# OBSERVATIONS ON THE EPIDEMIOLOGY OF TRICHOSTRONGYLOSIS OF CALVES

A. KLOOSTERMAN

# OBSERVATIONS ON THE EPIDEMIOLOGY OF TRICHOSTRONGYLOSIS OF CALVES

Dit proefschrift met stellingen van

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Landbouwkundig ingenieur, geboren te Leeuwarden, 9 augustus 1938, is goedgekeurd door de promotor, Dr. Th. Stegenga, hoogleraar in de Veeteeltwetenschap.

*De Rector Magnificus van de Landbouwhogeschool,*

J. M. POLAK

*Wageningen,* 3 mei 1971

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# OBSERVATIONS ON THE EPIDEMIOLOGY OF TRICHOSTRONGYLOSIS OF CALVES

*(with a summary in Dutch)*

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS, MR. J. M. POLAK,
HOOGLERAAR IN DE RECHTS- EN STAATSWETENSCHAPPEN
VAN DE WESTERSE GEBIEDEN
TE VERDEDIGEN TEGEN DE BEDENKINGEN VAN EEN
COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 28 MEI 1971 TE 16 UUR

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ABRAHAM KLOOSTERMAN

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# STELLINGEN

I

De belangrijkste mogelijkheden voor het voorkomen van maagdarmstrongylose zijn gelegen in landbouwkundige maatregelen.

II

Eitellingen hebben geen waarde voor de diagnostiek van maagdarmstrongylose bij kalveren in Nederland.

III

Het aantal eieren en larven dat op de weide overwintert is van belang voor de epidemiologie van maagdarmstrongylose bij kalveren.

IV

Bij de pogingen, in ons land te komen tot uniforme werkwijzen bij het parasitologisch mest-onderzoek, dient het gebruik van de McMaster-methode of een modificatie daarvan te worden overwogen.

V

Te betreuren valt dat het 'SWANN-report' blijk geeft van een eenzijdige beoordeling van het gebruik van antibiotica.

(Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine. H.M.S.O., LONDON, 1969).

VI

De afdeling Veeteelt van de Landbouwhogeschool is de aangewezen plaats om onderzoek te doen naar de invloed van genen op de resistentie van landbouwhuisdieren tegen enzoötische verwekkers van ziekten.

VII

De, althans in het verleden bestaande, neiging van zoölogen om de bestudering van economisch belangrijke dieren te vermijden is afkeurenswaardig.

H. D. CROFTON, "Nematodes", Hutchinson, LONDON, 1968.

VIII

Het publiceren van resultaten van opiniepeilingen op politiek gebied kan beschouwd worden als een niet ongevaarlijk middel tot beïnvloeding van het publiek.

IX

In de toekomstige nieuwbouw van het Veeteeltcomplex is het gewenst de kantine van (een) zo groot mogelijke tafel(s) te voorzien.

A. KLOOSTERMAN
Wageningen, 28 mei 1971

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# CONTENTS

# INTRODUCTION

General agreement exists among several authors that trichostrongylosis in cattle and sheep has to be looked upon as a management disease, caused by increasingly rapid changes in stocking-rate, grazing systems, number of cattle per unit of labour and other farm management factors.

Cattle husbandry in the Netherlands can be characterized by a very high-stocking rate (up to 2.5 cows per ha) which has been made possible firstly by the extensive use that is made of artificial fertilizers (up to 200 g pure N/ha) and secondly by application of rather intensive rotational grazing systems for milking cows. With the number of milking cows the number of calves per farm has also increased, but the rotational grazing is largly restricted to the first category while several calves are still grazing on the very same 'calf paddock' near the farm house.

Another point of importance is the use of permanent pasture (96% of the grassland) while the use of ley pastures is relatively small.

Finally the proportion of the total farm area that is mown at least once per year for hay- or silage-making is very high (up to 200%). From an epidemiological point of view this high mowing percentage might counterbalance the presumably unfavourable effects of high stocking-rates and permanent pastures. OOSTENDORP et al. (1965, 1968) showed that calf-grazing on aftermath (which becomes available as soon as early June) can be extremely useful in controlling trichostrongylosis in these animals.

Whereas considerable work has been done on the liverfluke-problem in the Netherlands and an extensive study was made of trichostrongylosis in sheep by WENSVOORT (1961), there is relatively little information on nematodes in cattle.

The present study is an attempt to get a better insight into the following subjects:

1. The excretion of eggs in the faeces by calves.
2. The infestation of pastures with infective larvae.
3. The incidence of the different nematode-species.
4. The effects of farm management on the epidemiology of the disease.

These points have not systematically been studied in the Netherlands, and abroad the first three have often been investigated under conditions of severe infection. Although gastro-intestinal nematodes are recognized to be widespread in cattle in the Netherlands, trichostrongylosis is not as serious a problem as it is in other parts of the world, where mortality and weight-loss among these animals can be relatively common. Therefore, in the study reported below, as large a number of farms as possible, among them farms with apparently healthy calves, have been under investigation.

Under field conditions the faecal egg-output is the only tangible result that can give an impression about the worm-population in the host. Besides that it gives information on the degree to which a pasture is contaminated. Although

recently much doubt has been thrown on the diagnostic value of faecal egg-counts, considerable labour is still spent on these counts in the Netherlands. Therefore the faecal egg-counts will in this study be evaluated both from the epidemiological and from the diagnostic point of view.

# 1. LITERATURE

## 1.1. General considerations

During the past century, particularly the last twenty years, an enormous number of papers has been published on gastro-intestinal nematodes in farm animals. Many of them deal with trichostrongylosis in sheep and with anthelmintic treatment of the disease in various domestic animals. Among them only those studies that are of general interest will be mentioned here.

A large proportion from the literature on trichostrongylosis in cattle appears to be of typical veterinary interest. In the survey presented below special attention will be given to methods of research and to the epidemiology of the disease as it occurs in the field. Wherever possible, use will be made of recently published review-articles.

Finally, we want to stress that it is not intended to give a complete review of the available literature.

## 1.2. The incidence of species

A list is given by Swierstra et al. (1959) of 13 species of gastrointestinal nematodes recorded from cattle in the Netherlands. No information is given on the relative abundance of these species, but according to Jansen (pers. comm). the situation resembles very much that reported by Rose (1968) for S. E. England. This author found the genera *Ostertagia, Cooperia, Nematodirus* and, to a lesser extent, *Trichostrongylus* to be most prevalent in calves. Within these genera *Ostertagia ostertagi, Cooperia oncophora, Nematodirus helvetianus* and *Trichostrongylus axei* were most frequently recorded. These findings are in good agreement with the reports from other authors studying natural infections in temperate climate regions: Roberts 1957a and Peterson, 1957 Australia; Anderson et al., 1965b, W. Scotland; Ross, 1965, N. Ireland; Burger et al., 1966, N. W. Germany; Rice and Smith, 1966, E. Canada.

Other species, *Oesophagostomum radiatum, Bunostomum phlebotomum, Haemonchus contortus, Haemonchus placei, Cooperia punctata, Cooperia pectinata* are regarded as important cattle parasites in tropical and subtropical climates (Porter, 1942, Southern U.S.A.; Roberts, 1951, Queensland, Australia; Lee et al., 1960, Nigeria; Alicata, 1960, Hawaii; Kuil, 1967, Suriname).

Of the species predominant in temperate climates, *Ostertagia ostertagia* is recognized by most authors as the main source of clinical disease and much research has been focussed on this species.

It must be pointed out here that estimates of the relative abundance of species can be made by two basically differing methods: by counting worms *post*

*mortem* or by counting and differentiation of eggs in faeces. These methods may yield quite different results.

ROBERTS (1957a), PETERSON (1957) and BÜRGER et al. found by faecal examinations that *Cooperia oncophora* was far more abundant in calves than *Ostertagia ostertagi*. CIORDIA et al. (1962a,b, 1964), ANDERSON et al. (1965), BÜRGER et al. (1966) and ROSE (1968), on the other hand, reported more *Ostertagia ostertagi* than *Cooperia oncophora* when counting worms P. M. These differences probably have to be attributed to differences in egg-laying capacity of the two species.

In the next sections the various species will be designated by abbreviations of the genus name, as *O. ostertagi*, *C. oncophora* and so on.

## 1.3. METHODS OF RESEARCH

The various methods in use for the study of trichostrongylosis can be divided into two main categories:

1. Observations on the host animal, e.g. clinical, biochemical, haematological and immunological observations, faecal examinations and worm-counts *post mortem*.
2. Observations on free living stages of the parasites, i.e. faecal examinations and estimations of pasture infection.

For the purpose of routine examinations on large numbers of farms, where subclinical infestations exist in the majority of cases, only the observations of the second category can be applied. Therefore only these methods will be considered here.

### 1.3.1. *Faecal examinations*

#### Sampling of faeces

Whatever the method of faecal examination may be, the counts are always made on a very limited amount of faeces which is supposed to be representative of all the faeces a certain animal has produced during a certain period of time. In another part of this chapter the variations between samples due to epidemiological causes, and the variation within samples due to subsampling of faeces or the technique used, will be considered.

The question we want to raise here is why, as a rule faecal samples are taken from individual animals. Very often (among others PITRE, 1967; DORSMAN, 1954) it is recommended that fresh samples should be taken from the animal's rectum, as older samples will not yield all the eggs because some of them may have reached a larval stage. Moreover, samples picked up from the soil might be contaminated with eggs and larvae of free-living nematodes. Another argument, though rather theoretical as yet, has been put forward by HUNTER and QUENOUILLE (1952) who stated that the dispersion of egg counts throughout a flock can be as important an epidemiological criterion as the mean level of infestation.

ROBERTS et al. (1952) found that the mean egg-count in a flock is a very effec-

tive aid to diagnosis, provided a sufficient number of animals is sampled and the disease is considered as affecting the herd rather than the individual animals. Many authors share this opinion. Furthermore, most of them advocate treatment of the whole flock in which disease occurs, instead of restricting treatment to individuals which show clinical signs. Nevertheless no studies have been made on the possibility of examining one single sample from a herd that has been collected by thorough-mixing either of separate rectal samples or of samples from freshly passed faecal pats on the pasture. Undoubtedly such procedures could save much time and labour.

## Counting of eggs

Counting of eggs in faeces is a widely used method in diagnostical and epidemiological investigations on trichostrongylosis. A great variety of techniques is known. Most of them can be regarded as modifications of the dilution technique of STOLL (1930), the direct centrifugal floatation (D.C.F.) technique of LANE (1924) and the MCMASTER-technique of GORDON and WHITLOCK (1939). Excellent reviews on various modifications are given by ECKERT (1963) and GIBSON (1965).

Comparison of the three mentioned basic techniques have led to the following general conclusions:

The most accurate are counts by the STOLL-technique and its modifications, as the MCMASTER-technique tends to some overestimation of the number of eggs per gram (= e.p.g.-value) (LEVINE et al., 1960; PETERS and LEIPER, 1940), and the D.C.F.-techniques may lead to serious underestimation, due to loss of eggs (LEVINE et al., 1960).

