Epidemiology and Control of Gastrointestinal Nematodes in First-Season Grazing Cattle in Sweden

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Abstract

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Gastrointestinal nematode parasites of first-season grazing cattle (FSGC) are ubiquitous and practically not feasible to eradicate. Heavy infections result in clinical parasitic gastroenteritis (PGE) characterized by diarrhoea, inappetence and weight loss. However, subclinical disease without obvious symptoms constitutes the majority of infections and may cause economic losses due to sub-optimal performance. This thesis is based on 4 separate publications that focus on epidemiology and control of gastrointestinal nematode infections in FSGC in Sweden.

Two grazing experiments were carried out over consecutive years. One 2-year study was performed on semi-natural pastures and a 3-year study was conducted on improved pasturelands. In each study, groups of 10 FSGC were subjected to various parasite control methods in comparison with anthelmintic bolus treated animals (maximum control), and untreated, set-stocked cattle (minimum control). In companion ecological plot experiments, larval availability and overwintering survival on pasture were investigated.

Results from the grazing trial on semi-natural pastures showed that nematode egg contamination of pastures by lightly infected animals during the first half of the season was sufficient to induce PGE the following spring. The rotation group suffered a weight gain penalty of the same magnitude as the untreated cattle (30 kg), compared with the bolus treated animals. The results were explained by the high degree of overwintering survival of the pre-parasitic stages from early season contamination, which was substantiated in the parallel plot study.

The 3-year grazing experiment on improved pastures included evaluation of 1) the nematophagous fungus *Duddingtonia flagrans,* 2) pasture rotation using a turnout pasture grazed the previous season by adult cattle in combination with a mid-summer move to aftermath and 3) copper supplementation. Excellent results were recorded in the pasture rotation group, with growth rates of cattle equal to, or exceeding, those treated with the anthelmintic bolus. In the last year of the trial, the difference between the untreated and the anthelmintic treated cattle was 65 kg. The group fed *D. flagrans* had a weight gain advantage of 45 kg compared with the untreated group, whereas the copper had no control effect. Again, the accompanying plot study provided information to explain the outcome of the grazing trial.

Keywords: *Ostertagia ostertagi*, *Cooperia oncophora*, cattle-nematoda, biological control, *Duddingtonia flagrans*, epidemiology, control, parasitic gastroenteritis

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Svensk sammanfattning

Epidemiologi och kontroll av mag-tarmnematoder hos förstagångsbetande kalvar i Sverige

Mag-tarmnematoder är parasitära maskar som förekommer allmänt i löpmage och tarm hos förstagångsbetande nötkreatur i hela Sverige. Det har visat sig att dessa i praktiken är omöjliga att utrota. Kraftigt infekterade djur insjuknar i parasitär gastroenterit (PGE) som karaktäriseras av diarré, aptitlöshet och dålig tillväxt. Av störst ekonomisk betydelse är dock lindrigare infektioner som ger nedsatt tillväxt hos djuren utan att tydliga sjukdomstecken ses och passerar vanligen djurägaren obemärkt förbi. Denna avhandling baseras på fyra publikationer och syftar till att förbättra kunskapen om mag-tarmnematodernas skadeverkningar och ekologi under svenska förhållanden samt att utvärdera utvalda alternativa kontrollstrategier, utan bruk av avmaskningsmedel.

Två fleråriga betesförsök ligger till grund för avhandlingen. Ett tvåårigt försök utfördes på hagmarksbeten medan det treåriga försöket genomfördes på åkermarksbeten. I de två studierna utvärderades effekten av olika typer av parasitkontroll genom att grupper om vardera 10 kalvar undersöktes regelbundet under olika betessäsonger. Behandlingseffekten av olika alternativa kontrollmetoder jämfördes mellan grupper av kalvar som antingen var avmaskade med vomkapsel (maximal kontroll) eller som var helt obehandlade och gick på permanent nötkreatursbete (minimal kontroll). I parallella försök på en intilliggande betesyta studerades förekomst, dynamik och övervintringsförmåga hos parasiternas frilevande larvstadier liksom nedbrytningshastigheten hos konstgjorda komockor som preparerats från kalvträck i betesförsöken.

Resultaten från hagmarksförsöket visade att ägg som utskiljts från låggradigt infekterade kalvar resulterade i en övervintrad smitta som var tillräcklig för att infektera de kalvar som släpptes i samma fålla (vårfålla) efterföljande vår. Trots att dessa djur flyttades till en annan fålla (höstfålla) i mitten av juli, var tillväxten i genomsnitt 30 kg sämre än hos de avmaskade kalvarna. Tillväxtbortfallet hos de flyttade djuren var till och med jämförbar med den hos obehandlade kalvar som gick kvar i samma fålla hela säsongen. Den mest sannolika förklaringen till det nedslående resultatet är att övervintringsförmågan hos parasitlarverna var mycket god. Denna slutsats styrktes av resultatet från det intilliggande överlevnadsförsöket med konstgjorda komockor. Eftersom äggutskiljningen var som högst under den första hälften av betessäsongen och då denna smitta dessutom övervintrade relativt sett bättre, utgjorde denna andel merparten av det totala antalet övervintrade larver. Faktum var att skillnaden mellan antalet övervintrade larver i den permanenta fållan och i vårfållan var obetydlig. Med andra ord, mönstret hos parasiternas äggutskiljning snarare än den totala tid som kalvarna använde fållan var avgörande för utfallet i detta betesförsök.

Det treåriga experimentet på åkermarksbete innefattade förutom maximal och minimal parasitkontroll enligt ovan även utvärdering av 1) rovsvampen *Duddingtonia flagrans* förmåga att minska betessmittan, 2) användning av ett

välkomstbete som året innan betades av äldre nötkreatur (sinkor) varefter kalvarna flyttades till ett återväxtbete i mitten av juli, och 3) tillskott av mineraliskt koppar via en kapsel som utsöndrade kopparjoner under de första 90 dagarna av betessäsongen. Resultaten visar att okontrollerade parasitinfektioner kan ge tillväxtbortfall på 50% jämfört med den hos avmaskade djur med tillgång till likvärdiga beten av samma kvalitet. Vid installning det sista året var viktskillnaden mellan de obehandlade och de avmaskade, ivermektinbehandlade kalvarna, i genomsnitt 65 kg. Den undvikande betesstrategi som utvärderades i detta experiment gav, till skillnad mot den som användes i hagmarksförsöket, en utmärkt parasitkontroll. Tillväxten hos de flyttade djuren var denna gång i klass med den hos de avmaskade kalvarna, eller till och med något bättre. Genom att kombinera ett låggradigt nedsmittat välkomstbete med en flytt till återväxtbete kunde både acceptabel parasitkontroll och effektivt betesutnyttjande uppnås under tre på varandra följande betessäsonger. Det bör poängteras att denna strategi förutsätter att det finns tillgång till såväl äldre nötkreatur som återväxtbeten.

Även rovsvampen reducerade betessmittan, men förutsätter att komockan är relativt intakt de första veckorna efter deponering. Den torra väderleken under andra betessäsongen (1999) medförde långsam nedbrytning av komockorna och gav således goda betingelser för svampen att förhindra parasitlarverna att vandra ut i betesgräset, vilket också resulterade i en låggradig övervintrad smitta. Följaktligen utsattes kalvarna i rovsvampsgruppen det sista året (2000) för en jämförelsevis låg infektionsdos vid betessläppning och de vägde vid installning det tredje och sista året 45 kg mer än de obehandlade djuren.

Under det avslutande årets regniga sommar visade sig svampen däremot inte alls fungera tillfredsställande, vilket sannolikt kan förklaras med att komockan bröts ned inom, i vissa fall, två veckor och därmed omintetgjordes svampens möjligheter att fånga larverna. Den sämre effekten kom dock inte att avspegla sig i högt antal parasitlarver i betsgräset förrän efter installning, och förklarar varför kalvarna i rovsvampsgruppen växte bättre än de obehandlade djuren. Under den påföljande våren, efter avslutat betesförsök (2001), genomfördes undersökningar för att bestämma den övervintrade smittan. Denna visade sig då vara lika stor i svampfållan som i den fålla där obehandlade kalvar gått hela föregående året. Kopparbehandlingen gav inga mätbara parasitkontrollerande effekter något av åren.

Resultaten från försöket med konstgjorda komockor visade att den betessmittan varierade under betessäsongen och att betesgräset på kort tid kan övergå från att vara låggradigt till höggradigt smittat. Den larvsmitta som djuren utsätts styrs framförallt av regnmängd, regnintensitet och komockans nedbrytningshastighet. Andelen övervintrade larver kan skilja sig åt tiofaldigt mellan olika år vilket innebär att en låggradig nedsmittning med ägg under föregående år inte nödvändigtvis behöver innebära att ett sådant bete är att betrakta som ”säkert” ur parasitologisk synvinkel påföljande år.

Det kunde inte påvisas någon skillnad i nedbrytningshastighet mellan komockor som hade sitt ursprung från kalvar som var obehandlade, behandlade med vomkapsel innehållande ivermektin eller hade givits rovsvampen *D. flagrans*. Kraftigt regn kort efter nedläggning resulterade i totalt försvinnande inom två veckor, medan långvarig torka fördröjde nedbrytningen i upp till 12 månader. Med andra ord, nedbrytningshastigheten var oberoende av om komockorna innehöll ivermektin, rovsvamp eller om komockorna kom från obehandlade kalvar. De tre årens varierande väderbetingelser visade däremot att nedbrytningen var beroende av nederbördsmängden och nederbördsintensiteten.

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Dimander, S.-O., Höglund, J., Spörndly, E. & Waller, P. J. 2000. The impact of internal parasites on the productivity of young cattle organically reared on semi-natural pastures in Sweden. *Veterinary Parasitology 90*, 271-284.

II. Dimander, S.-O., Höglund, J., Uggla, A., Spörndly, E. & Waller, P. J. 2003. Evaluation of gastrointestinal nematode parasite control strategies for first-season grazing cattle in Sweden. *Veterinary Parasitology 111*, 193-209.

III. Dimander, S.-O., Höglund, J. & Waller, P. J. 1999. The origin and overwintering survival of the free living stages of cattle parasites in Sweden. *Acta Veterinaria Scandinavica 40*, 221-230.

IV. Dimander, S.-O., Höglund, J. & Waller, P. J. 2003. Seasonal translation of infective larvae of gastrointestinal nematodes of cattle and the effect of *Duddingtonia flagrans*: a 3-year plot study. (submitted).

Abbreviations

ANOVA	analysis of variance
BC	biological control
COWP	copper oxide wire particle
CP	crude protein
CI	confidence interval
C. oncophora	*Cooperia oncophora*
D. flagrans	*Duddingtonia flagrans*
DM	dry matter
Epg	trichostrongyle nematode eggs per gram faeces
FSGC	first-season grazing cattle
GI	gastrointestinal
KRAV	kontrollförening för ekologisk produktion (the Swedish certification body for organic production)
L_3	infective third stage trichostrongyle nematode larva/-ae
NDF	neutral detergent fibre
O. ostertagi	*Ostertagia ostertagi*
PGE	parasitic gastroenteritis
SLU	Sveriges lantbruksuniversitet (Swedish University of Agricultural Sciences)
SPC	serum pepsinogen concentration
SRB	Swedish red and white breed
SSGC	second-season grazing cattle
SVA	Statens veterinärmedicinska anstalt (National Veterinary Institute)
SvDHV	Svenska Djurhälsovården (Swedish Animal Health Service)
SWEPAR	Department of Parasitology, SVA/SLU

Diarrhee

”Äro Fänaden mer än hästar underkastade, i synnerhet de betande, som förtära purgerande gräs

Ur ”Sjukdomslära – husdjurens inre sjukdomar” av Peter Hernquist (1726–1808), den svenska veterinärmedicinens fader

(In English: Said on **Diarrhoea**; *”Do cattle suffer from, more than horses, in particular the grazing cattle that consume purgative grass”*

Citied from ”Mechanism of disease – internal diseases of livestock” by Peter Hernquist, 1726–1808, the father of veterinary medicine in Sweden)

A first-season grazing SRB cattle in an experimental paddock at the Kungsängen Research Farm (SLU), Uppsala, Sweden. Photo: Sten-Olof Dimander

Introduction

The development of nematode parasite control strategies for first-season grazing cattle (FSGC) of today presents a challenge. The requirements not only have to apply to the changing and somewhat conflicting needs of the livestock producer (Nansen, 1987). Also, they have to be accepted by the increasingly urbanised consumers who have become gradually estranged from contacts with contemporary farm life. Fewer but larger farming units are under pressure to provide an increasing number of consumers with reliable food to a low cost on a competitive market, they also have to accomplish this with due recognition of animal welfare demands as well as to avoid the excessive use of agrochemicals (Sykes, 1994). It is obvious that these issues are conflicting.

The cattle is the economically most important livestock species in Swedish agriculture and the costs associated with gastrointestinal (GI) nematode infections can be substantial (Nansen, 1988; McLeod, 1995). Until recently, safe and effective drugs have been available for all important GI nematodes of livestock and these have had a major influence on the advancement of animal health and productivity of modern animal husbandry schemes (Zajac, Sangster & Geary, 2000). Nevertheless, this apparently happy state of affairs is now threatened.

Organic agricultural production is possibly the most fashionable topic on the modern farming agenda in Europe. This has been recently reviewed by Lund (2002) who concluded that animal health in organic farming is acceptable, however, with the important exception of parasitic diseases. Organic production is guided by the principles of a minimum use of drugs and feed additives, and the promotion of natural animal behaviour. These issues are now increasingly well recognised in Sweden and the goal is to have 10% of the cattle production organically reared by the end of 2005 (Andersson, 2001). Ruminants are kept outdoors for prolonged grazing periods and pasture should comprise at least 50% of the roughage intake (KRAV, 2002). The routine preventive use of anthelmintics is expressly banned and macrocyclic lactones are discouraged unless no alternative option is available.

