THE INTERACTION BETWEEN *COOPERIA* SPP. AND *OSTERTAGIA* SPP. (NEMATODA: TRICHOSTRONGYLIDAE) IN CATTLE.

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STELLINGEN

I

Interactie tussen *Cooperia oncophora* en *Ostertagia ostertagi* is epidemiologisch van weinig belang.

II

Wormlengte is een meer betrouwbare parameter voor de verkregen immuniteit van de gastheer dan het als zodanig veel gebruikte wormaantal.

III

Het feit dat *Ostertagia ostertagi* meer dan *Cooperia oncophora* voorkomt bij oudere dieren wordt niet veroorzaakt door het niet-immunogeen zijn van *O. ostertagi.*

IV

De ontwikkeling van vaccins tegen parasieten van landbouwhuisdieren dient hand in hand te gaan met genetische selectie van gastheren die op deze vaccins een goede respons vertonen.

V

De intensieve studie van het afweermechanisme van laboratorium dieren tegen parasitaire infecties heeft tot nu toe geen essentiële bijdrage geleverd aan het minimaliseren van schade door parasitaire infecties bij landbouwhuisdieren.

VI

Het meervoudig inclonen van het gen dat codeert voor het hormoon bovine somatotropine (BST) in het genoom van het rund met het doel diens melkproduktie te verhogen zal minder weerstand bij de consument opwekken dan het kunstmatig toedienen van dit hormoon.

VII

Volgens 'van Dale' wordt wormschade alleen door houtwormen veroorzaakt.

VIII

Het meesterschap van de hoogleraar ligt niet op het gebied van het onderwijs.

IX

Als grondstofleverancier voor de leerlooierij zou de veehouder zich het vel niet over de oren moeten laten halen.

X

Het gebruik van een tekstverwerker doet het aantal correcties zodanig toenemen dat de benaming tekstbewerker meer op zijn plaats is.

XI

Gezien de maatregelen die zijn genomen om tegemoet te komen aan het steeds intensievere gebruik van het Zodiac-gebouw ontbreekt het er nog maar aan dat dit gebouw wordt voorzien van roostervloeren; dit laatste zou in ieder geval de directe communicatie tussen de vakgroepen Veehouderij en Veefokkerij bevorderen.

K. Frankena

The interaction between *Cooperia oncophora* and *Ostertagia ostertagi* (Nematoda: Trichostrongylidae) in cattle.
Wageningen, 18 september, 1987.

De natuur gedoogt dat gij haar bespiedt,
niet dat gij haar ontraadselt.
(Pythagoras, 6e eeuw v. Chr).

VOORWOORD

Het onderzoek dat beschreven is in dit proefschrift is uitgevoerd bij de vakgroep Veehouderij van de Landbouwuniversiteit te Wageningen gedurende de periode maart 1984-januari 1987. Hoewel op het omslag alleen mijn naam staat vermeld hebben zeer velen een bijdrage geleverd aan het werk waarvan dit proefschrift het resultaat is. Speciaal de dagen waarop de kalveren geslacht moesten worden wil ik memoreren, aangezien de personeelsbezetting van het onderzoek op die dagen met 1500% toenam. Aan allen, begeleider, dierverzorger, student, analist(e), typist(e) of echtgenote, die het mogelijk hebben gemaakt, hetzij door het leveren van inspanning, hetzij juist door het zorgen voor ontspanning, om dit proefschrift te realiseren, zou ik willen zeggen: dank jullie wel!!. Zonder jullie hulp had ik me door heel wat meer moeilijkheden moeten wurmen dan nu het geval is geweest.

Klaas Frankena

CONTENTS PAGE

1 INTRODUCTION

Grazing cattle all over the world are afflicted with endoparasitic infections. Such infections might be of economical importance because they may lead to reduced production (milk, meat, progeny, labour), increased mortality and costs for medication and additional labour. In West-European countries most frequent occurring and abounding nematode parasites in cattle are *Cooperia oncophora* and *Ostertagia ostertagi* (Michel *et al.*, 1970; Borgsteede, 1977), nematodes which parasitize the small intestine and the abomasum, respectively.

In traditional systems, calves put on pasture in early spring and grazing calf paddocks, heavy infections with both species may occur. In such infections there seems to be a succession of both populations: *C. oncophora* is responsible for the majority of the faecal egg output during the first months after turnout while later on *O. ostertagi* eggs are most prevalent. The population dynamics of both parasite species are well known by means of experimental mono-infections. Very little is known, however, about influences that both nematode populations have upon each other when they parasitize the same host in concurrent or sequential infections, as is the case in natural infections. Such interaction might affect the parasitological characteristics of the parasite populations. When worm burdens or faecal egg output are influenced, interaction is of epidemiological* importance. Indications that interaction between both species do occur and are mediated by the host's immune response were found by Kloosterman *et al.* (1984), who observed a heterologous influence in sequential infections. This was the reason to start the present study on the interaction between *Cooperia oncophora* and *Ostertagia ostertagi*.

In this thesis, experiments are described in which immunizing primary infections with *C. oncophora* or *O. ostertagi* were given to helminth-free raised calves. Several levels and patterns of primary infections were applied because these might affect the host response. After treatment with an anthelmintic, challenge infections were carried out with either *C. oncophora* or *O. ostertagi* or a mixture of both. Interaction was monitored by means of parasitological parameters. Immunological parameters were used to assess the relation between the host's immune response and the occurrence of interaction.

* epidemiology and epizootiology are regarded as synonyms in this thesis

2 LITERATURE

2.1 THE PARASITES

2.1.1 *Cooperia oncophora*

This species has had little attention since its pathogenicity was regarded as moderate. In the sixties Herlich (1965a, 1965b) did some research with experimental infections. Since the late seventies this species has received more attention by the work of Coop *et al.* (1979), Borgsteede and Hendriks (1979), Kloosterman *et al.* (1978, 1984) and Albers (1981).

- Life cycle.

Cooperia oncophora is one of the most abounding species of nematode parasites in areas with a temperate climate. Despite this fact, its life cycle has not been described in detail until the study of Isenstein (1963). Under natural conditions animals are infected with this parasite by intake of infective third-stage larvae (L_3) with the grass. Following ingestion the L_3 exsheath in the abomasum and moult to fourth-stage larvae (L_4) in the small intestine, the final habitat. A last moult takes place ten days after ingestion, resulting in fifth-stage larvae (L_5). The first gravid females may occur 15 days after inoculation. Eggs produced by female worms may be found in the faeces within 20 days after ingestion. Eggs pass to the pasture with the faeces and develop into first-stage larvae (L_1) which hatch and moult to become second-stage (L_2) and third-stage (L_3) larvae. Under field conditions the development from egg to L_3 might take ten days to several weeks depending on temperature and rainfall. Moist conditions are necessary for the L_3 to migrate actively from the faecal pat to the grass where it is awaiting the grazing host. The life cycle is depicted in Figure 1. The L_3 is rather resistant to climatological conditions which are prevailing in West-European countries; it can overwinter on pasture and by doing so it can give rise to a new generation of the species the next year.

- Epidemiology.

In the Netherlands epidemiological work on bovine gastrointestinal nematodes has mainly been done by Kloosterman (1971) and Borgsteede (1977). After turnout, calves in their first grazing season are infected with overwintered larvae and a population of adult, egg producing worms will develop within a few weeks. The relative contribution of *C. oncophora* to the total level of nematode egg output is very high (> 60%) due to the high reproductive capacity of this species. Eggs develop into infective larvae and, if

climatological circumstances are suitable, this results in a mid-summer increase of larval infestation of the pasture. When calves are still grazing such pasture, a massive intake of larvae will occur at that moment. In general, however, no rise in faecal egg output is noticed after this massive intake as a result of the developing host resistance. From experimental infections Coop *et al.* (1979) concluded, using the egg output as a criterion, that the acquired immunity, a type of resistance, becomes effective within two months after primary inoculation. The acquired resistance is also related to the reduction of worm burdens, stunted growth and inhibited development of new incoming larvae (Albers, 1981). Because of this acquired resistance *C. oncophora* is less frequently found in older animals (Kloosterman, 1971; Borgsteede, 1982).

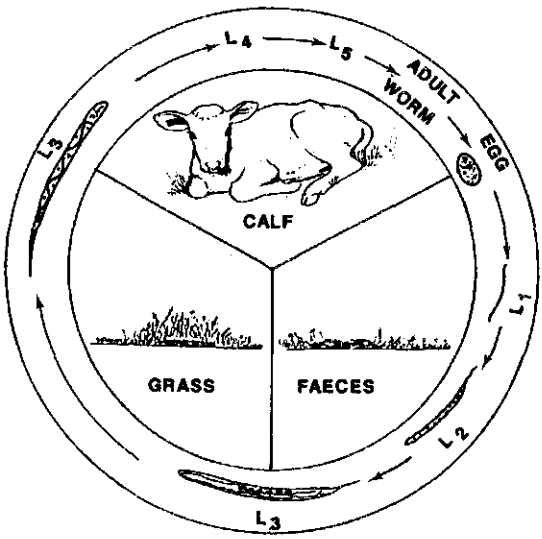

Fig. 1. Life cycle of *Cooperia oncophora.*

- Pathogenicity.

Cooperia oncophora is likely to be a lumen-dwelling parasite because Coop *et al.* (1979) have found, by histological examinations, no evidence of penetration by the parasite into the mucosa or the submucosa. Only limited compression and distortion of intestinal villi in contact with worms were noticed. The fact that no severe damage to the intestinal wall occurs might explain the moderate pathogenicity of this species. Herlich (1965b) infected calves, five to eight months old, with single doses of $350*10^3$ to $3*10^6$ larvae; only the highest dose gave severe enteritis, characterized by anorexia, diarrhoea and loss of weight. Coop *et al.* (1979) infected 4.5 months old calves daily with $5*10^3$, $10*10^3$ or $20*10^3$ larvae for 20 weeks; these infections reduced weight-gain

by 13.5%, but did not produce obvious clinical signs of trichostrongylosis; a slight hypoalbuminaemia occurred in the group receiving the highest dose. Three weeks after a single dose of $20*10^3$ larvae, Borgsteede and Hendriks (1979) noticed mild diarrhoea and a growth depression of about 15% at three months *post infectionem*. Albers (1981) observed a loss of appetite, diarrhoea and a loss of weight gain (20%) during secondary *C. oncophora* infections with $350*10^3$ larvae.

The low pathogenicity of *C. oncophora* infections might partially be explained by the rapid acquisition of immunity so that very large worm burdens usually do not exist under natural conditions.

2.1.2 *Ostertagia ostertagi*

Most of the work on this abomasal parasite was done by Michel and his co-workers (Michel, 1963; Michel *et al.*, 1970; Michel, 1970) and by the Glasgow-group (Anderson *et al.*, 1965, 1966, 1967; Armour, 1980).

- Life cycle.

The life cycle of *O. ostertagi* is quite similar to the one of *C. oncophora*, described in 2.1.1 (Fig. 1). Both parasite species have a direct life cycle and a free-living infective stage (L_3). The habitat of *O. ostertagi* is the abomasum.

- Epidemiology.

Overwintered *O. ostertagi* larvae are ingested by grazing cattle in spring and develop into adult worms in the abomasum. The egg production of an *O. ostertagi* population is low when compared to *C. oncophora* and it is, according to Michel (1969b), independent of the size of the worm burden and the type of infection (natural infections or experimental infections with single or repeated doses). After the midsummer increase of pasture contamination ostertagiasis may occur in late summer and autumn. Ostertagiasis may also occur in early spring when animals are still housed. Martin *et al.* (1957) suggested that the latter is due to maturation of larvae which are ingested as L_3 in the previous grazing season, but arrested their development at the early fourth stage (EL_4), presumably as a result of the host's acquired immunity. A series of experiments, reviewed by Armour and Ogbourne (1982), pointed out that the propensity to become arrested is a heritable trait of the larva itself and may occur in response to various adverse environmental stimuli such as declining temperature in late summer and autumn.

The host response to *O. ostertagi* does not seem to be completely effective, because Kloosterman (1971) found that 96% of cows older than 6 years were excreting *O. ostertagi* eggs. Borgsteede and van den Burg (1982) measured a mean abomasal worm burden

of almost 4,000 worms in older cattle; *O. ostertagi* was the most prevalent species (more than 85%), *Trichostrongylus axei* followed with 12%.

- Pathogenicity.

Following ingestion the larvae penetrate the gastric glands. At this stage no alteration in biochemical constitution of abomasal fluid or blood are detectable. From day 18 *post infectionem* onwards the young adult worms begin to emerge from the glands and, microscopically, hyperplasia and loss of cellular differentiation can be noticed. Consequences of these changes might be:

1. an elevation of the pH of the abomasal contents from 2 to 7 causing a failure to transform pepsinogen into pepsin, resulting in an impaired digestion of abomasal protein, and a loss of bacteriostatic capacity.
2. an enhanced permeability of the abomasal wall for macromolecules resulting in elevated plasma pepsinogen levels and hypoalbuminaemia.

In clinical infections, diarrhoea and a loss of appetite and bloom may occur, but the mechanism by which these symptoms are mediated, is not clearly understood. The cause of diarrhoea is possibly related to an increase of viable bacteria in the abomasal contents (Armour and Ogbourne, 1982). Diarrhoea and loss of appetite may result in a reduced weight-gain or even a considerable loss of weight. Michel *et al.* (1978a) measured a loss of weight of about 1 kg per day between the fifth and tenth week after the first inoculation when 8.5 month old calves were infected daily with increasing doses; a reduction in weight-gain was noticed already at three weeks after the first inoculation. Murray *et al.* (1970) inoculated ten week old calves with $3*10^5$ *O. ostertagi* larvae and noticed a loss of weight of about 5.5 kg during the fourth week. Kloosterman *et al.* (1984) measured a reduction in weight-gain of about 100 g/day during a period of five weeks using three month old calves infected with $100*10^3$ *O. ostertagi* larvae (resulting in subclinical infections).

2.2. INTERACTION BETWEEN PARASITE POPULATIONS

2.2.1 Mechanisms of interaction

It is common knowledge that interaction **within** one parasite population (intraspecific interaction - Fig. 2, arrow 1) does occur, especially in crowded situations (Silver *et al.*, 1980; Roberts, 1968). Interaction *between* parasites is also found and may result in reduced worm burdens and worm fecundity, premature expulsion and stunted growth and development, but also in delayed expulsion or prolonged longevity and fecundity. Interaction may occur either in a direct or an indirect way (Halvörson, 1976). The first one

occurs when parasites influence each other without involvement of host processes (Fig. 2 - arrow 2). Examples of direct interaction are predation, competition for food or habitat and outnumbering. Predation occurs, for example, when echinostome rediae are involved in multispecies trematode infections in molluscs. Competition for carbohydrates between the tapeworms *Hymenolepis diminuta* and *H. citelli* lead to stunted specimens in crowded situations (Read and Phifer, 1959). Differences in reproductive potential may lead to outnumbering of one species by another according to Hardin (1960); e.g. in mixed infections of two strains of trypanosomes the rate of disappearance of one strain could be calculated from the multiplication rates of both parent strains (von Brand and Tobie, 1960).

Indirect interaction means that host processes are involved in positive or negative influences that parasite species have upon each other. Interaction via the host has an immunological or physiological basis. Immunologically mediated interaction may either be specific or non-specific. The first originates from immunological cross-reactions. The immune response elicited by antigens of one species may cross-react with antigens of another species that parasitizes the same host at the same time or at a moment after the immunizing species has been expelled or removed (Fig. 2 - arrow 3); e.g. in rats the fecundity of *Trichinella spiralis* is negatively affected by a previous *Strongyloides ratti* infection (Moqbel and Wakelin, 1979). Suppression of the specific reaction, as an active process of the parasite to evade the host response, is also known to occur in some nematode infections. Immunosuppression caused by one species might interact with a second species (Fig. 2 - arrow 4); e.g. *Heligmosomoides polygyrus* (= *Nematospiroides dubius)* infections in mice result in prolonged longevity of a *Nippostrongylus brasiliensis* population (Jenkins, 1975) and delayed expulsion of *T. spiralis* worms (Behnke *et al.*, 1978). Allergic inflammation results from an immunologically mediated, non-specific reaction. The allergic reaction consists of an immunologically specific delayed hypersensitivity reaction between antigen and antigen sensitive T-cells followed by an immunologically non-specific inflammatory reaction (Larsh and Race, 1975). This kind of reaction elicited by one species may affect the other species; e.g. premature expulsion of *N. brasiliensis* occurs in concurrent infections with *T. spiralis* (Kennedy, 1980a) as a result of the inflammatory reaction elicited by *T. spiralis*.

Physiologically mediated interaction results from biochemical changes in the microenvironment induced by one species. Such changes might be detrimental (or beneficial) to another species. Mapes and Coop (1971) found that *Nematodirus battus* in the small intestine of lambs is negatively affected by massive *Haemonchus contortus* infections in the abomasum. The latter can cause an increased pH in the small intestine (Mapes and Coop, 1970) and thus less optimal conditions may arise for *N. battus*. *Trichostrongylus axei* (abomasum, cattle) may also induce an elevated intestinal pH in calves (Ross *et al.*,

1970). Jackson (pers. comm.) stated that the interaction between *Ostertagia circumcincta* and *Trichostrongylus vitrinus*, abomasal and intestinal nematodes of sheep, respectively, is mediated physiologically.

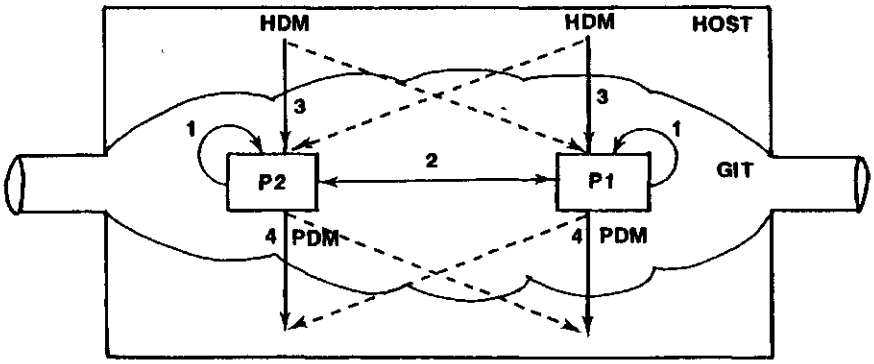

Fig. 2. Possible types of interaction. G.I.T. = gastrointestinal tract. P1, P2 = parasite populations 1 and 2. HDM = host defence mechanism. PDM = parasite defence mechanism.

1 = intraspecific interaction
2 = direct interspecific interaction
3 = indirect host-mediated negative interspecific interaction
4 = indirect host-mediated positive interspecific interaction

Localization of both parasite populations and timing of both infections are important features for measuring interaction. In case of interaction mediated by competition for habitat both parasite species should parasitize at the same site. As *C. oncophora* and *O. ostertagi* parasitize in the small intestine and the abomasum respectively, this type of interaction is of minor importance. Timing of the infections has proven to be very essential in a great number of interaction models. In case of sequential infections the lapse of time between immunizing and challenge infection might be important, because cross-reacting antibodies may have disappeared after a certain period. The same can be stated for immunosuppressive effects. Such effects may occur during a certain phase of the infection, for example, the immunosuppressive effect of *O. ostertagi* larval antigens during the prepatent period (Klesius *et al.*, 1984).

A synopsis of studies on interaction models is given in Table 1 (laboratory animals) and Table 2 (domestic animals). From these tables it can be concluded that negative interaction between parasite populations may result in reduced worm burdens, reduced fecundity, premature expulsion and stunted growth and development, while positive interaction, as a result from immunosuppression by one species, may lead to delayed expulsion, prolonged longevity and increased fecundity. Some authors assume that inter-

action results from a changed micro-environment. Kloosterman *et al.* (1984), however, did not find any influence of an *O. ostertagi* infection on a *C. oncophora* population that was present at the same time, although *O. ostertagi* infections may lead to an increased abomasal pH. This type of interaction possibly only occurs when massive abomasal infections are present. Most authors assume that interaction between parasite populations is mediated via the host immune system. Kloosterman *et al.* (1984) suggested that the interaction between *C. oncophora* and *O. ostertagi* is mediated immunologically since Keus *et al.* (1981) found cross-reacting antibodies when saline extracts of adult worms were used in the Enzyme-Linked-Immuno-Sorbent Assay (ELISA).

2.2.2 Parasitological parameters for measuring interaction

As was concluded from Tables 1 and 2 interaction between parasite populations affects the parasitological characteristics of one or both populations and are mediated via the host, either immunologically or physiologically. As the interaction between *O. ostertagi* and *C. oncophora* is believed to be immunologically mediated, parasitological parameters and immunological features of interaction (2.2.3) will be discussed briefly.

- Faecal egg counts.

This is the only parasitological parameter that can be obtained 'continuously' from the same animal during a gastrointestinal parasitic helminth infection. All other parasitological parameters require slaughtering of the animal. Faecal egg counts may reflect the fecundity of a parasite population because Albers (1981) found high correlations (0.84) between actual egg counts and number of eggs *in utero* of *C. oncophora* populations. Fecundity is defined by some authors as the number of eggs per gram of faeces (Jenkins, 1975) while others express it by the number of eggs *in utero* (Moqbel and Wakelin, 1979) or the number of larvae released per female per day (Silver *et al.*, 1980; Kennedy, 1980b; Moqbel and Wakelin, 1979). Positive effects of a *N. dubius* infection on the faecal egg output level and duration of a *N. brasiliensis* population were reported by Colwell and Wescott (1973). Kloosterman *et al.* (1984) found no interaction between *C. oncophora* and *O. ostertagi* in concurrent and sequential infections with regard to this parameter.

- Worm counts.

The most frequently used parameter for measuring interaction is the worm count. Interaction might affect the establishment (Mapes and Coop, 1971) and/or the moment of expulsion (Kennedy, 1980a; Jenkins, 1975; Lee *et al.* 1982), resulting in decreased or increased worm counts. In case of *C. oncophora* and *O. ostertagi*, Kloosterman *et al.* (1984) found a mutual negative interaction when worm counts are used as a criterion.

Table 1. Interactions between parasite populations in laboratory animals.

Parasites	Host	Type of infection	Results	(Assumed) Mechanism	Author(s)
N. brasiliensis *T. spiralis*	Mouse	immunizing infection with one species followed by a concurrent challenge	premature expulsion of *N. brasiliensis* and *T. spiralis;* impairment of growth and fecundity of *T. spiralis*	immunologically mediated non-specific inflammation	Kennedy (1980a)
N. brasiliensis *T. spiralis*	Rat	immunizing infections with *T. spiralis* followed by *N. brasiliensis* challenge	reduced numbers of *N. brasiliensis*	cross resistance	Kazacos (1975)
N. brasiliensis *S. ratti*	Rat	immunizing infection with one specie followed by a heterologous challenge	reduced numbers of the heterologous species	cross resistance	Kazacos and Thorson (1975)
N. brasiliensis *N. dubius*	Mouse	*N. brasiliensis* superimposed on or following a terminated *N. dubius* infection	prolongued fecundity and longevity of *N. brasiliensis*	reduced immunogenicity of *N. brasiliensis*	Jenkins(1975) Colwell and Wescott (1975)
T. spiralis *T. muris*	Mouse	immunizing infection with one species followed by a heterologous challenge	accelerated expulsion of heterologous species	cross immunity	Lee *et al.* (1982)
T. spiralis *S. ratti*	Rat	immunizing infection with one species followed by a heterologous challenge	reduced number, length and fecundity of heterologous species	cross immunity	Moqbel and Wakelin (1979)
T. spiralis *T. muris*	Mouse	single or both species superimposed on primary infections	simultaneous expulsion of *T. muris* during expulsion phase of *T. spiralis*	indirect cross immunity	Bruce and Wakelin (1977)
T. spiralis *H. diminuta*	Mouse	*H. diminuta* superimposed on *T. spiralis*	reduced establishment and growth of *H. diminuta*	disturbances in host diet by inflammatory response	Behnke *et al.* (1977)
T. spiralis *N. dubius*	Mouse	*T. spiralis* superimposed on *N. dubius*	delayed expulsion of *T. spiralis*	immunosuppressive effects of *N. dubius*	Behnke *et al.* (1978)
N. dubius *T. muris*	Mouse	*T. muris* superimposed on *N. dubius* expulsion of *T. muris*	delayed primary immune expulsion of *T. muris*	immunosuppressive effects of *N. dubius*	Jenkins and Behnke (1977)
F. hepatica *S. mansoni*	Rat	immunizing infection with one species followed by *F. hepatica* challenge	reduction of numbers and length of *F. hepatica by* heterologous priming	immunologically mediated cross resistance	El-Azazy (1985)

Table 2. Interactions between parasite populations in domestic animals.

Parasites	Host	Type of infection	Results	(Assumed) Mechanism	Author(s)
O. ostertagi *O. circumcincta*	lamb	immunizing infection with *O. ostertagi* followed by *O. circumcincta* challenge	reduced number of *O. circumcincta*	cross resistance	Coop *et al.* (1985)
H. contortus *O. circumcincta*	lamb	immunizing infection with *O. circumcincta* followed by *H. contortus* challenge	reduced numbers and faecal egg output *H. contortus*	alteration of abomasal pH	Blanchard and Wescott (1985)
H. contortus *N. battus*	lamb	immunizing infection with *H. contortus* or both species followed by *N. battus* challenge	reduced establishment and fecundity of *N. battus*	changes in alimentary physiology by massive *H. contortus* infections	Mapes and Coop (1971)
O. ostertagi *C. oncophora*	calf	immunizing infection with one or both species followed by heterologous challenge	reduction of numbers, fecundity and length of heterologous species	cross reacting antibodies	Kloosterman *et al.* (1984)
C. oncophora *C. pectinata*	calf/ lamb	immunizing infection with one species followed by heterologous challenge	reduced numbers of heterologous species	cross immunity	Herlich (1965b)
H. contortus *O. circumcincta* *T. axei*	lamb	single-, dual- and triple species infections (concurrent)	reduced numbers of *H.contortus* and *O. circumcincta* in concurrent infections	physiological disturbances of the abomasum by *T. axei*	Turner *et al.* (1962)
H. contortus *O. circumcincta* *T. axei* *T. colubriformis*	lamb	*H. contortus* or *T. axei* superimposed on a concurrent infection of all 4 species	'self cure' caused by the superimposed species results in concurrent expulsion of the other species	changes in environmental conditions at the time of 'self-cure' are detrimental	Stewart (1955)
O. circumcincta *T. vitrinus*	lamb	immunizing infection with *O. circumcincta* followed by concurrent challenge	reduced establishment of *T. vitrinus*	physiological disturbance of intestinal environment	Jackson (in press)
T. colubriformis *N. spathiger*	sheep	vaccination with irradiated larvae of *T. colubriformis* followed by challenge with one or both species	reduced numbers of *N. spathiger* in concurrent challenges	immunologically mediated non-specific reaction	Dineen *et al.* (1977)

The total worm burden can be differentiated in several ways. In the first place, the worm burden can be split according to the sex of the worms. Albers (1981) reported about an increasing sex-ratio (number of females/male) in homologous infections in the *C. oncophora* - calf system, presumably due to a selective loss of adult male worms. Crofton and Whitlock (1968) observed a gradual increase of the percentage female adult *H. contortus* worms in sheep which was due to a more rapid reduction of the male worm numbers. In a considerable number of interaction models changes in sizes of worm burdens were noticed, but no details have been reported about the female/male ratios. Kloosterman *et al.* (1984) did not find any significant alteration of the sex-ratios in the *C. oncophora* - *O. ostertagi* - calf model. Secondly, the total worm burden can be divided in various developmental stages. In homologous re-infections a large percentage of *C. oncophora* and *O. ostertagi* populations may consist of inhibited larvae and immature worms (Albers, 1981; Michel *et al.*, 1973). A third way to differentiate the total worm burden emerges from the fact that some species show polymorphism. According to Le Jambre and Ractliffe (1976) the ratio linguiform A/linguiform B vulval phenotypes reflects crowding of *Haemonchus contortus* cayugensis. Albers (1981) noticed that *C. oncophora* is more sensitive to expulsion than *C. surnabada* (two polymorphs according to Isenstein, 1971).

- Worm length.

Worm length is a very commonly used parameter to ascertain about growth and development of a parasite population. It is widely accepted that it is affected by the host response and this is the reason why worm length is one of the most important parameters in many interaction studies. Other parameters used for measuring worm development are width of the worms (Michel *et al.*, 1971), dry weight or biomass (Christie *et al.*, 1979) and length of spicula (Borgsteede and Hendriks, 1979).

- Vulval flap development.

According to Michel *et al.* (1972a) the development of the vulval flap of *O. ostertagi* females is negatively affected by the host response, which means that this parameter is very suitable for interaction studies. Kloosterman *et al.* (1984) found negative effects of previous *C. oncophora* infections on vulval flap development of *O. ostertagi* populations.

- Number of eggs *in utero*.

