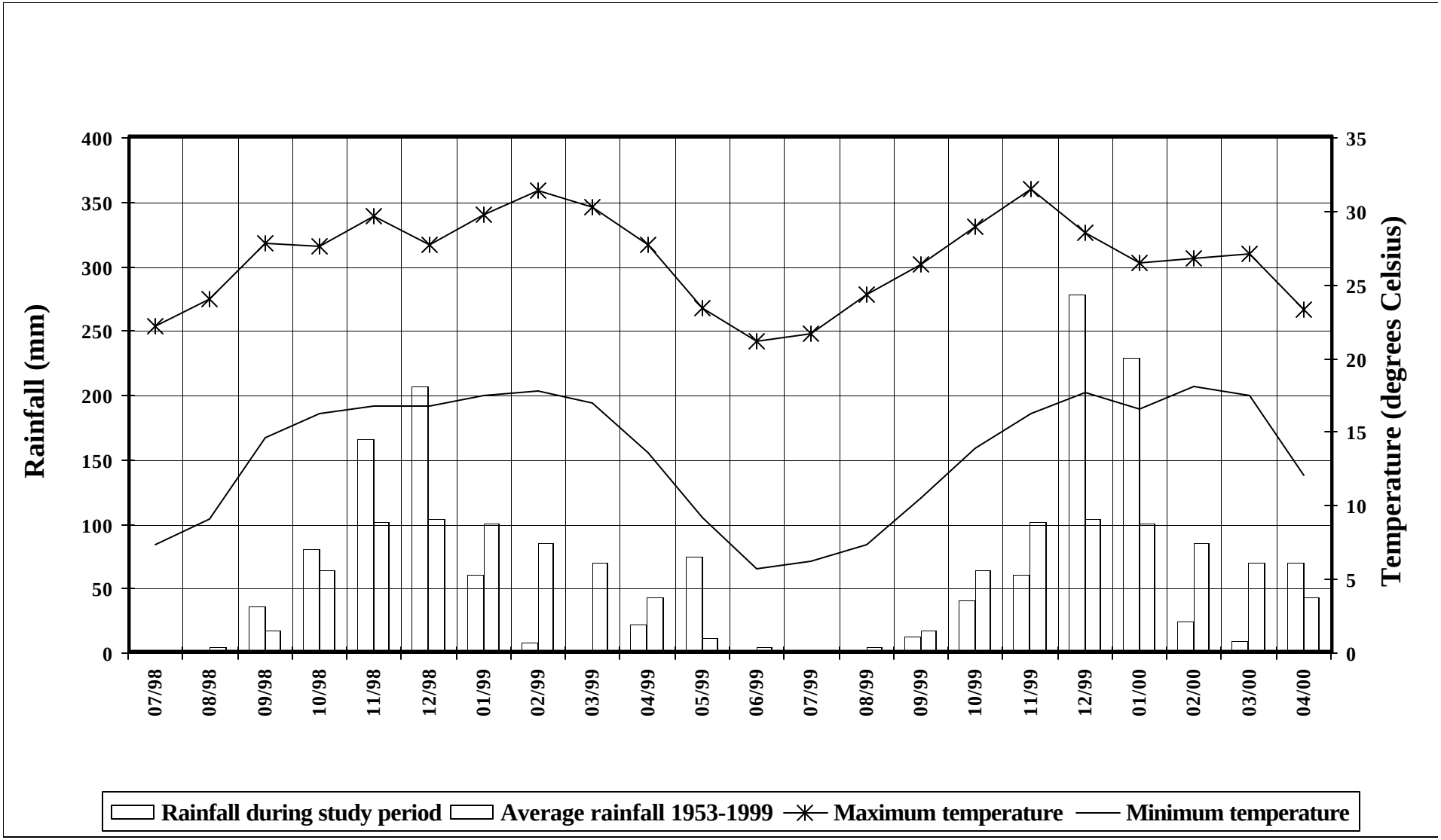


Fig. A1.2 : Climatic data for Impendle

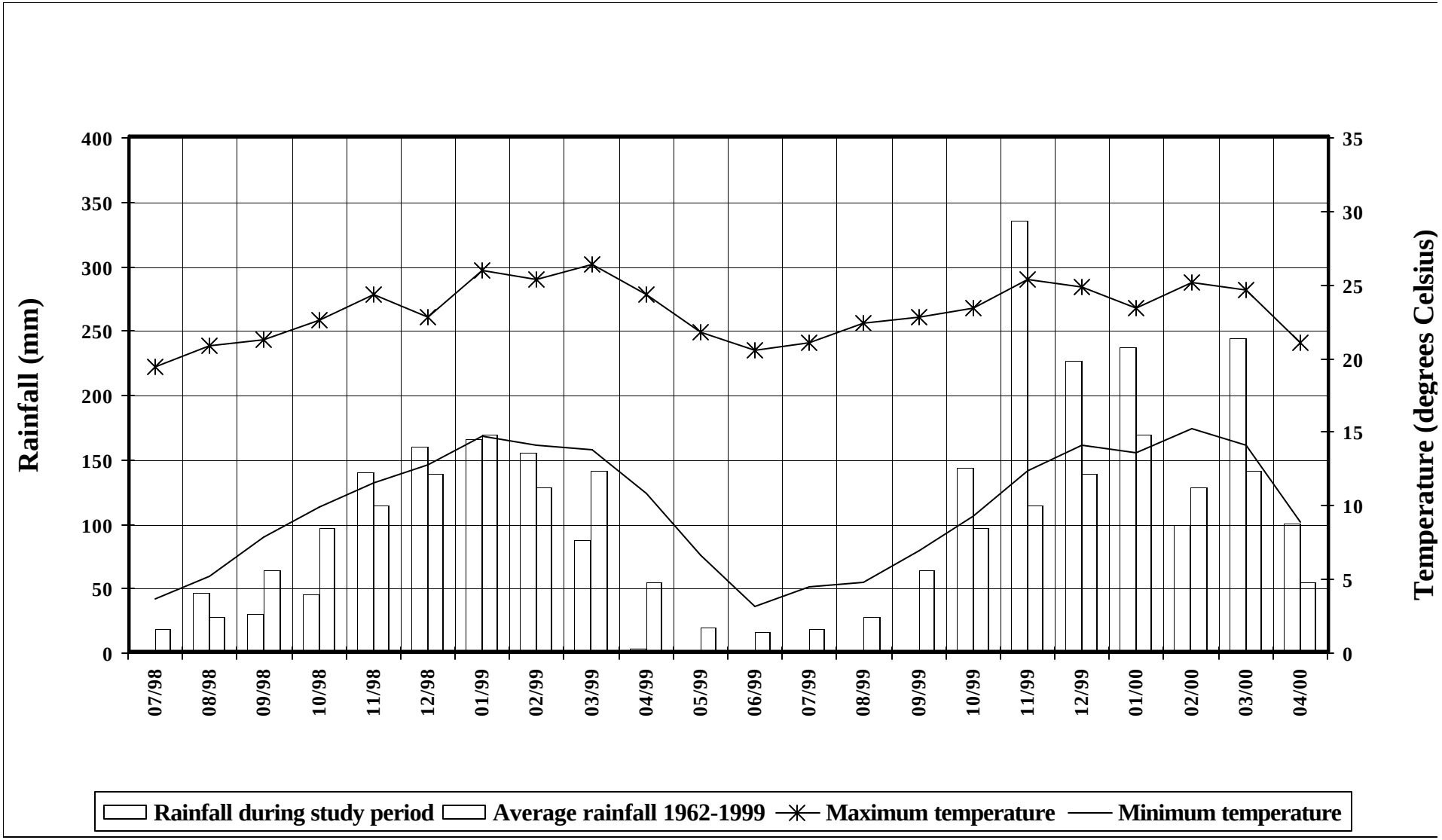
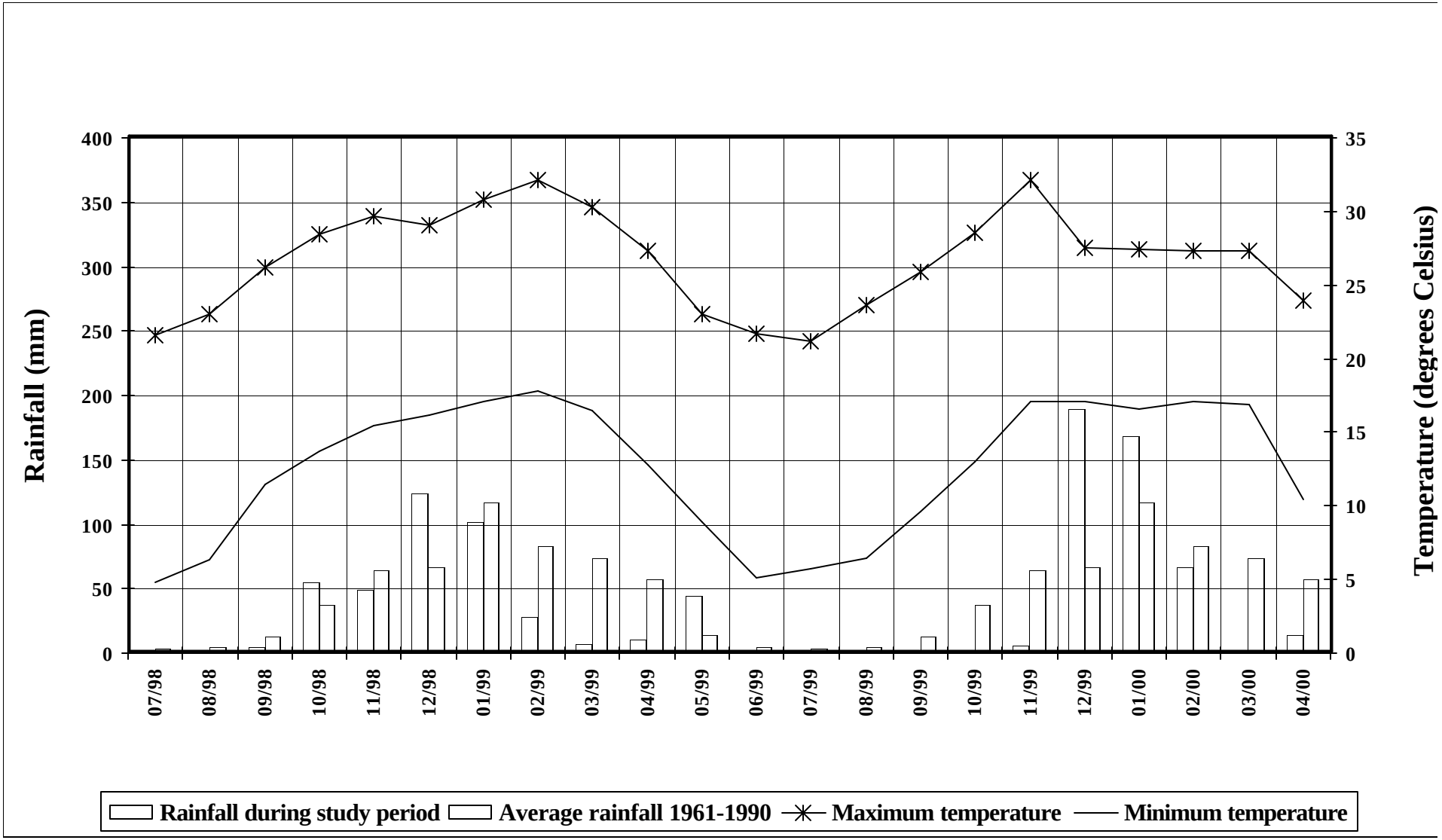