The worldwide emergence of anthelmintic resistance in virtually all the important nematode parasites of grazing livestock is alarming. For the small ruminant industries (sheep and goats), this situation has now reached a crisis point in some countries (Waller, 1997). Fortunately, anthelmintic resistance is presently not a concern in cattle parasites in Sweden (Waller & Höglund, 1998a) and there are good reasons for why available drugs will remain effective (Waller & Höglund, 1998b; Coles, 2002). However, anthelmintic resistance is not a serious threat for the cattle industry today, it would be unwise to count on anthelmintics as a last resort for the future, as research in anthelmintic pharmacology is devalued by governments, universities and the animal industry in general (Geary, Sangster & Thompson, 1999).

This thesis endeavours to investigate the epidemiology of GI nematode infection in FSGC in Sweden and to evaluate assorted alternative control strategies for these infections. Based on this, further studies of alternative parasite control methods and ecological investigations of the pre-parasitic stages of GI nematodes will be initiated.

Background

History

Modern helminth epidemiological thinking has its cradle in Australia, where Hugh McL Gordon conducted pioneering studies in sheep (Gordon, 1948, 1949). He stated that parasitic disease is characterised by an indefinite borderline between safe and dangerous worm burdens, where the manifestations of disease commonly are subclinical and insidious in onset. Gordon's approach stimulated other parasitologists to conduct further epidemiological work. Detailed investigations on the epidemiology of cattle GI nematode infections in temperate regions were conducted during the 1960's, particularly in the United Kingdom and mainly in Glasgow, Scotland, (Armour, 1980) and Weybridge, England (Michel, 1969b, 1976, 1985).

In Sweden, Hoflund & Koffman (1948) paid attention to the concept of GI parasite infections in cattle in a most sensible way. Although not referring to local scientific data, they pinpointed the subclinical nature of parasitic disease and the risk of other detrimental effects as a sequel to parasitism. He also recognised the substantial economic losses involved but underlined the difficult task to substantiate this in monetary units. Moreover, he mentioned the importance of specific epidemiological knowledge of parasitic infections and emphasised that actions for parasite control should be preventive rather than curative. As a consequence of Hoflund's work, there has for long been an awareness in Sweden of the potential impact of parasitism. However, the challenge still remains in distributing the message to farmers of how to adopt necessary precautions to avoid PGE in their herds.

During the 1970's and 1980's, different epidemiological investigations of GI nematode infections in cattle were performed in Sweden. Field experiments on naturally contaminated pastures were conducted (Nilsson & Sorelius, 1973; Olsson & Holtenius, 1980a; Törnquist & Tolling, 1983, 1987) where performance, parasitological parameters and larval availability on herbage were monitored. More specific studies, such as investigations on the ecology of the pre-parasitic stages of GI nematodes (Persson, 1974c) and including the use of tracer cattle (Olsson & Holtenius, 1980b) were carried out. Persson also performed several studies on the tolerance of the pre-parasitic stages in environments other than herbage (Persson, 1973a, b, 1974b, d, e, f, g). Recently, a questionnaire study (Svensson, Hessle & Höglund, 2000) and a field survey (Höglund, Svensson & Hessle, 2001) were conducted to obtain information on the control methods used in organic and

conventional dairy herds and to investigate the status of internal parasitism in cattle on such farms.

However, detailed investigations on the ecology and dynamics of the pre-parasitic stages of cattle nematodes, as companion studies to applied field grazing experiments, have never been executed in Sweden before. This would significantly improve the comprehensive evaluation and understanding of the results obtained from the parasite control measurements studied.

Current situation for Swedish cattle producers

Contemporary farming is conducted on a competitive market which requires versatile, informed and motivated managers who conduct their farming operations according to contemporary business principles. Agricultural policy is more complex than ever and subsidies for cattle meat production comprise 30 to 50% of the receipts in cost estimates, regardless of whether it comprises organic or conventional production systems (M. Alarik, Hushållningssällskapet, personal communication). Financial support is given for keeping defined classes of animals under defined conditions for defined periods of time (Anonymous, 2001). Unfortunately, the rules may even turn preferred grazing management regimes for parasite control aside, as the farmers are forced to keep grazing animals set-stocked. In addition, these directives may result in a compromised nutritional status of the animals.

Hence, it can be argued that it is irrational to have an inflexible system where as much as half of the receipts emanate from subsidies that support inefficient farming practises and thereby inhibit exclusive companies to take up the unique opportunities as they arise to turn their enterprise into the best. Time and skilled labour are depleted resources of modern farming, and, unfortunately, farmers are not being rewarded for what they generally do the best, namely efficient animal husbandry practise. Instead, the farmer needs to spend excessive time to conform to a standardising, wandering and obfuscating bureaucratic system that is inherent with the livestock production enterprises of member countries of the ever-expanding European Common Market.

First-season grazing cattle in Sweden

The absolute majority of detrimental GI nematode infections in cattle are contracted by FSGC (Sykes, 1994). However, apart from GI nematode infections, there are other pasture-borne infections that affect grazing cattle. The most important are coccidial and lungworm infections, and these have been studied in Sweden. *Eimeria* infections were investigated by Svensson (1994) who concentrated her work on the epidemiology and the significance of coccidial infections as a cause of diarrhoea in Swedish cattle during the first weeks after turnout. In the case of the bovine lungworm *Dictyocaulus viviparus*, work so far has been focussed on the transmission biology and in particular the role of wild ruminants as a possible reservoir (Höglund*, et al.*, 1999; Divina*, et al.*, 2000; Höglund*, et al.*, 2001).

The animals of primarily concern in this project were FSGC kept on pasture for the purposes of meat production (steers) and heifers aimed for recruitment in the dairy milk production.

Of the estimated 1,637,500 cattle in Sweden in June 2002, 5% (20,000) were reared organically (2001) according to the standards of KRAV (2002). There is a continuous structural change of the dairy enterprise with an unabated trend of a decreased number of animals reared on even fewer farm units, and hence, since 1995 the average number of dairy cows per farm has increased from 27 to 37 (SJV, 2002).

Furthermore, Sweden is characterised of large differences in climate and farm management due to the geography of the country and a highly variable landscape. These conditions necessitate caution before extrapolation of results, or experiences, obtained from one region to another, let alone from other countries. For instance, the normal grazing season comprise 4 months in the southern parts, but only 2 months in the north of Sweden (SJV, 2003). Consequently, differences in management may require some modifications to any standard approaches to parasite control.

Gastrointestinal nematodes of cattle in Sweden

Species and life cycles

In temperate regions, the most important nematode parasite species of cattle is the abomasal worm *Ostertagia ostertagi.* Of significance is also the small intestinal worm *Cooperia oncophora.* Typically, a mixture of *O. ostertagi* and *C. oncophora* are present under farm conditions. Both these hair-like bursate nematodes belong to the superfamily Trichostrongyloidea. Other members of this group are almost exclusively found in the alimentary tract of a range of ruminants. Closely related genera are *Haemonchus*, *Nematodirus*, *Trichostrongylus* and the lungworm *Dictyocaulus.*

Ostertagia ostertagi was first described by von Ostertag in 1890, but was renamed by Stiles (1892), while *C. oncophora* was first described by Railliet (1898) in France. Both of these parasites are the most important and prevalent species of GI nematodes of cattle also in Sweden (Nilsson & Sorelius, 1973). Apart from *O. ostertagi* and *C. oncophora*, 14 additional GI nematode species have been demonstrated in cattle in this country (Nilsson & Sorelius, 1973). Although the significance of these latter species is unknown, they are unlikely to be important even in the local context.

Ostertagia ostertagi and *C. oncophora* have direct life cycles (Fig. 1) as described in detail by Frankena (1987). Eggs are passed in faeces (1) and under optimal conditions the L_3 develop through the first (L_1), second (L_2) and third larval stages (L_3) within 2 weeks (2). However, the time for development from egg to L_3 is highly variable and the proportion of successful development is erratic (Ciordia & Bizzel, 1963). Before resumption of the life cycle, L_3 have to be transferred from the faecal environment to herbage and become available to the grazing cattle (3), *i.e.* translation (Rose, 1961). After ingestion of contaminated grass by susceptible

cattle, development takes place in the lumen of an abomasal gland (*O. ostertagi*) (4a), or in the small intestine (*C. oncophora*) (4b). The parasite moults twice and becomes sexually mature. The prepatent period is approximately 3 weeks, but if L_3 are subjected to stimuli in the external environment, the development of both *O. ostertagi* and *C. oncophora* may become arrested at the early fourth larval stage. The complex issue of inhibition has been thoroughly examined in a number of investigations (Armour*, et al.*, 1973; Armour & Bruce, 1974; Michel, Lancaster & Hong, 1975, 1978). In Sweden, arrested development of *O. ostertagi* was described by Olsson & Holtenius (1980b), and Olsson (1977) speculated that the period of inhibition of *O. ostertagi* may be prolonged under Swedish conditions as compared to the situation in areas with shorter winter periods.

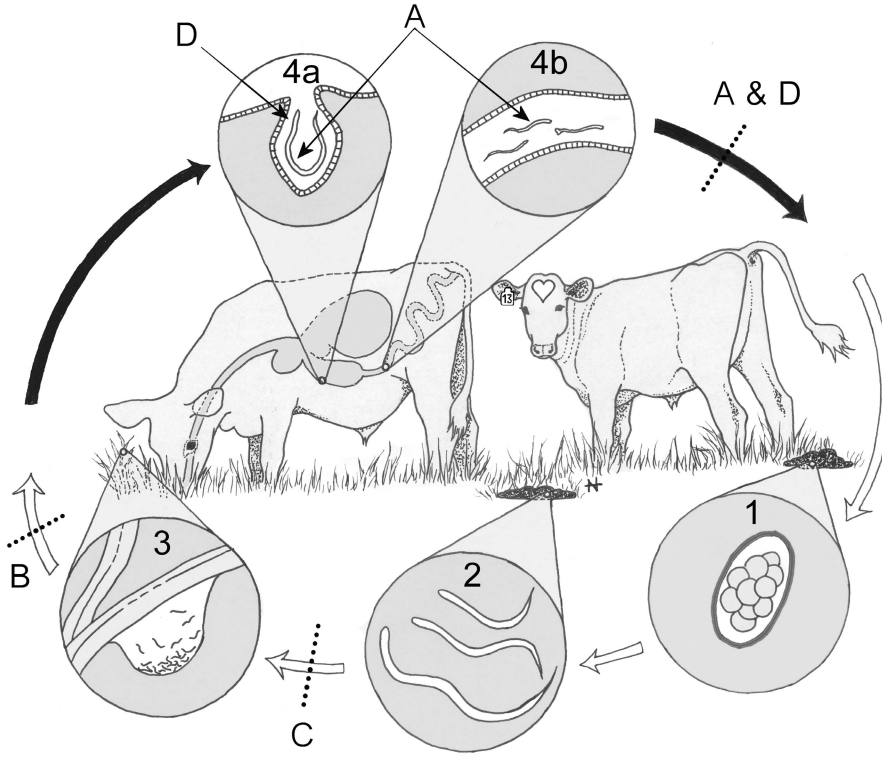

Fig. 1. The life cycles of *Ostertagia ostertagi* and *Cooperia oncophora* and the principles for parasite control. For description of the life cycle, see the text. The points of interference with the life cycle for the different modes of control evaluated in this thesis are indicated with letters and dotted lines (see pp. 27–30, "Control", for details). A) anthelmintics, B) grazing management, C) biological control (BC) and D) copper supplementation (COWP). The filled arrows represent the parasitic part of the life cycle, and the open arrows the pre-parasitic part. Drawing: Helena Nordenfors

Grazing, stocking rate and exposure to L_3

The grazing paddock is a combined kitchen and living room as well as a lavatory, and it thus provides an excellent environment for spread of infectious agents emanating from faeces (Fig. 2). As the source of parasite infection is contaminated herbage, grazing behaviour will influence the course of infection. Taylor (1954) stated that "a better knowledge of grazing habits would lead towards a better understanding of the epidemiology of parasitic disease".

The pre-parasitic stages are not evenly distributed on pasture, and logically, L_3 concentrate close to the faecal pat. That cattle keep away from grazing the rank tussocks surrounding faecal pats is a well-known phenomenon that reduces exposure (Durie, 1961; Rose, 1961; Greenhalgh & Reid, 1968; Gruner & Sauve, 1982). However, when the sward height declines or if stocking rates are high, animals will graze closer to the faecal pats (Rose, 1961; Morley & Donald, 1980). Furthermore, the spatial distribution of L_3 in the environment is dynamic, and above all, influenced by the presence of a water film necessary for active migration of L_3 (Crofton, 1954). This may have implications as grazing cattle prefer leaves before the stem of herbage (Chacon & Stobbs, 1978). Mechanical dispersal and rain splash will further enable distribution of L_3 to the close proximity of the faecal pat (Grønvold & Høgh-Schmidt, 1989). Although cattle to some extent avoid certain plants, *i.e.* buttercup (*Ranunculus* spp.), there is little evidence that the botanical composition has any influence on exposure of cattle to L_3 on pasture (Morley & Donald, 1980).

Understandably, as the L_3 are not evenly distributed on pasture, any systematic selection will enable lower or higher exposure to L_3, as illustrated by the avoidance of tufts around faecal pats. However, Michel (1955) observed that herbage samples plucked in direct proximity to where cattle grazed yielded fewer L_3 than randomly collected herbage (tussocks excluded) and concluded that bovines may discriminate beyond the evasion of herbage surrounding faecal pats.