Sometimes this parameter is used to assess the fecundity of a worm population. Impairment of fecundity in interaction models might result from immunological non-specific inflammatory processes (Kennedy, 1980a; Behnke *et al.*, 1977) or specific cross-immunity (Moqbel and Wakelin, 1979; Kloosterman *et al.*, 1984). Other parameters for

measuring parasite fecundity are the previous mentioned faecal egg count and the number of larvae released per female per day.

2.2.3 Immunological aspects of interaction

Resistance of a host to (re)infection has two main aspects: natural and acquired immunity. The first results from environmental conditions within the host which are a consequence of the host's structural, biochemical and physiological makeup (Wakelin, 1978). Natural resistance exists prior to contact with the pathogen and its action does not depend on any feature of the specific immune response. The acquired immunity originates from the previous exposure of the host to the same pathogen. Interaction between parasite populations may arise from this type of resistance if the acquired immunity elicited by one species is also acting against other species. Whether the acquired resistance is essential to the explanation of the interaction between parasite populations or not needs a thorough knowledge of the immune responses provoked by the separate species. Still little is known about the precise mechanism by which the immune response affects a parasite population, despite of considerable fundamental research, mainly in laboratory animals. The ultimate effect of such a response may consist of a migration away from the predeliction site (Moqbel and Denham, 1977; Kennedy, 1980b), a complete or incomplete expulsion (Murray *et al.*, 1970), reduced fecundity (Ogilvie and Jones, 1971), morphological evidence of damage (McLaren *et al.*, 1978; Mackenzie *et al.*, 1980) or inhibition of growth and development (Michel *et al.*, 1971; Michel *et al.*, 1972a).

As soon as a host is infected a variety of antigenic material is presented. The antigens might be of cuticular origin (Parkhouse *et al.*, 1981; Philipp *et al.*, 1981) but excretory and secretory products also proved to be immunogenic (Ogilvie *et al.*, 1973; Jenkins and Wakelin, 1977; Gamble *et al.*, 1983). Each developmental stage of the parasite may have different antigens, resulting in stage-specific immune reactions (Mackenzie *et al.*, 1978). Antigen absorption in the intestinal tract presumably takes place via the "M-cells" of the Peyer's patches (Husband and Watson, 1978), but also the increased mucosal permeability (Smith *et al.*, 1984a, 1984b) might be responsible for the uptake of antigen. The latter might be induced by damage to the gastrointestinal wall caused by the parasites themselves (Murray, 1969). Once the antigens have passed the epithelium of the gastrointestinal wall a complex host response is set to work. Cells differentiated from lymphoid and myeloid lineage are involved. The lymphoid lineage produces lymphocytes (B- and T-cells). Cells of myeloid origin are granulocytes (eosinophils, basophils and neutrophils), mast cells and monocytes. When pathogens enter the host B-cells differentiate into antibody-producing plasma cells whilst T-cells, when sensitized, act more

indirectly by cooperating with B-cells and by stimulating or recruiting components of the myeloid system.

The role of the different antibody classes (IgA, IgM, IgG_1, IgG_2, IgE) with regard to immunity against gastrointestinial parasites is not clearly understood. Immunity to helminths has always been associated with increased IgE-levels. The precise role of IgE is not known, but it presumably mediates mast cell degranulation (Jarrett and Miller, 1982) and thereby it takes part in the inflammatory response (Hurvitz, 1982; Schultz, 1982). IgG_1 appears to be essential to the expulsion of *N. brasiliensis* from rats (Ogilvie and Jones, 1971). IgA might be responsible for the expulsion of developing larvae, since Smith *et al.* (1983b) found that gastric lymph of immune sheep shows an increased level of IgA which coincide with a loss of developing *H. contortus* worms between day 6 and 10 *post* challenge infection. Charley-Poulain *et al.* (1984) observed a close temporal relationship between the rise in local anti-*H. contortus* IgA antibodies and the self-cure reaction, while IgG and IgM levels are less clearly associated with it. Sinclair *et al.* (1985) demonstrated a locally increased number of IgA-containing cells in the small intestine of lambs reinfected with *N. battus*. Challenge infections with *Trichostrongylus colubriformis* in immune sheep result in an increased number of IgA- and IgG_1-plasma-cells in the lamina propria of the small intestine, but IgA extracted from the intestinal lymph fails to transfer passive immunity against this parasite (Adams *et al.*, 1980). Lloyd and Soulsby (1978), however, passively protected mice against infection with *Taenia taeniaeformis* by intraduodenal injection of intestinal IgA from immune donors, while IgG had no protective effect when given in this manner. Befus and Podesta (1976) stated that IgA antibodies may directly act on the parasites, whereas antibodies of other classes act indirectly by recruiting and/or stimulating other components of the immune system.

As mentioned before, T-cells generally do not act upon nematodes directly. T-cells cooperate with B-cells and by doing so, they stimulate antibody production. In congenitally athymic (nude) mice, antibody production is impaired when *T. spiralis* infections are applied (Ruitenberg *et al.*, 1977). T-cell factors also might promote eosinophil reactions and goblet cell secretion (Mitchell, 1979). Cells of the myeloid lineage have a diverse action. Mast cells release a variety of effector molecules (e.g. histamine) which increase the permeability of the gastrointestinal wall. Increased permeability facilitates the action of other components of the host response. Eosinophils are attracted to the infection site by factors released from mast cells and parasites themselves. Eosinophils may kill worms directly in presence of antibody (McLaren *et al.*, 1978). Products of eosinophils, for example the major basic protein and hydrogen peroxide, are toxic to gastrointestinal nematodes (Butterworth *et al.*, 1979; Bass and Szejda, 1979). Some of the reaction of cells in the response to a worm infection is schemetically shown in Fig. 3.

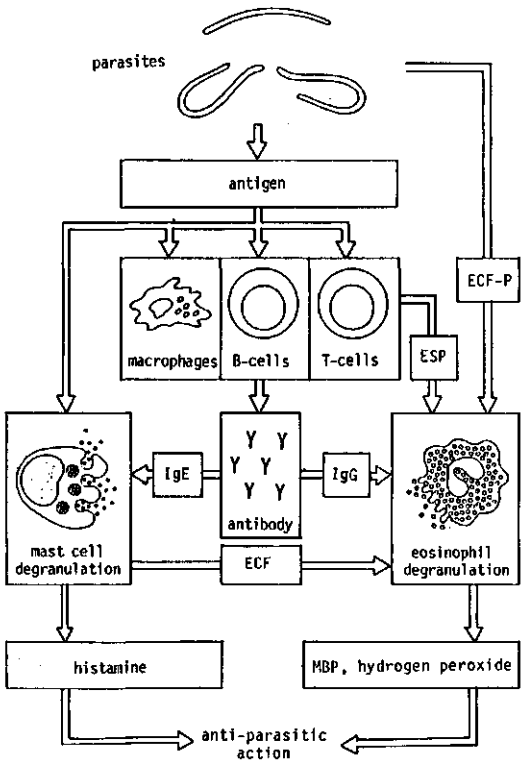

Fig. 3. Interaction of cells in the coordinated response to nematode infections (adapted from Roitt *et al.*, 1985). ECF(-P) = eosinophil chemotactic factors produces by mast cells (or parasites). ESP = eosinophil stimulation promotor. MBP = major basic protein.

Investigators pay most attention to the relation between expulsion and the immune response. According to Ogilvie and Love (1974) a direct antibody mediated damage of *N. brasiliensis* worms and a subsequent release of non-specific factors from sensitized T-cells are responsible for the expulsion of this parasite from rats. Expulsion of *T. spiralis* from mice might be the result of a specific reaction between sensitized T-cells and parasite antigens. This may cause tissue injury at the infection site and thereby trigger a non-specific inflammatory reaction (Larsh and Race, 1975). This inflammatory response of the gastrointestinal mucosa is characterized by infiltration with granulocytes, mast cells, globule leukocytes, plasmacells and macrophages. Increased concentrations of peroxidase, prostaglandin E_1 and histamine, released by the inflammatory cells, were also measured during the inflammatory reaction (Miller, 1984).

Interaction between parasite populations is often thought to be mediated immunologically (Table 1 and 2). As the immune response towards gastrointestinal nematodes is

far from being understood completely, it is not surprising that the supposed immunologically mediated interaction is not proven unequivocally in all cases. Moqbel and Wakelin (1979) put convincing evidence forward that negative interaction in the *Strongyloides ratti* - *Trichinella spiralis* - rat model exist and is immunologically mediated, but their conclusions are based on parasitological parameters only, as are the conclusions of most authors mentioned in Table 1 and 2. Behnke *et al.* (1977) and Kennedy (1980a) induced immunosuppression by cortisone treatment and could prevent premature expulsion of *H. diminuta* and *N. brasiliensis* from *T. spiralis* primed mice. These results indicate that the immune response of the host might be responsible for the influences that parasites have upon each other. Kloosterman *et al.* (1984) found negative interaction between *C. oncophora* and *O. ostertagi* and stated that this might be due to immunological cross-reactions, because Keus *et al.* (1981) found cross-reacting IgG antibodies *in vitro.* It is not known, however, whether such antibodies are directed towards relevant antigenic determinants or whether they exert a negative effect on another parasite species *in vivo* that, in addition, parasitizes at another site. *N. brasiliensis* larvae incubated *in vitro* in *T. spiralis* or *S. ratti* antiserum develop oral precipitates (Kazacos, 1975; Kazacos and Thorson, 1975), but no effects of these precipitates on larval survival or infectivity are reported. The ultimate evidence, that the immune system might be involved in parasite interaction was delivered by Lee *et al.* (1982). They achieved accelerated expulsion of *T. spiralis* worms in mice actively immunized by injection of soluble *T. muris* crude antigen and in mice adoptively immunized by transfer of mesenteric lymph node cells of mice infected with *T. muris*. These results were reciprocal.

3 MATERIAL AND METHODS

3.1 MATERIAL

3.1.1 Animals

For each experiment bull calves were bought on the market when they were less than one week old. Most of them were of the Dutch Friesian breed, but some were possibly cross-breds of Dutch- and Holstein Friesians. Although in practice most grazing calves are females, male calves were used in the experiments because they could be purchased more easily.

Calves were raised according to normal standards: milk replacer until weaning at about 10 weeks of age, hay and water *ad. lib.* from the very beginning and concentrates at a maximum of 2 kg per day. The animals were individually housed on a floor of rubber mats and some straw. At the age of about 2 months the calves were vaccinated against infectious bovine rhinotracheitis (IBR), parainfluenza (PI-3) and bovine respiratory syncytial virus (BRSV). Rearing calves under these helminth-free conditions was successfull, because no calves died and at 3 months of age no helminth infections could be detected.

3.1.2 Infective larvae

The strain of *C. oncophora* used in the present experiments was isolated in 1972 from naturally infected calves. From that moment on, it has been maintained at our laboratory (1 or 2 calf passages/year). The *C. oncophora* isolate contains about 30% *C. surnabada*. Isenstein (1971) found evidence for a polymorphic relationship between *C. oncophora* and *C. surnabada*. In this study Isenstein's conclusion is accepted and thus the strain is referred to as *C. oncophora*.

The strain of *O. ostertagi* also originated from naturally infected calves. It was isolated in 1972 and maintained as described for *C. oncophora*. This isolate consists of *O. ostertagi* (98-99%) and *O. lyrata* (1-2%). *O. ostertagi* and *O. lyrata* are, according to Jansen (1983), polymorphs. As the percentage of *O. lyrata* is very small and because of the polymorphic relationship, the isolate is referred to as *O. ostertagi*.

Larvae used in primary infections were cultured from faeces of experimentally infected donor calves. *O. ostertagi* larvae used for the challenge infection of exp. 1 were a mixture of larvae cultured at our laboratory and of larvae supplied by the Central Veterinary Institute, Lelystad, The Netherlands. Larvae cultured from faeces of primarily infected calves of the same experiment were also used for challenge infections. Larvae were cultured from faeces by incubating a mixture of fresh faeces and wood

shavings at 27°C for at least 7 days. Infective larvae were extracted from this mixture by baermannisation and stored in tapwater at 4°C for at most 3 months.

Larval doses were prepared the day before being used according to the following procedure. The larval suspension was mixed intensively, at room-temperature, with a vibromixer during 30 minutes. One ml of the larval suspension was diluted in tapwater until a concentration of about ten to twenty larvae/ml was reached. Ten ml of the diluted suspension was counted. Diluting and counting procedures were performed ten times. Coefficients of variation (standard deviation/mean*100) were less than 5%. Doses were prepared by pipetting the suitable amounts of the larval suspension into tubes. Prepared doses were stored at 4°C and put at room-temperature at least two hours before oral administration.

3.2 METHODS

3.2.1 Parasitological techniques

3.2.1.1 Faecal egg counts

Starting on day 16 or 17 *post infectionem* (p.i.) until treatment or slaughter faecal samples were taken from the rectum three times a week and examined by a modified McMaster technique (van den Brink, 1971). On day 0 p.i., on day 14 p.i. and 2 or 4 days after treatment all calves were checked whether any eggs were present or not by vibromixing a few grams of faeces with about 200 ml of saturated NaCl solution. Ten minutes after mixing, 2 ml was ladled from the surface and examined microscopically. A more sensitive check on treatment efficiency is to make larval cultures. However, the flotation technique was preferred because results of the cultures would not have been available at the time challenge infections were carried out.

As it is difficult to distinguish microscopically *C. oncophora* and *O. ostertagi* eggs, larval differentiations were made in faeces of calves infected with both species in order to assess the relative contribution of each species to the faecal egg count. Larval cultures were made by incubating a few gram of faeces, mixed with woodshavings, for at least 7 days at 27°C. However, the percentage of eggs that hatched was very low when this micro-technique was used. Therefore, results of larval differentiations are not discussed.

3.2.1.2 Worm counts

Animals were abstained from food twenty-four hours before slaughter. Worm counts in the small intestine were performed as described by Kloosterman *et al.* (1978): the small intestine was tied up at both ends and separated from the abomasum and the large intestine. It was made to a straight tube by cutting the mesentery and both ends were

untied. The worms were rinsed out by washing twice with 10-15 l tap water (30-35°C). The washing was sieved on a 0.150 mm mesh sieve with help of a firm waterjet (30-35°C); the debris was made up to 5 litres and stirred intensively by a vibromixer. Two 50 ml samples (fixated in 135 ml alcohol 96%) were counted. Originally (exp. 1 and 2) a 0.075 mm sieve was used. As inhibited larvae were rarely found, it was replaced by a 0.150 mm sieve to speed up the procedure.

Abomasal worm counts were determined according to Kloosterman *et al.* (1984): the abomasum was tied up at both ends and separated from the omasum and the small intestine. It was cut along the large curvature; the contents were washed into a bucket with warm tap water (30-35°C) and the abomasal wall was rubbed intensively while it was immersed into the bucket. The washing was proceeded as described above. As numbers of *O. ostertagi* worms in digested mucosae were very small (0-400) in exp. 1 and 2 and most of the worms were damaged by the pepsin treatment, these worms were not used for measurement of the length, the vulval flap score and the number of eggs per female.

Male and female worms were counted separately in order to obtain the sex-ratio. This ratio is expressed as the percentage of males. *Cooperia male* worms were differentiated into *C. oncophora* and *C. surnabada* polymorphs using the shape of the spicula (Isenstein, 1971).

3.2.1.3 Worm length

From counted and sex-differentiated worms a random subsample of at least 20 individuals, if present in the aliquots, was taken. In the subsample the lengths of all the worms were measured. A calf was excluded from calculation of worm length when less than 5 worms were present.

For measuring lengths, the median line of worms was put on paper, by the use of a drawing prism on a microscope at tenfold magnification. By tracing these lines on a graphics tablet (Apple Computer Inc.), attached to a computer, lengths were calculated. This method is more accurate and much faster than measuring with a curvimeter (Kloosterman *et al.*, 1978).

3.2.1.4 Number of eggs *in utero*

The number of eggs per female worm was determined according to the method described by Kloosterman *et al.* (1978): worms were desintegrated in a drop of hypochlorite solution in such a concentration that eggs stayed intact and could be counted. When available, at least 20 female worms were desintegrated. A calf was excluded from calculation when less than 5 female worms were present.

3.2.1.5 Vulval flap development

The development of the vulval flap was scored from 6 for fully developed flaps to 1 for completely reduced flaps (Michel, 1972b) in at least 20 *O. ostertagi* females, when available. A calf was excluded from calculation when less than 5 females were present in the aliquots.

3.2.2 Immunological techniques

3.2.2.1 Enzyme-Linked ImmunoSorbent Assay (ELISA)

The indirect antibody-detecting ELISA, as described by Voller *et al.* (1979), was being used.

- Principle

Antigens (Ag) are coated to the solid phase (polystyrene). Unbound proteins are washed out and sera samples are added. Antibodies (Ab), when present in the sample and when specific for the Ag, bind to the Ag. Unreacted material is washed out and a peroxidase labelled anti-Ab is allowed to react with the Ag-Ab complexes. Unbound labelled anti-Ab is washed out and a substrate sensitive to the peroxidase enzyme is added. The amount of Ab in the sample is related to the amount of substrate that is converted.

- Antigens

Secretory/Excretory (SE)-antigens were obtained by incubation (37°C) of adult worms in RPMI-1640 medium containing 10,000 U Penicilline, 7,500 U Streptomycine and 100,000 IU Nystatin per litre. Approximately 3,000 worms were incubated in 100 ml of medium in stoppered bottles. The medium was refreshed every 24 hours. In this way worms were kept alive during 4 to 5 days. Medium containing the antigens was centrifugated for 20 minutes at 10,000 g. The supernatant was sterilized by filtration (0.2 um Millipore filter) and stored at -80°C. SE-antigens were collected from both species. Antigen-coating procedure: antigens (0.100 ml) were coated to the solid phase by incubation for at least 17 hours at 4°C. As solid phase polystyrene microtitre-plates with 8*12 wells were used. Optimal antigen dilutions were determined by using serial dilutions of the SE-antigen in carbonate buffer (0.1 M, pH 9.6).

- Antibodies

During the experiments the calves were bled every 2 weeks from the jugular vein. Blood was allowed to cloth for 1 hour at 37°C and the sera were stored at -20°C until use. Standard positive samples were obtained from animals experimentally infected with one species. These samples were stored at -20°C in aliqouts of 1 ml. Antigen-antibody

reaction: 0.100 ml of serum, 1/40 and 1/160 diluted in dilution buffer (Phosphate Buffered Saline, PBS (pH 7.2), containing 0.5 ml Tween-20 and 6.7 ml normal horse serum per litre), was added to antigen coated wells. Serial ^{2}log dilutions of the standard positive serum were made with an initial dilution of 1/20 (1/20 - 1/40 - ..... - 1/2560). Serial dilutions of the standard positive were performed in duplicate on each plate. Antigen-antibody reaction was performed for 1 hour at 37°C.

- Conjugate

Peroxidase labelled rabbit-anti-bovine-IgG was purchased commercially (ICN Immunobiologicals, Lisle, Israel). Conjugate-reaction: optimal dilution of the conjugate was determined by chess-board titration with a standard positive sample. The dilution buffer was the same as described earlier for diluting the sera. Conjugate was added (0.100 ml) to each well and allowed to react for one hour at 37°C.

- Substrate

A 5-amino-2-hydroxy-benzoic-acid ($C_7H_7NO_3$) solution (80 mg $C_7H_7NO_3$ per 100 ml of aqua dest. of 70°C) was cooled down to 20°C and a 0.1 N NaOH solution was added until pH reached 6.0. Peroxide (30% stock solution of H_2O_2) was added in a concentration of 15 promille. Substrate reaction: substrate (0.100 ml) was added to each well and allowed to react for 1 hour at room-temperature.

- Measurement

Substrate conversion was measured spectrofotometrically (Titertek MCC multiskan) at 450 nm.

- Titre calculation

A mean standard curve was calculated from all standard positive serial dilutions. Extinctions that differed more than 2 standard deviations from the comparable mean were not used. From this mean curve a log-logit regression line was calculated (Ritchie *et al.* 1981). Sera dilution factors (20-40- ..... -2560) were log-transformed using formula 1, resulting in values of 1 to 8. Extinctions were logit-transformed according to formula 2.

$$f = {}^2\log(\text{dilution factor}/10) \quad \text{.....(1)}$$

$$f = \ln(Ext_i/(EMAX-Ext_i)) \quad \text{.....(2)}$$

Ext_i: extinction of sample measured at dilution factor i.
EMAXmaximal extinction of standard positive.

Presumptions:
- mean values of extinctions used to determine the regression line should be in the interval: 0.10*EMAX < EXT < 0.95*EMAX.
- prozone effects (i.e. EMAX is not at the 1/20 dilution but at a higher dilution) are not present. Extinctions of dilutions showing prozone effects are supposed to be equal to EMAX.
- valid regression lines are based on extinctions of at least 5 dilution factors and have a correlation coefficient of at least -0.96.
- the critical logit value (i.e. the logit value that determines the cut-off value) is calculated from the regression line at the 6.5 log-dilution.
- the titre of the sample (i.e. the ^{2}log dilution of the sample that shows a logit value equal to the critical value) is calculated from the dilution (1/40 or 1/160) that has an extinction nearest to 50% of EMAX.

3.2.2 Designs and aims of the experiments

Experimental designs were constructed in such a way that it was possible to compare primary infections (i.e. 'challenge' infections of primarily non-infected groups) with heterologous challenge infections. Homologous challenge infections were included just to have a standard of comparison. The effect of concurrent challenge infections was determined by comparing it with results of monospecies infections.

3.2.2.1 Experiment 1

- Design of experiment 1

Forty-eight male calves, born in the first week of January 1984, were allotted to 4 groups at three months of age. Groups had about equal mean liveweight (108.5 ± 4.5 kg at day -1 p.i.) and weight-gain (816 ± 89 g/day) during the 4 weeks before inoculation. Primary infections on day 0 consisted of a single dose of 0, $50*10^3$, $100*10^3$ or $200*10^3$ *C. oncophora* infective larvae (groups are labelled as N, C_{50}, C_{100} and C_{200} respectively). Five weeks later (day 35 p.i.) all groups were treated with an anthelmintic (Synanthic®, oxfendazole, 568 mg per calf). At day 42 p.i. each primary infection group was split into 3 challenge infection groups, based on liveweight and on weight-gain during the primary infection period. Each calf of the first group received $100*10^3$ *C. oncophora* larvae, those of the second $100*10^3$ *O. ostertagi* larvae and the ones of the third group received both these doses. Challenge groups are coded as C_{100}, O_{100} and MIX respectively. Subgroups are coded as N-C_{100}, C_{50}-C_{100}, C_{100}-C_{100} and so on. The experimental design is summarized in Table 3. Thirty-three days after challenge (75 days p.i.) all calves were slaughtered for *post-mortem* (p.m.) examinations.

Table 3. Design of experiment 1 with number of calves per group.

		PRIMARY INFECTION				
		N	C_{50}	C_{100}	C_{200}	TOTAL
CHALLENGE INFECTION	C_{100}	4	4	4	4	16
	O_{100}	4	4	3*	4	15
	MIX	4	4	3*	4	15
	TOTAL	12	12	10	12	46

*One calf in this group died accidentally within the first three weeks of the primary infection period and was excluded from the whole experiment.

- Aims of experiment 1

Reciprocal negative interaction between *C. oncophora* and *O. ostertagi* was found by Kloosterman *et al.* (1984): after challenge large negative effects of homologous primary infections were found, while heterologous primary infections showed minor negative effects; virtually no effects of concurrent infections were noticed. In this experiment the results of Kloosterman *et al.* (1984) were verified and possibly extended. The main purpose of exp. 1 was to investigate whether an *O. ostertagi* population was affected by a previous or a concurrent *C. oncophora* infection and, if so, whether the effects were depending on the primary immunizing dose of *C. oncophora* larvae or not. The magnitude of the effects was measured by comparing faecal egg output and p.m. results of primary infections (e.g. group $N\text{-}O_{100}$) with heterologous challenge infections (e.g. groups $C_{50}\text{-}O_{100}$, $C_{100}\text{-}O_{100}$ and $C_{200}\text{-}O_{100}$).

The time scheme of Kloosterman *et al.* (1984) was pursued. A primary infection period of 5 weeks is long enough to trigger the immune system. A time lapse of one week between primary and challenge infection was chosen, because immunological effects of the primary infection might disappear rather quickly when the antigenic stimulus has been removed. Necropsy was performed at 33 days p.c.i. At that moment the worm populations have developed to maturity and some animals may already have expelled their worm burdens. Waiting longer might result in a loss of almost the complete worm populations in the majority of calves. Sizes of the primary doses were based on results of Albers (1981): a primary dose of $100*10^3$ followed by a challenge dose of $350*10^3$ *C. oncophora* larvae showed in general no severe clinical signs of trichostrongylosis. Therefore, it was not expected that a primary dose of $200*10^3$ *C. oncophora* larvae would give much problems. The decision to start the experiment when the calves were 3 months old was based on the facts that in practice calves are put on pasture not before this age and that maternal antibodies in calves that have had colostrum during the first

few days of life have been catabolized (Banks, 1982). In addition, all experiments in our laboratory had been done with calves of this age (Albers, 1981; Kloosterman *et al.*, 1978; 1984).

3.2.3.2 Experiment 2

- Design of experiment 2

The basic set up of exp. 1 and exp. 2 was identical. The only difference between both experiments was that the primary infection of exp. 2 consisted of several doses of *O. ostertagi* larvae (0, $25*10^3$, $50*10^3$ or $100*10^3$ coded as N, O_{25}, O_{50} and O_{100} resp.). Calves in exp. 2 were born in the first week of June 1984, had a mean liveweight of 111.3 ± 6.3 kg at day 1 p.i. and had gained 956 ± 153 g/day during a pre-infection period of 4 weeks. No animals were lost during this experiment. Anthelmintic treatment was carried out on day 35 and day 37 p.i.

- Aims of experiment 2

The purpose of this experiment was to investigate whether a *C. oncophora* population was affected by a previous or by a concurrent *O. ostertagi* infection and, if so, whether the effects were depending on the primary dose of *O. ostertagi* larvae or not.

A pilot trial with a few calves showed that a primary dose of $200*10^3$ *O. ostertagi* resulted in severe clinical symptoms of ostertagiasis after three weeks. Therefore it was decided to use $100*10^3$ *O. ostertagi* larvae as the highest dose. Anthelmintic treatment was carried out twice, because *O. ostertagi* is a little less sensitive to oxfendazole than *C. oncophora* (Bairden and Armour, 1983). This repeated treatment also prevented underdosing since 1 bolus, containing 568 mg oxfendazole, was recommended for calves weighing 125 kg, while calves of exp. 2 weighed 138.8 ± 8.4 kg on day 35 p.i.

3.2.3.3 Experiment 3

- Design of experiment 3

The calves used in this experiment were born in the first week of January 1985, weighed 116.6 ± 6.7 kg at the age of 3 months and had gained 1025 ± 136 g/day during the 4 weeks before the first inoculation.

Forty-eight calves were allotted to three groups: group N was kept as non-infected control, group C_r received repeated doses of $7*10^3$ *C. oncophora* larvae and group O_r received repeated doses of $7*10^3$ *O. ostertagi* larvae. One calf of group C_r was excluded because it had shown repeated tympanitis during the primary infection period (this was due to anatomic abnormalities of the digestive organs as was seen by autopsy). Primary inoculation with a dose of $7*10^3$ larvae was repeated 14 times: 3 times a week on Monday, Wednesday and Friday. The last dose was given on day 30 p.i. In total each

calf of group C_r and O_r received about $100*10^3$ larvae. Primary infection was terminated by anthelmintic treatment on day 35 and 37 p.i. All calves were challenged with a single dose of $100*10^3$ *C. oncophora* larvae + $100*10^3$ *O. ostertagi* larvae (MIX) on day 42 p.i.

- Aims of experiment 3

The continuous uptake of larvae under field conditions was more or less imitated by repeated infection with small doses. Continuous uptake may result in a different immune reaction of the host, because of the continuous stimulus of larval and adult antigens, when compared to the reaction after a single dose.

The time scheme of this experiment was identical to the time scheme of exp. 1 and 2. From preliminary results of exp. 1 it was concluded that a mixed challenge infection was most suitable to detect negative interaction. Another reason for the use of only one type of challenge infection was to enlarge the number of animals per group since standard deviations of some parasitological parameters were rather high in exp. 1 and 2 and although group means differed considerably, statistical significance was often absent.

3.2.3.4 Experiment 4

- Design of experiment 4

Thirty-six calves born in the first week of December 1985, weighing 124.5 ± 5.3 kg when 3 months old and having gained 1042 ± 100 g/day during the second month of life, were divided into three groups of 12 animals each. Primary infection groups were the same as in exp. 3. Challenge infections were equal to those infections in exp. 1 and 2 (C_{100}, O_{100} or MIX) as was the time scheme.

- Aims of experiment 4

In this experiment the repeatibility of the results of the previous experiments was assessed.

3.2.3.5 Experiment 5

Techniques used in this experiment will be described very briefly. A more extensive description will be published elsewhere. This experiment was performed in cooperation with the Department of Pathology, Faculty of Veterinary Medicin, State University of Utrecht, The Netherlands.