Fig. A1.3 : Climatic data for Kraaipan



Appendix 2

Standard operating procedures

A2.1. McMaster method of faecal worm egg count

Extracted from Van Schalkwyk, P.C., Schröder, J., Malan, F.S. and Van Wyk, J.A., 1995. Worm Workshop : Recommendations on Worm Control. Division of Helminthology, Onderstepoort Veterinary Institute, Pretoria, pp. 18-19. Modifications made in the studies included in this dissertation are indicated in square brackets.

A2.1.1. Purpose

The presence of worm eggs in the faeces provides evidence that an animal is infected. The McMaster method is a simple method for obtaining a count of the number of nematode eggs per gram of faeces. The advantage of the faecal worm egg count lies in the fact that when conducted routinely, the pattern of worm infection can be determined and the success (or failure) of parasite control can be monitored.

A2.1.2. Apparatus

A2.1.2.1. In the field

a) Disposable plastic gloves.

b) Plastic bags.

c) Cooler box with ice pack.

d) Pen and labels.

e) Ear tags.

A2.1.2.2. In the laboratory

a) Scale/balance : Accuracy of 0,1g is required. [Triple beam balance, Ohaus Scale Corporation, Florham Park, N.J., USA.]

b) Microscope : A compound microscope capable of 80-100X magnification. The ideal combination is a 10X objective with 8X oculars (=80X). The field must be wide enough to cover one lane of the ruled grid of the McMaster slide.

c) McMaster counting chambers (slides). [Eggs-Acto™ McMasters, E. Krecek, South Africa.]

d) Blender/homogeniser (optional). [IKA® - Labortechnik, Janke and Kunkel, N.T. Laboratory Supplies, Johannesburg.]

e) Measuring cylinder (100ml capacity) or a syringe (>60ml capacity). [A dispenser was used – Zippette™, Jencons Scientific, England.]

f) Medical 100ml glass bottle with neck wide enough to fit over blender shaft.

g) Pasteur pipettes with a suction bulb or alternatively, cooldrink or artificial insemination (AI) straws, to fill the McMaster slide. [Liquipettes™, Elkay, Ireland or equivalent.]

h) Glass beaker of ca. 500ml capacity for composite samples.

i) Reagents : Amyl alcohol. [Substituted with polypropylene glycol (Polypropylene glycol P400™, Fluka) in January 2000 when a technician showed symptoms of a hazardous chemical injury due to amyl alcohol.]

Sugar solution (40 %).

A2.1.3. Method

A2.1.3.1. Collection of faeces

Faeces should be collected from the rectum and not picked up from the ground. Smaller animals such as young lambs can usually be induced to defaecate by inserting a finger into the rectum and

gently massaging until the sphincter relaxes. Collect the pellets in a disposable glove or place in a plastic bag.

A2.1.3.2. Individual counts

a) Weigh 2g (sheep) or 4g (cattle) of faeces and place in a 100ml glass bottle. Add 58ml (sheep) or 56ml (cattle) sugar solution and blend until the faeces is well dispersed (10 to 20 seconds).

b) Add six to 10 drops of amyl alcohol to the mixture and invert to mix. Leave for two minutes to allow air bubbles to break.

c) Wet the McMaster slide, shake well to remove excess water and wipe the outside dry with a paper towel. [This step was omitted.]

d) Mix the sample again by inversion or by blowing air through it with a cooldrink straw or AI pipette. [Samples were vigorously mixed with a Pasteur pipette.] Withdraw fluid without delay and fill the McMaster slide.

e) Fill two chambers of the McMaster slide and leave standing for at least two minutes before counting. Specimens should be examined within an hour after preparation.

f) Calculate the eggs per gram of faeces by multiplying the total number of eggs counted in the two chambers by 100 for sheep and by 50 for cattle.

Example : First chamber : 6

Second chamber : 8

Total : 14

Eggs per gram = 14 x 100 = 1400 (sheep)

Eggs per gram = 14 x 50 = 700 (cattle)

A2.1.3.3. Composite samples

From 10 to 15 specimens collected individually, weigh out 1g from each and pool them. Add 29ml sugar solution for each gram and blend as described above. Add 15 drops of amyl alcohol and proceed with the count as described above for individual samples. Calculate the result by the method for sheep above. A more detailed description of preparing composite faecal samples appears elsewhere in Van Schalkwyk et al. (1995).

A2.2. Trematode egg count

Extracted from Van Wyk, J.A., Schröder, J., Van Schalkwyk, P.C. and Horak, J.G., 1987. Tegnieke : Helmintologie (in Afrikaans). In : Schröder, J. (Ed.), Proc. Worm Resistance Workshop. Pretoria, South Africa, pp. 125-127. Translated here into English. Modifications made in the studies included in this dissertation have been indicated in square brackets.

A2.2.1. Purpose/Description

In contrast with the eggs of gastrointestinal roundworms, trematode eggs have such a high specific gravity that undamaged eggs do not float in flotation fluid.

The presence of eggs in faeces is thus determined by concentrating the eggs by means of sieving and/or sedimentation. In the process, some of the finer, the larger, as well as the lighter faecal particles are discarded, so that the eggs may be more easily seen. Addition of methylene blue stain increases the contrast between the eggs and the faecal particles, without making the identification more difficult.

A2.2.2. Apparatus

a) Helminth filter or sieves (64µm and 150µm).

b) Funnel sieve (with a 150µm sieve in the funnel). [150µm (United wire test sieve, Nigel, South Africa or equivalent) and 38µm (Labotec test sieve, Johannesburg or equivalent) metal sieves.]

c) 100ml [200ml] measuring cylinder.

d) 10mP [20ml] pipette.

e) Petri dish with parallel lines on the base. [70mm x 70mm gridded perspex container, E. Krecek, South Africa.]

f) Dissection microscope.

g) Methylene blue 1%.

h) 2P fruit jar [3P utilized when 2P not available].

i) Garden hose spray.

A2.2.3. Method

The technique that is used will depend on the available apparatus. For a quantitative diagnosis, the sample that is being concentrated for examination may be subdivided and only a portion of this examined.

A2.2.3.1. Helminth filter method

[This method was not utilised in the present study, but is included here since it is referred to in the sedimentation method described below.]

The apparatus consists of two sieves which fit into each other – the innermost one allows trematode eggs to pass through, and the outermost one does not allow eggs through, but is permeable for fine faecal particles.

The apparatus is relatively expensive, but will definitely be worth the trouble of purchasing if the practice is located in an area such as the Mpumalanga Province pan veld or the Free State Province, where trematode infection is common.

a) Weigh off 5g of faeces.

b) Place the faecal sample in the innermost filter.

c) Set the hose spray to give a small rosette stream, and not a thin stream.

d) Spray a strong stream of water over the faecal sample, until the water that flows out of the filter is clean.

e) Tap the contents of the outermost filter into a 100ml measuring cylinder.

f) Make up the cylinder contents to 100ml.

g) Mix the sample by covering the mouth of the cylinder with your hand and quickly inverting the cylinder a couple of times. Immediately hereafter withdraw 10ml of the suspension and transfer this to a Petri dish.

h) Examine the sample (aliquot) little by little in a gridded perspex Petri dish under a dissection microscope, at a magnification of approximately 25X. The contrast between the eggs and the faecal particles, which make up the background may be enhanced by adding 1% methylene blue to the sieved sample and allowing this to stand for five minutes before it is washed on a 38µm sieve and the material on the sieve collected for examination. [Methylene blue was not utilized in the current study.]

i) Count x 2 = worm egg count per gram of faeces.

Practical hints

a) Trematode egg counts are more time-consuming to carry out than those of roundworms. Try, therefore, first to establish whether the farm from which the samples originate poses a real danger of trematode infection (vleis or dams) before deciding to carry out a trematode egg count. Remember, however, that the animals may have been brought in from elsewhere, and that liver fluke infection may sometimes arise in drinking troughs.

b) The hose spray (which should preferable by connected with an instant clip-on attachment to the tap) is most effective when it is adjusted to provide a small rosette spray, instead of a thin spray, which tends to cause the sample to splash out of the sieve, and thus cause a falsely low count.

c) Move the spray continuously up and down in the innermost filter while the sample is being filtered, to spread out the whole wall of this filter, and to mash through the eggs.

d) The eggs of *Fasciola* spp. are oval, like those of *Paramphistomum* [*Calicophoron*], but are yellowish in colour, in contrast with the colourless transparent eggs of the latter. Sometimes cases occur where the eggs of *Paramphistomum* are light brown, but their appearance is more granular than those of *Fasciola* spp. The eggs of both types have a relatively clear operculum at one of the poles, and are undeveloped in fresh faeces – in other words, they do not contain a developed miracidium. In contrast, the eggs of *Schistosoma mattheei* are spindle-shaped, there is no operculum, and they contain a developed miracidium.

e) It is recommended that a reference sample of, for example, *Fasciola* eggs be kept to compare with samples from the field. The eggs can, for example, easily be obtained by washing the gall bladder contents of an infected animal on to a 38µm sieve.

f) The eggs of *S. mattheei* hatch quickly in water, and formalin must therefore be added before the faeces are processed to prevent hatching while the faeces are washed on the sieves or in the filter. These eggs sediment relatively slowly, and as a result the jar must be left to stand for up to 15 minutes during concentration of the eggs (see below) before the water is discarded.