The D.C.F.-techniques have a high sensitivity, i.e. they are able to detect lower numbers of eggs per gram of faeces, when compared with the other two, and of these, the MCMASTER-technique, being in a certain sense a combination of STOLL's and LANE's techniques, is more sensitive than the dilution-technique.

Of the three techniques the MCMASTER is reported to be the speediest which is probably one of the major reasons why this method is used by many authors, especially those working with sheep.

## Differentiation of larvae

Completing of faecal egg-counts by differentiation of the eggs at genus- or species-level may add considerable information, as it is known that the various species can greatly differ in pathogenicity, egg-laying capacity and resistance to adverse climatic conditions or anthelmintics. The eggs of *Nematodirus spp*, *Bunostomum spp*, *Trichuris ovis* and *Strongyloïdes papillosus* can be differentiated directly. Eggs of other genera are not easily distinguished from each other. Most work has been done on sheep-nematodes. KRUG and MAYHEW (1949) and HANSEN and SHIVNANI (1956) working on cattle nematodes, could separate the eggs of other genera into two groups: the *Haemonchus-Oesophagostomum*-group and the *Ostertagia-Cooperia-Trichostrongylus*-group. According to ECKERT (1963) direct egg-differentiations, despite the extensive studies made by

several authors, are of limited value for practical purposes. Under Dutch conditions, the first of the above mentioned group is probably not important in calves.

Differentiations are most frequently made by means of culturing and identification of infective larvae. A method of culturing 3rd stage larvae from eggs in faeces is described by ROBERTS and O'SULLIVAN (1950). Most of the methods used by various workers are essentially the same or slight modifications of it. (KEITH, 1953; HANSEN and SHIVNANI, 1956; CORTICELLI and LAI, 1964; THEODORIDES, 1964).

The temperature used in various studies varies from 25°C tot 28°C. This temperature-range is in good agreement with the opinion of WALLACE (1960), with the results of CIORDIA and BIZZELL (1963) and those of ROSE, (1961, 1963 a,b.)

Another important factor is the composition of the culture medium. ROBERTS and O'SULLIVAN (1950) concluded that ground dried sterilized cow-dung was the medium of choice out of several other substances they tested. CIORDIA and BIZZELL (1960) observed a favourable effect of sphagnummoss on the recovery of larvae from faecal cultures.

All the described procedures for extraction can be regarded as modifications of the original Baermann-funnel method (BAERMANN, 1917).

Although some authors (KAUZAL, 1940; DINABURG, 1942; DURIE, 1959) reported inconsistent results from this method, ECKERT (1963), using freshly cultured faeces, had good results with it. DURIE (1959) regarded periods of inactivity, particularly in aged larvae, as the main reason for the highly variable results with the Baermann-method. In many of the cited reports on different culture-media or extraction-procedures there is lack of statistical proof of the differences claimed.

KEITH (1953), HANSEN and SHIVNANI (1956) ,GEVREY et al. (1964) and CORTICELLI and LAI (1964) gave descriptions of morphological characters and of measurements of infective nematode larvae from cattle. Despite geographical variety of the countries where these authors were working, there was a good general agreement between their results. Most of them were using pure strains of nematode species; this means that the environments where the larvae came from must have been rather limited. Unfortunately, there are indications that environment (e.g. the diet of the donor-animal, the culture medium) may influence the measurements of larvae, (ECKERT, 1963) and therefore one cannot expect all the third stage larvae from faecal samples of a large host population to fall within the measurement ranges given by the above cited authors. The total length of the larvae and the extension of larval sheath beyond the tail of the larva are the two measurements by which, together with morphological characters, differentiation of larvae is possible. Logically, a positive regression between total length and extension of sheath might well be expected. Such regressions might be more useful than measurement-ranges, especially where overlapping of measurements occurs e.g. between *Cooperia* species (HERLICH, 1965) and between *O. ostertagi* and *Haemonchus contortus* (ECKERT, 1963). It appears however that no studies on these regressions have been made.

In order to avoid the nine-days culture time, which is a great disadvantage, particularly in diagnostic work, WHITLOCK (1956) described a method for the recovery and identification of first stage larvae of sheep nematodes. This method has as yet not been very much used, probably because of the time-consuming measuring procedure which is necessary, the possible confusion with free-living nematodes species, and the high vulnerability of this stage.

### Counting of larvae

Counts of 3rd stage larvae in faecal cultures have only rarely been practised. HANSEN and SHIVNANI (1956) applied this method and observed a good agreement with egg-counts, but for survey work they preferred the latter, because of their relative ease and speed.

ENIGK and STOYE (1963), cited by ECKERT (1963) used larval counts, because of their high sensitivity, as a check on egg counts. The possibility, to use for larval counts large amounts of faeces, is indeed a great advantage.

### 1.3.2. *The estimation of pasture infection*

Methods for the estimation of pasture infection or the numbers of larvae in pasture-samples have been described by TAYLOR (1939), CROFTON (1954), MICHEL and ROSE (1954), PARFITT (1955), STURROCK (1961), DURIE (1959, 1961) and DONALD (1967a).

### Sampling of pasture

TAYLOR (1939) described a sampling technique by which a bulk sample is taken by each of two persons who make a W-shaped traverse through the field, and make halts at regular distances, so that about one hundred halts are made. At each halt, pinches of grass are taken from four well defined different places. About eight ounces ($\pm$ 200 gr) of pasture was considered to be a suitably sized bulk-sample. CROFTON (1954) stated that, theoretically, in Taylor's method the number of traverses and pinches is much too low because the larvae are not randomly distributed and are not numerous in relation to the total number of pinches a pasture can provide. He agrees, however, that some compromise has to be made, as large bulk-samples make recovery techniques more difficult. Furthermore, this author prefers plucking of grass to cutting because in this way a larger part of the total larval population is sampled and the results will be less dependent on weather conditions. DURIE (1962) divided the pasture into four equal parts and sampled every week alternatively two diagonally opposite parts, along the diagonals of each subarea. Samples were taken by cutting the grass at ground level within an 8 inch square quadrat at intervals of three paces. Afterwards this sampling scheme was changed: the coordinates of the sampling points were determined on every occasion with aid of a random number table and numbered discs placed on two boundary fences at right angles. Tests indicated that the two methods yielded very similar results. The latter method was also used by DONALD (1967a).

## Separation of larvae from grass

The larvae are separated from the grass by washing of the grass in relatively large volumes of water. To improve the washing process, several measures can be taken.

1. Soaking of the grass for a certain period ranging from 2 hours (TAYLOR, 1939; PARFITT, 1955) to 24 hours (MICHEL and ROSE, 1954; DONALD, 1967a). This soaking can be repeated (DONALD, 1967a).
2. Repeated washing of the grass is applied by DURIE (1961), who used about one gallon of water 3 times, and by PARFITT (1955) who used two gallons of water twice.
3. Addition of a detergent which facilitates the wetting of the grass is applied by DURIE (1959) and ROHRBACHER (1957).
4. The use of warm water may activate the larvae and thus improve their separation from the grass. ROHRBACHER used water at approximately 40 °C. TARSHIS (1958), got better results with the BAERMANN-method when using water at 104 °F.

Generally the washings from a grass sample are bulked. In order to concentrate the larvae in a smaller amount of water the washings are left for a certain period, (usually overnight) to sediment. Thereafter the supernatant water is removed by sucking or syphoning off.

Two fundamentally different principles are used in the separation of larvae from the sediment.

1. The BAERMANN technique or modifications of it; here the separation of larvae from the substrate depends on the activity of the larvae. The method was used by TAYLOR (1939) KAUZAL (1940), ROHRBACHER (1957) TARSHIS (1958) and STURROCK (1961). According to DURIE (1959) the BAERMANN-method yields inconsistent results, especially in pasture samples, where activity of larvae is often decreased due to ageing of the larvae.
2. Techniques which make use of the difference in specific gravity of larvae and sediment. DURIE (1959) separated the larvae from the sediment by a constant upward water-flow in a funnel-shaped vessel, so that the flow rate gradually decreased. CROFTON (1954), MICHEL and ROSE (1954), PARFITT (1955) and DONALD (1967a) described methods by which larvae are separated from the sediment by floatation in a solution of high specific gravity.

## Counting of larvae

Counting of the larvae is done with aid of a microscope. In many of the techniques described this cannot be done until a laborious process has been worked through by which the larvae are concentrated in a very small volume.

In TAYLOR'S (1939) original method the larvae were counted under a dissection-microscope and removed if, rarely, high magnification was required. By so-doing larger amounts of suspension can be examinated. DURIE (1959) used a counting chamber in which aliquot samples of 1 ml from a 100 ml suspension were examinated. The resulting sensitivity is rather low and this may be a disadvantage (DONALD, 1967a).

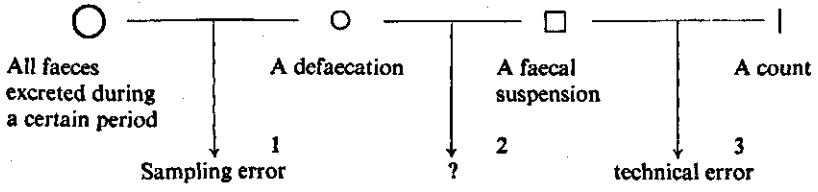
## 1.4. The interpretation of results

### 1.4.1. *Variations between faecal egg counts*

The first important condition with regard to the interpretation of results is knowledge about the variations between counts, particularly those caused by technical- and sampling error.

Before discussing the literature on the variations due to technical error and sampling error it should be pointed out that it is important to define accurately what is meant by a sample. As it is impossible, for routine purposes to count all the eggs an animal excretes during a certain interval of time, it is necessary to take one or a few defaecations for examination.

Once such a portion of faeces has come to the laboratory a sub-sample of one or a few grams is taken, the weight depending on the technique used. Within a sub-sample (or faecal suspension) one or more counts may be performed. The following scheme may be illustrative.




A count or an average of counts is considered to represent all the faeces passed during the time interval. As can be seen from the scheme, on three different occasions errors (denoted by 1, 2 and 3) may creep in. Error 2 may be seen either as technical error or as sampling error, this depending on what is called a sample. Usually the defaecation is seen as such; in that case error 2 should preferably be defined as technical error. A full description of the mixing- and subsampling -procedure and an estimate of the efficiency of that process should then be given. Unfortunately this appaers to be done rarely.

Hunter and Quenouille (1952) defined the faecal suspension as 'faecal sample'. In that case error 2 may be called sampling error.

With respect to error 1, observations were made by Peters and Leiper (1940), Spedding (1952, 1953) and Brambell (1963) on sheep; all found significant variations between defaecations even if these were taken from the same animal on the same day, or within a few days, so that variations due to epidemiological factors were not expected. Variations occur, that cannot be ascribed to some specific factor. This indicates that the production of eggs by female worms is not a continuous process, and the gastro-intestinal tract is not a very efficient mixing device.