- Design of experiment 5

Six calves were cannulated when they were about 10 weeks old. Cannulas (T-shaped, silicon rubber) were placed in the small intestine (about 3 metres behind the pylorus) and in the fundus region of the abomasum. Calves were divided into three groups of 2

animals each. At the age of four months the calves were infected according to the scheme of exp. 3. The two control calves were not infected during the challenge period. Challenge infection was terminated at day 28 p.c.i. by anthelmintic treatment.

Tissue fragments of the mucosa of small intestine and abomasum were taken at day -22, -1, 0, 7, 14, 28, 35, 42, 46, 49, 56, 70 and 84 p.i. Small intestinal biopsies were performed with a Sebus biopsy-capsule (Lamèris, Utrecht, The Netherlands). This capsule was connected to a 30 ml syringe via a tube of about 1.5 metres. Tissue fragments were taken by suction at a position of 4 - 4.5 metres behind the pylorus. Abomasal biopsies were performed by means of an endoscope equipped with biopsy forceps (Olympus). Two tissue fragments were immediately fixated in 0.6% formaldehyde-0.5% acetic acid and formalin-sublimat (6 g $HgCl_2$ dissolved in 100 ml of formaldehyde (4%)). Histological examinations concerned numbers of plasma cells (differentiated to antibody class), mast cells, globular leucocytes and eosinophils. For demonstration of cells, tissue fragments were cut perpendicular to the surface of the mucosa into 0.005 mm sections. The numbers of cells were enumerated with a 400x magnification. In all cases at least 4 fields of 20 x 1 mm^2 each were counted. Plasma cells were differentiated to antibody class (IgM, IgG_1, IgG_2, IgA) by use of an indirect peroxidase staining technique. Toluidin-blue (1% solution in 20% alcohol, pH 9.0) was used for demonstration of mucosal mast cells and globular leucocytes. Mucine constitution and crypt/villus ratios have also been recorded but will be published elsewhere.

- Aims of experiment 5

This experiment was aimed to give information about local responses occurring during gastrointestinal helminth infections. Changes in local responses induced by heterologous primary infections were of special interest. No statistics were done because of the limited number of animals.

3.2.4 Statistics

Statistical treatment of data was carried out by the use of the statistical software BMDP (Biomedical Programs, University of California) and SPSSX (Statistical Package of Social Sciences), implemented on a VAX-8600 configuration (Digital Equipment Corporation). Before analysis, data were checked for normality by inspection of skewness and kurtosis values. Homogeneity of variances was checked by Levene's test. The Brown-Forsythe test was used to draw conclusions about the significancy of factors in the model when Levene's test was significant ($p < 0.05$).

Analysis of variance was performed according to model (1) or model (2).

$$\underline{Y}_{ik} = u + P_i + \underline{e}_{ik} \quad \text{..... (1)}$$

$$\underline{Y}_{ijk} = u + P_i + C_j + (P^*C)_{ij} + \underline{e}_{ijk} \quad \text{..... (2)}$$

$\underline{Y}_{ik}$ and $\underline{Y}_{ijk}$ = observation on animal k (k = 1 to 4 in exp. 1, 2 and 4; k = 1 to 16 in exp. 3) that belongs to primary infection group i and challenge infection group j.)

u = fixed effect corresponding to the mean level.

P_i = fixed effect corresponding to primary infection group (i=1 to 4 in exp. 1, 2; i=1 to 3 in exp. 3 and 4).

C_j = fixed effect corresponding to challenge infection group (j = 1 to 2 or j = 1 to 3 in exp. 1, 2 and 4).

$(P^*C)_{ij}$ = fixed effect corresponding to interaction.

$\underline{e}_{ik}$ or $\underline{e}_{ijk}$ = random effect corresponding to error for animal k in group i or subgroup ij.

Data from the primary infection period (faecal egg counts, serum IgG titres) were analyzed according to model 1 (i = 1 to 4 in exp. 1 and 2; i = 1 to 3 in exp. 3 and 4). The primarily infected groups (exp. 1 and 2) were considered as a single group when the P-effect was not significant. Non-significant effects ($p > 0.05$) were excluded from the model. For analysis of variance Fisher's F-test was used to demonstrate significance. When F was significant differences between pairs of means were inspected by the test of Bonferroni (multiple range test).

The influence of the primary inoculation level in exp. 1 and 2 was tested by the use of the lineair regression model:

$$\underline{Y}_i = u + bX_i + \underline{e}_i \quad \text{.....(3)}$$

b = coefficient of regression

X_i = primary inoculation dose (i=1 to 3)

As significant regressions may arise from differences between primary infected and non-infected groups, the latter was not used in model 3. The primary inoculation levels were transformed on a logarithmic basis resulting in equidistant steps of the independent variable. The regression model was run for separated and combined challenge groups. These data were considered as a single group when regression lines of separate challenge groups did not differ significantly.

4 RESULTS

Results of exp. 1 to 4 are presented simultaneously whilst those of exp. 5 are given separately.

4.1 PARASITOLOGICAL PARAMETERS

4.1.1 Faecal egg counts

4.1.1.1 Faecal egg counts resulting from *C. oncophora* infections

- Primary infections

During the primary infections of exp. 1 four animals of the C_{200} group, 3 of the C_{100} group and 1 of the C_{50} group passed eggs already on day 14 p.i. These eggs were of an abnormal long-drawn shape and large vesicles could be seen inside. From primary and challenge infections of all experiments it could be concluded that almost all animals reached patency between day 16 and 19 p.i. Mean egg count curves of primary infection groups of exp. 1 are presented in Fig. 4A. Differences between primarily infected groups were statistically significant on day 17 and 19 p.i. only ($p < 0.05$). The absence of statistical significance during the rest of exp. 1 was due to very large within-group standard deviations that were often in the same order as the group means.

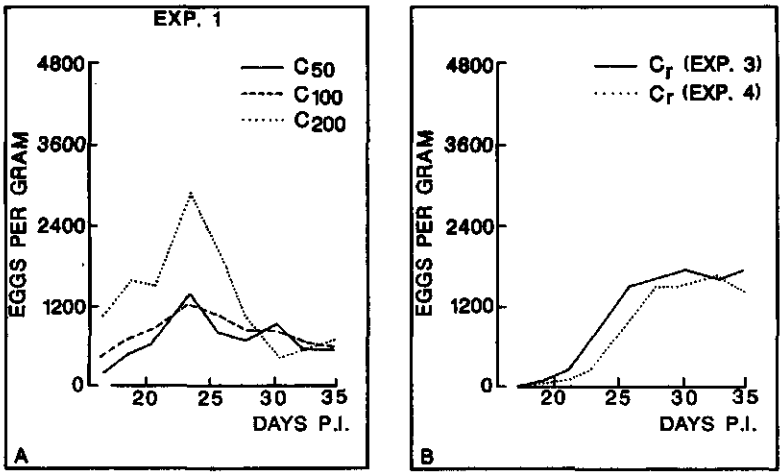

Fig. 4. Faecal egg count curves resulting from *C. oncophora* primary infections.

Individual egg count curves varied considerably. Within the primary C_{100} group three animals responded according to a pattern characterized by a rise of up to 3,000 or 4,000 eggs per gram of faeces on day 28 p.i. followed by a decline, while six others showed a

curve rising until day 21, dropping to almost zero on day 24 and starting to rise again on day 28. One animal of this group passed a few eggs only between day 17 and 21. Egg count curves of three calves, representative for the large variation between individuals, are shown in Fig. 5. Abnormal individual curves were also noticed in the primary C_{200} group. Within this group egg counts on day 24 p.i. varied from 0 to 10,500.

Repeated primary inoculations (exp. 3 and 4) resulted in a mean egg count curve rising slowly until a maximum level was reached on day 26; no decline was noticed until treatment on day 35 p.i. (Fig. 4B).

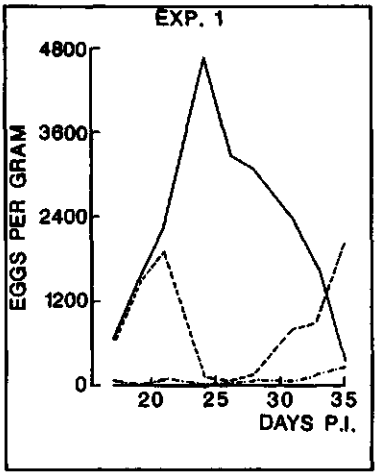

Fig. 5. Faecal egg count curves of three animals of the C_{100} group of exp. 1 showing the large individual variation.

- Challenge infections

Homologous challenge infections

Homologous reinfection in exp. 1 resulted in significantly reduced faecal egg counts (Fig. 6A) for primarily infected groups when compared to primary controls ($p < 0.05$ on day 17, 19 and 21 *post* challenge infection (p.c.i.); $p < 0.001$ during the rest of the experiment). These significances merely resulted from differences between primarily infected and non-infected groups. Differences between primarily infected groups were not significant, although mean egg counts of the C_{50} group was more than a fourfold of counts of the primary C_{100} and C_{200} group. In exp. 4 no reductions in faecal egg output were measured for this type of challenge infection (Fig. 6C). This was mainly due to one animal in the N-C_{100} group that showed very low egg counts (< 100 EPG) until day 23 p.c.i. and no egg count at all after that day. Two other animals of the same group expelled their worms presumably between day 23 and 26 p.c.i., because they showed a decrease in EPG from about 2,000 on day 23 to almost zero at the end of the experiment.

Heterologous challenge infections

Primary infection with *O. ostertagi* (exp. 2 and 4) did not affect the egg counts resulting from a pure *C. oncophora* challenge infection (Fig. 6B and 6C). As was stated earlier the curve of the N-C_{100} group in exp. 4 is disproportionally affected by one animal.

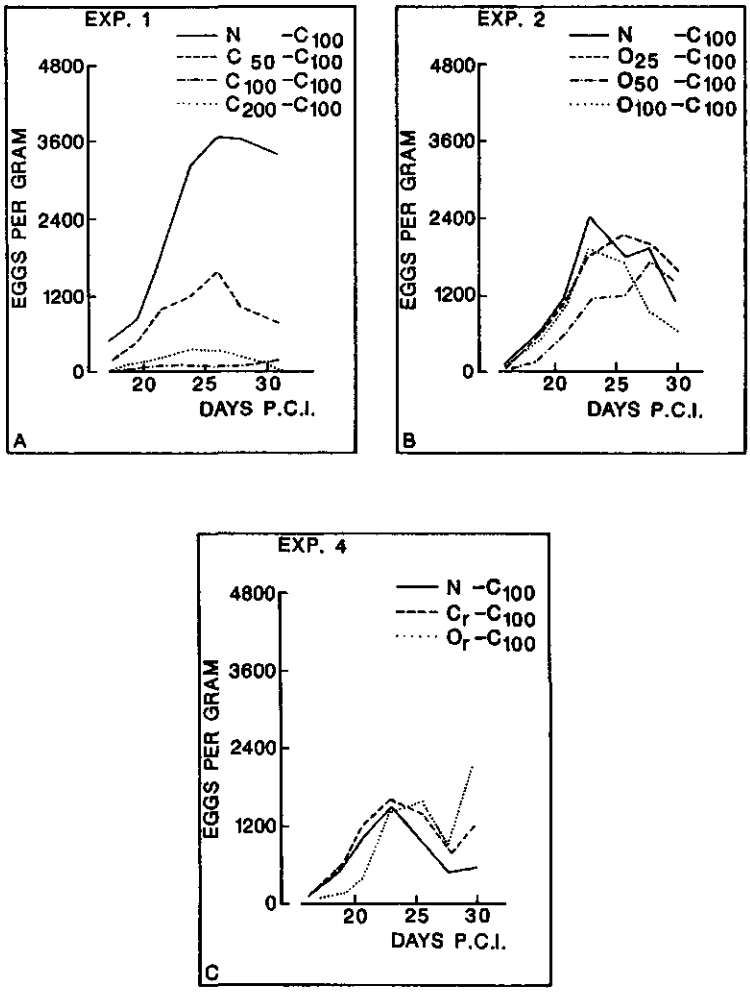

Fig. 6. Faecal egg count curves resulting from *C. oncophora* challenge infections.

4.1.1.2 Faecal egg counts resulting from *O. ostertagi* infections

- Primary infections

Only on day 19 p.i. a significant effect of the inoculation dose was present ($p < 0.01$) resulting in more eggs per gram of faeces when the dose increased (exp. 2 - Fig. 7A).

The faecal egg output reached its maximum already on day 21 p.i. for all groups. Differences between groups were significant ($p < 0.05$) at the end of the primary infection period (day 33 and day 35 p.i.). This was merely due to the relative high EPG of group O_{25}. Infection with repeated doses (exp. 3 and 4 - Fig. 7B) resulted in slightly different curves when compared to single dose infections of exp. 2.

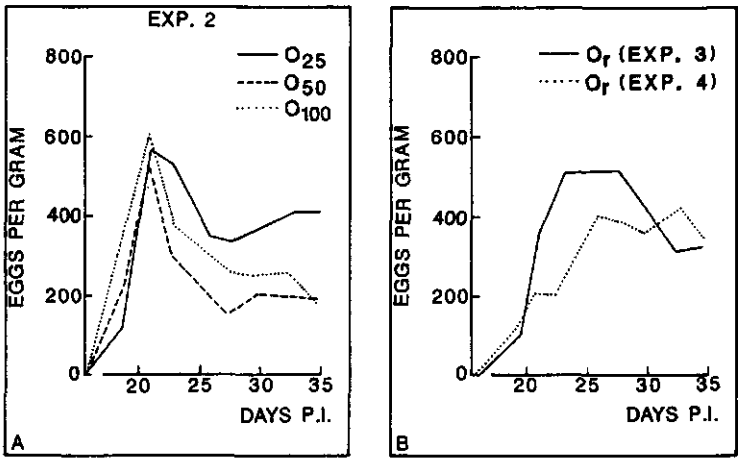

Fig. 7. Faecal egg count curves resulting from *O. ostertagi* primary infections.

- Challenge infections

Homologous challenge infections

O. ostertagi egg counts during the challenge infection of exp. 2 were positively affected by primary infections on day 21 and 23 p.c.i. ($p < 0.05$). This resulted from a low egg output of the control group (Fig. 8B) that showed an early decline on day 19 p.i. followed by an increase on day 23 p.i. Homologous reinfection in exp. 4 resulted in significantly reduced egg counts ($p < 0.05$) during the whole challenge period except on day 26 p.c.i. (Fig. 8C).

Heterologous challenge infections

Primary infection with *C. oncophora* did not affect the faecal egg output of pure *O. ostertagi* challenge infections in exp. 1 (Fig. 8A). As for the primary *O. ostertagi* infections in exp. 2, peak egg counts were measured early in infection (day 21 p.c.i.) and were followed by a rapid decline. Repeated priming with *C. oncophora* in exp. 4, resulted in egg counts that were intermediate between the EPG of the $N\text{-}O_{100}$ and $O_r\text{-}O_{100}$ group throughout the experiment, but differences between the $N\text{-}O_{100}$ and $C_r\text{-}O_{100}$ group were not significant as were the differences between the $O_r\text{-}O_{100}$ and $C_r\text{-}O_{100}$ group.

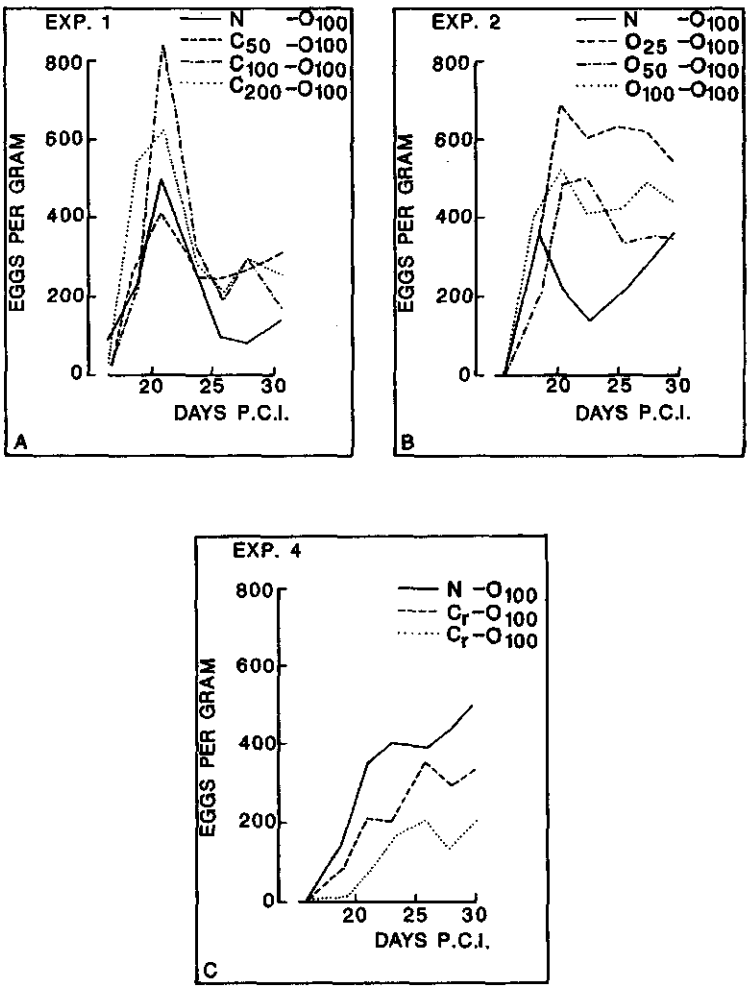

Fig. 8. Faecal egg count curves resulting from *O. ostertagi* challenge infections.

4.1.1.3 Faecal egg counts resulting from mixed infections

Mixed infections were only applied during the challenge period and were given as primary infections (N-MIX) or challenge infections following primary *C. oncophora* or *O. ostertagi* infections.

- Egg counts of mixed challenge infections following primary *C. oncophora* infections.

Calves primarily infected with *C. oncophora* larvae and challenged with larvae of both species showed reduced egg counts when compared to primary controls (Fig. 9A, 9C and 9D). Effects of primary dose-level were not present in exp. 1 with regard to this para-

meter. In this experiment egg counts of primarily infected animals were, when considered as one group, reduced significantly ($p < 0.05$) from day 24 to 28 p.c.i. (Fig. 9A). Repeated priming with *C. oncophora* showed about the same result as single dose priming. Significant differences between the N-MIX and C_r-MIX curves occurred in exp. 3 during the whole challenge period except in the beginning (day 16 p.c.i.) and at the end (day 30 p.c.i.). These differences were, however, not significant in exp. 4 (Fig. 9D). This was due to the large within-group variation of the C_r-MIX group and the small number of animals per group.

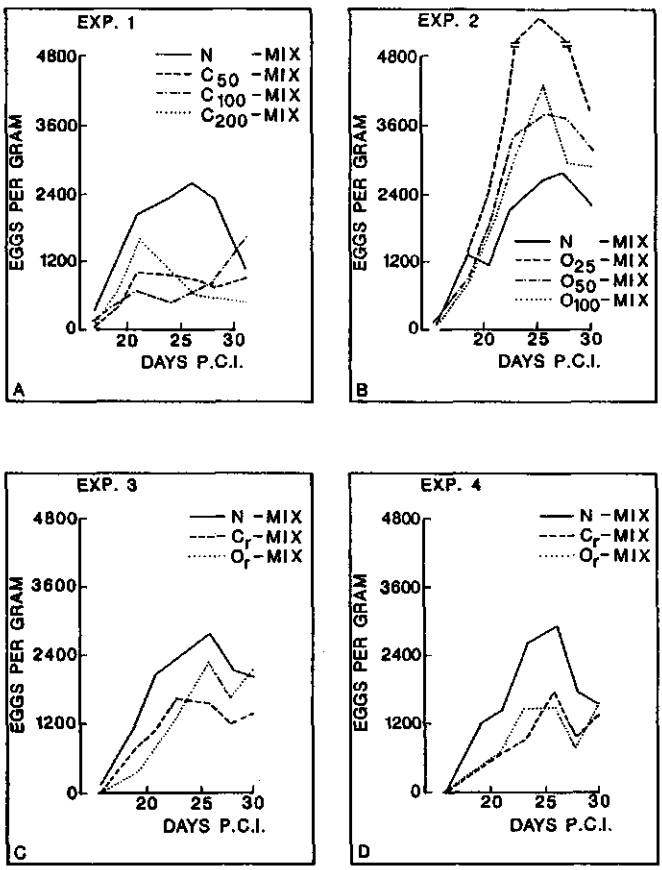

Fig. 9. Faecal egg count curves resulting from mixed challenge infections.

- Egg counts of mixed challenge infections following primary *O. ostertagi* infections.

Egg counts of a mixed challenge infection following a primary infection with *O. ostertagi* larvae are presented in Fig. 9B, 9C and 9D. The very high mean EPG (8,300) on day 26 p.c.i. of the O_{25}-MIX group was caused by one animal that showed an

EPG of 23,000 on that day. As was found in the monospecific challenge infection with *O. ostertagi* in exp. 2, the mean EPG of the control group was less than the mean EPG of the primarily infected groups (Fig. 8B and 9B), but due to large standard deviations and small numbers of animals per group, no significant differences occurred between primarily infected and non-infected groups. Egg counts of mixed challenge infections were affected negatively by repeated priming with small doses of *O. ostertagi* larvae (Fig. 9C and 9D). In exp. 3 these reductions were significant ($p < 0.01$) until day 26 p.c.i. In exp. 4 the egg counts of the N-MIX and the O_r-MIX groups did not differ significantly, although reductions were considerable (about 50%).

- Comparison of egg counts resulting from combined monospecific and mixed challenge infections.

As larval differentiations were not reliable, the relative contribution of each species to the faecal egg counts could not be assessed. When both parasite populations do not interact in concurrent infections, the EPG of a mixed challenge infection should be equal to the sum of egg counts of separate infections (C_{100}+O_{100}). In Fig. 10A to 10C EPG curves of mixed challenge infections and combined monospecific challenge infections are presented graphically. In exp. 1 and 4 the curve of the MIX group is almost identical to the curve of the combined C_{100} and O_{100} group. In exp. 2 the mixed infection showed a much higher EPG than the C_{100}+O_{100} group. This was merely due to the high contribution of the O_{25}-MIX group (Fig. 9B). The low level of the C_{100}+O_{100} egg counts in exp. 1 is due to the fact that most of the animals in this experiment had been primed with *C. oncophora*, resulting in reduced faecal egg counts (Fig. 6A).

4.1.2 Worm counts

In all four experiments inhibited larvae were found in a very limited number of animals and only in very small amounts, as could be expected from the results of Michel (1963) and Albers (1981): inhibition of larval development mainly occurs when continuous or repeated challenge infections are applied. As no systematic differences existed between experimental groups, these data were not analysed. For statistical analysis worm counts were transformed according to the formula $Y = \ln(X+1)$ in which X is the number of worms.

4.1.2.1 *C. oncophora* worm counts

Geometric means of *C. oncophora* worm counts found after slaughter are presented graphically in Fig. 11A to 11D. Differences between the four experiments with regard to worm recovery after challenge can be assessed by comparing the primarily uninfected groups. By doing so it appears that the percentage "take" measured on day 33 p.c.i. was

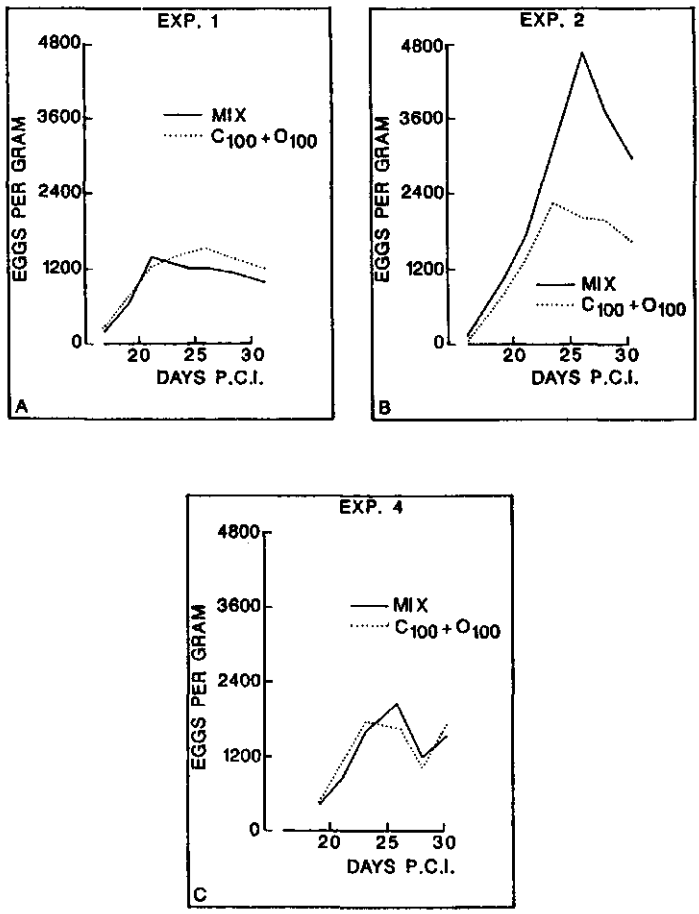

Fig. 10. Faecal egg count curves resulting from mixed (MIX) challenge infections and combined mono-specific ($C_{100} + O_{100}$) challenge infections.

31.7%, 32.2%, 50.2% and 7.6% for exp. 1 to 4, respectively. These percentages are based on retransformed worm counts of primarily uninfected animals challenged with C_{100} or MIX. The percentage take differed significantly between experiments ($p < 0.05$). This was merely due to the very low recovery in exp. 4.

- Homologous infections

Homologous reinfections in exp. 1 resulted in significantly reduced worm burdens when compared to the primarily uninfected groups (Fig. 11A). Reductions of worm burdens in this experiment tended to be dependent on the primary dose ($p < 0.10$); a reduction of 87% in total worm count was found for the primarily heaviest infected group (C_{200}).

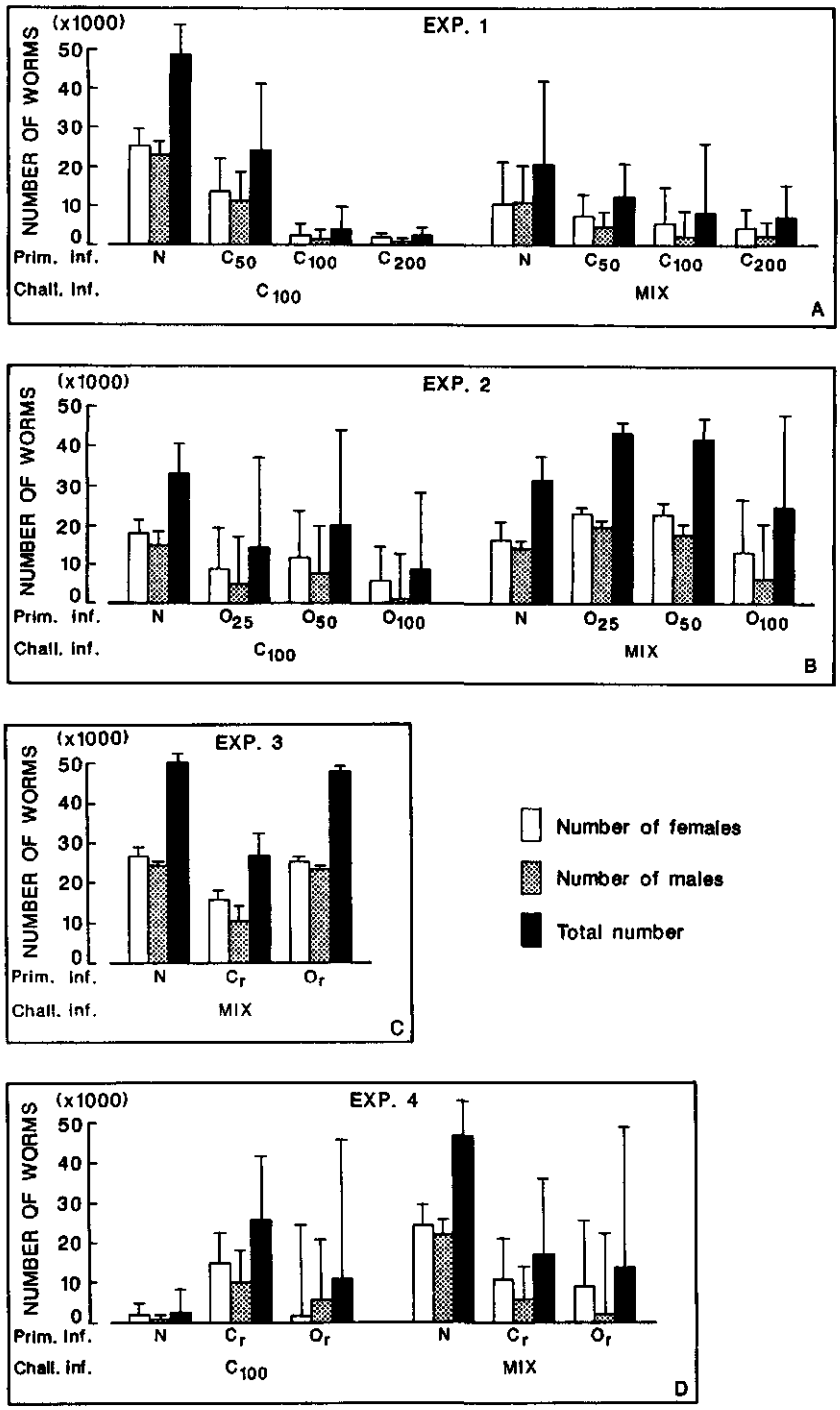

Fig. 11. *C. oncophora* worm counts (geometric means ± s.e.m.).