Although cattle present a highly variable grazing behaviour and larval availability is a dynamic phenomenon influenced by many factors, it can be assumed that high stocking rates or poor pasture allowance, or even worse, the combination of both, will increase exposure of L_3 to grazing cattle. If this further coincides with wet conditions that promote translation on a contaminated pasture, the risk of parasite disease is impending. The optimum would be to be able to ascertain the level of larval exposure to grazing animals at a given point in time. However difficult to attain, this is a critical issue in the epidemiology of PGE as the rate of infection, rather than the accumulated challenge will determine the magnitude of the worm burden and thus the risk of disease (Michel, 1969b).

Fig. 2. A grazing cattle in action (top) and a faecal pat (bottom). The grazing paddock is a combined dining-table and lavatory. As herbage is the source of GI nematode infections, and as the L_3 are not evenly distributed, stocking density and grazing behaviour will influence GI nematode exposure to the cattle. Photos: Sten-Olof Dimander (top) and Staffan Wiktelius (bottom)

Diagnosis

Animals

Grazing ruminants will always be exposed to parasite nematodes and the typically subclinical nature of parasitoses necessitates the aid of quantitative diagnostic tools to monitor worm burdens, or even better, the level of immunity. Thus, the question is not if FSGC are infected, but the level of their infections. Provided an adequate anamnesis, the relatively rare cases of clinical infections are in most instances easy to diagnose (Eysker & Ploeger, 2000).

There are both direct and indirect diagnostic methods available for GI nematode infections. Direct diagnosis is possible only through pathology and worm counts of dead or slaughtered animals. The obvious advantage is the possibility to demonstrate the parasites *in situ* as well as the proportion of inhibited stages. The performance of worm counts is a time and labour demanding task that requires experience and attention to detail to obtain valid results and correct conclusions. However, apart from the cost of animals, an inherent limitation with conducting worm counts is the great variability between animals in worm establishment, the fact that parasites tend to pass out of the gut of moribund animals, and that a worm count represents only an estimation at one point in time.

Faecal examination will only provide information of the egg output of the GI worms and give an indirect estimation of the worm burdens as well as the level of pasture contamination. Faecal egg count is an easily applicable and widely used diagnostic tool for both qualitative and quantitative diagnosis. The McMaster technique with a sensitivity of 50 eggs per gram (epg) (Urquhart*, et al.*, 1996) is perhaps the most widely used method. However, modifications of the methods are utilized, which make comparisons of results difficult. Although technically simple, faecal egg counts require careful preparation procedures and skilled labour for the reading and interpretation of the results. Despite thoroughness, repeated analyses from the same faecal sample may vary considerably. Moreover, faeces are characterised of a dynamic consistency (water content) and the faecal egg excretion may not be consistent. These are aspects that need to be brought in mind when results from faecal egg counts are under review (Anonymous, 1986).

Nevertheless, faecal egg counts in relation to infection levels in cattle seems to be valid approximately 2 months after turnout, but only if the initial levels of infection is low at the beginning of their first grazing season (Ploeger*, et al.*, 1994). One problem in the interpretation of faecal egg output is the similarity of the eggs from trichostrongylid nematode species of cattle. Considering the different pathogenicity, differences in fecundity, immunity and age-related resistance between *O. ostertagi* and *C. oncophora* (Kloosterman, Ploeger & Frankena, 1991), it is often desirable to further differentiate between the nematode species producing the eggs. Faecal larval cultures will enable additional separation between genera and their relative proportion. However, results from the faecal culture do not necessarily mirror the actual proportion off eggs in faeces, as this is only valid under the assumption of equal propensity of hatching between the egg producing species. It is known that yields from faecal larval cultures are never 100% and when, for some reason, the yield is low, the proportion of larvae developing may differ between species (Eysker & Ploeger, 2000). The fact that *C. oncophora* is

more fecund than *O. ostertagi* may affect the results from pooled cultures as it has been observed that very high egg counts are likely to comprise 80–90% *Cooperia* eggs (Kloosterman, Ploeger & Frankena, 1991). Thus, results from pooled faecal cultures may therefore bias towards an overestimation of the presence of *Cooperia* on a group basis.

Measurement of the serum pepsinogen concentration (SPC) is a quantitative diagnostic tool for parasitic gastritis in cattle infected with *O. ostertagi.* This method was first applied by Jennings*, et al.* (1966), but still the exact pathogenesis behind the development of hyperpepsinogenaemia remains to be explained. It is suggested that pepsinogen either enters the bloodstream due to increased permeability, or direct by a retrograde hypersecretion of pepsinogen-producing cells (Fox*, et al.*, 1989). A logical conclusion though is that the mechanisms behind the elevated SPC is more complicated than previously believed and may be multifactorial in origin (McKellar*, et al.*, 1986). Explicit levels of SPC using the method described by Berghen, Dorny & Vercruysse (1987) have been proposed for clinical and subclinical PGE, respectively (Hilderson*, et al.*, 1989), but implies no between laboratories variation. However, comparisons between laboratories are currently lacking and makes extrapolation of results from one laboratory to another questionable (Eysker & Ploeger, 2000) and specific levels given in different studies difficult to interpret (Shaw*, et al.*, 1998).

Elevated blood gastrin levels have also been correlated with patency in *O. ostertagi* infections, although this test is far less sensitive than that of SPC and also more expensive to perform (Eysker & Ploeger, 2000).

The demonstration of specific antibodies to *O. ostertagi* and *C. oncophora* indicates infection or previous exposure and has been used for research purposes and in epidemiological surveys (Nødtvedt*, et al.*, 2002; Sanchez & Dohoo, 2002; Sanchez*, et al.*, 2002), but currently, no such assays are commercially available. Acquired immunity can be assessed by the determination of different immunological parameters and Claerebout & Vercruysse (2000) recently reviewed the immunological methods available. However, because of the limited understanding of the mechanisms behind protective immune responses against GI nematodes in cattle, the best way to measure resistance is probably to estimate parasitological parameters after challenge infections.

Pasture

Herbage is the source of infection, and understandably, naïve cattle (tracer animals) or pasture larval counts will serve as a direct diagnostic aid to estimate the risk of PGE. Indeed, such information will be of value to indicate the level overwintering survival and to obtain data to study the seasonal dynamics of larval availability (Couvillion, 1993; Eysker & Ploeger, 2000). The use of tracer animals is expensive and labour intensive, but facilitate differentiation of the prevalent species of cattle nematodes and provide data on the actual exposure to grazing cattle, presumed normal grazing behaviour. As regards pasture larval counts, the analysis should not end with the quantification of L_3, but should also include differentiation to genus, as this will add further information about possible differences in the bionomics of the genera involved.

Translation is facilitated when wet conditions prevail and is limited under dry conditions (Rose, 1961). As discussed above, larval availability is highly dynamic and grazing behaviour is complex. When pasture samples are collected, it is not possible to imitate the grazing cattle and the between-samplers variation may be large (Eysker & Ploeger, 2000). In addition, while grazing is more or less a continuous process, collection of herbage will only allow point estimations. Consequently, results from pasture larval counts need to be interpreted in a rational way and the methods used should be maintained over the course of the trial. Pasture larval counts as a diagnostic tool were reviewed by Couvillion (1993) who concluded that comparison of results obtained from different experiments is questionable as the sampling and processing techniques are highly variable between laboratories. Moreover, pasture sampling and processing in the laboratory are extremely laborious (Eysker & Ploeger, 2000). Hence, it is difficult to design a statistically satisfactory protocol for pasture larval counts in a field situation.

Clearly, a number of diagnostic methods are available and these are often used in combination. However, all employed methods may not point in the same direction, but one has to be prepared for conflicting results (Claerebout & Vercruysse, 2000).

Epidemiology of gastrointestinal nematodes in cattle

Pathogenicity and clinical effects

Infections with GI nematodes in cattle lack pathognomonic signs and do not necessarily result in disease. Cattle GI nematodes are ubiquitous and PGE can be regarded as more or less a man-made problem (Sykes, 1994). Intensive production with high animal densities and the separate rearing of susceptible young stock inevitably increase the risk for detrimental impact of parasite infections (Nansen, 1987). Fortunately, clinical parasitic infections nowadays are a rarity in the developed world, mainly due to the prophylactic use of highly effective anthelmintics in combination with grazing management, but also as a result of improved animal nutrition. An animal in optimal nutritional condition *withstands* the effects of a defined level of infection better than an animal of poor status. The ability to withstand infection and the concept of resilience has been reviewed by van Houtert & Sykes (1996). However, since the majority of losses are seen in the absence of overt clinical signs, they are difficult to assess (Vercruysse & Claerebout, 2001).

The pathophysiological effects of PGE in cattle have been thoroughly reviewed (Holmes, 1987; Fox, 1993, 1997). Nematode infection in the GI tract gives rise to inappetence, impaired gastrointestinal function and alterations in protein, energy and mineral metabolism and changes in water balance. Consequences on this are changes in body composition and carcass quality (Fox, 1993).

Clinical PGE is characterised of watery diarrhoea, reduced weight gain or weight loss, dull hair coat, anorexia and a general loss of condition (Anderson*, et al.*, 1965). Subclinical PGE is the situation when overt clinical signs are absent but when infections are associated with production losses (Vercruysse & Claerebout, 2001). When PGE changes from being subclinical to clinical is a matter of subjective judgement. Michel (1985) concludes that the definition of subclinical PGE has been markedly altered over the years and claims that "the normal healthy animal some decades ago would today be recognised as suffering from clinical PGE". Even at present, by the aid of available diagnostic methods, subclinical PGE remains to be defined (Vercruysse & Claerebout, 2001).

Parasitic gastroenteritis may be viewed as starvation in the midst of plenty. Reduced feed intake is a well-known feature of trichostrongyle infections in ruminants (Coop & Holmes, 1996). Although the mechanisms behind this inappetence are still not fully understood, at least in chronic subclinical infections in sheep, reduction of feed intake can range between 15 and 20% (for review, see Coop & Kyriazakis, 1999).

Data on reduced feed intake due to GI nematode infections in cattle are scarce. However, Forbes*, et al.* (2000) performed a grazing experiment where he used an automatic system for measuring of foraging behaviour in free-ranging ruminants (Rutter, Champion & Penning, 1997). They observed that cattle treated with the ivermectin sustained-release bolus grazed, on average, 105 min longer per day than animals with evidence of patent nematode infections.

Although PGE is a multifactorial disease syndrome, by far the most work has been conducted on infections with *O. ostertagi* (Armour, 1980; Taylor*, et al.*, 1989). Despite the frequent occurrence of *C. oncophora,* this parasite has often been disregarded in favour of the more pathogenic *O. ostertagi* (Armour*, et al.*, 1987). However, work on artificial trickle infections with *C. oncophora* resulted in inappetence, weight loss and other pathological effects (Armour*, et al.*, 1987). Concurrent infections with *O. ostertagi* and *C. oncophora* were investigated by Parkins*, et al.* (1990) who found that these concomitant infections produced severe alterations in metabolism, which exceeded those produced by either species alone. They concluded that reciprocal establishment of *O. ostertagi* and *C. oncophora* resulted in a synergistic pathogenic effect. In contrast, Satrija & Nansen (1993) were unable to demonstrate any synergistic or antagonistic effect of *C. oncophora* on the course of a concurrent *O. ostertagi* infection. Nevertheless, in experimental infections, it is important to consider *O. ostertagi* and *C. oncophora* in combination and to make efforts to mimic the natural situation where larval exposure is continuous and dynamic.

Immunity

The immunological responses following GI nematode infections are complex. So far, only limited information is available about the mechanisms involved in the development of protective immunity in cattle (for reviews, see Vercruysse & Claerebout, 1997; Claerebout & Vercruysse, 2000). The processes amenable to regulation of the parasite burden within the host are establishment, egg production and death of established worms (Barger, 1987). However, these are highly dynamic events difficult to study unless helminthologically naïve animals are used. As such animals are rare in the field situation, the continuous exposure of L_3 in already infected cattle implies that several events take place concomitantly. Hence, the regulation and succession of the worm burden becomes a difficult scientific task to solve (Barger, 1987). Arrested larval development, which to some extent is thought to be influenced by host immunity, further complicates this issue (Schad, 1977).

Protective immunity develops more quickly against *C. oncophora* than against *O. ostertagi* (Armour, 1989; Dorny*, et al.*, 1997). The immunity to reinfection against *C. oncophora* is virtually absolute by the end of the first grazing season, or the second year of life in young cattle (Armour, 1989) whereas the protective immune responses against *O. ostertagi* are weaker and a much longer period of exposure is required to make them discernible (Claerebout & Vercruysse, 2000). In practise, this is reflected in adult cattle as they continue to harbour low numbers of mostly *O. ostertagi* despite a history of multiple grazing seasons (Agneessens*, et al.*, 2000; Borgsteede*, et al.*, 2000). The faecal egg counts of these adult cattle are normally low, but may perpetuate the presence of infective larvae on pasture. As the actual number of eggs deposited on pasture is also dependent on the amount of faeces produced, low egg counts in adult cattle do not exclude considerable dissemination of eggs on pasture (Stromberg, 1997).

Age resistance against *O. ostertagi* and *C. oncophora* in cattle were investigated by Kloosterman, Ploeger & Frankena (1991). Groups of cattle, 3, 6 and 9 months old, were artificially infected with 10,000 L_3 of each species 3 times a week for 6 weeks. After 9 weeks the animals were slaughtered. Although some conflicting results were observed, the general conclusions were that no age-related resistance was found against *O. ostertagi*, while there was evidence of such an effect against *C. oncophora*. These observations are in support of results presented by Michel, Lancaster & Hong (1973, 1979) and (Armour, 1989), for the 2 species, respectively.

