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GENETIC RESISTANCE TO EXPERIMENTAL *COOPERIA ONCOPHORA* INFECTIONS IN CALVES

(with a summary in Dutch)

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CONTENTS

1. INTRODUCTION

Gastro-intestinal nematode infections are among the most widespread causes of disease and exert their harmful effect on man and animals all over the world. The significance of nematode parasites in cattle has become clear from a vast number of epidemiological studies. KLOOSTERMAN (1971) and BORGSTEEDE (1977) studied the situation in the Netherlands and found it to be essentially similar to that in other parts of Europe and the rest of the world, at least in temperate climates.

In field infections there is large variation among infected animals, not only in faecal egg output and number of parasites harboured, but also in clinical signs of infection. Undoubtedly, differences in numbers and species of infective larvae are responsible for a part of this variation. However, as infection experiments show, even when the larval intake is the same, differences between animals can be enormous. In the present study, this variation in resistance is investigated, especially to see whether a part of it is genetically determined.

When studying the variation in resistance in a host-parasite system, three aspects may be taken into consideration:

- The course of infection: i.e. population dynamics. This can be studied by means of a number of parasitological techniques;
- The reaction of the host. This can be derived partly from parasite population dynamics, but one can also try to measure the host's reaction more or less directly by using immunological methods;
- The host's performance, including clinical signs of infection. The gastro-intestinal tract can be damaged; digestion and metabolism and therefore important performance parameters such as growth and feed utilization can be affected.

These three types of parameters were measured concurrently during the experimental infections reported below. Parasitological and immunological parameters to allow a selection of useful indicators of host resistance to infection; performance parameters to evaluate the consequences of infection and of different levels of resistance to infection for the productivity of the host as a farm animal.

The experiments were carried out on *Cooperia oncophora* infections in calves, as a continuation of the work reported by KLOOSTERMAN et al. (1978).

The influence of genetic factors was investigated by comparisons of half-sib groups of male calves, progeny groups of AI-sires. In three consecutive experiments several levels and patterns of infection were applied because this factor, exerting the most important influence on host resistance and its manifestations, might well interfere with the hereditary component.

2. LITERATURE

2.1. THE PARASITE: COOPERIA ONCOPHORA

Cooperia oncophora is one of the most abundant nematode parasites of cattle. For the Netherlands this was found in epidemiological studies by KLOOSTERMAN (1971) and BORGSTEEDE (1977). MICHEL (1976) – in a survey of the literature – cited several other authors who made similar observations in various parts of the world with a temperate climate.

This abundance of *Cooperia oncophora* in the field is in contrast to the relative paucity of reports on experiments carried out on this parasite. Epidemiological work has shown that the pathogenicity of *C. oncophora* is only moderate in comparison with e.g. the equally abundant parasite *Ostertagia ostertagi* (a.o. MICHEL, 1969a). Furthermore, field studies have revealed a rather fast development of immunity to *C. oncophora* (SMITH and ARCHIBALD, 1968).

It was not until 1963 that the first report on pure artificial infections with *C. oncophora* was published (ISENSTEIN, 1963). In this report ISENSTEIN gave a detailed description of the life history of the parasite. According to the description by ISENSTEIN, who made his observations in primary infections, infective larvae exsheath in the abomasum. At three days after infection all worms are found in the small intestine and are moulting to the fourth stage. At around ten days the last moult takes place. ISENSTEIN saw the first gravid female worms at 15 days after inoculation and observed a variation in prepatent period from 17 to 22 days.

Cooperia oncophora seems to be a lumen-dwelling parasitic nematode. COOP et al. (1979), by histological examination, found no evidence of any penetration of the parasite into the mucosa or submucosa. Such evidence also does not emerge from other sources in the literature or from our own observations.

This property of *Cooperia oncophora* might explain its moderate pathogenicity mentioned previously and confirmed first by experimental work of HERLICH (1965a). This author infected 5 to 8 month old calves with single doses of *C. oncophora* varying from 350,000 to 3,000,000 larvae. Only at the highest dose levels did he observe anorexia, diarrhoea and complete suppression of live weight gain. COOP et al. (1979) gave daily infections (5,000; 10,000 or 20,000 larvae for 5 days each week) during 20 weeks. They observed a 13.5% reduction of live weight gain in all three groups of infected calves. However, they saw no significant effects on feed intake nor any obvious clinical signs. BORGSTEEDE and HENDRIKS (1979) observed some mild diarrhoea after a single primary dose of 200,000 larvae; the infection caused a significant growth depression of around 20% which, however, was virtually compensated at 140 days after infection. Earlier work at our laboratory also showed mild growth depressions in *C. oncophora* infections given as a single primary dose of 100,000 larvae (KLOOSTERMAN et al., 1978).

The relatively low pathogenicity of *C. oncophora* might also be caused by the rapid acquisition of immunity by calves. HERLICH (1965b) found an almost completely immunizing effect – if egg output was taken as a criterion – of a single dose of 32,000 infective larvae. However, because the challenge dose was given about 30 weeks after the primary dose, a considerable age effect can not be excluded.

COOP et al. (1979) concluded from the egg output pattern that acquired resistance became manifest at about 8 weeks after the start of continuous infection with daily doses ranging from 5,000 to 20,000 larvae. BORGSTEEDE and HENDRIKS (1979) and KLOOSTERMAN et al. (1978) put forward more convincing evidence by observing reduced worm numbers at 6 to 8 weeks after primary infection with 100,000 and 200,000 larvae. KLOOSTERMAN (1971) concluded from epidemiological observations that, concerning the time needed to provoke a refractory state – as judged by faecal egg output –, *Cooperia* spp. are intermediate between *Nematodirus* spp. and *Ostertagia* spp..

As regards the relative pathogenicity of *C. oncophora*, this species seems to be less pathogenic for calves than the species of the same genus *C. punctata* (ALICATA and LYND, 1961) and *C. pectinata* (HERLICH, 1965a).

2.2. PARAMETERS FOR MEASURING RESISTANCE

As was already mentioned in the introduction, the parameters that can be used to measure the resistance of an animal to a parasitic infection might be divided into three groups according to the viewpoint from which the host-parasite interaction is studied. Parasitological parameters can be used to assess the course of the infection. Immunological measures are necessary if the reaction of the host towards the parasite population is taken as a criterion. Parameters which describe an animal's performance might reflect the ultimate effect of parasitic infection on the host.

2.2.1. *Parasitological parameters of resistance*

On this subject, this review will be restricted to reports of parasitological studies on gastro-intestinal nematode infections in cattle and sheep.

Faecal egg output. The observation that is most easy to carry out because it does not require slaughtering of the animal is to assess the faecal egg output. If repeated egg counts have rendered a complete egg output pattern, several parameters can be derived from this. The total or average egg output after an infection (DONALD et al., 1963; RIFFKIN and DOBSON, 1979), discards information on the pattern of output which produced this total or average. If this pattern is stereotyped, as suggested for *O. ostertagi* infections in calves by MICHEL (1969b), this is, however, not a serious disadvantage. Other characteristics of the egg output curve may contain equally valuable information. The length of the prepatent period (HARNESS et al., 1971; RIFFKIN and DOBSON, 1979) or, the most frequently used feature, the peak egg output (a.o. HERLICH, 1976;

Ross, 1970) are reported as measures in experiments on experimental comparison of resistance to infection. The use of the patent period as a parameter (Bailey, 1949) in most cases results in practical problems because of its length.

Excreted worms in faeces. The only other possible parasitological parameter in the living animal is to search for worms excreted in the faeces after rejection by the host. Chiejina and Sewell (1974) considered the recovery of exsheathed third stage larvae from faeces as a sign of resistance to the establishment of incoming infective larvae; Gibson and Everett (1976) did the same, whereas Goldberg (1973) recovered all parasitic stages from faeces thus obtaining an indication of worm loss too.

Size of worm burden. If animals are killed for *post mortem* examination, a number of other parameters can be measured. The most simple one is the size of the (undifferentiated) worm burden. Perhaps in infection experiments this is the most frequently used parameter to express an animal's resistance. Especially in host-parasite systems that are used as a model, the recovery of worms after a certain challenge infection is a very common criterion.

Various differentiations of the worm burden are possible: sex differentiation, stage differentiation (immature – adult; L_3–L_4–adult; early L_4–late L_4–adult). Differentiation of male and female worms can be carried out for adult worms and for immature stages that have developed far enough (mostly L_4). Although sex differentiation is widely carried out, reports of aberrant ratio's or changes of the sex ratio in the course of infection are scarce. Donald et al. (1964), studying *Nematodirus sphatiger* infections in sheep, found more than fifty percent females during the L_4 stage. The adult stage, in contrast, contained less than 50% (significantly less) female worms. Crofton and Whitlock (1968) observed a gradual increase of the percentage female adult worms due to a more rapid reduction of male worm numbers in *Haemonchus contortus* infections in sheep. Other authors frequently present the sex differentiation in the description of experimental results, but none of them seems to consider it a useful parasitological parameter.

The distinction between the various stages of parasitic development has of course attracted the most attention. The proportion of immature worms reflects the rate of inhibition of development. The feature 'arrested development' (Michel, 1974) can only be quantified by counting the number of parasites in the arrested stage.

Other ways to differentiate between the worms of a monospecific parasite burden emerge from the fact that some species have polymorphs or may show morphological aberrations. If the various morphs have different sensitivities to resistance mechanisms their proportions or ratio's may be used as parameters to measure the effect of the host upon the parasite population. Le Jambre and Ractliffe (1976) concluded that the ratio linguiform A: linguiform B vulvar phenotypes in *Haemonchus contortus cayugensis* reflected crowding of this species in a particular sheep. In contrast to this genetical polymorphism of flap type is the finding by Michel et al. (1972) that the development of the vulval flap of female *O. ostertagi* in calves is directly influenced by host resistance.

Body size of worms. It is widely accepted and shown in numerous experiments that host resistance affects body size of the parasite. The measure which is usually taken is body length, assessed separately for male and/or female worms because there is a marked difference between sexes in body size. Other measures related to body size which are sometimes used are body weight (RACTLIFFE et al., 1971; COADWELL and WARD, 1975) and body proportions (MICHEL et al., 1971).

Eggs in utero of female worms. Many authors report observations on the egg content of female worms. This feature is generally considered as a measure of the individual egg production of the worms, although very few authors have actually checked this assumption.

2.2.2. *Immunological parameters of resistance*

The use of immunological parameters to describe or measure the resistance of a certain host to its parasite population can only be supported by thorough knowledge of the immunological mechanisms that confer resistance to the parasite. Although a lot of research has been done in this field, this knowledge is still only limited. Most investigations are carried out in laboratory models of host-parasite systems. The four mammal-nematode systems that have received most attention are rat - *Nippostrongylus brasiliensis* (reviewed by OGILVIE and LOVE, 1974); rat/mouse - *Trichinella spiralis* (reviewed by LARSH and RACE, 1975); guinea pig - *Trichostrongylus colubriformis* (see e.g. ROTHWELL et al., 1974) and mouse - *Trichuris muris* (WAKELIN, 1975).

Most of the work in these systems has concerned the expulsion process in a primary single infection which limits the applicability of the results. For the rat - *N. brasiliensis* system OGILVIE and LOVE (1974) concluded that expulsion of the parasite is a two step mechanism consisting of a humoral and a cellular component. After specific antibodies have affected ('damaged') the worms, sensitized lymphocytes cause actual expulsion. WAKELIN (1975) drew essentially the same conclusions concerning the expulsion of *T. muris* from the mouse.

Although immunity to helminths is closely associated with high IgE-levels and helminth infections are known to exert a potentiating effect on any IgE response, the specific antibody needed in the first step appears to be IgG_1 (OGILVIE and LOVE, 1974).

DINEEN and KELLY (1973) found evidence that also bone marrow derived cells are essential for the expulsion of *N. brasiliensis*. These might be eosinophils because these cells are known to be attracted by products of several reactions that may take place at the site of infection such as T-lymphocyte-antigen interaction, immune complex formation, anaphylactic hypersensitivity reaction (BUTTERWORTH, 1977). Furthermore, eosinophilia is a striking feature of many parasitic infections.

LARSH and RACE (1975) stated that expulsion of *T. spiralis* from the mouse only requires a specific reaction between sensitized T-cells and parasite antigen. This reaction causes tissue injury and thereby triggers a non-specific inflammatory reaction to the injury, which results in tissue changes which are unfavourable to the parasite. LOVE et al. (1976) found evidence, however, that

antibodies were also necessary for expulsion of *T. spiralis* from the rat. Moreover PERRUDET-BADOUX et al. (1978) showed that 'Biozzi high responder' mice were more resistant to a primary *T. spiralis* infection than low responder mice, while the only difference between the two lines was their ability to produce antibodies.

The expulsion of *T. colubriformis* from the guinea pig is associated with pharmacologically active amines that are released by accumulated and degranulated eosinophils and basophils (ROTHWELL et al., 1974). Probably, this non-specific process is triggered by an immunologically specific step such as the action of antibodies or T-lymphocytes. Also in this system a contradictory result was found; CONNAN (1972) successfully transferred immunity to *T. colubriformis* with serum, although immunity in this case may have been associated with the transfer factor, a product of cell mediated immunity (see e.g. ROSS and HALLIDAY, 1978), and not with antibodies.

Thus a wide variety of host - parasite interactions have been found or suggested to participate in the expulsion mechanism. Furthermore, expulsion of parasites is only one of the manifestations of host resistance. From the above it seems likely, however, that in every system specific humoral and cellular components can be found. Based on this assumption a search for specific immunological tests of resistance to a certain nematode parasite seems to be promising.

A problem, however, for development of such a test, is the fact that a host reaction which is specific to a certain parasite, does not necessarily contribute to an animal's resistance to this parasite. For the humoral response, SOULSBY (1960) introduced the term 'functional' for specific antibodies that appear to have real protective value, i.e. contribute to mechanisms directed against the parasite. The complex antigenic structure of a nematode provokes an equally complex antibody response of the host (see a.o. CAPRON, 1968). Moreover a functional antibody response probably is only part of an entire reaction chain and thus can fulfil its function only if the other components of the chain are also present. These considerations indicate the difficulty of finding a satisfactory immunological parameter of host resistance to a nematode parasite. As MITCHELL (1979) states in his expert review on host protective immunity to parasites, a particular mechanism may be necessary but not sufficient for the expression of host resistance at its most efficient.

The continuation of this section will briefly review attempts reported in the literature to establish a relationship between immunological and parasitological parameters of host resistance in gastro-intestinal nematode infections in ruminants.

Some authors merely recorded a stimulating effect of gastro-intestinal nematode infection on the level of particular immunoglobulin classes: JENSEN and NANSEN (1978) in natural infections (mainly *Ostertagia*) in cattle, and CRIPPS and ROTHWELL (1978) in *T. colubriformis* infections in sheep. A number of other authors observed a specific antibody response to an infection: STEWART (1950) was the first in this respect when he reported the stimulating effect, especially of infectious *H. contortus* larvae, on the formation of complement fixing antibodies to this parasite in sheep. With other techniques, other authors demonstrated the

occurrence of specific antibodies in serum or at mucosal surfaces at the site of infection: AHLUWALIA (1975) in *C. curticei* infections in sheep; CURTAIN and ANDERSON (1972) and HOGARTH-SCOTT (1969) in *O. circumcincta* in sheep (the latter was the first to demonstrate homocytotrophic antibodies in sheep); ROTHWELL and MERRITT (1974) in *T. colubriformis* in sheep (antibodies to acetylcholinesterase, an enzyme secreted by this nematode).

Other authors attempted to associate the occurrence of immunological phenomena with manifestations of resistance or differences in resistance. ROSS (1970) did not succeed in this respect in an investigation on *T. axei* infestation of lambs. He observed very variable but generally rather low antibody titres. In *T. colubriformis* infections in sheep, CRIPPS and STEEL (1978) found that a rise of serum Ig-levels was coincident with the development of resistance to the parasite as indicated by the faecal egg output. In a vaccination trial in the same host - parasite-system, GREGG et al. (1978) concluded from a comparison of experimental groups that specific antibodies to worm acetyl-cholinesterase reflected the extent of antigenic exposure rather than resistance. The presence of globule leucocytes in the gut wall was associated with resistance. In *H. contortus* infections in ewes CHEN and SOULSBY (1976) observed a tendency for the blastogenic response of peripheral lymphocytes to worm antigen to decrease around the partus when also a rise of faecal egg output was recorded. From vaccination experiments with this parasite, SMITH and CHISTIE (1978, 1979) concluded that resistance was associated with the presence of antibodies and globule leucocytes in the intestinal mucosa. In field infections in cattle Ross et al. (1960) demonstrated higher levels of complement fixing antibodies in genetically more resistant cattle. HERLICH and MERKAL (1963) assessed antibody titres (passive haemagglutination with *H. contortus* homogenate as antigen) in *T. axei* infected calves. They observed an increase of titres due to infection but could not draw conclusions on a quantitative relationship because the experimental design was not appropriate. MICHEL and SINCLAIR (1969) showed that the administration of cortisone to calves infected with *O. ostertagi* reduced their resistance but also depressed their production of specific antibodies against the parasite.

A quantitative relationship between phenomena of host resistance and an immunological parameter was established by RIFFKIN and DOBSON (1979) in *H. contortus* infections in sheep. They found a significant positive relationship between the blastogenic response of peripheral lymphocytes of worm free sheep to *H. contortus* antigen and resistance to subsequent infection as measured by faecal egg output and parasite recovery. This is the only report of a real quantitative relationship between immunological and parasitological parameters of resistance that could be found in the literature.

2.2.3. *Performance parameters*

Numerous authors have reported the deleterious effects of gastro-intestinal nematode infections on performance of sheep or cattle. A wide variety of parameters to characterize the performance of animals has been used in these studies. This variety ranges from a rough quantitation of clinical signs of infection (e.g.

severe diarrhoea) to a very minute measurement of particular physiological parameters that may be affected during parasitic infection (e.g. liver protein synthesis). As an illustration two extremes can be referred to: WARWICK et al. (1949) communicated the results of a selection experiment of sheep on resistance to field infection (mainly *H. contortus*); as a criterion for resistance of genetically different groups of sheep they used the survival percentage. On the other hand, a considerable number of studies has been carried out to investigate the pathophysiology of gastro-intestinal nematode infections (reviewed by STEEL, 1974). Macro- and microscopic damage to tissues of the gastro-intestinal tract is commonly seen and may be due to direct damage by the parasite (e.g. blood-sucking nematodes) or may be caused by the response of the gastro-intestinal tissue to nematode infection.

Blood sucking nematodes of course may cause anaemia but also nematodes that do not ingest blood may cause increased leakage of plasma proteins into the intestine. SYMONS (1976) concluded that this feature is more important than reduced digestion and absorption of nutrients, because compensation of the latter functions occurs at sites distal to infection. The ensuing increased protein cycling must impair overall efficiency of amino acid use and causes increased energy costs of synthesis of protein. SYKES (1978), however, stated that the quantitative significance of these changes in protein metabolism is hard to measure in ruminants. The processes described above cause an increased requirement for nutrients (also depressed mineral retention is reported, see SYKES, 1978) which, exacerbated by the commonly seen effect of depressed food consumption (anorexia), causes a sequence of metabolic effects producing a syndrome analogous to undernutrition (SYMONS and STEEL, 1978).

We are still far from a full understanding of the aetiology of production loss in helminth infections (SYMONS and STEEL, 1978) but present knowledge already indicates the usefulness of a number of parameters for measuring the extent of parasatic damage to the performance of the host.

The most rough parameter has already been mentioned: mortality. Next there is a number of clinical signs of gastro-intestinal nematode infection (anorexia, reduced growth or weight loss, diarrhoea) which may be quantitated in production traits such as live weight gain and food consumption. Furthermore, with more or less effort observations may be carried out which measure features or processes that are basic to the earlier mentioned clinical manifestations of parasitic infection i.e. nitrogen-, energy and mineral retention. Finally, a number of blood parameters have proven to be indicative of parasitic damage to the abomasal wall (plasmapepsinogen, see review FORD, 1976) or damage to intestinal tissues which results in plasma leakage (e.g. albumin, see for review DARGIE, 1975). The blood sucking activity of nematodes such as *Haemonchus spp.* can be measured by assessing anaemia in terms of packed cell volume (PCV), haemoglobin content or the red cell count (DARGIE, 1975).

2.3. Resistance and performance

The term resistance can be used in two different ways: firstly, to indicate the ability of an animal to put some restraint on the parasite; secondly, to describe the capacity of an animal to withstand the pathogenic effects of infection.

Among others, Gordon (1960) recognized that these two should be distinguished because they not necessarily parallel each other. Such a parallelism would be based on the assumption that the extent to which the host is affected during a parasitic infection is determined purely by the parasite's activity and thereby reflects the host's ability to limit the activity of the parasite. As is stated, however, by Wakelin (1978) the extent to which the life processes of the host are affected by physiological disturbances caused by parasitic infection (e.g. disturbance of nutrient digestion caused by damage to the intestinal tissue) also depends on the host's ability to neutralize these disturbances.

Furthermore, the available literature indicates that a part of the physiological disturbances mentioned above may be caused by a defence response of the host to parasitic infection. Histopathological changes in the intestinal tissue due to an inflammatory response to infection may cause the plasma leakage which is responsible for reduced nutrient utilization by a parasitized animal (Symons and Steel, 1978).

Thus there are several indications that the relation between resistance to a certain infection and the performance of an animal during that infection is not a fully predictable one. In contrast to this is the virtual absence of reports in the literature on investigations designed to study this relation.

An exception is presented by Riffkin and Dobson (1979). These authors observed a significant negative correlation between resistance to a primary single *H. contortus* infection in sheep and the live weight gain of these sheep during five weeks after infection. Earlier, the work of Barger (1973) had provided some evidence that resistance to the parasite exaggerated the effects of infection. This author found an (unexpected) positive correlation ($P < 0.10$) between wool growth and worm numbers in sheep infected with two single doses of *T. colubriformis*.

To preclude any confusion of thought the term resistance will be reserved here to indicate the ability of an animal to put some restraint to the parasite.

2.4. Genetic resistance to gastro-intestinal nematode parasites in sheep and cattle

Genetic control of resistance to disease is receiving more and more attention. For a number of specific diseases the genetic control of resistance has been studied; moreover basic mechanisms which might reflect general disease resistance (e.g. the humoral antibody response) have been the subject of genetic studies (see reviews by Spooner et al., 1975 and Andresen, 1978).

Genetic control of resistance to parasitic infection has also been the subject of

a progressively increasing number of reports. As quite recently the latter subject was extensively reviewed by WAKELIN (1978) this section will be restricted to a short enumeration of observations in the literature on genetic control of resistance to gastro-intestinal nematode parasites in cattle and sheep.

Almost all the work on genetic resistance to nematodes in ruminants has been carried out in sheep. Reports on differences in resistance between sheep breeds are listed below. In the middle column are mentioned the criteria of resistance that appeared to be affected by genetic factors in the quoted study.

Type of infection	Criterion of resistance	Reference
field infection	faecal egg count	STEWART et al. (1937)
field infection	survival rate	WARWICK et al. (1949)
field infection	faecal egg count worm count (*Ostertagia* and *Nematodirus*)	SCRIVNER (1964a)
field infection	faecal egg count (*H. contortus*) worm count	LOGGINS et al. 1965)
field infection (predom. *H. contortus*	packed cell volume haemoglobin concentration faecal egg count	JILEK and BRADLEY (1969)
field infection	worm count (*H. contortus*)	KNIGHT et al. (1973)
field infection	faecal egg count number adult *H. contortus* number immature *H. contortus*	PRESTON and ALLONBY (1979)
field infection	faecal egg count worm count (*Nematodirus*) worm fecundity (epg/worm) haemoglobin concentration	YAZWINSKY et al. (1979)
exp. *H. contortus*	faecal egg count	SCRIVNER (1967)
exp. *H. contortus*	faecal egg count worm recovery packed cell volume	RADHAKRISHNAN et al. (1972)
exp. *H. contortus*	faecal egg count worm recovery	PRESTON and ALLONBY (1978)
exp. *H. contortus*	faecal egg count worm recovery packed cell volume serum albumin content iron turnover gastro-intestinal plasma loss	ALTAIF and DARGIE (1978a, b)
exp. *T. axei*	worm recovery lamb survival weight gain	ROSS (1970)

Type of infection	Criterion of resistance	Reference
exp. *Ostertagia* spp.	faecal egg count	SCRIVNER (1967)
exp. mixed infection	faecal egg count	YAZWINSKI et al.
	worm fecundity	(1979)

The listing shows that most results are based on studies of experimental *H. contortus* infections or field infections in which *H. contortus* played a major role. As it is the simplest parameter for resistance, the faecal egg count has been most often used as a criterion. The size of the worm burden also revealed breed differences in several cases. Breed differences were, in the case of *H. contortus* infections, also reflected in significant changes of several haematological parameters, which is not surprising because *H. contortus* is a blood-sucking parasite.

The occurrence of breed differences indicates also that within breeds genetic differences are very likely to occur. Indeed, within-breed differences in resistance to nematode parasitism have repeatedly been shown. This has been done by essentially two sorts of comparisons of sheep of the same breed: comparisons of groups constituted on the basis of family relations and, secondly, comparisons of groups of sheep with different haemoglobin types (homozygous A (AA), heterozygous (AB) or homozygous B (BB)). A listing of within-breed differences in sheep reported in the literature is given below for observations on field infections.

Type of comparison	Criterion of resistance	Reference
progeny groups	faecal egg count	GREGORY et al. (1940)
progeny groups	faecal egg count blood loss	WHITLOCK (1955)
progeny groups	haematocrit	WHITLOCK (1958)
progeny groups	faecal egg output haematocrit	WHITLOCK and MADSEN (1958)
progeny groups	faecal egg count	PIPER et al. (1978)
haemoglobin type	packed cell volume haemoglobin concentration body weight	ALLONBY and URQUHART (1976)
haemoglobin type	no differences	YAZWINSKI et al. (1979)

The results of experimental infections with *H. contortus* in sheep are presented in the following list.

Type of comparison	Criterion of resistance	Reference
progeny groups	faecal egg count haematocrit	LEJAMBRE et al. (1975)
mothers-daughters	lymphocyte stimulation index	RIFFKIN and DOBSON (1979)

Type of comparison	Criterion of resistance	Reference
haemoglobin type	faecal egg count	EVANS et al. (1963)
haemoglobin type	no differences	RADHAKRISHNAN et al. (1972)
haemoglobin type	no differences	LEJAMBRE et al. (1975)
haemoglobin type	faecal egg count worm count haematological parameters	ALTAIF and DARGIE (1978a, b)

Only one trial on experimental infection with another parasite has been reported: ALTAIF and DARGIE (1978c) found significant differences in resistance to *O. circumcincta* between haemoglobin types of sheep. They observed significant differences in number of adult worms, number of inhibited L4, serum albumin concentration and gastro-intestinal plasma leakage.

Again, with respect to within-breed genetic differences the majority of work has been done on *H. contortus* infections. A lot of workers have studied the influence of haemoglobin type: in a number of cases significant effects of haemoglobin type were found, but several authors failed to show an effect. ALTAIF and DARGIE (1978a), studying the influence of breed and haemoglobin type concurrently, concluded that genetic differences other than those related to haemoglobin type were much more important. For the Australian situation LEJAMBRE (1978) concluded that haemoglobin types were not genetically correlated with resistance, but that Australian sheep appear to have sufficient (within-breed) genetic variation to make a programme of selective breeding for resistance feasible.

Genetic resistance to gastro-intestinal nematodes in cattle has been the subject of hardly any study as far as could be determined from the available literature. The first observations on genetic differences in cattle were reported by ROSS et al. (1959). They saw differences in resistance to field infections between the progeny of one grand sire and the progenies of several other grand sires in a herd of Zebu-cattle in Nigeria. The resistant progeny passed fewer worm eggs in the faeces and had a higher live weight gain, probably as a consequence of this resistance. Further observations in the following season (ROSS et al., 1960) confirmed the first result and showed, moreover, that the progeny of the resistant grand sire had higher antibody titres against *H. contortus* antigen as measured by a complement fixation test.

Other field observations were reported by SEIFERT (1971). This author evaluated the resistance of various types of crossbreds in field infections; he only found a significant difference in faecal egg output due to *Oesophagostomum radiatum*.

So far, KLOOSTERMAN et al. (1978) have published the only report on genetic

resistance to experimental infection. Between progeny groups of sires they found significant differences in antibody titres and worm length in single infections with *Cooperia oncophora*. Differences in resistance between two extreme sire groups were confirmed to some extent in a field trial.

3. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. *Experimental animals*

Dutch Friesian bull calves were used as experimental animals. The calves descended from previously chosen A.I. sires that were expected to produce offspring in significant numbers during several years because they were expected to transmit high milk production to their progeny. Initially 16 sires were chosen, eight in the province of Friesland and eight in the province of Noord-Holland.

The calves for an experiment, which were born within a very limited period, were fed colstrum on the farm for at least a few days. Then they were collected and transported to the experimental farm.

Calves were reared according to normal standards: hay ad lib. from the very beginning; milk replacer until weaning at about 10 weeks of age; concentrates at a maximum of 2 kg per day; additional water by means of a bucket (expt. I) or drinking nipples (expts. II and III). Calves were housed individually on a floor of wooden slats.

Rearing of calves was quite succesful: out of 142 purchased calves only three died during the rearing period. In the calves of experiment II and unidentified virus infection caused a notable growth depression in the weeks prior to infection. However, at the time of infection all calves apparently had recovered.

3.1.2. *Infective larvae*

Larvae used in primary infections in these experiments were cultured from faeces of at least four donor calves which were three to five months of age. The infective larvae for primary infection in expt. II were a mixture of larvae cultured at our own laboratory and of larvae of the same strain supplied by the Central Veterinary Institute, Lelystad, The Netherlands. Larvae for secondary infections were cultured from faeces of primarily infected experimental calves.

Larvae were cultured from faeces of donor calves by incubating a mixture of fresh faeces and wood shavings at 28 °C for at least a week. Infective larvae were extracted from this mixture by means of a Baermann apparatus.

Calves were infected by administering gelatine capsules enclosing the infective larvae.

The strain of *Cooperia oncophora* used in these experiments contains about 30% *C. surnabada*. It is not clear whether *C. oncophora* and *C. surnabada* are different species or just two polymorphs of one species. ISENSTEIN (1971) found evidence for a polymorphic relationship. A differentiation can only be made in male adult worms. In this study we accept ISENSTEIN's conclusion and thus refer to *Cooperia oncophora* infections.

3.2. METHODS

3.2.1. *Observations*

3.2.1.1. Parasitological observations

– Egg counts

Frequency: Three times a week starting at day 14 after primary infection.
Method: Faecal samples were taken between 8.00 and 9.00 a.m.
Eggs were counted in 0.05 g of faeces, according to a modified McMaster technique described by VAN DEN BRINK (1971).

– Worm counts

Method: Adult worms in the small intestine were counted in two 1% samples as described by KLOOSTERMAN et al. (1978). In expt. I, after primary infection, a 150 μm mesh sieve was used; expts. II and III, after secondary infections, a 75 μm mesh sieve.
Larvae were counted similarly but in two 0.1% samples.
Male and female adult worms were counted separately. The percentage male worms was calculated only if more than 25 adult worms were found in the aliquots.

– Worm lenght

In expt. I for every calf the length of 20 male and 20 female worms was measured, if these numbers were present in the aliquots. In expt. II and III, if possible, 50 adult males, adult females and larvae were measured. If less than 10 worms were present, a calf was excluded from calculations for worm length.
Method: The method used was decribed by KLOOSTERMAN et al. (1978). For measuring larvae a fourfold magnification of the microscope was used.