The faecal consistency and the age and body weight of the animal are recognized by some authors as disturbing factors for which corrections might be introduced. (Levine and Clark, 1956; Riek et al., 1958; Brambell, 1963). Introduction of correcting factors, however, is only valuable if it can decrease sampling error significantly in relation to the existing over-all variance. Accord-

ing to BRAMBELL (1963) this is not the case when correcting for faecal consistency.

SPEDDING (1952) and BRAMBELL (1963) observed large differences between samples within sheep, within a day; they could not show a systematic pattern of variation. However, DORSMAN (1957) suggested there was some indication of a specific daily rythm in the egg output of cattle.

Regarding error 2, observations were made by PETERS and LEIPER (1940) and BESCH et al. (1960), who showed that significant differences between portions of sheep-defaecations are possible. No information on this point is available from cattle. Anyhow, it will be safe to mix the faeces; the efficiency of mixing should then be estimated.

On error 3 information has become available for the McMASTER-technique applied to sheep faeces. PETERS and LEIPER (1940), HUNTER and QUENOUILLE (1952) and BRAMBELL (1963) reported the McMASTER-counts within a faecal suspension to be POISSON-distributed. DUNN et al. (1967) however, working on faeces with a very high egg-content reported a deviation from it. Unfortunately these sorts of studies have not been made on D.C.F. techniques.

Apart from the variations mentioned, which are due to sampling and technical error, variations between counts may arise from seasonal or treatment effects and from differences between animals. These variations are the subjects of most of the investigations. Usually animals or groups of animals are sampled regularly at certain intervals of time. Variations between times of sampling will be considered in another part of this chapter.

Variations between animals within a herd are known to be quite large. Even if animals are experimentally infected with the same doses of larvae, large variations are observed. These differences can partly be ascribed to age, nutrition and genetical factors. Variations between counts of different sheep within flocks were estimated by HUNTER and QUENOUILLE (1952) on 13 occasions. They found that those counts followed a negative binomial distribution with k (= index of dispersion) = 0.7.

From the scheme given before, it may be seen that this k is partly a result from sampling error and not entirely from between-animal variation in resistance to worms. According to WHITLOCK (1961) the parameter k cannot be expected to be a constant for enteric nematodes. This author observed that counts from sib-groups of sheep follow the POISSON rather than the negative binomial distribution. So k is at least partly determined by the consanguinity of animals. NORTHAM and ROCHA (1958), cited by WHITLOCK loc cit. demonstrated a negative binomial distribution of worm counts in normal outbred chickens whereas a POISSON distribution was observed in inbred chickens.

Very little information is available on variation between egg counts from different herds in the field. For instance egg-counts from cattle on a number of farms were performed by ROBERTS et al (1951), ANDERSON et al. (1965) and BÜRGER et al (1966). Either the number of observations per farm or the number of farms often appears to be rather limited, so that statistical analysis of the results is not given. Moreover, in the majority of cases, suspected problemfarms

were selected for study, so that a true picture of the distribution of counts from various herds might not have been obtained.

1.4.2. *Variations between larval counts on pasture*

Similar to the situation with faecal egg-counts, variations are partly due to technical error and to sampling error. Here however, no trouble arises when these two must be defined and distinguished from each other.

Variations between counts within samples, are due to technical error. In many of the techniques reported, all the larvae that are washed from the grass, freed from the sediment and concentrated in a small volume, are counted. In such cases technical errors can only be estimated by determining the recovery-rate after a known number of larvae has been added to the grass-sample. TAYLOR (1939) recommended this method on every sampling occasion, by adding 1000 larvae to half of one sample. Two difficulties are likely to arise in this procedure. A dose of exactly 1000 is not easily prepared: if taken as an aliquot sample from a large suspension it is subject to a standard deviation of $\sqrt{1000}$. Secondly, an equal distribution of larvae between the two sample halves may not be assumed. On the other hand, the amount and nature of the pasture and sediment may vary considerably and thus influence the recovery-rate, so that recovery determined in a limited number of samples may be very misleading.

PARFITT (1955), STURROCK (1957), DURIE (1959) and DONALD (1967c) respectively reported recoveries of 43%, 80%, 76% $\pm$ 10%, and 93,8%. Some of these figures refer to the recovery of larvae from a suspension, others to the whole process. The number of samples involved in these examinations, if mentioned, appear to be low, 20 samples being the largest number, reported by DURIE. STURROCK and DURIE counted the larvae in aliquot samples of 1 ml taken from 100 ml larval suspension. A POISSON-like distribution between counts may be expected in such cases; this was not tested or at least not reported by the authors.

The recovery rate is not the only factor determining the reproducibility of a technique. The sensitivity must also be taken into consideration. According to DONALD (1967a) the technique of DURIE (1959), though it works well in routine-examinations, has a low sensitivity. This, however, depends on the number of aliquot samples counted. DONALD (loc cit.) had no reason to increase the sensitivity, because of the very large variations observed between pasture samples.

Concerning the between-sample variation, CROFTON (1952) observed that the counts from a great number of small samples taken from an evenly grazed part of a sheep pasture fitted well to NEYMANS Contagious Distribution Type A (NEYMAN, 1939). The same author (CROFTON, 1954), showed however, that defaecations are not randomly distributed on a pasture and he concluded that the type of distribution mentioned would hold only for small areas of pasture. TALLIS and DONALD (1964) constructed theoretical models for the distribution of infective larvae on sheep pastures. In these models various components were

built, in, which are representative of such biological data as time, number of sheep, distribution of faeces, egg numbers per defaecation, developmental and mortality rate of the free living stages. No use of these models have been made till now, as far as we know. DONALD (1967b) reported observations on the frequency distribution of infective larvae in a large number of small samples from a sheep-pasture. He concluded that the distribution was highly overdispersed and could best be fitted, empirically, to the truncated log-normal distribution. It may be concluded that large variations exist between samples taken on the same occasion and therefore observations may be subjected to considerable sampling error. DONALD (1967c) stressed that the interpretation of larval counts from herbage samples should be very cautious if no estimate of error is made. This estimate is possible if duplicate samples on each occasion are collected and examined separately.

Variations between pasture samples if not attributable to sampling error can arise from two sources. Firstly during periods where a rise or fall in pasture infection (due to season, introduction or removal of animals or mowing) can be expected, the time of sampling may be a source of variation. This variation will be considered in another part of this chapter.

Secondly, there may be, within a certain interval of time, large differences between pasture fields. As far as is known no purposeful study has been made on this sort of variation.

### 1.4.3. *Statistical treatment of results*

The use of non-parametric methods is often advocated if the distribution of the data cannot be defined exactly. See for example SIEGEL (1961). WHITLOCK (1961) had a personal preference for treating his data with these methods but he states that some information is sacrificed. BARTLETT (1947) put it this way: 'It has been suggested by various writers that even when measurements are available it may be safer to analyze by use of ranks. It might however be remembered that if we discard the original measurements apart from their order we are throwing away the original scale and all quantitative transformations of it, one of which may well be relevant for estimating quantitative treatment effects and measuring interactions; such wholesale jettisoning should be avoided if possible'.

Probably therefore most of the workers in the field of parasitology have decided to use the 'classic' parametric methods. In order to make an analysis of variance it is necessary to transform counting data which usually are not normally distributed.

WHITLOCK (1961), working with sib-groups of animals where the distribution is of the POISSON-type, transformed the egg-counts according to the equation $y = \sqrt{0.2}\, x$. in which y = the transformed value and x is the number of eggs per gram of faeces. HUNTER and QUENOUILLE (1952), observing a negative binomial distribution of egg counts from different sheep, transformed them according to the expression given by BEALL (1942): $y = k^{\frac{1}{2}} \sinh^{-1} x^{\frac{1}{2}} k^{-\frac{1}{2}}$ and provided a table for easy transforming the counts, based on the assumption that if k cannot be estimated from the experimental results, it is save to take k =

0.7. The transformation $y = \ln x$ or $y = \log_{10} x$ or if zero counts occur frequently: $y = \ln (x + 1)$ or $y = \log_{10} (x + 1)$ is used by DONALD et al. (1964), DINEEN et al (1965a, b, 1966a, b), DONALD (1967b) also used this transformation for larval counts on pasture.

1.4.4. *Some other factors, important for the interpretation of results*

BARTLETT (1947) gives an account of the conditions that should be fullfilled before an analysis of variance is applied, and transformations suitable for the various distributions which can be met with in data from research work.

Even if sufficient information has been collected on variation between egg counts in faeces and larval counts on pasture the interpretation of results may still be very difficult.

Egg counts are often made for diagnostic purposes. Till now it is the only method which can be used on a fairly large scale in live animals without disturbing the host-parasite relationships. Whereas larval intake and worm counts *post mortem* are reasonably well related to each other and to the clinical state and/or weight gain of animals (GOLDBERG and LUCKER, 1960; CIORDIA et al. 1962a, b, 1964; HERLICH, 1962; ANDERSON et al., 1966; MICHEL, 1966; KEITH, 1967), faecal egg counts do not always give a good picture. Certain e.p.g. values are suggested by several authors which would be indicative of clinical trichostrongylosis. TAYLOR (1939b) suggested 300–600 e.p.g. and LEVINE and AVES (1956) 300 e.p.g. of mixed strongyles to be the 'border-line of disease'. ROBERTS et al. (1951) gave estimates of these values for the separate genera or species, values varying from 10.000 e.p.g. of *Cooperia spp.* to 300 e.p.g. of *Bunostomum phlebotomum.* DORSMAN (1954) and PITRE (1967) discussing this material, suggested that faecal egg-counts of much lower levels may be considered dangerous. As LEVINE and AVES (1956) stated, much depends on the recovery-rate of the technique used.

Some authors on the other hand did not observe any relationship between egg-counts and weight gain or clinical signs (MAYHEW, 1949; KEITH, 1967). Others could not demonstrate a correlation between egg counts and larval intake (MICHEL, 1966) or worm counts P. M. (ANDERSON et al, 1966).

Two different reasons evidently disturb the relationship between egg-counts and the performance of the animal. Firstly it is known that the various species differ greatly in egg-laying capacity. GORDON (1967) reviewed the information that has become available from sheep. DEWHIRST and HANSEN (1961) obtained evidence that comparable differences between genera occur in cattle. Therefore differentiations of eggs should always be made, unless the infection-type (i.e. the relative abundance of species or genera) is rather constant from one animal to another, from one herd to another, or from season to season.

Secondly, due to the build-up of resistance by animals, the association between egg-counts in faeces and numbers of worms can be disturbed, even within species. The ratio between immature worms and adults for instance is subject to much variation especially in *O. ostertagi* (ANDERSON et al., 1965, ROSS, 1965) but also in other species (DONALD et al., 1964; SOULSBY, 1965). Furthermore, the

ratio between male and female adults may be influenced by the host-response (DONALD et al., 1964). Finally, the egg production per female worm may be directly suppressed (DONALD et al., 1964; MICHEL, 1967a). ROBERTS (1957b) found that egg counts are usually an accurate index of infestation, but when resistance occurs the egg counts are of little value for this purpose. A further indication that resistance plays an important role are the findings of HERLICH and MERKAL (1963) who observed an inverse relationship between the level of circulating antibodies and the faecal egg counts of calves infected with *T. axei*. MICHEL (1967a) stated that only two situations in which the egg output differs greatly from the expected pattern, allow us to draw conclusions about the worm burden: very low counts in the second month after exposure indicate few worms; persisting high counts in calves exposed for several months are indicative of severe helminthiasis.