The influence of sex and reproductive status and the susceptibility to nematode parasite infections was reviewed by Barger (1993). Without going into detail, differences in susceptibility to infection between sexes and the status of reproduction seem to exist. In one study, bulls were reported to have higher egg counts than steers and heifers had the lowest when grazing together. The observed disparity may not solely be due to differences in susceptibility but may also depend on a sex-related variation in behaviour that result in differences in exposure (Barger, 1993). A periparturient relaxation of immunity at the time of parturition is well documented in ewes, and also to a lesser extent in cows (Michel, Lancaster & Hong, 1972).

The mechanisms behind the regulation of trichostrongyle GI nematode infections in cattle were reviewed by Barger (1987). Considerable work was carried out on *O. ostertagi* in Weybridge (for review, see Michel, 1976), where it was concluded that the egg production in continuously infected cattle presents a stereotypical egg count trajectory with an early peak around 30 days after initial infection followed by an exponential decline. Under natural conditions with mixed infections, most eggs during the first part of the grazing season are shed by *C. oncophora*. In contrast, the proportion of *O. ostertagi* increases towards the end because immunity develops slower against this parasite and as its fecundity is lower (Eysker & Ploeger, 2000). Thus, as the first grazing season proceeds, a gradual shift in egg production from predominantly *C. oncophora* eggs to *O. ostertagi* is seen as a result of the suppression of *C. oncophora* egg production due to the strong and rapid development of immunity.

Larval dynamics and faecal egg excretion – the Swedish situation

Overwintering L_3

The overwintered generation of L_3 (see Fig. 3 A) is the key factor in the epidemiology of bovine GI nematode infections in Sweden, and may be sufficient to induce clinical disease in FSGC early in the grazing season (Nilsson & Sorelius, 1973). The reason for the enhanced overwintering survival of L_3 in Sweden compared with southern parts of Europe is not known, but a protective effect of snow cover has been proposed as one explanation (Nilsson & Sorelius, 1973). High levels of overwintered L_3 are unusual in the more southern parts of Europe (*e.g.* England), despite earlier turnout (Armour*, et al.*, 1973). In areas with low levels of overwintered L_3, the role of early season infection is rather to infect animals to a level that ensures "seeding" of the pasture (Armour*, et al.*, 1973).

Faecal egg excretion

The initial exposure of naïve animals to infective larvae on pasture will generate egg production approximately 3 weeks after turnout (B). The faecal egg counts in FSGC normally follows a definite pattern and, within limits, seems independent of the larval exposure at turnout in spring (Nilsson, 1973). It is not uncommon in Sweden to observe a peak in faecal egg counts within the first month following turnout with an ensuing decline to low levels during the rest of the season. However, alterations of this general pattern may occur due to the level of overwintered larvae, the timing of turnout, and weather conditions (Nilsson & Sorelius, 1973). The 6-year trial by Törnquist & Tolling (1987) substantiated this and showed that a between-years variation does exist in Sweden. On the other hand, in central and western Europe, the peak in faecal egg counts often occurs approximately 2 months after turnout (Shaw*, et al.*, 1997; Shaw*, et al.*, 1998).

Second generation L_3

The second generation of L_3 derived from the current season contamination (C) is not regarded as important to cause disease in Sweden. Despite the early peak of

faecal egg excretion, the process of translation does not result in high levels of the numbers of L_3 on herbage until later in the season, at, or even after, housing. Indeed, the generally shorter grazing season in Sweden contributes to this difference. Törnquist & Tolling (1987) claimed that climatic conditions influenced the build-up of pasture larval contamination during the later part of the grazing season and elevated levels were present in late July in only one of the 6 years of their study.

This is in contrast to the situation in other parts of Europe where disease primarily is caused due to infection with L_3 derived from early season contamination apparent from July and onwards (Vercruysse & Claerebout, 2001). Based on this knowledge, the "dose and move" system for control of PGE was advocated in the United Kingdom (Michel, 1969a, 1976) as well as in other European countries, like the Netherlands (Eysker*, et al.*, 1998).

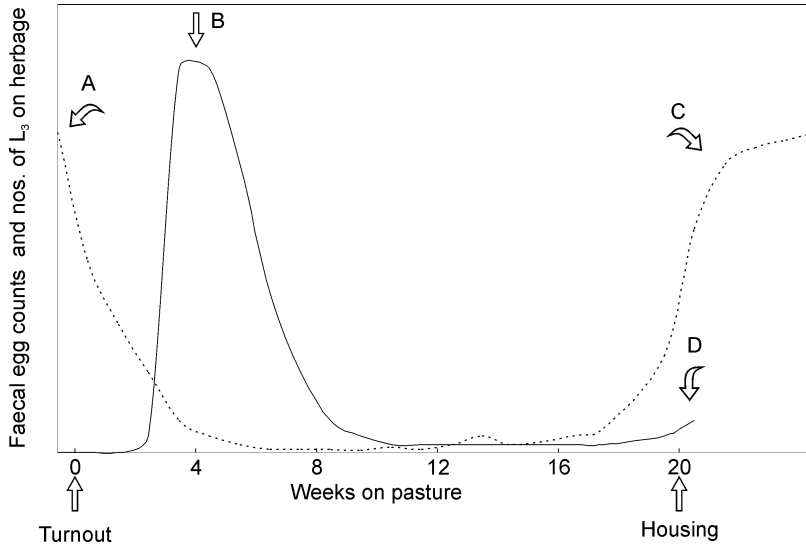

Fig. 3. A schematic graph representing the typical dynamics of faecal egg excretion (solid line) of FSGC and pasture larval availability (broken line) during a normal 20-week grazing season in Sweden. The time of turnout and housing are indicated, while letters represent points discussed in text above (adapted from Swedish data).

Control

General concepts

As Gordon (1973) succinctly describes, “control means restraining the biotic potential of a parasite at a level compatible with the biological requirements of economic husbandry”, and he concludes that in practise, parasite control in livestock inevitably is a compromise between the level of control and the cost of these measures. The purpose of any preventive control method is to break or restrain the life cycle of the parasite at designated times, based on the epidemiology of infection when the parasite population is most vulnerable. Anthelmintics reduce egg output of infected animals, whereas grazing management strategies lower the level of L_3 exposure to animals on pasture. Complete control is only achievable by total separation of faeces from the feed of livestock.

Virtually all first-season grazing cattle acquire GI nematode infections in the absence of anthelmintic “blanket” cover. The question is not if, but when, the infections take place and the rate and thus the magnitude of exposure. It is a fallacy to view the grazing animal as a vacuum cleaner whereby the parasites progressively accumulate and eventually exceed a detrimental threshold independently of when and where grazing takes place (Michel, 1985). As the epidemiology of nematode infections largely is determined by management measurements, not always made for reasons related to parasite control, and factors in the external environment that act on the pre-parasitic stages, the epidemiology may vary from country to country, from region to region and from one year to another. Hence, local epidemiological research is justified to provide farmers and veterinarians with control strategies based on scientific knowledge that also can be used to refine and explain the success, or otherwise, of empirically practised control methods (Stromberg & Averbeck, 1999).

Control methods that primarily depend on the dynamics and survival of the pre-parasitic stages, *i.e.* grazing strategies, runs the risk of being unsuccessful under exceptional conditions that may occur irregularly. The principles behind the control methods used in this project will be summarised briefly below. Apart from the methods evaluated here, additional potential approaches for parasite control are available, though beyond the scope of this assignment. Throughout the world, research activities are ongoing in the area of host genetic selection, helminth vaccines and nutritional approaches, inclusive of herbage species with anthelmintic properties. Although these areas of research show interesting results and should be continued, no miraculous alternative solutions for parasite control in grazing livestock can be expected within the near future. The fields of novel approaches to parasite control were reviewed and discussed recently at the third international workshop conference on this matter (Anonymous, 2002).

Anthelmintics and anthelmintic resistance

Twenty years ago, anthelmintics in cattle were used to salvage clinically affected animals. Today, they are primarily used to maximise profit (Craig & Wikse, 1995). The introduction of safe and effective drugs enabled the livestock industry to intensify production without endangering the economy of livestock enterprise, or animal health due to GI parasitism (Nansen, 1987).

Anthelmintics remove established worms or prevent reinfection of treated animals (Fig. 1, A). Provided no resistance of the egg producing worms, egg output is eliminated for the duration of the anthelmintic activity of the drug, and seeding of pasture is reduced for several subsequent weeks. In 1981, the macrocyclic lactone endectocide, ivermectin, was introduced on the market. Ivermectin represented a novel class of animal antiparasitic drugs that combined a wide spectrum of activity against endo- and ectoparasites, as well as having excellent safety, duration and potency. The development of the sustained-release bolus device for administration provided veterinarians and farmers with a highly effective tool for nematode parasite control of cattle. Active drug is released for 135 days and maintains the treated cattle virtually worm-free for most of the grazing season. It has been argued that the development of immunity may be compromised, as the exposure to L_3 may be too low to generate an adequate level of acquired immunity during the first grazing season (Armour, 1989). However, this fear has not been convincingly verified in the field situation.

As mentioned in the introduction, anthelmintic resistance of cattle GI nematodes in Sweden is not a matter of immediate concern (Waller & Höglund, 1998a). The principal reasons for this were considered by Coles (2002) who concluded that it is possible to delay or prevent the development of resistance on the assumption that a management is practised, which permits sufficient numbers of worms to be in refugia, *i.e.* unexposed to selection by anthelmintics. However, to take this fortunate state as a pretext for the dismissal of novel or alternative approaches to parasite control would be unwise. Reports of anthelmintic resistance have emerged from the humid regions of southern Latin America (Echevarria & Pinheiro, 2001; Fiel*, et al.*, 2001) and to a lesser extent in Australasia (Vermunt, West & Pomroy, 1995) where intensive anthelmintic treatment regimes are practised.

Moreover, farm management practises and the established means of anthelmintic application are not static factors, but may change. This is exemplified by the fact that an anthelmintic formulation with no withdrawal time for milk is presently available (*i.e.* eprinomectin). Treatment of dairy cows in Canada was recently demonstrated to improve milk yield (Nødtvedt*, et al.*, 2002; Sanchez & Dohoo, 2002) and reproduction (Sanchez*, et al.*, 2002). If such treatment strategies become accepted and adapted among Swedish cattle producers and veterinarians, the prerequisites for a continuation of the privileged Swedish situation as regards anthelmintic resistance is altered. Thus, the favourable cost-benefit calculations of using anthelmintics to increase milk production of dairy cows may well be unsustainable and should not be advocated from the standpoint of ultimate selection for anthelmintic resistance (Coles, 2002). *Modus operandum* should be "mindful rather than mindless anthelmintic use" (Vercruysse & Dorny, 1999).

Grazing management

The common fundamental principal behind the employment of grazing management strategies for parasite control is to mitigate the exposure to L_3 in grazing cattle (Fig. 1, B). These strategies may be applied in a variety of ways although Michel (1985) classified grazing strategies as either being *preventive*, *evasive* or *diluting*.

Preventive strategies imply the provision of clean pastures, *i.e.* a pasture not contaminated by cattle nematodes during the previous season. If clean pastures are not accessible, prevention is obtained by suppression of egg output by anthelmintic treatments until the initial exposure of L_3 has decreased to a very low level.

Evasive strategies are simply directed at avoidance of grazing cattle to existing larval exposure. Movement to aftermath before the appearance of the second generation of L_3 on herbage is a commonly used evasive management practise.

Dilution strategies exploit helminthologically inert animals of the same or different species for mixed grazing. The effect is achieved by both a reduction of existing larval availability through ingestion of contaminated herbage by the helminthologically inert animals, and a reduced output of faecal eggs by the animals at risk because of their lower stocking density.

Biological control

Biological control (BC) of nematodes of grazing livestock is no longer a novelty of mainly basic research interest, but has developed into an area of applied research with the potential to become a part of the battery of control options available for nematode parasites (Larsen, 1999). Although nematode parasites of livestock theoretically can be controlled by different nematode-destroying micro-fungi, current research is almost exclusively associated with the species *Duddingtonia flagrans*. Important reasons for choosing *D. flagrans* are its ability to survive gut passage through the resistant resting stage, the chlamydospores and its capability to grow rapidly in freshly deposited dung. The propensity to produce a three-dimensional nematode-trapping network in the faecal pat environment is the key feature for nematode parasite control. The characteristics for *D. flagrans* have been shown to occur for the same environmental conditions as those that promote larval development and translation (for review, see Larsen, 1999).

The faecal pat environment is the target with this mode of nematode control (Fig. 1, C), which thus is fundamentally different from anthelmintics and grazing management. The principle is to achieve a reduction of the numbers of pre-parasitic larvae by means of the specialised hyphal structures that capture and destroy the nematodes before they migrate to herbage and become available to grazing animals (Fig. 4). Thus, already existing larvae on pastures grazed by animals subjected to BC are not affected, nor is the worm egg production due to any contracted infection. Consequently, the benefits of using *D. flagrans* is not instant, but may be viewed as an investment in parasite control for the future in terms of a reduced translation of L_3 derived from eggs voided in faeces during the period of fungal administration.

In the cattle studies so far, the fungus has been fed in troughs once or twice a day. If a fungal sustained-release bolus would become available in the near future this should have obvious advantages over the currently recommended daily-supplementary feeding regimens. Although some progress along these lines has been made (Waller, Faedo & Ellis, 2001), there is no clear indication that a testable product will soon be available. The prospects for controlling parasitic nematodes of cattle by predacious micro-fungi and a summary of accomplished research in this area is presented in Larsen*, et al.* (1997) and Larsen (2000).