– Percentage *Cooperia surnabada* male worms

Method: If possible, 100 adult male worms, randomly chosen from the aliquots. were examined microscopically to differentiate between *C. oncophora* and *C. surnabada* according to the description given by ISENSTEIN (1971). In expts. II and III some specimens could not be identified due to their small size; these were withheld from the computation of the *C. surnabada*-percentage. A number of 25 male worms was considered as the minimum that allowed computation of a reliable percentage.

– Number of eggs per female worm

In expt. I two samples of 10 females were examined as a whole. In expts. II and III, if present, the eggs in 50 female worms were counted in every single worm. If less than 10 females were present, this parameter was considered as missing.
Method: KLOOSTERMAN et al. (1978) described the method that was used.

3.2.1.2. Immunological observations

– Immuno Fluorescent Antibody Test (IFAT)

Frequency: Blood samples were taken every week and tested all together after finishing the experiment.
Method: KLOOSTERMAN et al. (1978) gave the detailed procedure of this test.

– Indirect HaemAgglutination test (IHA)

Frequency: This test was performed on a fraction of the serum of the same weekly blood samples that were used for the IFAT.
Method: Also for this technique the method was described by KLOOSTERMAN et al. (1978).

– Intra Dermal Test (IDT)

Frequency: In expt. I this test was performed only three times: at days 28, 40 and 47 after infection. In expt. II and III the IDT was carried out regularly at two week intervals starting two days before the first infection.
Method: The IDT is based on the immediate hypersensitivity reation after intradermal injection of antigen. The antigen used in these experiments was a saline extract of adult worms with an N-content of about 0.2 percent, prepared as described by KLOOSTERMAN et al. (1978). Except for the second and third test in expt. III, the same batch of antigen was always used.

Doses of 0.25 ml. were injected intradermally in the neck region. Dilutions were injected alternately on the left and right side of the calf.

The reaction of the calf was assessed 45 minutes after antigen injection by measuring with a marking gauge the two axes of the usually, elliptical wheal which developed at the injection site. The reaction criterion was obtained by multiplying these two lengths. In expts. I and II six different concentrations of antigen were injected; undiluted; 1:10; 1:100; 1:1,000; 1:10,000; 1:100,000. This was done because a small pilot study had indicated that the wheal resulting from an intradermal injection was related to the quantity of antigen injected. This relation however seemed to be not linear (on a logarithmic scale) but more complicated. Furthermore on the basis of this pilot study an effect of the side of the neck of the calf, that was used for injection, was suspected. Analysis of results of expts. I and II however allowed no clear conclusions on these points (the response curve of a dilution series and the injection site effect), probably due to the large variations in reactions that were seen. These variations were large

both between calves on the same test day and between different test days for the same calf. The latter source of variation suggests that the experimental techniques used were not very accurate. For this reason it was decided for the third experiment to inject only two antigen dilutions which had shown the highest correlations with all other dilutions injected. In the analysis of all results of the IDT, these dilutions (1:100 and 1:1,000) were used.

3.2.1.3. Other observations

- Live weight

Frequency: Calves were weighed at two week intervals from the moment of arrival until five weeks prior to infection. From this time calves were weighed every week.
Method: Weighing was always done at the same time of day by means of a mobile balance with an accuracy of 1 kg.

- Feed intake

Frequency: In expt. I feed intake was not recorded. In expt. II intake of hay and concentrates was recorded daily in 17 calves from 17 days prior to the first infection until the end of the experiment. In expt. III daily records were made of all calves from four days before the secondary infection onwards.
Method: During one day calves were given a known amount of concentrates (2 kg.) and hay. The next morning the hay and/or concentrates that had remained uneaten were collected and weighed again.

- Diarrhoea

Frequency: Only in expts. II and III, all calves were checked every day for the occurrence of diarrhoea from the day of the first infection onwards.
Method: Consistency of faeces was observed visually. A value 1 was given if the faeces had a normal consistency, a value 3 when the faeces was very thin (diarrhoea) and a value 2 if the consistency of faeces was intermediate.

3.3. Experimental designs

The designs of all experiments are summarized in table 1, which is included as a fold-out on the last page.

3.3.1. *Experiment I*

3.3.1.1. Description of design

In expt. I 46 calves, sons of 16 sires ($14 \times 3; 2 \times 2$) were infected at a mean age

of 91.6 ± 2.2 days and a mean live weight of 107.9 ± 9.3 kg. All calves were given a single dose of 100,000 infective *Cooperia oncophora* larvae. On three occasions, 34; 41 and 48 days after infection, calves were slaughtered for *post-mortem* (P.M.) examination. The three necropsy groups contained 16, 16 and 14 animals respectively. For each necropsy group one calf was chosen at random from each sire group.

The calves in this experiment were born between 16 and 22 November 1975; the infection was given on February 18, 1976; calves were slaughtered in March and April, 1976.

3.3.1.2. Aims of design

One of the purposes of this experiment was to provide information for choosing sires whose offspring would show extreme resistance or susceptibility to *C. oncophora* infections. The consideration that a large number of small sire groups gives the greatest chance to find extremes led to the choice of the actual design. (A group size of two would make a second calf useless if one of them died).

The calves were infected at three months of age. This was done for a number of theoretical and practical reasons. To save labour and money experiments in very young calves would be most advantageous. However, at three months of age the calves had, as in practice, only been weaned for two or three weeks and were only recently accustomed to a normal diet of hay and concentrates. Moreover, in practice calves are virtually never put on to pasture and thereby into contact with gastro-intestinal parasites before the age of three months. A more theoretical consideration was that maternal immunity, if present at all, can be expected to have faded out at three months.

Because this experiment was designed as a screening trial an infection dose which causes maximum variation in reaction of calves was the most suitable. Previous infection experiments in our own laboratory (KLOOSTERMAN et al., 1978) and by other authors (HERLICH, 1965a, b; BORGSTEEDE and HENDRIKS, 1979) had made it likely that a dose of 100,000 infective larvae would give satisfactory results in this respect.

The times of necropsy were chosen to cover the period of decrease of worm numbers. To prevent a dramatic reduction of group sizes and to have every sire represented in each group, the number of necropsy groups had to be limited to three.

3.3.2. *Experiment II*

3.3.2.1. Description of design

In this experiment 44 calves were used comprising five sire groups of 11; 11; 9; 7 and 6 animals respectively. At 96.5 ± 5.8 days of age and at a live weight of 106.3 ± 10.7 kg all calves were infected with a dose of 100,000 *C. oncophora* infective larvae. Seven weeks later, at day 49 p.i., all calves received a single, secondary dose of 350,000 larvae. Calves were slaughtered for P.M. examinations in four groups of eleven at 18; 25; 32 and 39 days after secondary

infection. Members of each sire group were randomly divided over necropsy groups.

The calves in this experiment were born in October/November 1976, received their first infection on January 21, 1977 and were slaughtered in April and May 1977.

3.3.2.2. Aims of design

The preliminary results of expt. I had not shown highly significant differences between sire groups, although some of the differences observed were quite large (see chapter 4). To reduce the risk of choosing the wrong sires, two groups of extreme sires were chosen as an intermediate selection before the ultimate selection of two extreme sires in expt. III.

Selection of sires was based on egg counts and IFAT-titres, two correlated characteristics, of their offspring in expt. I. Two 'resistant' sires (low egg counts and high titres) were chosen; each of them provided 11 calves. As 'susceptible' sires three A.I. breeding bulls were chosen instead of two because of the limited number of offspring available. Because calves for expt. II were purchased at the start of the calving season when there were relatively few available, they were not as uniform in age as animals in the other experiments.

Expt. I had shown that host reactivity is not determined by hereditary factors to a very high degree. Therefore it was impossible to predict accurately the reactivity of a certain animal solely by its descent. Because the purpose of this experiment was to investigate resistance to a re-infection in calves with a known reactivity, it was decided to apply a 'screening' infection before the re-infection. Therefore all calves were given the same primary single dose as in expt. I to screen their reactivity.

The second infection was given after seven weeks because, as expt. I and previous experiments (KLOOSTERMAN et al., 1978) had shown, the animals would have recovered by then and the worm population could be expected to have reached a more or less steady level. Furthermore this time was needed to culture infective larvae from the primary infection.

The calves of expt. II were necropsied on four occasions to get information on the course of the worm population after re-infection. Because it was expected that worms would be expelled earlier than after primary infection, the first group was slaughtered 18 days after re-infection, i.e. as soon as possible after the worms could have reached maturity.

3.3.3. *Experiment III*

3.3.3.1. Description of design

Experiment III was carried out on a total of 49 calves, consisting of two sire groups of 25 and 24 animals. Seven animals were assigned to a control group which was not infected and not necropsied; otherwise they were treated exactly the same as infected animals. Forty-two remaining animals were given a primary dose at a mean age of 92.8 $\pm$ 2.8 days when their mean live weight was 103.5 $\pm$7.2 kg.

Twenty eight calves received 20,000 *C. oncophora* infective larvae and fourteen were given 100,000 larvae, as primary doses. Beginning seven weeks after primary infection, half (fourteen) of the calves given the lower primary dose and all calves which had received the higher dose, were infected three times a week (at Monday, Wednesday and Friday) with a dose of 75,000 larvae each. The remaining 14 calves that were primarily infected with 20,000 larvae received a single secondary dose of 350,000 larvae seven weeks after the primary dose.

All infected calves were slaughtered for P.M. examination in two equal groups at 38 and 40 days after the (first) secondary infection.

The calves in expt. III were born around April 1, 1977. The primary dose was administered on July 1, 1977. Calves were slaughtered on September 26 and 28, 1977.

3.3.3.2. Aims of design

When the calves of expt. III had to be purchased, only limited information on the results of expt. II was available. On the basis of egg counts and IFAT antibody titres of alternate weeks, two sires were chosen to be the fathers of offspring in expt. III. The 'resistant' sire was denoted 'ABH', the 'susceptible' sire was called 'PAN'.

The aim of expt. III was to investigate, in calves with a known reactivity, the reaction to continuous infection. Expt. II, however, had shown that a screening dose of 100,000 larvae had a huge immunizing effect and thereby prevented a lot of variation between animals after secondary infection. It was decided to give a lower screening dose of 20,000 larvae to 28 calves in this experiment. Fourteen animals received the same primary dose as in expts. I and II to allow comparisons between experiments. For the same reason of comparability, 14 of the calves which received a primary dose of 20,000 larvae were given the same re-infection as in expt. II: a single dose of 350,000 larvae. The other calves were given doses of 75,000 larvae three times a week as a feasible simulation of continuous infection. The number of larvae per dose was based on the number of larvae that could be cultured.

Subdivision of the total number of calves into different groups (sire groups and infection groups) had already reduced group numbers to seven. It was decided therefore to slaughter all calves within the shortest possible period to minimize the variation due to time of necropsy. Calves were slaughtered therefore in two groups, two days apart. The infection in repeatedly dosed animals was continued; therefore the calves of the second necropsy group were given one more dose of 75,000 larvae than animals of the first necropsy group. The time of necropsy was chosen so that calves could have shown the detrimental effects of infection but still could be compared with one of the necropsy groups in expt. II.

Transformations

When parametric statistical methods were applied, data were subjected, if necessary, to a logarithmic transformation. This necessity arose when dealing with results of parasitological counts (worms, eggs) and immunological data (titres).

Faecal egg counts

Observations on faecal egg output were treated by fitting a three parameter exponential function to individual egg count curves. This function is characterized by the following formula

$$E_t = \exp(A) \cdot \exp\left(\frac{TM - t}{K}\right) \cdot \exp\left\{1 - \exp\left(\frac{TM - t}{K}\right)\right\}$$

In this formula E_t is the egg count at day t after infection. This egg count can be described as a function of A, the peak egg count; TM, the time at which this peak egg count occurs, and K, which is a factor indicating the width of the curve. Thus if K is very small the curve rises very fast to a peak and decreases dramatically after the peak.

This function was fitted to the data by an iterative non-linear least-squares technique included in the computer program BMDX85 (DIXON, 1973).

Test on significance of treatment effects

The effects of treatments (experiment, sire, dose level, time of necropsy) were analysed by analysis of variance. For necropsy data in expt. I Tukey's test for nonadditivity was applied first. This test revealed no significant interaction between sire effect and effect of time of necropsy. Therefore a normal analysis of variance was also valid in this case.

Correlations

Correlation coefficients were in most cases computed as non-parametric rank correlation coefficients as given by Spearman. In a number of cases, when the distributions of data were suitable, a parametric product-moment correlation (Pearson) was calculated. Significance of correlation coefficients was judged by a two-tailed test.

Levels of significance

Generally in the description of results three levels of significance will be indicated, sometimes by asterisks. The levels used and the asterisk notation assigned to each are:

$P < 0.05$ *
$P < 0.01$ **
$P < 0.001$ ***

4. RESULTS

4.1. General comment on comparison of results between experiments

This study is based on the results of three experiments, carried out within a period of two years. Although all experimental procedures and conditions were kept as uniform as possible, considerable differences in infection results were found between experiments.

An important explanation for these unexpected differences has been found by combining the results of this study with previous work on experimental infections in calves with the same parasite (Kloosterman et al., 1978): There are highly significant differences in infection results (i.e. egg counts, worm length) between calves born in autumn and spring-born calves, when infected with a dose of 100,000 larvae at three months of age. Spring-born calves infected in early summer turned out to be the most susceptible (Albers et al., in prep.).

Expts. I and II of this study were carried out on autumn born calves; in expt. III spring-born calves were used.

Therefore between experiments results can only be compared very cautiously.

4.2. Primary infections

4.2.1. *Parasitological observations*

4.2.1.1. Results

4.2.1.1.1. Egg counts

The egg count curves after a primary infection showed a typical pattern, at least if group means are considered (fig. 1a, b). One should be aware, however, of the large variation between individual calves; this variation is shown in fig. 2 by the curves of two extreme calves in expt. I; the mean for all calves is depicted as a reference.

Although the frequency of observations does not allow a very accurate estimate of the prepatent period, it can be concluded that there was rather small variation in the time of patency of infection. Of 132 infected calves none had eggs in its faeces at day 14 p.i. In expt. I, 24 of 46 infected calves passed a very small number of eggs in their faeces on day 16; except for three calves the remainder joined them on day 19. Of 86 infected calves in expt. II and III, 81 passed worm eggs for the first time on day 17, irrespective of the dose level. So it can be concluded that virtually all primary infections reached patency at day 16 or 17.

After patency a rise in egg production occurred until a peak was reached around the 28th day. After this peak the egg counts declined very rapidly when a

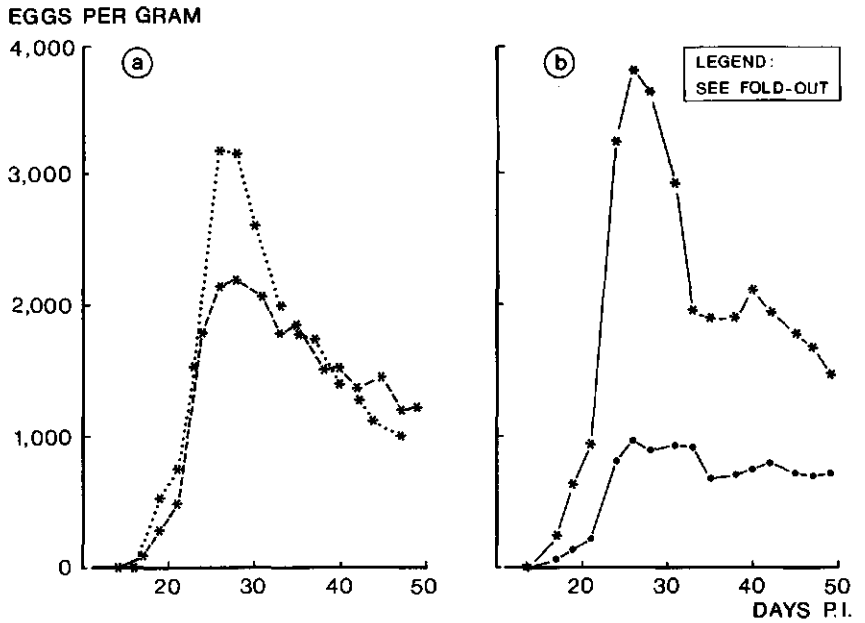

FIG. 1. Faecal egg counts following primary single doses of 100,000 infective larvae in expts. I and II (a) and doses of 20,000 and 100,000 in expt. III (b).

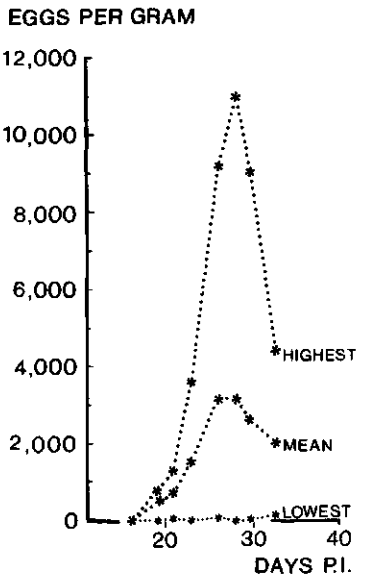

FIG. 2. Faecal egg counts of the calves with highest and lowest egg output and mean egg counts of 46 calves in expt. I.

dose of 100,000 larvae was given. Following a dose of 20,000 a much smaller decline, if any, was seen.

To make the information, contained by the individual egg count curves, more readily available, the three parameter function, as described in section 3.4, was fitted to the egg count data. The success of this procedure was evaluated by calculating for every individual calf the average of squared deviations of the estimated egg output from the observed egg output (i.e. unexplained mean square). This error estimate was related to the peak egg count to correct for scale. This 'deviation percentage' was less than 15% in 90 of 131 animals, lower than 30% in 125 calves. In only 6 of 131 animals a serious deviation of more than 30% was found. The curves of these calves, by showing two distinct peaks and generally low egg counts, were in essential disagreement with one of the basic assumptions for fitting the function, namely that only one peak occurs. Fig. 3 illustrates the findings described above by presenting observed and fitted curves of two calves, representing the two described categories. Fig. 3a shows the curves of a calf for which the fitting of the curve was satisfactory ('deviation percentage': 10.4). Fig. 3b presents a case in which the deviation percentage was 69.2.

The parameters resulting from these calculations are given in table 2. An additional value is given in this table: A divided by TM. This value, the peak egg count divided by the time needed to reach it, indicates the steepness of the rising part of the egg count curve. An estimate of total egg production following primary infection is given in table 2 by the sum of log-transformed egg counts during 7 weeks after primary infection.

The differences between expts. I, II and III, reflected by three groups of calves that received 100,000 larvae as a primary dose are only significant for A/TM

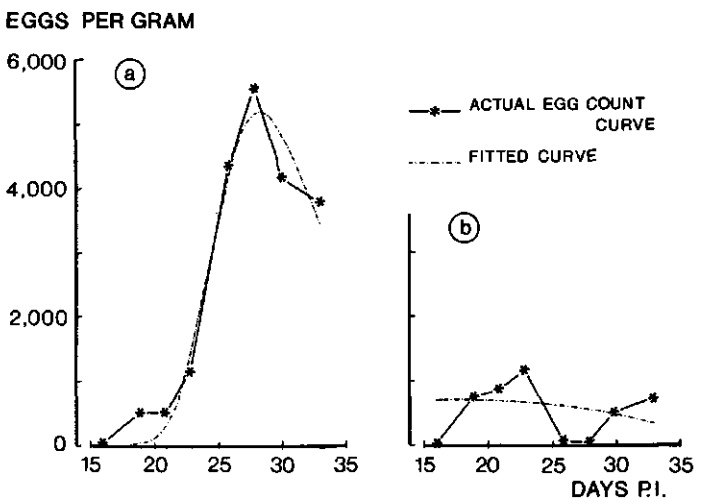

FIG. 3. Actual and estimated faecal egg count curves. An example of successful fitting (deviation % 10.4) (a) and an example of the opposite (deviation % 69.2) (b).

TABLE 2. Mean ± standard deviations of parameters of egg output after primary infections.

Dose level	100,000			20,000
Experiment (*n*)	I (46)	II (44)	III (14)	III (28)
A (peak egg count)	4.79 ± 1.32	4.74 ± 0.61	5.19 ± 0.61	3.89 ± 0.49
TM (time of peak egg count)	27.6 ± 3.7	28.3 ± 2.3	27.2 ± 2.2	29.2 ± 1.8
K (width of curve)	4.41 ± 4.89	5.03 ± 1.77	4.62 ± 1.28	6.67 ± 1.59
A/TM (steepness of increase)	0.18 ± 0.05	0.17 ± 0.03	0.19 ± 0.03	0.13 ± 0.02
Total egg output (log count during 7 weeks p.i.)	–	24.1 ± 5.4	26.7 ± 4.1	21.0 ± 2.4

($P<0.05$). When combined with results of other experiments, however, the difference in peak egg count (*A*) also proved to be significant and due to a seasonal effect (ALBERS et al., in prep.).

Because of this influence of season, the two primary dose levels can only be compared within expt. III. As table 2 and fig. 1 show, the egg counts following a dose of 20,000 larvae rose slower (*A/TM*; $P<0.001$), reached a lower peak (*A*; $P<0.001$) at a later stage of infection (*TM*; $P<0.001$) and resulted in a flatter curve (*K*; $P<0.001$) when compared with egg counts after a 100,000 dose. Total egg excretion of calves dosed with 20,000 larvae was significantly lower than in calves dosed with 100,000 larvae ($P<0.001$). Table 3 contains the Spearman rank correlation coefficients between the egg count parameters in expt. II. The results in other experiments were similar. From these correlations it can be concluded, that a higher total egg production resulted when *A*, *TM* and *K* were higher, i.e. when the surface under the egg count curve was larger. The correlation between *TM* and *K* is rather high; both were correlated with *A/TM* negatively: a flat curve had a slow rising part and a late peak. A flat curve, however, resulted in a higher total egg production. This is possible because there

TABLE 3. Correlation coefficients between egg output parameters after primary infection with 100,000 larvae in expt. II ($n = 44$).

	TM	*K*	*A/TM*	Total egg output
A	0.12	0.13	0.73***	0.72***
TM		0.62***	–0.51***	0.35*
K			–0.34*	0.51***
A/TM				0.35*

(*: $P < 0.05$; **: $P < 0.01$); ***: $P < 0.001$).

TABLE 4. Average results (± s.d.) of worm counts at three necropsies in expt. I.

Days p.i. (*n*)	34 (16)	41 (16)	48 (14)
No. of male worms	19,575 ± 7,763	14,669 ± 10,664	9,746 ± 8,071
No. of female worms	22,675 ± 8,257	18,113 ± 10,979	11,989 ± 8,621
Total no. of adults	42,250 ± 15,859	32,782 ± 21,412	21,736 ± 16,557
Percentage male worms	45.4 ± 4.1	41.5 ± 9.8	43.7 ± 9.0
Percentage *C. surnabada*	29.1 ± 8.3	32.6 ± 11.2	32.5 ± 10.7

is no relation of A with TM or K: a flat curve still could have a high peak egg count.

Although large differences in egg counts between sire groups were seen, most of these turned out to be non-significant due to the large variation within these groups. Only the peak egg count (A) was significantly different for the five selected sires that were represented by sons in expts. I and II ($P < 0.05$).

4.2.1.1.2. Worm counts

Necropsies after a primary infection were only carried out in expt. I following a dose of 100,000 larvae. Table 4 presents the results of worm counts in this experiment. Firstly it is clear that mean worm numbers declined steadily from day 34 to day 48 p.i. This decline is statistically significant ($P < 0.05$). However, the variation between calves was so large that the range of worm counts was almost the same at the three dates of necropsy. This is shown by histograms for each date of necropsy in fig. 4. At the first date the distribution is skewed to the left, at the last date the frequency distribution is skewed to the right. This feature explains the decline of the mean worm count.

The figures for the percentage of male worms found at each date of necropsy (table 4) suggest that the loss of worms was a selective process: male worm numbers decreased more than female worm numbers. This effect, however, is not significant, in contrast to the increase of the percentage *Cooperia surnabada* males which was found ($P < 0.05$). Apparently *C. oncophora* males were expelled more rapidly than *C. surnabada* males. This selectivity of worm loss for *C. oncophora* and for male worms is confirmed by the correlation between these parameters: their correlation was negative and became larger as time (and loss of worms) proceeded: -0.24 (N.S.), -0.42 ($P < 0.10$) and -0.62 ($P < 0.05$) for the three consecutive necropsy groups. Further support for this conclusion is found in the negative correlation between number of worms present at a certain time after infection and the percentage *C. surnabada* in this population (pooled r is

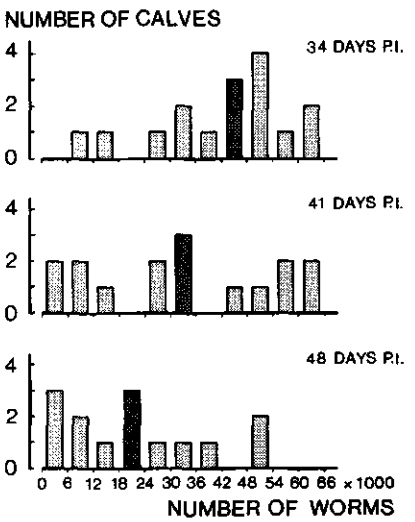

FIG. 4. Frequency distributions of calves according to worm burden in expt I. Shaded bars contain the average worm burden at a certain time of necropsy.

-0.47 ($P < 0.01$)). The relation between number of worms and the percentage male worms within necropsy groups was weaker: $+0.23$ (N.S).

Although the samples were not purposely examined for the presence of fourth stage larvae, the fact that none were found justifies the conclusion that larvae were not present in significant numbers.

No genetic effect on worm counts was found in expt. I: significant differences between sire groups were absent.

TABLE 5. Length measurements (mm) (mean ± s.d.) in expt. I for separate necropsy groups and total.

		34 days p.i. (16)	41 days p.i. (16)	48 days p.i. (13)	Total (45)
Females	average length	12.29 ± 0.81	12.15 ± 0.88	12.44 ± 0.55	12.29 ± 0.76
	s.d. of length	0.79 ± 0.18	0.85 ± 0.35	0.85 ± 0.22	0.83 ± 0.26
Males	average length	9.45 ± 0.57	9.29 ± 0.42	9.62 ± 0.37	9.44 ± 0.48
	s.d. of length	0.55 ± 0.10	0.61 ± 0.17	0.55 ± 0.09	0.57 ± 0.13
Male *C. onc.*	average length	9.58 ± 0.62	9.53 ± 0.39	9.81 ± 0.35	9.63 ± 0.48
Male *C. surn.*	average length	9.12 ± 0.48	8.92 ± 0.40	9.19 ± 0.31	9.06 ± 0.42

4.2.1.1.3. Worm length

As for worm counts, data on worm length in a primary infection are only available for a dose of 100,000 larvae (expt. I). Detailed results of worm length measurements are given in table 5.

A first conclusion from statistical analysis of these data is that female worms were much longer than male worms. Furthermore *C. oncophora* males were longer than *C. surnabada* male worms ($P < 0.001$).

The time of necropsy did not significantly affect worm length, although for male worm length the difference between necropsy groups was almost significant ($P < 0.06$). Because, however, no definite trend was seen, this finding probably is accidental.

Length variation between individual worms within calves was not different between necropsy groups and (relatively) the same for males and females (table 5).

A close relationship was found between male and female worm length: the correlation, $+0.77$, is significant at $P < 0.001$.

The relation between mean worm length and within calf standard deviation of worm length was very weakly negative and not statistically significant.

No significant effect of sire group on worm length was found in expt. I. However, some tendency was seen: male worm length was affected by sire group at a significance level of $P < 0.10$.

4.2.1.1.4. Number of eggs per female worm

The results of egg counts per female worm for expt. I are given in table 6. This number was markedly lower at the second date of necropsy, although the difference from the other dates is not quite significant ($P < 0.10$).

No genetic (sire group) effect on egg content of females was found, which is not surprising if the between calf variation is taken into account (table 6).

4.2.1.1.5. Relations between parasitological parameters

These relations, e.g. between egg counts and worm numbers, can only be studied in expt. I for a primary dose of 100,000 larvae.

It seems reasonable to suppose that a high egg output is the result of a large adult worm burden when at least the parasite population has an uninhibited opportunity to function. This was clearly not the case in expt. I: worms were being expelled during the period when calves were slaughtered. Table 7 shows however that the correlation between peak egg count and adult worm numbers

TABLE 6. The number of eggs per female worm (mean ± s.d.) for each necropsy group in expt. I.

Days p.i (*n*)	34 (16)	41 (16)	48 (13)
No. of eggs per female worm	29.2 ± 17.5	18.9 ± 18.6	29.7 ± 15.5

TABLE 7. Correlation coefficients between egg output and necropsy determinations in expt. I.

	A (peak egg count)				Actual egg count			
	34	41	48	total	34	41	48	total
No. of worms	0.37	0.81***	0.41	0.63***	0.52*	0.61*	0.34	0.61***
No. of eggs per female worm	0.39	0.26	–0.20	0.28	0.84***	0.78***	0.70**	0.72***
No. of worms × no. of eggs per female worm	0.41	0.64**	0.31	0.52***	0.91***	0.89***	0.68**	0.85***

was still positive. Apparently expulsion of worms had not proceeded so far that an initial difference in number of mature worms had completely disappeared.

The correlation between number of worms and 'actual' egg count (i.e. the last egg count before slaughter) was not higher than with peak egg count (table 7). An explanation for this is found in the fact that actual egg production correlates much better with the number of eggs per female worm, and therefore probably with egg production per female worm (table 7).

The very high correlations between actual egg count and the product of worm number and number of eggs per female show that the number of eggs per female is a good indication of the egg output per female. The number of eggs per female apparently did not vary in accordance with the number of worms present. It even seems that, when worm numbers had been decreasing for some time, individual egg production rose again as a compensation (table 6). The tendency of the correlation between worm numbers and number of eggs per female to change from positive to negative (0.35, 0.22 and –0.16 in the three consecutive necropsy groups) suggests the same.

Table 8 shows that mean worm length, which probably is constant after worms have matured, was greater in calves that reached a higher peak egg count. Apparently worm length is determined by the same or closely associated factors which allow a certain peak egg count to be reached. Worm length is also positively correlated with the numbers of worms present (table 8).

The negative correlations between worm length and percentage *C. surnabada* can have two explanations. Firstly *C. surnabada* males were shorter than *C. oncophora* males: average male worm length will be smaller if a larger proportion of (shorter) *C. surnabada* males contribute to this average. An analogous effect is likely to exist in female worms. However the length difference between the two polymorphs cannot be the only explanation. When the lengths of *C. surnabada* and *C. oncophora* males are correlated separately with the percentage *C. surnabada* these correlations are still negative, although somewhat lower. Thus it can be concluded that there is a true negative correlation between worm length and the percentage *C. surnabada*.

TABLE 8. Correlation coefficients between worm length and other parasitological observations at three necropsies in expt. I.

Days p.i.	34	41	48
A peak egg count) and			
male worm length	0.79***	0.62**	0.56*
female worm length	0.59*	0.43	0.63*
Total no. of worms and			
male worm length	0.39	0.73**	0.19
female worm length	0.46	0.57*	0.71**
Percentage *C. surnabada* and			
male worm length	–0.51*	–0.68**	–0.81***
male *C. surnabada* length	–0.37	–0.26	–0.62*
male *C. oncophora* length	–0.36	–0.56*	–0.63*
female worm length	–0.47	–0.61*	–0.78**

4.2.1.2. Discussion

Faecal egg production in a primary single infection of *C. oncophora* appears to be very variable. However, the same basic pattern could be recognized in all individuals. The length of the prepatent period hardly showed any variation between calves within groups which received the same dose level nor between groups which received different doses. This finding was in agreement with an earlier experiment designed to screen differences (in hours) in prepatent period, that showed a remarkably constant length of this period (unpublished).