Apart from their value for diagnostic work, faecal egg counts may however answer many epidemiological questions e.g. the infectiousness of a pasture. MICHEL (1967a) suggested that within wide limits the contamination of a pasture will not be influenced by the worm-burdens of the calves which graze this pasture. This may be true in cases of paddocks which are continuously grazed by heavily infected animals.

With regard to the interpretation of larval counts on pasture some remarks can be made. Firstly these counts may be expressed either as larvae per unit of area or as larvae per unit of weight of grass. Expression as larvae per unit of weight is preferred by most authors. This can be best related to larval intake because the daily intake of grass is also a matter of weight and not of area grazed. The number of larvae per unit of weight, unfortunately, is subjected to variations due to growth of grass, the so-called 'dilution-effect' (TAYLOR, 1939). ROSE (1964) showed the vertical distribution of larvae, to depend partly on the height of the grass, the larvae being relatively better available in tall pasture. This factor might partly counterbalance the 'dilution-effect'

The availability of larvae depends on a complex of several factors. Firstly the vertical and lateral movements of the larvae are important. In addition, the grazing behaviour of the animals may profoundly influence the intake of larvae. For example, the avoidance by cattle of the contaminated herbage surrounding faecal pats (ROBERTS, O'SULLIVAN and RIEK (1951) may be influenced by grazing-intensity.

## 1.5. THE EPIDEMIOLOGY OF TRICHOSTRONGYLOSIS OF CALVES

### 1.5.1. *The course of egg-output*

Generally, soon after an experimental infection has become patent the egg-output rises to a peak, followed by a more or less gradual decrease to low levels. (MAYHEW 1941, 1948, 1949; ROBERTS, 1957a, 1962; MAYHEW et al., 1960; MICHEL, 1966; RITCHIE et al., 1966). The pattern seen in natural infections usually conforms with this course. (ROBERTS et al., 1952; RIEK et al., 1953;

ROBERTS, 1957a, b; PETERSON, 1957; BÜRGER et al., 1966). The fall of egg counts is regarded as a phenomenon of resistance in the calves. The rise to the peak may be rapid or more gradual. This may be caused by a different reaction of various species. On the other hand it must be born in mind that in most of the studies cited, rather severe infections were involved. In the field the initial larval intake may be insufficient to evoke a host-reponse.

That the ideal curve is not always observed has been shown by ROBERTS (1957b) who studied natural *H. contortus* infections of calves. The departure of the curves could partly be ascribed to seasonal variation in larval uptake, but individual resistance of calves was also assumed to be very important.

MICHEL (1969c) concluded that the *O. ostertagi* egg-output of naturally infected Ayrshire calves followed the same stereotyped pattern as in calves that were experimentally infected either by a single dose or by continuous daily doses. A peak was reached about 50 days after first exposure and followed by a logarithmic decrease. This course appeared to be independent of worm burdens and this had two important consequences. Firstly, the formerly held theory that parasite populations show an exponential increase, is not applicable to Ostertagiosis of calves. Secondly, low egg-counts in late summer or autumn may occur in animals harbouring a harmful worm burden.

However, in field observations MICHEL et al. (1970) found more pluriform egg-output curves, which were considered to conform to one of three typical patterns. One of these shows the expected early peak followed by the logarithmic decrease. The second type of curve, which is associated with rather low residual infections, shows its peak somewhat later. The peak is lower than in the first type of curve, but the high level is maintained for some time. The third pattern is associated with very low residual herbage infestations in spring, and a peak in egg-output is not reached until autumn. Therefore, the uniform eggcount curves found by MICHEL (1969b,c) in experimental work may not be entirely representative for field conditions. The levels of infection, the breed of the experimental calves and the parasite species used, may be responsible for this discrepance.

### 1.5.2. *The ecology of free living stages*

Knowledge of the translation-process (defined by ROSE, (1960) as the process whereby eggs in faeces become infective larvae on herbage, available for the host) is extremely important for the understanding of the epidemiology of trichostrongylosis.

Three major components of this process can be distinguished: development, migration and survival.

For development sufficiently high temperatures are required. The reader is referred to the laboratory experiments of CIORDIA and BIZZELL (1963) and of ROSE (1961, 1963a, b, 1966) who worked with *O. ostertagi*, *C. oncophora*, *C. punctata*, *N. helvetianus*, *H. contortus*, *T. axei* and *T. colubriformis*. The latter author verified the results from laboratory experiments also in the field, with infected faeces placed on grass-plots. These observations were in good agreement with the results at the laboratory.

Optimum development was observed at approximately 25 °C. At that temperature the majority of larvae reaches the infective stage in 7–9 days, except *N. helvetianus*. No development of this species is seen beyond the gastrula-stage at 10–11 °C (ROSE, 1966). Other species fail to develop further if temperatures are below 6 °C (CIORDIA and BIZZELL, 1963). These authors found also that at high temperatures (> 32 °C) mortality of pre-infective stages is high.

Besides suitable temperatures, the faeces must stay sufficiently moistened. ROSE (loc. cit.) noted that generally calf-faeces do so, forming a crust in hot and dry weather, which prevents the larvae from migrating out of the faeces, but keeps the inner part moist until development to the infective stage is completed.

Aëration is another requirement for optimal development. WALLACE (1960) called attention for the fact that there is a lack of direct measurements on $O_2$-tension. KUTZER (1967) stated that variation in aëration of faecal pats may be caused by earth-worms, beetles and other organisms. ROSE (1963a) recorded 7 to 13 months for the complete desintegration of artificial pats from calf-faeces. This might also be an important factor for aëration.

Migration of larvae is a further step in the process of translation. Many studies have been made on the migration. Presence of thin water-films seems to be necessary. Rainfall or artificial spraying has been shown to enhance the migration of larvae out of the faeces to the grass (ROSE, 1964, 1966; DURIE, 1961; ROBERTS et al., 1952; KUTZER, 1967), but heavy rainfall may be the cause that larvae on the herbage cannot readily be demonstrated (SPINDLER, 1936). About the horizontal distribution of larvae in the grass, which is extremely important for the availability of larvae to the host, it is clear from the literature that larvae can migrate horizontally for appreciable distances (TARSHIS, 1958; DURIE, 1961) but most of the larvae remain in the immediate surroundings of the faeces (ROSE, 1961, 1963a, b; DURIE, 1961).

Vertical migration which is also important for the availability of larvae to cattle has been studied by many authors. WALLACE (1960) reviewed the literature on this point and he concluded that it was not entirely clear whether vertical movements are random, or the result of specific responses like negative geotropism or positive fototropism.

Besides active migration passive migration may occur for instance by the legs of grazing animals, by birds or by insects. KUTZER (1967) stated that the slope of a field can cause such passive migration. Also the dissemination of larvae over long distances (< 2 m) by sporangiae of *Pilobolus spp.* fungi (BIZZELL and CIORDIA, 1965; ROBINSON, 1962) may be important.

The survival of larvae, finally, is an important factor in the epidemiology. The infective larvae are far better resistent to freezing and dessication than pre-infective stages (ROSE, 1961, 1963a, b, 1966). *H. contortus* survived at −6 to −4 °C only for 12 days but *O. ostertagi*, *C. oncophora* and *N. helvetianus* did so for 14, 25 en 32 weeks respectively. At 6–7 °C survival for these 4 species was 87 weeks, 134 weeks, > 24 months and > 20 months respectively, and at 24–25 °C 35 weeks, 29 weeks, 13 months and 5 months. Probably survival is influenced indirectly by temperature, by way of the activity of the larvae and

depletion of food-reserves. KUTZER (1967) also observed that *C. oncophora* survived better than *O. ostertagi*. The latter species was more active, particularly at lower temperatures.

1.5.3. *The seasonal course of pasture infection*

From the work on ecology of free living stages it is clear that large variation is seen in the time needed for the translation-process. MICHEL (1969d) presented the results of extensive studies over a period of seven years, where the larval herbage infestation was measured in grazing experiments. The pattern appeared to be very constant through the various years. The infestation is low at the beginning of the grazing season (April, May, June). This is considered to be the result of increased mortality of overwintered larvae, the rapid growth of grass and the low rate of development of newly passed eggs into infective larvae. During July a rapid rise is observed if weather conditions are suitable. This rise is enhanced by the decreasing rate of herbage growth and increasing consumption of grass by the calves. During autumn and winter a high level is generally maintained, depending on weather conditions, followed by a decrease to low levels in spring.

In the Netherlands most calves are housed during the winter and young calves are turned out in spring being practically free of worms. MICHEL (1969d, 1970) did not consider overwintered infections to be dangerous in England. In his experiments, however, *O. ostertagi* was the pre-dominant species. SMITH and ARCHIBALD (1969) in East Canada have shown that overwintered infections of *C. oncophora*, *O. ostertagi* and *N. helvetianus* can be the cause of clinical disease even if animals are put on pasture as late as late June.

In Australia, where animals are grazed throughout the year, the highest levels of pasture infection were also observed during the period of least herbage growth. In such situations the great influence of rainfall can be clearly shown (DURIE, 1962). Other reports on seasonal fluctuations are scarce. Particularly there is lack of information on pastures which are not continuously grazed by infected animals, for instance pastures alternatively grazed by calves and older animals, or calf-pastures that are intermediately cut for silage or hay.

## 1.6. FARM MANAGEMENT IN RELATION TO THE EPIDEMIOLOGY OF TRICHOSTRONGYLOSIS

1.6.1. *The management of grassland*

Modern grassland exploitation, as it is practised in the Netherlands, is characterized by very high stocking rates, rotational grazing of lactating animals and a high rate of mowing for the production of hay and silage.

This pasture-management is made possible by the extensive use of fertilizers. From literature it is clear that several components of the complex may influence the epidemiology of trichostrongylosis.

High stocking rates are considered to increase the chances of infection

(CIORDIA et al. 1962, SILVERMAN and CAMPBELL, 1957). Rotational grazing has, in the past, been advocated with the aim of avoiding as much as possible the auto-infection of animals, but observations on the ecology of free living stages (ROSE, 1961, 1963a, b, 1965) and on pasture infections (MICHEL, 1969d, and DONALD, 1967c) have shown that high herbage-infestations may well occur on the very moment when animals return to the pasture. Also from direct experiments concerning the effect of rotational grazing, no favourable influence could be observed (CIORDIA et al., 1964; LEVINE and CLARK, 1955; LEVINE et al., 1956).