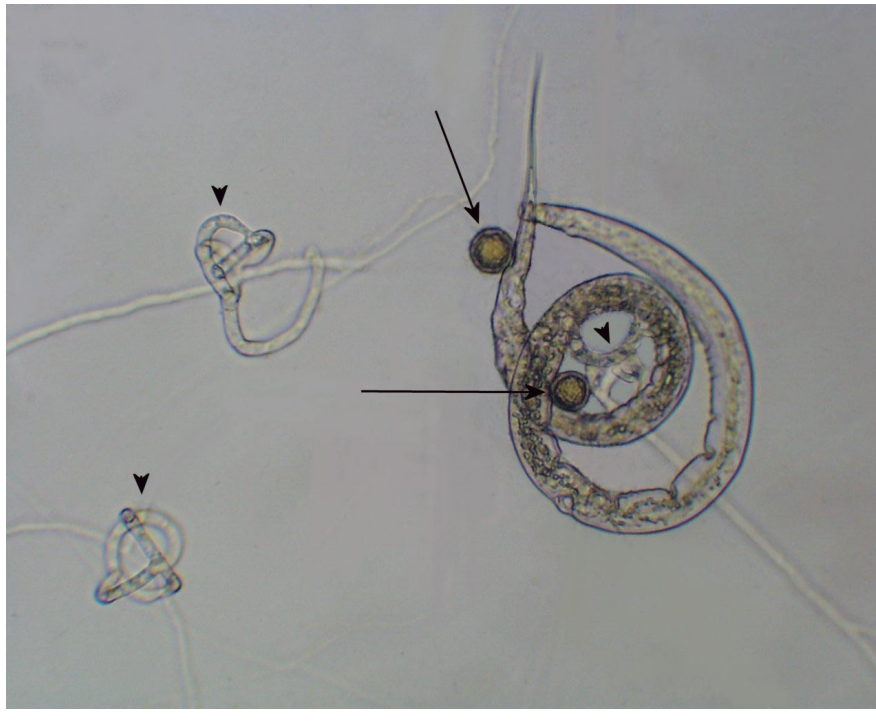

Fig. 4. A dead and disintegrating L_3, trapped in the loops of the biocontrol agent *D. flagrans*. The resting spores (chlamydospores) are indicated with arrows and the three-dimensional trapping networks are marked with arrowheads. Photo: Sten-Olof Dimander

Copper supplementation

Copper oxide wire particles (COWP) administered as a capsule to sheep have proved effective on reducing the establishment and fecundity of abomasal parasites (Fig. 1, D) of sheep in New Zealand (Bang, Familton & Sykes, 1990) and Australia (Knox, 2002). The reason for this effect on abomasal parasites of sheep is not known, but Bang, Familton & Sykes (1990) concluded that ionic copper liberated by the acid secreting mucosa of the abomasum is responsible for the anthelmintic activity in this gut site. The concentration necessary for an effect, and the exact mechanism behind this effect, are critical questions yet unanswered. Reasonably, a similar effect would also be expected against abomasal nematodes in cattle, but according to available literature no such investigation has previously been done.

Parasite control in Sweden

Parasite control methods in Swedish dairy herds were investigated with the objectives to compare the strategies used on organic and conventional farms and to form an opinion of the magnitude of parasite infections. From the questionnaire study (Svensson, Hessle & Höglund, 2000) it was found that a majority of the conventional farmers (58%) relied on prophylactic anthelmintic treatment of their FSGC. However, this is lower than the results obtained from an earlier Scottish survey where 86% reported prophylactic use of anthelmintics (Gettinby*, et al.*, 1987). Although anthelmintics were widely used on conventional cattle farms, the owners also employed strategic nutritional supplementation in the spring and in the autumn. Notably, Michel's "dose and move" strategy is not commonly used in Sweden and is, accordingly, rarely advocated by the Swedish Animal Health Service (SvDHV). This is mainly due to the recommendation of early season treatment and to the use other and more convenient anthelmintic preparations.

With regard to organic cattle producers in Sweden, they often combined different control methods that comprised a variety of grazing managements and nutritional supplementation. In a field survey on 15 organic farms, the internal parasitism was monitored during 2 consecutive years (Höglund, Svensson & Hessle, 2001). From this study it was concluded that the organic farmers managed to control internal parasitism to low or moderate levels without prophylactic anthelmintic treatments. However, it was revealed that lungworm *(D. viviparus)* infection was prevalent on 12 (80%) of these organic farms and on 2 of these, clinical dictyocaulosis was observed.

Environmental impact of parasite control

Outside the scope of this thesis but, nevertheless, an important supplementary part of this project were environmental impact assessments of some of the parasite control treatments. This work included a range of scientific disciplines and collaboration with experts from Sweden, Denmark and New Zealand. While environmental impact may sound negative, in this context the impact does not necessarily have to be deleterious. For instance, it must be recognised that grazing in itself promotes a variable botanical composition and a faunistic diversity. For example, certain plant species and invertebrates threatened by extinction in fact depend upon grazing by livestock to persist (Bernes, 1994; Gärdenfors, 2000). It is likewise important not to overlook that grazing animals are a widely appreciated part of the rural landscape and constitute an essential component of country life and a natural element of the historic, human-modified environment.

Particular emphasis was given in this study to comparing the use of the ivermectin sustained-release bolus and the nematophagous fungus *D. flagrans*. The possible adverse impact on beneficial organisms in pastureland ecology that may follow the deployment of these parasite control measures are critical issues in their comprehensive environmental evaluation. Above all, environmental impact studies should preferably be carried out over consecutive years to allow the observation of any long-term effects and interactions with climatic variability. With these objectives in mind, 4 independent trials complementary to the main grazing study described in this thesis (study II) were conducted. They investigated the potential for adverse environmental consequences on pasturelands arising from the use of *D. flagrans* and the ivermectin sustained-release bolus in comparison with pastures grazed by cattle not receiving parasite control agents.

Saprophagous soil nematodes are important and beneficial in the processes of nutrient recycling on grazed pastures (Yeates, 1984; Ingham*, et al.*, 1985; Griffiths, Young & Caul, 1995). Any perturbation of the number or composition of these organisms may disturb the overall ecological balance of the pasture-soil community. Soil represents the source of most nematodes that invade the faecal pats and is ultimately the repository of faecal residues following dung pat degradation.

The impact of the use of ivermectin sustained-release bolus and *D. flagrans* spores on free-living saprophytic soil nematodes was assessed in 2 trials over 3 consecutive years. One was conducted on pastureland that was grazed by the cattle receiving the treatments (Yeates*, et al.*, 2002), and the other was carried out as a companion plot study where soil samples were taken directly underneath the dung pats derived from the animals in the grazing trial (Yeates*, et al.*, 2003). The results of these investigations showed no overall effect of either treatment on total numbers, diversity or functional groups of soil nematodes. The possible spread of *D. flagrans* from deposited faecal pats containing chlamydospores to surrounding soil was investigated by Faedo*, et al.* (2002) during the second year (1999) of the 3-year plot trial. The results showed no dissemination of the fungus to the surrounding soil and are in accordance with observations from Australia (Knox, Josh & Anderson, 2002).

As regards ivermectin, certain developmental stages of some coprophilic invertebrates (dung beetles and flies) are particularly sensitive to ivermectin residues in ruminant dung (Strong*, et al.*, 1996). For this reason there is great concern associated with the use of avermectins in Sweden (Wiktelius, 1996) and elsewhere (Strong, 1993; Edwards, Atiyeh & Römbke, 2001). In the plot trial, the influence of ivermectin and *D. flagrans* on faecal pat disintegration was also monitored (Dimander, Höglund & Waller, 2003). As dung colonising invertebrates play an important role in the process of dung disintegration, an obvious effect would be a delayed breakdown of faecal pats (Wall & Strong, 1987). However, the effect of *D. flagrans* on dung disappearance has not previously been investigated. The results showed that, apart from the significant influence of prevailing weather conditions, there was no effect on dung disappearance, neither of ivermectin or *D. flagrans* treatment (Fig. 5). Notably, the absence of an observed treatment effect on dung disintegration does not exclude the possibility of an underlying detrimental effect on specific components of the dung insect fauna assemblage. Nevertheless, this possible negative effect was overruled by other components involved in dung disintegration in the study area and under the prevailing conditions.

Publications derived from the environmental impact part of the project

Dimander, S.-O., Höglund, J. & Waller, P. J. 2003. Disintegration of dung pats from cattle treated with the ivermectin anthelmintic bolus, or the biocontrol agent *Duddingtonia flagrans*. (submitted).

Faedo, M., Larsen, M., Dimander, S.-O., Yeates, G. W., Höglund, J. & Waller, P. J. 2002. Growth of the fungus *Duddingtonia flagrans* in soil surrounding faeces deposited by cattle or sheep fed the fungus to control nematode parasites. *Biological Control 23*, 64-70.

Yeates, G. W., Dimander, S.-O., Waller, P. J. & Höglund, J. 2002. Environmental impacts on soil nematodes following the use of either the ivermectin sustained release bolus or the nematophagous fungus *Duddingtonia flagrans* to control nematode parasites of cattle in Sweden. *Acta Agriculturæ Scandinavica, Section A, Animal Science 52*, 233-242.

Yeates, G. W., Dimander, S.-O., Waller, P. J. & Höglund, J. 2003. Soil nematodes beneath faecal pats from cattle treated with either the ivermectin sustained-release bolus or the nematophagous fungus *Duddingtonia flagrans* to control nematode parasites. (submitted).

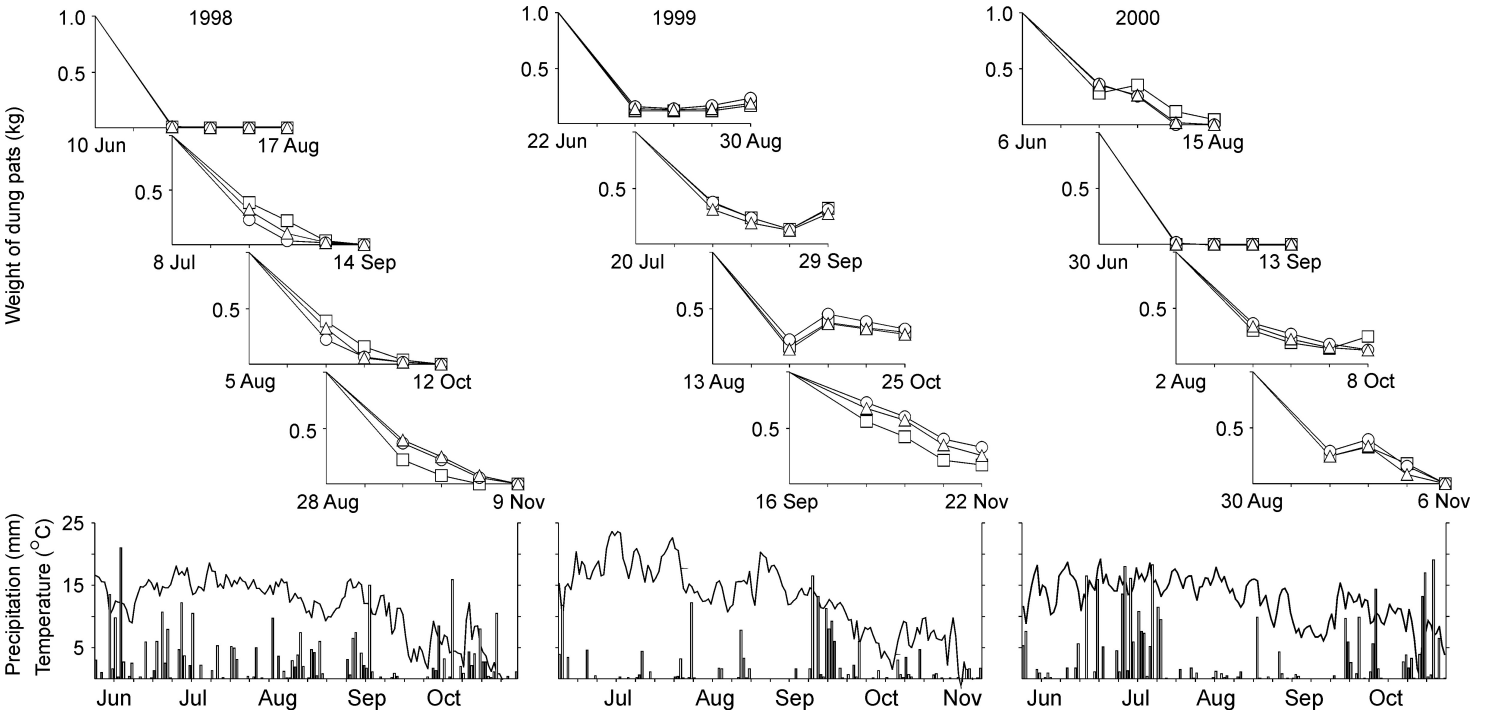

Fig. 5. The change of mass of original artificial 1 kg dung pats derived from cattle that were either untreated, Control (triangle), treated with the ivermectin bolus, IVM Bolus (square) or fed the fungus *Duddingtonia flagrans*, Fungus (circle). The dung pats were protected from birds and deposited on a nylon mesh on 4 occasions during the grazing seasons 1998–2000, respectively. The year-to-year pat location for the different treatments and deposition occasions were repeated during the 3-years of the trial. Weighing of the pats was performed 4, 6, 8, and 10 weeks after deposition where the nylon mesh supporting the dung pat was lifted on to a scale and immediately afterwards replaced to its original position. The corresponding daily mean temperature (solid line) and precipitation (bars) for the study periods are displayed below. (see Dimander, Höglund & Waller, 2003).