Reduction percentages for all groups are given in Table 14 (page 63). Differences between types of challenge infection, i.e. C_{100} versus MIX, were statistically not significant. This was due to very large within-group variations; e.g. within the N-MIX group the total number of worms recovered from the small intestine varied from 3,550 to 68,600. Repeated primary infection with *C. oncophora* in exp. 3 caused a significant reduction ($p < 0.05$) of 47% in worm burden after challenge when compared to primarily uninfected animals (Fig. 11C).

No significant reductions were noticed in homologous challenge infections of exp. 4 (Fig. 11D). This was due to the very low mean number of worms in the N-C_{100} group. Three animals of this group showed a total worm count less than 1,500 while 53,500 worms were found in the fourth animal.

- Heterologous infections

Heterologous challenge infections never resulted in a significant reduction of *C. oncophora* worm counts. The mean number of *C. oncophora* worms within the challenge C_{100} group of exp. 2 is clearly reduced when compared to the MIX group (Fig. 11B), but large within-group variations prevented statistical significance.

4.1.2.2 *O. ostertagi* worm counts

The number of *O. ostertagi* worms are presented graphically in Fig. 12A to 12D. The mean percentages of worms recovered from the primarily uninfected groups varied considerably between the experiments. Respectively 20.5%, 28.8%, 40.6% and 8.6% of the inoculation dose was recovered as adult worms in exp. 1 to 4. This percentage of take differed significantly between the experiments ($p < 0.01$).

- Homologous infections

Homologous challenge infections in exp. 3 and 4 resulted in significantly reduced ($p < 0.001$) abomasal worm burdens when compared to the primary control group (Fig. 12C and 12D). Reduction percentages were 33% and 47%, respectively (Table 15 - page 64). Primarily infected groups showed significantly more *O. ostertagi* worms than primary controls in exp. 2. This was partly due to the low number of *O. ostertagi* females in the latter (Fig. 12B). The sex-ratio, expressed as the percentage of males, in this group was 60% while normal ratios are about 50%. Concurrent challenge infections with *C. oncophora* did not affect the number of *O. ostertagi* worms when compared to mono-species challenge infections with *O. ostertagi* only (Fig. 12B and 12D).

- Heterologous infections

Neither heterologous primary infections nor mixed challenge infections had significant effects on *O. ostertagi* worm counts.

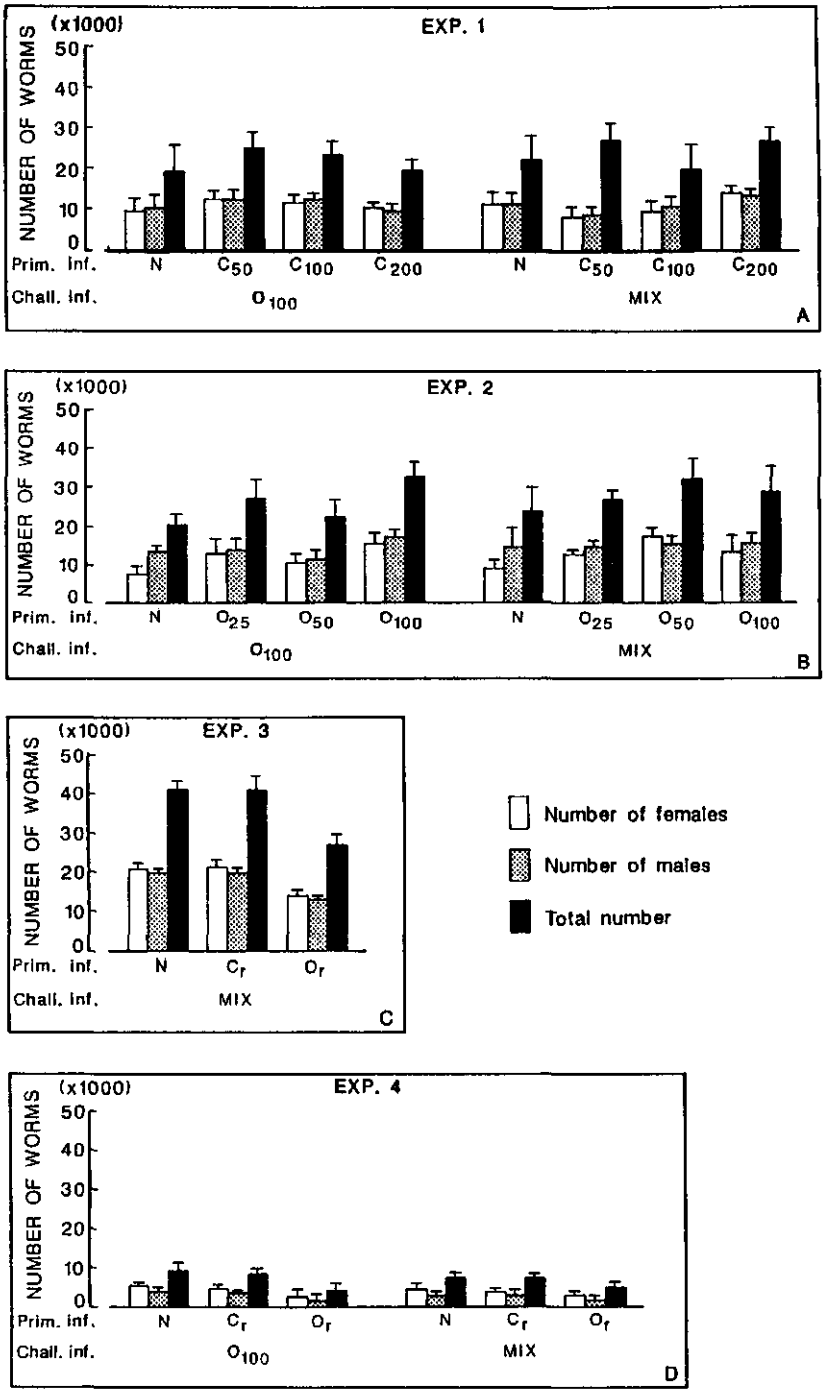

Fig. 12. *O. ostertagi* worm counts (geometric means ± s.e.m.).

4.1.3 Worm differentiation

4.1.3.1 Percentage of males in *C. oncophora* infections

Results of sex-differentiation, expressed as the percentage of males, found in *C. oncophora* infections are presented in Table 4. The type of challenge infection, C_{100} or MIX, did not affect this parameter significantly. Mean percentages of males in primarily uninfected groups challenged with C_{100} or MIX (Table 4 - group 1) varied from 45.4% to 48.8% when the first three experiments are considered. The percentage of males was reduced significantly in animals primarily infected with *C. oncophora* (exp. 1: $p < 0.01$; exp. 3: $p < 0.05$).

Results of this parameter are given in detail for exp. 4 because of their aberrancy (Table 5). The low percentage of males in the N-C_{100} group (21.1%) was caused by three animals that showed extreme low worm burdens (less than 1,500). Individual percentages of males in this group were 45.6%, 29.7%, 5.3% and 3.7%. The high mean percentage of males in the O_r-C_{100} group (59.8%) was due to one animal. From this calf only male worms were recovered. The low percentage of males in the O_r-MIX group could be explained by one calf that showed only female worms. These extreme values are reflected in large standard errors.

Heterologous primary infection in exp. 2 reduced the percentage of males to some extent, but these reductions were not significant. Heterologous priming in exp. 3 did not affect this parameter.

Table 4. Mean percentage of *C. oncophora* males resulting from primary infection groups.

	PRIMARY INFECTION GROUP*				
EXP	1	2	3	4	MEAN
1	48.8	42.2	32.6	28.2	38.1
2	45.4	42.2	41.4	40.3	42.3
3	47.5	41.2	47.7	----	45.6
4	34.2	38.2	47.8	----	40.0

* 1-4: groups N-C_{50}-C_{100}-C_{200} for exp. 1
groups N-O_{25}-O_{50}-O_{100} for exp. 2
1-3: groups N-C_r-O_r for exp. 3 and 4

Table 5. Mean percentage of *C. oncophora* males (± s.e.m.) for all subgroups of exp. 4.

	PRIMARY INFECTION GROUP		
CHALL. GROUP	N	C_r	O_r
C_{100}	21.1 ± 10.1	40.7 ± 4.9	59.8 ± 13.5
MIX	47.4 ± 0.6	35.6 ± 5.6	35.8 ± 12.0

4.1.3.2 Percentage of males in *O. ostertagi* infections

Sex-differentiation of *O. ostertagi* worms is given in Table 6. The type of challenge infection did not affect the percentages of *O. ostertagi* males and the primary infection was only of significant importance ($p < 0.001$) in exp. 2. This significance was entirely due to the extreme high percentage of males in the control group (61.2%). The percentages of males in the primarily infected groups of exp. 2 did not differ much from the percentages found in exp. 1 and 3. Aberrant sex-ratios in the primarily non-infected group of exp. 2 might be caused by a selective expulsion of female worms between day 19 and 23 p.c.i., because a considerable drop in egg count was noticed during that period. Rather low percentages of males were found in exp. 4 when compared to other experiments.

Table 6. Mean percentage of *O. ostertagi* males resulting from primary infections.

	PRIMARY INFECTION GROUP*				
	1	2	3	4	
EXP					MEAN
1	51.1	50.7	51.6	48.6	50.4
2	61.2	52.5	48.9	53.1	53.9
3	49.1	47.7	47.6	----	48.1
4	43.4	43.9	42.9	----	43.4

* 1-4: groups N-C_{50}-C_{100}-C_{200} for exp. 1
groups N-O_{25}-O_{50}-O_{100} for exp. 2
1-3: groups N-C_r-O_r for exp. 3 and 4

4.1.3.3 Differentiation *C. oncophora*/*C. surnabada*

The mean percentages of *C. surnabada* (± standard deviations) were 32.6% ± 8.7, 27.9% ± 10.8, 30.7% ± 11.9 and 27.1% ± 13.0 for exp. 1 to 4 respectively. This parameter was not affected significantly by any type of infection in exp. 1 to 3. However, in exp. 4 the percentage of *C. surnabada* was significantly lowered (16.1%) within *O. ostertagi* primed groups; values of 24.4% and 34.1% were found for the primary control group and the *C. oncophora* primed group, respectively.

4.1.4 Worm length

4.1.4.1 *C. oncophora* worm lengths

Mean length of the *C. oncophora* females varied from 11.0 to 12.0 mm between experiments when primarily non-infected groups are considered. The mean male length varied from 8.4 to 9.1 mm for those groups (Table 7). Differences between the experiments were significant ($p < 0.05$ and $p < 0.01$ for female and male length, respectively).

Table 7. Mean lengths (mm) ± standard deviation of *C. oncophora* worms of primarily non-infected groups.

	EXPERIMENT			
	1	2	3	4
FEMALES	11.0 ± 0.4	12.0 ± 0.2	11.7 ± 0.1	11.1 ± 0.4
MALES	8.4 ± 0.2	9.1 ± 0.1	9.0 ± 0.1	8.5 ± 0.2

- Homologous priming

The results of *C. oncophora* length measurements are presented in Fig. 13A to 13D. Primary infection with *C. oncophora* reduced the length of male and female worms after challenge. Reduction percentages are given in Table 14. Average reductions of up to 13% were assessed when single dose priming was applied (exp. 1) while repeated priming (exp. 3 and 4) resulted in reductions of 3 to 6%.

Homologous primary infections reduced significantly lengths of both male ($p < 0.001$) and female ($p < 0.01$) worms in exp. 1 and 3. Worms were smaller when the primary inoculation dose increased (exp. 1), but analysis of regression showed that primary dose level did not affect worm length significantly. The reduction percentages in exp. 4 were small and the lengths were not affected significantly by homologous priming. The type of challenge infection was of some importance for female length in exp. 1, resulting in smaller worms when concurrent infections were applied ($p = 0.06$).

Relative frequency distributions of female length for primary infection groups of exp. 1 are given in Fig. 14. From these distributions it can be concluded that the decreased mean length for homologously primed groups was merely due to a reduced percentage of large worms (>12 mm). The percentage of small worms (<8 mm) was only slightly increased. This is reflected in a significantly ($p < 0.05$) decreased mean within-calf standard deviation for female length (Table 8). Within-calf standard deviations (WC_{SD}) and coefficients of variation (CV=standard deviation/mean*100) could be assessed, because the lengths of individual worms were measured. The WC_{SD} of male length was not affected significantly by primary infection.

- Heterologous priming

Priming with *O. ostertagi* reduced the length of *C. oncophora* worms (Fig. 13B, 13C, 13D; Table 14). In exp. 2 the average reduction in length increased when the primary infection dose increased, but despite this fact the worms that were recovered from primarily infected animals were not significantly smaller than the worms collected from primarily uninfected animals. This was also found in exp. 4. In exp. 3 worm length was significantly affected by heterologous priming ($p < 0.001$); *C. oncophora* worms were more reduced by heterologous priming than by homologous priming in this experiment.

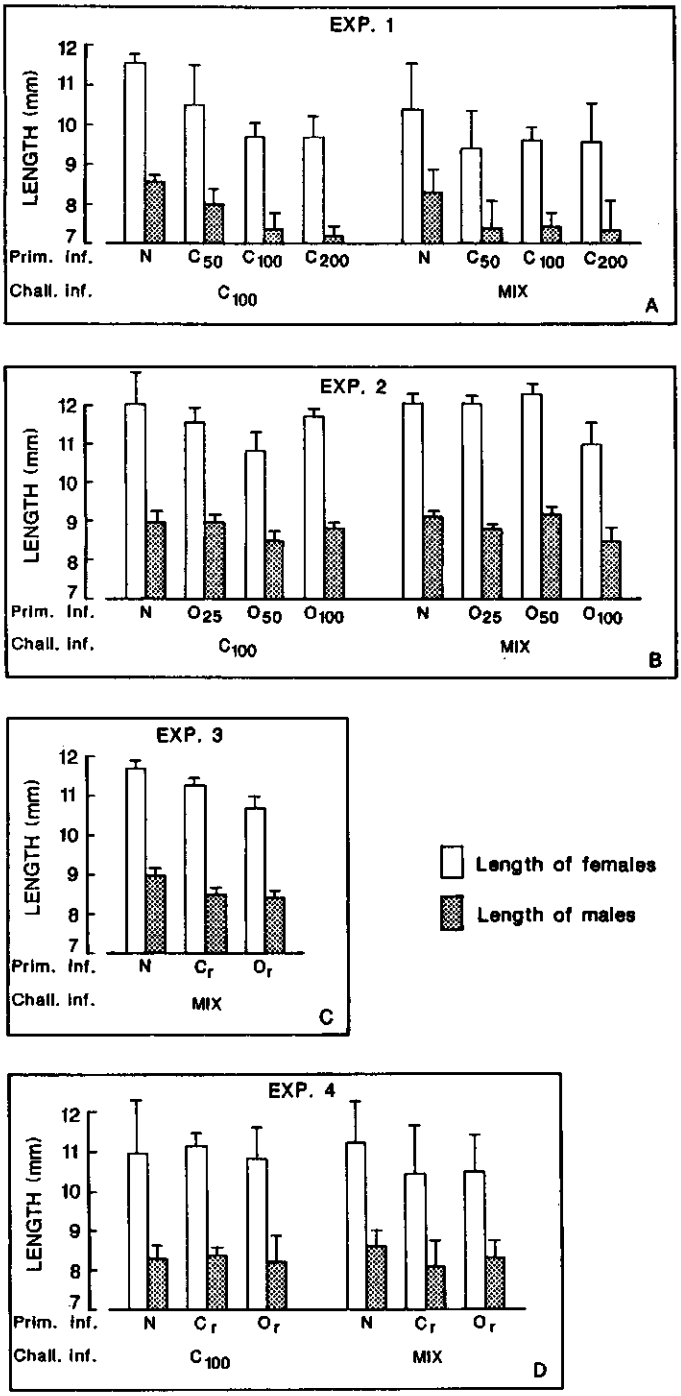

Fig. 13. Length of *C. oncophora* male and female worms (mean ± s.d.).

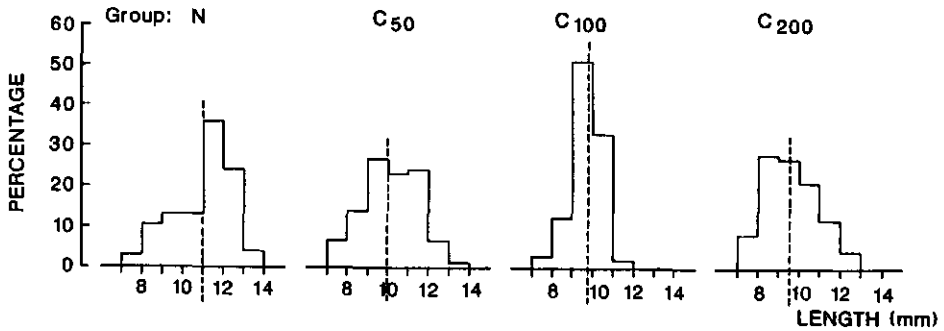

Fig. 14. Relative frequency distributions of the length of *C. oncophora* females from primary infection groups of exp. 1 (the vertical broken lines indicate the group means).

Table 8. Mean within-calf standard deviations (± s.e.m.) and mean coefficients of variation (± s.e.m.) of *C. oncophora* male and female lengths for primary infection groups of exp. 1.

	PRIMARY INFECTION GROUP			
	N	C_{50}	C_{100}	C_{200}
MALES				
WC_{SD}(mm)	0.63 ± 0.05	0.56 ± 0.06	0.63 ± 0.06	0.63 ± 0.05
VC (%)	7.6 ± 0.6	7.4 ± 1.1	8.0 ± 0.7	8.6 ± 0.8
FEMALES				
WC_{SD}(mm)	1.13 ± 0.10	0.85 ± 0.10	0.68 ± 0.08	0.99 ± 0.07
VC (%)	10.5 ± 1.2	8.8 ± 1.3	7.3 ± 0.6	10.9 ± 0.8

4.1.4.2 *O. ostertagi* worm lengths

The mean lengths of *O. ostertagi* male and female worms are shown in Fig. 15A to 15D for exp. 1 to 4 respectively. Considering only the primarily uninfected groups, the mean length of *O. ostertagi* female worms varied from 8.5 to 8.9 mm between the experiments, while the mean length of male worms varied from 6.9 to 7.2 mm. The length of male worms differed significantly between experiments ($p < 0.05$).

- Homologous infections

Homologous priming in exp. 2, 3 and 4 resulted in significantly reduced lengths of both male and female worms after challenge ($p < 0.001$). Neither the level of primary inoculation dose (exp. 2) nor the type of challenge infection contributed significantly to the reduction of length. Reduction percentages are given in Table 15. The average reduction of the length of female worms was 9.3%, 11.2% and 10.2% in exp. 2, 3 and 4, respectively, while the lengths of male worms were reduced with 7.7%, 10.0% and 8.7% in those experiments.

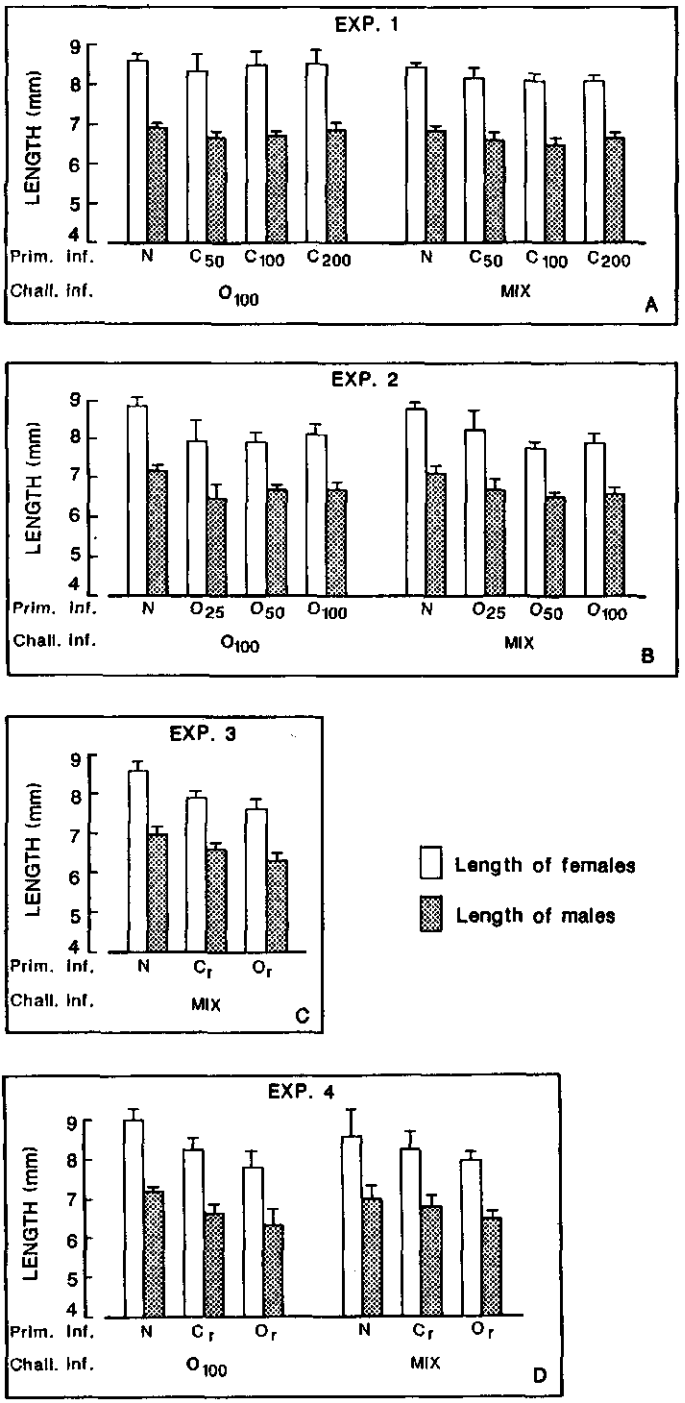

Fig. 15. Length of *O. ostertagi* male and female worms (mean ± s.d.).

Relative frequency distributions of *O. ostertagi* female length are given in Fig. 16 for primary infection groups of exp. 2. Reduction of length concerning primarily infected groups was due to a decreased percentage of large worms (>9 mm) and an increased percentage of small worms (<7.5 mm). Neither within-calf standard deviations nor variation coefficients (Table 9) differed significantly between groups.

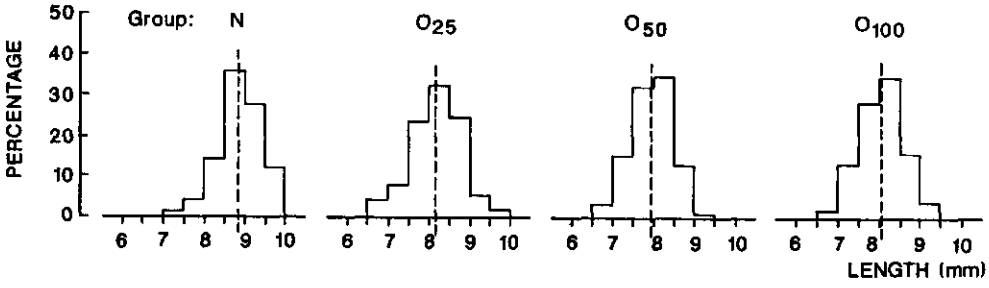

Fig. 16. Relative frequency distributions of the length of *O. ostertagi* females from primary infection groups of exp. 2 (the vertical broken lines indicate the group means).

Table 9. Mean within-calf standard deviations (± s.e.m.) and mean coefficients of variation (± s.e.m.) of *O. ostertagi* male and female lengths for primary infection groups of exp. 2.

	PRIMARY INFECTION GROUP			
	N	O_{25}	O_{50}	O_{100}
MALES				
WC_{SD} (mm)	0.38 ± 0.03	0.34 ± 0.01	0.40 ± 0.02	0.34 ± 0.02
VC (%)	5.2 ± 0.4	5.1 ± 0.2	6.0 ± 0.3	5.1 ± 0.2
FEMALES				
WC_{SD} (mm)	0.49 ± 0.03	0.47 ± 0.03	0.49 ± 0.03	0.45 ± 0.03
VC (%)	5.6 ± 0.3	5.8 ± 0.4	5.9 ± 0.5	5.6 ± 0.3

- Heterologous infections

The lengths of *O. ostertagi* worms recovered from heterologously primed animals were intermediate between the lengths of worms collected from non-primed and homologously primed animals (Fig. 15A, 15C, 15D; Table 15). Priming with *C. oncophora* reduced the length of *O. ostertagi* male worms significantly (exp. 1: $p = 0.01$, exp. 3 and 4: $p < 0.01$). In exp. 1 male worms were also significantly smaller when mixed challenge infections were applied ($p < 0.001$). The same results were obtained for *O. ostertagi* females with the exception of the primary infection of exp. 1.

4.1.5 Number of eggs in utero

4.1.5.1 Eggs *in utero* of *C. oncophora* females

The mean numbers of eggs per *C. oncophora* female are shown in Fig. 17A to 17D for exp. 1 to 4, respectively. Considerable between-experiment variations were found for this parameter. When primarily uninfected groups are considered the mean number of eggs per female (± s.d.) were 50.9 ± 22.0, 60.3 ± 39.5, 36.6 ± 14.6 and 27.5 ± 22.1 for exp. 1 to 4, respectively. As the worms were desintegrated individually in exp. 1 and 2, within calf standard deviations and mean variation coefficients could be assessed. For primarily uninfected groups the mean within calf standard deviation (± s.d.) was 18.5 ± 5.0 in exp. 1 and 19.0 ± 4.8 in exp. 2, while the mean variation coefficients (± s.d.) were 42.8% ± 16.5 and 44.3% ± 36.1, respectively.

- Homologous challenge infections

Within the C_{100} challenge group of exp. 1 the mean number of eggs per female was reduced significantly by primary infection ($p < 0.05$). This reduction resulted from an increase of females with very few eggs *in utero* as is shown by relative frequency distributions (Fig. 18). In some primarily infected animals almost all females were non-gravid. The mean reduction was about 45% (Table 14). No effect of primary inoculation level was present. Within the MIX challenge group this parameter was slightly increased by primary infection (Fig. 16A). This was entirely due to the low number of eggs per female within the N-MIX group. It also caused an almost significant interaction ($p \approx 0.06$) between the main effects of the statistical model. Homologous primary infection did not affect this parameter in exp. 3 and 4.

- Heterologous challenge infections

Primary infection with *O. ostertagi* never affected the number of eggs per *C. oncophora* female significantly. In exp. 2 the type of challenge infection was of significant importance resulting in more eggs per *C. oncophora* female when mixed challenge infections were applied ($p < 0.05$).

4.1.5.2 Number of eggs *in utero* of *O. ostertagi* female worms.

The mean numbers of eggs per *O. ostertagi* female are presented in Fig. 19A to 19D for exp. 1 to 4, respectively. For primarily uninfected groups of exp. 1 to 4 the mean number of eggs (± s.d.) per *O. ostertagi* female were 18.8 ± 2.5, 20.2 ± 3.1, 13.9 ± 3.3 and 19.5 ± 6.3, respectively. The mean within-calf standard deviations (± s.d.) for these groups were 5.9 ± 1.3 and 5.5 ± 1.2 in exp. 1 and exp. 2 while the mean variation coefficients amounted to 31.6 ± 7.4 and to 27.8 ± 7.7, respectively. The reduction percentages due to homologous and heterologous primary infections are given in Table 15.