Grassland-management of permanent pastures may, on long term, change the botanical composition and this may in turn influence the availability of infective larvae (CROFTON, 1948; RIEDEL, 1955) but it is not likely that management brings forth such extreme changes in botanical composition that the epidemiology of trichostrongylosis is affected.

The alternate grazing and cutting of pastures, which is advocated from the point of view of pasture utilization, may well have a favourable result with respect to the epidemiology of trichostrongyle infections. Not only the spelling time of pasture is increased, presumably also a considerable part of the infective larvae is removed and an unfavourable environment for the remaining infection is left behind after mowing. ENDREJAT (1954) observed good preliminary results from aftermath grazing with sheep and so did SONNEVELD (unpubl.) in more extensive studies. For calves this system has been tested both by many grazing experiments and on practical farms, through several years by OOSTENDORP et al., (1965) and OOSTENDORP and HARMSEN (1968), who obtained consistently excellent results with it. However, the favourable results from aftermath grazing cannot only be ascribed to lower worm infections.

MICHEL (1966, 1968, 1969d, 1970) developed a grazing-system for calves which was based on extensive epidemiological studies. Some new views emerging from his work (that the parasite population increase is not an exponential one, that the generation time of the parasites is so long that only one generation is completed in a grazing season, and that disease is produced by the mid-season increase in pasture infestation) led him to the system of grazing a clean pasture until July and after an efficient anthelmintic treatment the animals are then brought to another clean pasture.

It has been shown that in the Netherlands this system yields not as satisfactory results as the systematic grazing on aftermath (Proefstation voor de Akker- en Weidebouw, unpubl. results).

1.6.2. *The management of animals*

An important factor in our climate is the housing of cattle during the winter season. Another factor which may be of importance is the raising of the calves separated from older cattle. These two factors together imply that infection of calves is likely to result from the infection that has overwintered on pasture. A third factor, the concentration of calves on a paddock near the farm-house may greatly intensify this process. ANDERSON et al., (1965) and BÜRGER et al., (1966) considered the calf-paddocks which are seen frequently in W.Scotland and

N.W. Germany as a major cause of trichostrongylosis in calves in the field. The situation in the Netherlands, is likely to be similar. TALSTRA (1966) observed in the province of North-Brabant that on 68 % of the farms the calves were grazed on one single paddock near the house all the season. In other parts of the country the situation may be somewhat better in the sense that calves are removed from the paddock by late summer, but exact information on this point is not available. A number of factors in calf raising interfere with the resistance of the animals either to the parasites or to the effects of parasitism. As GORDON (1964) states this distinction is very important. It is also important to distinguish between natural or innate resistance and acquired resistance. Particularly for age resistance it is necessary to separate age resistance *per se* and the with age increasing ability of the host to make an immune response (MANTON et al., 1962; URQUHART et al., 1966).

Several criteria for resistance have been used by various authors. Usually the rate of establishment of worms is the most important criterion but also other criteria are used, for instance: the titre of circulating antibodies (STEWART and GORDON, 1953; HERLICH and MERKAL, 1963), the length of the prepatent period (HERLICH, 1960; BRUNSDON, 1962a) the length of the patent-period (HERLICH, 1960) the degree of reduction of egg-production (BRUNSDON, 1962a) the degree of inhibition in the fourth larval stage (DONALD et al., 1964) and the lengths of worms and incidence of females without vulval flaps (MICHEL, 1967).

Three factors that influence resistance are mentioned here: age, nutrition and heredity.

## Age

This is not the place to give details of the large amount of work on age resistance. From the literature it seems doubtful whether there is any influence of age on the establishment of worms given by one single infection (STEWART and GORDON, 1953). In case of repeated or natural infections, however, a negative relation between these two may be seen (HERLICH, 1960; BRUNSDON, 1962a, b; VEGORS et al., 1955).

## Nutrition

A similar situation exists if the relationship between nutrition and resistance is considered. If worm-free calves are given a single infection no effect of feeding can be expected on establishment of worms (GOLDBERG, 1959, 1965; FRASER and CAMPBELL, 1966; STEWART and GORDON, 1953), unless there is a direct influence of the diet on the environment where the worms penetrate, e.g. the influence of milk on the pH of the abomasum (ROHRBACHER et al,, 1958). If, however, sheep have experienced previous infections, a low feeding level favors the establishment of a new infection (STEWART and GORDON, 1953; BRUNSDON, 1964).

Feed components like protein and vitamin A may be of particular importance in parasite-infections (HUNTER, 1953), but deficiencies in the feed may also, to some extent, be unfavourable for the worms (RICHARD et al., 1954; DOWNEY, 1965).

The role of supplementary feeding of pastured animals is very difficult to interprete. Not only an enhanced resistance, but also reduced intake of contaminated herbage may result in fewer worms (VEGORS et al., 1955; VEGORS et al., 1956; CIORDIA et al., 1962a; SPEDDING et al., 1963). Apart from this, the effect of a given worm burden, may exist of inappetance, and may reduce the digestion efficiency (SPEDDING, 1951; GIBSON, 1955; GOLDBERG, 1959, 1965; GORDON, 1964; HOLDER, 1964), and therefore supplementary feeding may mask the effects of parasitism, i.e. clinical signs and reduction of weight gain.

Genetic factors

In many reports on experimental infections a wide variation between animals that received the same larval dose has been found, which could not be attributed either to age or nutrition. Generally this variation has been ascribed to genetic differences between animals. Evidence for this was not only got from differences between breeds (ROSS et al., 1959; ROSS et al., 1960; SCRIVNER, 1964), but also from variation within breeds. (WHITLOCK, 1955, 1958, 1961, 1963; WHITLOCK and MADSEN, 1958).

Although for several reasons genetical variation cannot in the near future be regarded as a management tool for controlling trichostrongylosis in calves, the other two mentioned factors age and nutrition may be of practical value.

The distinction as drawn here between grassland-management and animal management is, admittedly very artificial. For instance rotational grazing can be regarded as a grassland management- and animal management-factor as well. The same is true for the mixed grazing of animals.

The grazing of sheep together with cattle may be advantageous to sheep. (ROBERTS, 1942; SMITH and ARCHIBALD, 1965). Also grazing of pastures with older stock that has become resistant has been suggested as a means of keeping down the infection on pasture. (TAYLOR, 1957; BAXTER et al., 1959).

Summarizing, management measures taken against strongylosis can be divided into measures that limit the intake of larvae and measures that raise the resistance of animals.

The intake of larvae may be limited either by restricting the intake of grass or by keeping the infestation of grass with larvae as low as possible. Restricting the intake of grass is a very common practice, as on almost every farm the calves on pasture are given some supplementary feeding. Keeping the calves inside on a hay concentrate ration is perhaps another possibility. Suppression of the numbers of larvae might be achieved by the use of new leys, by intermediate mowing and by the rotational grazing of calves on as large an area as possible, preferably without returning to a paddock previously grazed in that season.

In order to increase the resistance of animals they might be turned out to pasture at an older age and their nutrition might be raised to a higher level.

Even then the larval intake should be kept as low as possible, and it should be stressed that restriction of larval intake by keeping down the infection-level of pastures is the first prevention method to think of. Whether any method of prevention is an economie one or not, depends on the value of calves and the cost of extra feed and labour involved.

# 2. METHODS AND MATERIALS

*General remarks*

In this chapter the methods of sampling and counting will be described briefly and, if necessary, the reasons for choosing any particular method or procedure will be given.

The experiments carried out, can be divided into two main groups: one group of small laboratory experiments that were done to test the efficiency of techniques (experiments 1 to 15), and a second group of experiments or surveys, concerning calf-pastures and calves during their first grazing season. (Experiments I to VIII).

Only the latter experiments will be described here. The purpose, lay-out and methods used, will be reported. The smaller experiments will be described briefly where the results of the tests are reported.

## 2.1. METHODS

### 2.1.1. *Sampling of faeces*

Samples from individual animals were taken from the rectum with the aid of a plastic bag; if necessary defaecation was stimulated by gentle massage of the rectal wall. Samples were taken between 9 a.m. and 11 a.m. Occasionally it was necessary to return in the afternoon to an animal that had failed to produce faeces in the morning.

Herd samples were taken from freshly passed faecal pats. After removing the skin that has been formed on the faeces even after a few hours, approximately 5 g of faeces were taken from the centre of the pat with the aid of a spoon. The number of pats sampled was three times the number of calves in the herd, varying from at least 20 to 50.

Individual samples were processed immediately in the laboratory. Subsamples were taken after the faeces had been mixed by kneading the plastic bag. Herd samples were sent to the laboratory after removing the air from the plastic bag and knotting it tightly. They were processed within 48 hours. Before subsamples were taken the faeces was put into a dish and mixed for 1 minute with a mixer for kitchen-use.

### 2.1.2. *Counting of eggs*

A direct centrifugation-floatation-technique was used which can be described as follows:

A 1 g subsample is put into a 250 ml Erlenmeyer flask and 100 ml tap-water is added. After standing for at least 2 hours (if necessary the suspension is shaken thoroughly now and then), it is checked to see that the faeces are completely suspended. A 10 ml centrifugetube is filled quickly after vigorous shaking of the

flask. It is centrifuged for 3 minutes at 2500 r.p.m. The supernatant is sucked off and the sediment resuspended in Zinc-sulphate solution (s.g. 1,33). This should be done gently to avoid the formation of small air-bubbles. After filling the tube to a slightly positive meniscus with the aid of an eye-dropper, a 18 × 18 mm coverslip is laid on top of the tube. After recentrifuging (3 minutes, 2500 r.p.m.) the coverslip is removed by a careful vertical movement and put on a microscope-slide. All the eggs in the preparation are counted with a microscope (magnification 40–100 x).

A slight modification of this method, applied by HONER (1966, personal communication) was used in the 1967 experiments. It consisted of the application of the coverslip after the second centrifugation instead of before. After the second centrifugation, the meniscus is made slightly positive, and a coverslip placed on it. After waiting 1 minute the slip is removed and the eggs are counted as usual.

In the following pages the first method and its modification are referred to as method $E_1$ and method $E_2$ respectively.

2.1.3. *The preparation of cultures for larval counts in faeces*

A 10 g subsample of faeces is put into a petri-dish (diameter 9 cm, height 1,5 cm), and mixed thoroughly with approximately 5 g of fine sawdust using a spatula. After sprinkling about 5 ml of tap water into the culture, it is placed in an incubator at 27 °C. (The lid of a petri-dish is provided with three projections so that continuous air exchange is possible). After 9 days exactly the cultures are taken out for further processing.

2.1.4. *The counting of larvae in faecal cultures*

Two methods were used for the counting of larvae: $L_1$ and $L_2$. $L_1$ was used in the 1965 and 1966 experiments and it can be described as follows: The contents of the Petri dish are placed in a 2 litre measuring can. Dish and lid are washed manually with approximately 100 ml of tap-water at about 40 °C. The washings are added to the can. The culture is left to soak for 2 hours. Then 1500 ml of water (40 °C) is added and a kitchen mixer is run in it for 1 minute in order to obtain a complete desintegration of the culture.