Aims of the study

The main objectives of this thesis were:

- To evaluate GI nematode control strategies for FSGC in Sweden through consecutive years field experiments on both improved and semi-natural pasturelands. In comparison with anthelmintic bolus treated animals (maximum control), and untreated, set-stocked cattle (minimum control), 2 different grazing management strategies without any anthelmintic treatment were evaluated, as well as treatment with the nematophagous fungus *Duddingtonia flagrans*.
- To perform ecological investigations on the free-living stages of cattle nematodes in Sweden to obtain a better understanding of the epidemiology and dynamics of GI nematode infections. Knowledge about the ecology of the pre-parasitic stages is fundamental for the development of any control regime.

Methodological considerations

Detailed information is presented in the Material and Methods section of each publication included in this thesis.

Experiments

General description

Animals

First-season grazing cattle (FSGC) of Swedish red and white breed (SRB) was the primary class of interest in this project. The animals originated in study I from the Kungsängen Research Centre and the second year from a private farm on the outskirts of Uppsala. In study II they were all first-season grazing heifers and steers originating from the Kungsängen Research Centre. However, in both grazing trials, adult cattle contributed by indirect means. Second-season grazers (heifers) were used as accompanying grazers to facilitate normal grazing behaviour of the tracer cattle in the tracer tests in study I and II. As part of the rotation grazing strategy in study II, SRB dairy cows during their dry period were used to graze the paddock that subsequently served as turnout pasture for the FSGC in the rotation group each year.

The tracer cattle were all parasite naïve pen raised SRB animals between 6–9 months old. These were held on pasture for 3 weeks and were then housed on slatted floor for 2 weeks (study I) and 3 weeks (study II) before slaughter.

Pasturelands and plot area

The semi-natural pastures (hagmark) used in study I were situated on the slopes along the banks of the Fyris river, south of the city of Uppsala. The experimental paddocks formed a contiguous row along the river and contained many of the features associated with hagmark pastures of central Sweden. In trial II, the grazing area consisted of a suite of contiguous uniform paddocks of improved pastures of flat topography and belonging to the Kungsängen Research Centre, Uppsala.

The plot trials were conducted on an enclosed area of pasture comparable to the improved pasturelands used in grazing experiment II and were situated approximately 500 m from the grazing study.

Artificial infection

In study I, all animals received a single ”priming” dose of approximately 10,000 fresh infective trichostrongyle larvae at turnout. *C. oncophora* (> 90%) comprised the larval dose the first year, whereas in the second year, the same number of larvae were given to the animals at turnout, but approximately 5,000 *O. ostertagi* infective larvae were also included in each dose. This latter dose was also given to the animals in study II prior to turnout the first year whereas no further artificial larval dosing was applied the 2 remaining years of study II.

The trichostrongylid mixture of L_3 originated from cattle from a farm outside Uppsala and was sustained in pen raised, artificially infected young cattle at the Kungsängen Research Centre. The pure *O. ostertagi* isolate was obtained from the Danish Veterinary Laboratory, Copenhagen, Denmark.

Fungal material

The fungal material used in study II was kindly provided by Christian Hansen BioSystems A/S, Copenhagen, Denmark. Single fungal spore consignments from the same batch were used each year. The fungal material was administered daily mixed in 1 kg concentrate per animal between weeks 3 and 16 (90 days). A differentiated fungal spore dose was used during the study. In 1998, a dose of 1×10^6 spores/kg body weight/day was used whereas in 1999 and 2000 the dose was halved to 0.5×10^6 spores/kg body weight/day.

Diagnostic methods used

Faecal samples

Presence of trichostrongyle eggs in faeces was analysed based on 3 g faeces using the method described by Gordon & Whitlock (1939) with a sensitivity of 50 epg. Pooled faecal cultures were set up for each group on all sampling occasions. Faeces were mixed with vermiculite, and following incubation for 14 days at 25 °C and > 90% RH, L_3 were harvested with the Roberts and O´Sullivan technique (Anonymous, 1986).

Individual faecal cultures were prepared in experiments II and IV to evaluate the trapping effect of *D. flagrans* under constant optimal conditions. The procedure described by Henriksen & Korsholm (1983) was used for culturing and harvesting. The harvested L_3 were stored in flasks in a refrigerator at 8 °C before differentiation according to Borgsteede & Hendriks (1974).

Blood samples

Serum pepsinogen estimations were carried out using the micro-method described by Dorny & Vercruysse (1998). The levels of serum antibodies specific to *D. viviparus* were measured by the Ceditest (ID-DLO, Lelystad, the Netherlands) (Cornelissen, Borgsteede & van Milligen, 1997) on all sampling occasions on all animals in study I and at housing each year in study II.

Due to unexpectedly high SPC in study II during 1998, assorted sera were rerun to identify possible problems with the test. The repeated analysis showed remarkably similar results but to further investigate this matter it was decided to proceed with an inter-laboratory comparison. Sera from 1998 were divided into 3 groups (low, medium and high) according the serum pepsinogen concentrations attained with the Uppsala assay. From these 3 groups, 10 samples were randomly chosen together with the control sera used in the Uppsala assay. The 32 samples were numbered without relation to the results in the Uppsala assay and equally divided into 3 tubes. By kind permission and in agreement of the purpose of the analysis, one set of samples was sent to Department of Parasitology, Faculty of

Veterinary Medicine, University of Ghent, Belgium, and one to Division of Parasitology and Tropical Veterinary Medicine, University of Utrecht, the Netherlands, for an inter-laboratory comparison of the micro-method. The results were statistically evaluated using the Bland-Altman method for analysis of measurement method comparison data (Bland & Altman, 1986) and are displayed in Fig. 6. It was found that the values from Uppsala and Ghent had a mean SPC difference of 0.221 (95% confidence interval (CI); 0.078–0.364), the ones from Uppsala and Utrecht 1.524 (95% CI; 1.032–2.015) and the mean SPC difference between Ghent and Utrecht was 1.303 (95% CI; 0.887–1.718).

Before the development of the micro-method (Dorny & Vercruysse, 1998), available serum pepsinogen methods were laborious and comparisons between laboratories were difficult (Berghen*, et al.*, 1993; Scott, Stear & McKellar, 1995). This was realised by Shaw*, et al.* (1998) who were unable to make any comparative analysis of SPC results obtained from different experiments. It was concluded that unless a simplified method was used, any relative comparison of SPC obtained from different countries would be of little value.

Although the simplified and less expensive micro-method is widely adopted and thus has improved the prerequisites for the between-laboratory validity, this small comparison highlights differences that after all may be considerable. The Uppsala and Ghent results agreed reasonably well, but the Utrecht results showed approximately 50% lower values than the Uppsala and Ghent values at all levels of SPC (low, medium and high). The obvious conclusion drawn from this is that the proposed levels of SPC for subclinical and clinical ostertagiosis, respectively, should be interpreted with great caution unless an inter-laboratory test has been performed between the reference laboratory and the laboratory that analyses the samples in question. For instance, of the 10 “high” SPC samples in the Uppsala assay (> 5.0 U tyr) in the current laboratory comparison, 8 were classified as > 5 U tyr when examined in Ghent, whereas none of the samples analysed in Utrecht were found as being > 5.0 U tyr, indicative of clinical ostertagiosis. Consequently, the elaboration of achieved results in different experiments would be justified and more relevant if further and more comprehensive comparisons are encouraged. This may apply also for parasitological methods other than the micro-method for estimation of SPC.

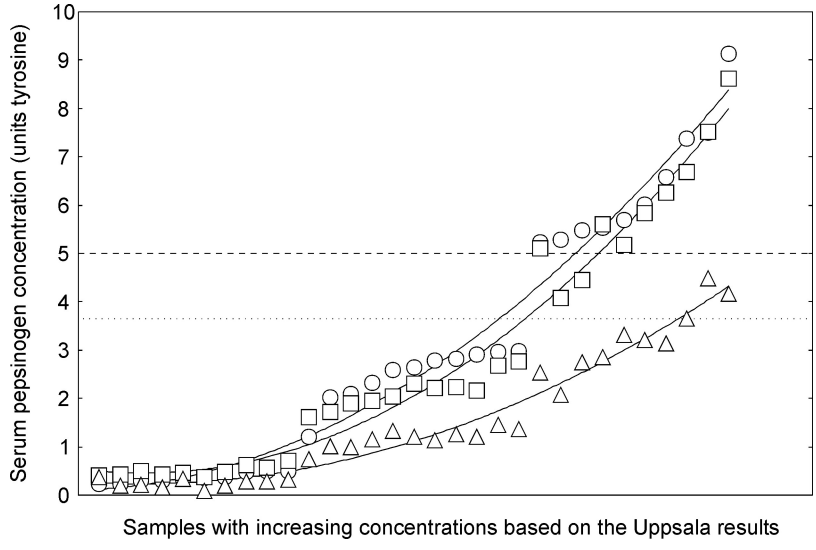

Fig. 6. Comparison of serum pepsinogen concentrations between the laboratories in Uppsala (circles), Ghent (squares) and Utrecht (triangles). The 2 lines indicate the proposed cut-off concentrations for subclinical (dotted) and clinical ostertagiosis (dashed), respectively (Hilderson, *et al.*, 1989). A trend-line is included for each laboratory.

Tracer worm counts

Abomasum and small intestine were collected from the tracer animals at the abattoir, and the process was in general accordance with the procedures described by Donald*, et al.* (1979). However, from 4 l of abomasal and small intestine contents, respectively, 20 ml subsamples were instantly frozen at –20 ºC instead of preserved in formalin. After thawing, nematodes were identified, counted and differentiated to species according to Barth & Visser (1991).

Pasture larval counts and pasture estimations

Herbage samples were collected by hand according the method described by Taylor (1939). The L_3 were recovered in large Baermann funnels according to procedures outlined by Persson (1974a). Total recoveries of nematodes were concentrated into 20 ml water, and from each sample duplicate 1 ml subsamples were stained with Lugol's iodine solution and L_3 were counted and expressed as per kg dry matter of herbage (L_3/kg DM). Additionally, in 2000 and 2001 the L_3 were differentiated into genus.

To estimate herbage availability, sward height was measured with a sward stick (Barthram, 1986) every 2 weeks with at least 30 readings per paddock following a "W" shaped path. Herbage for estimation of the nutritive value of pasture was collected at every 5 readings and dried before analysis. Crude protein (CP) content was determined in a fully automated Kjelldahl procedure while metabolisable energy was analysed as described by Lindgren (1979) and expressed as megajoules per kg dry matter (MJ/kg DM). The amount of neutral detergent fibre (NDF) was assessed according to the method of Goering & Van Soest (1970).

Meteorology

During the whole period of this project, continuous weather data was recorded at the Bäcklösa meteorological station, located approximately 2.5 km from the experimental areas. Information based on daily mean temperatures, daily precipitation and information on snow depth and the extent of snow cover were presented in various forms in all studies conducted.

Statistical methods

Data were summarised and the raw figures were prepared in Excel (Microsoft). The figures were subsequently completed in Canvas (Macintosh) or CorelDraw (Windows).

Statistical analyses were performed with the software packages StatView® 4.5 (Abacus Concepts, 1996) for Macintosh (Apple computers) or Intercooled Stata 7.0 for Windows NT (Stata Corporation, College Station, Texas, USA, 2002). The dependent variables measured from the FSGC in the grazing studies I and II were analysed in individual repeated ANOVA models. Various modes of transformation were required to fulfil the ANOVA assumptions of normal distribution and equal variances. The bolus treated animals were excluded from the analysis of faecal egg counts due to zero egg counts on almost all sampling occasions. If a significant treatment effect was found in weight gain differences in the repeated ANOVA model, one-way ANOVA was used for analysis of weight gain differences at housing. The Bonferroni method was applied to adjust for multiple comparisons. Data from study III were analysed using the non-parametric Kruskal-Wallis and Mann-Whitney U tests, as the data were not transformable to normal distribution and equal variances. Two-way ANOVA was employed to analyse the results from study IV. The significance level was set to $p < 0.05$.

Results and discussion

GI nematode infections and performance

For those who were of the opinion that GI nematode infections in cattle in Sweden are merely a matter of academic curiosity, I am sorry to disappoint you with this thesis. Results from experiment II show that uncontrolled GI nematode infections in FSGC can reduce the potential performance under set-stocked conditions by 50% compared with animals treated with the ivermectin sustained-release bolus. Moreover in untreated FSGC, if appropriate grazing management strategies are implemented, in combination with access to nutritionally high-quality pasture, this difference may be even larger. Translated to weight gain differences over a normal length of the Swedish grazing season (approximately 20 weeks), this corresponded to a weight gain penalty for the untreated cattle of 65 kg in comparison with the anthelmintic treated cattle. If compared with cattle subjected to grazing management only, this difference may be as large as 85 kg. The grazing experiment on hagmark (1997–1998) and the trial on improved pastures (1998–2000) together enable a comparison between the positive and negative control groups over 5 grazing seasons under 4 different sets of weather conditions. The average daily weight gains for the bolus treated cattle in study I were 0.82 and 0.87 kg during 1997 and 1998, respectively, and for the untreated 0.84 and 0.66 kg, respectively (Fig. 7 A). In trial II, the average daily weight gains during 1998–2000 were 0.94, 0.70 and 1.0 kg for the treated and 0.76, 0.66 and 0.52 kg for the untreated, respectively. Thus, the productivity in the anthelmintic treated group varied between 0.70 and 1.0 kg weight gain per day and between 0.52 and 0.84 kg per day in the untreated group. Interestingly, the range of the maximum and minimum daily weight gains in the treated and untreated control groups during the 5 grazing seasons were approximately the same (0.30 and 0.32 kg, respectively).