A2.2.3.2. Sedimentation method

[Modified as described in Chapter 2 for use in the present study.]

Through repeated dilution of the faecal suspension, and sedimentation of the eggs (which are heavier than most of the faecal particles), the faeces in the sample are reduced and the eggs concentrated so that they are more easily observed. The process can be made very much easier if the faeces are initially sieved through a 150µm sieve.

a) Weigh off 5g of faeces, and crush these finely.

b) If possible, sieve with water through a 150µm funnel sieve, which is placed above a container to collect the filtrate, which passes through the sieve. [In the present study, the faeces were sieved through a 150µm metal sieve (United wire test sieve, Nigel, South Africa or equivalent) into a 38µm metal sieve (Labotec test sieve, Johannesburg or equivalent), using water sprayed from a nozzle at high pressure. The sediment remaining on the 38µm sieve was washed into a 2 or 3P fruit jar.]

c) Place the finely crushed faeces or the filtrate from the 150µm sieve into a 2P fruit jar, and fill with water.

d) Allow to stand for four minutes (longer for *Schistosoma* – see above) so that the eggs sink to the bottom of the jar. [In the present study, the samples were allowed to stand for at least 15 minutes.]

e) Pour off as much of the supernatant as possible, without losing any of the sediment.

f) Repeat this process of sedimentation and discarding of the supernatant several times until the supernatant is clear within a few moments of the jar having been refilled.

g) Discard the supernatant a last time after a standing time of four minutes, and pour the sediment into the measuring cylinder.

h) Mix, take an aliquot, and examine microscopically as described for the filter method.

Practical hints

a) More often than not the sample is ready for examination after it has been sedimented and decanted three times.

b) If the bottle stands for too long after water has been added, too much of the faeces sediments out, and the eggs are not effectively concentrated. On the other hand, sedimentation for less than four minutes will cause eggs to be discarded before they have reached the base of the jar.

c) Use of a water vacuum pump (a cheap apparatus which is fitted to a water tap) to remove the supernatant, will speed up the process of sedimentation, since a larger proportion of the supernatant may be removed each time, and together with it also a larger proportion of the faecal particles.

A2.3. Faecal larval culture

Extracted from Van Wyk, J.A., Schröder, J., Van Schalkwyk, P.C. and Horak, J.G., 1987. Tegnieke : Helmintologie (in Afrikaans). In : Schröder, J. (Ed.), Proc. Worm Resistance Workshop. Pretoria, South Africa, pp. 123-125. Translated here into English. Modifications made in the studies included in this dissertation have been indicated in square brackets.

A2.3.1. Purpose/Description

The *eggs* of the majority of the most common gastrointestinal roundworms differ morphologically so little from each other that they cannot be differentiated microscopically from each

other. Consequently, with a few exceptions, it cannot be determined from a faecal egg count which worm types are involved.

In contrast, the *infective larvae* of these worm types may be differentiated to genus level, and faecal cultures may thus be used in the live animal to determine with which worm types the animals are infected. It is certainly not easy to differentiate between the larvae, but the techniques to recover larvae are described here so that larvae may be recovered and sent to an expert for identification. It is hoped that this may stimulate more veterinary practitioners to learn the identification of larvae so that they may provide a better service to their clients.

Faeces are mixed with a medium which keeps them moist and aerated so that worm eggs may hatch and the resulting larvae may develop to the third stage. For the collection of the larvae, use is made of their instinctive tendency to migrate upwards in the presence of moisture and light, away from the faecal culture in which they hatch.

Two methods are described – the first for the collection of relatively small number of larvae (only for diagnostic purposes), and the other for the collection of larger numbers, e.g. for the infection of animals to enable the identification of the worm types to species level.

A2.3.2. Apparatus

A2.3.2.1. Diagnostic method (small numbers of larvae)

a) 1 small wide-mouthed bottle of heavy glass, ± 3cm in diameter x 2cm high.

b) 1 larger wide-mouthed bottle, ± 5cm in diameter x 4-5cm high.

A2.3.2.2 Collection of large numbers of larvae

a) 1P fruit jar.

b) 2 wooden dowelling rods, approximately 3cm in diameter x 40cm long.

c) Plastic flat-bottomed mixing bowl, approximately 50cm x 30cm.

d) McMaster canvas bags for collection of the faeces [not utilized].

A2.3.2.3. Both methods

a) Vermiculite, or other culture medium, such as newspaper.

b) Plastic washbottle.

c) 100ml measuring cylinder.

d) Counting container with lines on the base of the container.

e) Standard microscope.

f) Stereo dissection microscope.

g) Pasteur pipette with suction bulb.

h) Glass slides.

i) Cover slips (22mm x 40mm).

j) Lugol's iodine.

A2.3.3. Method

A2.3.3.1. Diagnostic larval culture

[This method was not utilised in the present study, but is included here since it is referred to in the section on "Collection of large numbers of larvae" described below.]

a) Collect the faeces from the animal as for a worm egg count.

b) Sample approximately 10g of faeces.

c) Break the sheep faeces into fine pieces by flattening the pellets.

d) Mix cattle dung or unformed sheep faeces with vermiculite (approximately equal amounts of each), and moisten the mixture without it becoming soft.

e) Fill the small bottle (3cm x 2cm high) level with the faeces mixture.

f) Place the small bottle in the larger one, and add water to the larger bottle up to the brim of the small one.

g) Screw on the lid of the larger bottle and incubate the sample for seven to 10 days at ±27°C (Reinecke, 1983).

h) Thereafter remove the smaller bottle with a tissue forceps and pour the fluid from the larger bottle into a test tube (Reinecke, 1983).

i) Allow the tube to stand for 20 minutes [15 minutes or longer in the present study] so that the larvae settle and with the help of a Pasteur pipette [Liquipettes^{TM}, Elkay, Ireland or equivalent] carry a few drops of the sediment over to a glass slide (Reinecke, 1983).

j) Add a drop of iodine, cover with a cover slip, and identify the larvae (±10X objective lens).

Practical hints

a) As with all similar samples, labelling and identification of the samples is essential.

b) Dry, sterilised faeces, charcoal or even old newspaper may be used in the place of vermiculite. The aim of the substance is to aerate wet faeces, otherwise the worm eggs will not hatch.

c) If there are too little faeces to fill the small bottle, cotton wool may be placed in the bottom of the bottle and wetted before the faeces are placed on top of it (Reinecke, 1983).

d) In the case of cattle faeces, rather make two cultures since there are often few eggs, and hence larvae, in the faeces (Reinecke, 1983).

e) Should the identification of the larvae take a long time, or if this cannot be done immediately, evaporation can be prevented by smearing petroleum jelly on the edges of the cover slip before it is placed on the drop of larval suspension on the slide (Reinecke, 1983).

A2.3.3.2. Collection of large numbers of larvae

[Used in the present study.]

a) Place a McMaster faecal bag on the sheep or bovine to collect sufficient faeces.

b) Break the sheep faeces into fine pieces.

c) Mix the faeces with vermiculite (approximately equal amounts of each).

d) Hold one of the wooden dowelling rods in the middle of the fruit jar while the faecal mixture is placed little by little in the bottle and pressed lightly down around this rod with the second rod up to a maximum height of 5-7,5cm (Reinecke, 1983).

e) With a tissue, wipe off excess faeces on the inside of the jar (Reinecke, 1983). If this is not done, the larval suspension may be contaminated with faeces and pieces of vermiculite during harvesting of the larvae.

f) Rinse the inside of the fruit jar with the washbottle down to the surface of the compacted faeces.

g) Adjust the moisture content until it is damp but not too soft.

h) Screw on the lid of the flask lightly and incubate for seven to 10 days at 27-30°C (Reinecke, 1983).

i) Thereafter flush the inside of the flask down to the surface of the culture and place this in indirect sunlight in the laboratory (Reinecke, 1983). [This step was omitted in the present study.]

j) After one to two hours the larvae migrate up the sides of the flask. Collect the larvae by holding the flask upside down and by flushing the larvae off the sides and allowing these to run into a 100ml measuring cylinder.

k) Allow the larvae to sediment out and examine a sample thereof as for the diagnostic method.

Practical hints

a) Ensure that the dowelling rods are thoroughly cleaned between faecal samples, otherwise cross-contamination may occur.

b) Sheep faeces may be crushed finely by stamping the faeces lightly before they are removed from the bag.

c) The faecal mixture in the flask is only compacted enough so that it withstands handling without crumbling during collection of the larvae.

d) Often only a portion of the larvae in the larval culture migrate out of the culture initially with the first flushing and the flushing and collection may be repeated a couple of times to collect more larvae.

e) *Nematodirus* eggs do not hatch within seven days in the culture. The culture may be left for 14 days, but then funga1 growth may create problems. Another method must thus usually be used for the collection of these eggs.

f) The eggs of certain worm types, such as those of *Trichuris* and *Toxocara*, do not hatch in the cultures. These worm types can however be identified from the egg morphology to the genus level.

g) Hookworm larvae (*Bunostomum*, *Gaigeria* and *Ancylostoma*) sometimes apparently do not migrate upwards out of the culture. In such a case, the larvae may be collected by filling the jar with water and by inverting it as follows : fill the jar until the water meniscus bulges above the edge of the jar, place a large Petri dish upside down on the jar, and turn the jar and dish upside down so that the fruit jar stands upside down in the Petri dish. Now add water to the Petri dish to a depth of 2-3cm. Slide a microscope slide under the lip of the fruit jar to leave a small space between the bottom of the dish and the edge of the flask. After one to two hours the water is collected from the Petri dish and worm larvae are to be found therein (Reinecke, 1983).