The suspension is filled up to 2 litres and rapidly poured over a sieve (meshes ± 1 mm) to remove the sawdust. The sievings are caught in a bucket. From the suspension an aliquot sample of 200 ml is taken in 2 centrifuge tubes of 100 ml each. These are centrifuged for 3 minutes at 2000 r.p.m. and the supernatant is poured over a filter-paper in a Büchner-funnel, which is put under vacuum by a water-jet pump. With a spatula the sediment is resuspended in 25 ml of Zinc-sulphate solution s.g. 1,33. After 3 minutes centrifugation (2000 r.p.m.) the supernatant is poured over the same filter. After sucking off the $ZnSO_4$ solution, the filter is washed through with 50 ml of tap-water, removed from the funnel and put upside down into a petri-dish containing 10 ml water at 40 °C. During 1 minute the larvae are enabled to free themselves, then the filter is removed while being rinsed with a fine jet of water from a syringe flask. The petridish is pro-

vided with 5 concentric lines and one radiant line, engraved on the bottom. All larvae are counted under a dissecting microscope.

Method $L_2$, which was used during 1967, can be considered as a modified Baermann-technique. Originally it was used by us as a non-quantitative method for collecting larvae for differentiation. Its results were rather promising for quantitative use, and after comparison with $L_1$ it was adopted as a quantitative method. The procedure can be described as follows:

The culture dishes are saturated with water (40 °C) of which a part has been used for cleaning the lid of the petri-dish. Then the dish is placed upside down in a larger petri-dish (⌀ 16–20 cm). This can be done easily by first putting the large dish as a lid on the smaller one and thereafter inverting the two together. The large dish is partly filled with water. The culture is allowed to stand for 2 hours. Than the small dish with its contents is removed carefully. The suspension is transferred to a 100 ml measuring cylinder. After filling this up to 100 ml an aliquot sample (10 ml) is taken with the aid of a pipette. A homogeneous distribution of larvae in the suspension is achieved by blowing air bubbles through it just prior to taking the 10 ml. The pipette contents are run into a 'counting dish'. In this procedure a counting dish was used which consisted of a spiral-groove engraved into a round transparent perspex-plate, the groove having a total capacity of 12 ml.

2.1.5. *Sampling of pasture*

A pasture field was sampled by taking pinches of grass between thumb and forefinger, after every 10 paces on the diagonals of the field. As two samples were taken on every occasion, the pinches were taken every 5 paces and alternatively put into two plastic bags.

As it is desirable that samples do not differ too much in size (due to area of the field or growthstage of pasture), the size of pinches is adapted to a certain extent. In very large fields, also the number of paces between two pinches may be increased.

Theoretically it might be better to take samples along one of the diagonals and a number of tracks parallel to it.

However, due to the uneven distribution of faecal pats through a field it is desirable to have all four corners adequately represented in the sample. A further advantage is that the track can easily be determined. In the case of a triangular shaped field, samples are taken along the gravity-lines from each of the three corners.

2.1.6. *Counting of larvae in pasture samples*

If possible the pasture samples were processed immediately after they had been collected. Then the following procedure was carried out:

The samples are weighed and put into a 12 l bucket containing 4 l of water at 40 °C. The plastic bag is rinsed with some (100 ml) water which is added to the bucket. After the grass has been thoroughly wetted and dispersed in the water, it is left soaking for 2 hours. Then the grass is washed and removed by small

handfulls. The washings are poured over a coarse sieve in order to sieve off the remaining grass, and are caught in a second bucket. This washing-process is repeated twice in about 3,5 l water, which is added to the first washings. It is left to sediment for 3 hours. Than the supernatant is poured off, leaving 2 l of suspension behind. After mixing the suspension an aliquot sample of 400 ml is taken. The rest of the counting process is the same as that of method $L_1$ for faecal samples, except for the addition of 20 ml HCl-solution (HCl: water = 1:30) which is poured on the filter in the Büchner-funnel and left there for 1 minute, before, as usual, the filter is washed through with water. By doing this, the majority of free living soil- and plant-nematodes that are present on the grass, is killed, so making the counting process much easier.

The above mentioned methods were chosen because of their ease and speed. From this point of view the McMaster-egg counting technique might be preferable to the D.C.F.-technique. A high sensitivity of the method was desirable however, because low counts may be expected from subclinically infected calves. A second reason for using the D.C.F. technique was that it is used by most of the other workers in this country.

2.1.7. *Differentiation of eggs and larvae*

Eggs could be divided directly into the following categories:

1 Eggs of the trichostrongylid type
2 Eggs of *Nematodirus* spp.
3 Eggs of *Trichuris ovis*
4 Eggs of *Strongyloïdes papillosus.*

Eggs in the first category were differentiated by the identification of third stage larvae according to the method of KEITH (1953) whose data have been extended by other authors in other parts of the world (see review of literature). After a preliminary investigation (KLUVERS, 1966) it was considered possible to place the larvae into genera, restricting the time-consuming measuring process to doubtful specimens only.

The larvae were taken from $\pm$ 20 g faecal cultures, which were incubated simultaneously with the cultures for larval counts during the 1966 experiments. From 1967 onwards, when the new larval counting method $L_2$ was introduced, they were taken from the larval suspension, after counts had been performed on it. In this way 100 larvae were differentiated from each sample. Due to very low egg counts ($<$ 10 e.p.g.), it was not always possible to examine 100 larvae. This occurred in relatively few samples taken from older animals, or taken just after an infection had become patent. These samples did not show systematic differences with respect to generic composition from others taken at the same time from other animals or herds, so there was no reason to exclude them from analyses.

## 2.2 Description of the experiments

### 2.2.1. *Experiment I. The course of pasture infection, winter 1964–1965*

The purpose of this experiment was to obtain preliminary data on the validity of the technique and the nature of variability of larval counts from pasture samples. Moreover the course of pasture infection during the winter season was of interest.

A field of 0.8 ha, that had carried sheep during the spring of 1964, was grazed by yearling cattle during several periods of the summer. In September 1964, as a result of close grazing, the so-called tussocks of grass around faecal pats were clearly discernable. One hundred of these places were marked. Starting on October 5, grass samples were taken from these places every fortnight. 8 samples were examined each time and 2 counts per sample were performed.

### 2.2.2. *Experiment II. Grazing experiment University Farm, 1965*

The purpose of experiment II was to evoke different levels of infection on two pastures, and to evaluate such differences both by direct larval counts from pasture samples and by introduction of young test-calves. The validity of the counting and sampling techniques for pasture- and faecal-samples was also to be checked.

The pasture described in experiment I was divideed into two equal parts by a fence. Part 1 was grazed by 6 heifers from April 27 to May 13 and from May 25 to June 4. These heifers were on the average excreting 120 eggs per gram of faeces. On May 18 the grass on part 2 was mown and removed immediately. From June 10 to August 24 both parts were grazed by calves. September 14 both fields were mown and the grass removed.

20 Calves, of various breeds and ages and of both sexes were divided into 2 equivalent groups as illustrated by the following scheme:

| | Pasture (Group) 1 | | Pasture (Group) 2 | |
|---|---|---|---|---|
| Breed | 5 F.H. | 4 M.R.Y. 1G. | 5 F.H. | 5 M.R.Y. |
| Sex | 8 ♂ | 2 ♀ | 8 ♂ | 2 ♀ |
| | range | average | range | average |
| Age (June 10) days | 79–192 | 105.9 | 80–174 | 106.5 |
| Weight (June 2) kg | 64–174 | 95.0 | 64–160 | 94.4 |

These groups grazed the two fields from June 6 to August 24. By that time severe signs of gastro-enteritis had developed in group 1, and both groups were housed. During the grazing period the calves received max. 2 kg of concentrate/head/day as supplementary feed. The same amount was given when the animals were housed and hay of good quality fed ad libitum. On September 14 group 1 was treated with an anthelmintic by the veterinary practitioner.

The following observations were made:

1. The pasture infection was determined by sampling every two weeks, from April 12 to October 11. As in all other routine examinations of pasture, two samples were taken, on each of which two counts were carried out.
2. The egg output of calves was determined by method $E_1$ carried out on individual samples taken every two weeks from May 18 to October 5. On the same days, from June 16 to August 24, herd samples were taken, in which larvae were counted by method $L_1$. The herd samples were taken *in duplo*, two subsamples were taken from each sample and two counts per subsample were performed.
3. The live-weight of the calves was assessed on May 14, June 2, July 7, August 11, August 24, August 27, September 9 and October 6.
4. The hay intake per group was recorded daily from August 25 to September 26.

2.2.3. *Experiment III. Grazing experiment on the experimental farm 'De Vlierd' 1965*

General purpose, lay-out and results of the experiments on this farm are described by OOSTENDORP et al. (1965, 1968).

Our main purpose was to verify the effects of various grassland management factors by larval counts from pasture samples. In addition the validity of herd-sampling of faeces could be tested. We had the opportunity to do observations on pasture infection and egg output of calves. In the 1965 experiment 5 groups of 10 Friesian bull-calves born early February were treated as follows:

Group 1 Was rotated weekly on 4 paddocks of 0.25 ha each. It received 2 kg of concentrates/head daily.

Group 2 As group 1, with 1 g/kg phenothiazine added to the concentrate.

Group 3 Was grazed on aftermath. Weekly rotated, 2 kg concentrate fed. When a new paddock was provided, this had been mown previously for hay or silage. The total area occupied was 8 paddocks of 0.25 ha each.

Group 4 As group 3. Received 1 kg of concentrate.

Group 5 Was kept indoors, loose housing. The animals received freshly mowed grass *ad libitum* and 2 kg concentrates

The following observations were made:

1. The pasture infection was determined every two weeks for each of the total areas occupied by groups 1 to 4, from April 13 to October 5. This means that grass-samples were taken from the total areas of 1 ha. for group 1 and 2, and for group 3 and 4 from 2 ha., irrespective of the question which of the 4 or 8 paddocks was grazed by the calves at the moment of sampling.
2. The egg output of the 5 groups was determined every two weeks from June 22 to September 28, by $L_1$ counts in herd-samples. A hiërarchical sampling scheme as in experiment II was followed, i.e. one observation, two samples, four subsamples, eight counts.

*Experiment IV. Observations on experimental farm 'C.R. Waiboerhoeve', 1965*

The possibility of fattening bulls and steers on grassland and grassland-products is one of the subjects studied on this experimental farm. Every year some 50 bull-calves are purchased and kept for approximately 2½ years. So there are three age-categories: calves, yearling steers and 2 year old steers. During the season, all these animals are rotationally grazed on 15 fields with a total area of 24.33 ha, as separated age groups. Preferably every field is mowed once a year for hay or silage. The most important objective of our observations was to obtain data from pasture fields on which calves were not introduced at all or only for short periods as is the case with the majority of the fields in practice. Therefore the following observations were made:

1. The pasture infection was determined every two weeks, from April 22 to October 7, on 4 fields for which an interesting management had been planned.
2. The egg-output of each of the three age-groups was determined by $L_1$ counts from herds-amples. From April 22 to June 6 the 2-year-old steers were sampled, from April 22 to October 7 the yearling steers, and from June 6 to October 7 the calves. The same sampling scheme as for experiments II and III was followed.