This uniformity is in sharp contrast to the large variation among calves after patency. The general success, however, of fitting a relatively simple function to individual egg count curves, shows that all these curves were variations on one basic theme: a logarithmic increase from day 16 till a peak was reached (in most calves around day 28), followed by a logarithmic decrease of egg counts. Furthermore it appears that in calves that produced large numbers of eggs, which usually coincided with a high peak egg count, a large burden of long worms was found. Calves that produced less eggs harboured less and shorter worms. An obvious qualification for the latter combination of characteristics is the term 'resistance'.

Egg production and the number of worms are determined initially by the success of establishment of the infection. At a certain stage of infection however, adverse host effects become visible when, after a peak, egg counts decline and worm numbers decrease. Senility could also play a role here, but an experiment on the effect of immunosuppression shows that host responses at least contribute significantly (KLOOSTERMAN et al., 1974).

The results of expt. I show that egg production depends on two basic features: the number of (female) worms present and the egg production per worm. Apparently these two can vary differently. It is possible therefore that the initial decrease of egg counts is a result of a reduced egg production per worm. It is not clear at which stage of infection expulsion of worms starts and contributes to the

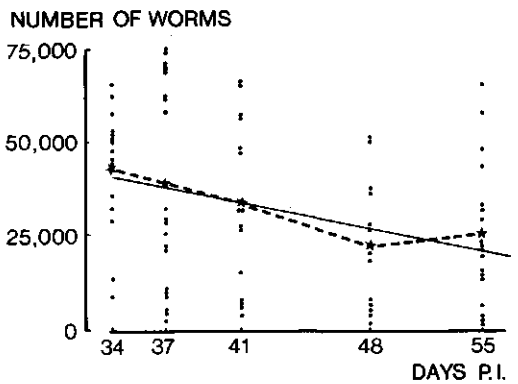

FIG. 5. Numbers of worms at different times after a primary single dose of 100,000 larvae in expt. I and previous experiments (KLOOSTERMAN et al., 1978).

decline of egg counts. The fact, however, that some calves showed a sudden drop of egg output to nearly zero at 26 or 28 days p.i. suggests very strongly that expulsion can take place as early as one week after patency. It cannot be concluded from expt. I whether the average worm burden had reached a more or less steady level at the last date of necropsy (48 days p.i.). A combination of the results of expt. I with results of previous experiments (KLOOSTERMAN et al., 1978) suggests that in most animals no dramatic changes in worm burden occur after this time (fig. 5). However differences between calves were still very large.

The egg counts after a dose of 20,000 larvae showed hardly any decline, once a certain level was reached. BORGSTEEDE and HENDRIKS (1979) also found this constant level of egg production with the same infection dose. They saw hardly any absolute decline of worm numbers during the first 8 weeks of infection. This proves that senility of worms is out of the question at least until 7 weeks p.i. Furthermore the threshold level of antigenic stimulation which provokes an operative host reaction (DINEEN, 1963) apparently was not reached in most animals infected with a dose of 20,000 larvae.

The process of expulsion seems to be selective in two ways: *C. oncophora* males were expelled more rapidly than *C. surnabada* males. Furthermore female worms seem to be more persistent than males; BORGSTEEDE and HENDRIKS (1979) observed the same phenomenon. Possibly the percentage *C. surnabada* and the percentage male worms could give an indication of the extent to which expulsion has affected the worm burden. In expt. I in three consecutive necropsy groups the proportions of calves that harboured more than 30% *C. surnabada* males were 31%, 50% and 69% respectively. One crucial question, however can not be answered: can the percentage *C. surnabada* males and the sex ratio also be influenced by the establishment rate or do they depend only on the expulsion rate. A strong indication that the expulsion rate largely or even completely determines the percentage *C. surnabada* is found after secondary infections (section 4.3): Although the establishment of a secondary infection was much lower than that of a primary dose the percentage *C. surnabada* in worms that did

establish was about the same as in the first necropsy group of expt. I (primary infection).

This finding is important when the generally negative correlations between worm length and percentage *C. surnabada* are considered. If the percentage *C. surnabada* reflects worm expulsion, these correlations (negative at the first necropsy) mean that expulsion was already taking place at day 34 p.i.

These correlations (negative and greater as time and expulsion proceeded) show anyhow that the most vigorous expulsion had occurred in animals that harboured the shortest worms. Thus host resistance seems to be a phenomenon that is expressed as several distinct, but maybe not independent, characteristics of the worm population (worm length, egg production, expulsion). Whether the establishment of incoming larvae in a primary infection is also related to the host's resistance is an open question that will be discussed later.

Egg output is the characteristic of the worm population in which all possible adverse effects exerted by the host can accumulate. A low establishment, retarded growth of worms, depression of individual egg production and an early and pronounced expulsion can all be expressed in a lower total egg output. Thus egg production could well be a very sensitive indicator of the parasite's wellbeing or its counterpart: host resistance. This sensitivity of egg production as a resistance parameter holds, of course, only when average egg production reaches a level sufficient to allow the expression of variation.

The above considerations might explain why the peak egg count is the parasitological parameter that appears to be most sensitive to genetic effects. However, the general conclusion from these experiments must be, that in primary single infections hardly any genetic effect on host resistance as measured by parasitological parameters was found.

4.2.1.3. Conclusions

- The prepatent period after an infection with 20,000 or 100,000 *C. oncophora* larvae was almost invariably 16 or 17 days.
- Egg output following these infections showed large individual variations, but a basic pattern of logarithmic increase and a subsequent logarithmic decrease could be seen in most calves at the 100,000 dose level.
- After a dose of 20,000 larvae egg output was generally much lower; after a peak was reached the egg counts remained at that level until at least 7 weeks p.i.
- In infections with 100,000 larvae, worms were expelled during the period 5 to 7 weeks after infection.
- Expulsion was selective in two ways: among male worms *C. surnabada* was more persistent than *C. oncophora* and, in the whole worm population, females were more persistent than males.
- The parasite population compensated for worm loss by increasing individual egg production, after an initial reduction.
- Female worms were longer than male worms (12.3 mm as against 9.4 mm).
- *C. oncophora* male worms were longer than *C. surnabada* male worms (9.6 mm as against 9.1 mm).

– Resistant calves showed a low egg output that was the result of a small burden of short worms.
– The genetic contribution to host resistance in primary single *C. oncophora* infections was found to be only very small in these experiments.

4.2.2. *Immunological observations*

4.2.2.1. Results

4.2.2.1.1. Immuno Fluorescent Antibody Test (IFAT)

The results of this technique, which was performed weekly, are presented graphically in fig. 6 for all experimental groups. The general form of antibody titre curves appeared to be very similar in all groups and experiments. None of the curves start at zero on the day before the first infection. In most of the calves a certain level of 'antibodies' was found before infection. Because these non-specific reactions were rather variable between calves it was decided to include the pre-infection titres in the analysis of results. Furthermore, it appeared that individual antibody titre curves could quite satisfactorily be characterized by the mean titre during the post infection period and the steepness of the rising part of the curve, expressed as the regression coefficient of antibody titre on time in days p.i. This was concluded from inspection of a matrix of correlations between these parameters and the individual antibody titres.

For the calculation of the three parameters the actual titres (the antibody titre is the reciprocal of the final serum dilution which shows antibody activity) were transformed first by taking the logarithm to base two. The mean titre was

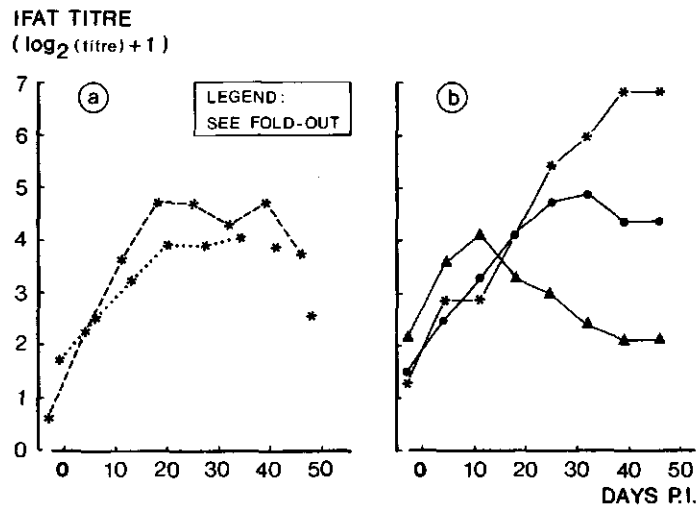

FIG. 6. IFAT titres after primary single doses of 100,000 larvae in expts. I and II (a); after doses of 20,000 and 100,000 and in control calves in expt. III (b). (Points that are not joined represent means of different numbers of calves.)

TABLE 9. IFAT parameters (mean ± s.d.) following a primary infection with 100,000 larvae in three experiments and the significance of differences between experiments for ABH and PAN calves.

Experiment (n)	I (46)	II (44)	III (14)	Significance of differences
Pre-infection titre	1.72 ± 1.42	0.61 ± 1.10	1.29 ± 1.20	***
Mean titre	–	3.58 ± 1.75	5.96 ± 1.81	**
Titre increase	6.71 ± 10.53	10.91 ± 8.73	13.29 ± 6.35	N.S.

TABLE 10. IFAT parameters (mean ± s.d.) as affected by the dose level in expt. III.

Dose level (n)	100,000 (14)	20,000 (28)	Control (7)
Pre-infection titre	1.29 ± 1.20	1.50 ± 1.40	2.14 ± 1.07
Mean titre	5.96 ± 1.81	4.94 ± 2.19	3.69 ± 0.97
Titre increase	13.29 ± 6.35	10.03 ± 5.51	– 0.47 ± 6.94

TABLE 11. Correlation coefficients between IFAT pre-infection titre and other IFAT titre parameters in all primarily infected groups and in control calves.

Dose level	100,000			20,000	Control
Experiment (n)	I (46)	II (44)	III (14)	III (28)	III (7)
Pre-infection titre mean titre	–	0.35*	0.31	0.67***	–0.22
Pre-infection titre titre increase	–0.03	–0.27	–0.49	–0.05	–0.87**

calculated from all observations during primary infection (7) and the pre-infection titre. The titre increase was derived from the pre-infection titre and the titres during the first five weeks of infection. The resulting value was multiplied by 100 to get workable numbers.

In fig.6 it can be seen that the reaction levels following primary infections varied considerably between experiments, even when the same infection dose was given. This is also illustrated in table 9 which shows the means of each

experiment of the three parameters that describe the IFAT titre curve. When tested only for those calves the sires of which were represented in all experiments, the differences between experiments were significant for the pre-infection titre ($P < 0.001$) and the mean titre ($P < 0.01$). The regression coefficient, which is not related to the overall level, was not significantly different between experiments.

This means that only comparisons within experiments are valid. A comparison of the effect of primary dose level can be made within expt. III. Table 10 shows that the uninfected control calves generally showed a decrease of IFAT titres during the period before secondary infection, whereas the infected groups showed a marked increase of titres. In the infected groups the increase of IFAT titres was somewhat higher in calves infected with a dose of 100,000 larvae ($P < 0.10$). Although the mean titre was higher for calves given 100,000 larvae than for those given 20,000 larvae, this difference was not significant.

Table 11 shows that in most calves a high pre-infection titre was followed by a high average IFAT titre level in infected groups. This is probably caused by an autocorrelation between these two parameters, which arises from the inclusion of the pre-infection titre in the calculation of the average titre level. The negative correlation between pre-infection titre and titre increase in the infected groups that showed the highest increases can similarly be caused partly by an autocorrelation. The result is that the highest increase is seen in calves with the lowest pre-infection titre. A similar tendency in the opposite direction is seen in control calves: titres decreased most in those with the highest pre-infection titre.

However, differences between sire groups, may have influenced these phenomena. The sires, which were selected partly on IFAT-titre increase also showed differences in pre-infection titre: in expts. II and III the sire effect on pre-

TABLE 12. Differences in IFAT parameters between ABH and PAN calves following a primary dose of 100,000 larvae in three experiments.

Expt.	Sire	(*n*)	Pre-infection titre	Mean titre	Titre increase
I	ABH	(3)	2.33 ± 1.15	–	17.96 ± 2.83
	PAN	(3)	3.00 ± 0.00	–	– 1.50 ± 9.08
II	ABH	(11)	0.00 ± 0.00	3.48 ± 1.93	14.84 ± 9.92
	PAN	(7)	1.29 ± 1.25	4.00 ± 1.34	10.03 ± 4.47
III	ABH	(7)	0.71 ± 1.11	5.33 ± 2.28	13.18 ± 7.16
	PAN	(7)	1.86 ± 1.07	6.59 ± 0.99	13.41 ± 6.00
Overall significance of difference between sires			***	–	*

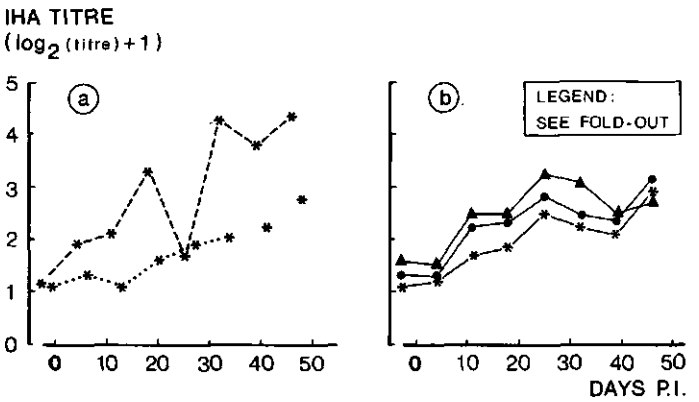

FIG. 7. IHA titres after primary single doses of 100,000 in expts. I and II (a); after doses of 20,000 and 100,000 and in control calves in expt. III (b).

infection titres was highly significant. Table 12 illustrates this for two sires that were ultimately selected. For mean IFAT-titres, differences between sire groups were smaller. IFAT titre increase was clearly affected by the sire within expt. I ($P < 0.01$); within expt. II this sire effect was not significant; within expt. III again a tendency was seen ($P < 0.10$) if all infected groups were included. Overall, as table 12 shows, in 100,000 infected group titre increase was higher in ABH calves than in PAN calves, although analysis of variance showed, by indicating a significant interaction between experiment and sire, that such a general conclusion is not without hazard.

4.2.2.1.2. Indirect HaemAgglutination test (IHA)

The results of the IHA test are presented graphically in fig. 7 for all experimental groups. Because this technique is based on the reaction of calf antibodies to adult stage antigens, the antibody response curves for this test were expected to be different from the results of the IFAT test (fig. 6). The following differences between the two tests occurred (cf. fig. 6 and 7):

- already at 4 days p.i. a marked increase in IFAT titres was seen whereas IHA titres increased later;
- the absolute titres reached were higher for the IFAT;
- the variation between calves was higher for the IFAT;
- the variation between experimental groups within expt. III was smaller for the IHA test;
- in expt. II large differences occurred in the mean IHA titres in successive weeks. This last phenomenon could be explained by the fact that the sera of weeks that gave higher titres had been thawed once before the IHA was performed.

As an overall impression at first sight it seems that the IHA test was neither as sensitive nor as specific as the IFAT procedure.

To perform a more detailed analysis of results, analogous parameters were derived from IHA antibody response curves as was done for IFAT curves (pre-infection titre, mean titre and titre increase). In the first place it was necessary to test for differences between experiments. Table 13 shows that these differences were considerable when the results of a primary dose of 100,000 larvae are compared in ABH and PAN calves in all three experiments. The pre-infection titres were the same in all experiments; however, the increase of IHA titres and therefore the mean titre after primary infection were very different ($P < 0.001$).

When, however, IHA titres are compared for groups that received different primary infections (table 14) none of the differences are significant. Moreover,

TABLE 13. IHA parameters (mean ± s.d.) following a primary infection with 100,000 larvae in three experiments and the significance of differences between experiments for ABH and PAN calves.

Experiment (*n*)	I (46)	II (44)	III (14)	Significance of differences
Pre-infection titre	1.10 ± 0.59	1.14 ± 0.84	1.11 ± 0.35	–
Mean titre	–	2.83 ± 0.61	1.96 ± 0.45	***
Titre increase	3.00 ± 2.78	6.74 ± 3.60	4.07 ± 2.47	***

TABLE 14. IHA parameters (mean ± s.d.) as affected by the dose level in expt. III.

Dose level (*n*)	100,000 (14)	20,000 (28)	Control (7)
Pre-infection titre	1.11 ± 0.35	1.36 ± 0.67	1.57 ± 0.67
Mean titre	1.96 ± 0.45	2.24 ± 0.58	2.54 ± 0.36
Titre increase	4.07 ± 2.47	4.37 ± 2.18	6.03 ± 3.32

TABLE 15. Correlation coefficients between IHA pre-infection titre and other IHA parameters in all primarily infected groups and in control calves.

Dose level	100,000			20,000	Control
Experiment (*n*)	I (46)	II (44)	III (14)	III (28)	III (7)
Pre-infection titre mean titre	–	0.62***	0.46	0.74***	0.00
Pre-infection titre titre increase	–0.28	–0.47**	–0.01	–0.26	–0.62

the differences that did occur were the reverse of those expected: the highest response was seen in uninfected controls and the highest primary dose caused the lowest rise in IHA titres. In the infected groups, the calves which had a higher pre-infection titre also had a high mean titre after primary infection and in most cases a lower titre increase than calves which had a low start titre (table 15).

Genetic differences in IHA antibody response were not found in primary infections.

4.2.2.1.3. IntraDermal Test (IDT)

The response criterion of the IDT test was simply defined as the total estimated wheal surface resulting from injection of two dilutions of antigen. The response is expressed in square centimeters (see section 3.2.1.2.). When this response is plotted against the date of the test, response curves result which are comparable with the curves of the other immunological tests (IFAT and IHA) (fig. 8).

For the analysis of results similar parameters were derived from these curves: the response just before the start of primary or secondary infection, the mean response and the increase of response during primary or secondary infection. Expt. I is omitted from the analysis because no regular observations were done from the start of the experiment.

The most prominent difference in response is seen between experiments (fig. 8). Table 16 confirms this clearly: the pre-infection response of calves primarily infected with 100,000 larvae was the same in expts. II and III. The increase during this primary infection and the mean response during this time were, in spite of the large variation, significantly different between experiments at $P < 0.001$. However, no difference was seen due to the dose level: in expt. III the response of control calves seemed to decline somewhat but roughly the reactions of control calves and calves dosed with 20,000 or 100,000 larvae were the same (table 17).

In primary infections a highly significant genetic effect was found in expt. II: the increase of IDT response differed between sire groups ($P < 0.01$). However,

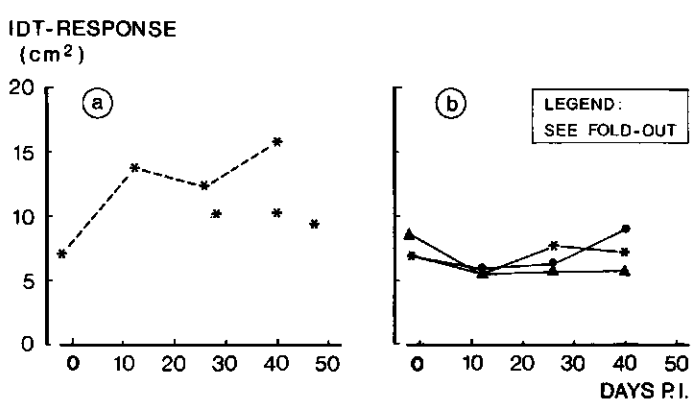

FIG. 8. IDT response after primary single doses of 100,000 larvae in expts. I and II (a); after doses of 20,000 and 100,000 and in control calves in expt. III (b).

TABLE 16. IDT parameters (mean ± s.d.) after a primary dose of 100,000 larvae in two experiments and the significance of differences between experiments for ABH and PAN calves.

Experiment (*n*)	II (44)	III (14)	Significance of difference
Pre-infection response	6.90 ± 6.11	6.95 ± 3.35	-
Mean response	12.20 ± 5.12	6.84 ± 1.88	***
Response increase	18.11 ± 21.93	− 0.11 ± 14.68	***

TABLE 17. IDT response parameters as affected by the primary dose level in expt. III.

Dose level (*n*)	100,000 (14)	20,000 (28)	Control (7)
Pre-infection response	6.95 ± 3.35	6.84 ± 6.61	8.60 ± 2.39
Mean response	6.84 ± 1.88	6.98 ± 4.20	6.48 ± 2.91
Response increase	− 0.11 ± 14.68	4.96 ± 16.31	− 5.80 ± 4.58

TABLE 18. Correlation coefficients in control calves between responses measured by three different immunological techniques.

		IHA		IDT	
		Pre-infection titre	Titre increase	Pre-infection response	Response increase
IFAT	Pre-infection titre	–0.22	–0.29	0.14	0.43
	Titre increase	0.55	–0.18	–0.04	–0.79*
IHA	Pre-infection titre			0.07	–0.89**
	Titre increase			–0.29	0.64

ABH- and PAN calves did not differ significantly in IDT response in any of the infected groups.

4.2.2.1.4. Relations between immunological parameters

In infected groups only incidentally a significant but low correlation was found between the responses measured by the three different immunological techniques. The highest correlations, however, were found in the uninfected control calves in expt. III (table 18). Only a few were significant due to the low

number of control calves. The table suggests a positive relation between IDT- and IHA response increase and a negative relation between IDT- and IFAT response increase. IFAT and IHA appear to be unrelated.

4.2.2.2. Discussion

The results of immunological observations suggest that only one of the methods used is of any value in studying primary infections: the IFAT-method. The IHA and IDT method did not discriminate between groups of calves which received different dose levels, or between infected calves and uninfected controls. An explanation for this finding could be that these methods do not reflect the presence of infection-induced antibodies (humoral and homocytotropic). However, the clear increase of IHA-titres in all groups including controls remains unexplained.

This increase could be caused by a reaction of the IHA method with some serum constituent that is not related to infection but which happened to increase in concentration for some unknown reason. Another explanation, which is more likely because it is not based on mere coincidence, could be an unintentional interaction between the test methods that were applied. The IDT method was based on intradermal injection of adult worm antigen at two week intervals. This procedure could have served as a kind of artificial immunization. Because the IHA was performed using exactly the same adult worm extract as antigen, it seems possible that the IHA in fact has measured the response to this immunization by the IDT injections. The positive relation between IHA and IDT response in the uninfected control calves in expt. III seems to support the latter explanation. It is not clear, however, how the IFAT method and its negative correlation with IDT response in control calves, can be fitted within this hypothesis. This issue will be discussed later when the results of secondary infections are presented.

The IFAT method seems to be of more value: a rise of titres occurred that was clearly dependent on the larval dose level (0; 20,000; 100,000). Already in the first week after infection a response was seen in all groups: apparently antibody production started very soon after dosing with larvae. Although in control calves the same increase was seen (fig. 6) the titre increase in infected calves was probably not due only to induction of a response by intradermal injection of antigen because in expt. I, in which the calves were not injected with antigen before day 28 p.i., the same rise of titres was seen. However antigen injection may have played a role: the steepest rise was seen in expt. II, the slowest rise was seen in expt. I; expt. III was intermediate. This correlates positively with the amount of antigen that was injected to perform the IDT method (see section 3.2.1.2.).

In expts. I and II no increase of IFAT titres was seen from around day 20 p.i. onwards, suggesting that the mature adult stage of the parasite was not an important antigenic stimulus. In expt. III, especially in the 100,000 group the peak IFAT titre had not been reached at 3 weeks p.i. However, from this time only a relatively small and slow increase occurred. The later finding that secondary infection boosted IFAT-titres very strongly confirms that antigenic stimu-

lation must have faded out in the course of primary infection.

Whether the overall differences between experiments, especially in IFAT titre level, were due to the test methods or reflected true (seasonal) differences in calves and/or course of infection must remain an open question.

Because selection of sires was partly based on IFAT titre increase, it was expected that sire differences for this parameter would increase in consecutive experiments. This result was not found, at least in primary infections, although overall ABH and PAN, the sires ultimately selected, were different in this respect. The effect of selection of sires appeared to be much larger in an indirect way for a correlated parameter: the IFAT titre before infection.

This raises the question whether this pre-infection titre is as non-specific as it was supposed to be. The best answer to this question can be given by investigating the relation between IFAT-titres and parasitological observations, as will be done in section 4.2.4.

4.2.2.3. Conclusions

- There were large differences between experiments in the results of immunological observations.
- These differences may have reflected true differences in host reaction due to seasonal factors or may have been caused by the test procedures applied.
- The IHA and IDT methods did not detect differences between calves given different levels of infection or between uninfected and infected calves.
- Some results indicated that the intradermal injection of antigen in the IDT method had provided an antigenic stimulus.
- The IHA titres may partly have reflected this unintentional immunization procedure.
- IFAT titres did discriminate between groups given different levels of infection: the response was higher following a higher larval dose.
- A firm increase of IFAT titres was seen from the first observation after infection until the parasite population had reached maturity.
- Genetic selection on IFAT titre increase following a primary infection proved not to be very successful. However, genetic differences for this parameter did clearly exist, including between the two sires that were ultimately selected.
- An indirect effect of selection was found for the IFAT titre before infection.

4.2.3. *Other observations*

4.2.3.1. Results

4.2.3.1.1. Live weight gain

When the growth curves of the experimental groups are compared it is clear at first sight that the infections had no dramatic effect on live weight gain (fig. 9). When a more detailed analysis of live weight gain is made, however, some effect can be seen. Fig. 10a, b presents live weight gains of the experimental groups from 5 weeks prior to infection until the day of necropsy (expt. I) or the day of

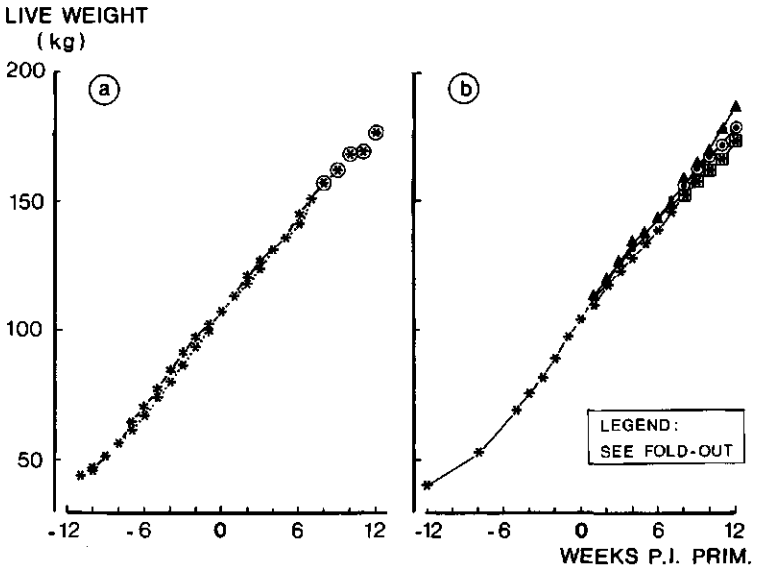

FIG. 9. Live weight curves of experimental groups of calves in expts. I and II (a) and in expt. III (b).

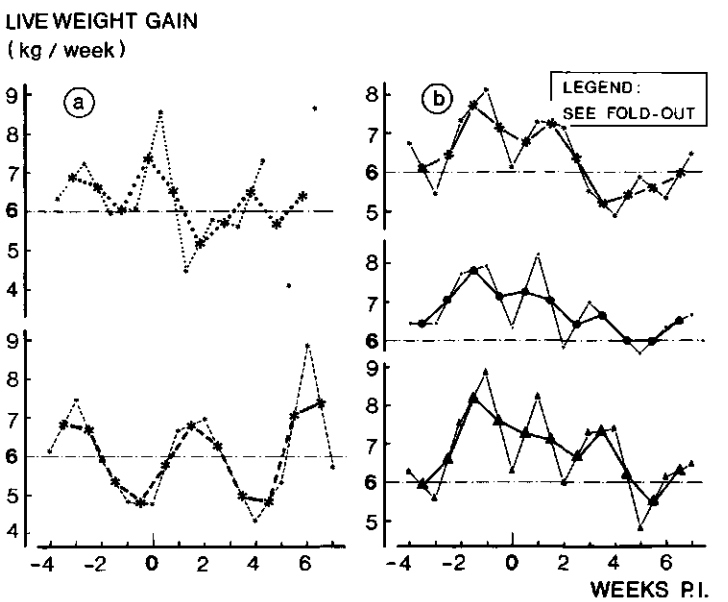

FIG. 10. Weekly live weight gains before and after primary infection in expts. I and II (a) and in expt. III (b). Smaller symbols represent original observations, larger symbols are graphical interpolations.

TABLE 19. Live weight gains (grams/day) (mean ± s.d.) in calves given 100,000 larvae in three experiments.

Experiment (*n*)	I (46)	II (44)	III (14)
5 weeks before infection	984 ± 134	838 ± 153	971 ± 88
4 weeks p.i.	836 ± 178	852 ± 157	895 ± 123
7 weeks p.i.	–	894 ± 129	873 ± 104

TABLE 20. Live weight gains in grams/day (mean ± s.d.) as affected by the primary dose level in expt. III.

Dose level (*n*)	100,000 (14)	20,000 (28)	Control (7)	Significance of differences
5 weeks before infection	971 ± 88	997 ± 94	988 ± 54	–
4 weeks p.i.	895 ± 123	982 ± 124	1036 ± 105	*
7 weeks p.i.	873 ± 104	943 ± 102	942 ± 99	$P < 0.10$

secondary infection (expts. II and III). Because live weight gain per week, calculated as the difference between two consecutive weekly weighings, appeared to be a very variable parameter, a simple form of smoothing of curves has been applied in fig. 10: New points were constructed by interpolating between two consecutive points.

In expt. II a rather serious growth depression occurred a few weeks before infection. This phenomenon was due to an unidentified respiratory disease, which caused clinical signs in a large number of calves.

In both expts. I and II a depression of growth was seen in the post infection period: in expt. II around the fourth week; in expt. I the depression was less clear and more spread in time.

In expt. III (fig. 10b) a comparison of the effect of different dose levels can be made. It seems that a larger growth depression occurred in calves given 100,000 larvae than in those given 20,000 larvae (significant in the third and sixth week p.i. at $P < 0.05$). Until 4 weeks p.i. the growth of control calves was slightly better than of infected calves. After that time, however, growth of control calves seemed to be depressed too. Especially in the fifth week weight gain of these control calves was very low (4.8 kg).

The above findings are confirmed by tables 19 and 20 in which live weight gains per calf per day are presented for the 5 week pre-infection period and for

periods of 4 and 7 weeks after infection. Expt. III gives the most complete information on the effect of infection on live weight gain: growth of infected calves can be compared with their own growth in the pre-infection period and with the growth of control calves. From these comparisons the conclusion can be drawn that an infection of 100,000 larvae reduced growth by 70 g per day during a period of 7 weeks after infection. If there was any effect on growth from a dose of 20,000 larvae this was virtually compensated within 7 weeks p.i. In expt. III the effect of dose level on live weight gain was significant at $P < 0.05$ for the 4 weeks post infection period and also at $P < 0.05$ for the period of 7 weeks after infection.

It is well known that live weight gain is partly genetically determined. In expt. I, which used a random sample of bull calves from the Dutch Friesian population, genetic differences were indeed found ($P < 0.01$) for live weight gain prior to infection. However, these genetic differences did not continue in the post infection period, suggesting that infection somehow masked the genetic growth potential.