A2.4. Identification of infective third-stage nematode larvae of small stock and cattle

The keys described here are those referred to in Van Wyk, J.A., Alves, R.M.R. and Michael, L.M., 1997a. A novel key for identifying nematode infective larvae (L_3) from domesticated ruminants. In : Proc. 16th International Conference of the World Association for the Advancement of Veterinary Parasitology, Sun City, South Africa.

The easiest way to identify infective L_3 of small stock and cattle is to compare the lengths of the free sheaths (sheath tail from the posterior tip of the tail to the tip of the sheath) of different genera with one another.

For instance, measure the length of the sheath tail of *Trichostrongylus colubriformis* L_3 with the aid of a compound microscope graticule and at a 100X magnification, and let this equal "X". Then the free sheath tail of *Haemonchus contortus* is 2"X".

The following tables provide keys to the identification of the more common L_3 in South Africa.

Please note, however, that the measurements of some helminth field strains differ somewhat from the tables (e.g. *H. contortus* with a free sheath tail shorter than 2"X"). Remember also that only the measurements of the most common worm species are included; the tail sheaths of *Trichostrongylus falculatus* L_3 are, for instance, somewhat longer than those of *T. colubriformis*.

It remains difficult to identify nematode larvae, and this manual is only intended as a guide after a person has already had practical training. In other words, it is unlikely that someone without practical training will be able to identify larvae accurately using this manual. Furthermore, even the most experienced person may become confused after long sessions of larval identification and become hesitant about even the most common worm species. The best advice in this case is to stop identifying

the larvae until one's mind is fresh again. It is also essential to have a standard set of I_3 of pure helminth strains, so that comparisons can be made when larvae in field samples are difficult to identify.

It is most important particularly in the period immediately after a practical training course, to practise larvae identification or else it may be difficult to perceive the subtle differences between the various larvae.

Preparing larvae for identification

Transfer a small aliquot of larvae (harvested from faecal cultures) using a Pasteur pipette, to a microscope slide, add a drop of iodine and cover with a coverslip. Examine microscopically using the following keys.

Table A2.4.1

A key for the identification of nematode third-stage larvae of small stock

No.	Description	Identification
1.	Sheath tail absent	2
	Sheath tail present	3
2.	Sheath tail absent	
2.1	Oesophagus ± 40% of total length of larva; without a bulb; larva very thin; "head"* relatively bullet-shaped; tail appears blunt, like a sheath tail that has broken off. *Examine carefully: it often appears as if the larva does have a sheath*	*Strongyloides*
2.2	Oesophagus < 40% of length of larva and rhabditiform (double bulb); thick, cigar-shaped larva with a long tail that may appear to have a sheath; tip of "head" has a definite "structure" and is reasonably flattened	Free-living nematode
3.	Sheath tail present; oesophagus < 40% of total length of larva	
3.1	Oesophagus with a single bulb; larva stains ± uniformly with iodine	5.3 + 8
3.2	Oesophagus without a bulb; larva stains light anteriorly and dark posteriorly with iodine	4
4.		
4.1	Sheath tail invisible; larva lethargic and has a protuberance on the "head"	*Dictyocaulus*
4.2	Sheath tail visible; fresh larva very active; no protuberance on the "head"	5
5.	Sheath tail length	
5.1	Short, without filament	6
5.2	Medium, with short filament	7
5.3	Medium, with long filament	8, 9
5.4	Long, with long filament	10,11
6.	Length of sheath tail "X"; head somewhat flattened; sheath tail narrows fast to a point (resembles a pencil point); no filament	
6.1	Larva tail smooth when exsheathed	*Ostertagia/Teladorsagia, T. axei* and *Marshallagia*
6.2	Exsheathed larva tail unevenly serrated (digitate)	Intestinal *Trichostrongylus*
7.	Length of sheath tail 2"X"; "head" ± bullet-shaped and tapers relatively fast to a point (± slanted sides); sheath filament short (± 15% of sheath tail length)	*Haemonchus*

No.	Description	Identification
8.	Length of sheath tail 3“X”; short larva which stains uniformly with iodine; “head” bullet-shaped; sheath filament long (± 50% of sheath tail length); oesophagus with single bulb	*Gaigeria*
9.	Length of sheath tail 3“X”; “head” broad and flattened; sheath filament long (±25% of sheath tail length); 28-32 intestinal cells (rectangular shape) in *fresh* larva	*Chabertia*
10.	Length of sheath tail 4,5“X”; “head” broad and flattened (with nearly parallel sides); 18-22 intestinal cells (triangular shape) in *fresh* larva; sheath filament long (± 50% of sheath tail length)	*Oesophagostomum*
11.	Length of sheath tail 8“X”; “head” is bullet-shaped but somewhat flattened; sheath filament is very long (± 50% of free sheath length); larva tail with finger-like projection (only clearly visible in exsheathed larva)	*Nematodirus*

* “Head” refers to the cranial/anterior tip of the larva.

Bear in mind that the L_3 of many genera look as though the “head” is flattened when the larvae are partially or totally exsheathed.

Appendix 3

Third-stage nematode larvae results

The results of the differential larval counts were analysed statistically with reference to the null hypothesis that states that each worm genus should occur with the same frequency within a culture. A Chi-square test, where $P<0.05$ was considered significant, was used to indicate significant differences in occurrence of a particular genus. Only significant peaks in a particular worm genus are indicated by means of an asterisk in the figures. Although the figures display the incidence of the worm genera in terms of a percentage, the actual differential larval counts were used in the statistical analysis.

Fig. A3.1 : Generic composition of nematode third-stage larvae recovered from cultures of faeces from goats at Rust de Winter

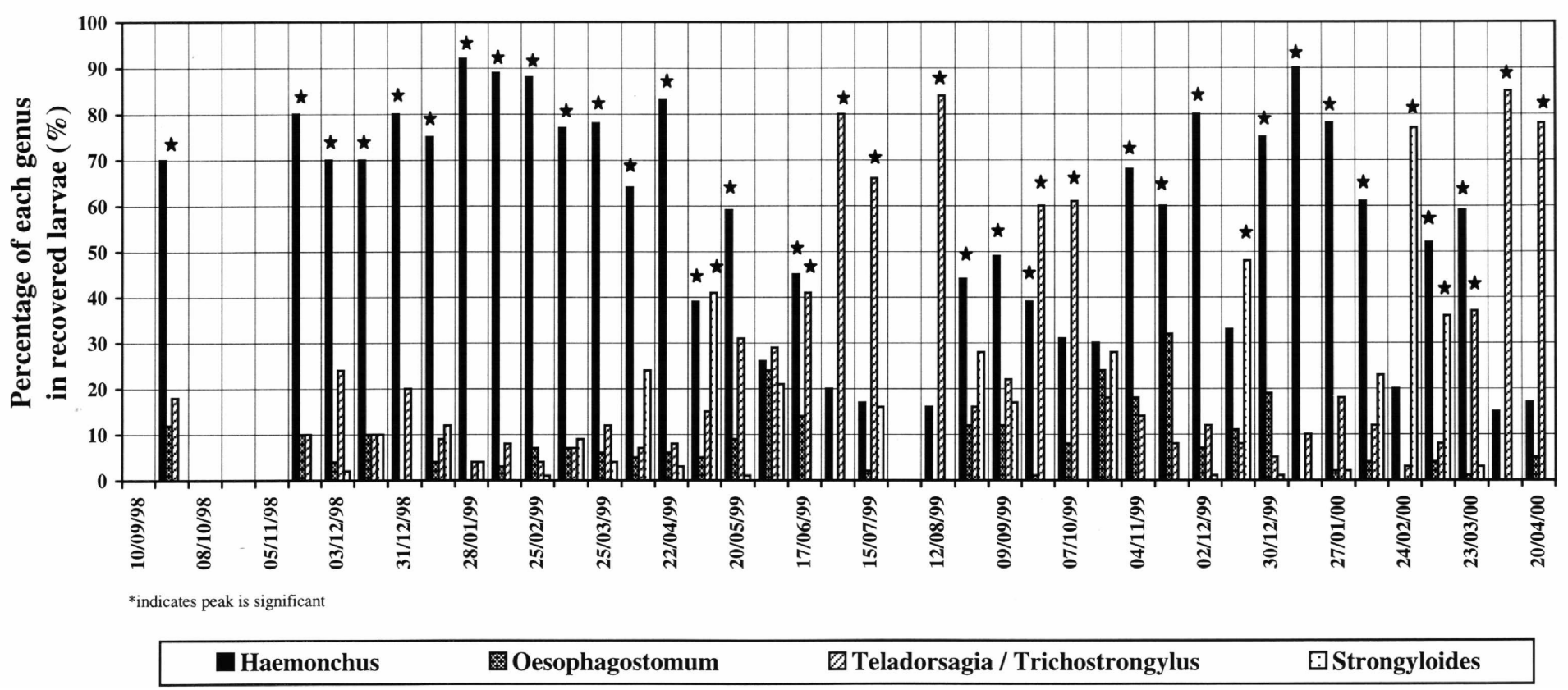


Fig. A3.2 : Generic composition of nematode third-stage larvae recovered from cultures of faeces from sheep at Rust de Winter