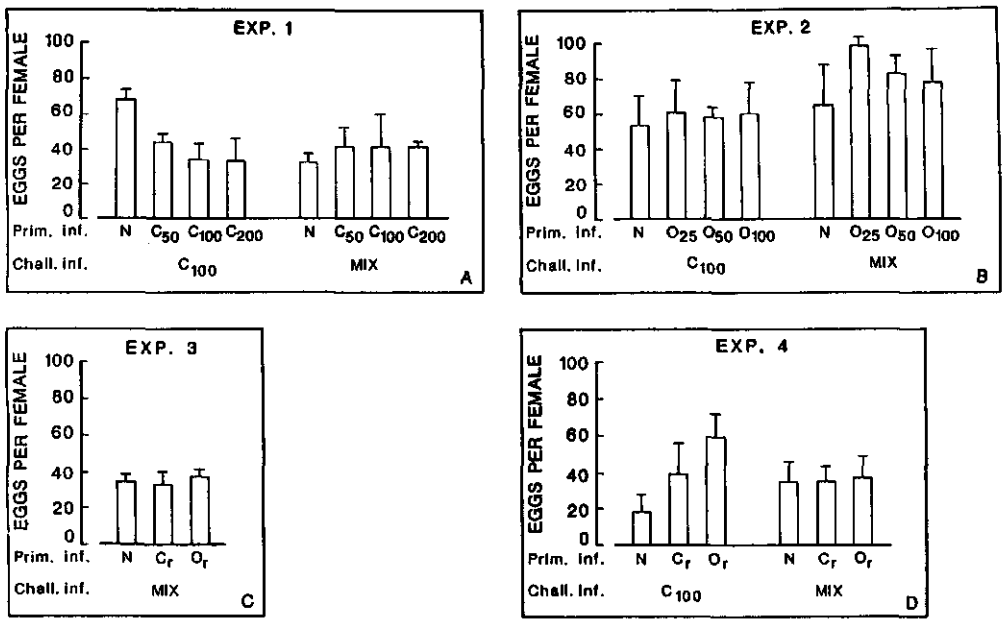

Fig. 17. Number of eggs *in utero* of *C. oncophora* females (mean ± s.d.).

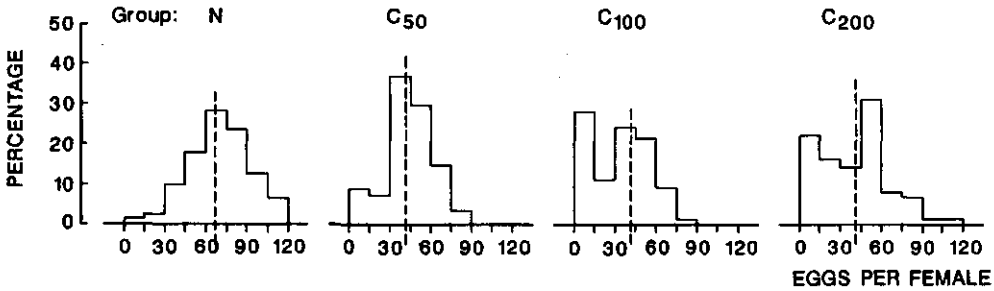

Fig. 18. Relative frequency distributions of the numbers of eggs *in utero* of *C. oncophora* females from primary infection groups of exp. 1 (the vertical broken lines indicate the group means).

- Homologous infections

Homologous priming resulted in significant reductions of the number of eggs per female ($p < 0.001$ in exp. 2, 3 and 4). In exp. 2 this parameter was negatively affected by the primary inoculation dose ($p < 0.001$). The reductions resulted from an increase of females with moderate numbers of eggs *in utero* (Fig. 20). In contrast to *C. oncophora*, non-gravid females were sporadically found. Reductions resulting from homologous priming were about 30% in exp. 2 and 3 and more than 50% in exp. 4. The type of challenge infection did not contribute to the model significantly.

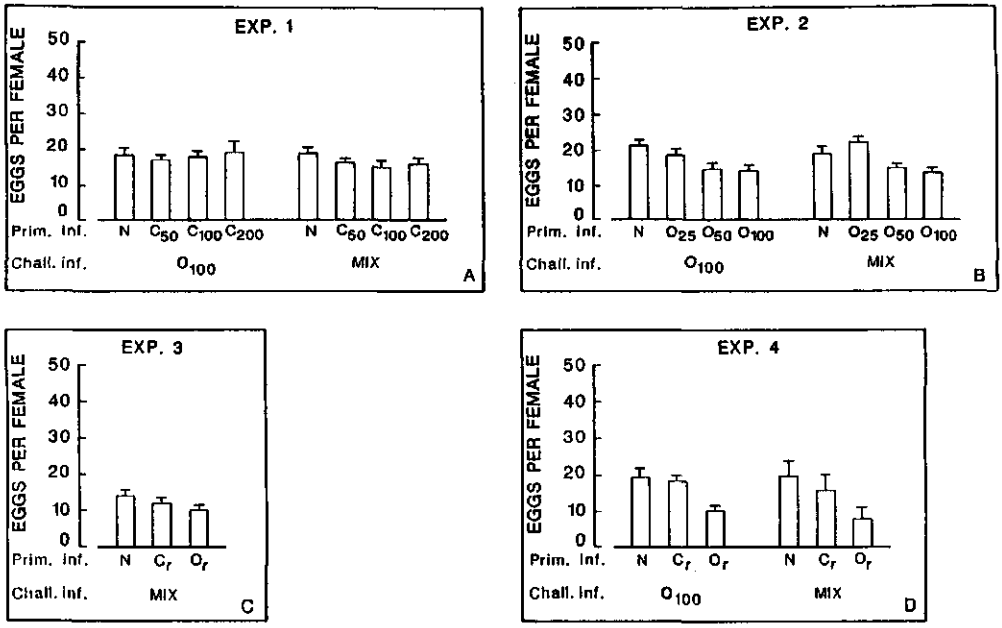

Fig. 19. Number of eggs *in utero* of *O. ostertagi* females (mean ± s.d.).

- Heterologous infections

Heterologously primed animals always showed intermediate fecundity of *O. ostertagi* females when compared to non-primed and homologously primed animals (Table 15). However, the differences between non-primed and heterologously primed animals were statistically not significant.

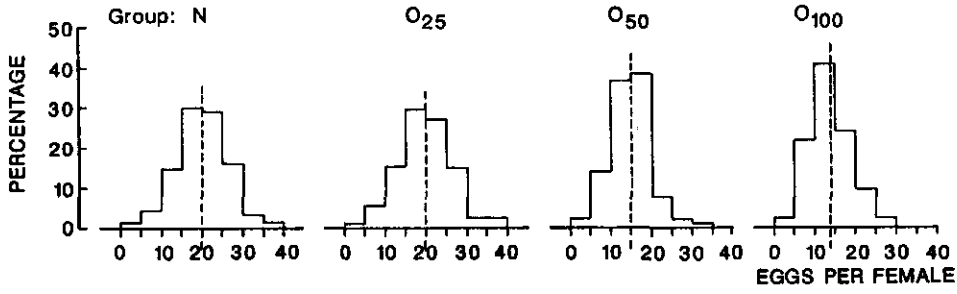

Fig. 20. Relative frequency distributions of the numbers of eggs *in utero* of *O. ostertagi* females from primary infection groups of exp. 2 (the vertical broken lines indicate the group means).

4.1.6 Vulval flap development

This parameter only concerns *O. ostertagi* populations. The mean scores (± s.e.m.) for primarily uninfected groups were 4.9 ± 0.1, 5.1 ± 0.1, 5.4 ± 0.1 and 5.0 ± 0.2 for exp. 1 to 4, respectively. The differences between the experiments were significant. Vulval flap scores of all the experiments are presented in Fig. 21A to 21D.

- Homologous priming

Homologous priming always resulted in significantly lowered flap scores ($p < 0.01$ in exp. 2 and 3; $p < 0.05$ in exp. 4). In exp. 4, however, a significant interaction was found between the main effects of the statistical model. This was entirely due to the relative high flap scores in the O_r-MIX group. The reduction percentages due to homologous priming were about 20 to 30% (Table 15). Relative frequency distributions are given in Fig. 22 for primary infection groups of exp. 2. It appears that worms with fully developed flaps were found in all groups.

- Heterologous priming

Priming with *C. oncophora* also resulted in a reduced vulval flap development (Fig. 21A, 21C, 21D; Table 15). The difference between the primary control group and the *C. oncophora* primed group was significant in exp. 3 ($p < 0.001$) and approached significance in exp. 4 ($p < 0.06$).

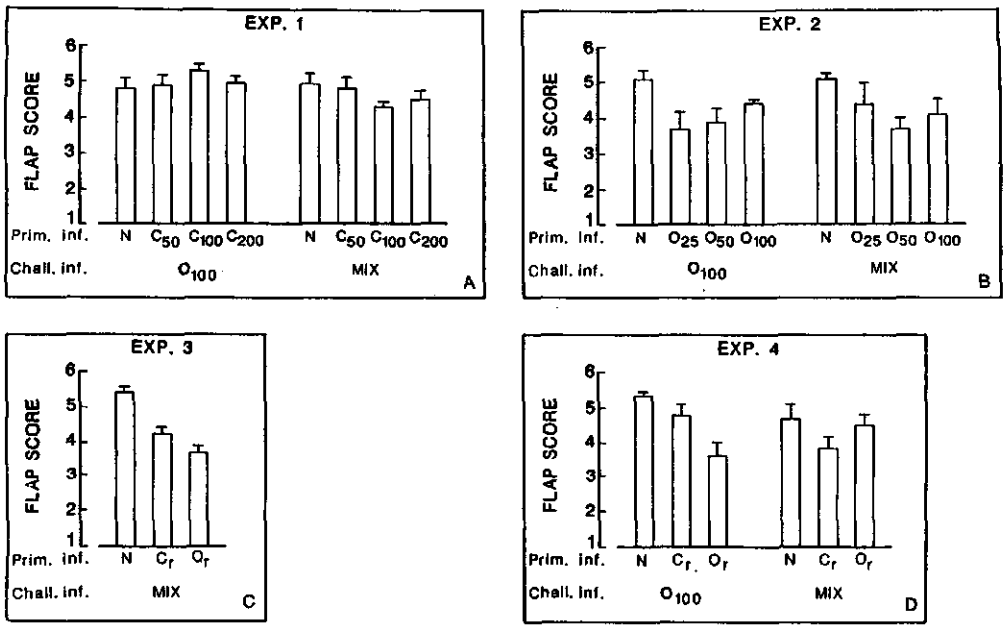

Fig. 21. Vulval flap scores of *O. ostertagi* females (mean ± s.e.m.).

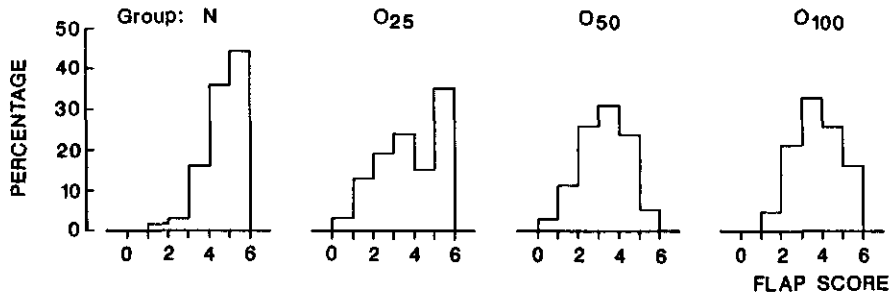

Fig. 22. Relative frequency distributions of vulval flap scores of *O. ostertagi* females from primary infection groups of exp. 2.

4.1.7 Relations between parasitological parameters

The relations between parameters are presented by means of Pearson correlation coefficients. As correlations might arise from differences between group means, an average within-group correlation (r_w), i.e. an overall correlation calculated when individual values have been transformed by subtraction of the group mean, has also been calculated.

4.1.7.1 Relations between *C. oncophora* parameters.

The relation between parasitological parameters of *C. oncophora* are given in Table 10. The correlations between *post mortem* parameters are significantly positive in all experiments. In fact, this means that no crowding, characterized by stunted growth and inhibited development, occurs when less than 50,000 adult worms (exp. 3) are present in the small intestine. Correlations between actual EPG (the EPG two or three days before necropsy) have been calculated within the challenge C_{100} groups only, because no correction could be made for the contribution to the EPG of the *O. ostertagi* populations when mixed challenge infections were applied. The actual EPG was related positively to both number of females and number of eggs *in utero*. The product of both parameters correlated best with the actual EPG, but hese correlations were statistically not significant because of the small group sizes.

4.1.7.2 Relations between *O. ostertagi* parameters.

The relations between the p.m. parameters of *O. ostertagi* are less obvious than those of *C. oncophora* (Table 11). The correlation coefficients between worm numbers and lengths were significantly positive in exp. 3 and 4. These correlations mainly resulted from differences between groups, because within-group correlation coefficients did not differ significantly from zero. In exp. 2 a negative relation between numbers and lengths of females was assessed, indicating that the larger worms were found in animals with a

Table 10. Overall correlations coefficients (r) and average within-group correlation coefficients (r_w) between parasitological parameters of *C. oncophora*.

PARAMETERS		EXPERIMENT 1		2		3		4	
		r	r_w	r	r_w	r	r_w	r	r_w
No. of F**	length of F	0.60[b]*	0.47[b]	0.44[b]	0.47[b]	0.31[a]	0.39[b]	0.49[a]	0.58[b]
No. of M**	length of M	0.66[b]	0.48[b]	0.38[a]	0.42[a]	0.52[b]	0.54[b]	0.51[b]	0.55[b]
No. of F	ova/F	0.26	0.02	0.45[b]	0.41[a]	0.47[b]	0.51[b]	0.68[b]	0.75[b]
Ova/F	length of F	0.52[b]	0.39[a]	0.53[b]	0.65[c]	0.32[a]	0.43[b]	0.52[b]	0.65[b]
No. of F	actual EPG	0.70	0.39	0.57	0.76	-----	-----	0.57	0.70
Ova/ F	actual EPG	0.64	-0.13	0.72	0.57	-----	-----	0.91	0.89
No. of F x Ova/F	actual EPG	0.82	-0.03	0.80	0.83	-----	-----	0.92	0.93

* a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$
** F = females; M = males

Table 11. Overall correlations coefficients (r) and average within-group correlation coefficients (r_w) between parasitological parameters of *O. ostertagi*.

PARAMETERS		EXPERIMENT 1		2		3		4	
		r	r_w	r	r_w	r	r_w	r	r_w
No. of F**	length of F	-0.14	-0.18	-0.44[a]*	-0.03	0.39[b]	0.17	0.58[b]	0.25
No. of M**	length of M	-0.17	-0.23	-0.01	0.05	0.31[a]	-0.26	0.51[a]	0.12
No. of F	ova/F	0.10	0.07	-0.09	0.29	0.49[b]	0.38[b]	0.52[b]	0.15
No. of F	flap score	0.06	0.06	0.43[a]	-0.22	0.28	-0.15	0.52[b]	0.27
Ova/F	length of F	0.19	-0.12	0.59[b]	0.45[b]	0.46[b]	0.18	0.51[a]	0.19
Ova/F	flap score	0.06	-0.09	0.37[a]	0.22	0.38[b]	-0.06	0.41[a]	0.24
Flap sc.	length of F	0.38[a]	0.15	0.80[b]	0.69[b]	0.88[b]	0.37[a]	0.73[b]	0.65[b]
No. of F	actual EPG	0.44	0.39	0.52	0.51	-----	-----	0.42	-0.21
Ova/F	actual EPG	0.65	0.78	0.36	0.50	-----	-----	0.59	0.10
No. of F x Ova/F	actual EPG	0.70	0.79	0.46	0.57	-----	-----	0.60	0.10

* a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$
** F = females; M = males

low number of females.

Fecundity, expressed as number of eggs per female, was related positively to length and vulval flap scores, but these correlations were also mainly due to differences between groups.

The vulval flap scores and lengths of female worms were correlated positively after correction for group means indicating that within all groups the larger worms have more completely developed flaps. The actual EPG was related positively to the worm numbers and the numbers of eggs *in utero;* these correlations were lower than those of *C. oncophora* populations.

4.2 IMMUNOLOGICAL PARAMETERS

4.2.1 Enzyme-Linked-ImmunoSorbent Assay

The composition of SE-antigen solutions which were being used has not been studied in detail. The protein contents of these solutions were very low (<20 ng/ml) when measured according to the BioRad assay (dye binding technique, modified from Bradford (1976)). It appeared that immunogenic antigens were proteins because treatment with hydroxyapatite (calcium phosphate hydroxide that binds proteins) resulted in a total loss of antigenic activity of the SE-solutions. Treatment of the hydroxy-appetite-protein complexes with a salt solution, resulting in free proteins, restored antigenic activity. Gel electrophoresis did not give any bands. This was due to the very low protein contents.

4.2.1.1 Titre counts using *C. oncophora* SE-antigens

Optimal antigen dilution was determined to be 1 to 50; optimal conjugate dilution as 1 to 3,500. As the between-microtitreplate variation was about equal to the within-plate variation, a mean curve (n > 20) of the standard positive was calculated over all plates (Fig. 23A). The lineair regression line (Y = 4.87 - 0.79X; r = -0.99), resulting from logit transformed mean extinctions is shown in Fig. 23B.

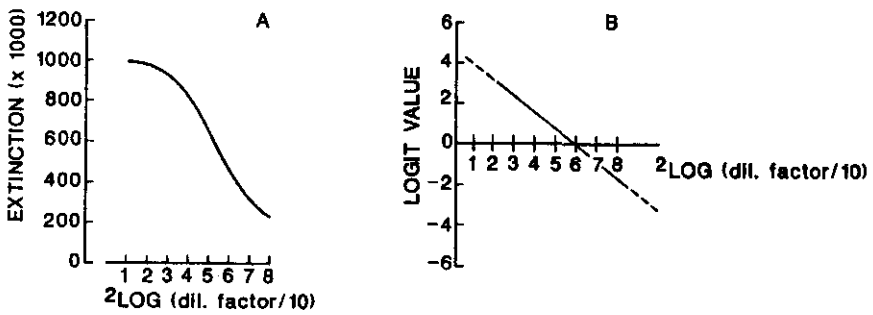

Fig. 23. A. Mean standard positive curve using *C. oncophora* antigens.
B. Linear regression line resulting from A.

- Primary infections

The mean anti-*C. oncophora* antibody titre counts (C-TC) during the primary infection periods of exp. 1 to 4 are presented in Fig. 24A to 24D. At the start of the experiments the mean C-TC of all primary infection groups was between 0 and 0.5. During the primary infections the C-TC of non-infected groups rose slightly to values between 0.5 and 1. The C-TC of groups infected with *C. oncophora* increased to a level of 3 to 4 at the end of the primary infection period except in exp. 3. Four weeks after the first inoculation already, significant differences ($p < 0.001$) occurred between *C. oncophora* infected and *O. ostertagi* or non-infected groups. A dose-response relationship was, however, not found in exp. 1.

Repeated priming with *O. ostertagi* resulted in a significantly increased C-TC on day 41 p.i. when compared to the non-infected groups ($p < 0.001$). Such an increase was not noticed when single dose priming with *O. ostertagi* had been applied (Fig. 24B).

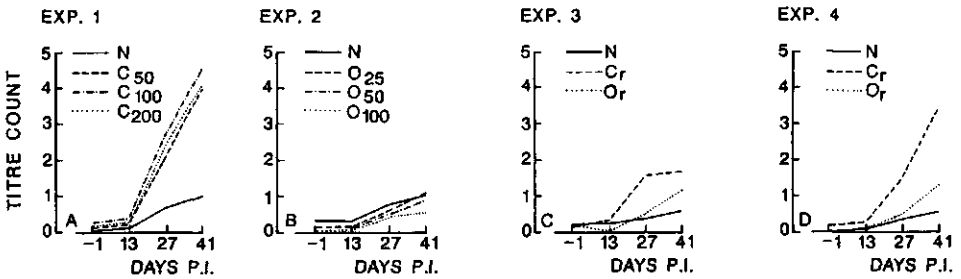

Fig. 24. Anti-*C. oncophora* titre counts resulting from primary infections of exp. 1 to 4.

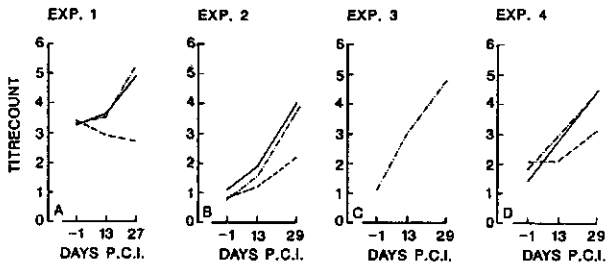

Fig. 25. Anti-*C. oncophora* titre counts resulting from challenge infections.
(——— C_{100}; ------ O_{100}; -·-·-·- MIX).

- Challenge infections

The results of *C. oncophora* titre counts during the challenge infections are given in Fig. 25A to 25D. Challenge infection groups C_{100} and MIX responded almost equally in exp. 1, 2 and 4, while the C-TC of the challenge O_{100} groups declined or increased slightly. Significant differences between the C_{100} and MIX group at one side and the

O_{100} on the other occurred in exp. 1 and 4 on day 13 p.c.i. already. During the challenge infection periods the C-TC is still affected significantly by the primary infections ($p < 0.01$) in exp. 3 and 4.

4.2.1.2 Titre counts using *O. ostertagi* SE-antigens

Optimal antigen and conjugate dilutions were determined to be 1 to 5 and 1 to 2,500, respectively. A lineair regression line was calculated from all standards (equation: $Y = 2.20 - 0.53X$; $r = -0.96$, $n > 20$).

- Primary infections

The mean anti-*O. ostertagi* antibody titre counts (O-TC) during the primary infection periods of exp. 1 to 4 are presented in Fig. 26A to 26D. The mean pre-infection level was between 1.5 and 2. During the primary infection period the titre count of the non-infected group increased with 1 to 1.5 unit. The primary infection with *O. ostertagi* larvae resulted in significant differences between *O. ostertagi* infected and non-infected or *C. oncophora* infected animals from 4 weeks after the first inoculation ($p < 0.05$). In exp. 2 the O-TC on day 41 p.i. was affected significantly by the inoculation dose ($p < 0.05$).

Primary infection with *C. oncophora* affected the O-TC positively (Fig. 26A, 26C, 26D) in exp. 1 and 4, but only in the latter the O-TC differed significantly from the non-infected group.

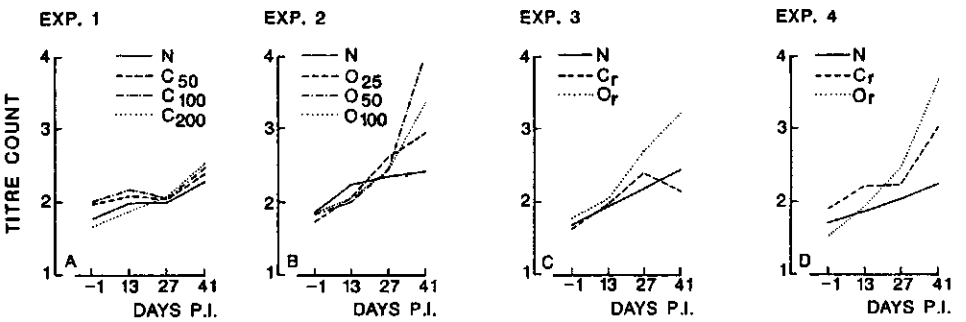

Fig. 26. Anti-*O. ostertagi* titre counts resulting from primary infections of exp. 1 to 4.

- Challenge infections

The results of *O. ostertagi* titre counts after challenge are shown in Fig. 27A to 27D. Effects of primary infection were still present on day 29 p.c.i. in exp. 2 and 4 ($p < 0.01$). The type of challenge infection was of significant importance on day 27 p.c.i. in exp. 1 ($p < 0.05$) and on day 13 p.c.i. ($p < 0.05$) and day 29 p.c.i. ($p < 0.01$) in exp. 2 and 4.

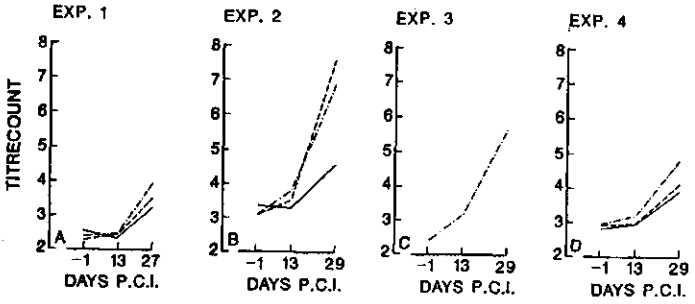

Fig. 27. Anti-*O. ostertagi* titre counts resulting from challenge infections. (——— C_{100}; - - - - - O_{100}; - · - · - · MIX).

4.3 RELATIONS BETWEEN PARASITOLOGICAL AND IMMUNOLOGICAL PARAMETERS

In this chapter the terms homologous and heterologous titres are used. Homologous titres are defined as serum IgG titre counts using SE-antigens of a species (e.g. C-TC) when related to parameters of that same species (e.g. number of *C. oncophora* worms). Heterologous titres are serum IgG titre counts using SE-antigens of a species (e.g. C-TC) when related to parameters of another species (e.g. number of *O. ostertagi* worms).

4.3.1 Titres related to C. oncophora p.m. parameters

Table 12 presents, by means of correlations, the relations that have been found between parasitological parameters of *C. oncophora* and homologous and heterologous titres just before and during the challenge infection.

- Homologous titres

In general, *C. oncophora* p.m. parameters were negatively correlated with the anti-*C. oncophora* antibody titre in exp 1 and 3. Significant negative relations existed already before challenge was carried out. This indicates that these antibodies are involved in the process that affects worm populations negatively or that they are a reflection of such a process. The absence of significant negative relations in exp. 2 is presumably due to fact that no primary *C. oncophora* infections were carried out and thus pre-challenge anti-*C. oncophora* antibody titres were low in this experiment. Although antibody titres increased rapidly after challenge (Fig. 25B), relations were not significantly negative at the end of the experiment. The absence of a significant negative correlation between worm numbers and C-TC in exp. 4 is due to extreme low number of worms in the

N-C_{100} group (Fig. 11D). Within the challenge C_{100} group of this experiment the correlation amounted to 0.39 (day 71 p.i.) while within the MIX group the correlation was -0.30. Worm length showed a significant negative relationship with the C-TC in exp. 1, 3 and 4. Numbers of eggs per female were negatively correlated with the C-TC in exp. 1 ($p < 0.05$) at the end of the experiment. The correlation coefficient (r) between worm numbers and titre counts at day 71 p.i. was very low (0.15) when the primarily non-infected calves of all experiments are considered as one group. Within this group a large variation of worm numbers was found but this was not expressed in a clear relation with the serum IgG titre count.

Table 12. Correlations between *post mortem* parameters of *C. oncophora* and homologous and heterologous titre counts during challenge infection.

	Homologous titers of experiment				Heterologous titres of experiment			
	1	2	3	4	1	2	3	4
	Day 41 p.i.				**Day 41 p.i.**			
No. of F**	-.51b*	.12	-.35a*	-.01	-.21	.03	.03	-.22
No. of M**	-.49b	.11	-.35a	.00	-.22	.10	.02	-.12
Total no.	-.52b	.12	-.35a	-.04	-.22	.05	.02	-.15
Sex-ratio	-.32	.12	-.30a	-.06	-.21	.16	.01	.17
Length of F	-.60b	.09	.00	-.27	-.26	-.31	-.23	-.40a
Length of M	-.65b	.08	-.13	-.31	-.25	-.35a	-.22	-.25
No. of ova/F	-.27	.09	-.09	-.11	-.21	-.02	-.17	.15
	Day 55 p.i.				**Day 55 p.i.**			
No. of F	-.52b	.02	-.49b	-.04	-.43a	.11	.13	-.06
No. of M	-.55b	-.02	-.46b	.14	-.39a	.15	.13	-.15
Total no.	-.54b	.01	-.48b	.04	-.43a	.11	.13	-.11
Sex-ratio	-.47b	-.12	-.37b	.21	-.22	.14	.11	-.12
Length of F	-.55b	.08	-.55b	-.21	-.41a	-.14	-.23	-.44a
Length of M	-.63b	.15	-.62b	-.38a	-.34a	-.32	-.20	-.43a
No. of ova/F	-.25	.11	-.08	.07	-.40a	.21	.08	-.09
	Day 71 p.i.				**Day 71 p.i.**			
No. of F	-.39a	-.12	-.38b	.13	-.24	.32	-.11	-.06
No. of M	-.34	-.17	-.33a	.00	-.25	.28	-.11	-.18
Total no.	-.37a	-.14	-.36a	.09	-.25	.31	-.11	-.11
Sex-ratio	-.16	-.23	-.23	-.12	-.20	.21	-.09	-.14
Length of F	-.49b	-.23	-.38b	-.32	-.56b	.17	-.04	-.60b
Length of M	-.46b	-.21	-.43b	-.38a	-.38a	.01	-.04	-.60b
No. of ova/F	-.42a	-.32	-.14	-.16	-.46b	.49b	.06	-.10

* a: $p < 0.05$; b: $p < 0.01$.
** F = Females; M = Males.

- Heterologous titres

Correlations between pre-challenge titre counts using *O. ostertagi* antigens (O-TC) and the *C. oncophora* length were negative in all four experiments (Table 12), but correlations were only significantly negative in exp. 2 and 4 ($p < 0.05$). The number of *C. oncophora* worms tended to be negatively related to the O-TC at day 71 p.i. in exp. 1 ($p < 0.10$), but the same correlations tended to be positive in exp. 2 ($p < 0.10$). These results could be expected when Fig. 11B and Fig. 27B are considered. Although the *C. oncophora* worm burden was not significantly affected by the type of challenge infection, the mean number of small intestinal worms within the challenge C_{100} group was considerably lower when compared to the MIX group (Fig. 11B) while the O-TC of the C_{100} group was lower than the one of the MIX group (Fig. 27B). This, of course, results in positive correlations between both parameters when all data are considered as a single group. The fecundity of the *C. oncophora* population, expressed as the number of eggs *in utero* was also positively related with the O-TC in exp. 2 ($p < 0.01$).