2.2.5. *Experiment V. Grazing experiment University farm, 1966*

The purpose of this experiment was to investigate whether the differences between the fields of experiment II would be prolonged into another grazing season.

The pasture fields described for experiment II were also used in this experiment. During the first weeks there was an excess of grass, because no animals had been grazing the pasture during the winter season. In order to get rid of this extra grass, 3 heifers were grazed on each of the paddocks from April 29 to May 16. In the faeces of these heifers no eggs could be demonstrated by method E1. Larval counts, however, proved to be slightly positive. There were no differences between the two groups.

Two groups of 8 calves of different breeds, sexes and ages were formed. The scheme given below shows that the groups were equivalent.

| | Group 1 | | Group 2 | |
|---|---|---|---|---|
| Breed | 4 F.H. | 3 M.R.Y. 1G | 5 F.H. | 3 M.R.Y. |
| Sex | 5 ♂ | 3 ♀ | 5 ♂ | 3 ♀ |
| | range | average | range | average |
| Age on April 29 (days) | 47–141 | 92.1 | 44–145 | 91.3 |
| Weight on April 18 (kg) | 36–120 | 87.5 | 52–164 | 87.5 |

There were some difficulties to cope with. Firstly, when the groups were turned out to pasture, on April 29, the two youngest calves, one from each group, were considered to be too young. They were kept indoors till May 16, and than added to their respective groups. Secondly, on July 29 the oldest bull-

calf was removed from each of the groups and stalled, because they were mounting and driving the younger heifer calves almost continuously. Thirdly, by August 19 there was a shortage of grass. The groups were put together in a pasture which had been mown previously and had not been grazed by calves during the 1966 season. They were rotated weekly. By this procedure a very low intake of infective larvae could be expected. The complete groups, including the two older bull-calves, returned to the experimental pastures on September 9. The bull- and heifer-calves within each group were separated now by a fence, and each of the two parts of the paddock was grazed alternately by the male and female calves every week. On September 27 a bull-calf from group II died; it had been showing severe signs of parasitism for several weeks. Large numbers of *O. ostertagi* worms were seen in the abomasum. Both groups were supplementally fed 2 kg of concentrates/head/day.

The following observations were made:

1. The pasture infection was determined every two weeks from February 25 to October 12. If possible, the larvae were differentiated at the generic level.
2. Egg-output of individual calves was estimated by $E_1$ and $L_1$, every two weeks from April 12 to September 27.

$L_1$ counts were performed *in duplo* from duplicate subsamples. The larvae were differentiated by genera, These examinations were also carried out on herd-samples, which had been collected *in duplo* from the pastures at the same dates.

3. The live-weight of the animals was assessed every two weeks from April 4 to October 10.

2.2.6. *Experiment VI. Observations on practical farms, Friesland, 1966*

The first purpose was to investigate whether larval counts from pasture samples, taken early in the season, might be indicative of the severity of trichostrongylosis later in the season.

Secondly we wanted an impression of variations in egg output that exist between calf herds in practice. Finally it was necessary to check, whether the relative abundance of species found in experiments on the University farm showed reasonable conformity to that found on other farms.

In cooperation with the Animal Health Service of the province of Friesland, four veterinary practitioners and the Agricultural Advisory Service, 43 farms were selected for this study.

In addition to farms that had experienced more or less severe trichostrongylosis, apparently healthy farms were also investigated. Furthermore, no sheep or only a small number of them were held on these farms, as large numbers of sheep would probably offer difficulties in the interpretation of larval counts from grass-samples. On most of the farms use was made of a calf paddock near the farm-house, for a varying period.

It was decided to test a new anthelmintic on some of the calf-herds, based on clinical state and egg-output of calves.

The following observations were made:

1. Very early in the season, the pasture infection was determined on the field(s) where the farmer expected to introduce his calves for their first grazing. Grass samples were taken during two relatively short periods: March 10–16 and April 12–15. If a sufficient number of larvae were present they were differentiated into genera.
2. The egg-ouput of calves was assessed by applying method $E_1$ and $L_1$ to herd-samples that were taken during 5 periods: June 13–16, July 11–15, August 8–12, September 5–8 and November 21–December 3. During the last-mentioned period, calves had been stalled and samples were taken from the dunging gutter behind the animals. $L_1$ counts were carried out *in duplo* on duplicate sub-samples.
3. During the last faecal sampling-period (November 21–December 3) the chest-girth of all calves belonging to the herds studied, was measured. The exact birth-dates of the calves were registered from the identification cards of the a.i.-centre or Animal Health Service.

Apart from these observations several other data were collected. A form was given to every farmer whereon data from the calf herd and from the calf-pastures could be recorded. Data from the calf herd included age distribution, introduction of young animals and removal of older ones and the grazing management. Data from the pastures included area, periods of grazing by the calves and the previous history of the field during the 1966 season. Adequate completion of the form was checked every time when a farm was visited.

Other data were collected by verbal inquiry, such as: Total farm area, type of soil, average number of milk-cows, heifers, calves, horses and sheep, the area usually mown for silage- and hay-making, the feeding scheme in use for the young calves, and the supplementary feeding given to the calves at pasture. Generally the farmers gave very good cooperation, which is gratefully acknowledged here.

2.2.7. *Experiment VII. Grazing experiment University Farm, 1967*

This experiment served several purposes. The first objective was to test the improved counting methods $E_2$ and $L_2$ on individual faeces-samples. Secondly, experiment VI indicated that the initial pasture infection picked up by animals during the first weeks of grazing determined almost completely the level of egg-output during the whole grazing-season, and that reinfection was of little importance. Therefore in this experiment, the opportuinity for reinfection was varied by removing animals from the pasture every three weeks, throughout the grazing season. Thirdly during the earlier experiments (II and V) rather large differences were seen between animals presumably due to varying ages, sexes and breeds. Therefore for this experiment bull calves of the F. H. breed, born in the first week of March 1967 were purchased. Moreover these calves were all sons from two bulls intensively used for artificial insemination by A.I.-Centre 'Meppel', namely 'Bernhard' and 'Adema 653'. Based on a more or less subjective judgement in previous years of the conformation of calves at the age of 1 year, the calves of 'Bernard' were characterized as rather poorly muscled

whereas the off-spring of 'Adema 653' were above average with respect to this point.

The same field as was used for the experiments I, II and V, was used in this experiment. Now however, it was divided into two parts by a fence that was at a right angle to the fence used during the 1965 and 1966 experiments. From March 10 to 28 three heifers were grazed on each part, those on field 1 excreting an average of 25 eggs/gram of faeces and those on field 2 an average of 2 eggs/gram of faeces. The egg-output was assessed several times in individual samples with method $E_2$.

23 Calves, of which 12 were sons of 'Bernhard' and 11 were 'Adema 653'-sons, were purchased from the original farms immediately after birth. None of the calves was from a first calving cow. They were raised on the original farm for 3 weeks and than brought to our farm. Here they were raised further uniformly. Two groups of 10 calves each were formed, based on age and live weight. Each of the groups consisted of 5 'Bernhard' and 5 'Adema' sons. The groups were introduced to the two fields on May 22. The remaining 3 calves were kept indoors. Every 3 weeks (i.e. on June 13, July 4 and 25, August 15 and September 5) two calves (one 'Bernhard' and one 'Adema') from each group were removed and stalled. Because the pasture infection and egg-excretion of calves from the two fields did not differ significantly, the 4 calves were stalled as one group. Before the experiment started a complete scheme for the stalling of calves had been fixed.

The calves at pasture received 2 kg of concentrates per head daily. The housed calves received hay *ad libitum*, and during late summer and autumn they were given silage and hay *ad lib*. In addition they received 2½ kg of concentrates per head daily. The stallboxes were cleaned weekly, but in such a way, that auto-infection and cross-infection was not entirely prevented. One animal stalled on July 25 had been losing weight for several weeks and showed severe signs of trichostrongylosis. On August 3 it was excluded from the experiment and was, by mistake, treated with hexachlorophene. It died, presumably from intoxication. Large numbers of *O. ostertagia* worms were found in the abomasum. No lesions were seen.

The following observations were made:

1. Pasture infection was determined twice weekly from March 14 to September 12. Thereafter it was determined on two occasions, September 26 and October 10. If possible differentiations of larvae were made.
2. The egg-excretion was assessed by methods $E_2$ and $L_2$ on individual samples and herd samples (from the calves at pasture). Individual samples were taken weekly from May 16 to October 10. Herd samples were taken from June 13 (when the onset of egg-output could be expected) to August 15 (when only 4 calves remained per herd).

Larval differentiations were carried out every two weeks.

3. The live-weight of the animals was assessed on the following dates: April 4, 25–May 9, 22– June 13 – July 4, 25 – August 15 – September 5, 28 and October 10.

4. The hay and silage intake of each group of stalled calves was recorded daily.
5. On November 28, 4 animals were slaughtered and the gastro-intestinal tract examined for the presence of mature and immature worms. 2 Animals were from the first stalled group (June 13) and 2 from the group stalled September 5. The P. M. examinations were carried out by Dr. J. Jansen at the Institute of Veterinary Parasitology, Utrecht, whose kind cooperation is gratefully acknowledged.

2.2.8. *Experiment VIII. Observations on egg-output of calves, on practical farms where prevention of trichostrongylosis by grazing management was tested. {P.A.W. serie 751}.*

On 51 farms (including a few experimental farms), located all over the Netherlands the prevention of trichostrongylosis by grazing on aftermath was tested (OOSTENDORP et al., 1968).

Our observations served three main purposes: Firstly, testing of the methods $E_2$ and $L_2$ on herd -samples; secondly testing whether herd-samples if taken by various persons, deviated in some way from herd-samples taken by ourselves. Finally it offered an opportunity to check whether the larval differentiations found in the experiments V, VI and VII could be regarded as representative for other regions or not.

On 4 or 5 occasions herd-samples of faeces were taken and $E_2$ and $L_2$ counts and larval differentiations were performed. Several other data which were of interest, e.g. live-weight gain, total farm area, type of soil, breed of cattle, number of cows, heifers, calves and sheep, grassland management and supplementary feeding were collected by OOSTENDORP et al. (1968) and kindly put at our disposal.

# 3. RESULTS

## 3.1. THE STATISTICAL TREATMENT OF RESULTS

As the most suitable statistical treatment depends on the results proper, this subject is the first one to be dealt with.

For two reasons it was decided to apply to the majority of results the classic parametric methods. Firstly, because one of the objectives of our studies was to provide quantitative estimates of certain variations instead of testing only their significance; these variations include interactions, which are most easily handled by analysis of variance. Secondly various authors state that some information may be lost if parameter-free methods are used (see review of literature).