The weight gains obtained in the present groups of treated and untreated groups of FSGC may be compared with results from a combined analysis of 85 studies involving approximately 2,000 FSGC in western Europe (Shaw*, et al.*, 1998). They found that the average weight gains of untreated FSGC classified as suffering from clinical or subclinical PGE were 0.38 and 0.53 kg per day, respectively. The corresponding results from the anthelmintic treated animals were 0.600 and 0.690 kg per day, respectively. Included in this investigation were results from the 6-year study from Sweden (Törnquist & Tolling, 1987) where the average weight gain in the untreated and anthelmintic treated cattle were 0.45 kg per day (range 0.32 to 0.55 kg) and 0.60 kg per day (0.46 to 0.75 kg), respectively.

However, the grazing seasons of the best and poorest performance, respectively, did not coincide for the treated and untreated groups. That is, the year of 2000 (Fig. 7 B) was the season of best performance in the bolus treated group (1.0 kg per day) but the year of the poorest performance in the untreated group (0.52 kg). Hence, not even lush pasture of good quality and supplementary feeding (part of BC strategy) could mitigate against the effects of parasites during this year. From the pasture estimations, 2 major conclusions with regard to performance can be drawn.

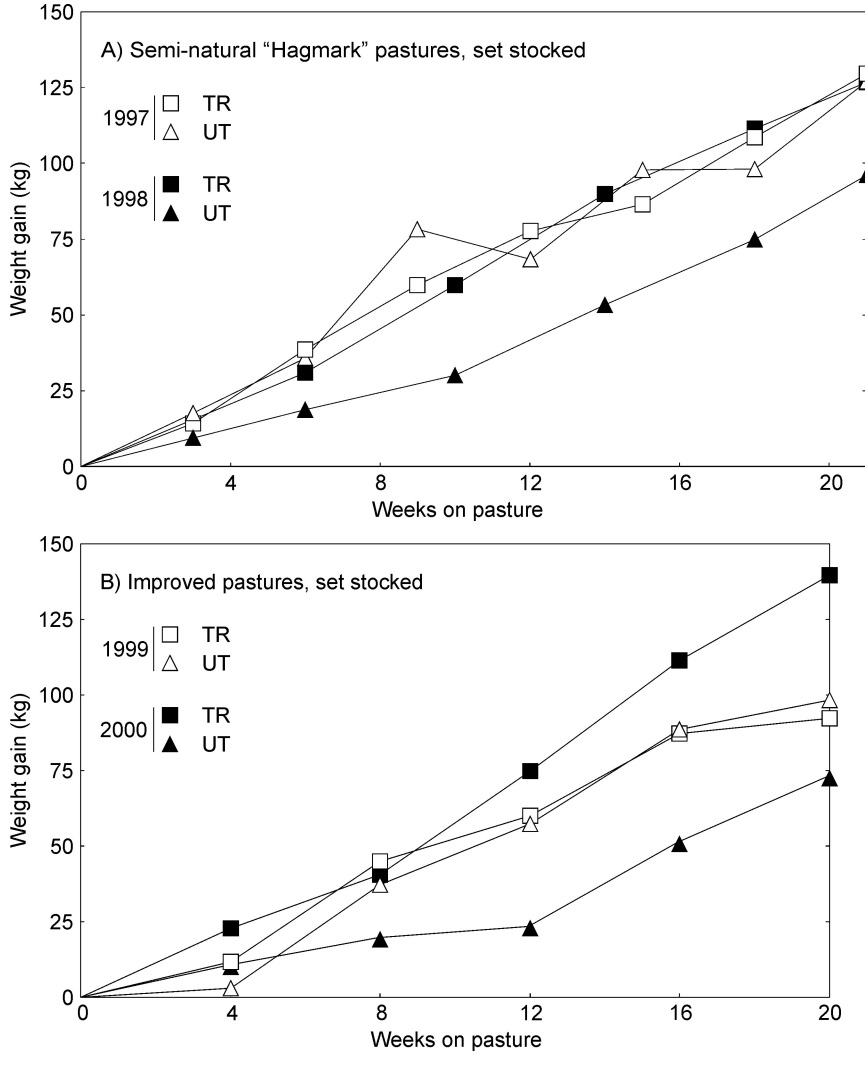

Fig. 7 A and B. The performance of set-stocked FSGC on A) “semi-natural” (hagmark) pastures (1997 and 1998), and B) improved pastures (1999 and 2000) that were either treated with the ivermectin sustained-release bolus (TR; squares) or untreated (UT; triangles).

(i) Results from trial II show that excellent production of set-stocked FSGC was possible if they were presented lush pastures and if protected from GI nematode infections. In 2000, weather conditions promoted pasture growth and the daily weight gain in the bolus treated set-stocked group was on an average 1.0 kg. Additionally, if untreated FSGC were offered a parasite safe turnout pasture (cow pasture) of sufficient herbage allowance in combination with a mid-July move to aftermath, even better performance was possible as the rotation group grew on average 1.12 kg per day. The most reasonable explanation for the better performance of the rotation group cattle compared with the bolus treated animals is a nutritional effect from grazing the non-fouled aftermath (Spörndly, 1996). In

other words, it is important to note that low levels of infection acquired prior to the move to aftermath had no apparent effect on productivity when animals were provided with an aftermath ley of high-quality nutrition and without an anthelmintic treatment prior to the swap.

(ii) A delayed turnout followed by a dry summer and early autumn, as in 1999, resulted in overgrown herbage of low nutritional value and a subsequent impeded pasture re-growth. From the parasitological standpoint, 1999 was of little significance with initially very low levels of overwintered exposure. This was reflected by very low egg counts, low SPC and poor conditions for L_3 translation. Consequently, GI nematode infections could largely be ruled out as a production-limiting factor this year. Despite the scarce pasture availability, 1999 was not the year with the poorest performance in the untreated group. The weight gains this year were 0.66 kg per day but only 0.52 kg in 2000, albeit at a time with lush pasture conditions. For the bolus treated cattle, the reverse results were observed as they performed the poorest in 1999 (0.70 kg) but the best in 2000 (1.0 kg). In other words, poor performance in the untreated group in 1999 was exclusively explained by the lack of feed and not because of GI nematode infections as substantiated by the fact that there was no statistical difference in weight gains between the 4 set-stocked treatments in 1999.

Overwintering and seasonal dynamics

To obtain a better understanding of key factors in the epidemiology of GI infections in Sweden, detailed ecological investigations are required. To accomplish this, plot studies III and IV were conducted in parallel with grazing trials I and II, respectively. The design enabled detailed evaluation of the significance of the timing of faecal pat deposition during the grazing season under the identical set of weather conditions as for the grazing trials. Results from studies III and IV confirmed the established view that GI cattle parasites can successfully overwinter in relatively large numbers in Sweden (Persson, 1974c). In study I, the poor results from the evasive grazing strategy could be explained by the significant carry-over effect of contamination from the previous year. Results from the accompanying plot study, where faeces with a known number of eggs were deposited at 3-week intervals throughout 1997, demonstrated the relative importance of deposition at different times during the season in the number of available L_3. It was observed that early season deposition in 1997, comprised 60% of the overwintered population that cattle encountered following turnout in 1998.

The high level of overwintering capacity of pre-parasitic stages of GI nematodes, observed in study III was confirmed in study IV. However, contamination from 1999 season overwintered in exceptionally high numbers. When compared with 1997, 1998 and 2000 contaminations, the level of overwintering was in fact 10-fold higher from 1999. Notably, this was the season when faecal egg counts were low compared with the other 3 years. Nilsson & Sorelius (1973) hypothesized that long winters and extended periods of consistent snow cover, which are the situation in many parts of Sweden, enhances the ability of the free-living stages of parasites to overwinter. Normally, the number of days with consistent snow cover in the

Uppsala region is 102 days, however, during the 4 winters of the study, the number of days with snow cover were only 44 (1997–1998), 78 (1998–1999), 38 (1999–2000) and 35 (2000–2001), respectively. Thus, it can be assumed that the limited snow cover during the winter of 1999–2000 was unlikely to be an important factor to account for the high levels of overwintering survival during this time. Rather, it is proposed that the enhanced overwintering survival can be attributed to the prevailing weather conditions during the 1999 grazing season that above all influenced the faecal pat disintegration. During most of the grazing period of 1999, total rainfall recorded was only 82.7 mm, which was divided into 18 days of rain of > 2.0 mm, until heavy rains eventually fell in late September. This should be compared with the normal precipitation of the corresponding period of 229 mm. The practical implications of this were demonstrated in plot study IV where the faecal pats from the July, August and September depositions disintegrated slowly as a result of the dry conditions (Fig. 5). Indeed, remnants of the faecal pats deposited throughout the grazing season still remained on the plot area at the time of turnout in May 2000.

Although there were occasional showers of rain during the summer of 1999, these were not sufficient to thoroughly soak the faecal pats to enable L_3 translation and to facilitate faecal pat disintegration. Consequently, development from egg to the infective larva advanced under cover of the intra-faecal pat environment that provided a shield during the critical phase through the sensitive first and second larval stages. Thus, the faecal pat was gradually converted into a "ticking bomb" of L_3, close but yet so far away from the grazing cattle. When heavy rainfall eventually fell in late September, the environmental conditions drenched the faecal pats and provided films of water necessary for larval translation. A similar phenomenon has been described by Barger, Lewis & Brown (1984) in Australia and Nansen*, et al.* (1989) in Denmark.

Not only was the pasture in study II converted from parasite "safe" to parasite "dangerous" within a few days, these larvae were obviously well prepared for overwintering. The detrimental impact of the exceptional overwintering capacity from 1999 deposition was mirrored in the poor performance in the untreated group in 2000, despite adequate nutritional supply. One can only speculate as to what would have happened if turnout of FSGC instead had occurred at the normal time of the year on a pasture with "normal" overwintered numbers of L_3. The ensuing higher numbers of faecal eggs, than was the case in study II in 1999, combined with similar weather conditions as in that summer, may well have caused a disaster in terms of clinical PGE and poor performance.

Results from study IV showed that the highest proportion of overwintered recovery of L_3 was 0.52%, recorded from the August 1999 deposition. A problem with the plot situation is its inherited "semi-natural" nature and thus makes a complete extrapolation to the field situation difficult. However, this large recovery was in turn reflected on larval availability estimations in the aftermath paddock used in grazing trial II. In the set-stocked situation, it is not possible to determine whether the origin of overwintered L_3 from previous season comes from early or late deposition. The rotation treatment in study II though, enabled an estimation of the importance of early (turnout pasture) and late (aftermath) contamination under

field conditions. From this it can be concluded that despite very low egg counts (50–100 epg) in the rotation animals during their period of 10 weeks on aftermath grazing, this lightly contaminated pasture generated a peak recovery of 20,000 L_3 per kg dry herbage in mid-winter and an overwintered larval availability of approximately 5,000 L_3 per kg dry herbage in early May.

Non-anthelmintic control of GI nematodes

It is evident from this project that sufficient GI nematode control in cattle in Sweden may be achievable without the use of anthelmintics. It was obvious that a reduction in the exposure of FSGC to the overwintered population of larvae at the start of the grazing season is an important factor to consider for the implementation of adequate control of GI nematode infections in Sweden. Based on the variable weather conditions and different management practises that were integral parts of this project, both success and failure was experienced in the efforts to protect young cattle from the overwintered larval population. The copper supplementation evaluated in this project showed no effect on establishment of *O. ostertagi* in FSGC. This was supported by the parasitological data, and more importantly, by poor animal performance in all 3 years of the experiment. The copper treatment will not be discussed further.

Grazing management

The evasive grazing strategy on semi-natural pasturelands evaluated in study I proved unsuccessful. In this case, the idea with a 2-paddock rotation system was tested, whereby the cattle were moved in mid-July to an autumn paddock that had been spelled during the first half of the grazing season. The second year of the trial, a new set of animals were turned out on the pasture contaminated the previous first half of the season by FSGC, that were only mildly infected from a priming dose of approximately 10,000 L_3. Hence, this pasture had been ungrazed from late summer to turnout the following spring, inclusive of the whole autumn of the previous season as well as the intervening winter. The results demonstrated that the untreated and the moved groups performed as well as the anthelmintic treated animals the first year (1997). However, during the second year of the trial (1998), cattle in both the rotation and the untreated set-stocked group suffered from clinical and subclinical PGE. In other words, despite contamination for only the first half of the season, the level of overwintered larval availability was equally high for the rotation animals as for the animals in the untreated set-stocked paddock. Clearly, apart from the priming dose of 10,000 L_3 at turnout the second year, the FSGC encountered overwintered larvae of the same magnitude as the untreated set-stocked animals that resulted in an average weight gain penalty of 30 kg compared with the ivermectin treated cattle. This was further substantiated, both through the companion plot study (III) where faeces derived from the first year of the trial was deposited at regular intervals throughout the grazing season, and a tracer test performed the following season. The conclusion is that contamination of a pasture with faeces with moderate levels of GI nematode eggs for only a short period early in the season may render such pasture unsafe at turnout the following year.