In expt. II no sire effect on growth before or after infection was found. This may have been due to a correlated selection effect: as table 21 shows, the sires of expt. II, which were selected only for their extreme IFAT titres and egg counts, in fact all had a very similar and high genetic growth potential. This effect probably reduced the between sire variation in growth potential. For expt. III a similar selection was made. In this experiment no sire effect on live weight gain was found before infection. After infection a difference was present and significant at $P < 0.01$ (table 22) but the same difference was seen in control calves over the 7 week p.i. period. During the first 4 weeks after infection the sire group difference

TABLE 21. Average live weight gains per sire group (kg.) during 5 weeks prior to infection and ranking of sires according to this in three consecutive experiments.

	Expt. I		Expt. II		Expt. III	
Sire	L.w.gain	Rank	L.w. gain	Rank	L.w. gain	Rank
BM	39.0	1				
KA	38.0	2				
VI	38.0	2	29.0	3		
AG	37.7	4				
BJ	37.0	5	28.2	4		
PAN	36.7	6	32.6	1	34.8	1
SB	36.5	7				
ABH	36.0	8	30.8	2	34.4	2
KT	34.0	9	26.1	5		
SKO	33.7	10				
P55	33.0	11				
NG	32.7	12				
BL	32.7	12				
BS	31.7	14				
FG	30.0	15				
TSK	25.0	16				

TABLE 22. Live weight gain in grams/day (means ± s.d.) before and after infection as affected by the sire in expt. III.

Dose level	100,000		20,000		Control	
Sire (*n*)	ABH (7)	PAN (7)	ABH (14)	PAN (14)	ABH (4)	PAN (3)
5 weeks before infection	951 ± 90	992 ± 88	1002 ± 76	992 ± 113	971 ± 40	1009 ± 72
4 weeks p.i.	852 ± 84	939 ± 147	931 ± 92	1033 ± 133	1018 ± 118	1060 ± 103
7 weeks p.i.	840 ± 54	907 ± 134	899 ± 73	987 ± 111	905 ± 130	980 ± 61

seems to be larger in infected calves than in control calves (table 22), but this was not significant.

As a general conclusion it can be stated that live weight gain is determined partly by genetic factors. Following infection, additional genetic factors may play a role.

4.2.3.1.2. Feed intake

Only in expt. II, feed intake was recorded before and during a primary infection. Fig. 11a presents means and standard deviations of hay intake of 17 calves calculated as a mean per week of daily observations. Because these calves

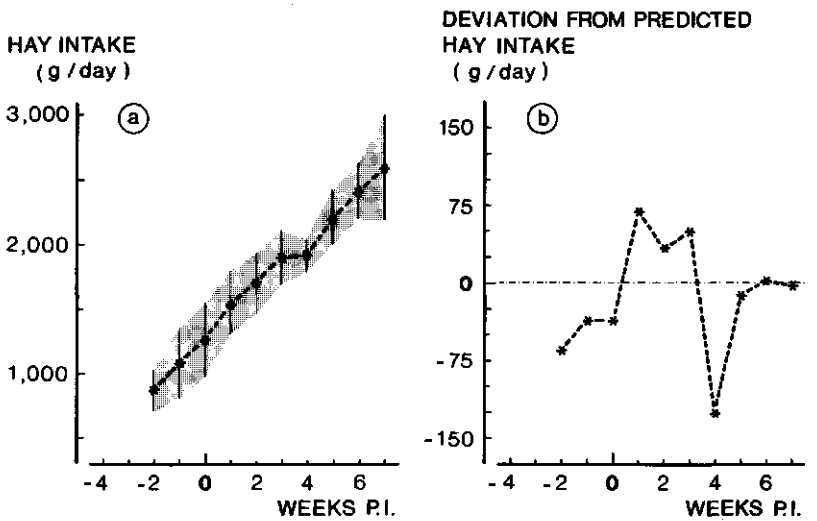

FIG. 11. Absolute hay intake (mean ± s.d.) of 17 calves before and after a primary dose of 100,000 in expt. II (a).
Hay consumption of the same calves expressed as the deviation of actual from predicted hay intake (b).

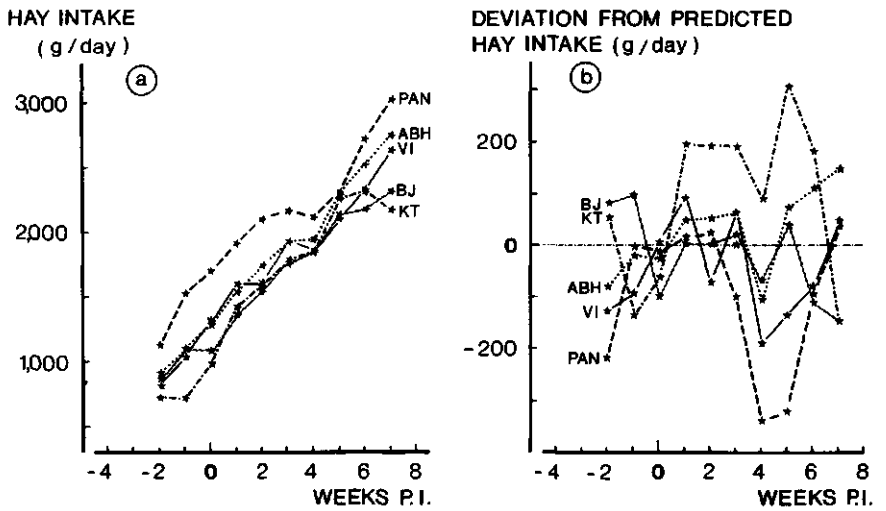

FIG. 12. Average hay intakes of five sire groups in expt. II (a). Average hay intakes of five sire groups in expt. II expressed as deviation of actual from predicted intake (b).

(around 3 – 4 months of age) were in a period of fast growth and because intake of concentrates was kept at a maximum of 2 kg. per calf per day, the steady increase of hay intake could be expected. This increase was quite constant, about 180 g. each week, except for the fourth week after infection, when the same amount of hay was eaten as in the week before. The variation in hay intake was relatively small and showed a typical pattern: the standard deviation decreased from the day of infection to a very low level in week 4 p.i. and increased again after that time.

Fig. 11b gives the means of deviations of observed hay intake from the intake predicted by the following regression equation:

$$y = a + 184.820\,x$$

in which y is the predicted hay consumption; x is the week after infection and a is the hay intake in the week of infection. This a was calculated for individual calves as the average hay intake in weeks -1; 0 and $+1$. The regression coefficient was calculated from hay consumption data of the 17 calves in expt. II in the period 2 weeks before to 3 weeks after primary infection and of 6 control calves in expt. III during the period 7 to 12 weeks after primary infection (fig. 33). In these 'unaffected' calves the correlation between predicted and observed hay consumption was 0.998. All these calculations were carried out on average intake data per week.

Generally fig. 11b confirms the conclusion from fig. 11a: a striking depression of hay intake occurred in the fourth week after infection.

Because hay intake was recorded for only 17 calves in expt. II, the number of calves per sire group was very limited (2; 3 or 4 animals). However, highly significant differences between these sire groups were found. Fig. 12a shows

TABLE 23. Levels of significance for sire group differences in hay intake and intake-deviation before and during primary infection in expt. II.

Week p.i.	Hay intake	Deviation from predicted hay intake
-2	.01	.05
-1	.001	.05
0	.01	ns
1	.05	ns
2	.001	ns
3	.10	ns
4	.05	.10
5	ns	.10
6	.001	ns
7	.05	ns

average hay intake per sire group each week. The results of analysis of variance (table 23) show that sire differences were significant in 8 out of 10 weeks, nearly significant in week 3 and not significant in week 5. The figure suggests that the largest depression of hay intake occurred in the calves that were at the highest level. Therefore, average hay intakes per sire group converged in the critical period after infection (cf. fig. 12a). Fig. 12b shows that indeed differences in intake depression varied greatly in time. In table 23 it can be seen that sire differences in intake depression were almost significant in week 4 and 5, whereas in other weeks differences were much smaller.

Sire differences in intake depression before infection were probably caused by a respiratory disease that occurred at that time.

As has been mentioned, every calf was given 2 kg. of concentrates every day. Fig. 13a shows for each week the number of calves that did not eat these

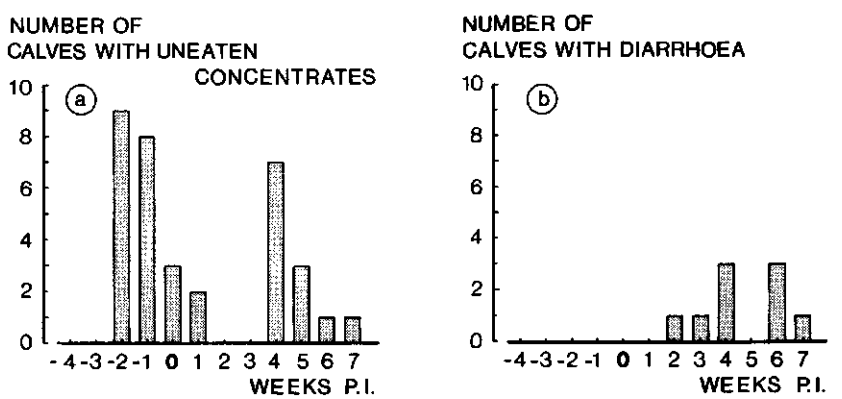

FIG. 13. a) Number of calves out of a total of 17 in expt. II that left concentrates uneaten before and during a primary infection of 100,000.
b) Number of calves out of a total of 44 that showed severe diarrhoea in a primary infection of 100,000 in expt. II.

concentrates completely on one or more days in that week. In the weeks before infection and the first week p.i., feed requirement had apparently not yet reached the level at which all concentrates were eaten. In the second and third week however, all calves consumed their 2 kg. of concentrates every day. In the 4th week after infection, the week of depressed hay intake, 7 out of 17 calves left variable amounts of concentrates uneaten on one or more days. This same phenomenon was seen in a decreasing number of animals in subsequent weeks.

4.2.3.1.3. Faecal consistency

The consistency of faeces was recorded daily in expts. II and III. It was hard to distinguish between normal and subnormal faeces, so only the incidence of evident diarrhoea was used as a reliable parameter.

Fig. 13b shows the numbers of calves with diarrhoea in expt. II. Only 10 out of 44 calves showed scours on one or more days, most of them in week 4 or 6 after infection. In expt. III only two cases of diarrhoea were recorded: one calf in the third week after an infection of 20,000 and one calf in the fourth week after an infection of 100,000 larvae.

4.2.3.2. Discussion

Cooperia oncophora infections are known to have only moderate pathogenicity. This is confirmed by the results of this study in which primary infections had only very small effects on weight gains and general levels of feed intake.

However, it cannot be denied that some effects were seen: a primary single dose of 100,000 larvae caused a mild growth depression, a measurable loss of appetite and in some calves a short period of conspicuous diarrhoea. All of these phenomena coincided at around the fourth week after infection.

A dose of 20,000 larvae had no significant effect on live weight gain.

From the various observations the conclusion seems to be justified that at 7 weeks after infection all infected calves had recovered completely: growth and hay intake were at a normal level again.

The analysis of differences between sire groups shows that in calves growth and feed intake were determined partly by genetic factors. It seems that the detrimental effects of infection were also related to the genetic background of the animals: infection masked differences that were present before infection (live weight gain, expt. I; hay intake, expt. II) or caused differences that were not present without infection (live weight gain, expt. III; deviation from expected hay intake, expt. II). The results of expt. II even suggest that the largest effect of infection is seen in calves with the highest genetic potential for feed intake. Of course this may only be a coincidence caused by the selection of these particular five sire groups.

4.2.3.3. Conclusions

- A primary single infection of 100,000 *C. oncophora* larvae caused only a slight but significant growth depression.
- This growth depression coincided with a notable loss of appetite.

– In a number of calves diarrhoea was seen.
– The critical period in which these phenomena occurred was around four weeks after infection.
– A dose of 20,000 larvae caused hardly any perceptible effects.
– In calves unaffected by infection, growth, and to a greater extent feed intake, appeared to be genetically determined.
– The detrimental effects of infection masked existing genetic differences or caused genetic differences to arise when they were not seen before.

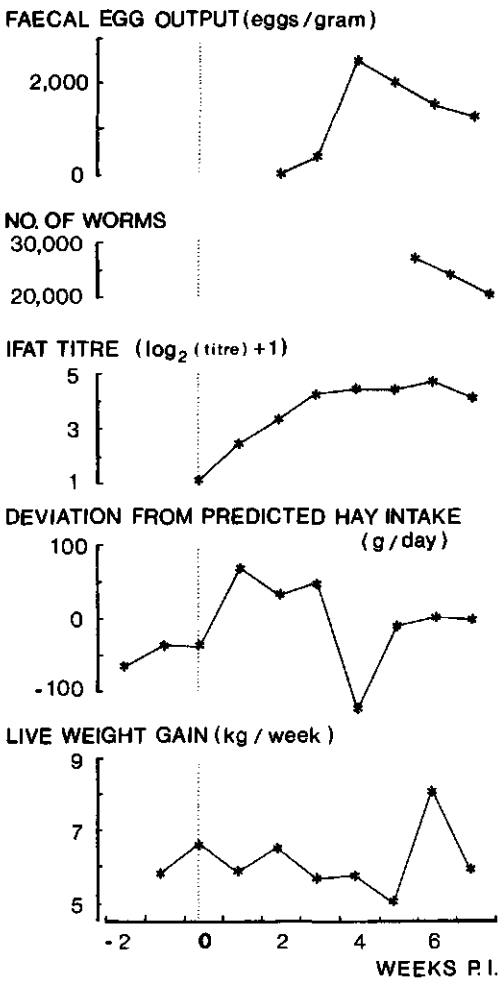

FIG. 14. The course of various parameters in calves infected with a primary single dose of 100,000 larvae.

4.2.4. *Relations between parasitological, immunological and other parameters*

4.2.4.1. Results

Fig. 14 summarizes on one time scale the levels and course of various parameters which were measured in these experiments. All lines represent weekly averages of all calves that had received a primary dose of 100,000 larvae and in which a certain parameter was measured. Therefore the lines are based on different numbers of calves. The most striking conclusions from a comparison of different parameters during the course of infection are the following: IFAT titres had reached their maximum level when egg excretion started to rise (week 3). The depression of hay intake was most clear when egg output was at a maximum and had started to decline again. The most serious growth depression was seen somewhat later than intake depression and coincided with the initial decline of egg output. During week 6 p.i. a conspicuous compensatory live weight gain occurred while hay intake had reached a normal level again. Although the animals seemed to have recovered in this respect, the egg output and the number of worms harboured was still decreasing significantly.

Thus host performance was affected at the very moment that host reaction against the parasite seemed to be maximal (IFAT titres) and had started to act on the parasite population (egg excretion, worm numbers). It cannot be determined from fig. 14, however, whether this is a quantitative relationship or just a coincidence. Therefore, a quantitative analysis of the mutual relationships between the various parameters was carried out by calculating correlations.

An analysis of the relations between parasitological and immunological parameters could give information on the value of the applied immunological me-

TABLE 24. Correlation coefficients between peak egg count (*A*) and IFAT titre parameters in primary infections.

Expt.	Dose level	*n*	Pre-infection titre	Titre increase
I	100,000	46	0.13	–0.46**
II	100,000	44	0.21	–0.07
III	100,000	14	0.12	–0.18
III	20,000	28	–0.16	0.16

TABLE 25. Correlation coefficients between peak egg count (*A*) and IFAT titre increase in the various sire groups.

		Sire group				
Expt.	Dose level	ABH	VI	KT	PAN	BT
II	100,000	–0.53	–0.22	–0.12	0.43	0.49
III	100,000	–0.10			–0.02	
III	20,000	0.54*			0.27	

thods as a tool for a direct assessment of host reaction to the parasite. As far as the IDT and IHA methods are concerned, no consistent significant correlations with any of the parasitological observations in any of the primary infections was found. Results of the IFAT method apparently were correlated with egg output but in a rather complex way. Table 24 presents Spearman correlations between IFAT parameters and peak egg count. Only in expt. I was a significant negative correlation found between IFAT titres (increase) and peak egg count. A more detailed analysis however showed that the relation between IFAT increase and peak egg count was dependent on the sire group. Table 25 illustrates this for the various sire groups in expts. II and III. In expt. II an interesting difference between sire groups is seen: ABH and VI calves, selected as resistant groups, showed negative correlations, whereas especially PAN and BJ calves, selected as susceptible groups, showed positive correlations between peak egg count and IFAT titre increase. (In KT calves, however, which were also selected as susceptible, a slight negative correlation was found). These differences between sire groups, although not significant, explain why the overall correlation between peak egg count and IFAT titre increase in expt. II was virtually zero (table 24). In expt. III differences between sire groups were smaller. When a dose of 20,000 was given, the positive correlation between peak egg count and IFAT titre increase was significant in ABH calves.

Thus the relation between egg counts and IFAT titres seems to depend on the extent to which a reaction against the worm population is displayed: after a low dose the relation is positive in all calves; after a higher dose (100,000) the relation is still positive in susceptible groups but negative in more reactive animals. Because IFAT titres, at least partly, reflected differences in reactivity (see differences between sire groups and differences between dose levels) this leads to the conclusion that the relation between egg counts and IFAT titres varies according to the IFAT titre level. Indeed, regression analysis revealed a significant curvilinear relationship between peak egg count and IFAT titre increase. In this regression analysis the regression line fitted to the experimental data could be described by the following formula: $A = A_c + b(I - I_c)^2$, in which A is the peak egg count, I is IFAT titre increase. A_c stands for the constant, i.e. the peak egg

TABLE 26. Results of fitting a quadratic regression line to describe the relation between peak egg count and IFAT titre increase in various primarily infected groups.

Expt.	Dose level	(n)	Fitted regression line	Significance of		
				total regression	linear component	quadratic component
I	100,000	(46)	$A = 5.31 - 0.0033\,(I - 0.14)^2$	$P < 0.001$	n.s.	$P < 0.10$
II	100,000	(44)	$A = 4.98 - 0.0031\,(I - 10.86)^2$	$P < 0.01$	$P < 0.05$	$P < 0.01$
III	100,000	(14)	$A = 5.35 - 0.0039\,(I - 10.91)^2$	n.s.	n.s.	n.s.
Pooled	100,000	(104)	–	$P < 0.001$	n.s.	$P < 0.01$
III	20,000	(28)	$A = 3.99 - 0.0019\,(I - 14.93)^2$	n.s.	n.s.	n.s.

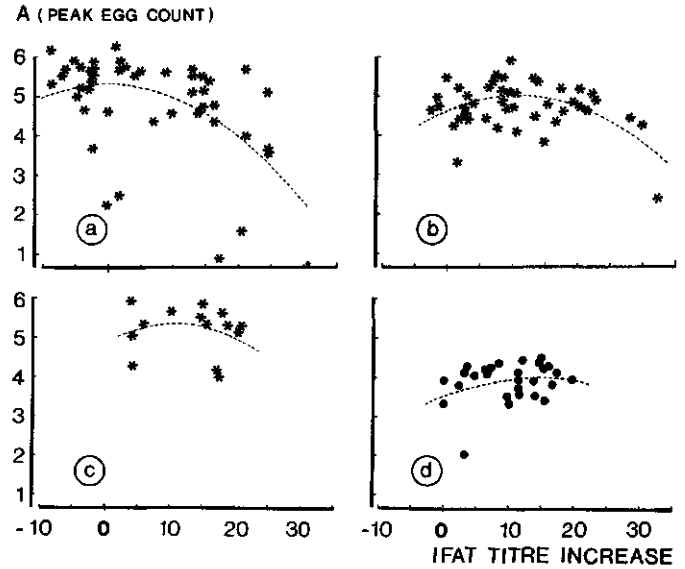

FIG. 15. The relation between A (peak egg count) and IFAT titre increase after a primary dose of 100,000 larvae in expt. I (a) expt. II (b), expt. III (c) and after a dose of 20,000 larvae (d).

count if IFAT titre increase equals I_c, which is the titre level at which the relation turns from positive into negative; b is the regression coefficient. The formula describes a function which is symmetrical around the inflection point. Table 26 presents the results of this analysis for various primary infection groups. A graphical presentation is given in fig. 15, a – d. It can be seen that in all groups convex regression lines fit best. The quadratic component of regression is significant at $P < 0.01$ in expt. II and nearly so in expt. I ($P < 0.10$). When the results of fitting all 100,000 dose groups are pooled it can be concluded that the quadratic term of regression significantly ($P < 0.01$) contributes to the description of the relation between peak egg count and IFAT titre increase (table 26).

In 20,000 infected calves no significant relation was found. It is, however, interesting to see (fig. 15d) that most calves are along the rising part before the inflection point of the (non significant) regression line.

A significant relation was also found between worm length and IFAT titre increase in expt. I. The correlation coefficient between male worm length and IFAT increase was –0.41 ($P < 0.01$); the correlation between female worm length and IFAT increase was –0.38 ($P < 0.01$). This relation was not significantly deviant from linear.

As was seen in section 4.2.1.1.5. (table 8) worm length was quite well related to peak egg count in expt. I. However, regression analysis showed that IFAT titre increase explained a significant part of the variance in peak egg count that could not be explained by worm length. This means that in some calves egg output was significantly depressed by a high IFAT increase although long worms were present. This phenomenon is illustrated in fig. 16, that shows estimated (on the

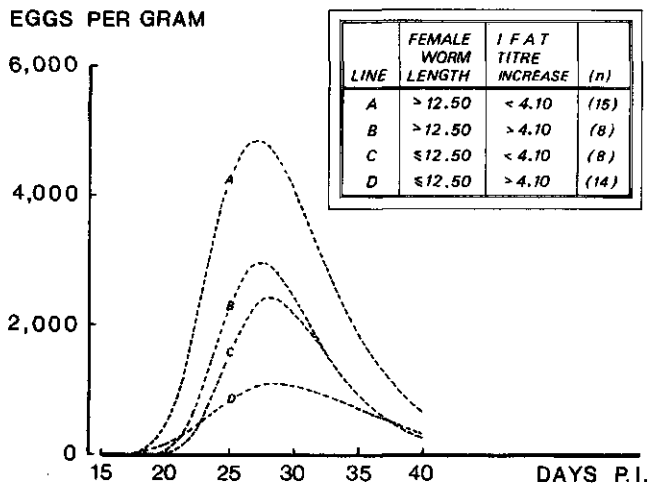

FIG. 16. Estimated faecal egg count curves of groups of calves with combinations of high and low values (above and below median) for female worm length and IFAT titre increase (expt. I).

basis of averaged parameters) egg count curves of groups of calves with combinations of high and low values for worm length and IFAT titre increase. Apparently worm length is determined at an earlier stage of infection than peak egg count and therefore is less affected by a host response which is measured by means of IFAT titres. More evidence for this conclusion is found in table 27: if IFAT increase did not reach the mean level of 6.71 in expt. I, worm length correlated very well with the steepness of the rise in egg counts (A/TM) and with peak egg count (A). If, however, IFAT increase was higher than 6.71, worm length was still well correlated with A/TM, the correlation with peak egg count was lower and no longer highly significant.

IFAT titres were not related to parasitological findings other than egg counts and worm length.

Evidence for interrelationships between parasitological and immunological parameters on the one hand and weight gain or feed intake on the other, was found only in expt. I. As table 28 shows, the results suggested that calves that responded better to the parasites (higher IFAT titre increase, shorter worms,

TABLE 27. Correlation coefficients between worm length and egg output parameters in calves with low and high IFAT titre increase in expt. I.

IFAT increase		n	A/TM	A
	Male worm length	24	0.68***	0.70***
< 6.71	Female worm length	24	0.51*	0.63***
	Male worm length	21	0.64**	0.42
> 6.71	Female worm length	21	0.55**	0.44*

TABLE 28. Correlation coefficients between some infection parameters and live weight gain in the critical period in expt. I (n = 30).

	Live weight gain during week 5 p.i.	Minimal live weight gain in weeks 3 to 6 p.i.
IFAT titre increase	–0.32	–0.44*
Male worm length	0.39*	0.33
Female worm length	0.47**	0.51**
A (peak egg count)	0.29	0.16

lower egg counts) were the ones of which the live weight gain in the critical period was affected the most. This finding was not confirmed by other experiments.

4.2.4.2. Discussion

The results described in section 4.2.2. suggested that only the IFAT method was related to infection, because differences were seen between groups of calves that were given different infection doses. Section 4.2.4.1. indeed revealed a relation between IFAT titre increase and worm length and peak egg count. No relation with worm numbers was found. This finding, together with the fact that IFAT titres reached a peak level when egg counts rose, suggests that the IFAT method measures the reaction of the host in an early stage of infection. This was not surprising because the IFAT method was based on attachment of antibodies to 4th stage larvae.

Although egg counts were quite well related to worm length and both were related to IFAT increase, these interrelationships did not completely parallel each other: low peak egg counts could be found in calves with high titres and long worms. An explanation for this phenomenon could be that worm length is determined during the prepatent period. In some calves a reaction against the worm population was already effective during this period, in others the worms were affected by the host only after patency of the infection.

The curvilinear relationship between egg counts and IFAT increase suggests the existence of a kind of threshold level: below this threshold calves do react to the parasites by producing antibodies, they produce even more antibodies if the worm population is larger (higher egg counts); beyond this threshold level antibody production is correlated to antiparasitic activity of the host.

Because necropsies were carried out at a late stage of infection, expulsion had greatly influenced worm counts. The absence of a relation between IFAT titres and any of the worm count parameters therefore indicates that host factors which are not related to IFAT titres, play a dominant role in expulsion of worms.

The finding of most serious growth impairment in those calves which showed the 'best' (measured) host reaction against the parasite in expt. I was not repeated in subsequent experiments. An explanation for this difference between experiments might be derived from the fact that live weight gain as well as worm length (although less evident) and IFAT titres appeared to be genetically determined in expt. I. Selection of sires for subsequent experiments influenced the genetic

predisposition of experimental calves for IFAT titres and worm length but also, although unintentionally, for growth potential. The absence of a correlation between live weight gain and resistance parameters in expts. II and III might therefore be an artefact due to genetic selection. The opposite explanation, i.e. that genetic predispositions for low growth potential and high resistance accidentally occurred in combination with each other in expt. I, is improbable because of the number of sires (16) and the fact that these sires were a random sample from the Dutch Friesian population.

4.2.4.3. Conclusions

- No relation was found between the results of the IHA or IDT method and any of the parasitological infection parameters.
- Shorter worms were found in calves that showed a higher IFAT titre increase.
- Evidence was found for a curvilinear relationship between peak egg count and IFAT titre increase.
- Below a certain threshold level of IFAT increase a positive relation between peak egg count and titre increase was found; beyond this level the relation turned into a negative one.
- The results indicated that most calves which received 20,000 larvae and some of those given 100,000 larvae, especially those in sire groups selected as susceptible ones, did not reach this threshold level.
- IFAT titre increase was not related to worm numbers or to expulsion of worms.
- In expt. I growth impairment appeared to be larger in those calves which showed a higher IFAT titre increase and harboured shorter worms.

4.3. Secondary infections

4.3.1. *Parasitological observations*

4.3.1.1. Results

4.3.1.1.1. Egg counts

Fig. 17a shows that a secondary dose of 350,000 larvae caused no perceptible rise of egg counts after a primary dose of 100,000 larvae (group 2). After a primary dose of 20,000, however, a significant rise of egg counts could be seen (fig. 17b). The increase of egg output started at day 17 after re-infection (group 4). Repeated dosing after a primary 100,000 dose (group 3) hardly produced any rise of egg counts; repeated dosing after a primary dose of 20,000 (group 5) resulted in a marked rise of egg output which seemed to be somewhat lower, however, than in group 4 (single secondary dose).

The prepatent period can not easily be determined because egg output was still continuing at the time of secondary infection. As an estimate, however, the day of patency is defined as the day after secondary infection at which the first of at

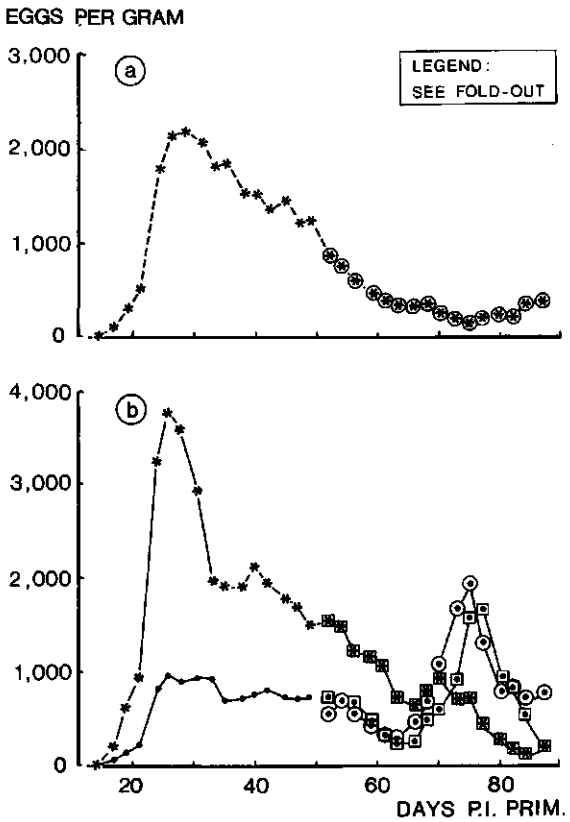

FIG. 17. Faecal egg counts during primary and secondary infection in group 2 (a) and groups 3, 4 and 5 (b).

least two consecutive egg counts exceeded the preceding count. Table 29 shows that in secondary infections the day of patency was delayed considerably compared to primary infections. In particular, repeated infections (groups 3 and 5) markedly prolonged the prepatent period.

TABLE 29. Patency* of secondary infection in various infection groups.

Infection group	*n*	Prim. inf/ sec. inf. dose (thousands)	Average prepatent period (days)	% of calves patent before 20 days p.i. sec.
2	11	100/350	20.3	73
3	14	100/17 × 75	25.6	29
4	14	20/350	20.1	57
5	14	20/17 × 75	23.8	36

* Definition: see text.

TABLE 30. Means ± standard deviations of parameters of egg output after secondary infections.

Infection group	2	3	4	5
Primary dose level	100,000		20,000	
Secondary dose(s)	350,000	17 × 75,000	350,000	17 × 75,000
(*n*)	(11)	(14)	(14)	(14)
A	–	2.70	3.77	3.36
(peak egg count)		± 1.76	± 1.49	± 1.62
TM	–	26.5	28.2	29.5
(time of peak egg count)		± 5.8	± 5.2	± 4.1
K	–	6.93	5.62	3.27
(width of curve)		± 6.03	± 3.32	± 2.27
A/TM	–	0.12	0.14	0.12
(steepness of increase)		± 0.09	± 0.07	± 0.07
Total egg output	7.0	6.6	10.9	8.2
after sec. infection	± 2.2	± 5.3	± 5.3	± 6.4
(until slaughter)				

TABLE 31. Correlation coefficients between egg output parameters following primary and those following secondary infections.

Infection group	2	3	4	5
Primary dose level	100,000		20,000	
Secondary dose(s)	350,000	17 × 75,000	350,000	17 × 75,000
A	–	0.57*	0.41	0.33
(peak egg count)				
A/TM	–	0.35	0.01	0.21
(steepness of increase)				
Total egg output	0.02	0.61*	0.38	0.45

Table 30 presents the egg count parameters that were, in the same way as in primary infections, calculated from the observations on egg output. The table confirms the conclusions from fig. 17. Statistical analysis of these parameters showed that the variation between individual calves (group members) was very large; so large that none of the differences between groups 3, 4 and 5 is significant. Analysis of variance shows that the effect of primary dose level (in groups 3 and 5) nor the effect of different secondary infection (in groups 4 and 5) is statistically significant.