4.3.2 Titres related to O. ostertagi p.m. parameters

Relations between *O. ostertagi* p.m. parameters and serum IgG antibody titre counts are given in Table 13.

- Homologous titers

It is obvious from Table 13 that in all four experiments the number of *O. ostertagi* worms is not significantly related to the homologous titre at the end of the experiment. When the primarily non-infected animals of all four experiments, showing large variations in abomasal worm burdens, are considered as one group, a significantly positive correlation coefficient ($r = 0.54$; $p < 0.01$) is found between worm counts and O-TC on day 71 p.i. This indicates that during a primary infection the O-TC depends on the numbers of worms present as could be expected from results of exp. 2 (Fig. 26B). Worm length and flap scores were negatively related to the pre-challenge O-TC in exp. 2, 3 and 4. These parameters were related in the same way with the O-TC on day 55 p.i. Fecundity of the *O. ostertagi* populations was negatively related to the pre-challenge O-TC in exp. 2. Both parameters were negatively related to each other throughout exp. 4.

- Heterologous titres

O. ostertagi p.m. parameters that indicate growth and development of the worm population are negatively related to the C-TC throughout the experiments although significantly negative correlations were only found during exp. 1 and 3. Abomasal worm burdens were not related to the heterologous titre count.

Table 13. Correlations between *post mortem* parameters of *O. ostertagi* and homologous and heterologous titre counts during challenge infection.

	Homologous titers of experiment				Heterologous titres of experiment			
	1	2	3	4	1	2	3	4
	Day 41 p.i.				**Day 41 p.i.**			
No. of F**	.08	.32	-.24	-.38	.15	-.15	-.17	.08
No. of M**	.13	.15	-.23	-.32	.15	-.05	-.26	.08
Total no.	.11	.24	-.24	-.35	.15	-.12	-.21	.08
Sex-ratio	.11	-.28	.04	.11	.00	.16	-.23	-.01
Length of F	-.25	-.52b*	-.27	-.62b	-.33	.00	-.39b	-.18
Length of M	-.25	-.56b	-.34a*	-.62b	-.41a	-.07	-.28	-.21
No. of ova/F	.17	-.59b	-.17	-.53b	-.20	-.09	-.26	.01
Flap score	-.28	-.37a	-.34a	-.44a	-.13	-.01	-.30a	-.18
	Day 55 p.i.				**Day 55 p.i.**			
No. of F	.21	.35a	-.35a	-.37	.21	.04	-.13	-.02
No. of M	.30	.19	-.38b	-.29	.30	.02	-.17	.00
Total no.	.26	.28	-.37b	-.34	.26	.02	-.15	-.01
Sex-ratio	.17	-.42a	-.09	.16	.17	-.03	-.12	.05
Length of F	-.20	-.46b	-.49b	-.41a	-.20	-.24	-.66b	-.24
Length of M	-.10	-.52b	-.57b	-.40a	-.10	-.17	-.57b	-.19
No. of ova/F	.29	-.17	-.25	-.43a	.29	-.03	-.22	-.20
Flap score	-.21	-.29	-.59b	-.45a	-.21	-.03	-.62b	-.13
	Day 71 p.i.				**Day 71 p.i.**			
No. of F	-.01	.23	-.08	-.16	-.16	-.05	.06	-.28
No. of M	.08	-.06	-.01	-.13	-.12	-.03	.03	-.33
Total no.	.03	.09	-.05	-.14	-.15	-.01	.04	-.31
Sex-ratio	.20	-.52b	.17	.05	.12	.11	-.08	-.17
Length of F	-.37a	-.41a	-.14	-.44a	-.59b	-.16	-.42b	-.31
Length of M	-.31	-.43a	-.20	-.42a	-.49b	-.12	-.39b	-.30
No. of ova/F	.14	-.14	.03	-.38	-.26	-.10	-.01	-.28
Flap score	-.36a	-.13	-.17	-.56b	-.42a	-.12	-.48b	-.12

* a: $p < 0.05$; b: $p < 0.01$
** F = Females; M = Males

4.4 RESULTS OF EXPERIMENT 5

4.4.1 Faecal egg counts

Eggs were found in the faeces of all infected animals after primary infection. This indicates that adult worm populations were present (Fig. 28) and that the host was exposed to larval and adult worm antigens. The egg output of *C. oncophora* primed animals was reduced after challenge. Totally different egg count curves were found

after challenge within the O_r-MIX group. Calf no. 75 showed a very low egg output which may indicate that the intestinal worm burden was small in this animal.

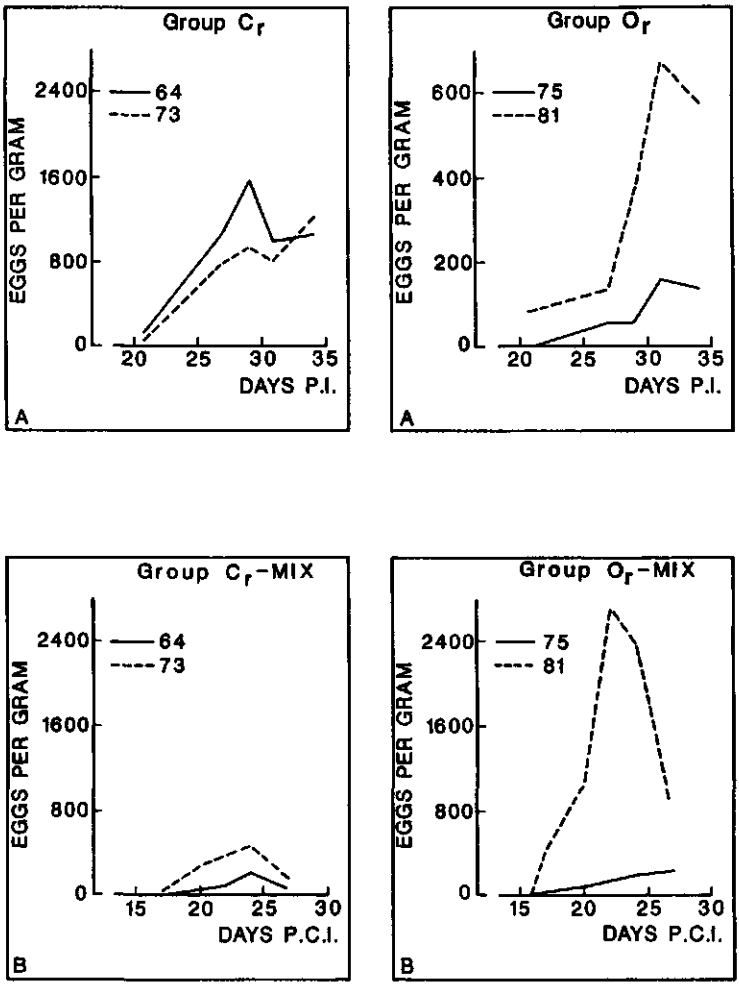

Fig. 28. Faecal egg counts resulting from primary and challenge infections of exp. 5.

4.4.2 Titre counts

The anti-*C. oncophora* antibody titres are shown in Fig. 29A. During the primary infection a marked increase of the titre count was found in *C. oncophora* infected animals. These titre counts exceeded those of the non-infected animals on day 28 p.i. After challenge a rapid and early increase of anti-*C. oncophora* antibody was found in *O. ostertagi* primed animals despite of the fact that these calves did not experience *C. oncophora* before.

The anti-*O. ostertagi* antibody titres remained very low during the primary *O. ostertagi* infections (Fig. 29B). Homologously reinfected calves (O_r-MIX) only showed elevated anti-*O. ostertagi* antibody titre counts after challenge.

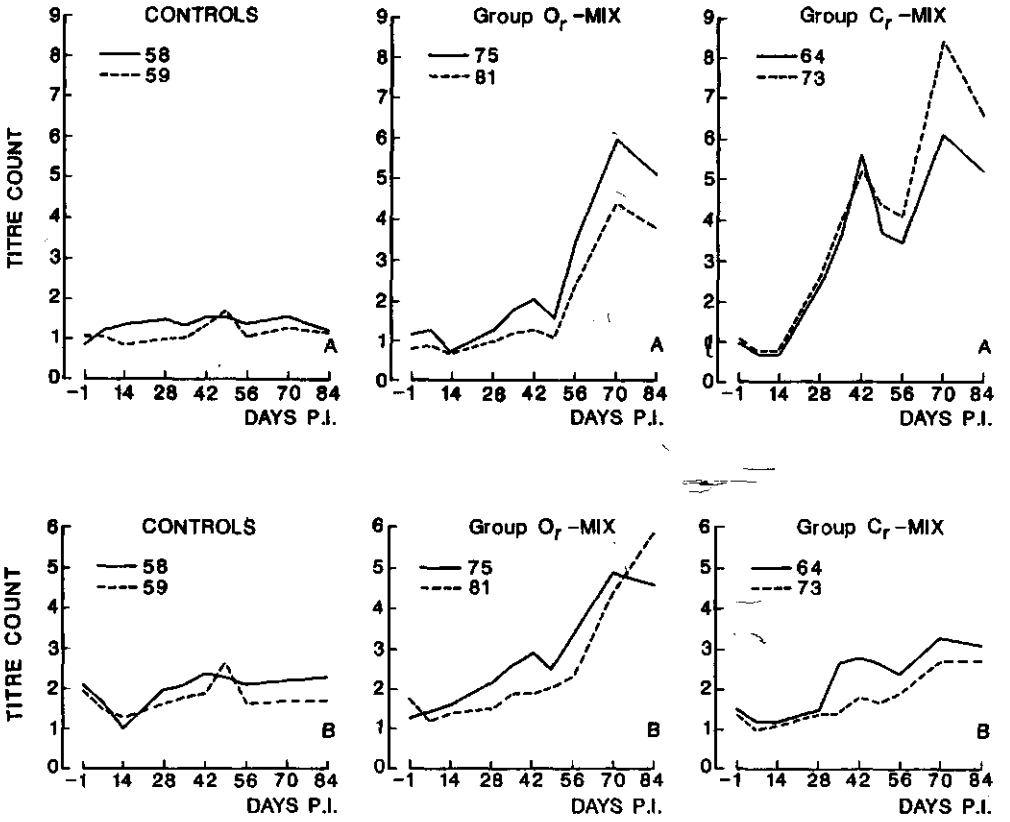

Fig. 29. A. Anti-*C. oncophora* titre counts during exp. 5.
B. Anti-*O. ostertagi* titre counts during exp. 5.

4.4.3 Local responses

The numbers of mast cells, globular leucocytes and IgG_1 plasma cells are not presented because no clear response to infection was measured for these cell types.

- Small intestine

The numbers of eosinophils and IgM-, IgG_2- and IgA plasma cells that were found by histological examination of small intestinal tissue fragments are presented in Fig. 30. During the primary infection only *C. oncophora* infected calves showed increased numbers of plasma cells and eosinophils. Calf 64 showed a weak IgM-, a late IgA-, but

an early IgG_2 plasma cell response when compared to calf 73. The number of plasma cells and eosinophils declined rapidly after anthelmintic treatment, with exception of the number of IgG_2 plasma cells in calf 73. During the challenge infection the non-infected calves showed a small IgG_2 plasma cell peak. The reason for this response is not known, but possibly a short-lived bacterial or viral infection occurred. Both animals of the C_r-MIX group showed about equal patterns during the primary and the challenge infection, but responses occurred earlier during the challenge infection. Animal 73 of the C_r-MIX group showed an increased number of IgG_2 plasma cells on day 84 p.i., 2 weeks after worms had been removed by anthelmintic treatment. Whether this rise is a consequence of the parasitic infection or of some other infection is not known, but it should be noticed that this calf responded very late during the primary infection. Animal 81 of the O_r-MIX group showed early responses of eosinophils and all types of plasma cells after challenge. An increased number of cells was found on day 7 p.c.i. already. Animal 75 of the same group did not show any IgM or IgG_2 plasma cell response, while the number of IgA plasma cells and eosinophils was about equal to calf 81. The absence of IgM and IgG_2 responses in calf 75 might be related to its low faecal egg counts. Reduced faecal egg counts may reflect a low establishment and this results possibly in a reduced antigenic stimulation.

- Abomasum

The numbers of plasma cells and eosinophils in the abomasal mucosae are presented in Fig. 31. Non-infected and *C. oncophora* infected animals did not show a single response during the primary infection. Repeated infection with *O. ostertagi* resulted in an increased number of eosinophils and IgA- and IgG_2 plasma cells. Virtually no IgM response was measured. The number of cells declined rapidly after anthelmintic treatment.

The results after challenge for the O_r-MIX group resembled those after priming. Calf 75 showed consistently lower responses than calf 81. One animal from the C_r-MIX group showed an IgM response. In fact, this was the only IgM plasma cell response that was found in the abomasum. The number of IgG_2 plasma cells remained very low in this group. An early increase of IgA plasma cells was found on day 7 p.c.i. in both primarily infected groups. The increased number of IgG_2- and IgA plasma cells on day 84 p.i. in calf 59 is undoubtedly due to the transplant, via the abomasal cannula, of about 5,000 adult *O. ostertagi* worms on day 77 p.i. Eggs from transplanted worms were found in the faeces within 48 hours. The EPG was about 1,200 for over 10 days which is rather high when it is considered that a relatively small worm burden was transplanted.

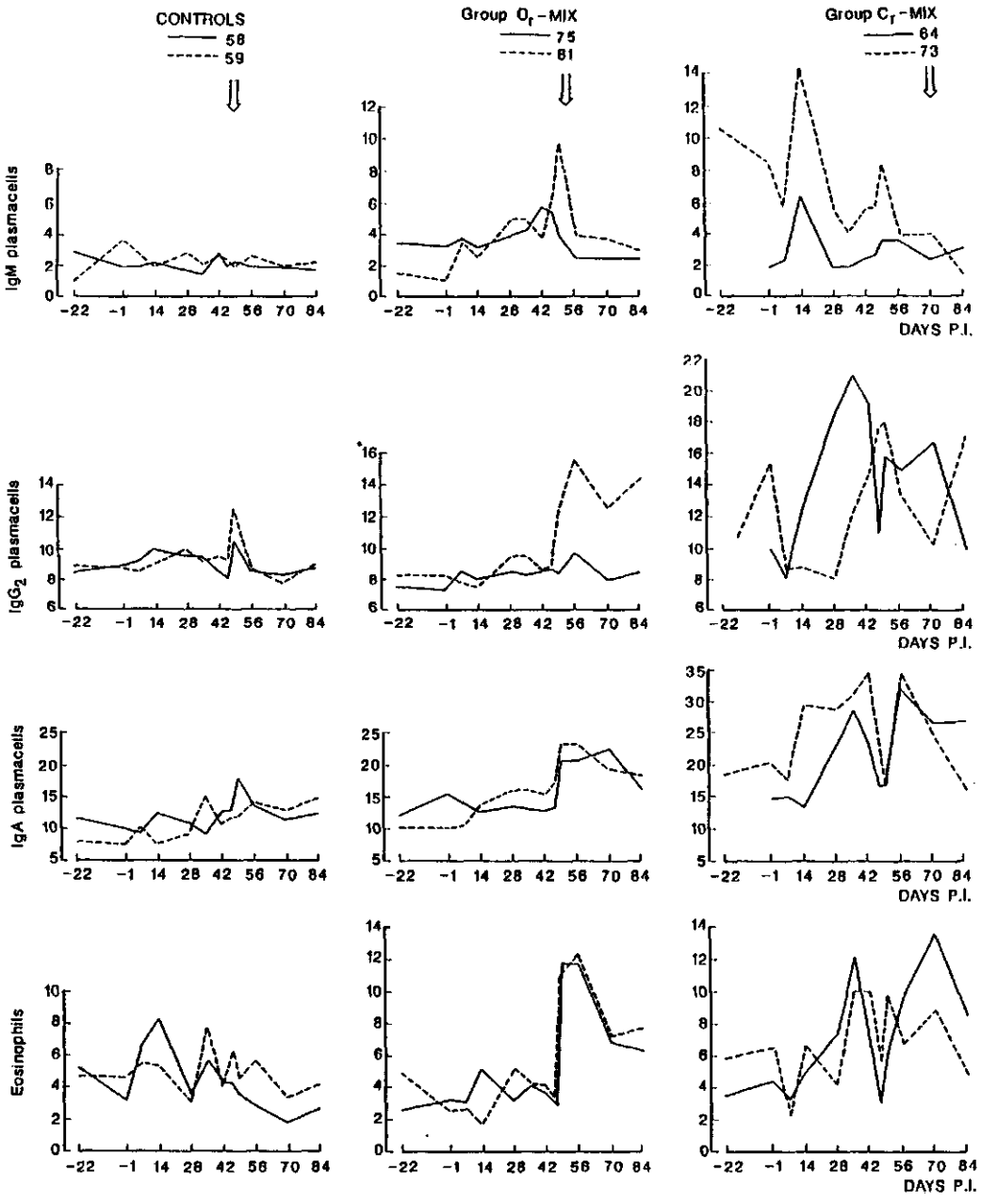

Fig. 30. Numbers of IgM-, IgG_2 and IgA plasma cells and eosinophils in tissue fragments of the small intestinal mucosa.

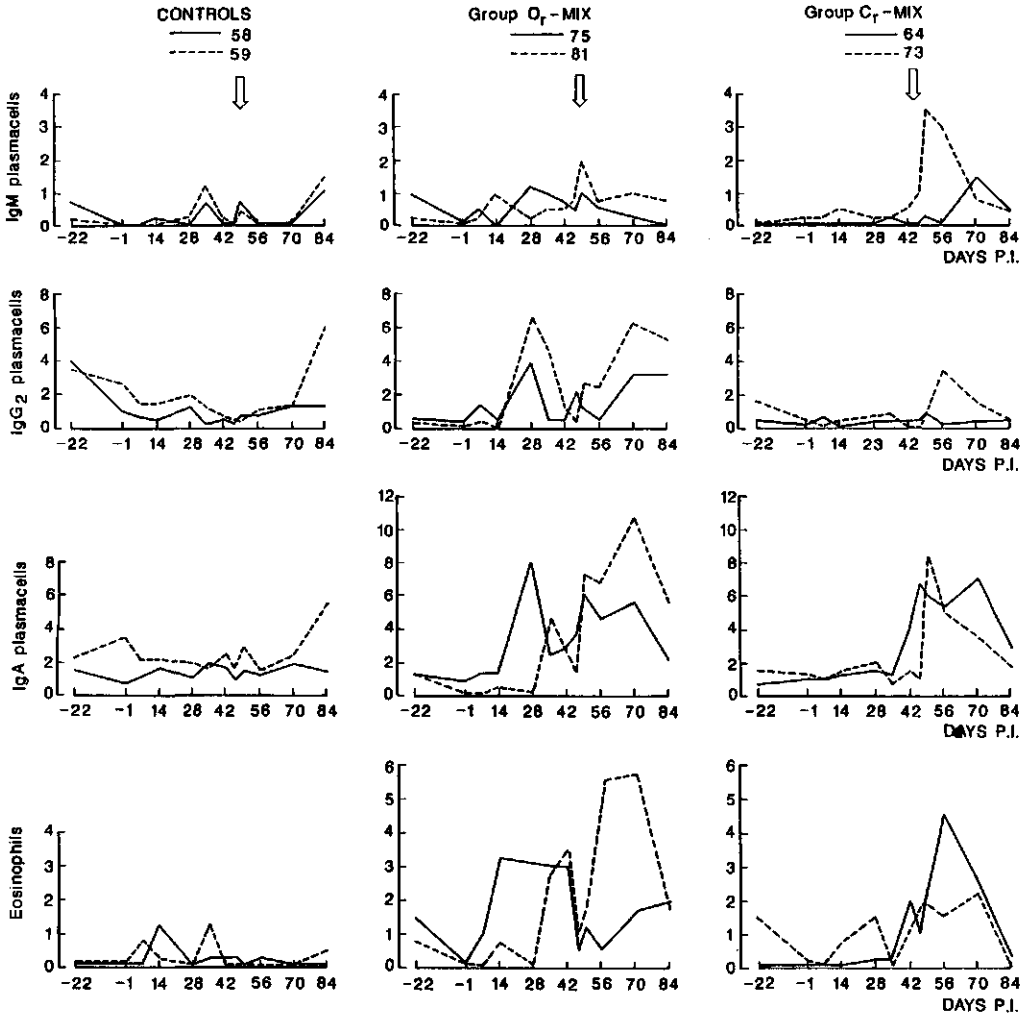

Fig. 31. Numbers of IgM-, IgG_2 and IgA plasma cells and eosinophils in tissue fragments of the abomasal mucosa.

Table 14. *Cooperia oncophora:* reduction percentages of parasitological parameters for separate and combined challenge groups when compared to primary controls. A negative sign means an increase instead of a reduction.

		Primarily infected groups of experiment:									
		1			2			3		4	
PARAMETER		C_{50}	C_{100}	C_{200}	O_{25}	O_{50}	O_{100}	Cr	Or	C_r	O_r
No. of F**	C_{100}	48	90	92	52	31	68	-	-	-539	18
	MIX	26	45	55	-36	-39	21	42	6	55	64
	Average	38	78	82	19	-2	50	-	-	-69	46
No. of M**	C_{100}	52	95	98	68	48	92	-	-	-2503	-1455
	MIX	53	79	76	-39	-29	20	54	5	73	91
	Average	53	90	93	33	18	75	-	-	-164	-16
Total number	C_{100}	50	91	95	56	37	72	-	-	-736	-252
	MIX	39	60	65	-37	-34	21	47	5	63	69
	AVERAGE	45	82	87	22	8	54	-	-	-76	-4
Length of F	C_{100}	8.6	15.3	16.3	3.4	10.2	2.2	-	-	-2.3	1.4
	MIX	9.6	7.7	8.2	0.4	-2.0	8.6	4.0	8.4	7.8	6.8
	AVERAGE	9.1	11.6	12.5	i*	i*	i*	-	-	2.8	4.3
Length of M	C_{100}	6.2	14.0	15.0	-0.3	5.3	2.2	-	-	-0.6	1.0
	MIX	10.5	10.1	11.0	2.8	-1.2	6.3	6.1	6.4	6.0	3.6
	Average	8.4	12.1	13.0	1.3	2.1	4.5	-	-	3.3	2.9
No. of ova/F	C_{100}	37	51	49	-10	-6	-2	-	-	-118	-228
	MIX	-31	-31	-30	-50	-28	-19	5	-6	2	-4
	Average	i*	i*	i*	-32	-18	-14	-	-	-39	-73

* Significant interactions were found between the main factors of the statistical model. Hence it is not allowed to combine both groups.

** F = females; M = males.

Table 15. *Osteragia ostertagi:* reduction percentages of parasitological parameters for separate and combined challenge groups when compared to primary controls. A negative sign means an increase instead of a reduction.

PARAMETER		Primarily infected groups of experiment: 1 C_{50}	C_{100}	C_{200}	2 O_{25}	O_{50}	O_{100}	3 Cr	Or	4 C_r	O_r
No. of F**	O_{100}	-35	-24	-10	-50	-25	-77	-	-	11	57
	MIX	26	16	-24	-38	-90	-48	5	31	3	33
	Average	0	-2	-17	-44	-54	-6	-	-	7	46
No. of M**	O_{100}	-26	-20	5	-3	17	-25	-	-	9	56
	MIX	23	9	-17	2	-5	6	1	35	2	37
	Average	2	-5	-5	-1	7	-15	-	-	5	48
Total number	O_{100}	-30	-22	-3	-22	0	-45	-	-	10	57
	MIX	24	12	-21	-13	-36	-21	-2	33	3	35
	Average	1	-4	-11	-17	-16	-33	-	-	7	47
Length of F	O_{100}	2.1	0.4	0.3	10.0	9.4	8.6	-	-	8.0	13.0
	MIX	3.0	4.7	4.1	5.9	11.5	10.0	7.8	11.2	4.2	7.3
	Average	2.6	2.5	2.2	8.0	10.4	9.3	-	-	6.2	10.2
Length of M	O_{100}	3.0	2.2	0.8	9.1	7.6	7.3	-	-	7.4	10.4
	MIX	3.2	5.2	3.2	5.6	8.7	7.7	5.9	10.0	2.7	7.0
	Average	3.0	3.7	2.0	7.4	8.1	7.5	-	-	5.1	8.7
No. of ova/F	O_{100}	10	2	-1	12	30	32	-	-	9	50
	MIX	26	19	15	-13	21	31	12	27	20	60
	Average	18	11	7	0	25	32	-	-	15	55
Flap score	O_{100}	-2.2	-10.1	-3.5	28	24	15	-	-	10	32
	MIX	2.7	13.2	8.2	15	28	20	22	32	19	5
	Average	0.3	1.7	2.4	21	26	18	-	-	i*	i*

* Significant interactions were found between the main factors of the statistical model. Hence it is not allowed to combine both groups.

** F = females; M = males.

5 DISCUSSION

This chapter is divided into four parts. In the first place some remarks will be made about the marked differences that were found between equally treated groups of the different experiments. Secondly, the value of the various parameters for measuring resistance in the homologous situation are discussed. In part 3 and 4 the interaction between *C. oncophora* and *O. ostertagi* and its possible consequences for the epidemiology will be dealt with.

5.1 COMPARISON OF EXPERIMENTS

Despite of the fact that all experiments were carried out under standardized conditions, rather large between experiment variations occurred when comparable groups are considered. For example, the mean egg output of groups challenged with $100*10^3$ *C. oncophora* larvae varied from 1,200 (exp. 4) to 3,600 (exp. 1). The percentage of take also differed considerably. In exp. 3 the recovery of *O. ostertagi* worms was about 40% while it amounted to 9% in exp. 4. These variations might be caused by factors such as: larval infectivity, 'quality' of the host (i.e. to which extent the host can meet nutritional and environmental requirements of the parasite), presence of disease or unknown factors. All these factors will be considered here briefly.

Systematic differences in larval infectivity may have been present, because different batches of larvae were used for each experiment. It was tried to standardize larval infectivity by storage at low temperature and by using less than three months old larvae. Weak infectivity of a batch of larvae is reflected in low worm burdens in all infected animals. Within experiments, differences between doses with regard to numbers of larvae and larval infectivity were minimal as a consequence of the dose preparation procedure. Therefore, the low recovery of *O. ostertagi* in exp. 4 is most likely due to a weak infectivity. However, the low percentage of take of *C. oncophora* in the same experiment cannot be explained by weak larval infectivity, because the variation in worm burden was considerable between individuals.

The quality of the host depends partly on its nutritional status. A good nutritional status may affect parasite populations in two ways. In the first place, a 'happy host, happy parasite' relationship is thought to exist. Snider *et al.* (1985) suggested that relatively high *O. ostertagi* and *T. axei* counts in calves are due to a good health and nutritional status. Such a good status, however, may also affect parasites negatively because the development of host resistance may be related positively to the plane of nutrition (Holmes, 1985). Liveweight and weight-gain differed considerably between the

experiments, but no consistent relation between parasitological or immunological parameters and the nutritional status could be demonstrated. The quality of the host is also determined by its genetic constitution. For example, the number of *C. oncophora* worms in experimentally infected calves differs significantly between half-sib groups (Albers, 1981). This is undoubtedly related to the fact that the immune response is controlled by genes of the major histocompatability complex (Wassom *et al.*, 1984; Wakelin, 1985). Between experiments no systematic differences in genetic make up of groups can be expected, but it is obvious with the limited number of animals per group that such differences might have been present by chance.

Presence of other diseases might also be of importance. In the present study very few clinical signs of disease were noticed. Only in exp. 1 and 2 most of the calves showed some nasal discharge, conjunctivitis and incidental coughing during a few weeks. Presumably these were symptoms of respiratory viral diseases, although the animals had been vaccinated against bovine respiratory syncytial virus (BRSV), infectious bovine rhinotracheitis virus (IBR) and parainfluenza virus (PI-3). The symptoms were never that severe that intervention was necessary. These unpleasant adventitious circumstances are not uncommon when susceptible calves are housed at high density. Snider *et al.* (1985) and Armour (pers. comm.) noticed the same.

Of course, significant differences between experiments are not desirable, but it is not absolutely necessary to have identical levels for a certain parameter to draw similar conclusions from two or more experiments. Large within-group variations, however, have insuperable consequences for the parameter involved as a suitable one for measuring resistance or interaction. When all the experiments of the present study are considered it appears that anomalies are found with regard to the mean faecal egg output of *C. oncophora* infected groups, the percentages of take of both *C. oncophora* and *O. ostertagi* infections and the sex-ratio of *O. ostertagi* in the primarily non-infected group of exp. 2.