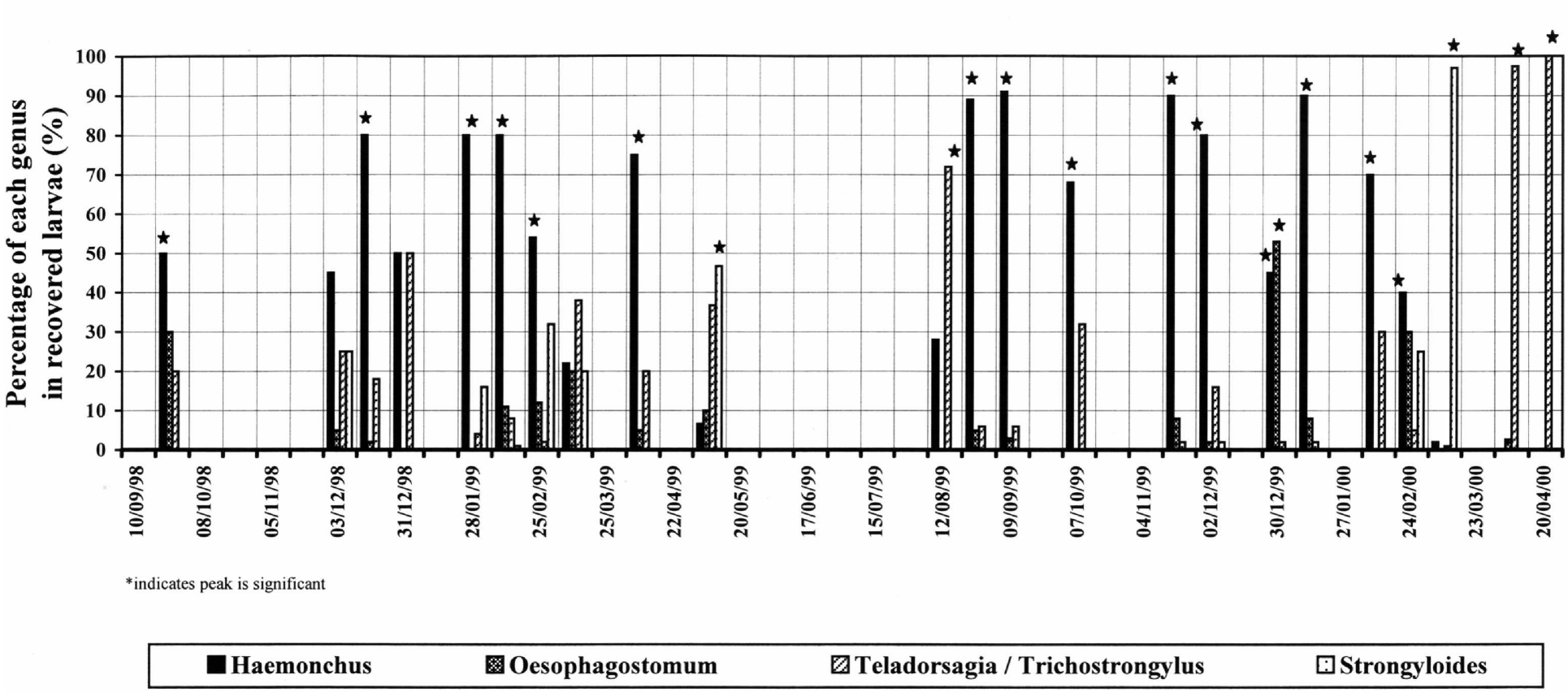


Fig. A3.3 : Generic composition of nematode third-stage larvae recovered from cultures of faeces from goats at Site 1, Impendle

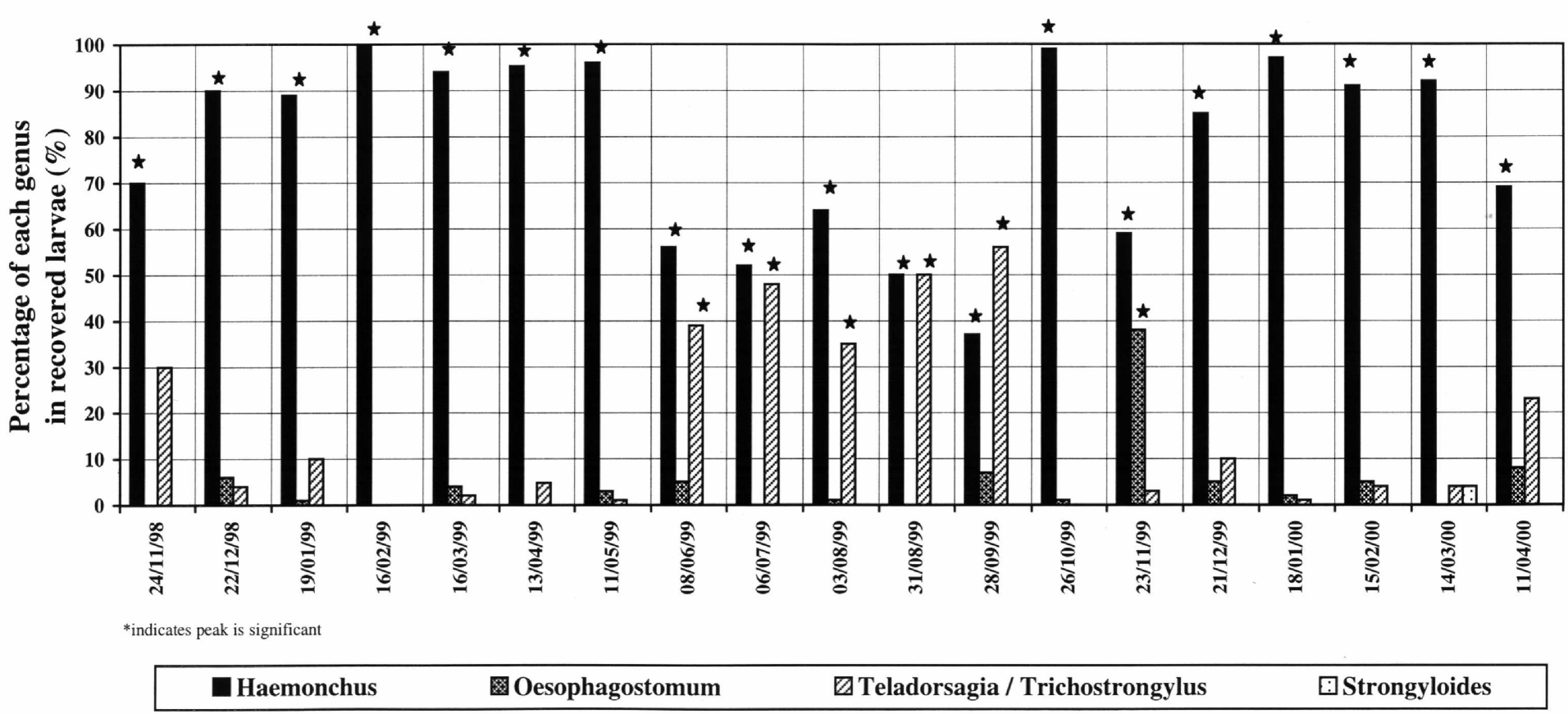


Fig. A3.4 : Generic composition of nematode third-stage larvae recovered from cultures of faeces from goats at Site 2, Impendle

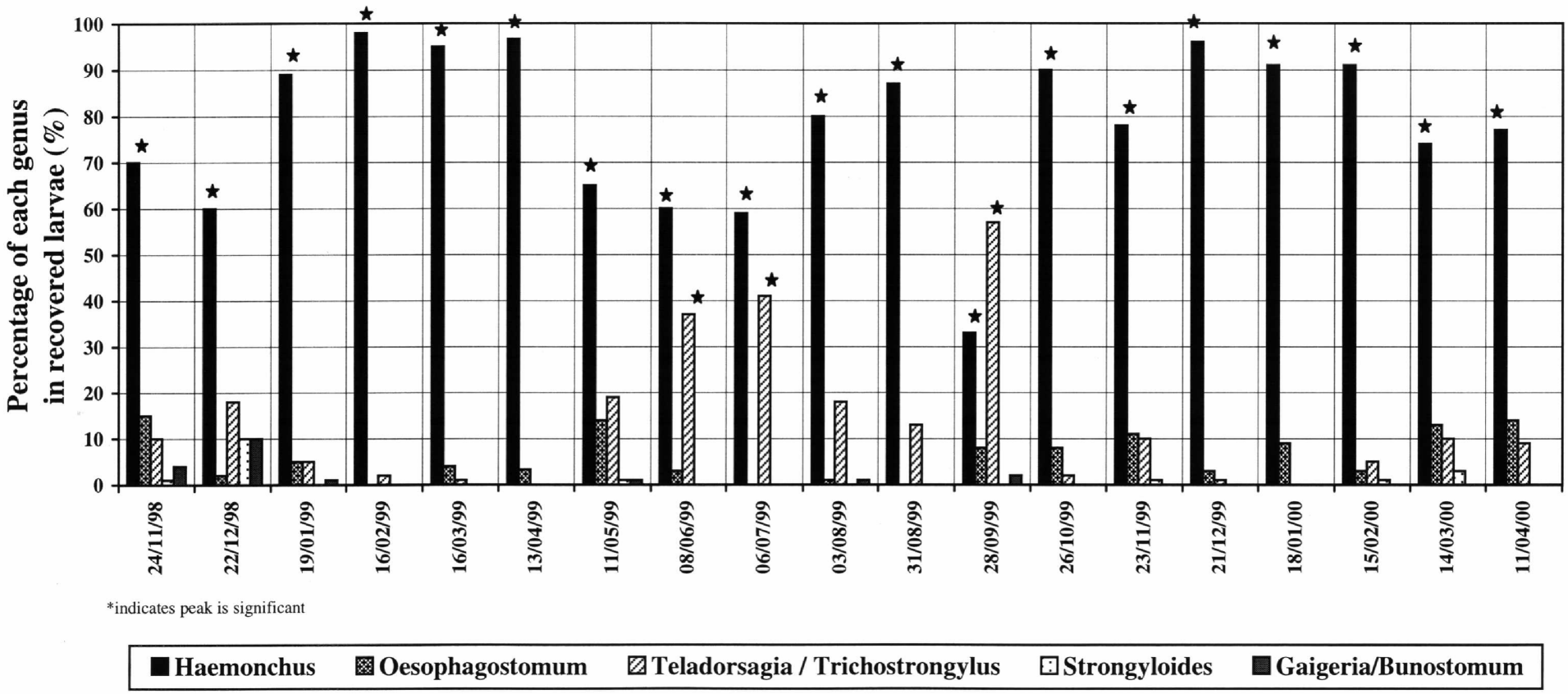


Fig. A3.5 : Generic composition of nematode third-stage larvae recovered from cultures of faeces from goats at Kraaipan