On the other hand, in study II, another evasive grazing management strategy was tested. Here, a pasture grazed the previous year by adult cattle (cow pasture) served as turnout pasture and then the FSGC were moved to a silage aftermath in mid-July. In all 3 years of the experiment, these cattle grew as well, or better, when compared with the ivermectin bolus treated set-stocked animals. Obviously, the cow pastures used in study II could be considered as "safe", in support of parasitological data and excellent productivity, but in particular, by the tracer test carried out in May 2001. The tracer animals that grazed a cow pasture equivalent to those used in study II had contracted a worm burden of 4,000 worms (> 90% *O. ostertagi*). This should be compared with burdens of 1,700 worms in the tracers that grazed the paddock with a history of 3 consecutive years of ivermectin bolus treated animals, and 40,000 in the tracers that grazed the paddock with a 3 year history of untreated, set-stocked cattle. Although these results are promising, we cannot declare all cow pastures in Sweden as completely safe in terms of low levels of larval availability. In the Netherlands, Eysker*, et al.* (2002) recently investigated the exposure of grazing livestock to L_3 on cow pastures and concluded that the safety of such pastures for FSGC may be questionable.

Based on these results, a control strategy that assumes a low level of L_3 exposure to FSGC at turnout as, for instance, Michel's "dose and move" system (Michel, 1969a), has poor prospects of success in Sweden. This system was developed under the assumption of a lower level of exposure of FSGC to overwintered larvae than is attributable to Swedish conditions, and thus rests on a different epidemiology of GI nematode infections. In fact, the combination of a low level of L_3 exposure, as provided by the cow pasture, and a mid-summer move to aftermath without an anthelmintic treatment proved successful. Consequently, no efforts should be spared in providing FSGC in Sweden with a safe pasture at turnout, and if this is combined with a move to good quality aftermath, excellent productivity is possible in the absence of remedial anthelmintic treatments.

Although the impact of second generation L_3 was less important in the present experiments, late season PGE should not be disregarded in Sweden. Especially if the grazing season is prolonged and if scarce herbage availability is not supplemented with extra feed.

Biological control

For the first time, a consecutive year field evaluation was conducted whereby the biocontrol agent *D. flagrans* was used to control nematode parasites in FSGC (II). A prerequisite for BC to become a practically feasible option for farmers is that the administration of the fungal spores is not too laborious and that the animals receive approximately the same dose of chlamydospores. Currently, animals need to be trough fed the fungus on ideally a daily basis through an admixture of fungal material and concentrate. The experience from this experiment is that the concentrate was readily consumed all through the fungal feeding period and that the 0.5 m trough space available for each animal was enough to allow them to eat at the same time. Although this was just a visual observation, it was supported objectively by the results of the presence of nematode trapping fungi in all of the individual faecal cultures.

To be able to evaluate the sustainability of deployment of *D. flagrans* and to estimate the possible beneficial carry-over effects between grazing years, it is necessary to conduct consecutive year studies. This also applies for the comprehensive assessment of any perturbations in the soil nematode community conducted in this project, as summarised in the above section of environmental impact of parasite control. Single year studies have previously been conducted in Denmark (Wolstrup*, et al.*, 1994; Larsen*, et al.*, 1995; Nansen*, et al.*, 1995; Fernández*, et al.*, 1999) and Lithuania (Šarkūnas*, et al.*, 2000). However, in contrast to study II, these experiments did not include a group of anthelmintic treated cattle to enable a maximum productivity comparison. Furthermore, as the single year studies only allow of estimating the reduction of the second generation L_3, these trials are difficult to put into a broader context and to translate to the Swedish situation (see pp. 25-26). Nevertheless, all these studies reported promising results, either in terms of significant weight gain differences, reduced numbers of L_3 on herbage, significantly lower SPC or prevention of PGE towards the end of the grazing season. Unfortunately, the overwintered numbers of L_3 were not assessed, neither of the Danish or the Lithuanian experiments.

In the last year of study II, the animals in the BC group performed 45 kg better than the untreated control animals. This was explained by the reduced exposure to L_3 at turnout, in support of the lower SPC demonstrated in the BC treated animals. Apparently, the reduction of the numbers of L_3 on herbage was transferred from the second to the last year of the trial. This observation was supported in studies II and IV by the results on the numbers of L_3 found on herbage at turnout the last year of the trial. This substantiated the importance of the overwintered numbers of L_3 for the epidemiology of GI nematode infections in Sweden. The 45 kg advantage of the animals in the BC group could be explained by the turnout on a relatively safe pasture the last year, and consequently, not because of a reduction of the second generation of L_3 derived from within season contamination. In fact, *D. flagrans* worked poorly the last year of the trial, but this was not evident until after housing and at turnout the following year.

Biological control with *D. flagrans* exerts its effect in the highly variable external environment and largely within the faecal pat. Thus, the fungus needs to be closely evaluated under different sets of weather conditions with a direct influence on different characteristics of the faeces, *e.g.* faeces dry matter content (DM). Usually, soft faeces are voided by FSGC during the first 2–3 weeks after turnout, mainly due to the change from stable feed to lush grass of high nutritional value and to the common occurrence of coccidial infections in such animals (esp. *Eimeria alabamensis*) (Svensson, Uggla & Pehrson, 1994). Soft faeces, if deposited at a time of regular rainfall, also result in a rapid disintegration of the faecal pats (Dimander, Höglund & Waller, 2003). In study II it was found that occasions of poor performance of BC coincided with wet weather conditions.

The observations made on the combination of early season high precipitation and rapid dung disintegration has not been reported before. In the Danish trials (Wolstrup*, et al.*, 1994; Larsen*, et al.*, 1995; Nansen*, et al.*, 1995) and the Lithuanian trial (Šarkūnas*, et al.*, 2000), low early season precipitation was reported. The impact of low precipitation and an assumed slow faecal pat

disintegration probably contributed to the promising fungal effects observed in those trials.

In studies II and IV, we also evaluated the dose rates of spores that were considered by the producer (Chr. Hansen Biosystems) to be economically realistic. Of course this is a key issue. It is no point in pursuing the evaluation of a novel technology unless it can be produced and marketed at a competitive price for the end-users. The doses used in study II were 1×10^6 spores/kg body weight/day 1998 (high) and 0.5×10^6 spores/kg body weight/day 1999 and 2000 (low). Both the higher and the lower dose rates have been tested in Denmark (Fernández*, et al.*, 1999) and Lithuania (Šarkūnas*, et al.*, 2000) and they found a higher suppression of the numbers of L_3 on herbage when calves were fed the higher dose rate. In study IV, results from the individual faecal cultures incubated under constant optimal conditions showed no significant difference in development between the high and low dose rate. Neither was there any significant dose-dependent difference in the effect of *D. flagrans* of the numbers of L_3 on herbage in study IV. However, significant differences in reduction were observed between years.

Rather, the year of the poorest fungal effect was the last year when the higher dose rate was used, but the year when wet weather conditions prevailed during the period of the highest egg excretion. Moreover, the overwintered numbers of L_3 the following time of turnout (2001) was not lower in the BC paddock compared with the paddock where untreated, set stocked animals had been grazed for 3 consecutive years (II, tracer test).

Logically, the poor effect under wet conditions is likely to be due to a dilution effect because of the physical separation of the fungal spores and the free-living stages of nematodes. In this instance, the property of *D. flagrans* as being a weak competitor in the pasture bio-community (Faedo*, et al.*, 2002; Knox, Josh & Anderson, 2002) is a disadvantage, but nevertheless beneficial as regards environmental impact aspects. Under the conditions experienced the last year of trial II, it may be speculated that a higher dose rate would have resulted in a better performance of *D. flagrans*. However, the evaluation of a higher dose rate under such field conditions has not yet been made. Inversely, if the fungus is allowed to operate under conditions when the faecal pat is relatively intact and persist for a number of weeks, the dose rate seems to be of lesser importance (studies II and IV). At least when the dose rates of 1×10^6 spores/kg body weight/day and 0.5×10^6 spores/kg body weight/day are used.

In conclusion, any control strategy would take advantage of the knowledge and experiences acquired from the work on the ecology of the pre-parasitic stages of cattle nematodes. We deal with biology and potentially pathogenic organisms that have an obligatory pre-parasitic part of their life cycle in a highly variable external environment that will affect their biological potential considerably. Because of the indefinite sets of conditions exposed to the pre-parasitic stages of GI nematodes of cattle, package solutions cannot be provided that are applicable to every farmer in all years under all conditions. Rather than to advocate elaborate control regimes that are difficult to conform to, the flagrant pitfalls in poor parasite control management should be avoided.

Summary and concluding remarks

Professional farming enterprise is a business that produces food commodities with you and me as the customers. In a market economy, the strongest and most efficient means of bringing pressure to bear on the producer is the consumer. If the consumer wants to buy cheap food of top quality produced with a minimum use of chemicals, this might be cumbersome to comply with unless all parties involved show an understanding attitude and have some patience. Farmers have to realise that parasite control in the absence of anthelmintics is possible, but requires from them considerable efforts in animal management and planning, as well as their continued commitment to these activities. Committed farmers in turn need support and clear-cut advice from informed veterinary practitioners, animal scientists, and extension workers, on relevant, scientifically based strategies for parasite control. Because of the applied nature of this project, it was recognised from the outset to alert farmers and advisers to the presence, relevance and insidious nature of GI nematode infections and to distribute information on our results. To accomplish this, special emphasis was also given to farmer meetings, informal talks and easily understood articles in the farming press.

At the other end of the spectrum, consumers need to understand that modern agriculture has inborn characteristics that make it very difficult (and in most cases impossible) to raise healthy, highly productive livestock in the complete absence of chemicals and drugs. Anything else would be naïve.

Finally, this thesis cannot provide Swedish farmers with the final solution for appropriate GI nematode parasite control in FSGC, and neither was this the intention. It is further not possible to present all the important epidemiological determinants of cattle GI nematode infections in context of their relative importance under any given set of environmental and management conditions. This, however, is not a reason to abandon this cause. On the contrary, let us proceed with a whetted appetite and the motto:

"We have to learn to live with the GI nematodes and while we do, we can learn about them, and hence, develop and advocate better control strategies"

As a result of the efforts at setting the benchmark for the epidemiology and control of GI nematode infection in FSGC in Sweden, the following major conclusions and remarks can be made for the benefit of the Swedish cattle and their producers and for future studies:

- Adequate nematode parasite control may be achievable without the use of anthelmintics (study II)
- Uncontrolled GI nematode infections resulted in a 50% lower weight gain compared with anthelmintic treated cattle. This difference was even larger when pasture management and pasture supply was optimal (rotation) (II)
- Importantly, the weight gain penalty occurred without overt clinical signs (II)
- Overwintering survival of GI nematode larvae was the key factor in the epidemiology of PGE in Sweden (I–IV)
- A dry summer and the subsequent poor disintegration of faecal pats provided the pre-parasitic stages of the nematodes with an environment protected from detrimental external factors. This in turn was a beneficial prerequisite for a high degree of overwintering survival of infective larvae (IV)
- Even contamination of pastures with very low faecal egg counts during a dry season rendered such pasture heavily infected and unsafe to turn out FSGC onto the subsequent year (II and IV)
- The availability of L_3 on herbage is dynamic and may change rapidly from parasite “safe” to parasite “dangerous” (II and IV)
- Contamination of a pasture for only the first half of the grazing season resulted in high levels of overwintered larvae the following season (I and III)
- Biological control with the nematophagous fungus *D. flagrans* proved effective in reducing larval availability, but this effect was impaired when rapid dung disintegration occurred due to wet weather conditions and during the first half of the grazing season (II and IV). Unfortunately, this is the period when the egg output normally is the highest

Future research

Agricultural policies and management strategies of the livestock enterprise are dynamic, as is the biology of parasitic nematodes. These entities act together and deserve current attention if livestock are to be maintained in a "post-anthelmintic" era of parasite control. Different management strategies may require altered control strategies, and parasitic nematodes may present a different appearance that may change the epidemiology of GI nematode infections. Research in the area of applied helminthology should continue with an interdisciplinary approach where traditional parasitologists collaborate with molecular biologists, animal scientists, biologists and environmentalists.

This project has suggested that the following areas of research would be highly worthwhile:

- Large-scale field experiments for the evaluation of 1) strategic supplementary feeding with concentrate and roughage and 2) a grazing management strategy with a mid-summer move of FSGC from the turnout pasture to aftermath, and the use of second-season grazing cattle (SSGC) to use this contaminated turnout pasture. This pasture will be used the following year as a turnout pasture for a new set of FSGC.
- Monitoring of SSGC on faecal egg excretion, faecal cultures and SPC to assess the impact of grazing the pastures contaminated by FSGC prior to their move to aftermath
- Monitoring of FSGC on performance, faecal egg excretion, faecal cultures and SPC during the housing period and the subsequent second grazing season following different modes of parasite control of their first season
- Ecological investigations by means of tracer tests the dynamics of the pre-parasitic stages including the propensity for arrested development of GI nematodes
- Evaluation of the interaction between GI nematode and coccidial (*Eimeria* spp.) infections

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Magnus, who crossed my doorstep with the intention to stay for a couple of months. Your well-timed arrival cheered me up from a less than cheerful departure. Although it feels like 4 months, you were my flatmate for 4 years. I taught you how to make béchamel – twice by the way – and you taught me how to make apple crumble. I miss our outstandingly pleasant three-course weekend meals with alternating master chefs Dimander and Åkerblom. We almost got through the folder of delicious recipes. There are still some left to try out and more to come. Magnus, everybody needs a person he can bring up any subject for discussion with, at any time. You are this person for me. Five years of struggling have passed for both of us as we eventually brought our projects to a successful close. I was your pacemaker at some times, you were my pacemaker at other times; somebody always kept the machinery going. Boy, you know a lot about me that nobody else knows. I guess that you and I have what is called a lifetime friendship. Congratulations on your degree, engineer Åkerblom!

Fritz and **Aska**, you never let me down.

My family. **Mother**, **father**, **brothers** and **sisters**. You all provide me with a rest haven.

In conclusion: the funding is spent, a pleased PhD has been produced and a small amount of knowledge has been added to science.

Over and out!