The relation between egg output after primary and after secondary infection is presented in table 31 by means of correlations, for three egg count parameters calculated on egg count data, between primary and secondary infection. (In group 2 most animals were killed too early to allow enough observations on egg

TABLE 32. Egg count parameters (mean ± s.d.) for sire groups ABH and PAN following secondary infections in expt. III.

Infection group	Prim. inf./ sec. inf. (thousands)	Sire	(*n*)	*A* (peak egg count)	*A/TM* (steepness of increase)	Total egg output
		ABH	(7)	2.36	0.10	5.6
3	100/17 × 75			± 1.92	± 0.09	± 5.5
		PAN	(7)	3.04	0.13	7.6
				± 1.67	± 0.09	± 5.2
		ABH	(7)	2.80	0.09	7.6
4	20/350			± 1.19	± 0.03	± 3.5
		PAN	(7)	4.75	0.19	14.2
				± 1.09	± 0.06	± 4.8
		ABH	(7)	2.70	0.09	5.5
5	20/17 × 75			± 1.68	± 0.06	± 5.7
		PAN	(7)	4.02	0.15	10.8
				± 1.38	± 0.06	± 6.4
Overall significance of difference between sires				**	**	**

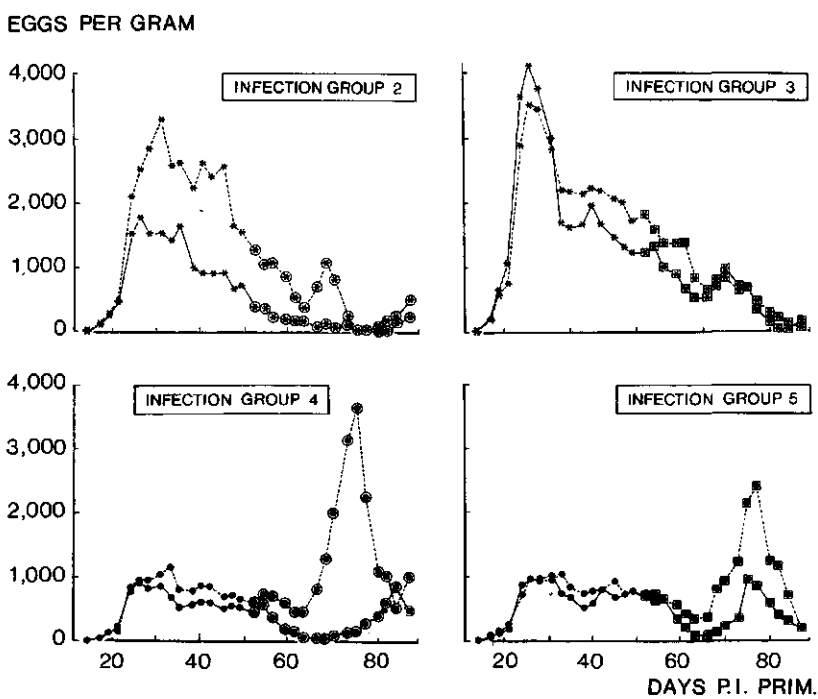

FIG. 18. Faecal egg counts in sire groups ABH (solid line) and PAN (dotted line) in infection groups 2, 3, 4 and 5.

production). From table 31 it can be concluded that the egg output of an animal after secondary infection was positively related to its egg count after primary infection: all calculated correlations were positive although only two were significant.

When genetic differences in egg counts are analysed it appears that differences between sire groups were more clear after secondary infection than after primary infection, at least in groups which showed any increase of egg output after secondary infection. Table 32 and fig. 18 present egg count data after secondary infection for the two sire groups in expt. III, ABH and PAN. From both it can be concluded that egg output was higher in PAN calves. When the overall sire effect is evaluated statistically, it appears that in ABH calves after secondary infection the rise of egg counts was slower (A/TM; $P < 0.01$), a lower peak was reached (A; $P < 0.01$) and total egg production was lower ($P < 0.01$).

4.3.1.1.2. Worm counts

The results of worm counts in expt. II (group 2) are presented in table 33. The first necropsy in this experiment was done at the time that the worms of the second dose could have reached maturity. Apparently at least some of them had reached the adult stage, because the number of adult worms was considerably larger than the number that could have persisted from the primary dose (cf. table 4). However, one week later, adult worm numbers had decreased to approximately 20,000 and stayed there during subsequent necropsies. Thus it is not clear when the process of worm loss had begun, but it apparently had terminated in most calves much sooner than in a primary infection.

The decrease in the percentage of male adults was not significant; the increase in the percentage *C. surnabada* was nearly so ($P < 0.10$). At day 18 a considerable

TABLE 33. Results of worm counts (mean ± s.d.) at four necropsies in expt. II (primary dose 100,000; secondary dose 350,000 larvae).

Days p.i.sec. (n)	18 (11)	25 (11)	32 (11)	39 (10)
No. of male worms	29,489 ± 28,516	7,983 ± 10,125	8,995 ± 13,832	8,455 ± 12,861
No. of female worms	34,798 ± 33,542	11,753 ± 11,856	13,284 ± 15,910	12,561 ± 15,280
No. of adult worms	64,287 ± 62,024	19,735 ± 21,309	22,279 ± 28,997	21,016 ± 27,913
No. of larvae	15,427 ± 28,321	16,797 ± 20,767	2,835 ± 8,820	3,674 ± 8,380
Percentage male worms	45.3 ± 3.5	39.1 ± 11.3	33.7 ± 13.3	37.0 ± 16.8
Percentage *C. surnabada*	30.9 ± 9.1	36.9 ± 7.6	35.6 7.1	35.2 ± 9.3

number of larvae was present. Apparently the development of worms had been retarded considerably. In contrast to adult worms these larvae were still present one week later. Between days 25 and 32 after secondary infection the majority of larvae was lost. This suggests that the control of the levels of larval and adult worm numbers is based on different mechanisms or at least different stimuli. An alternative explanation might be that larvae are more resistant than adult worms to the mechanism that caused their loss.

TABLE 34. Results of worm counts (mean ± s.d.) at two necropsies in infection group 3 (primary dose 100,000; secondary infection: repeated doses of 75,000 larvae).

Days p.i. sec. (*n*)	38 (8)	40 (6)	Mean
No. of male worms	14,616 ± 21,530	6,581 ± 5,756	10,599
No. of female worms	21,357 ± 27,134	13,147 ± 8,533	17,252
No. of adult worms	35,973 ± 48,397	19,728 ± 14,115	27,851
No. of larvae	44,958 ± 41,199	47,847 ± 41,032	46,403
Percentage male worms	34.0 ± 12.5	29.7 ± 9.7	31.8
Percentage *C. surnabada*	25.5 ± 11.8	36.2 ± 9.7	30.9

TABLE 35. Results of worm counts (mean ± s.d.) at two necropsies in infection group 4 (primary dose 20,000; secondary dose 350,000 larvae).

Days p.i. sec. (*n*)	38 (6)	40 (8)	Mean
No. of male worms	37,596 ± 30,581	11,088 ± 17,396	24,342
No. of female worms	46,853 ± 34,857	16,856 ± 17,856	31,855
No. of adult worms	84,449 ± 64,933	27,944 ± 34,703	56,197
No. of larvae	2,659 ± 3,076	188 ± 530	1,424
Percentage male worms	42.6 ± 10.2	31.7 ± 21.8	37.2
Percentage *C. surnabada*	31.4 ± 17.8	41.3 ± 13.1	36.4

TABLE 36. Results of worm counts (mean ± s.d.) at two necropsies in infection group 5 (primary dose 20,000; secondary infection: repeated doses of 75,000 larvae).

Days p.i. sec. (*n*)	38 (7)	40 (7)	Mean
No. of male worms	33,396 ± 35,912	12,951 ± 12,600	23,714
No. of female worms	43,049 ± 42,603	22,717 ± 19,434	32,883
No. of adult worms	76,446 ± 78,441	35,668 ± 31,936	56,057
No. of larvae	61,183 ± 59,871	32,817 ± 26,583	47,000
Percentage male worms	42.4 ± 5.9	30.5 ± 8.4	36.4
Percentage *C. surnabada*	26.6 ± 6.1	35.1 ± 6.4	30.9

TABLE 37. Numbers of adult worms at 39 days after various combinations of primary and secondary infections.

		Secondary infection	
		350,000	17 × 75,000
Primary infection	20,000	56,197*	56,057*
	100,000	21,106	27,851*

(* mean of necropsies at 38 and 40 days)

Tables 34, 35 and 36 give the results of worm counts in the three groups (3, 4 and 5) in expt. III respectively. The mean number of adult worms seems to be lower in group 3 (high primary dose) than in groups 4 and 5 (low primary dose). The effect of primary dose on the number of adult worms found after repeated secondary infection is not statistically significant, however ($P < 0.15$). When the results of expts. II and III are combined (table 37) a clear confirmation of this effect of primary infection results: after a primary dose of 20,000 considerably more adult worms were found in a secondary infection than after a dose of 100,000. On the other hand, as table 34, 35 and 36 show, the average number of larvae found after secondary infection was not affected by the primary dose level. Analysis of variance within expt. III however showed, that the number of larvae found depended largely on the type of secondary infection given, i.e. single vs. repeated ($P < 0.001$). Table 38 shows that this finding is in agreement with the results of expt. II: after repeated secondary infection massive numbers of larvae were found irrespective of the primary dose; after a single secondary dose there were only a few thousand.

TABLE 38. Numbers of larvae at 39 days after various combinations of primary and secondary infections.

		Secondary infection	
		350,000	17 × 75,000
Primary infection	20,000	1,424*	47,000*
	100,000	3,674	46,403*

(* mean of necropsies at 38 and 40 days)

Although the two necropsy groups in expt. III were slaughtered only two days apart, the differences in worm counts were very large (table 34 to 36). Worm numbers apparently were not yet at a stable level. This is in contrast to expt. II, where average adult worm number had already stabilized at day 25 after secondary infection. Whether this very rapid fall of worm numbers was natural or somehow artificially induced will be discussed later. An interesting feature in expt. III is that worm loss seems to be proportional to the number present: groups 4, 5 and 3, which harboured 84,449; 76,446 and 35,973 adult worms at the first necropsy retained 33%, 47% and 55% of these numbers respectively, at the second necropsy.

The number of larvae showed a significant decrease between the two times of necropsy in group 4 only ($P < 0.05$). In group 3 the number of larvae at the second time of necropsy was even larger than at the first time.

The results of expt. III allow a clear conclusion on the selectivity of worm loss. When tested in an overall analysis of variance it appeared that male worms had

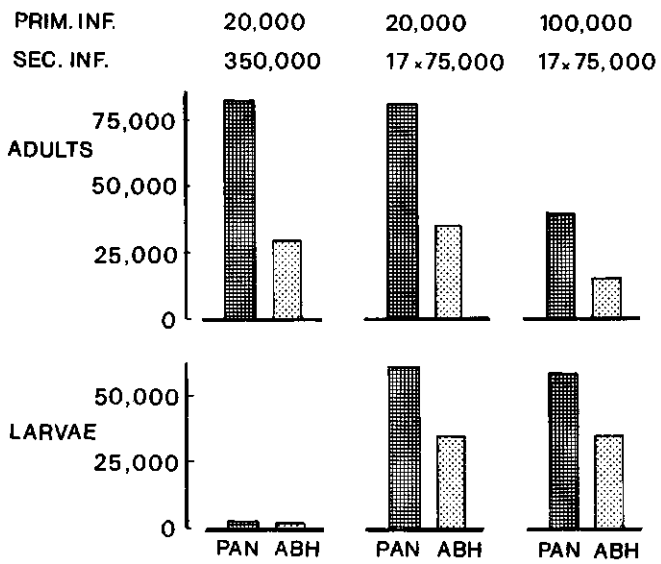

FIG. 19. Mean parasite counts in sire groups ABH and PAN in three infection groups in expt. III.

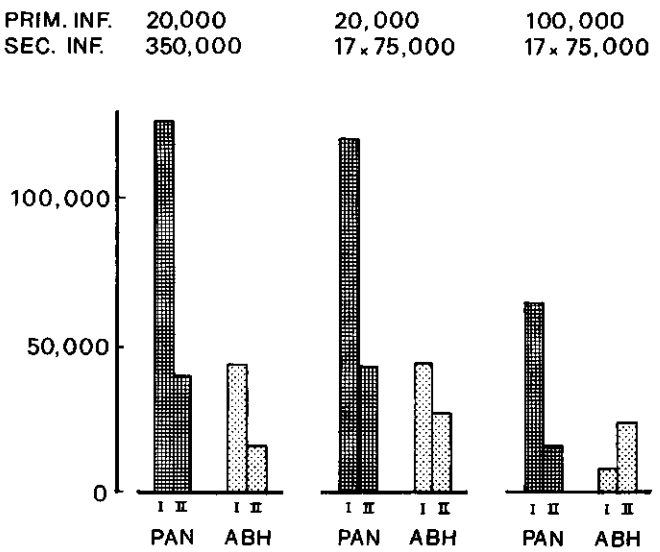

FIG. 20. Mean adult worm numbers at two times of necropsy (I: 38 days p.i. sec.; II: 40 days p.i. sec.) in sire groups PAN and ABH in three infection groups in expt. III.

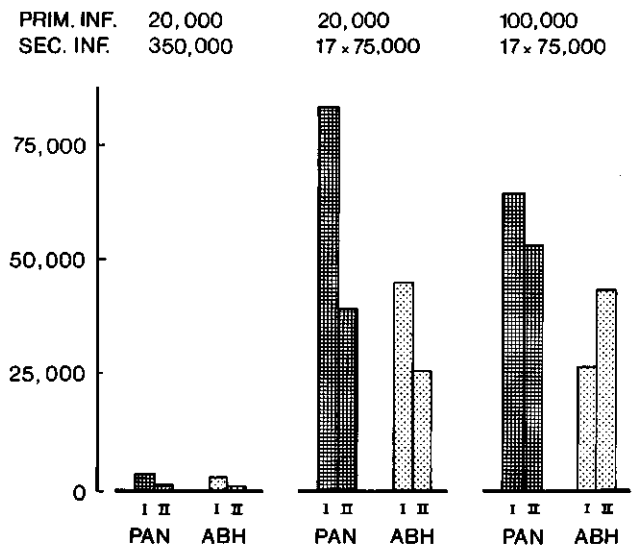

FIG. 21. Mean numbers of larvae at two times of necropsy (I: 38 days p.i. sec.; II: 40 days p.i. sec.) in sire groups PAN and ABH in three infection groups in expt. III.

been expelled to a larger extent than female adults ($P < 0.05$). Furthermore *C. oncophora* numbers decreased more than *C. surnabada* numbers ($P < 0.05$).

Except for a different percentage *C. surnabada* ($P < 0.05$), no differences in worm counts between the 5 sire groups of expt. II could be found.

The two sires that were ultimately selected in expt. III (ABH and PAN) showed some notable differences in worm counts. Fig. 19 displays adult worm numbers for these sire groups in infection groups 3, 4 and 5. Overall the difference between the two groups is nearly significant ($P < 0.06$). This difference is the result of a significant difference in number of females ($P < 0.05$) and a non-significant difference in number of male adult worms. Although similar differences in number of larvae between these sires were present (fig. 19) these were not significant.

Fig. 20 shows that PAN calves tended to display more worm loss than ABH calves but this feature was not significant. Larval counts again showed similar features (fig. 21) but even less clearly.

4.3.1.1.3. Worm length

Separate assessments of length of *C. oncophora* and *C. surnabada* male worms were not included in P.M. examinations in secondary infections. Average male and female worm lengths after secondary infection are shown in table 39. Generally, adult worms were much shorter than after primary infection (cf. table

TABLE 39. Length (mean ± s.d.) of male and female worms (in mm) in various secondary infections.

Infection group	Prim. inf./ sec. inf. (thousands)	Sex	Mean length	Mean length at necropsy on day					
				18	25	32	38	39	40
2	100/350	♀	9.55 ± 1.26	9.72 ± 1.50	9.07 ± 1.42	9.83 ± 1.18		9.65 ± 1.18	
		♂	7.55 ± 0.87	7.32 ± 0.90	7.38 ± 1.07	7.82 ± 0.82		7.74 ± 0.65	
3	100/17 × 75	♀	8.81 ± 1.37				8.27 ± 1.14		9.56 ± 1.40
		♂	6.56 ± 1.11				6.33 ± 0.77		6.83 ± 1.38
4	20/350	♀	10.91 ± 0.96				10.83 ± 1.32		10.96 ± 0.67
		♂	8.22 ± 0.57				8.11 ± 0.73		8.31 ± 0.45
5	20/17 × 75	♀	9.31 ± 0.74				8.96 ± 0.74		9.66 ± 0.60
		♂	6.93 ± 0.85				6.88 ± 0.79		6.97 ± 0.98

5). Both primary dose level and the type of secondary infection (single or repeated) affected worm length. As table 39 shows, the repeated infection (groups 3 and 5) produced shorter worms than the single secondary dose (groups 2 and 4) (significant within expt. III). After a primary dose of 20,000 larvae longer worms were found than after a primary dose of 100,000. This effect of primary dose level is not significant within expt. III, but the results of expt. II agree very well with group differences within expt. III in this respect (table 40). A (reducing) seasonal effect on worm length in expt. II can, however, not be excluded (see section 4.1).

TABLE 40. Female worm length (mm) following various combinations of primary and secondary infections.

		Secondary infection			
		(None)	350,000	17 × 75,000	Mean
Primary infection	20,000	(–)	10.90	9.31	10.11
	100,000	(12.29)	9.57	8.92	9.25
	Mean		10.24	9.12	

TABLE 41. Within calf standard deviations (mean ± s.d.) of male and female worm length in various secondary infections (mm).

Infection group	Prim. inf./ sec. inf. (thousands)	Sex	St. dev.	St. dev. at necropsy on day					
				18	25	32	38	39	40
2	100/350	♀	1.52 ± 0.57	1.72 ± 0.58	1.85 ± 0.65	1.23 ± 0.42		1.30 ± 0.41	
		♂	1.22 ± 0.43	1.41 ± 0.43	1.36 ± 0.43	0.98 ± 0.41		1.07 ± 0.37	
3	100/17 × 75	♀	1.68 ± 0.39				1.77 ± 0.40		1.54 ± 0.38
		♂	1.11 ± 0.25				1.14 ± 0.21		1.06 ± 0.32
4	20/350	♀	1.15 ± 0.37				1.32 ± 0.40		1.02 ± 0.29
		♂	0.76 ± 0.28				0.93 ± 0.36		0.63 ± 0.10
5	20/17 × 75	♀	1.77 ± 0.42				1.83 ± 0.33		1.72 ± 0.52
		♂	1.29 ± 0.21				1.25 ± 0.19		1.33 ± 0.24

TABLE 42. Average within calf standard deviations (mm) of female worm length at 39 days after various combinations of primary and secondary infections.

		Secondary infection			
		(None)	350,000	17 × 75,000	Mean
Primary infection	20,000	(–)	1.15*	1.77*	1.46
	100,000	(0.83)	1.30	1.68*	1.49
	Mean		1.23	1.73	

* Mean of calves necropsied at 38 and 40 days

The variation of adult worm length within calves is characterized by the standard deviations (table 41), which were also markedly affected by secondary infection. From a comparison with primary infection (cf. table 5) it can be concluded that, although average worm length was lower in secondary infections, length variations increased dramatically. For female worms for instance the standard deviation after repeated reinfection (groups 3 and 5) was about double that in the primary infection (1.77 mm as against 0.8 mm). Expressed in the coefficient of variation, the increase was almost threefold (7% to 19%). If calves had received a secondary infection, the secondary infection regimen (single vs. repeated) apparently was the factor determining length variation of worms; the primary dose level was of less importance. After a primary dose of 20,000, repeated dosing resulted in a larger variation than a single secondary dose ($P < 0.001$). A combination of results of expt. II and III (table 42) suggests a similar effect in calves that received 100,000 larvae as a primary dose. The difference between group 3 (primary dose 100,000) and group 5 (20,000) was not significant.

The combined effects of infection on average adult worm length and on length variation of worms emerge when relative frequency distributions of worm length are drawn. This is done in fig. 22 for female worm length in all infection groups. As a general trend it appears that frequency distributions were flatter and had a lower peak when the total amount of infective larvae given was higher. In groups 3

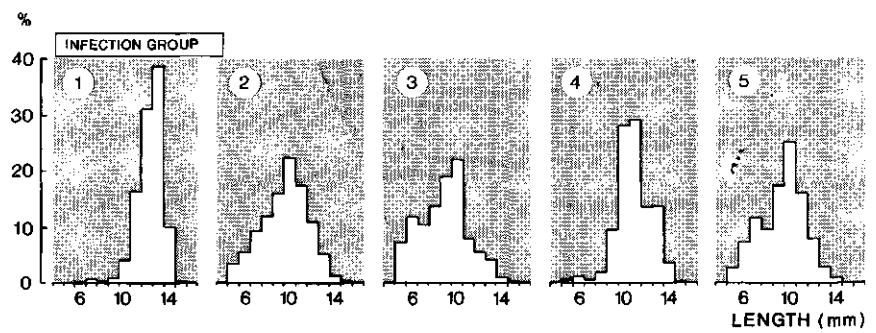

FIG. 22. Relative frequency distributions of female worm length in infection groups 1 to 5.

and 5, given repeated doses as secondary infection, two peaks seemed to occur: the highest is found in approximately the same length class that showed a peak in calves that received a single secondary infection. There is some indication of a small extra peak in the class of 6 mm (group 3) or 7 mm (group 5). Table 39 gives average worm lengths for separate necropsy groups. In all groups, but especially in groups 3 and 5, there is some tendency for average worm length to increase from the first to subsequent necropsy groups. This trend was significant ($P < 0.05$) in female worms in groups 3 and 5, which were both given repeated secondary doses. In male worms in these groups, and in both male and female worms in other groups, this effect was much less clear and not significant.

The variation of adult worm length within calves was also influenced by the time of necropsy but, in the opposite direction (table 41). In expt. II this trend was significant for female worms ($P < 0.05$) and nearly so for males ($P < 0.10$). In expt. II overall, the effect was almost significant for both ($P < 0.10$), and was most clearly shown in group 4 ($P < 0.05$). Thus it seems that during the course of secondary infections a decrease of length variation of adult worms occurred, which was most prominent in groups that received only a single secondary infection.

Larvae were not found in sufficient numbers to estimate their length in all infection groups. Table 43 gives the results of observations on length of fourth stage larvae in calves of expts. II and III. Generally, the variation of larval length was very small; this holds for length variation between infection groups, between calves within infection groups and between larvae within calves. This conclusion is confirmed by the relative frequency distributions of larval length that are shown in fig. 23. In groups 2, 3 and 5 respectively 50%; 72% and 85% of the

TABLE 43. Length and length variation (mean ± s.d.) in mm. of fourth stage larvae after various secondary infections.

Infection group	Prim. inf/ sec. inf. (thousands)		Mean	On day					
				18	25	32	38	39	40
2	100/350	Mean length	2.35 ± 0.22	2.32 ± 0.30	2.32 ± 0.16	2.29 ± 0.00		2.53 ± 0.18	
		St. dev. of length	0.48 ± 0.13	0.45 ± 0.18	0.49 ± 0.10	0.46 ± 0.00		0.54 ± 0.04	
3	100/17 × 75	Mean length	2.19 ± 0.16				2.15 ± 0.11		2.23 ± 0.22
		St. dev. of length	0.38 ± 0.16				0.35 ± 0.14		0.42 ± 0.19
5	20/17 × 75	Mean length	2.09 ± 0.12				2.09 ± 0.07		2.10 ± 0.16
		St. dev. of length	0.23 ± 0.22				0.25 ± 0.16		0.20 ± 0.28

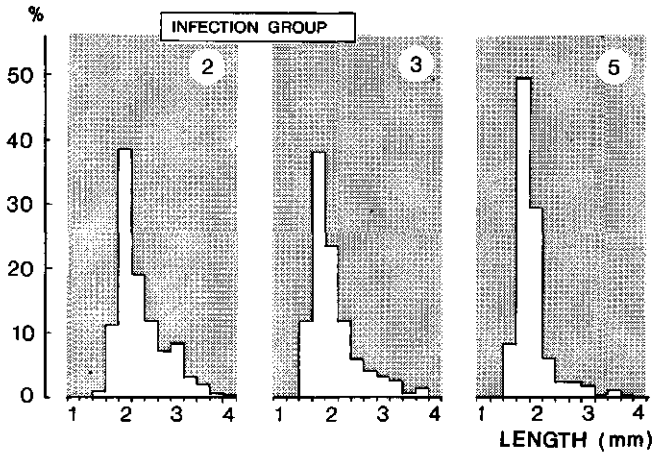

FIG. 23. Relative frequency distributions of length of fourth stage larvae in infection groups 2, 3 and 5.

TABLE 44. Mean length and length variation (within calf standard deviation) of female worms in ABH and PAN calves in expt. III (mm).

Infection group	Prim. inf./ sec. inf. (thousands)		Sire		Significance of difference
			ABH	PAN	
3	100/17 × 75	Mean length	8.83	8.90	N.S.
		St. dev. of length	1.52	1.82	N.S.
4	20/350	Mean length	10.33	11.47	$P < 0.05$
		St. dev. of length	1.28	1.07	N.S.
5	20/17 × 75	Mean length	8.98	9.62	$P < 0.10$
		St. dev. of length	1.52	2.02	$P < 0.05$

larvae belong to the classes 1.50; 1.75 or 2.00 mm. (In group 4 too few larvae were found to allow a comparison.)

Genetic differences in worm length were not found in expt. II. In contrast to this, large differences in worm length occurred in expt. III between the two sires (ABH and PAN) that in previous infections had been shown to be quite similar. In group 4, ABH-calves had significantly shorter adult worms than PAN calves ($P < 0.05$) (table 44); in group 3 ABH and PAN calves showed hardly any difference; in group 5 the difference was intermediate and nearly significant ($P < 0.10$) (table 44). For length of larvae, no genetic differences were seen.

As regards length variation of adult worms, significant sire differences were found in groups 2 and 5 for both male and female worms ($P < 0.05$). In group 5 length variation of worms in PAN calves was higher than in ABH calves (table 44), although average worm length was also higher. This seems to be in contrast

to the general finding that a high average length is correlated negatively with the standard deviation.

Genetic differences for length variation of larvae were not found.

4.3.1.1.4. Number of eggs per female worm

In expts. II and III the counts of the number of eggs *in utero* were made for individual worms in order to get information on between-worm variation within calves. As table 45 shows, this approach revealed that sometimes a considerable number of females did not carry any eggs. Female worms that did harbour eggs *in utero* are called gravid females.

In expt. II, the number of eggs per female worm increased with time significantly ($P < 0.01$). As table 45 shows this increase was caused by an increase of both the percentage gravid females and the number of eggs per gravid female worm. In expt. III (groups 3, 4 and 5) generally the same trend is seen, although this increase was not significant.

TABLE 45. The mean number of eggs *in utero* of female worms as determined by percentage of gravid females and number of eggs per gravid female in the various secondary infection groups.

Infect. group	Prim. inf./ sec. inf. (thousands)		Days after secondary infection					
			18	25	32	38	39	40
2	100/350	Percentage gravid females	30.6 ± 35.5	42.6 ± 34.5	78.6 ± 28.6		83.2 ± 16.4	
		Eggs per gravid female	22.8 ± 10.8	31.1 ± 16.1	38.6 ± 24.9		36.9 ± 15.2	
		Eggs per female present	8.0 ± 12.8	12.6 ± 11.7	30.6 ± 25.9		30.8 ± 14.7	
3	100/17 × 75	Percentage gravid females				27.6 ± 14.6		53.4 ± 38.8
		Eggs per gravid female				15.8 ± 8.3		28.4 ± 14.4
		Eggs per female present				4.9 ± 4.4		17.6 ± 19.1
4	20/350	Percentage gravid females				71.2 ± 26.8		73.2 ± 36.1
		Eggs per gravid female				22.5 ± 11.1		41.9 ± 19.3
		Eggs per female present				17.4 ± 11.9		33.6 ± 25.0
5	20/17 × 75	Percentage gravid females				33.2 ± 8.0		47.3 ± 21.6
		Eggs per gravid female				15.5 ± 6.4		15.1 ± 10.0
		Eggs per female present				5.2 ± 2.4		7.2 ± 5.7

As regards the mean group levels in expt. III, a difference ($P < 0.01$) was found between groups 4 and 5 in favour of group 4. Comparison with the level of expt. II at the same time of infection (day 39) suggests that, at this stage of infection, egg numbers per female worm were higher when the secondary infection was given as a single dose. Again, if a difference between groups occurred in expt. III, this was due to a difference in the number of eggs per gravid female, as well as in the percentage gravid females.

In none of the four groups that received secondary infections were there significant differences between sire groups.

4.3.1.1.5. Relations between parasitological parameters

As in primary infection, the actual egg output at necropsy was very well correlated with the product of number of adult worms and number of eggs per female worm (table 46).

Also, average individual egg production increased as a kind of compensation

TABLE 46. Correlation coefficients between actual egg output and necropsy determinations after various secondary infections at different necropsies.

Infec. group	Prim. inf./ sec. inf. (thousands)		Days after secondary infection						Total
			18	25	32	38	39	40	
2	100/350	No. of worms	0.86***	0.46	0.59		0.88***		0.62***
		No. of eggs per female worm	0.60	0.19	0.41		−0.18		0.39*
		No. of worms × no. of eggs	0.93***	0.75**	0.80**		0.59		0.86***
3	100/17 × 75	No. of worms				0.56		0.64	0.53
		No. of eggs per female worm				0.54		0.90*	0.81**
		No. of worms × no. of eggs				0.70		0.70	0.78**
4	20/350	No. of worms				0.60		0.71	0.63*
		No. of eggs per female worm				0.60		0.60	0.50
		No. of worms × no. of eggs				0.94**		0.93***	0.97***
5	20/17 × 75	No. of worms				0.47		0.56	0.52
		No. of eggs per female worm				0.47		0.25	0.40
		No. of worms × no. of eggs				0.68		0.77*	0.67**

TABLE 47. Correlation coefficients between worm length and other parasitological observations at different necropsies and for entire infection groups in expt. III.

	Infect. group	Days after sec. inf. 38	40	Total
A (peak egg count) and male worm length	3	0.43	0.60	0.46
	4	0.83*	0.43	0.62*
	5	0.54	0.77	0.70**
female worm length	3	0.57	0.60	0.73*
	4	0.60	0.90*	0.71**
	5	0.64	0.54	0.64*
Number of worms and male worm length	3	–0.60	–0.10	–0.49
	4	0.60	–0.02	0.30
	5	0.07	0.49	0.18
female worm length	3	–0.54	–0.10	–0.42
	4	0.54	0.52	0.33
	5	0.14	0.21	0.00
Percentage *C. surn.* males and male worm length	3	0.50	0.30	0.62
	4	–0.49	–0.14	–0.22
	5	–0.07	0.10	0.20
female worm length	3	0.60	0.30	0.70*
	4	0.54	–0.14	0.20
	5	0.18	0.10	0.40
Percentage male worms and male worm length	3	–0.89*	–0.50	–0.72*
	4	0.49	–0.45	–0.21
	5	0.00	–0.14	–0.20
female worm length	3	–0.96***	–0.50	–0.80**
	4	–0.31	–0.50	–0.34
	5	–0.11	–0.36	–0.54

during the stage of infection in which expulsion of worms occurred (see table 45, cf. tables 33–36).