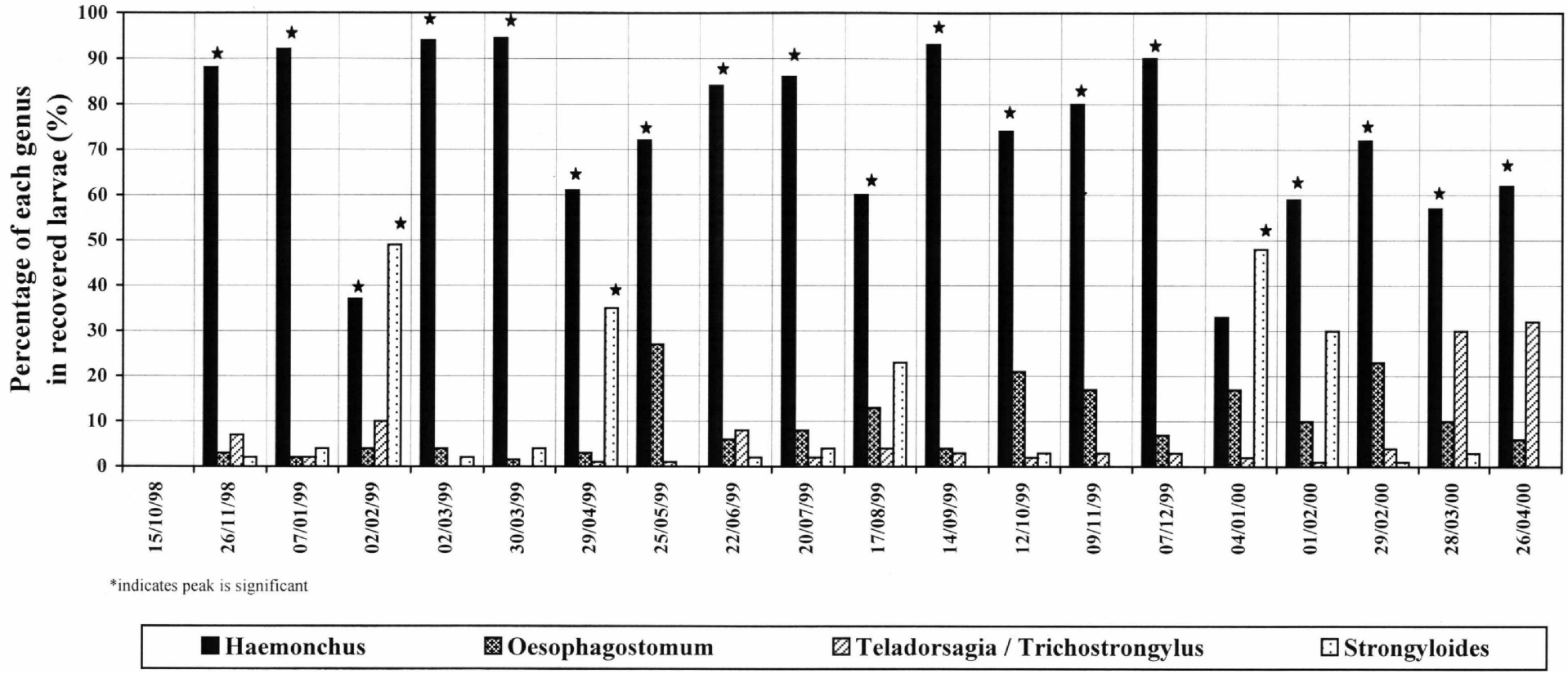
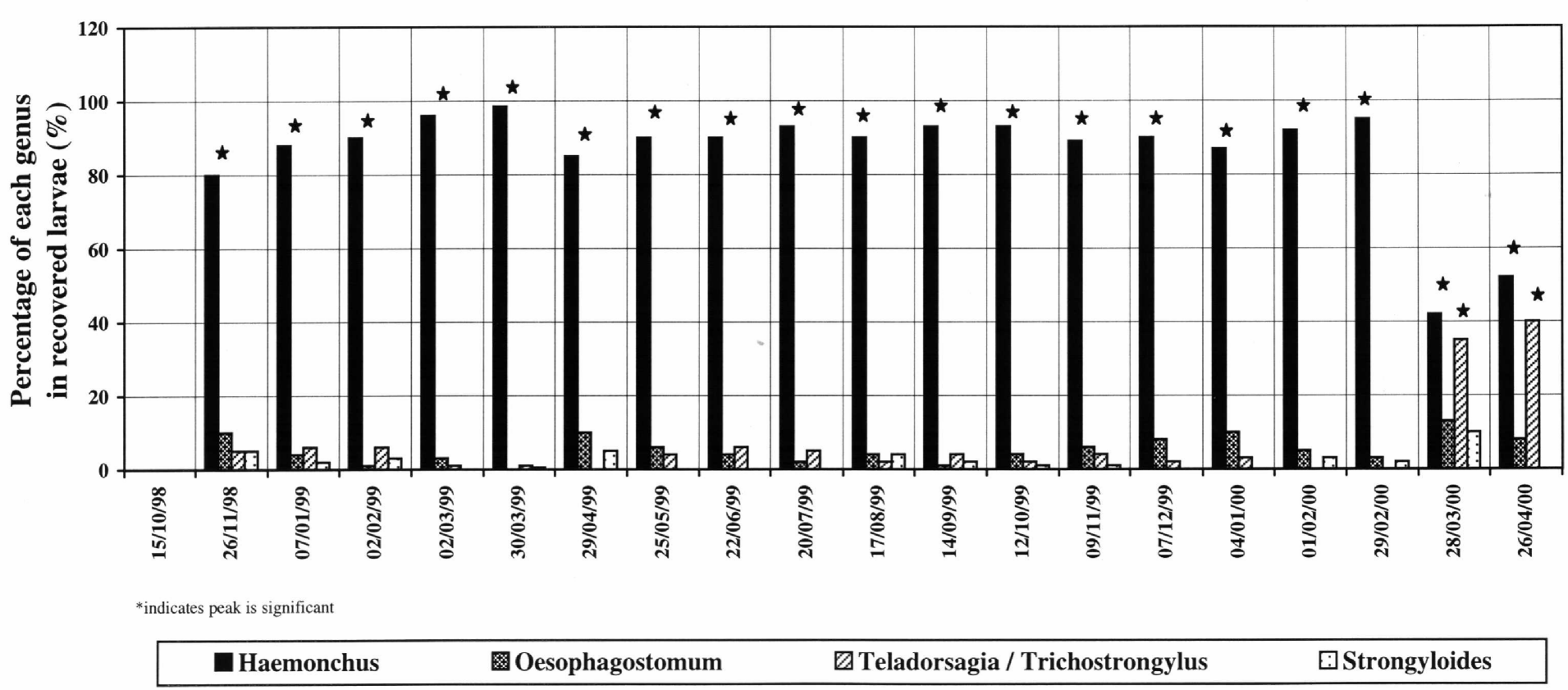


Fig. A3.6 : Generic composition of nematode third-stage larvae recovered from cultures of faeces from sheep at Kraaipan



Appendix 4

Tables of numbers of animals in each FAMACHA© category over time for the three study sites

Table A4.1

Numbers of animals in each FAMACHA© category over time for goats at Rust de Winter

FAMACHA© category	10-Sep-98		24-Sep-98		8-Oct-98		22-Oct-98		5-Nov-98		19-Nov-98		3-Dec-98		17-Dec-98		31-Dec-98		14-Jan-99		28-Jan-99	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	4	9	1	2	0	0	1	2	5	14	12	26	4	11	6	16	1	3	2	6	1	3
2	16	34	15	35	8	19	25	60	17	47	21	46	18	50	12	32	10	30	12	39	11	37
3	27	57	24	56	24	57	11	26	13	36	12	26	9	25	17	46	16	48	15	48	15	50
4	0	0	3	7	10	24	5*	12	1	3	1	2	5	14	2	5	4	12	2	6	3	10
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	6	0	0	0	0
Total no. treated	47	.	43	.	42	.	42	.	36	.	46	.	36	.	37	.	33	.	31	.	30	.
	0	0	3	7	10	24	5	12	1	3	1	2	5	14	2	5	6	18	2	6	3	10

No.: number of adult and weaner animals examined at each visit.
*animals were in fact not treated because anthelmintics were not available.