5.2 VALUE OF PARAMETERS

In this study the presence of interaction between *C. oncophora* and *O. ostertagi* has been investigated in concurrent and sequential infections. As concurrent infections did not differ from mono-specific infections in the majority of the cases, emphasis is laid on the results of sequential infections. The value of parasitological and immunological parameters is determined by the answers to the following questions: (1) does the parameter give a clear and consistent reflection of the resistance of the host and (2) is the parameter related to other potential resistance parameters. Resistance is defined here as the negative effect that a previous exposure of the host has upon the challenge infec-

tion. These negative effects may result in a reduced worm burden, a lowered faecal egg count, stunted growth and a reduced fecundity. First, both questions will be answered for homologous situations on the basis of literature and the present experimental results. From the latter it was already seen that for some parameters there is a lot of controversy. Fortunately there are also parameters that show some degree of consistency. When a certain parameter does not give a measure of increased resistance in the homologous situation, it is reasonable not to expect that it measures some degree of resistance to the heterologous species.

5.2.1 Worm counts

In experimental infections this is the most frequently used parameter to express an animal's resistance. Host resistance may lead to reduced worm burdens in the intestine of calves (*C. oncophora:* Albers, 1981), rats (*N. brasiliensis:* Ogilvie, 1965) and mice (*T. spiralis:* Kennedy, 1980b). Resistance also leads to reduction of the abomasal worm burdens in calves (*O. ostertagi:* Michel *et al.*, 1973; Snider *et al.*, 1981) and sheep (*H. contortus:* Adams, 1983).

Worm burdens at a certain moment after (re)inoculation depend on: (1) the number of larvae administered, (2) the larval infectivity and (3) the number of worms lost. Loss of worm is either due to intraspecific competition for limited resources (nutrients, site) and/or immunological and physiological processes generated by the host (Anderson and Michel, 1977). In the present study no indication was found that worm loss as a consequence of crowding occurred, because worm numbers and parameters that measure parasite growth and development were positively related to each other. Therefore, loss of worms in our experiments was merely due to host regulated processes which result in expulsion of worms.

The rather large between-experiment variation with regard to the worm counts of both species and the large within-group variation of *C. oncophora* worm counts needs a closer examination. When within-group variations are small, but between-experiment variation is considerable, it is most likely that the latter is due to differences in larval infectivity. Thus it is thought that the low *O. ostertagi* worm counts in exp. 4 are due to poor quality of the larvae despite of the fact that larval culture and storage was performed under standardized conditions. The hypothesis of Snider *et al.* (1985), good health and nutritional status positively affects the percentage of larvae that develop into adults, does not hold for the *O. ostertagi* - calf model in the present experiments: calves of exp. 4 had a mean weight and weight-gain that was highest of all experiments but nevertheless a very low recovery was assessed. *C. oncophora* larvae had a more constant quality. In all the present experiments high individual worm counts were found, but large variations occurred between individuals. For example, in the $N\text{-}C_{100}$ group of

exp. 4 the small intestinal worm burden varied from 1,500 to 53,500. In the present study the number of larvae per dose and the larval infectivity was about equal for all animals within the same group. Therefore, differences in worm burdens of animals in the same group are due to variations in the proportion of worms that is lost. It is thought that these differences are due to the time of onset of expulsion, a factor which is known to be under genetic control (Wassom *et al.*, 1984; Wakelin, 1985) and thus individual differences may easily occur. The time of onset of expulsion can be obtained by sequential slaughtering, but because of the considerable individual variation, large numbers of animals are needed for drawing appropriate conclusions. Faecal egg counts may give some information about the moment of expulsion. Rapid expulsion, i.e. expulsion of the developing parasites within a few days after they have reached the predeliction site (Miller *et al.*, 1981; 1983), results in very low egg counts during the whole infection period. Expulsion during the patent period is characterized by a rapid decline of the EPG to very low levels. The moment of expulsion may be an important factor when necropsy is performed at a particular day after challenge. At that day some animals may have expelled their worms very recently, whereas equally treated others would have done so very soon. Thus individual differences in worm counts might be due to a difference of only a few days in the time of onset of expulsion.

- *C. oncophora* worm counts

From small intestinal worm counts it is concluded that homologous primary infections may reduce the number of *C. oncophora* worms after challenge, but this finding was not consistent because no reduction was found in exp. 4. Priming with a large single dose (exp. 1) is possibly more effective in reducing worm burdens than priming with repeated small doses (exp. 3). The sex-ratio changed to lower percentages of male worms when worm burdens were reduced. Presumably, male worms are more sensitive to the host response than female worms. This was also concluded by Albers (1981). The conclusion of this author, *C. oncophora* is more sensitive to this reponse than *C. surnabada*, is not confirmed in the present experiments.

As mentioned before, reductions in worm numbers may be due to expulsion of developing larvae or adult worms. Expulsion of worms is known to be affected by the immune response of the host. Expulsion of *N. brasiliensis* from the small intestine of rats only occurs when lymphocytes are present: irradiation, thymectomie and treatment with an anti-lymphocyte serum prevented expulsion (Keller and Keist, 1972). The process of expulsion could be restored by injection of normal mesenteric lymph node cells. The expulsion of *T. spiralis* is also affected in artificially T-cell depleted rats (Ruitenberg *et al.*, 1979) and congenitally athymic mice (Ruitenberg *et al.*, 1977). The immunosuppressive

drug dexamethasone results in an increased number of adult *H. contortus* worms in sheep on day 21 p.c.i. (Adams, 1982); it was shown that this is due to a suppression of responses acting against establishment of infection. In exp. 1 and 3 primary infections with *C. oncophora* resulted in elevated serum antibody titres at the moment when the challenge was carried out. These pre-challenge IgG titres were negatively related to the worm numbers at necropsy. This suggests strongly that antibodies are involved in the process which results in reduced worm burdens. According to Ogilvie and Jones (1971) rat IgG antibodies damage *N. brasiliensis* adult worms. This damage, combined with a subsequent reaction of sensitized lymphocytes, results in the expulsion of *N. brasiliensis* from the gut. Maybe pre-challenge anti-*C. oncophora* IgG also affects establishment or expulsion of *C. oncophora.*

- *O. ostertagi* worm counts

Abomasal worm counts after challenge were negatively affected by repeated priming (exp. 3 and 4). Reduced abomasal worm burdens after challenge were also noticed by Michel *et al.* (1973) and Snider *et al.* (1981). According to Michel *et al.* (1973) such reductions are due to a lowered establishment and not to an increased loss of adult worms. Single dose priming affected worm counts positively in exp. 2. Klesius *et al.* (1984) stated that *O. ostertagi* larval antigens have immunosuppressive capacities *in vitro* when lymphocytes of primed animals are used. Immunosuppression by larval stages might increase the proportion of larvae that establish and thus increase the adult worm burden. Adams (1982) induced immunosuppression chemically during the prepatent period of *H. contortus* in immune sheep and found that establishment increased 6-fold in treated animals. It is not likely, however, that immunosuppression acted positively on worm numbers in exp. 2, because other parameters that are known to be affected by the immune response (worm length, worm fertility, vulval flap development) were negatively influenced in the same group. Therefore, it is more likely that the higher numbers of worms in primed animals are due to a loss of worms in the primarily uninfected group rather than to an increased establishment in primed animals. The egg count curve of the $N\text{-}O_{100}$ group indicates that a loss of worms has taken place between day 19 and 21 p.c.i. At that moment a relatively large proportion of male worms is found in the mucosa when compared to the female population (Kloosterman and Armour, unpublished, 1987). Thus, when a loss of worms only occurred in the population that has already been emerged from the mucosa, it is possible that a change in sex-ratio occurred. However, the reason why this selective loss only took place in the primarily uninfected group of exp. 2 is not known.

When compared to *C. oncophora*, *O. ostertagi* worm counts are less sensitive to individual host influences. This might be due to a less dramatic expulsion which means that

O. ostertagi worms are not expelled all at once, but are lost slowlier. The question whether this is due to the parasitized site, the inflammatory reaction is not expressed in its most effective form by the abomasal wall, or to a relative insensitivity of *O. ostertagi* towards such a reaction, is difficult to answer. In any case, it is not due to a low immunogenicity of *O. ostertagi* worms, because antigen specific antibodies are found in the serum within four weeks after primary infection and local responses in the abomasal mucosa occur within 5 weeks after inoculation. However, there was no clear relation between the abomasal worm counts and the pre-challenge serum IgG titre counts.

- Conclusion:

From the present study it is concluded that the *C. oncophora* worm count is a rather weak parameter to indicate the previous experience to this nematode. Especially the fact that non-primed animals may expell *C. oncophora* very rapidly contributes to this conclusion. It is not clear whether *O. ostertagi* worm counts are adequate as a parameter measuring resistance or not. In the present study, reduction due to previous experience is not found when single dose priming was carried out. This was also found in the experiment of Kloosterman *et al.* (1984). Michel *et al.* (1973) measured substantial reductions in *O. ostertagi* worm burdens after repeated priming. This is confirmed in the present study. Possibly, the number of *O. ostertagi* worms is only reduced when repeated priming has been applied, i.e. when the host has been exposed to larval antigens for a longer time.

5.2.2 Faecal egg counts

This is a very commonly used parameter of resistance in field experiments because it is very easy to assess and it does not require slaughtering of the animal. Results from Coop *et al.* (1979), Borgsteede and Hendriks (1979) and Albers (1981) show that a large individual variation exists between the *C. oncophora* larval intake and the subsequent faecal egg output. During primary *C. oncophora* infections the faecal egg output reflects to some extent the inoculation level when group means are considered (Kloosterman *et al.*, 1974a; Albers, 1981). Egg counts resulting from *O. ostertagi* infections also appear to be weak indicators of the infection rate when the results of Michel (1969a, 1969b) are considered.

The EPG depends on three factors: (1) the size of the worm population, (2) the number of eggs released per female per unit of time and (3) the faeces production of the host. The third factor is often considered as constant, but in cases of diarrhoea or obstipation it may affect the EPG to some extent. A high EPG indicates that a large worm burden is present, but the opposite is not necessarily true, because the process of

egg production and release might be impaired. As was seen in the previous paragraph, the size of the worm population was a weak indicator of resistance and therefore the faecal egg output, which is related to this worm burden, is not expected to be a parameter that reflects the host resistance consistently.

- Faecal egg counts of *C. oncophora* populations

According to Albers (1981) a primary infection with $100*10^3$ *C. oncophora* larvae in 3 month old calves results in an egg count curve rising from day 16 p.i. till day 28 p.i. on which day a peak level of about 2,500 to 3,000 eggs per gram of faeces is reached. Egg counts decline rapidly after this peak. Rather large individual deviations from this basic pattern were noticed by the same author. During the primary infection of exp. 1 a relatively large proportion of animals showed an abnormal faecal egg count curve that was characterized by a temporal suppression one week after patency was reached. This might have been the reason that no relation between the faecal egg count and the inoculation dose was found. Albers (1981) stated, using the individual egg count as a criterion, that expulsion can take place as early as one week after patency has been reached and that the parasite population can compensate for worm loss by increasing the egg production per female worm. Thus a drop in the egg output to almost zero might be followed by a slight increase. Whether in exp. 1 a large proportion of the animals expelled their worms very early or not is not known because no necropsies were carried out during the primary infection period, and thus no worm burdens could be assessed.

Repeated infection with small doses of larvae gave another pattern of the faecal egg output. This curve is characterized by a gradual increase until a maximum level is reached on about day 26 p.i. After that day hardly any decline was noticed. This pattern resembles very much the pattern that was found when low single dose infections of $20*10^3$ *C. oncophora* larvae were applied (Borgsteede and Hendriks, 1979; Albers, 1981). Possibly the treshold level of antigenic stimulation that provokes an operative host reaction (Dineen, 1963) is not reached in such low dose infections. In case of repeated infections with small doses this treshold level might be reached later, because the number of adult worms gradually increases with the number of doses administered. The rather stable EPG may also arise from a continuous turn-over: older worms are constantly replaced by young, more fertile ones as was found to occur for *O. ostertagi* (Michel, 1970).

After homologous challenge reduced faecal egg counts were found. Reduction of faecal egg output is generally believed to be under the control of the host's immune response because immunosuppression by administration of corticosteroids increase the faecal egg output (Michel and Sinclair, 1969; Jansen, 1973; Kloosterman *et al.*, 1974b). As was

mentioned above, a reduced EPG results either from a reduced worm burden or worm fecundity. Both the numbers of *C. oncophora* worms and the numbers of eggs *in utero*, as a parameter indicating worm fecundity, were reduced on day 33 p.c.i. so it is most likely that the reduction in egg output is a combination of factors as was found for the larval output of *T. spiralis* in mice (Kennedy, 1980b). Homologous challenge infections in exp. 4 did not result in reduced egg counts, but this is due to the early expulsion of worms in the $N\text{-}C_{100}$ group, which results in very low egg counts.

- Faecal egg counts of *O. ostertagi* populations

According to Michel (1969b) the typical *O. ostertagi* egg count curve is characterized by an early peak on about day 25 p.i. followed by a logarithmic decline. The faecal egg output of the primary *O. ostertagi* infections in exp. 2 corresponded fairly well to this pattern. The peak egg count occurred somewhat earlier. In exp. 2 the egg count curves after primary inoculation were not affected by the number of larvae per dose. This indicates that the *O. ostertagi* egg count is independent from the number of worms present, because it is reasonable to state that a larger dose results in a larger worm burden. Michel (1970) found that the worm burden was positively related to the inoculation level, although the proportion of larvae that established was lower when the dose increased. The $N\text{-}O_{100}$ group of exp. 4 either showed, in spite of the very low worm burdens, no abnormal low egg count when compared to the other experiments. This is in agreement with the results of Michel (1969a, 1969b, 1969c). According to Michel the egg output pattern of an *O. ostertagi* population follows a stereotyped pattern independent from the type of infection (natural infections, experimental single or repeated infections), the inoculation dose and the numbers of worms present in the abomasum. Michel concluded that the egg output of an *O. ostertagi* population was restricted to a limit which depended on the previous egg production of the population. The mediator of this self-regulating mechanism is still not known. From results of Michel and the present study it can be concluded that the faecal egg counts of *O. ostertagi* infected animals have no predictive value for the abomasal worm burdens, although within the experiments a positive relationship was found between the actual faecal egg counts and the worm counts.

Homologous challenge infection in exp. 2 did not show any reduction in the faecal egg counts when compared to the primarily uninfected group. The early decrease and subsequent increase in the curve of the latter might be due to a selective expulsion of females after day 19 p.i., because abnormal sex-ratios were found at necropsy. If this has indeed happened, the rise in the faecal egg output after day 23 p.c.i. supports the hypothesis of Michel that the egg production of an *O. ostertagi* population is self-regulating and independent from the number of worms present. In exp. 4 repeated

priming resulted in reduced egg counts after homologous challenge. According to Michel and Sinclair (1969) the host's immune response is involved in the reduction of the faecal egg count. They achieved very high egg counts after cortisone treatment. Egg counts of treated and control groups differed from day 30 p.i. onwards, the moment when in the present study, the host response is reflected in increased serum IgG titre counts. Snider *et al.* (1981) also found that homologous priming reduced faecal egg output after challenge.

- Conclusion:

The faecal egg output of *C. oncophora* infections reflects to some extent the previous experience of the host to the same species, at least, when group means are considered. From the present experiments it is difficult to draw a conclusion about the suitability of *O. ostertagi* egg counts as a parameter of resistance.

5.2.3 Worm length

This parameter is sometimes used to assess the growth and development of a parasite population. Host resistance leads to a reduced length of *C. oncophora* (Albers, 1981) and *O. ostertagi* (Michel *et al.*, 1978b) worms. These reductions might be due to (1) an inhibited growth, (2) a selective expulsion of relative large worms or (3) a shrinkage of worms during the infection. The latter is thought to occur in the *S. ratti* - rat model (Moqbel and Denham, 1977). The mean length of adult *S. ratti* worms decreased from 2.8 mm on day 11 p.i. to 0.9 mm on day 26 p.i. during the primary infection. However, as reduction in length was found to start at the same moment that worm numbers decreased, it might also result from selective expulsion of large worms. This is only possible when short worms are present during the whole infection period, but unfortunately Moqbel and Denham (1977) reported no within-rat standard deviations. Michel *et al.* (1978b) noticed a decrease in length of about 8% of *O. ostertagi* worms at the moment when most of the worms were expelled. Although this was most likely due to a more rapid loss of large worms, the possibility of shrinkage could not entirely be excluded. Albers (1981) measured *C. oncophora* female lengths after primary and challenge infections. During the challenge infections the worm length was significantly decreased when compared to the primary infection, but within the challenge period no reduction in the worm length was noticed between day 18 and day 39 p.c.i., although the worm numbers decreased from 65,000 to 20,000. Thus for *C. oncophora* it seems likely that a reduction in length is caused by an inhibited growth rather than to a selective expulsion of large worms or a shrinkage of worms.

- Length of *C. oncophora* worms

Kloosterman *et al.* (1984) found reductions in length of about 7.5% for this species when both primary and challenge infections were carried out with a single dose of $100*10^3$ larvae. In the present experiments the reductions that were found are similar or even greater. Albers (1981) noticed a threefold increase in the coefficient of variation when single dose priming was followed by a challenge with repeated doses. When the latter was carried out with a single dose it increased to a much lesser extent. In case of challenge infections with repeated doses the later inoculated larvae may develop less well than the first ones, resulting in an increased within-calf standard deviation of worm length. This is also reflected in the dramatically increased number of fourth-stage larvae that is found at necropsy after challenge with repeated doses (Albers, 1981). In the present experiments the mean coefficients of variation were quite similar for primarily infected and non-infected groups, while the within-calf standard deviations of female worm length were lower for primarily infected animals. From these parameters it can be concluded that challenge infection with a single dose results in smaller worms, but the percentage of extremely short worms is not increased. As the worm length is positively related to the worm numbers, this inhibited growth is not due to intraspecific interactions (crowding). It is more likely that the observed stunted growth is immunologically mediated, because the worm length is negatively correlated with serum antibody titres. Comparable correlations between these two parameters were found by Kloosterman *et al.* (1984). The mechanism by which the immune response induces stunted growth is not known. As the antibody titre was measured by using secretory/excretory products of living worms, it is possible that the immune response blocks or inhibits the activity of exo-enzymes that are necessary for normal development. Very little is known about enzymes excreted by nematodes. Some attention is paid to acetylcholinesterase (AChE) (Ogilvie *et al.*, 1973; Rothwell *et al.*, 1976). This enzyme might act as a biological 'holdfast' in the maintenance behaviour of the parasite (Miller, 1984). AChE modulates the host's cholinergic nerve transmission and thereby it interferes with Goblet cell secretion or with the smooth muscle contractions in the gastro-intestinal tract. AChE of *O. ostertagi* was found to be immunogenic by Axelsson and Luthman (1983).

- Length of *O. ostertagi* worms

According to Michel *et al.* (1971) the reduction in length of *O. ostertagi* worms is mainly due to two factors: initial worm burden and time. Michel *et al.* (1971) found an inverse relationship between the initial worm burden and the worm length when measured on day 30 p.i. Such a relation was not found in the present experiments when primarily uninfected groups, necropsied on day 33 p.c.i., are considered. The number of abomasal worms recovered from animals of these groups significantly differed between

experiments, but this was neither reflected in significantly affected length nor in significant negative correlations between both parameters. However, results of Michel *et al.* (1971) might be criticised, because the infection rate (i.e. the number of larvae administered per day - varying from 200 to 1,600 in Michel's experiment) might affect development, resulting in a gradual increase in young, and thus small, stages when the infection rate increases. In fact this has happened in that particular experiment, because the percentages of early and late L_4 stages increased with the inoculation dose (Michel, 1970). Hence, it is very likely that the number of young, relatively small, adults was increased, resulting in a decreased mean length when compared to repeated low dose infections. Therefore, it is rather difficult to draw conclusions about length of adult worms in populations that are not uniform of age. From the present experiments it can be concluded that no density dependent decrease in worm length occurs before day 33 p.c.i. when worm burdens are less than $40*10^3$ individuals (the largest mean number of abomasal worms recovered in this study).

A second factor thought to be of importance to the reduction of worm length is time. Michel *et al.* (1971) showed that mean worm length decreases with time when daily infections are applied. They stated that a constant turn-over occurs, i.e. older worms are constantly replaced by new incoming larvae, but the latter do not develop to the same length as the old, expelled worms, presumably due to host mediated effects. Michel *et al.* (1978b) also demonstrated that after single dose infections the mean length of *O. ostertagi* worms decreases with time. They measured a reduction in length of about 8% on day 50 p.i. when compared to length on day 20 p.i.; it was suggested that this reduction was due to a more rapid loss of larger worms or a shrinkage of worms. This implies, however, that the within-calf standard deviation decreases when worms are lost. As the reduction in worm burdens of homologously infected animals of exp. 3 and 4 is not reflected in a decreased within-calf standard deviation of length, no selective loss of large worms occurred in the present study and thus the time effect of Michel *et al.* (1978b) is not effective before day 33 p.c.i. Mechanisms involved in induction of inhibited growth must have been present in an early stage of the secondary infection, because maturity of the whole population is reached soon after patency when single infections are applied (Michel *et al.*, 1978b; Albers, 1981). As the pre-challenge homologous IgG titre counts are negatively related to the worm length, it is very likely that stunted growth is immunologically mediated, at least partly.

- Conclusion:

For both *C. oncophora* and *O. ostertagi* it appears that the worm length reflects very consistently the previous experience with the same species and therefore it is a very suitable parameter of resistance.

5.2.4 Number of eggs per female

This parameter is generally considered as a measure of the individual egg production of the worms, although very few authors have actually checked this assumption. The number of eggs *in utero* depends on (1) the number of eggs produced per unit of time and (2) the number of eggs released per unit of time.

- Number of eggs in the uterus of *C. oncophora* females

In general, this parameter is positively related to the number of *C. oncophora* females found at necropsy and the length of those females. Thus crowding effects did not occur. On the contrary, these findings show that when a larger percentage of the dose is established, the length and the fecundity of individual worms is also increased. These positive relationships possibly are caused by intrinsic properties of the host: a more suitable calf carries a larger, better developed and more productive worm population than other, less suitable calves. Homologous priming was only of significant importance in exp. 1 and the type of challenge infection only in exp. 2. This is not surprising when it is considered that a large variation between worms within the same calf (expressed as the within-calf standard deviation) is measured. Kloosterman *et al.* (1984) also found that the fecundity of *C. oncophora* was not significantly affected by previous exposure of the host to the same species.

- Number of eggs in the uterus of *O. ostertagi* females

When compared to *C. oncophora*, the *O. ostertagi* females contained less eggs *in utero*. The mean within-calf standard deviations and the mean coefficients of variation were lower. This is reflected in a much lower faecal egg count of calves infected with *O. ostertagi* when compared to *C. oncophora*.

Decreased fecundity was found after homologous challenge. This resulted from a reduction of the number of highly productive females as can be concluded from the frequency distributions. According to Michel (1963) a decreased number of eggs *in utero* is due to interference with the process of ovulation. An inhibited ovulation might be regulated by density dependent mechanisms or by host-mediated factors. Michel (1969b) found an inverse relationship between the infection rate - and thus initial worm burden - and the female egg content: at higher infection rates the females contained less eggs than their potential maximum. However, this relationship was only found in the beginning of that experiment (necropsy on day 28 p.i.). In the present experiments no negative correlations between worm numbers and number of eggs *in utero* were assessed. Between experiment variations in abomasal worm burdens do not either imply such a relation. The *O. ostertagi* females in exp. 4, present in low numbers, showed about equal numbers of eggs *in utero* when compared to the females of the other experiments. The

fact that the faecal egg count of the $N\text{-}O_{100}$ group at the end of exp. 4 is, in spite of the low numbers of females, higher than the EPG of the $N\text{-}O_{100}$ groups of exp. 1 and 2 suggests that in exp. 4 the process of egg production and release is much faster. Therefore, egg production is better reflected by the number of eggs released per unit of time than by the number of eggs in the uterus, but the first one is difficult to determine, because egg production readily stops when worms are kept *in vitro* (unpublished results).

The positive relation between the length and the number of eggs per female suggests that the worms contain few eggs merely because they are small. However, when corrected for group differences the positive relation has almost disappeared. Michel (1963) found no relation between the length and the fecundity of the *O. ostertagi* worms of the same calf. Thus within-calves and within-groups there is no obvious relation between the fecundity and the length present. Therefore, it is likely that between group differences in growth and fecundity sprang from an identical cause, presumably host-mediated factors. Correlations between serum IgG titres and number of eggs per female do not suggest that the host response is involved, but one should be aware of the fact that the immune response directed to helminths acts very locally and that IgG is only one of the immunological factors involved. Michel and Sinclair (1969) showed that immunosuppression increased the faecal egg count eight- to ten-fold and the number of eggs *in utero* two-fold. As in that experiment the numbers of female worms were slightly increased and the faeces production of calves slightly decreased by cortisone treatment, the egg laying per female must have been increased at least fivefold. Cortisone treatment of rats infected with *N. brasiliensis* prevented the usual inhibition of ovulation (Ogilvie, 1965). From these results it is clear that the host's immune response is involved in regulating the egg output of a parasite population, but serum IgG titre counts do not reflect this response adequately in the *O. ostertagi* - calf model.

- Conclusion:

The fecundity of *C. oncophora* worm populations is not consistently affected by the previous experience of the host to the same species and therefore it can not be regarded as a suitable paramater of resistance. Quite the contrary is true for *O. ostertagi*. Homologous priming significantly affected the number of eggs *in utero* in all experiments of the present study. These reductions were not related to the serum IgG titre count, however. The reduced fecundity of *O. ostertagi* did not necessarily lead to a reduced faecal egg output which indicates that the resultant of egg production and release, expressed by the number of eggs *in utero*, is stabilized at a lower value in the *O. ostertagi* worms of primed animals.

5.2.5 Vulval flap development

Michel *et al.* (1972a) suggested that the vulval flap development of *O. ostertagi* females was negatively affected by host-mediated factors. After homologous challenge they found a threefold increased percentage of females with reduced flaps when compared to primary infections and the proportion of worms with reduced flaps did not change appreciably after day 24 p.c.i. until the end of the experiment (day 62 p.c.i.). As a loss of worms did not occur before day 24 p.c.i., this reduction was not due to the selective loss of worms with fully developed flaps (Michel *et al.*, 1972a). The use of an immunosuppressive drug significantly increases the proportion of worms with well developed flaps (Michel and Sinclair, 1969). Thus it is very likely that vulval flap development is affected by the immune response of the host. Results of the present experiments are in agreement with the results of Michel *et al.* (1972a). Significant reductions of vulval flap scores occurred when the animals had previously experienced *O. ostertagi*. Michel *et al.* (1972a) found no relationship between flap scores and worm length, but in the present experiments they were strongly positively related, even within groups. Both parameters are possibly affected by the same factor(s). As mentioned for the *O. ostertagi* worm length, the factors that influence growth and development must have been effective early in the infection and again the relation of the flap score with the pre-challenge IgG titre count suggests that this influence is immunologically mediated.

- Conclusion:

Previous experience of *O. ostertagi* resulted in a reduced vulval flap development and because of its consistency it may be regarded as a parameter that indicates a degree of resistance. The fact that it is related to pre-challenge IgG titres suggests that the host's immune response is involved.

5.2.6 Serum antibody titre counts

The SE-antigens used did not show a total genus specificity. After single dose priming no increase of the heterologous titre count was found, but when repeated priming was applied, the heterologous titre count increased. Therefore, these cross-reacting antibodies might be elicited by the antigens of larval origin which are continuously supplied when repeated inoculations are carried out. Keus *et al.* (1981) showed that larval antigens were not genus specific and thus it is possible that antibodies raised by such antigens do not show genus specificity either.

Antigen-specific serum IgG titre counts were significantly increased already on day 28 p.i. As SE-antigens of adult worms were used, the host was exposed to those antigens for only ten days unless these antigens are not stage- specific and were also presented by larval stages. Gamble *et al.* (1983) used larval SE-antigens of *T. spiralis* and noticed

an increased antigen-specific serum IgG level in pigs ten days (i.e. on day 15 p.i.) after worms reached maturity. In the experiment of Gamble *et al.* (1983) the kinetics of IgG antibodies depended on the inoculation dose: when low dose infections were applied, it took about 5 weeks before positive animals could be detected. Concerning *C. oncophora*, the increase of the titre count was not related to the inoculation dose. As no necropsies were carried out during the primary infection of exp. 1, it is not known whether there was a positive relation between the inoculation dose and the size of the resulting worm population. The primarily non-infected groups of exp. 1 and 4 showed large differences in worm numbers, but these differences were not reflected in significant correlations between worm numbers and titre counts. Therefore, the titre count is independent from the amount of antigen (= worm numbers) which is present during the first 30 days after inoculation.

Quite the contrary is true for *O. ostertagi*. The O-TC on day 41 p.i. of exp. 2 increased when the inoculation dose was larger. According to Michel (1970) and Anderson and Michel (1977) the number of *O. ostertagi* worms present depends on the number of larvae administered. When the primarily non-infected groups of all four experiments, which showed a large variation in worm numbers, are considered, it appears that a low percentage of take results in a low titre count, thus indicating that, at least between day 30 and 40 after infection, anti-*O. ostertagi* titre counts are positively affected by the amount of antigen present.