Although worm length in secondary infections was subject to changes, it was still positively related to the peak egg output (table 47) similarly to the finding in primary infections (table 8). This was not merely the consequence of differences between sire groups as shown by the correlations in group 3, where sire group differences were absent.

Only some of the correlations between worm length and the various worm count parameters were significant (tables 47 and 48). A striking feature however is, that especially in groups 2 and 3 which had received a high primary dose of 100,000, the sign of the correlations was generally opposite to that found in primary infection (table 8). From the (significant) correlations in table 47 it can be concluded that those group 3 calves which harboured long worms, also

TABLE 48. Correlation coefficients between worm length and other parasitological observations for separate necropsy groups and for all calves together.

	Days after secondary inf.				Total
	18	25	32	39	
Total egg output and					
male worm length	–	–	–	–0.13	–0.13
female worm length	–	–	–	0.38	0.38
Number of worms and					
male worm length	–0.15	–0.43	–0.55	–0.37	–0.37*
female worm length	–0.02	–0.47	0.08	0.45	–0.04
Percentage *C. surn.* males and					
male worm length	–0.23	–0.22	–0.37	0.21	–0.18
female worm length	–0.22	–0.03	–0.09	0.57	–0.04
Percentage male worms and					
male worm length	0.27	–0.06	–0.26	–0.33	–0.06
female worm length	0.55	–0.11	0.01	–0.72*	–0.10

contained a high percentage *C. surnabada* males and a low percentage of male worms. Moreover these calves tended to harbour less worms. Because a high percentage *C. surnabada* and a low proportion of male worms are the result of a high degree of expulsion, it must be concluded that in group 3 the most vigorous expulsion had occurred in calves that harboured the longest worms.

This conclusion is exactly the reverse of the statement for primary infection (section 4.2.1.2.). The change in sign of correlations between worm length and percentage males or percentage *C. surnabada* in four subsequent necropsy groups in group 2 between day 18 and day 39 after secondary infection (table 48), might reflect a gradual transition from the situation after primary infection into the situation after secondary infection.

4.3.1.2. Discussion

Generally, egg output after secondary infections was rather low, thus showing the sensitizing effect of primary infection. The variation between calves was, however, even larger than after primary infection as can be seen from the standard deviations in tables 2 and 30. Besides this, the 'prepatent' period was also very variable after secondary infection; in particular, repeated doses (groups 3 and 5) delayed the day of patency.

The interpretation of egg counts after secondary infections is complicated by the unknown role played by adult worms that were left from primary infections. From primary infection data it was concluded earlier that at the time of secondary infection a number of adult worms was still present. These worms therefore could have contributed to the egg output pattern. Although the evidence is not conclusive, it is probable that the 'old' worms were not expelled completely soon after secondary infection: no sudden drop of egg output nor any clinical sign of

TABLE 49. Number of gravid female worms in relation to number of long females ($\geq$ 11 mm) in the course of secondary infection in expt. II (primary dose 100,000, secondary dose 350,000 larvae).

		Days after secondary infection			
(*n*)*		18 (9)	25 (9)	32 (9)	39 (7)
Number of gravid females		15,725	3,931	11,471	10,396
Number of females $\geq$ 11 mm		16,854	2,902	5,938	3,378
Number of females $\geq$ 11 mm as percentage of number of gravid females	mean % of group members	261	449	72	38
	% for group means	107	74	52	32

* group sizes: see text.

expulsion (diarrhoea) was seen. Furthermore, the length variation among adult worms in the first necropsy groups of expt. II was so very large because of the relatively high frequencies in low as well as in high length classes. The role of old worms in total egg output after secondary infection could have been derived from joint estimates of length and of number of eggs *in utero* of individual female worms. Unfortunately this combination of observations was not carried out. Eggs *in utero* were however counted per individual female worm. In the first necropsy group of expt. II only 30.6% of females carried any eggs in their uteri; the females that did carry eggs however, harboured a mean of 23 eggs. This feature could have been due to the presence of a gravid population of old worms remaining from the primary infection and a group of non-gravid young females from the secondary dose.

Although this hypothesis cannot be proven, its validity is supported by the analysis of worm numbers as presented in tables 49 and 50. (Group sizes in these tables are different from those in other tables. Because these special worm numbers could only be calculated for calves that harboured enough females to measure their length and to assess their gravidity, some calves had to be left out of calculations. In order to keep all necropsy groups comparable, the same number of animals was left out from necropsy groups within the same infection group. Calves that harboured the lowest female worm numbers were dropped.) If it is assumed that nearly all female worms which had established from a primary dose had reached a minimum length of 11 mm (as was seen in expt. I) an estimate can be made of the number of old worms found in calves after secondary infection. At the first date of necropsy in expt. II, on average the number of old females roughly equalled the number of gravid females. In subsequent necropsy groups the ratio long females: gravid females gradually decreased, suggesting that more and more shorter, young females became gravid. In other words, at 18 days after secondary infection probably the majority of eggs was produced by primary dose worms. Due to expulsion of these worms and growth of young

TABLE 50. Number of gravid female worms in relation to number of long females ($\geqslant$ 11 mm) in the course of secondary infection in expt. III.

Prim. inf./sec. inf. (thousands)		20/350		20/17 × 75		100/17 × 75	
Days after sec. inf.		38	40	38	40	38	40
(n)*		(5)	(7)	(6)	(6)	(7)	(5)
Number of gravid females		37,648	11,634	14,725	12,694	4,778	8,770
Number of females $\geqslant$ 11 mm		34,722	7,509	11,983	10,000	1,850	4,645
Number of females $\geqslant$ 11 mm as percentage of number of gravid females	Mean % of group members	80	250	73	108	47	41
	$\bar{x}$	179		90		44	
	% for group means	92	65	81	80	39	53
	$\bar{x}$	84		81		47	

* group sizes: see text.

worms, egg production was gradually taken over by female worms originating from the secondary dose.

In expt. III there is one difficulty in trying to confirm this hypothesis: a primary dose of 20,000 had only a very weak sensitizing effect thus allowing secondary dose worms to reach higher lengths and thereby interfering with the 11 mm criterion. But also in this experiment the ratio long worms: gravid worms decreased with time. Besides, this ratio fell as the primary or secondary infection dose rose. In summary: it seems probable that after secondary infections only some of the 5th stage females contributed to the total egg production of the worm population. These were constantly being expelled and replaced by newly grown worms that became gravid.

In table 45 it can be seen that when the reservoir of fourth stage and young fifth stage females approached exhaustion (in groups 2 and 4 where no further larvae were taken in), most females ultimately became gravid. In groups 3 and 5, due to continuous intake of infective larvae, only a relatively small proportion of females was gravid at one time.

Thus egg production of the total worm burden was determined by two sources of variation: the proportion of gravid females and the number of eggs per gravid female. As can be derived from table 45 these parameters were correlated. However, for the ultimate result (measured as number of eggs per female present) the proportion of gravid females was much more important than the number of eggs per female worm.

The regulation of worm numbers after secondary infections was more complex than after primary infection by the presence of larvae as well as adult worms. Various differences were seen between the regulation of the larval burden and the adult worm burden: in section 4.3.1.1.2. it was concluded that the number of

larvae depended on the infection regimen; the number of adult worms was determined by infection experience and genetic constitution of the calf.

Another difference between the regulation of adult worm and larval burdens concerns the effect of the expulsion process. The decrease in the number of larvae was not simultaneous with (expt. II), or much smaller (expt. III) than the decrease in the number of adult worms. Two explanations can be proposed for this difference: Firstly, it is possible that fourth stage larvae were not expelled at all but resumed development by growth to the fifth stage, similar to the course of events proposed by MICHEL (1963) for the *Ostertagia ostertagi*-calf system. A second explanation might be that fourth stage larvae, being in an inhibited stage (see later), were less susceptible to the expulsion process than adult worms.

Expulsion of adult worms caused dramatic changes in adult worm numbers within a short time. In expt. II this expulsion occurred as early as between 18 and 25 days p.i., which was faster and probably earlier than after a primary dose in expt. I. Also in group 3 most expulsion had occurred before day 38 (the low percentage male worms shows that expulsion must have occurred there). Generally, in expt. III expulsion seemed to occur later in groups 4 and 5 vs. group 3 and later in PAN calves than in ABH animals. Apparently the immune status of calves, as determined by the level of the primary dose and the genetic background of the animal, influenced the time of expulsion of adult worms.

In expt. III a very large expulsion was seen within a period of only two days. It is very likely, that the sharp decrease of worm numbers was a case of normal expulsion because the differences in parasitological findings between the two necropsy groups very much resembled the effects of normal expulsion as seen in other experiments (decrease of percentage male worms, increase of percentage *C. surnabada* male worms, increase of number of eggs per female worm). Although it is not likely, this expulsion may have been artificially induced somehow. E.g. the removal (for the first necropsy) of half of the experimental calves after 24 hours of starvation may have disturbed the remaining calves.

Expulsion of adult worms appeared to be proportional to the number that had been present. The expulsion rate was – in other words – higher in calves that had harboured more worms. This explains the finding that in calves which had shown a high egg output and harboured long worms (signs of a large adult worm burden), a higher percentage *C. surnabada* and a lower percentage male worms (signs of more expulsion) were observed.

Worm length was very much affected by previous infection experience and by the actual, secondary infection regimen. Besides this, and in contrast to primary infection, worm length changed in subsequent necropsy groups.

Expt. II offers the best opportunity to study this phenomenon: absolute frequency distributions of length of all worms (fourth stage larvae and male and female adults) can be constructed here at four times after secondary infection (see fig. 24). Frequency distributions of subsequent necropsy groups were significantly different (χ^2-test) except for groups 3 and 4. A closer look at these frequency distributions might suggest how changes in the total worm population may have affected worm length: between the first and the second date of

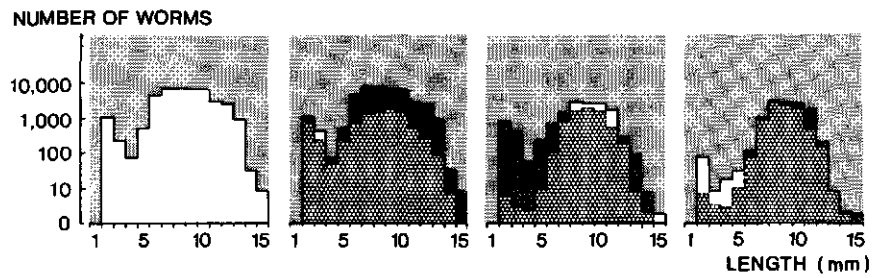

FIG. 24. Absolute frequency distributions (based on logarithm transformed counts) of worm length (adults and larvae) at four consecutive necropsies in infection group 2. (Darker areas represent distribution at previous necropsy).

necropsy an expulsion of adult worms took place (see also table 33), which apparently was not selective with respect to worm length: in all length classes in which adults could be present (4 to 15 mm), about the same decrease of frequencies occurred. The number of larvae did not change (table 33). The length classes that contain developing larvae or very young adults contained more worms than would be expected if only expulsion of adults had taken place. Apparently some early fourth stage larvae had developed.

This same feature suggests an explanation for the difference in frequency distributions between necropsy groups 2 and 3. Most larvae had gone at this stage of infection (see also table 33), less worms in developing stages were present but more worms are found in classes 8 to 11 mm although some expulsion seems to have taken place (lower frequencies in length classes 12 to 14 mm). Thus it seems that between 25 and 32 days after re-infection, considerable growth of larvae and young adults had taken place. It cannot be determined whether the decrease in the number of larvae was due only to this development, or to direct expulsion of fourth stage larvae at the same time.

Between the third and fourth dates of necropsy, no marked changes in frequency distribution of worm length occurred.

From this analysis of frequency distributions it might be concluded that after expulsion of adult worms (early) fourth stage larvae started to develop into adult worms. The sequence of events as described above is analogous to the description of population dynamics of a continuous *O. ostertagi* infection in calves by MICHEL (1963). He introduced the term 'turnover of worm populations' which also applies in our situation.

The changes in worm length in expt. III were very similar to those described for expt. II: a generally increasing worm length (table 39) and decreasing variation of worm length (table 41) might be explained by a similar turnover of worm populations. This would require a considerable growth of worms (up to 1.3 mm in two days in group 3 (table 39)), but the work of ISENSTEIN (1963) shows that such a growth rate is not exceptional for *Cooperia oncophora*.

Turnover of worm populations as described above can be a continuing process

if continuous infection provides a constant replacement of (early) fourth stage larvae, as in the classical example of MICHEL (1963) and our groups 3 and 5. In group 2 the turnover process ended with the depletion of the reservoir of fourth stage larvae. In group 4 this reservoir may even have never existed because all established larvae had the opportunity to continue their development into adult worms without interruption.

The length of fourth stage larvae, in contrast to the length of adult worms, in a sense was a very simplistic characteristic. Most larvae were found in only a few length classes (1.50 – 2.00 mm) (fig. 23), that were described by ISENSTEIN (1963) as early fourth stage larvae. It is very likely that these larvae were temporarily inhibited at this stage of development, until they received an opportunity (after expulsion of adult worms) to resume development. MICHEL et al. (1970) showed that in a situation of seasonal arrest of development, development of *Cooperia oncophora* was interrupted at the early fourth stage.

In infection groups 3 and 5 (continuous dosing) some exsheathed third stage larvae were also found. The data on third stage larvae were not analysed because of their low numbers and the possible inaccuracy of these data. A number of third stage larvae may have escaped observation due to their small size. Furthermore, some third stage larvae probably had not yet reached the intestine.

Genetic differences in parasitological parameters after secondary infections were sometimes very clear; in other cases they were completely absent.

In groups 4 and 5, in which egg output significantly increased after secondary infection, a significantly larger egg output was observed in PAN calves than in ABH calves. Thus previous sensitization caused genetic differences to become clearly visible. The positive relation between egg output after primary and secondary infection (table 31) shows that calves which were better able to resist a primary dose, were sensitized by this dose to a higher degree than less reactive calves.

Genetic differences in worm counts very much resembled differences in egg counts. In groups 2 and 3, in contrast to groups 4 and 5, no significant differences were seen; probably expulsion had proceeded too far here. In group 2, however, a significant sire effect on percentage *C. surnabada* males was found. This is an indication that expulsion had affected adult worm burdens here to a different degree in different sire groups. In group 2 PAN calves had the highest average *C. surnabada* percentage (43 %), whereas ABH calves had the lowest (29 %). Therefore more expulsion had taken place in PAN calves and initially PAN calves must have harboured more adult worms than ABH calves, just as in groups 4 and 5.

4.3.1.3. Conclusions

– In secondary infections egg output was severely depressed due to the immunizing effect of the primary dose.
– The higher primary dose exerted more effect than the lower dose.

– Egg output after secondary infection was positively related to egg production after the primary dose.
– Repeated secondary doses delayed the moment of patency of secondary infection.
– Total egg output and average egg production per female worm depended on the proportion of gravid females and the egg production per gravid female.
– At 18 days after secondary infection in expt. II all gravid females probably originated from the primary dose.
– After that time these 'old' female worms were expelled and partly replaced by shorter, gravid females originating from the secondary dose .
– At 18 days after secondary infection in expt. II all gravid females probably actual egg output. Only if, after a single secondary dose, the reservoir of fourth stage and young fifth stage females became exhausted did the percentage gravid females rise much above fifty.
– The number of adult worms found after secondary infection depended on the primary dose level and was not influenced by the secondary infection regimen.
– In contrast, the number of larvae at 6 weeks after infection was purely a function of the secondary infection regimen.
– Adult worms were expelled earlier and more rapidly after secondary infection than after primary infection.
– Expulsion of adult worms took place later in groups that had received a lower primary dose or were genetically more susceptible.
– Expulsion caused a decrease of adult worm numbers that was proportional to the number present.
– The expulsion process probably did not affect directly the number of fourth stage larvae, or at least not to the same extent as the number of adults.
– Expulsion of adult worms in the presence of fourth stage larvae was followed by a resumption of development of the latter.
– These turnover processes caused significant variations in worm length.
– However, calves that had shown a higher peak egg output still harboured longer adult worms.
– Especially in heavily infected groups (2 and 3) high peak egg counts and long worms were correlated with a high expulsion rate of adult worms.
– Also in secondary infections expulsion of worms was selective in two respects: male worms were expelled more vigorously than females; *C. oncophora* numbers decreased more than *C. surnabada* numbers.
– On average, shorter adult worms were found if the primary dose level had been higher or if the secondary infection had been repeated doses rather than a single dose.
– Within-calf variation of worm length was greatly increased after secondary infection due to the mixture of 'old' and 'new' worms and due to turnover processes in the worm populations.
– Length variation of fourth stage larvae was rather small: the vast majority of larvae had accumulated in a very limited length traject and might be regarded as inhibited early fourth stage larvae.

– A significant sire effect on egg output was seen in secondary infections only after the low primary dose.
– Therefore, previous sensitization enlarged genetic differences in egg output between offspring of the sires used in the experiment.
– The genetic effect on adult worm numbers very much resembled the effect on egg output.
– In expt. III the expulsion rate significantly differed between sire groups, whereas worm numbers did not differ. Sire PAN calves, that excreted more eggs and harboured more worms in expt. III showed a significantly higher expulsion rate than ABH calves. Also in expt. II PAN calves initially must have contained more worms than ABH calves.
– Sire differences in number of larvae generally were much smaller and not significant, probably because the number of larvae was a function of the infection pattern rather than of the quality of the calf.
– Worm length differed between sire groups only if the growth of worms was not too much retarded due to the infection pattern, i.e. after a low primary dose and especially after the combination of low primary dose with a single secondary dose (group 4).

4.3.2. *Immunological observations*

4.3.2.1. Results

4.3.2.1.1. Immuno Fluorescent Antibody Test (IFAT)

Fig. 25 presents IFAT curves of all experimental groups in expts. II and III. The curves depicted here cover both primary and secondary infection to show that the titre at the start of secondary infection depended largely on the response after primary infection. Thus this starting point was higher after primary infections with 100,000 larvae (groups 2 and 3) than after infections with 20,000 (groups 4 and 5). Also the starting level in expt. III was higher than in expt. II (group 3 vs. 2). Fig. 25 shows, furthermore, that secondary infection boosted IFAT titres in all infected groups. The level that was ultimately reached was about the same in all groups. An unexpected result was that IFAT titres of control calves, which were successfully kept wormfree, also showed a considerable increase. This increase was statistically significant ($P < 0.01$) and will be discussed later.

To allow statistical analysis of results, similar parameters were derived from IFAT curves as in primary infections. The first titre after secondary infection (day 53) was accepted as the initial titre. The increase from day 53 to day 74 was represented by the regression coefficient of IFAT titre on time (in days after secondary infection). Furthermore a mean titre was calculated on the basis of all observations after secondary infection. Table 51 gives these parameters for groups 2 to 6. Generally, these parameters confirm the conclusions already drawn from the IFAT titre curves.

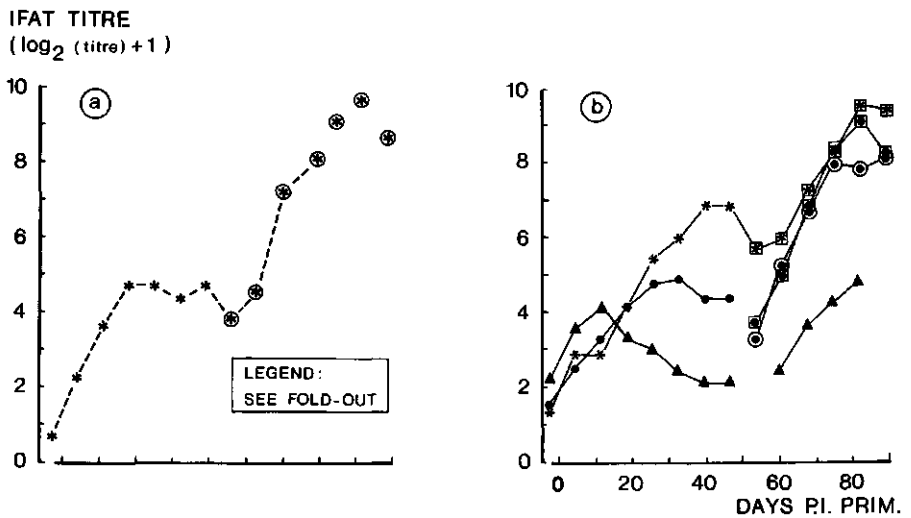

FIG. 25. The course of IFAT titres in primary and secondary infections in expt. II (a) and III (b).

TABLE 51. IFAT titre parameters following various secondary infections.

Infect. group	Prim. inf./ sec. inf. (thousands)	Initial titre	Mean titre	Titre increase
2	100/350	4.55 ± 2.57	7.63 ± 2.54	16.10 ± 8.75
3	100/17 × 75	5.71 ± 2.02	7.71 ± 1.66	14.29 ± 8.57
4	20/350	3.29 ± 1.54	6.55 ± 2.23	16.94 ± 9.74
5	20/17 × 75	3.71 ± 1.86	6.90 ± 2.20	20.41 ± 7.16
6	0/0	4.00 ± 1.41	– –	8.57 ± 0.00

TABLE 52. Correlations between IFAT titre parameters of primary and secondary infections.

Infection group	Prim. inf./ sec. inf. (thousands)	Coefficients for		
		Initial titre	Mean titre	Titre increase
2	100/350	0.04	0.69*	–0.16
3	100/17 × 75	–0.35	0.57*	–0.56*
4	20/350	–0.03	0.44	0.34
5	20/17 × 75	0.49	0.75**	0.32

TABLE 53. IFAT titre response in ABH and PAN calves in secondary infections preceded by a dose of 20,000 larvae.

Infection group	Prim. inf./ sec. inf. (thousands)	Sire group	Initial titre	Mean titre	Titre increase
4	20/350	ABH	3.57	7.53	20.82
		PAN	3.00	5.57	13.06
5	20/17 × 75	ABH	4.14	7.81	23.67
		PAN	3.29	6.00	17.14
Significance of difference between sire groups			–	*	*

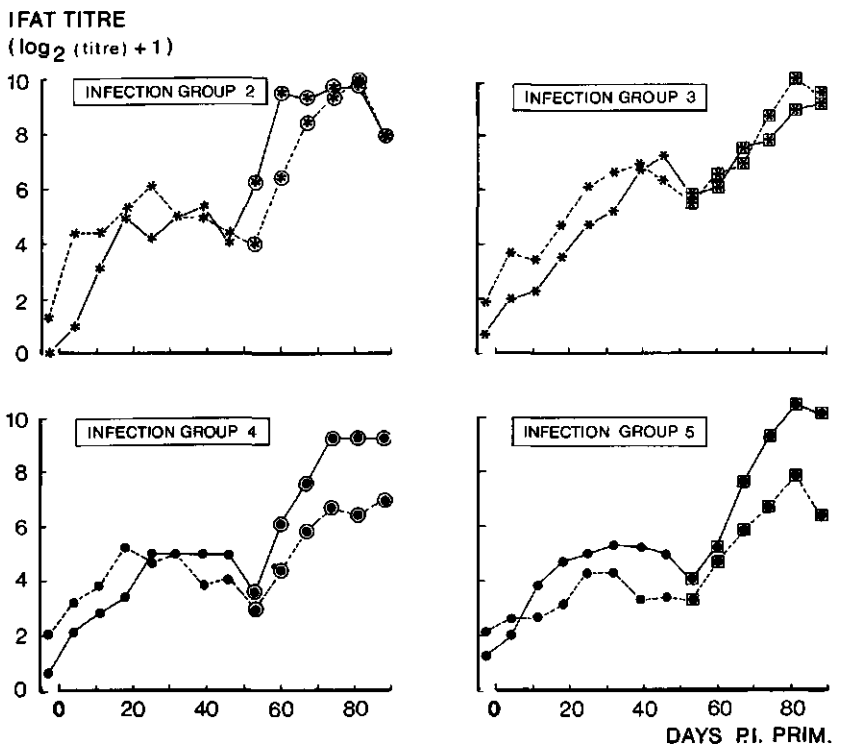

FIG. 26 The course of IFAT titres in primary and secondary infections in infection groups 2 to 5 for ABH calves (solid line) and PAN calves (dotted line).

The relation between IFAT titres after primary and secondary infections can be derived from table 52. The correlation between initial titres is not significant in any of the groups. Mean titres were positively correlated in primary and secondary infection. The correlation for IFAT titre increase was negative in the group which had the highest secondary starting titre (group 2), and this steadily changed to positive as the mean group starting titre fell (cf. table 51). Thus, when a high antibody level was reached after primary infection, group members seemed to converge to a certain plateau level. When primary infection had induced lower levels (primary dose 20,000, groups 4 and 5), differences between calves in one experimental group tended to become larger.

Sire differences following secondary infection were significant only when a low primary dose had been given in expt. III. Initial titres did not differ between ABH-and PAN calves, but the increase thereafter, and therefore also the mean titre, were different at $P < 0.05$ (table 53).

Fig. 26 gives a graphical presentation of these sire differences.

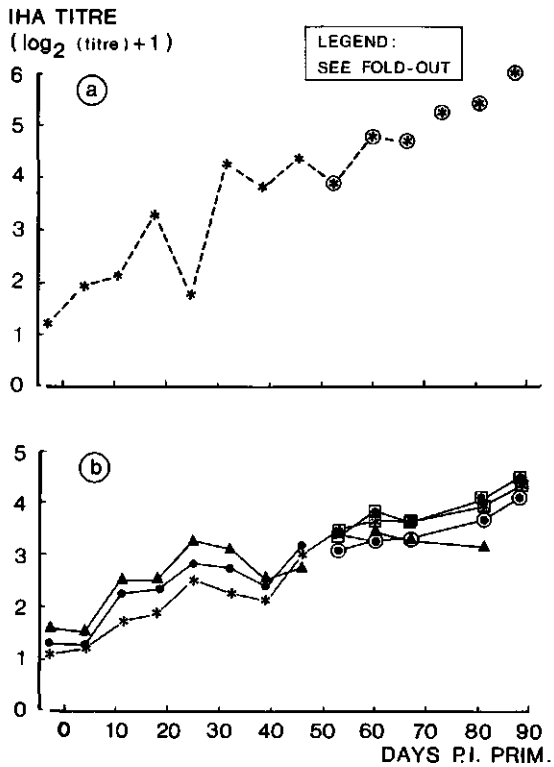

FIG. 27. The course of IHA titres in primary and secondary infections in expt. II (a) and III (b).

4.3.2.1.2. Indirect HaemAgglutination test (IHA)

The results of the IHA test in secondary infections are presented graphically in fig. 27. The observations at day 78 p.i. in expt. III were excluded from graphs and calculations because they were very deviant (probably due to incorrect treatment of the blood samples). Three parameters were derived from IHA curves as for IFAT curves. These are presented in table 54. As table 54 and fig. 27 show, the results of the IHA test after secondary infections were quite similar to those after primary infection. A steady increase was seen in all groups; titres in expt. II were generally higher than in expt. III. IHA titres of experimental groups within expt. III, including the uninfected control group, hardly showed any difference. Secondary infection did not boost IHA titres.

In contrast to the findings in primary infections, significant differences between sire groups were found in secondary infections in expt. III. As is illustrated in table 55 and fig. 28, the initial IHA titre was not different for ABH and PAN

TABLE 54. IHA titre parameters following various secondary infections.

Infection group	Prim. inf./ sec. inf. (thousands)	Initial titre	Mean titre	Titre increase
2	100/350	3.84 ± 1.08	5.05 ± 1.11	5.39 ± 4.45
3	100/17 × 75	3.43 ± 0.62	3.79 ± 0.69	1.60 ± 2.27
4	20/350	3.04 ± 0.77	3.46 ± 0.78	2.07 ± 2.18
5	20/17 × 75	3.32 ± 0.54	3.86 ± 0.55	2.27 ± 2.53
6	0/0	3.75 ± 0.35	–	–

TABLE 55. IHA titre response in ABH and PAN calves after secondary infections in expt. III.

Infection group	Prim. inf./ sec. inf. (thousands)	Sire group	Initial titre	Mean titre	Titre increase
3	100/17 × 75	ABH	3.43	3.99	2.59
		PAN	3.43	3.53	0.61
4	20/350	ABH	3.14	3.73	2.48
		PAN	2.93	3.19	1.66
5	20/17 × 75	ABH	3.36	4.11	3.70
		PAN	3.29	3.60	0.85
Significance of difference between sire groups			–	*	**

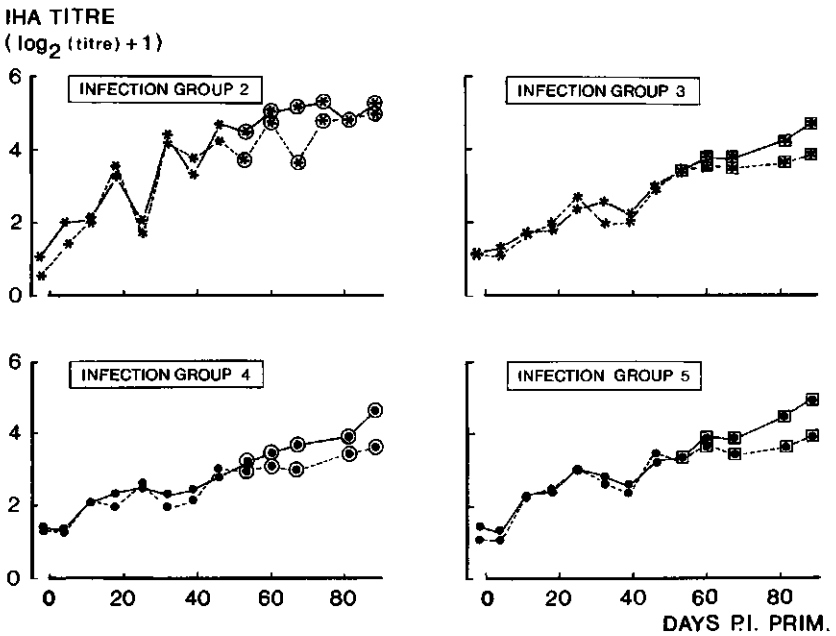

FIG. 28. The course of IHA titres in primary and secondary infections in infection groups 2 to 5 for ABH calves (solid line) and PAN calves (dotted line).

calves. Titre increase, and therefore the mean titre, were significantly higher for ABH calves than for PAN calves ($P < 0.01$ resp. $P < 0.05$). Too few observations on control calves were available to allow conclusions for this group.

4.3.2.1.3. Intra Dermal Test (IDT)

Fig. 29 and table 56 present the results of the IDT in secondary infections. The three parameters in table 56 were derived from response curves by the same procedures as in primary infections; the first test result after secondary infection, at day 54 p.i., was accepted as the initial response. As was seen in primary infections, the response level in expt. II was much higher than in expt. III. None of the group differences between infection groups within expt. III was significant.

A striking phenomenon in this experiment was the sudden and steep rise of IDT response in uninfected controls from day 54. At the last test date these controls had reached the same level as the infected groups. The increase of IDT response in control calves was therefore larger than of infected groups during this period ($P < 0.01$).