Table A4.1 continued

FAMACHA© category	11-Feb-99		25-Feb-99		11-Mar-99		25-Mar-99		8-Apr-99		22-Apr-99		6-May-99		20-May-99		3-Jun-99		17-Jun-99		1-Jul-99	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	2	6	0	0	3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	10
2	23	68	16	52	22	73	12	40	6	20	13	43	24	80	21	68	22	71	29	94	22	73
3	8	24	13	42	5	17	11	37	19	63	11	37	6	20	10	32	9	29	2	6	5	17
4	1	3	2	6	0	0	7	23	4	13	6	20	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	34	.	31	.	30	.	30	.	30	.	30	.	30	.	31	.	31	.	31	.	30	.
	1	3	2	6	0	0	7	23	5	17	6	20	0	0	0	0	0	0	0	0	0	0

Table A4.1 continued

FAMACHA© category	15-Jul-99		29-Jul-99		12-Aug-99		26-Aug-99		9-Sep-99		23-Sep-99		7-Oct-99		21-Oct-99		4-Nov-99		18-Nov-99		2-Dec-99	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	0	0	0	0	2	7	1	4	0	0	1	3	3	9	0	0	0	0	1	3	0	0
2	20	67	17	57	21	72	19	68	20	63	13	42	13	41	10	30	10	30	16	47	20	61
3	9	30	10	33	5	17	8	29	11	34	8	26	13	41	16	48	18	55	15	44	10	30
4	1	3	3	10	1	3	0	0	1	3	9	29	3	9	7	21	5	15	2	6	3	9
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	30	.	30	.	29	.	28	.	32	.	31	.	32	.	33	.	33	.	34	.	33	.
	1	3	3	10	1	3	0	0	1	3	9	29	3	9	7	21	5	15	2	6	3	9

Table A4.1 continued

FAMACHA© category	15-Dec-99		30-Dec-99		13-Jan-00		27-Jan-00		10-Feb-00		24-Feb-00		8-Mar-00		23-Mar-00		6-Apr-00		20-Apr-00	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	18	55	19	58	19	59	14	44	14	45	14	48	10	50	16	76	14	70	13	62
3	13	39	12	36	10	31	15	47	16	52	8	28	9	45	5	24	5	25	8	38
4	2	6	2	6	2	6	3	9	1	3	7	24	1	5	0	0	1	5	0	0
5	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	33	.	33	.	32	.	32	.	31	.	29	.	20	.	21	.	20	.	21	.
	2	6	2	6	3	9	3	9	1	3	7	24	1	5	0	0	1	5	0	0

Table A4.2

Numbers of animals in each FAMACHA© category over time for sheep at Rust de Winter

FAMACHA© category	10-Sep-98		24-Sep-98		8-Oct-98		22-Oct-98		5-Nov-98		19-Nov-98		3-Dec-98		17-Dec-98		31-Dec-98		14-Jan-99		28-Jan-99	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	3	50	0	0	0	0	0	0	1	17	3	50	3	43	3	43	4	57	1	20	5	100
2	3	50	5	83	0	0	3	50	1	17	3	50	4	57	3	43	2	29	3	60	0	0
3	0	0	1	17	3	60	2	33	3	50	0	0	0	0	0	0	1	14	1	20	0	0
4	0	0	0	0	2*	40	1*	17	1	17	0	0	0	0	1	14	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	6	.	6	.	5	.	6	.	6	.	6	.	7	.	7	.	7	.	5	.	5	.
	0	0	0	0	2	40	1	17	1	17	0	0	0	0	1	14	0	0	0	0	0	0

No.: number of adult and weaner animals examined at each visit.
*animals were in fact not treated because anthelmintics were not available.

Table A4.2 continued

FAMACHA© category	11-Feb-99		25-Feb-99		11-Mar-99		25-Mar-99		8-Apr-99		22-Apr-99		6-May-99		20-May-99		3-Jun-99		17-Jun-99		1-Jul-99	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	4	80	0	0	4	80	2	40	1	20	1	20	2	40	3	60	4	80	5	100	5	100
2	1	20	5	100	0	0	3	60	4	80	4	80	3	60	2	40	1	20	0	0	0	0
3	0	0	0	0	1	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A4.2 continued

FAMACHA© category	15-Jul-99		29-Jul-99		12-Aug-99		26-Aug-99		9-Sep-99		23-Sep-99		7-Oct-99		21-Oct-99		4-Nov-99		18-Nov-99		2-Dec-99	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	4	80	1	20	5	100	4	80	0	0	0	0	0	0	0	0	1	20	1	20	0	0
2	1	20	4	80	0	0	1	20	4	80	1	20	5	100	3	60	3	60	4	80	2	40
3	0	0	0	0	0	0	0	0	1	20	4	80	0	0	1	20	1	20	0	0	3	60
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	20	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	20	0	0	0	0	0	0

Table A4.2 continued

FAMACHA© category	15-Dec-99		30-Dec-99		13-Jan-00		27-Jan-00		10-Feb-00		24-Feb-00		8-Mar-00		23-Mar-00		6-Apr-00		20-Apr-00	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	40	0	0
2	2	40	4	80	3	60	5	100	5	100	5	100	5	100	4	80	3	60	5	100
3	3	60	1	20	2	40	0	0	0	0	0	0	0	0	1	20	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.	5	.
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A4.3

Numbers of animals in each FAMACHA© category over time for goats at Site 1, Impendle

FAMACHA© category	24-Nov-98		22-Dec-98		19-Jan-99		16-Feb-99		16-Mar-99		13-Apr-99		11-May-99		8-Jun-99		6-Jul-99		3-Aug-99	
	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%
1	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	10	45	12	55	9	41	9	43	12	57	8	38	10	48	9	43	16	76	7	35
3	7	32	7	32	13	59	11	52	6	29	8	38	8	38	11	52	4	19	8	40
4	4	18	3	14	0	0	1	5	3	14	5	24	3	14	1	5	1	5	4	20
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
Total no. treated	22	.	22	.	22	.	21	.	21	.	21	.	21	.	21	.	21	.	20	.
	4	18	3	14	0	0	1	5	3	14	5	24	3	14	1	5	1	5	5	25

MG : monitor group.

Table A4.3 continued

FAMACHA© category	31-Aug-99		28-Sep-99		26-Oct-99		23-Nov-99		21-Dec-99		18-Jan-00		15-Feb-00		14-Mar-00		11-Apr-00	
	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	8	40	3	16	6	32	12	75	7	50	6	43	3	21	6	43	3	27
3	12	60	9	47	9	47	4	25	7	50	2	14	7	50	3	21	7	64
4	0	0	7	37	4	21	0	0	0	0	6	43	3	21	5	36	1	9
5	0	0	0	0	0	0	0	0	0	0	0	0	1	7	0	0	0	0
Total no. treated	20	.	19	.	19	.	16	.	14	.	14	.	14	.	14	.	11	.
	0	0	7	37	4	21	0	0	0	0	6	43	4	29	5	36	1	9

Table A4.4

Numbers of animals in each FAMACHA© category over time for goats at Site 2, Impendle

FAMACHA© category	24-Nov-98		22-Dec-98		19-Jan-99		16-Feb-99		16-Mar-99		13-Apr-99		11-May-99		8-Jun-99		6-Jul-99		3-Aug-99	
	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%
1	5	20	0	0	3	14	0	0	0	0	0	0	0	0	0	0	2	11	1	5
2	13	52	17	68	10	45	5	24	9	43	7	35	10	50	17	89	13	68	14	74
3	5	20	8	32	9	41	14	67	8	38	10	50	10	50	2	11	3	16	4	21
4	2	8	0	0	0	0	2	10	4	19	3	15	0	0	0	0	1	5	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	25	.	25	.	22	.	21	.	21	.	20	.	20	.	19	.	19	.	19	.
	2	8	0	0	0	0	2	10	4	19	3	15	0	0	0	0	1	5	0	0

MG : monitor group.

Table A4.4 continued

FAMACHA© category	31-Aug-99		28-Sep-99		26-Oct-99		23-Nov-99		21-Dec-99		18-Jan-00		15-Feb-00		14-Mar-00		11-Apr-00	
	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%
1	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	10	53	12	63	7	37	4	21	13	68	5	28	7	41	5	29	5	36
3	8	42	7	37	10	53	13	68	5	26	11	61	5	29	7	41	7	50
4	0	0	0	0	2	11	2	11	1	5	2	11	4	24	5	29	2	14
5	0	0	0	0	0	0	0	0	0	0	0	0	1	6	0	0	0	0
Total no. treated	19	.	19	.	19	.	19	.	19	.	18	.	17	.	17	.	14	.
	0	0	0	0	2	11	2	11	1	5	2	11	5	29	5	29	2	14

Table A4.5

Numbers of animals in each FAMACHA© category over time for goats at Kraaipan

FAMACHA© category	15-Oct-98		26-Nov-98		7-Jan-99		2-Feb-99		2-Mar-99		30-Mar-99		29-Apr-99		25-May-99		22-Jun-99		20-Jul-99	
	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%
1	1	4	4	19	1	5	0	0	1	5	0	0	0	0	1	6	0	0	0	0
2	6	26	12	57	7	33	16	76	11	52	7	37	10	56	10	56	12	67	11	61
3	12	52	4	19	6	29	5	24	8	38	8	42	7	39	6	33	5	28	6	33
4	4*	17	1	5	6	29	0	0	1	5	4	21	1	6	1	6	1	6	1	6
5	0	0	0	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	23	.	21	.	21	.	21	.	21	.	19	.	18	.	18	.	18	.	18	.
	4	17	1	5	7	33	0	0	1	5	4	21	1	6	1	6	1	6	1	6

MG : monitor group.
*animals were in fact not treated because anthelmintics were not available.

Table A4.5 continued

FAMACHA© category	17-Aug-99		14-Sep-99		12-Oct-99		9-Nov-99		7-Dec-99		4-Jan-00		1-Feb-00		29-Feb-00		28-Mar-00		26-Apr-00	
	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%
1	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	9	53	7	41	17	100	6	35	6	35	8	50	6	55	5	50	3	30	5	50
3	7	41	10	59	0	0	8	47	9	53	7	44	4	36	3	30	6	60	4	40
4	0	0	0	0	0	0	3	18	2	12	1	6	1	9	2	20	1	10	1	10
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	17	.	17	.	17	.	17	.	17	.	16	.	11	.	10	.	10	.	10	.
	0	0	0	0	0	0	3	18	2	12	1	6	1	9	2	20	1	10	1	10

Table A4.6

Numbers of animals in each FAMACHA© category over time for sheep at Kraaipan

FAMACHA© category	15-Oct-98		26-Nov-98		7-Jan-99		2-Feb-99		2-Mar-99		30-Mar-99		29-Apr-99		25-May-99		22-Jun-99		20-Jul-99	
	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%
1	3	14	16	76	12	57	14	64	13	59	5	23	9	45	0	0	2	11	3	17
2	12	55	5	24	7	33	7	32	9	41	15	68	11	55	17	89	16	89	15	83
3	7	32	0	0	1	5	1	5	0	0	2	9	0	0	2	11	0	0	0	0
4	0	0	0	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	22	.	21	.	21	.	22	.	22	.	22	.	20	.	19	.	18	.	18	.
	0	0	0	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0

MG : monitor group.