Antibodies raised by the primary infections were negatively related to the parasitological parameters after challenge which indicates that the immune response is involved in the resistance to reinfection.

- Conclusion:

It is very likely that IgG antibody titre counts reflect a process which influences parasitological parameters. Especially the fact that the amount of pre-challenge antibody is negatively related to these parameters suggests this very strongly.

5.2.7 Local responses

Two aspects of the results of exp. 5 will be considered: first, the nature of the local responses and second, the meaning of these responses to resistance and interactions.

The repeated primary infections with either *C. oncophora* or *O. ostertagi* resulted in increased numbers of eosinophils and plasma cells, except that no IgM plasma cells were found in the abomasal mucosa. The plasma cells were not checked on antigen specificity. This means that these cells might originate from other infections in the intestinal tract. In fact, this was the reason for keeping two calves uninfected during the whole experiment. As control calves showed no local responses, with the exception of a small IgG_2

peak, it is reasonable to state that the responses measured in infected animals are due to the parasitic infection.

The absence of a clear IgG_1 plasma cell and mast cell response is remarkable, because Ogilvie and Love (1974) found that IgG_1 is a major component in the expulsion of *N. brasiliensis* from the intestine of rats. Adams *et al.* (1980) found an increased number of IgG_1 containing plasma cells in sheep infected with the intestinal nematode *T. colubriformis* in sheep whilst the numbers of IgG_2 plasma cells were not affected. Mast cells might be important for an effective expulsion of *T. spiralis* from mice (Larsh and Race, 1975) although mast cell deficient animals can expel quite normally this nematode (Uber *et al.*, 1980). In exp. 5 the number of IgM plasma cells in the small intestinal mucosa was increased within two weeks after the first inoculation, but returned very quickly to normal levels. This is in agreement with the generally accepted view that IgM acts as a 'first line defence' and thus occurs early in the infection. The numbers of IgG_2- and IgA plasma cells were increased in the fourth week of the primary infection whilst increased numbers of eosinophils were found one week later. This lapse of time between the primary inoculation and the observation of a local response is in agreement with the results of Smith *et al.* (1983a). They did not find an obvious IgA plasma cell or eosinophil response in gastric lymph during the first three weeks after primary inoculation of sheep with $50*10^3$ *O. circumcincta* larvae. The two calves that were primarily infected with *C. oncophora* did not show equal responses. The animal that showed a weak IgM response, possibly compensated for this by an early IgG_2 response. These differences in local response were not reflected in different egg count curves. Smith *et al.* (1983a) also found large individual variations in degree and timing of the local response, but these variations did not affect the worm burdens.

Anthelmintic treatment was very efficient in decreasing the number of IgA plasmacells and eosinophils. This is another strong indication that these cells were increased because of the antigenic stimulus of the parasites.

The responses after challenge were about equal to the primary responses, except that they occurred earlier. Some cell types increased within one week after the challenge dose was administered. This rapid response might be due to three factors: the age of the animals, the inoculation dose or the effect of the primary infection. The calves were primarily infected when they were four months old and therefore, it is not likely that these animals were not fully immunocompetent at that age whilst they were fully competent six weeks later. The type of infection, repeated infection with small doses *versus large* single doses, may have been of importance. A large dose presents a relative large amount of antigenic material to the animal when compared to a small dose and this may provoke a more rapidly occurring immune response. The third factor that might explain the rapid increase in immunocompetent cells after challenge emerges from the memory

aspect of the immune response. This memory is generally believed to be responsible for the 'booster' effects that are found during secondary infections. Smith *et al.* (1983b) found increased numbers of IgA plasma cells in the gastric lymph within 5 days after challenge of immune sheep with $50*10^3$ *O. circumcincta* larvae, but the percentage of eosinophils was not increased. Challenge of immune sheep with $15*10^3$ *T. colubriformis* larvae also showed increased numbers of IgA containing cells within 5 days in the intestinal lymph (Adams *et al.*, 1980). In exp. 5, however, no discrimination can be made between the effects due to the type of infection and the effects due to priming because of the experimental design.

The importance of the local responses to the host resistance is not exactly known. According to Befus and Podesta (1976) IgA might directly act upon the parasites whilst antibodies of other classes act indirectly by recruiting or stimulating other components of the immune system. Antibodies might interfere with exo-enzymes released by adult worms. These enzymes might be necessary for worm nutrition or maintenance behaviour (Miller, 1984). Eosinophils, attracted to the infection site by chemotactic factors released by the parasites themselves or by mast cells, are capable of killing intestinal parasites *in vitro* (McLaren *et al.*, 1978). Eosinophils produce subtances (major basic protein, hydrogen peroxide) that are toxic to larvae of *Schistosoma mansoni* and *Trichinella spiralis* (Butterworth *et al.*, 1978; Bass and Szejda, 1979; Wassom and Gleich, 1979).

From the faecal egg counts it can be concluded that the establishment in the *C. oncophora* primed groups is low after challenge. As low faecal egg counts might also occur in non-primed animals, see exp. 1, it is not clear whether the rapid occurring immune response was responsible for the low faecal egg counts or not, the results very strongly suggest this. Smith *et al.* (1983b) stated that immunity to *O. circumcincta* in sheep, negatively affected the establishment and the development of the parasites, measured by worm length. According to them IgA might be of significant importance in mediating these effects of resistance (Smith *et al.*, 1984). Charley-Poulain *et al.* (1984) observed a close temporal relationship between the amount of local IgA antibodies in the abomasal mucosa of immune sheep and the self-cure reaction of those sheep in *H. contortus* infections. Thus there are some indications that the local immune response is a major factor in the occurrence of resistance to challenge infections. The question how the local response affects the heterologous species in sequential infections of *C. oncophora* and *O. ostertagi* is not answered by this experiment. Primed animals showed an early response of IgA plasma cells in the organ that was parasite-free during the primary infection. This response, however, might be caused by an effect of the inoculation dose. Results of Smith *et al.* (1983a) indicate that large doses of *O. circumcincta* larvae in naieve sheep did not show marked responses within three weeks after inoculation. If

the early occurrence of the immune response is due to a heterologous primary, it is rather strange that it only affects parameters that indicate growth and development of the worms, while worm numbers and fecundity are not affected.

5.2.8 General conclusion about the value of the parameters

In the present experiments it is shown that only parameters which indicate the growth and the development of worms, and worm fertility for *O. ostertagi*, consistently express the previous exposure of the host to the same species. The other parameters, worm counts, faecal egg counts and *C. oncophora* worm fertility, were affected by some factors which could not easily be controlled and this renders them unsuitable as parameters of resistance. The fact that the growth and the development of parasites are negatively affected by the previous exposure of the host to the same species indicates that the micro-environment is less optimal during the challenge infection. This might originate from the primary infection in two ways: either the environment is not fully restored at the moment challenge is carried out or a process with a memory mechanism is involved. Because of this memory, the host's immune response may be responsible for the resistance that is found after challenge. One of the effects of this response may consist of blocking enzymes that are necessary for worm nutrition. The fact that secretory-excretory products appear to be immunogenic and that antibodies directed towards these antigens are negatively related to the parameters which indicate growth and development, are in favour of this thought.

5.3 INTERACTION

Interaction between parasite populations that have different habitats is thought to be mediated via physiological and/or immunological processes of the host. In the present experiments a number of parasitological and immunological parameters were used to study the interaction between *C. oncophora* and *O. ostertagi*. Interaction was quantified by using an experimental set-up that made it possible to compare primary infections with heterologous reinfections. Homologous reinfections were included just to have a standard of comparison for effects found after sequential heterologous reinfections.

In the previous paragraph it was shown that parameters which indicate growth, development or fecundity (*O. ostertagi* only) are suitable parameters of resistance. The other parasitological parameters did not indicate resistance in the homologous situation and therefore, it is hard to expect that they will indicate a degree of resistance that is elicited by other species. The results of the present experiments show indeed that worm counts, faecal egg counts and worm fecundity (*C. oncophora* only) were not affected by the previous exposure of the host to the heterologous species. This is in contrast with

the results of Kloosterman *et al.* (1984) who found reduced worm burdens after challenge of heterologously primed animals. In the present study the parameters that indicated resistance to the homologous species also consistently reflected heterologous resistance. As was found by Kloosterman *et al.* (1984) heterologous priming negatively affected the lengths of *C. oncophora* and *O. ostertagi*. Reduction of length after heterologous priming has also been found in other host-parasite models. The length of *Fasciola hepatica* flukes is negatively affected by priming with *Schistosoma mansoni* in rats (El-Azazy, 1985). Moqbel and Wakelin (1979) found a mutual negative effect of heterologous priming in the rat - *T. spiralis* - *S. ratti* model with regard to this parameter. Kloosterman *et al.* (1984) suggested that interactions could be mediated by cross-reacting antibodies, because such antibodies were found by Keus *et al.* (1981) in the ELISA-test. The negative relation between worm lengths and the heterologous antibody titres implies that the immune response of the host is involved. In the heterologous situation the levels of these parameters were, in general, intermediate between those of the primary non-infected group and the homologously primed group.

Interaction between parasite populations may occur in a direct or an indirect way. When parasites directly act upon each other they must be present in the same host at the same time. Direct influences between *C. oncophora* and *O. ostertagi* are very unlikely, because both species do not compete for food or habitat and nothing is known about the production of toxic substances that are detrimental to other species. Indirect interactions might also occur during concurrent infections. This type of interaction is due to interference with physiological or immunological processes of the host. Physiologically mediated influences of *O. ostertagi* on *C. oncophora* were not inconceivable, because *O. ostertagi* has a marked effect on the abomasal contents. An *O. ostertagi* population of about 15,000 adult worms is capable of increasing the pH of abomasal fluid to almost neutral levels (McKellar *et al.*, 1986). Such an altered pH might have an adverse effect on the small intestinal micro-environment as was shown to occur in calves infected with the abomasal nematode *T. axei* (Ross *et al.*, 1970) and in sheep infected with *H. contortus* (Mapes and Coop, 1970). Immunosuppression is one of the mechanisms by which parasites can evade the host response. When one of both species is acting immunosuppressively during concurrent infections, positive interactions might occur. Suppression of bovine lymphocyte proliferation is known to occur *in vitro* by products of *O. ostertagi* (Klesius *et al.*, 1984) and *Oesophagostomum radiatum* (Gasbarre *et al.*, 1985). Suppressive capacities of *C. oncophora* have not been reported. As no positive interaction was found in the present study, immunosuppressive capacities, if present *in vivo*, were not expressed. Interaction due to an immunologically mediated inflammatory reaction, effective in expulsion of worms (Larsh and Race, 1975), might also occur during concurrent infections, resulting in a concurrent expulsion of all para-

site species present in the gastro-intestinal tract (Stewart, 1955, Kennedy, 1980b). However, in the present experiments concurrent infections were very rarely of significant importance and therefore, the above mentioned mechanisms of interaction have little value in explaining influences that *C. oncophora* and *O. ostertagi* have upon each other.

The fact that interaction occurred in a sequential infection model strongly suggests that a process with a memory mechanism is involved. Cross- reacting antibodies and the fact that the local responses occur earlier when animals were primed with the heterologous species imply a possible role for an immunologically mediated interaction. The exact mechanism of this interaction is not known. This, however, is not surprising when it is considered that host responses against nematodes are very complex and are expressed locally. This complex and local character needs sophisticated techniques to elucidate the way the host response act on gastro-intestinal nematodes.

Thus it can be concluded that interaction between *C. oncophora* and *O. ostertagi* does occur, although the most important parameters, worm numbers and faecal egg output, are not affected.

5.4 INTERACTION AND ITS CONSEQUENCES FOR THE EPIDEMIOLOGY

When interaction between nematode species leads to reduced worm burdens or reduced faecal egg output, they are of epidemiological importance. In lambs such an interaction was found between *O. circumcincta* and *T. vitrinus* (Jackson, in press), between *H. contortus* and *N. battus* (Mapes and Coop, 1971) and between *H. contortus, O. circumcincta, T. axei* and *T. colubriformis* (Stewart, 1955). In calves a mutual negative influence between *C. oncophora* and *O. ostertagi* was found by Kloosterman *et al.* (1984) with regard to worm numbers. These reduced worm numbers, however, did not result in a reduced faecal egg output. In the present study, being a follow-up of the experiment of Klooosterman *et al.* (1984), no evidence was found that interaction between *C. oncophora* and *O. ostertagi* influences parastiological parameters that are important for the epidemiology and thus it is concluded that the interaction between both species is of no importance from the epidemiological point of view. However, when parameters indicating growth and development of a parasite population are being used in the *C. oncophora* - *O. ostertagi* - calf model, one should consider that these parameters are negatively affected by a previous exposure of the host to the heterologous species.

6 SUMMARY

In this study the presence of interaction between *Cooperia oncophora* and *Ostertagia ostertagi*, nematodes which parasitize the small intestine and the abomasum of cattle, respectively, has been investigated. Interaction is of epidemiological importance when it leads to a reduced worm burden or a lowered faecal egg output. As there were some indications that interaction between *C. oncophora* and *O. ostertagi* is immunologically, mediated experiments were carried out in which calves were given some degree of immunity by inoculation with one of both species. By means of challenge infections it was examined whether this immunity affected the heterologous species or not. The effect of concurrent infections was also investigated.

During the experiments the course of infection was monitored by faecal egg counts. Other parasitological parameters, worm numbers, worm length, number of eggs *in utero* (indicating the fecundity and vulval flap development of *O. ostertagi* females, were determined *post mortem*. By making use of the Enzyme-Linked-ImmunoSorbent Assay (ELISA), the relative amount of serum IgG was measured to examine whether the interaction is immunologically mediated or not. Additionaly, the numbers of some effector cells were counted in tissue fragments of the small intestinal and abomasal mucosa.

Experiment 1 concerned 48 calves that were divided into 4 groups of 12 calves each. One group was kept as non-infected controls whilst the other groups received a single dose of $50*10^3$, $100*10^3$ or $200*10^3$ *C. oncophora* larvae, respectively. These primary infections were terminated by anthelmintic treatment on day 35 p.i. One week later the 4 groups were split into 3 subgroups of 4 animals each. These subgroups were infected with $100*10^3$ *C. oncophora* larvae, $100*10^3$ *O. ostertagi* larvae or a mixture of both doses, respectively. All calves were slaughtered on day 33 *post challenge* infection for *post mortem* examinations. The aim of exp. 1 was to investigate whether the acquired immunity against *C. oncophora* influenced *O. ostertagi* or not and, if so, whether this influence depended on the primary inoculation level or not. From the results of exp. 1 could be concluded that some degree of immunity was present against *C. oncophora*. This could be demonstrated by the reduced worm burdens, worm fertility and worm length in primarily infected groups when compared to primary controls. The negative relation between serum IgG titre counts and the parasitological parameters indicated that the host's immune response was involved. This immunity against *C. oncophora* however, had marginal affects on a subsequent *O. ostertagi* infection. Only the length of the abomasal worms was significantly reduced by a previous *C. oncophora* infection. This length was also negatively influenced by concurrent infections.

The design of exp. 2 was equal to the one of exp. 1 except that the primary infection

was carried out with several doses of *O. ostertagi* larvae ($25*10^3$, $50*10^3$ or $100*10^3$). The aim of this experiment was to examine whether a previous *O. ostertagi* infection affects a subsequent *C. oncophora* infection or not. The results of this experiment demonstrated that some degree of acquired immunity against *O. ostertagi* existed after priming. This resistance led to reduced fertility, stunted growth and inhibited development (vulval flap) of *O. ostertagi*, but did not negatively affect the worm numbers. Immunity directed to *O. ostertagi* had little influence on *C. oncophora*. Only the length of the small intestinal worm was reduced, but due to the small group sizes this reduction was statistically not significant.

Therefore, it was decided to enlarge the groups sizes in exp. 3. In this experiment priming was performed with repeated (3 times a week during 30 days) small doses of $7*10^3$ *C. oncophora* larvae (16 calves) or $7*10^3$ *O. ostertagi* larvae (16 animals); a third equally sized group was kept as control. Repeated dosing was carried out to imitate the natural situation. After anthelmintic treatment all calves were challenged with $100*10^3$ larvae of both species. The results showed that resistance to homologous species was present: in the homologous situation the worm numbers and the growth and the development of the worms were reduced. The fecundity was also negatively affected for *O. ostertagi*. Reciprocal cross resistance was found for the worm growth and the development whilst the fecundity of *O. ostertagi* was also lowered by *C. oncophora* priming.

In the fourth experiment the repeatibility of the previous experiments was assessed. The calves were allotted to 3 groups of 12 animals each. The primary infection was equal to the one in exp. 3. After anthelmintic treatment the primary groups were split into 3 equally sized subgroups. These subgroups were challenged as in exp. 1 and 2. With regard to *C. oncophora* there seemed to be no effect of priming. This was merely due to a loss of worms from primarily non-infected calves. The results from *O. ostertagi* infections were about equal to the results obtained in exp. 3.

A fifth experiment was carried out to come to a conclusion about the relation between the local responses in the gastrointestinal tract and the occurrence of interaction. Cannulas were placed in the small intestine and the abomasum of six calves. This made it possible to collect tissue fragments of the small intestinal and the abomasal mucosa. The infections were equal to the ones in exp. 3 except that the primarily non-infected calves were also kept as controls during the challenge period. Histological examinations of the tissue fragments revealed that several cell types were involved in the local reactions. The numbers of eosinophils and IgM-, IgG_2- and IgA plasma cells increased during the primary and the challenge infection. No response of these cells was found in the non-infected organ during the primary infections. This indicates that a response in the abomasal mucosa is not expressed at the small intestinal level and *vice versa*. After the mixed challenge a very rapid response was seen in both organs when compared to

the primary infection. Whether these accelerated reactions were due to priming or not could not be unequivocally proven, because they might also originate from the size of the dose, the repeated *versus the* single inoculation or the age of the host at the moment of inoculation.

From the results of exp. 1 to 4 can be deduced that interaction between *C. oncophora* and *O. ostertagi* exists. This interaction is expressed in a stunted growth and development of worms, but it does not affect the worm burden or the faecal egg output.

The fact that interaction was only found in a sequential infection model implies that the host's immune response is involved by mediation of this interaction. This is also obvious when the negative relation between parasitological parameters and heterologous antibody levels are considered. The mechanism by which immunoglobulines affect parasitic nematodes is not known, but since they have affinity to secretory/excretory antigens, it is possible that antibodies interfere with enzymes that are necessary for normal growth and development.

In many parasite-host models interaction between nematode species is of epidemiological importance, because it has a negative or positive influence on the size of the worm population or the faecal egg count.

The results from experiments described in this thesis do not imply that such importance is present in the *C. oncophora-O. ostertagi*-calf model. However, when parameters indicating growth and development of worms are used as parameters that measure resistance it should be considered that these parameters are influenced by a previous heterologous infection.

SAMENVATTING

Dit onderzoek heeft zich toegespitst op de vraag of er een interactie bestaat tussen *Cooperia oncophora* en *Ostertagia ostertagi*, nematoden die parasiteren in respectievelijk de dunne darm en de lebmaag van het rund. Een interactie tussen beide soorten is van epidemiologisch belang wanneer dit leidt tot een veranderde wormlast of ei-uitscheiding. Aanwijzingen dat de interactie tussen *C. oncophora* en *O. ostertagi* via de immuun respons verloopt, leidden tot experimenten waarin aan kalveren de gelegenheid werd gegeven om immuniteit op te bouwen tegen een van beide soorten. Vervolgens werd nagegaan of deze immuniteit van invloed was op de andere soort. Ook de invloed die beide soorten op elkaar uitoefenen bij gelijktijdige aanwezigheid in dezelfde gastheer werd onderzocht.

Tijdens de experimenten werd het verloop van de infecties gevolgd door bepaling van het aantal wormeieren in de mest. Na slachting van de kalveren werden de wormen uit de organen verzameld. Diverse parameters werden bepaald, zoals: aantal wormen, lengte van de wormen, aantal eieren in de uterus van de vrouwelijke wormen (als maat voor de fertiliteit) en de ontwikkeling van de vulva flap bij *O. ostertagi* vrouwtjes. Om de rol van de immuun respons bij de eventuele interactie na te gaan, werd elke twee weken de relatieve hoeveelheid immunoglobuline G in het serum bepaald met behulp van de Enzyme Linked Immunosorbent Assay (ELISA). Bovendien werd de verandering, ten gevolge van de infectie, in de aantallen van de bij de afweer betrokken cellen in de slijmvliezen van de maag- en darmwand bepaald.

In experiment 1 werden 48 kalveren in eerste instantie verdeeld in 4 groepen van 12 dieren elk. Drie groepen kregen, respectievelijk, een eenmalige dosis van $50*10^3$, $100*10^3$ of $200*10^3$ *C. oncophora* larven; de vierde groep werd nog niet geinfecteerd. Deze primaire infecties werden na 5 weken beeindigd door het toedienen van een anthelminticum. Een week later werden de 4 groepen elk opgedeeld in 3 subgroepen van 4 dieren. Subgroepen werden geinfecteerd met $100*10^3$ *C. oncophora* larven, $100*10^3$ *O. ostertagi* larven of een mengsel van beide. *Post mortem* onderzoek vond 33 dagen na deze herinfectie plaats. Doel van exp. 1 was om na te gaan of een immuniteit opgebouwd tegen *C. oncophora* een effect heeft op een *O. ostertagi* populatie en, zo ja, of dit effect afhankelijk is van de primaire dosis *C. oncophora* larven. Uit de resultaten van exp. 1 bleek dat er een zekere mate van immuniteit was opgebouwd tegen *C. oncophora*. Dit uitte zich in verlaagde wormaantallen en -fertiliteit en een verminderde wormlengte in primair geinfecteerde dieren ten opzichte van primair niet geinfecteerde dieren. De weerstand was in het algemeen niet afhankelijk van de primaire dosis. De negatieve correlaties tussen serum IgG titers en parasitologische parameters impliceren dat de immune respons van de gastheer bij deze weerstand is betrokken. Deze immuniteit tegen

C. oncophora had echter marginale effecten op *O. ostertagi*. Alleen de lengte van de maagwormen werd significant verminderd door de immuniteit tegen de darmwormen. Deze lengte werd bovendien beinvloed door het gelijktijdig aanwezig zijn van beide soorten in dezelfde gastheer.

Het tweede experiment was qua opzet gelijk aan exp. 1. De primaire infectie bestond nu echter uit verschillende doses *O. ostertagi* larven ($25*10^3$, $50*10^3$ of $100*10^3$). Doel van dit experiment was om na te gaan of een immuniteit opgebouwd tegen *O. ostertagi* ook een effect heeft op een *C. oncophora* populatie. Resultaten uit dit experiment gaven aan dat ook tegen *O. ostertagi* een immuniteit optrad. Deze weerstand uitte zich wel in een geremde groei, ontwikkeling en fertiliteit van de maagwormen, maar niet in een gereduceerde wormlast. Immuniteit opgebouwd tegen *O. ostertagi* had weinig effect op *C. oncophora* wormen. Alleen de groei van de darmwormen werd gereduceerd, maar door het geringe aantal dieren per groep was deze reductie niet significant.

Om die reden werd in exp. 3 met grotere groepen dieren gewerkt. Om de natuurlijke situatie enigzins na te bootsen werden de dieren herhaalde malen (3 maal per week gedurende 30 dagen) geinfecteerd met $7*10^3$ *C. oncophora* larven (16 dieren) of $7*10^3$ *O. ostertagi* larven (16 dieren). Een even grote derde groep werd nog niet geinfecteerd. Na behandeling met een anthelminticum werden alle dieren geherinfecteerd met $100*10^3$ larven van beide soorten. In dit experiment kwam de immuniteit tegen de homologe soort zeer duidelijk naar voren. Zowel aantallen wormen als groei en ontwikkeling van die wormen waren gereduceerd. Fertiliteit van de wormen was bovendien gereduceerd voor *O. ostertagi*. Reciproke kruisimmuniteit werd gevonden voor groei en ontwikkeling van de wormen, terwijl de fertiliteit van *O. ostertagi* ook werd verlaagd door een heterologe primaire infectie.

In exp. 4 werd getracht de voorgaande experimenten min of meer samen te vatten. De kalveren werden verdeeld in 3 groepen van 12 dieren elk. De primaire infectie was dezelfde als in exp. 3. Na behandeling met een anthelminticum werden de primaire groepen gesplitst in 3 subgroepen van 4 dieren, die geherinfecteerd werden als in exp. 1 en 2. Ten aanzien van *C. oncophora* kwam uit dit experiment geen significante immuniteitsopbouw naar voren. Dit was voornamelijk te wijten aan afdrijving van wormen in de primaire controle groep, hetgeen het totale beeld vertroebelde. Voor *O. ostertagi* waren de resultaten, ook wat interactie aangaat, gelijk aan die van exp. 3.

In exp. 5 werd getracht om op orgaan niveau een verklaring te vinden voor het optreden van interactie. Hiertoe werden 6 kalveren voorzien van canules in dunne darm en lebmaag, waardoor het mogelijk werd om biopten van de slijmvliezen te nemen. Het infectieschema was gelijk aan dat van exp. 3, behalve dat de primair niet geinfecteerde groep ook tijdens de herinfectie niet geinfecteerd werd. Histologisch onderzoek van de biopten toonde aan dat bij de lokale afweerreactie verscheidene celtypen betrokken zijn.

Zowel tijdens de primaire als de challenge periode werden IgM-, IgG_2- en IgA plasmacellen in verhoogde aantallen aangetroffen. Ook de aantallen eosinofiele granulo-cyten namen sterk toe. In het heterologe orgaan werd tijdens de primaire infectie geen verhoging van het aantal bij de afweer betrokken cellen gevonden. Dit geeft aan dat een respons in de mucosa van de lebmaag niet tot expressie komt in de mucosa van de dunne darm en omgekeerd. Na challenge met beide soorten werd een versnelde respons waargenomen in beide organen, dus ook in het orgaan dat primair niet geinfecteerd is geweest. Of deze versnelde reacties het gevolg waren van de primaire infectie staat niet onomstotelijk vast. Het kan niet uitgesloten worden dat de versnelde reacties een gevolg waren van de grootte van de dosis en het eenmalig danwel meermalig toedienen van die doses of de leeftijd van de gastheer.

Uit de resultaten van de experimenten kan afgeleid worden dat er interactie tussen *C. oncophora* en *O. ostertagi* aanwezig is. Deze interactie veroorzaakt een geremde groei en ontwikkeling van de wormen, maar heeft geen invloed op het aantal wormen en de faecale ei-uitscheiding. Het feit dat interacties slechts gevonden werden in een sequentieel infectie model impliceert dat de immuun repons van de gastheer een rol kan spelen bij deze interacties. Dit blijkt ook uit de negatieve relatie tussen parasitologische parameters en heterologe antistoffen. De wijze waarop de immunoglobulines aangrijpen op de parasitaire nematoden is niet bekend, maar aangezien de aangetoonde antistoffen gericht waren tegen secretoir antigeen is het mogelijk dat antistoffen interfereren met enzymen die nodig zijn voor een normale groei en ontwikkeling van de wormen. Overigens moet men er op bedacht zijn dat het serum IgG niveau waarschijnlijk een zwakke reflectie is van de lokaal optredende afweerreactie in het geparasiteerde orgaan, gezien het feit dat meerdere typen plasmacellen en ook granulocyten bij de afweer betrokken zijn.

In een groot aantal gastheer-parasiet modellen heeft interactie tussen wormsoorten een effect op de wormlast en/of de faecale ei-uitscheiding en is dus van epidemiologisch belang. De experimenten die beschreven zijn in dit proefschrift wijzen er echter niet op dat een dergelijk belang aanwezig is in het *C. oncophora* - *O. ostertagi* - kalf model. Wanneer echter parameters die groei en ontwikkeling van de wormen weergeven gebruikt worden als resistentie parameter moet bedacht worden dat deze parameters beinvloed worden door een voorgaande heterologe infectie.

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Curriculum vitae

Klaas Frankena werd geboren op 19 december 1956 te IJlst. In 1975 werd het Atheneum-B diploma behaald aan het Johannes Bogerman College te Sneek. Omdat hij werd uitgeloot voor de studie Diergeneeskunde volgde hij de parkeerstudie Biologie aan de R.U. te Groningen. Hiervan werd in 1976 het propaedeuse-examen behaald. Van 1976 tot 1982 volgde hij de studie Zoötechniek aan de Landbouwhogeschool te Wageningen. In 1982/1983 werd de militaire dienstplicht vervuld bij de Koninklijke Landmacht. In maart 1984 begon hij met een 3-jarig ZWO-project dat werd uitgevoerd bij de vakgroep Veehouderij van de Landbouwuniversiteit, hetgeen resulteerde in dit proefschrift. Per januari 1987 is hij verbonden aan bovengenoemde vakgroep als universitair docent in de epidemiologie.