Table 57 and fig. 30 show that in secondary infections the mean response level generally was slightly higher in ABH calves than in PAN calves; this difference was not quite significant ($P < 0.10$). The increase of IDT response was affected differently by the sire in different infection groups. A significant interaction term between primary dose level and sire ($P < 0.05$) was caused by the fact that

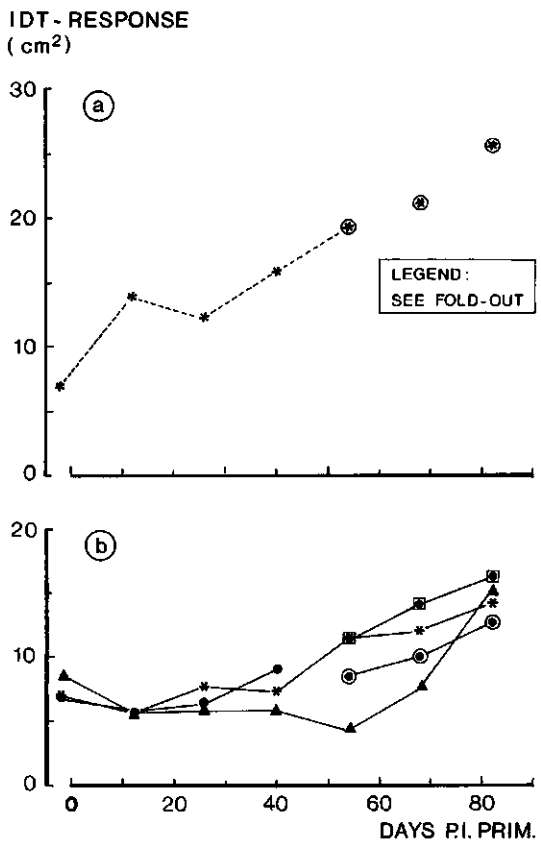

FIG. 29. The IDT responses in primary and secondary infections in expts. II (a) and III (b).

TABLE 56. IDT response parameters following various secondary infections.

Infection group	Prim. inf./ sec. inf. (thousands)	Initial response	Mean response	Response increase
2	100/350	19.27 ± 9.74	22.86 ± 8.72	19.90 ± 22.01
3	100/17 × 75	11.35 ± 4.54	12.52 ± 4.11	10.08 ± 20.10
4	20/350	8.49 ± 7.51	10.38 ± 6.27	14.92 ± 25.00
5	20/17 × 75	11.24 ± 5.83	13.84 ± 4.20	17.58 ± 13.48
6	0/0	4.32 ± 1.91	9.07 ± 3.67	39.17 ± 27.92

TABLE 57. IDT response in ABH and PAN calves after secondary infections in expt. III.

Infection group	Prim inf./ sec. inf./ (thousands)	Sire group	Initial response	Mean response	Response increase
3	100/17 × 75	ABH	10.74	12.86	21.22
		PAN	11.96	12.18	- 1.07
4	20/350	ABH	10.83	12.24	12.81
		PAN	6.16	8.52	17.04
5	20/17 × 75	ABH	13.16	15.61	14.44
		PAN	9.33	12.08	20.71
6	0/0	ABH	3.90	10.26	54.82
		PAN	5.15	6.68	7.86

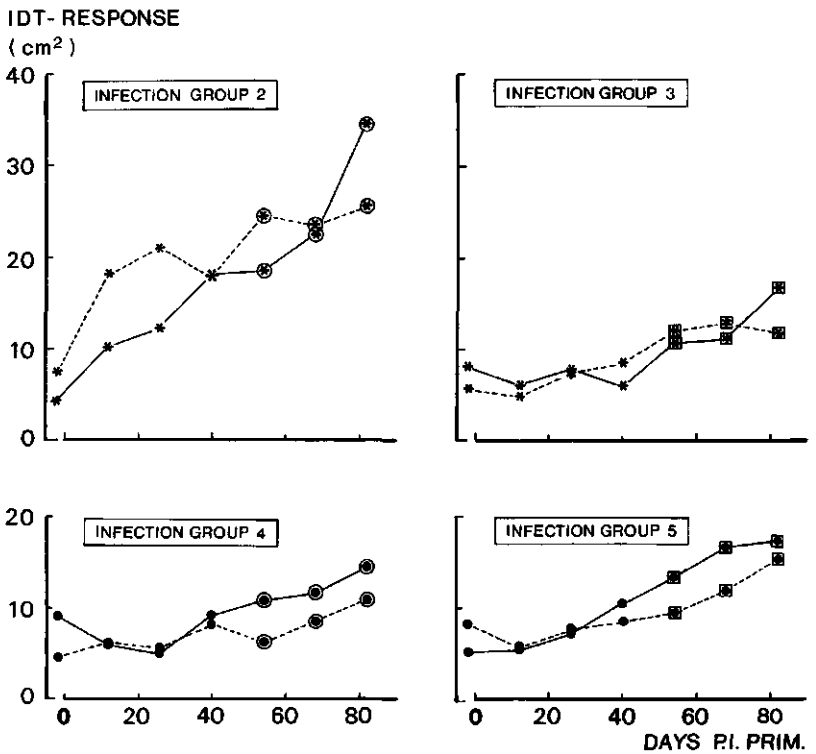

FIG. 30. The IDT responses in primary and secondary infections in infection groups 2 to 5 for ABH calves (solid line) and PAN calves (dotted line).

TABLE 58. Correlation coefficients between immunological parameters after secondary infection in groups 4 and 5 (primary dose 20,000).

			IHA		IDT	
		Infec. group	Initial titre	Titre increase	Initial response	Response increase
IFAT	Initial titre	4	0.58*	–0.14	0.27	0.38
		5	0.34	–0.12	0.51	–0.57*
	Titre increase	4	0.12	–0.12	0.42	–0.32
		5	0.34	0.32	0.77**	–0.54*
IHA	Initial titre	4			0.15	0.51
		5			0.26	–0.24
	Titre increase	4			–0.42	–0.08
		5			0.28	–0.14

response increase was higher for PAN calves than for ABH calves after a primary dose of 20,000 (groups 4 and 5). In group 3, however, after a primary dose of 100,000 the opposite occurred: response increase was higher in ABH calves than in PAN calves. The increase of IDT response in control calves was significantly larger in ABH calves than in PAN calves ($P < 0.05$).

4.3.2.1.4. Relations between immunological parameters

Only a few significant correlations were found between the various immunological parameters measured after secondary infection. Most of them were found in infection groups 4 and 5; these are presented in table 58. However, even in these groups correlations were rather low and do not indicate a clear relationship.

In group 5 there is some indication of a negative relation between IFAT and IDT response. In control calves too few observations were available to check the conclusions from the 'primary infection period'.

4.3.2.2. Discussion

The value of the IFAT method for assessing the reaction of calves to infection is, in a sense, confirmed by the finding that secondary infections boosted IFAT titres. This indicates that the calves were subjected to a discontinuous antigenic stimulation; which strongly suggests that the IFAT method detected specific antibodies against fourth and/or third stage larvae.

After secondary infection mean group titres converged, so that they all ultimately reached about the same level. Probably at this stage of infection a maximum level of antibody production was reached.

Genetic differences were not found in groups 2 and 3 which had received the

high primary dose. In group 4 and 5 however differences between PAN and ABH calves were significant. Fig. 26 shows that in groups 4 and 5 the ultimate level of IFAT titres was lower in PAN calves than in ABH calves; in groups 2 and 3 both sire groups reached the same level of about $1 : 2^{10}$, which may indicate a kind of biological maximum.

The significant rise of IFAT titres in control calves can only be explained if it is assumed that repeated IDT tests stimulated the production of antibodies detected by the IFAT test (see also section 4.2.2.2.). As figure 29 shows, the IDT results were increasing during the same period.

After secondary infection IHA titres continued to increase. However in expt. III the increase in ABH calves was higher than in PAN calves. This might have been caused by infection, but it seems more likely that this difference reflected a difference in reactivity to the antigens injected by the IDT, for the IDT results in control calves reveal the same sire group difference.

Thus several results strongly suggest that intradermal injection of antigen provoked an antibody response in control calves. Whether this also happened in infected calves and whether it interfered with the effects of infection cannot be checked because all infected calves were tested with the IDT method. Such an interference is very unlikely in view of the numerous reports of failures to immunize animals against gastro-intestinal nematode infections by means of injecting worm extracts (URQUHART, 1980). However, the control calves from expt. III, when infected a few months later with a single dose of 200,000 larvae in an other experiment, showed extremely low egg outputs (unplublished). The greater age (8 months) of these animals and the higher infection dose they were given may have contributed to their apparent resistance.

Although IHA and IDT results were useless as infection parameters, the genetic differences for the results of these tests are interesting. In several groups the ABH calves showed a stronger reaction than PAN calves. This suggests that, in a more general sense, ABH calves had a higher genetic potential to react to antigenic stimulation.

4.3.2.3. Conclusions

- Secondary infections boosted antibody titres as measured by the IFAT test.
- Generally, the IFAT response to primary infection was positively correlated with the response to secondary infection.
- Ultimately about the same level was reached in all groups suggesting a plateau level of maximum antibody titre.
- This plateau level was reached by ABH calves in all groups; PAN calves however, that had received a low primary dose (groups 4 and 5) did not reach this level.
- A significant sire effect on IFAT antibody response was found only in secondary infections which had been preceded by a low primary dose of larvae.
- The IHA and IDT methods showed steadily increasing responses but did not detect differences between infection groups.
- In control calves, IFAT titres and IDT response showed a sharp increase.

Probably, sensitization by repeated intradermal injection of worm extract was responsible for this.
– Sire differences for the results of the IHA and IDT tests suggested that ABH calves had a higher potential for antibody production than PAN calves.
– No clear relations between the results of the three immunological methods were found.

4.3.3. *Other observations*

4.3.3.1. Results

4.3.3.1.1. Live weight gain

Fig. 9 presents average live weight curves for all experimental groups. Infection did affect live weight of the animals, but only marginally. The largest effect occurred after secondary infections.

Fig. 31 provides more detailed information on the effect of secondary infection on live weight gains. This figure presents, for each infection group, average weekly gains in kgs, estimated on the basis of weekly weighings and

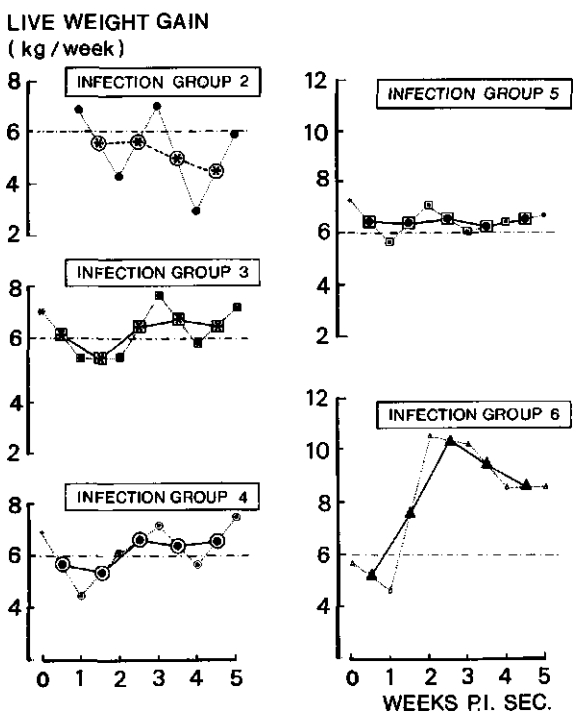

FIG. 31. Weekly live weight gain after secondary infection and in control calves at the same time. Smaller symbols represent original observations, larger symbols are graphical interpolations.

TABLE 59. Daily live weight gains (g/day, mean ± s.d.) of the various infection groups in three periods (differences were tested by Student's t-test).

Experiment	I	II	III			
Infection group	1	2	3	4	5	6
5 weeks before infection	984 ± 134	838 ± 154			988 ± 87	
PRIMARY DOSE	100,000	100,000	100,000	20,000		0
5 weeks after primary infection	767 ± 163	834 ± 148	886 ± 107	948 ± 119		957 ± 145
significance of difference	***	n.s.	**	n.s.		n.s.
SECONDARY DOSE	–	350,000	17 × 75,000	350,000	17 × 75,000	0
5 weeks after secondary infection	–	803 ± 201	788 ± 158	837 ± 212	808 ± 129	1279 ± 169
significance of difference with 5 weeks after prim. inf.	–	n.s.	*	*	**	**
significance of difference with 5 weeks before inf.	–	n.s.	***	**	***	***

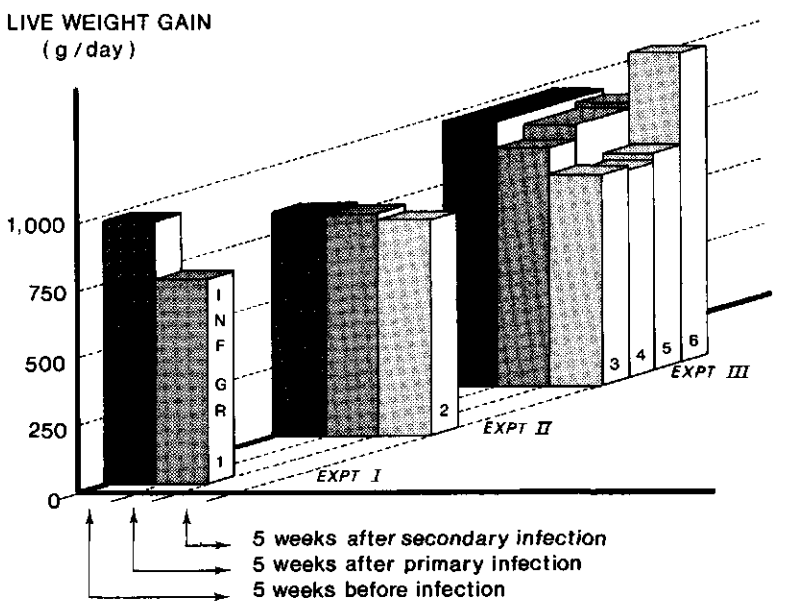

FIG. 32. Average daily live weight gains of the various infection groups in three experimental periods in all experiments.

corrected for the varying genetic constitution of groups. Although there were differences between groups, especially between infected and uninfected animals, these were never significant at $P < 0.05$. Only in the second week after secondary infection was the group difference within expt. III significant at $P < 0.06$, due to the difference between control calves and the infected groups.

When a comparison is made, however, between three experimental periods (i.e. before infection, after primary infection and after secondary infection) the conclusion can be drawn that during secondary infection live weight gain of infected calves was significantly depressed. This comparison is illustrated in table 59 and fig. 32. In all groups the average live weight gain after secondary infection was around 800 grams per day. In expt. III this was significantly lower than the live weight gain of comparable groups before, and even after, primary infection. Control calves on the contrary, gained even more weight than in the pre- and post-primary infection periods. In expt. II live weight gain after secondary infection was the same as in expt. III. Within expt. II, however, live weight gain

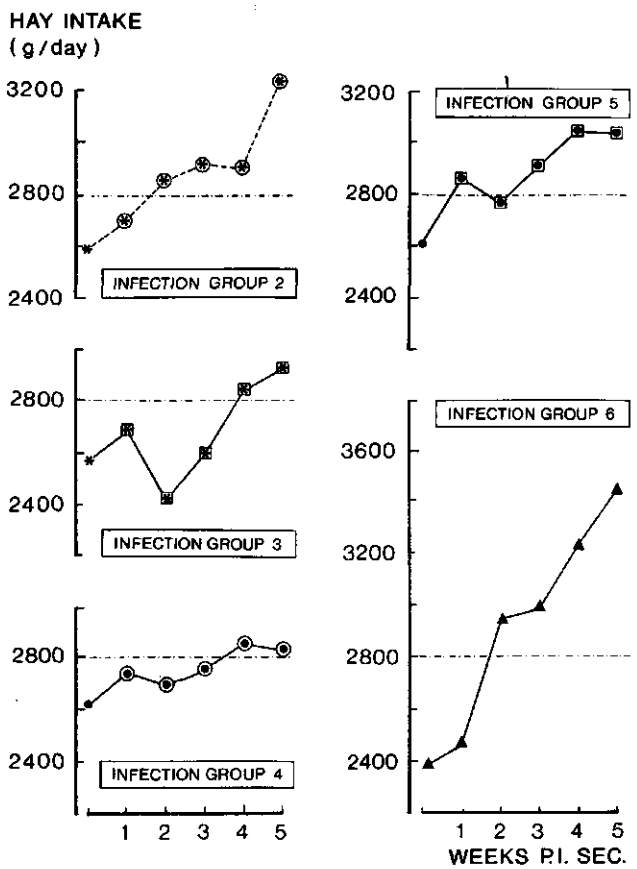

FIG. 33. Daily hay intakes after secondary infection and in control calves at the same time.

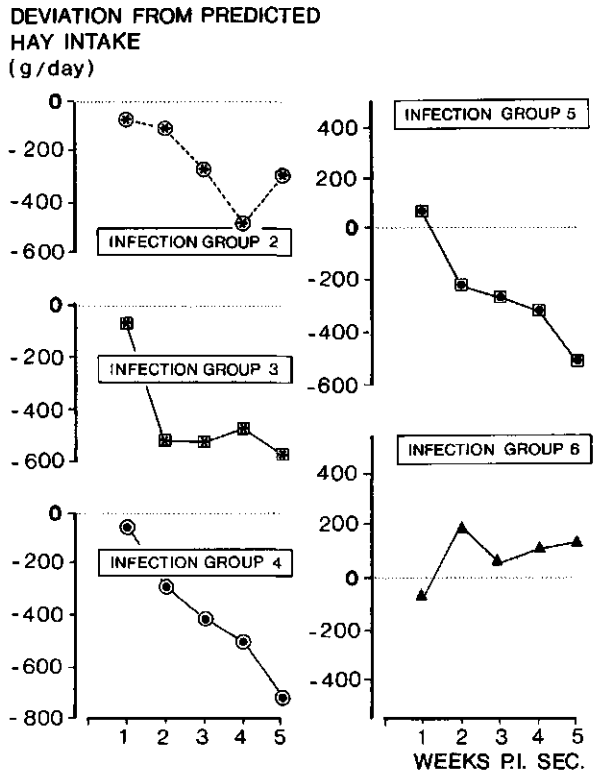

FIG. 34. Deviations of actual from predicted daily hay intake after secondary infections and in control calves at the same time.

did not differ significantly between experimental periods due to the poor gains before infection caused by a respiratory disease (see section 4.2.3.1.1.).

No significant sire effect on live weight gain after secondary infection was found.

4.3.3.1.2. Feed intake

Fig. 33 shows average hay intakes after secondary infection for the various experimental groups. Though some differences were seen, especially between uninfected and infected calves, these were not significant.

If however hay consumption was calculated as the deviation of actual from predicted intake (for calculation of this parameter see section 4.2.3.1.2.), some notable and significant differences were seen (fig. 34). In weeks 2 and 5 p.i.sec. the differences between groups within expt. III were significant at $P < 0.05$. In weeks 3 and 4 p.i. sec. the differences were less clear ($P < 0.10$). As fig. 34 shows the largest differences were between all infected groups compared with uninfected controls.

Only a few calves in the secondary infection period refused concentrates. Therefore, this characteristic did not show enough variation to compare different groups. Control calves ate all the concentrates offered in this period.

After secondary infections no significant differences between sire groups were found in average daily hay intake or deviation of actual from predicted hay intake, although in expt. II a strong tendency was present.

4.3.3.1.3. Faecal consistency

Table 60 presents, for all experimental groups after secondary infection, the numbers of animals that showed diarrhoea in a certain week. In expt. II most cases of diarrhoea occurred in the 4th and 5th week after secondary infection: around 30% of the remaining calves were affected. In expt. III there was no evident concentration of cases of diarrhoea in a particular period although most cases were seen in week 5. Most cases occurred in group 4, which suggests that a single secondary dose (groups 2 and 4) provided a stronger stimulus than repeated doses. The absence of any case of diarrhoea in the control group confirms that the occurrence of diarrhoea was due to the infection.

4.3.3.2. Discussion

During secondary infection a significant depression of live weight gain of calves in the infected groups was seen. Average live weight gain was around 800 grams daily, whereas a daily gain of about 1000 grams is possible under these circumstances, as pre-infection gains and the gain of control calves showed. All

TABLE 60. Cases of diarrhoea after secondary infection. The numbers present the number of calves that showed diarrhoea in a certain week. Between brackets are the total numbers of calves present at that time.

Infection group	2	3	4	5	6
Primary dose	100,000		20,000		0
Secondary dose	350,000	17 × 75,000	350,000	17 × 75,000	0
Week after secondary infection					
1	1(44)	0(14)	0(14)	0(14)	0(6)
2	0(44)	2	2	0	0
3	2(33)	1	2	0	0
4	7(22)	0	1	1	0
5	3(11)	2(14)	2(14)	2(14)	0(6)
Total no of calves that showed diarrhoea at any time	9	4	6	3	0

infected groups were affected to the same extent, suggesting that, ultimately, the different infection regimens all had the same effect on the growth of calves.

The observations on hay intake in fact lead to the same conclusion: the infected groups did not differ significantly but all together they deviated significantly from what they were expected to eat under normal conditions.

Diarrhoea occurred simultaneously with a depression of feed intake and live weight gain. On average, in expts. II and III calves that showed diarrhoea in a particular week had a live weight gain in that week of 0.4 ± 3.7 kg. The occurrence of diarrhoea was preceded in most cases by a serious depression of hay intake. One calf only ate 180 g of hay two days before he started scouring. After the consistency of faeces had become normal, calves recovered and resumed their normal intake level. Apparently in a number of calves the function of the digestive tract was disturbed thoroughly for a short period; this disturbance ended with diarrhoea. Unpublished observations at our laboratory suggest that this disturbance was accompanied by a constipation which might have caused the depressed feed intake.

4.3.3.3. Conclusions

- Live weight gain after secondary infection was significantly depressed in all infected groups by about 200 grams per day, which is about 20%.
- Hay intake of infected calves was significantly lower than the intake level expected under normal conditions.
- In all infected groups a number of calves showed diarrhoea (on average 30%). The highest incidence was in groups which had been infected with a single secondary dose of larvae.
- Diarrhoea occurred simultaneously with a depression of feed intake and live weight gain.
- After secondary infections no genetic differences in feed intake or live weight gain were found.

4.3.4. *Relations between parasitological, immunological and other parameters*

4.3.4.1. Results

Table 61 presents, by means of correlations, the relations that were found between the results of immunological techniques (expressed as the average response level after secondary infection) and two parasitological parameters (peak egg count after secondary infection and worm length). Generally, the highest (negative) correlations were found between worm length and mean IFAT titres. In groups 4 and 5, that had received a low primary dose, the peak egg count also was negatively correlated with IFAT titres. In these groups the IDT response also was negatively related to the two parasitological parameters. IHA titres were not significantly related to worm length or egg count.

There were no clear relations between worm count parameters and immunological findings.

IFAT titres, worm length and peak egg count apparently describe a coherent

TABLE 61. Correlations between peak egg count and worm length after secondary infection and average response levels of three immunological techniques.

Infection group	Prim. inf./ sec. inf. (thousands)		*A* (peak egg count) after secondary infection	Length of worms	
				males	females
		IFAT	–	0.59	−0.16
2	100/350	IHA	–	−0.01	0.40
		IDT	–	−0.18	−0.12
		IFAT	−0.05	−0.75**	−0.66*
3	100/17 × 75	IHA	−0.29	0.01	−0.24
		IDT	−0.25	0.27	0.20
		IFAT	−0.54*	−0.58*	−0.75**
4	20/350	IHA	−0.34	−0.30	−0.30
		IDT	−0.59*	−0.63*	−0.58*
		IFAT	−0.39	−0.58*	−0.47
5	20/17 × 75	IHA	−0.52	−0.49	−0.40
		IDT	−0.33	−0.68**	−0.54*

TABLE 62. Correlation coefficients between live weight gain after secondary infection and various other parameters.

Infection group	Prim. inf./ sec. inf. (thousands)	*A* (peak egg count)	Length of worms		IFAT (mean titre)
			males	females	
2	100/350	–	0.08	−0.12	0.26
3	100/17 × 75	−0.60*	−0.18	−0.59*	0.24
4	20/350	−0.14	−0.20	−0.10	0.51
5	20/17 × 75	−0.43	−0.53	−0.57*	0.47

part of a calf's response to infection. Table 62 shows how these parameters were correlated with the live weight gain of calves during the first five weeks after secondary infection. All but one of the correlations between live weight gain and worm length and egg count were negative; three of them were significant at $P < 0.05$. The correlations between IFAT titres and live weight gain were positive in all infection groups, although never significant. Thus the live weight gain of calves that showed a stronger response to infection was generally higher. In groups 2 and 3 (high primary dose) the relation between live weight gain and IFAT titre was very weak. In groups 2 and 4 (single secondary dose) the correlation between parasitological parameters and live weight gain was nearly absent (table 62).

Expulsion of worms was correlated with the occurrence of diarrhoea. This can be concluded from table 63 which presents a comparison of calves which had and calves which had not shown diarrhoea in groups 2 and 4. The higher percentage

TABLE 63. The relation between the occurrence of diarrhoea and several parameters in two different infection groups.

	Last necropsy group infection group 2 (100/350)		Infection group 4 (20/350)	
(*n*)	diarrhoea (5)	no diarrhoea (6)	diarrhoea (6)	no diarrhoea (8)
Percentage *C. surnabada* males	40.4 ± 1.2	33.8 ± 8.6	45.9 ± 17.2	29.2 ± 10.1
Percentage male worms	22.5 ± 18.0	46.7 ± 5.7	26.9 ± 21.0	43.4 ± 12.5
Length male worms	7.9 ± 0.9	7.6 ± 0.5	8.1 ± 0.4	8.3 ± 0.7
Length female worms	10.5 ± 0.5	9.0 ± 0.6	10.8 ± 0.5	11.0 ± 1.2
A (peak egg count) after prim. inf.	4.8 ± 0.2	4.5 ± 0.5	3.9 ± 0.4	4.0 ± 0.3
A (peak egg count) after sec. inf.	–	–	3.2 ± 1.6	4.2 ± 1.4
Number of adult worms	27,373 ± 35,775	16,778 ± 24,097	26,992 ± 41,661	71,037 ± 59,914
Live weight gain 5 weeks after sec. inf.	25.8 ± 9.0	30.0 ± 5.0	25.5 ± 7.9	32.6 ± 6.4

C. surnabada males and the lower percentage male worms indicates a higher expulsion rate in diarrhoeic calves. As was concluded earlier (4.3.1.3.) however, expulsion and diarrhoea in infection group 2 had occurred in calves that harboured longer worms and had shown higher egg counts, i.e. initially had harboured more worms. This phenomenon explains why, in group 2, calves that had shown diarrhoea harboured even more worms than calves that had not. Infection group 4 on the contrary showed a pattern which seems very similar to the findings in primary infection. In both groups however, calves that had scoured had a lower live weight gain in the five week period after secondary infection, as was seen earlier in section 4.3.3.1.

4.3.4.2. Discussion

The relations between parasitological and immunological parameters after secondary infection were similar to those found after primary infection with 100,000 infective larvae. A different result was the negative correlation in groups 4 and 5 between IDT response and worm length and peak egg count. This shows that, whatever the reason for the increasing IDT response, the calf's response to infection was related to its ability to respond to *C. oncophora* antigenic material.

The occurrence of diarrhoea was clearly associated with expulsion of adult

worms in infection groups 2 and 4. Probably in these groups, more than in groups 3 and 5 that were given repeated secondary doses, the simultaneous maturation of a large number of worms established from the same infection dose provoked a more intense host reaction which manifested itself in diarrhoea.

4.3.4.3. Conclusions

- The results of the IHA method were not related to any of the other parameters.
- Calves in expt. III that showed higher IFAT titre levels after secondary infection harboured shorter worms and had a lower egg output, especially in the infection groups that had received a low primary infection of 20,000 larvae.
- In these groups the IDT response also was negatively related to worm length and egg counts, which shows at least that this method somehow measured the ability of calves to respond to infection.
- Worm count data were not related to any of the immunological parameters.
- In groups that were given repeated secondary doses live weight gain was higher in calves that harboured shorter worms, excreted less eggs and showed higher IFAT titres.
- The occurrence of diarrhoea was clearly associated with massive expulsion of worms.
- In contrast to group 4 (low primary dose), diarrhoea and expulsion in group 2 (high primary dose) particularly took place in the calves that harboured long worms and, probably, initially had contained the largest worm burdens.

5. GENERAL DISCUSSION

5.1. RESISTANCE: MECHANISMS AND EFFECTS

In the experiments reported here a number of parasitological and immunological parameters have been used to assess the course of infections and to measure the reactions of calves. Most of these parameters have been reported in the literature (chapter 2) as being affected by resistance mechanisms. Only little is known about the interactions between these two.

Experiment I gives an unequivocal demonstration of how host resistance is expressed in parasitological parameters of infection. Mutual positive correlations between peak egg count, worm length and worm number show a coherent picture of resistance in parasitological terms: a resistant calf has a low egg output. that is produced by a small burden of short worms. In the light of numerous parasitological studies on resistance, this is not an unexpected conclusion.

In a primary infection increase of body size of parasites is only very small or completely absent after patency (SOMMERVILLE, 1960; COADWELL and WARD, 1975). Thus, worm length in primary *Cooperia oncophora* infections is probably completely determined in approximately the first two weeks of an infection.

The peak egg output is, on average, reached at day 28 of primary infection. At this time an active host resistance apparently balances the potential of the parasite population to increase egg production to an even higher level. This must be concluded from experiments on immune suppression by administration of immunosuppressants (MICHEL and SINCLAIR, 1969; KLOOSTERMAN et al., 1974) or whole body irradiation (DUNSMORE, 1961). These experiments show that egg output continues to rise and reaches a higher peak at a later time.

Expulsion of adult worms, which determined variation in worm counts at the times of necropsy in expt. I, is the final expression of host resistance.

The mutual correlations in expt. I between the three parameters mentioned above (worm length, peak egg count and worm number) suggest that the parasite populations during different stages of infection is affected by only one host mechanism or at least by closely associated, host-induced, processes. Thus, calves that had previously been wormfree seemed to be able to interfere actively with development and functioning of the parasite. This conclusion is made inevitable by the negative relation that was found between worm length and peak egg count, and the humoral antibody response of the calves as measured by the IFAT-technique.

This active interference of the host with development of the parasite so early in a primary infection complicates the separation of two commonly accepted elements of resistance, namely 'innate' and 'acquired' resistance. Innate resistance is defined as resistance comprised by characteristics, mechanisms or factors of the host, which exist prior to contact with the parasite. Acquired resistance is the

result of (a) defence response(s) which arise(s) only after previous experience of infection (WAKELIN, 1978).

In contrast to innate resistance, acquired resistance is specific and is based on an adaptive response to an antigenic stimulus. This response is comprised of several elements: specific proliferation of sensitized cells, synthesis of specifically reacting antibody and the establishment of the so called immunological memory (ROITT, 1977).

In expt. I we found significant relations between the production of specifically reacting antibody (IFAT) and development and functioning of the worm population as measured by worm length and peak egg count. Thus, according to the definitions mentioned above, it must be concluded that in our case acquired resistance played a significant role in an early stage of a primary infection. This contrasts with the common use of the term 'acquired resistance', because it is mostly used to designate resistance to a secondary infection. Such a use of the term is only correct if resistance is explicitly defined and measured as the ability to prevent the establishment of a new infection (challenge). If, however, the term resistance is used to designate any ability of a host to restrain or prevent any part of development and/or functioning of an invading or pre-existing parasite population, a concept of resistance which is also accepted here, it must be concluded that the specific host response seen in primary infections in our experiments, was a manifestation of acquired resistance.

Innate resistance must have played a role too; whether innate and acquired resistance are correlated cannot be concluded from our experiments. Because they are based on different factors and mechanisms (for general review; see SPRENT, 1969), there is reason to expect a negative relation. A low innate resistance may favour the establishment and growth of a large worm population which in turn will produce a violent antigenic stimulation of the immune apparatus. Such a negative relation between innate and acquired resistance may be a reason for the low correlation between IFAT antibody titre and parasitological findings: e.g. a lower innate resistance may result in longer worms but also lead to a high IFAT titre.