Table A4.6 continued

FAMACHA© category	17-Aug-99		14-Sep-99		12-Oct-99		9-Nov-99		7-Dec-99		4-Jan-00		1-Feb-00		29-Feb-00		28-Mar-00		26-Apr-00	
	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%	MG	%
1	7	39	1	6	0	0	1	6	0	0	0	0	0	0	0	0	2	33	1	17
2	11	61	12	71	16	94	11	65	12	71	14	88	6	86	5	83	4	67	5	83
3	0	0	4	24	1	6	5	29	5	29	2	13	0	0	1	17	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	1	14	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total no. treated	18	.	17	.	17	.	17	.	17	.	16	.	7	.	6	.	6	.	6	.
	0	0	0	0	0	0	0	0	0	0	0	0	1	14	0	0	0	0	0	0

Appendix 5

Statistical comparison between goat and sheep strongyle faecal egg counts and haematocrits at Kraaipan

Given that the goats and sheep at Kraaipan were kept under very similar if not the same managemental conditions (e.g. same access to pasture, herded together, kraalled together at night, more-or-less the same numbers of each, same samples taken on same dates), it seemed appropriate to compare the data between species. This appendix supplements the data presented in Chapters 4 and 7.

The Wilcoxon non-parametric two-sample procedure was used to compare the means for the strongyle faecal egg count (FEC) and haematocrit data of Kraaipan to test for statistical significance between the goat and sheep values. While Chapters 4 and 7 present the proportional strongyle FECs, the Wilcoxon procedure was applied to the total FECs. Values for the two-sided probability derived by the normal approximation to the test were examined.

Throughout the period of investigation, the egg counts and haematocrits of the sheep remained higher than those of the goats did (Fig. A5.1). On 12 out of the 20 visit dates, the sheep showed significantly higher egg counts than the goats did (using the Wilcoxon two-sample test) (Table A5.1). On almost all occasions, the mean haematocrits of the goats were lower than those of the sheep were. On eight occasions, this difference is statistically significant (Table A5.2).

The lower egg counts in the goats at Kraaipan are probably due to the differences in eating habits between the two species. In contrast to the goats, the sheep were observed to prefer grazing to browsing. They would therefore ingest more larvae than the goats, and would develop higher worm burdens. The lower haematocrit values in the goats are probably an inherent species difference. Schalm's Veterinary Hematology (Jain, 1986) sets the normal haematocrit range for sheep at 24-50% while that for goats is 19-38%. Dorny et al. (1995) have shown that in goats and sheep grazed under the same conditions, the mean haematocrit of the goats was almost always lower than that of the sheep. The mean haematocrit range of the sheep and goat flocks grazed together in their study were 22.3 – 29.2 and 19.1 – 24.8, respectively. The current results, then, seem to confirm the lower haematocrit range for goats when compared with sheep.

Fig A5.1 : Strongyle faecal egg counts and haematocrits for small ruminants at Kraaipan

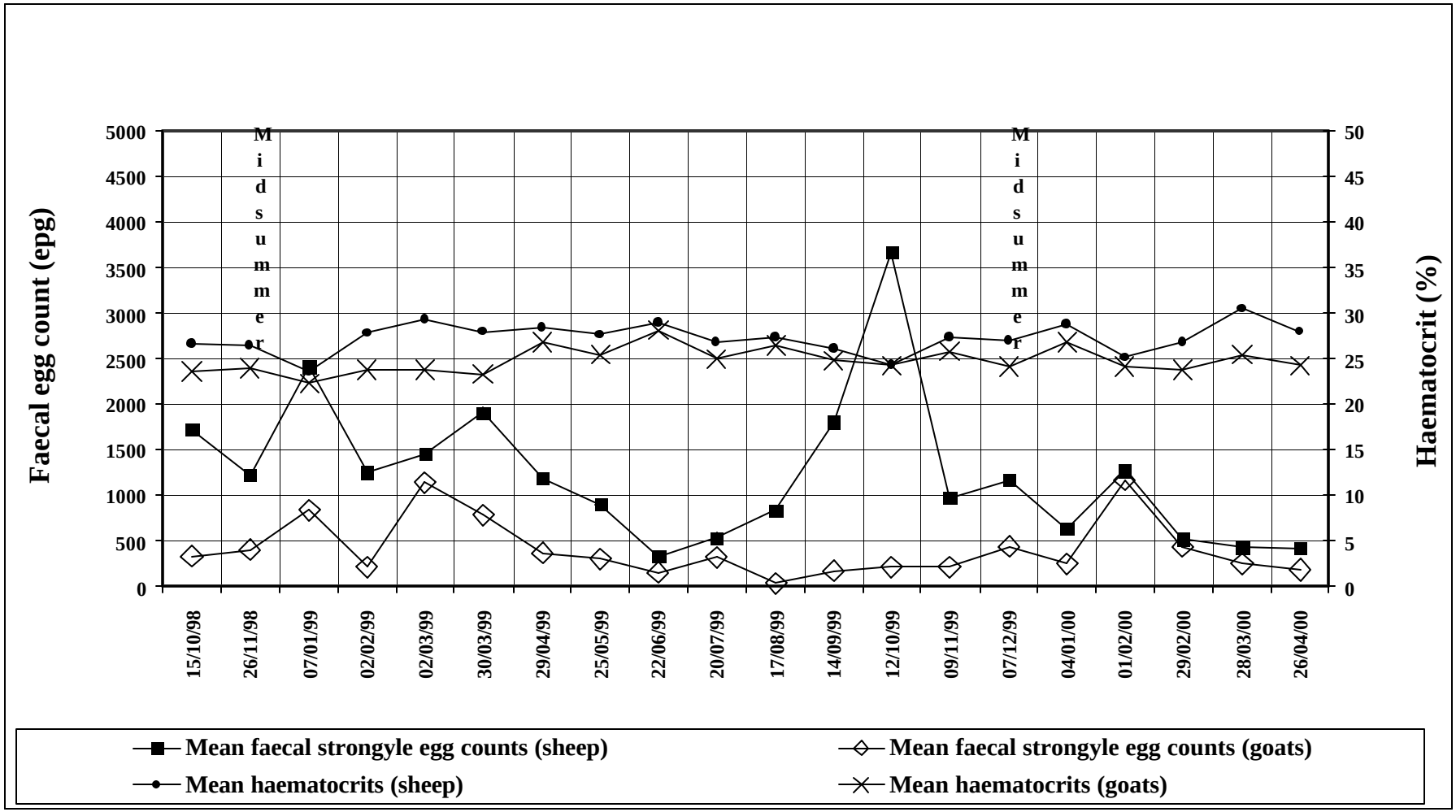


Table A5.1

Results of Wilcoxon two-sample test for comparing mean faecal strongyle egg counts between goats and sheep of Kraaipan

Date	Goats			Sheep			Probability
	Mean	SD	n	Mean	SD	n	
15-Oct-98	339	304	23	1,729	1,554	21	<0.0001
26-Nov-98	395	338	20	1,222	886	18	<0.01
07-Jan-99	829	996	17	2,400	2,033	4	<0.05
02-Feb-99	211	270	18	1,256	1,211	18	<0.0001
02-Mar-99	1,144	1,236	18	1,459	1,629	17	NS
30-Mar-99	786	788	14	1,900	1,519	17	<0.05
29-Apr-99	371	576	14	1,183	1,182	12	NS
25-May-99	306	496	17	900	857	15	<0.05
22-Jun-99	153	255	17	335	304	17	NS
20-Jul-99	317	477	18	541	555	17	NS
17-Aug-99	385	65	13	835	737	17	<0.001
14-Sep-99	171	149	17	1,800	1,387	15	<0.0001
12-Oct-99	224	217	17	3,669	3,127	16	<0.0001
09-Nov-99	212	209	17	964	1,112	14	<0.01
07-Dec-99	429	455	17	1,163	1,436	16	<0.05
04-Jan-00	247	314	15	631	497	13	<0.05
01-Feb-00	1,173	1,021	11	1,271	1,469	7	NS
29-Feb-00	440	591	10	517	436	6	NS
28-Mar-00	250	190	10	433	731	6	NS
26-Apr-00	190	185	10	417	436	6	NS

SD: standard deviation.
n: sample size.
NS: not significant.

Table A5.2

Results of Wilcoxon two-sample test for comparing mean haematocrits between goats and sheep of Kraaipan

Date	Goats			Sheep			Probability
	Mean	SD	n	Mean	SD	n	
15-Oct-98	23.5	3.2	23	26.8	2.8	21	<0.01
26-Nov-98	23.9	2.3	20	26.1	2.4	18	<0.01
07-Jan-99	22.0	3.3	17	23.3	6.5	4	NS
02-Feb-99	23.8	3.2	18	27.7	3.5	18	<0.01
02-Mar-99	23.8	3.4	18	28.9	2.4	17	<0.001
30-Mar-99	23.3	2.8	14	27.9	2.9	17	<0.001
29-Apr-99	26.8	2.1	14	28.4	3.1	12	NS
25-May-99	25.5	3.2	17	27.5	4.1	15	NS
22-Jun-99	28.1	3.6	17	29.0	2.2	17	NS
20-Jul-99	25.0	2.5	18	26.7	2.6	17	NS
17-Aug-99	26.5	3.1	13	27.0	2.6	17	NS
14-Sep-99	24.7	3.2	17	26.1	3.2	15	NS
12-Oct-99	24.3	3.3	17	24.3	3.7	16	NS
09-Nov-99	25.8	2.5	17	27.2	4.2	14	NS
07-Dec-99	24.1	2.8	17	26.9	2.8	16	<0.01
04-Jan-00	26.7	3.3	15	28.5	2.4	13	NS
01-Feb-00	24.0	4.0	11	25.1	4.1	7	NS
29-Feb-00	23.8	2.6	10	26.7	3.2	6	NS
28-Mar-00	25.4	1.8	10	30.4	1.7	6	<0.01
26-Apr-00	24.2	3.5	10	28.0	2.4	6	<0.05

SD: standard deviation.
n: sample size.
NS: not significant.

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