There are other reasons for the generally low, although significant, correlations between IFAT antibody titre and parasitological results: the IFAT technique measures only a part of the immune response, namely the presence of certain antibodies to worm material. This type of parameter has the following shortcomings:

- If antibodies of various specificities are detected it is not certain that all are functional (SOULSBY, 1960) i.e. are part of a reaction chain that has protective value for the host.
- The IFAT technique, due to its particular procedure, possibly measures only some of the antibodies that may be produced against the parasites, namely circulating IgG antibodies against fourth stage larval cuticular structures.
- The antibody response is probably only a part of the specific immune response to an invading parasite population. A cellular response, i.e. proliferation of antigen-reactive T-lymphocytes has been shown to play an essential role in

parasite expulsion in several intensively studied host-parasite model systems (see e.g. OGILVIE and LOVE, 1974; WAKELIN, 1975).

The very fact that the humoral antibody response is only a part of the total host response, might explain the rather complex relation between egg output and IFAT antibody response in the various primary infection groups in our experiments. It was concluded that antibody production was proportional to the level of egg output of a particular calf, unless a certain critical level of egg output was reached. Above this level a negative correlation between IFAT antibody response and peak egg output of individual animals was seen. This remarkable feature might be explained in two ways:

- The antibodies measured by the IFAT technique do not play a functional role in protection of the host, but reflect the degree of stimulation of the entire immune system by antigenic information from the parasite population. Apparently, the stimulation of the functional response requires more antigenic information than the stimulation of the IFAT antibody response.
- IFAT antibodies do have protective value, but only in collaboration with other activated components of the immune system. Resistance mechanisms become operative only if all parts of the reaction chain are present. Apparently antibodies – measured by the IFAT technique – are not the component that needs most antigenic stimulation.

In both cases mechanisms other than a humoral antibody response are essential to effect actual impairment of the parasite's development or functioning.

Significant correlations were found between IFAT titre increase on one hand, and worm length and peak egg output on the other. No significant correlation, however, was found between IFAT antibody response and worm numbers. This suggests that the immune mechanisms that regulate worm numbers (especially expulsion of worms) are less directly dependent on the humoral immune response than host responses which act against development and egg production of the worms. The available literature offers no clarification on this point. As was discussed in chapter 2, a lot of experimental work has been carried out on the expulsion mechanisms but not on resistance mechanisms against developing parasite populations. One of the few examples of such work (LOVE and OGILVIE, 1975) showed that in *N. brasiliensis* infections in the rat the immune reaction to larval stages in immune hosts was different from the one that expells adult parasites. This difference might, however, have been only quantitative.

Thus, a number of results show the complexity of the phenomenon 'resistance': on one hand the complexity of its mechanisms on the other hand the variety of effects that it may bring about on the parasite population.

5.2. THE VALUE OF VARIOUS PARAMETERS OF RESISTANCE

The level of resistance in a certain host at a certain time is determined by a large number of factors (see chapter 2). In our experiments all of them are kept as constant as possible, except two: infection experience and genetic background of

the host. Apart from a study of the influence of these factors, such a set-up, by providing experimental groups with different levels of resistance, allows an evaluation of parasitological and immunological parameters for their quality in measuring resistance. The experimental lay-out allows a comparison of three levels of infection experience – at the start of a certain infection regimen –: no experience (worm-free), a previous infection with one dose of 20,000 larvae, a previous infection with one dose of 100,000 larvae. On the basis of genetic differences in resistance, resistant and susceptible half-sib groups of calves were selected.

The results of the three experiments indeed show a marked similarity in the effects of the 'primary dose factor' and the 'genetic factor': egg output was lower, worms were shorter and less adult worms were found if it was a secondary infection in contrast with a primary infection or if, in the case of a secondary infection, the primary dose had been higher, or if the calf belonged to a half-sib group of a more resistant sire (ABH in expt. III). Furthermore it appeared from this comparison that the sensitivity (i.e. the ability to demonstrate variation between animals) of the various parameters for resistance was different: at a certain level of resistance all variation in a certain characteristic may have disappeared because the maximum level is reached in this respect (resistance is complete).

Thus, after a primary dose of 100,000 larvae resistance of all calves was so high that no significant rise of egg output could be brought about by a secondary dose, and therefore no variation in resistance could be expressed by this parameter. A similar result was found for the IFAT antibody response: in expt. III a difference in IFAT antibody response was seen between ABH- and PAN-calves after a primary dose of 100,000 larvae but not after the succeeding secondary infection, a kind of plateau level having been reached.

The number of worms, on the other hand, can vary without being expressed in variation in egg output and apparently this was the case in infection group 2 (expt. II). In this group, a significant sire effect on the percentage *C. surnabada* male worms at necropsy showed that sire group differences in adult worm numbers must have been present in an early stage of secondary infection (see section 4.3.1.2.).

In fact only the number of larvae seems to be unrelated to an animal's resistance; in section 4.3.1.1.2. it was concluded that the number of larvae at around 6 weeks after secondary infection depended only on the secondary infection regimen. Of course, this conclusion can not simply be generalized to other infection regimens. It is not clear, for instance, whether the larval burden in infection group 5 is the result of a lower establishment rate and a lower turnover rate, compared to infection group 3 where the larval burden was of approximately equal size. A lower establishment rate may indeed produce the same larval burden if the turnover rate, and therefore growth of larvae into fifth stage worms, is also lower.

As a general conclusion from the above it can be stated that the finding of differences in resistance depends on the level of resistance induced in the expe-

rimental animals and the sensitivity of the parameter that is used.

As an answer to the general question of how to compare the resistance of calves against *C. oncophora* infection, it can be stated that if all animals have had the same treatment, then egg output, worm length, adult worm number and parameters derived from these, are useful parasitological parameters to describe an animal's resistance. As an immunological parameter for resistance, the IFAT antibody titre is useful. These parameters are mutually correlated but rather weakly and the unexplained individual variation is still large. Therefore it is desirable to record as many characteristics as possible.

If, in contrast to the situation mentioned above, nothing is known about infection history, a more universal diagnostic is needed. In that case the number of useful parasitological tools to describe an animal's resistance to *C. oncophora* infection decreases dramatically. In fact only worm length expresses an animal's resistance quite independently of the time of necropsy. Our experiments show, indeed, that worm length is generally lower if there is more experience of infection (higher primary and/or secondary dose) or if the animal is genetically more resistant. If, however, turnover takes place, this characteristic also becomes somewhat uncertain.

The IFAT antibody titre is also a rather independent characteristic. Its sensitivity seems to be a bit different from the parasitological measures mentioned above; the IFAT antibody titre, as a parameter of resistance, is more sensitive at lower levels of resistance.

5.3. The effect of infection experience and genetic constitution on resistance

As was stated earlier, the experimental set-up allows a study of the effects of infection experience and genetic constitution on the resistance of calves to *C. oncophora* infection.

The immunity that is derived from previous infections has been studied in numerous investigations. Our results quite clearly confirm the immunizing effect of a previous infection: expts. II and III show that a primary dose of 100,000 instead of a dose of 20,000 larvae results in a significantly higher resistance to secondary infection. The immunizing effect of a primary dose of 100,000 larvae is even so high that a secondary infection can hardly result in significant egg production. This was also found in earlier work on the same host-parasite system (Kloosterman et al., 1978). Herlich (1965b) – the only author that reports comparable infections – saw an immunizing effect on egg output at 30 weeks after a single dose of 32,000 *C. oncophora*. In our experiments egg output seemed to be more sensitive than worm number; in *O. ostertagia* infections Herlich (1976) recorded the same phenomenon. This difference in sensitivity has been discussed earlier.

Following a re-infection, specific resistance mechanisms apparently can act earlier and more strongly: the IFAT antibody response is boosted as in the

classical example of a secondary response after the establishment of an immunological memory. The result is that in secondary infection a large variation in prepatent period is caused by specific resistance. In primary infections, patency of infection was apparently reached too soon to allow significant interference by specific resistance mechanisms. The finding of differences in prepatent period, as for instance by BRUNSDON (1962) in *Nematodirus* spp. infections in sheep, can probably be attributed to non-specific (innate) resistance mechanisms.

In repeated infections – as in our experiments or more frequent or even continuous infection as in the field – the spreading of larvae over more doses offers the opportunity for the host to develop a higher level of resistance to larvae ingested later. In our own experiments worm length was indeed smaller in infection groups that were given repeated doses (groups 3 and 5) than in groups receiving a single secondary dose (infection groups 2 and 4). A similar result was found by ELLIOT (1974), who investigated this question very systematically, although in a different host parasite system (sheep-*Ostertagia* spp.). He found that if the same number of infective larvae was given in twenty five daily doses instead of one, significantly more immature worms were found at necropsy. This effect was only seen if the infection rate was high enough to provoke a significant resistance.

In *Cooperia oncophora* infections in the calf resistance develops rather fast, as was concluded earlier. Therefore, differences in adaptive immunity – also genetic differences – must become visible in a relatively short period. In our experiments it appeared that genetic differences existed but only in special cases, i.e. for a certain parameter only if a certain level of antigenic stimulation had been present. The largest differences between sire groups were seen in infection group 4; it seems that this particular situation was about the optimum infection regimen to provoke genetic differences in resistance.

An intriguing question is whether the small sensitizing dose of 20,000 infective larvae was of crucial importance for differences in resistance to the secondary dose. It is clear, in the first place, that a certain level of infection must be present to provoke a host response and thereby to allow any genetic difference in host response to appear. Once this minimum necessary level of antigenic stimulation is reached in a primary infection (single dose), the development of the parasite population has already been unaffected for a certain time and the opportunity for the host to influence the parasite population has decreased. Therefore it seems likely that the largest genetic differences can be demonstrated in animals that are sensitized by homologous infection to such an extent that only a minimum additional stimulation is needed to provoke an effective response.

This hypothesis is supported by the results of previous experiments at our laboratory (KLOOSTERMAN et al., 1978). In sire groups selected for resistance/susceptibility, they found significant but smaller genetic differences in a primary single infection of 100,000 larvae, especially for worm length, than in infection groups 4 and 5 of our experiment III. Furthermore, KLOOSTERMAN et al. (1978) found in their field trial with two genetically different groups of calves,

differences in egg counts between the two groups in the period of 10 to 16 weeks after the start of the grazing period and not in the first ten weeks of infection.

As was seen in chapter 2, genetic differences in resistance to nematodes have been found previously in a number of laboratory host – parasite models; especially between inbred lines but also within outbred lines. In domestic animals of economic importance most of the work has concerned comparisons of breeds and haemoglobin types in sheep. Reports on within breed genetic differences in resistance to nematode parasites of domestic animals are very scarce. As far as cattle are concerned, apart from the work of KLOOSTERMAN et al., (1978) – of which the experiments reported here are a continuation – only ROSS et al. (1959) described a case of genetic variability in resistance to natural infections within the Zebu breed in Nigeria.

The present results are – after the results reported by KLOOSTERMAN et al. (1978) – a second and independent proof that in a random sample of Dutch Friesian bull calves a part of the variation in resistance to *Cooperia oncophora* infections has a genetic origin.

The magnitude of this genetic part of variation in resistance (heritability) cannot be calculated simply. Firstly, because the experimental procedures included a selection of extreme sire groups and therefore disturbed the initial situation of normal genetic variation. Secondly, because it appears to be impossible to measure resistance as one simple parameter. A third insoluble problem is the fact that (genetic) differences in resistance appear to depend very much on the actual infection regimen. Heritability estimates would therefore apply only to calves that are treated and infected in the same way as the animals in our experiments (i.e. bull calves kept indoors, fed hay and concentrates and given the same primary and secondary infections).

Although in our experiments the opportunity for genetic differences to be revealed were maximized (discriminating infection patterns in otherwise standardized circumstances), genetic differences that were found were quite moderate in size, even if it is kept in mind that members of half sib groups share only one quarter of their genetic information. On the other hand, it could not be expected that resistance in a natural host – parasite system would be determined genetically to a high degree: resistance is a very complex phenomenon and partly the result of a number of immune mechanisms.

One of these mechanisms, the humoral antibody response, has been studied very extensively by BIOZZI and co-workers. They found that in mice the humoral antibody response to heterologous red blood cells was indeed genetically determined; the heritability estimate was only about 0.2 (BIOZZI et al., 1975). Besides this, in general the heritability of resistance to specific diseases is found to be between 0.0 and 0.3 (ANDRESEN, 1978).

The finding, however, that resistance to a nematode parasite in cattle clearly has a genetical basis undoubtedly bears importance for further experimental work.

5.4. Resistance and performance

To evaluate the importance of the heritability of host resistance for the practice of animal husbandry, another question has to be answered first, namely how resistance, as discussed above, is related to the actual performance of a calf.

As was concluded from the review of the literature, the distinction between resistance to the parasite and resistance to the harmful effects of parasite infection is explicitly recognized by a number of authors (see section 2.3.). Others implicitly assume that such a distinction is not necessary because resistance – by exerting harmful effects on the parasite – can only have favourable consequences for the host itself. Examples in the literature (see section 2.3.) have shown that the latter assumption is not always justified.

The host – parasite system that is the subject of our studies may not be the most suitable one to study the relation between resistance and performance. *Cooperia oncophora* is known to exert only a very moderate pathogenic effect on the calf (see section 2.1.). We found, however, significant depressions of live weight gain, in the same range as observed by Herlich (1965a), Borgsteede and Hendriks (1979), Coop et al., (1979). Besides this, other clinical signs of infection, such as anorexia and diarrhoea, were incidentally recorded. It must be added here that the experimental calves were all kept under good circumstances and on a relatively high plane of nutrition. It is probable that under field conditions the effect of infection would have been more pronounced.

Nevertheless, in primary infections a dose of 100,000 larvae significantly affected calf performance; a dose of 20,000 larvae did not, but neither did such a dose provoke any effective host response. In the 100,000 infection, the depression of live weight gain and feed intake was concentrated at the time that egg output started to decline and expulsion of adult worms commenced. Thus the depression of host performance coincided with a host response to the parasite. But apparently this was not just a coincidence: the correlations between live weight gain and resistance parameters, such as egg count and IFAT antibody response, show that the generation of a protective host response to the parasite was the very reason for the depressed feed intake and live weight gain. In other words, the reaction of the calf against the parasite in a primary single infection of 100,000 infective larvae was, at least in the short term, unfavourable to it.

In secondary infections the situation appeared to be quite different. Compared to primary infections with 100,000 larvae the infections given were heavier, host responses were stronger and the effects on performance were somewhat more pronounced. Sons of the resistant sire (ABH) gained not less – as in primary infection – but even slightly more weight than sons of the susceptible sire (PAN). Indeed, within infection groups 3 and 5 a significant positive correlation was found between live weight gain and resistance (as measured by peak egg count and worm length).

This is in sharp contrast to the findings in a primary infection with 100,000 larvae. This contrast might be explained by the fact that in a secondary infection the calves – being sensitized by a primary dose – have the opportunity to prevent

the establishment of the parasites. More resistant calves will thereby never harbour as many adult parasites as less resistant calves, nor will they have to expell these larger numbers of worms. The expulsion of larger numbers of worms was the very reason for the depressed performance of resistant animals in the primary infection. Groups 3 and 5 gave the clearest demonstration of this phenomenon, because in these repeatedly infected calves prevention of establishment of incoming larvae gave the largest favourable effect.

Thus, in the long run resistance to *Cooperia oncophora* infection may be a favourable characteristic of an animal; whereas in a primary infection the building-up and/or display of resistance impairs host performance.

Such a conclusion is not directly supported by similar ones in the available literature on resistance to gastro-intestinal nematodes. The review of relevant literature in chapter 2, however, presents circumstantial evidence: the unfavourableness of a protective response to the parasite may be derived from the pathophysiological implications of inflammatory reactions in the intestinal tissues (harmful physiological disturbances) that are controlled by the host and not – at least not directly – by the parasite.

Only two reports provide some direct support for our conclusion. RIFFKIN and DOBSON (1979) found a significant negative correlation between resistance to a primary single *H. contortus* infection and live weight gain of sheep during five weeks after this infection. BARGER (1973) recorded a negative correlation between resistance to *T. colubriformis* after the administration of two single doses of larvae and wool growth during ten weeks after primary infection. BARGER (1973) had not expected this result; RIFFKIN and DOBSON (1979) suggested that the inverse relationship between resistance and performance is not absolute. The results of the present study strongly support this suggestion: after prolonged infection in both cases the inverse relationship very likely would have turned into a positive one.

6. SUMMARY

The variation in resistance of cattle to gastro-intestinal nematode infection was investigated in three experiments. Bull calves, aged three months and reared under uniform conditions, were artificially infected with infective larvae of *Cooperia oncophora*, a moderately pathogenic but very abundant parasite of the small intestine. The study aimed to detect a possible genetic effect on resistance and to evaluate the consequences of this resistance for the performance of the animals.

Resistance to infection was measured by a number of parasitological and immunological techniques. The course of infection was determined by monitoring faecal egg output and by *post mortem* examination of the worm burden and characteristics of individual worms. Three immunological tests were used to measure immunological reactions of the calves: an immunofluorescent antibody test (IFAT) on fourth stage larvae and an indirect haemagglutination test (IHA) using adult worm extract as the antigen to monitor humoral antibodies in the serum; an intradermal test (IDT) to detect homocytotropic antibodies. Performance of the experimental calves was assessed by recording clinical signs of infection, live weight gain and, for some animals during a limited period, feed intake.

In the first experiment 46 calves, comprising 16 half-sib groups (sons of A.I. sires) were infected with a single dose of 100,000 infective larvae. Faecal egg output after this infection, although very variable, showed a basic pattern of logarithmic increase and subsequent logarithmic decrease in most calves. The decrease was due to an initial reduction of individual egg production of female worms and a subsequent expulsion of worms. This expulsion was selective in two ways: female worms were more persistent than males and, among male worms, the polymorph *Cooperia surnabada* was more persistent than *Cooperia oncophora*.

Resistant calves showed a low faecal egg output that was the result of a small burden of short worms. Egg output and worm length were related significantly to the humoral antibody response of calves as measured by the IFAT technique. There were no relations between the immunological response measured by the IHA test or IDT technique and any of the parasitological parameters. The relation between IFAT antibody level and faecal egg output was curvilinear, suggesting a threshold level of antigenic stimulation which provokes antiparasitic activity of the host.

A primary single infection with 100,000 infective larvae caused a mild but significant growth depression which, in the second experiment, appeared to coincide with a notable loss of appetite. However, in experiment I, growth impairment was significantly larger in those calves which showed a higher level of resistance to infection.

Analysis of variances between and within sire groups in experiment I showed that the IFAT antibody response was partly genetically determined.

From the 16 sires which had provided offspring for expt. I five, which had shown extreme resistance (2) or susceptibility (3) as judged by IFAT antibody titres and faecal egg counts, were chosen for expt. II. Similarly, for expt. III an ultimate selection was made of two extreme sire groups, one being the most resistant and one the most susceptible.

Except for a control group, all 93 calves in expts. II and III received a primary dose of either 20,000 or 100,000 infective larvae and, seven weeks later, a secondary infection of either 350,000 larvae as a single dose or multiple doses of 75,000 larvae given at a rate of three doses per week.

In expts. II en III the primary infection appeared to have an immunizing effect which was largest for the high primary dose. Thus egg output after secondary infection was severely depressed, mainly due to the low number of gravid female worms present. Generally, adult worms were shorter and were expelled earlier and more rapidly due to this immunizing effect.

In contrast to the adult worm burden, the number of fourth stage larvae was greatly enlarged if repeated doses of larvae instead of one single dose were given as secondary infection, irrespective of the primary dose level. The decrease of the number of fourth stage larvae in the course of secondary infection was probably not mainly due to direct expulsion but to a turnover process which consists of development of worms from the early fourth into the adult stage following expulsion of adult worms.

As regards the immunological techniques, the IHA and IDT methods proved to be of no value as indicators of resistance of the calves to infection. IFAT antibody titres, on the contrary, were boosted by secondary infection and appeared to be negatively correlated to faecal egg output and worm length.

Live weight gains after secondary infection were significantly depressed by about 20%; feed intake was lower than expected in the absence of infection. Diarrhoea, associated with massive expulsion of worms, occurred simultaneously with a depression of feed intake and live weight gain.

In contrast to the findings in a primary infection with 100,000 larvae, resistance to secondary infection (as measured by peak egg output, worm length and IFAT titres) was associated with higher weight gains. As an explanation for this contrast it is suggested that expulsion of adult worms was one of the causes of depressed performance. In primary infection a high level of (acquired) resistance caused a large expulsion, in secondary infection the opposite was true because then a high level of resistance already prevented the establishment of a large worm burden and thereby the necessity to expel it.

Thus, in the long run, resistance of calves to *Cooperia oncophora* infection seems to be a favourable characteristic, whereas in a primary infection it impairs host performance.

Genetic differences after secondary infections were found for egg output,

worm numbers and worm length, but only if the primary infection had been the small dose of 20,000 larvae rather than the larger dose of 100,000. With regard to IFAT antibody titres a similar difference appeared between the two extreme sire groups ultimately selected. In secondary infections after the small primary dose only the sons of the resistant sire reached a plateau level of maximum antibody titre, whereas after the large primary dose calves of both extreme groups reached this level.

Thus, genetic differences in resistance were found only in special cases i.e. for a certain parameter only if a certain level of antigenic stimulation had been present. This result was very similar to the finding that the appearance of differences in resistance due to different levels of infection experience depended on the level of resistance induced and on the sensitivity of the parameter that was used.

Nevertheless, this study has clearly shown that genetic differences in resistance of calves to *Cooperia oncophora* infections do exist. The experimental procedures did not allow an estimation of the heritability, although the results suggest only a minor role of genetic factors. Furthermore, it should be kept in mind, that the expression of genetic differences depends largely on the infection regimen and that resistance to infection is not invariably reflected in a better performance.

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SAMENVATTING

De weerstand van kalveren tegen maagdarmwormen wordt gekenmerkt door een grote individuele variatie. Deze studie beschrijft een drietal experimenten dat werd uitgevoerd om deze variatie te onderzoeken. Onder uniforme omstandigheden opgefokte stierkalveren werden hiertoe op een leeftijd van drie maanden besmet met infectieuze larven van *Cooperia oncophora*. Deze nematode, die weliswaar zeer algemeen voorkomt, maar niet erg pathogeen is, parasiteert de dunne darm van het rund. Het doel van het onderzoek was, vast te stellen of de weerstand van kalveren tegen *Cooperia oncophora* voor een deel erfelijk bepaald is en of deze weerstand, uit zoötechnisch oogpunt bezien, een gunstige eigenschap is.

De weerstand van de kalveren tegen besmetting werd gemeten door middel van een aantal parasitologische en immunologische technieken. Zo werd het verloop van de infectie gevolgd door bepaling van de ei-uitscheiding in de mest en door diverse bepalingen na slachting van de proefdieren zoals van het aantal wormen, de differentiatie naar stadium en sexe, de lengte van wormen en het aantal eitjes in de uterus van vrouwelijke wormen. De immunologische reactie van de kalveren werd gemeten met drie technieken. Voor de bepaling van antilichamen in het serum werden een indirecte immunofluorescentie test (IFAT) op basis van vierde stadium-larven en een indirecte haemagglutinatietechniek (IHA) op basis van volwassen worm extract gebruikt. Om de aanwezigheid van homocytotrope antilichamen te kwantificeren werd een intradermale techniek (IDT) toegepast. Om de mogelijke nadelige invloed van infectie op het kalf vast te stellen werd het voorkomen van diarrhee geregistreerd en werden groei en – weliswaar in beperkte mate – voeropname vastgelegd.

De eerste proef werd uitgevoerd met 16 groepjes van 2 of 3 zonen van eenzelfde KI-stier, in totaal 46 kalveren. Alle proefdieren werden met een dosis van 100.000 infectieuze larven besmet. De individuele variatie in ei-uitscheiding na deze infectie was zeer groot. De meeste kalveren echter vertoonden hetzelfde patroon: een logarithmische stijging naar een duidelijke top, gevolgd door een logarithmische daling. De daling van de ei-uitscheiding werd veroorzaakt door, aanvankelijk, een daling van de eiproduktie van individuele wormen, later gevolgd door afdrijving van wormen. Het afdrijvingsproces was selectief in twee opzichten: mannelijke wormen bleken gevoeliger dan vrouwelijke en mannelijke wormen behorende tot *Cooperia surnabada*, een polymorf van *C. oncophora*, handhaafden zich beter dan de andere mannelijke wormen.

Kalveren die een lage ei-uitscheiding hadden bleken een kleine wormlast bestaande uit korte wormen te herbergen. Bovendien was er een significant verband tussen ei-uitscheiding en wormlengte enerzijds en de antilichaamrespons bepaald door middel van de IFAT methode anderzijds. Het verband tussen ei-uitscheiding en IFAT antilichaamrespons was kromlijnig, hetgeen wijst op een drempelniveau van antigene prikkeling dat overschreden moet worden voor

de gastheer actie tegen de parasiet onderneemt. De resultaten van de IHA of IDT techniek bleken niet gerelateerd aan resistentie tegen de parasiet.

Een primaire, eenmalige infectie met 100.000 larven veroorzaakte een geringe maar significante groeidepressie. Deze bleek in een volgend experiment overigens samen te gaan met een verminderde eetlust van de kalveren. Opmerkelijk was, dat in proef I de groeivertraging juist bij de meer resistente kalveren het grootst was.

Analyse van varianties binnen en tussen stiergroepen in proef I wees uit, dat de antilichaamrespons gemeten door middel van de IFAT-methode, voor een deel genetisch bepaald was.

Van de 16 KI-stieren die nakomelingen hadden geleverd voor proef I werden er, op basis van IFAT antilichaamtiters en ei-uitscheiding, 5 uitgekozen om kalveren te leveren voor proef II. Van twee stieren hiervan hadden de nakomelingen zich in proef I gemiddeld het meest resistent getoond, terwijl de andere stieren de meest gevoelige groepen hadden geleverd. Voor proef III werd andermaal een selectie uitgevoerd; slechts zonen van de meest resistente en de meest gevoelige stier werden hier ingezet.

Met uitzondering van de controledieren werden alle 93 kalveren in proef II en III besmet met een primaire dosis van 20.000 of 100.000 infectieuze larven. Zeven weken later kregen ze allemaal een secundaire infectie van, óf 350.000 larven ineens, of doses van 75.000 larven in een tempo van drie doses per week.

In proef II en III bleek, dat de primaire infectie een duidelijk immuniserend effect had. Dit effect was het grootst voor de hoge primaire dosis. Zo was de ei-uitscheiding na secundaire infectie sterk verlaagd, vooral als gevolg van een reductie van het aantal wormen dat eieren produceerde. In het algemeen resulteerde het immuniserende effect van primaire infectie in kortere wormen die eerder en sneller werden afgedreven.

Het aantal volwassen wormen was dus afhankelijk van de primaire infectiedosis. Het aantal vierde stadium-larven daarentegen werd juist beïnvloed door de aard van de secundaire infectie: ongeacht de primaire dosis werden na de herhaalde infecties veel grotere aantallen larven gevonden dan na de eenmalige secundaire infectie. De daling tijdens het verloop van de secundaire infectie die ook bij het aantal vierde stadium-larven optrad, was waarschijnlijk niet het gevolg van rechtstreekse afdrijving, maar van een 'turnover'-proces. Dit hield in, dat larven uit het vroege vierde stadium zich ontwikkelden tot volwassen wormen, telkens nadat een hoeveelheid volwassen wormen was afgedreven.

Wat de immunologische technieken betreft, bleken de IHA noch de IDT-methode gerelateerd aan de weerstand van kalveren tegen secundaire infectie. De respons gemeten met de IFAT echter vertoonde een klassieke 'booster' na secundaire infectie en was negatief gecorreleerd met de ei-uitscheiding in de mest en de lengte van de volwassen wormen.

De groei van de proefkalveren was onder invloed van secundaire infecties met ongeveer twintig procent verlaagd, terwijl ook de voeropname nadelig

beïnvloed werd. Een daling van voeropname en groei ging in een deel van de gevallen gepaard met diarrhee. Deze diarrhee trad vaker op bij kalveren waarbij sprake was van afdrijving van grote aantallen volwassen wormen.

Een hogere weerstand van kalveren tegen secundaire infectie (dat wil zeggen een lagere ei-uitscheiding, geringere wormlengte en hogere IFAT titers) ging gepaard met een hogere groei. Dit in tegenstelling tot de situatie na primaire infectie, waarin een negatief verband tussen weerstand en groei werd gevonden. Als verklaring voor deze tegenstelling wordt de mogelijkheid geopperd dat met name het afdrijvingsproces met een verminderde voeropname en groei gepaard gaat. In primaire infecties gaat een hogere weerstand gepaard met meer afdrijving van wormen; in secundaire infecties laat een hoger niveau aan reeds verworven weerstand een wormlast van beperktere omvang tot ontwikkeling komen waardoor er juist minder intensief afdrijving plaatsvindt in kalveren met een hogere weerstand.

Als antwoord op de vraag of een hogere weerstand van een kalf tegen *Cooperia oncophora* een gunstige eigenschap is, kan dus gesteld worden dat dit op langere termijn zeker het geval is, maar dat na een primaire infectie eerder het tegendeel waar lijkt.

Erfelijk bepaalde verschillen werden na secundaire infecties gevonden voor de ei-uitscheiding in de mest, de aantallen wormen en de wormlengte; uitsluitend echter wanneer de primaire infectie de lage dosis van 20.000 larven was geweest. De IFAT antilichaamrespons leverde een soortgelijk resultaat op. In secundaire infecties na een lage primaire dosis bereikte alleen de groep zonen van de als meest resistent geselecteerde stier een plateau van maximale antilichaamtiters; na de hoge primaire dosis bereikten beide groepen, ook de gevoelige, dit niveau.

Aldus kwamen erfelijk bepaalde verschillen in weerstandsvermogen alleen tot uiting in specifieke situaties, namelijk voor bepaalde kenmerken alleen na een bepaalde hoeveelheid antigene prikkeling. Een soortgelijke conclusie kon getrokken worden ten aanzien van verschillen in weerstand veroorzaakt door verschillende infectieniveau's. De expressie van deze verschillen hing immers ook af van het bereikte weerstandsniveau en de gevoeligheid van het kenmerk waarin die weerstand werd uitgedrukt.

Niettemin heeft dit onderzoek duidelijk het bestaan van genetisch bepaalde verschillen in resistentie van kalveren tegen *Cooperia oncophora* aangetoond. Hoewel een schatting van het relatieve belang van deze erfelijke factoren op basis van dit onderzoek niet mogelijk is, lijkt hun rol beperkt. Bovendien moet bedacht worden dat de feitelijke infectiekansen grotendeels bepalend zijn voor het tot uiting komen van een bepaalde genetische aanleg. Tenslotte is een hoog weerstandsvermogen niet zonder meer een gunstige eigenschap van een dier.

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TABLE 1. Summary of experimental designs.

Number of experiment	Total number of		Primary infection dose	(*n*)	Secondary infection	(*n*)	Time of necropsy days after		Infection group	Symbols used in figures	
	calves	sires					prim. inf. (*n*)	sec. inf. (*n*)		in primary infection	in secondary infection
I	46	16	100,000		–		34 (16)	–			
							41 (16)	–	1		–
							48 (14)	–			
II	44	5	100,000		Single dose		–	18 (11)			
					of 350,000		–	25 (11)	2		
							–	32 (11)			
							–	39 (11)			
III	49	2	100,000	(14)	Repeated	(14)	–	38 (8)			
					doses of 75,000		–	40 (6)	3		
			20,000	(28)	Single	(14)	–	38 (6)			
					dose of 350,000			40 (8)	4		
					Repeated	(14)	–	38 (7)			
					doses of 75,000		–	40 (7)	5		
			0	(7)	0	(7)	Not necropsied		6		