In order to use the classic methods transformation of counts may be necessary. A transformation that is universally applicable was suggested by BEALL (1942): $y = k^{-\frac{1}{2}} \operatorname{Sin} h^{-1} \{k.x\}^{\frac{1}{2}}$ in which y = the transformed count, x = the original count and k is an index measuring dispersion of a negative binomial type distribution. The author states that the transformation embraces both the square-root and logarithmic transformations. Our data permitted the estimation of value's of k, both for counts from faecal samples and from pasture samples.

### 3.1.1. *Faecal examinations*

The value of k was estimated by BEALL (loc. cit.) as $k = \frac{\Sigma s^2 - \Sigma \bar{x},}{\Sigma \bar{x}^2}$ in which $s^2$ is the variance of any set of counts and $\bar{x}$ is the mean of it. Some other estimates of k are discussed by ANSCOMBE (1949). From his paper it appears that the above mentioned estimate is reasonably efficient for our data.

It may be useful to distinguish between values of k within samples and between samples, because the former depend largely on the procedure of subsampling and counting, and the latter are probably connected with the resistance of host animals and the parasite-species.

Table 1 presents values of k within samples that could be estimated from experiments where duplicate counts were performed on duplicate subsamples. (The experiments 9 and 10 are described briefly on page 42).

From the table it can be seen that k within-samples takes values between 0 and 1. This indicates that analysis of variance after either the square-root transformation or the logarithmic transformation may lead to false conclusions. It is well known that k is highly variable; the highest value of k (= 0,199) is an illustration of this. It was brought about by one single sample. If this sample is excluded, the k-value is well within the range of the others. The lowest value of k (= 0,000) found for $L_2$ counts within subsamples is strongly suggestive of a

TABLE 1. Values of $k = (\Sigma s^2 - \Sigma\bar{x})/\Sigma\bar{x}^2$, estimated from experiments where duplicate counts were performed in duplicate sub-samples.

| Experiment | Number and nature of samples | Method | Values of k | |
|---|---|---|---|---|
| | | | within samples | within sub samples |
| 9 | 63 indiv. | $E_1$ | 0.037 | 0.043 |
| 10 | 30 indiv. | $E_2$ | 0.035 | 0.033 |
| 9 | 63 indiv. | $L_1$ | 0.199 | 0.006 |
| 10 | 30 indiv. | $L_2$ | 0.019 | 0.000 |
| II, III, IV | 154 herd | $L_1$ | 0.060 | 0.007 |
| V | 151 indiv. | $L_1$ | 0.024 | 0.007 |
| VI | 214 herd | $L_1$ | 0.044 | 0.016 |

POISSON distribution of these counts within subsamples, which is quite conceivable in view of the counting procedure.

Furthermore it may be stated that there is no indication of a relation between within-sample k-values and either the counting method or the origin of samples (individual vs. herd samples).

Finally, as in experiments II through VI fairly large numbers of samples are involved, a common k-value of k = 0,034 was estimated after the results had been pooled. Therefore, k = 0,03 was chosen for application in BEALL's transformation prior to analysing the within sample-variance.

Concerning the variation between samples, k-values were estimated from means and variances within sampling-occasions. (Usually sampling-occasions are identical with dates of sampling; in the experiments VI and VIII however, each sampling-occasion extended over a period of some days or even longer).

The results are presented in table 2. It appears that values of k are subjected to considerable variation; in particular occasional values for *Nematodirus spp*

TABLE 2. Values of $k = (\Sigma s^2 - \Sigma\bar{x})/\Sigma\bar{x}^2$, between samples, based on variation between samples, within sampling occasions.

| Experiment | Group | Number of animals | Number of herds | Number of sampling occasions | Values of k | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Tricho-strongylid eggs | Nemato-dirus eggs | T.ovis eggs | Total eggs | Larvae |
| II | 1 | 10 | – | 11 | 2.18 | 1.38 | 4.21 | 2.21 | – |
| | 2 | 10 | – | 11 | 2.24 | 0.67 | 2.22 | 0.64 | – |
| V | 1 | 7* | – | 13 | 1.05 | 1.64 | 3.16 | 0.99 | 1.02 |
| | 2 | 7* | – | 13 | 0.79 | 2.61 | 1.37 | 0.65 | 0.64 |
| VI | – | – | 43 | 5 | 1.06 | 5.56 | 2.26 | 1.05 | 0.94 |
| VII | 1 | 2–10 | – | 16 | 0.49 | 1.31 | 0.40 | 0.56 | 0.30 |
| | 2 | 2–10 | – | 16 | 0.37 | 0.90 | 0.79 | 0.46 | 0.26 |

* From each group the youngest animal, which was put on pasture 2 weeks later, was excluded.

eggs and *Trichuris ovis* eggs were very high, due to a rather high proportion of zero counts in some of the samples. Values of k are rather low in experiment VII. This is probably due to the homogeneity of the host -population with respect to age, sex and genetical origin.

It becomes clear from this table that, for analysing between sample variance, another value of k (e.g. $k = 1$) may be preferable to $k = 0.03$ used for the variance within samples. BEALL's transformation becomes almost identical to the logaritmic transformation if the value $k = 1$ is used. The $y = \ln x$ transformation is, according to ANSCOMBE (1949) wellknown and common where the standard deviation of the counts appears to be roughly proportional to the mean. Examination of a part of our results revealed that this situation is also met with in our data.

The efficiency of any transformation can be judged from the degree to which the distribution of counts is normalized, and also from the degree to which the variance of counts is made homogeneous and independent of the mean. The latter is the most important requirement (BARTLETT, 1947). We have compared the effect of different transformations namely:

A: $y = \sqrt{x + 0{,}5}$
B: $y = k^{-\frac{1}{2}} \operatorname{Sinh}^{-1} \{k.\ (x + 0{,}5)\}^{\frac{1}{2}}$, $k = 0{,}03$
C: $y = \ln (x + 0{,}5)$
D: $y = k^{-\frac{1}{2}} \operatorname{Sinh}^{-1} \{k(x + 0{,}5)\}^{\frac{1}{2}}$, $k = 1$

The transformation of BEALL (B and D) was slightly modified as proposed by BARTLETT (1947).

The square-root and logarithmic transformation have been used by various authors working on nematode egg counts and have for that reason been included in our comparisons.

Figure 1 presents the relationship of the original counts to the transformed counts. This may help the reader to get an impression of how values are changed by the various transformations, and for easily reconverting transformed counts into original counts.

In table 3 the results are given of tests for normality of the distributions, independence of variance and mean, and homogeneity of variances of egg counts of the trichostrongylid type, as affected by various transformations in five of the larger experiments.

Only those observations were considered that had a reasonable chance of yielding positive counts. This means that observations falling within the prepatent period were not included.

From the table it can be concluded that none of the transformation succeeds in making the distributions of counts normal for each of the five experiments. Comparison of the transformations indicates that transformation B gives the best results. The effect of transformation on normality is illustrated by fig. 2, which shows the relative frequency distributions. From each experiment the counts were placed into 10 classes, the width of the classes being 1/10 of the highest value ever observed, obtained after each of the transformations.

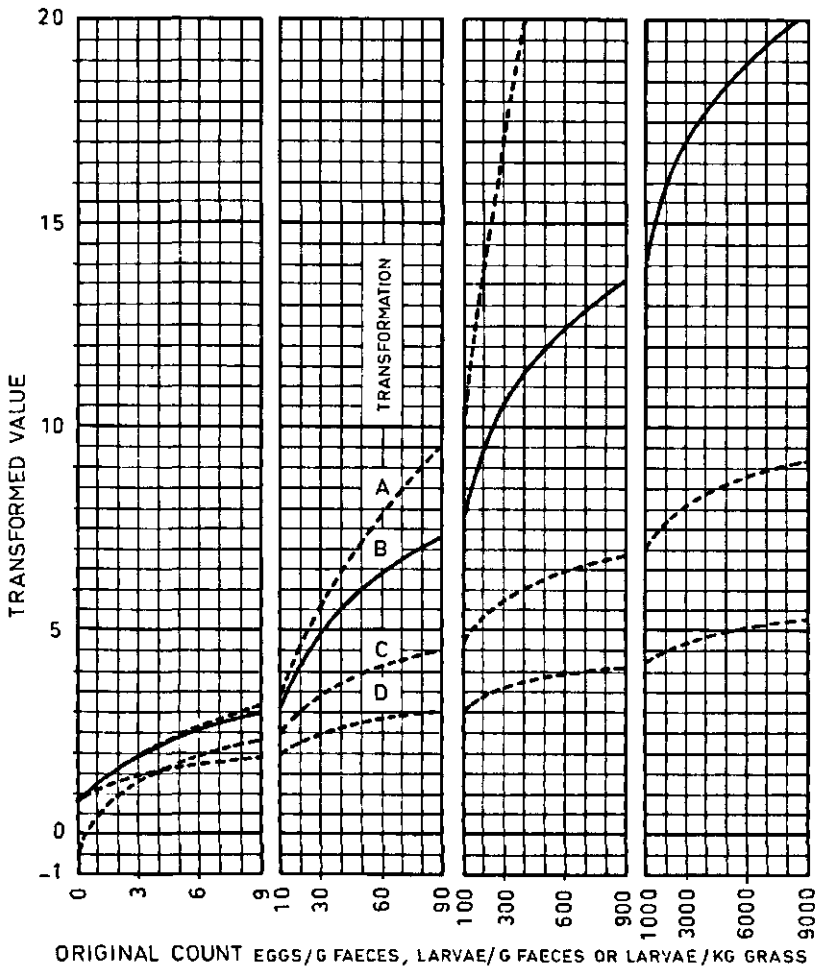



FIG. 1. Transformed values for egg- and larval-counts in relation to original counts (For transformations A, B, C and D see text).

2570 e.p.g., observed in experiment II, was the highest untransformed value ever seen in these five experiments.

Two additional remarks should be made about figure 2. The distribution of counts from experiment II appears to be very different from that of other experiments, especially after transformation. This is because of the heterogeneity of the material, due to the lay-out of the experiment. Counts from group 1 calves had reached a high level, whereas those of group 2 remained low for a long period. Strictly the distribution of counts should have been given separately for the two groups. Here however, the only purpose was to compare the effect of various transformations. Differences between groups are considered elsewhere.

TABLE 3. The effect of various transformations as judged by normality of the distribution and independence and homogeneity of variances.

| Test | Experiment | Degrees of freedom | Untrans-formed | Transformation | | | |
|---|---|---|---|---|---|---|---|
| | | | | A | B | C | D |
| FISHER'S | II | 2 | 5403.59** | 272.73** | 11.62** | 8.23* | 7.12* |
| normality | V | 2 | 2099.96** | 47.65** | 3.73 | 17.89** | 9.01* |
| test | VII | 2 | 1245.58** | 4.06 | 49.73** | 225.84** | 191.65** |
| ($\chi^2$ values) | VI | 2 | 1060.28** | 64.90** | .19 | 97.26** | 47.30** |
| | VIII | 2 | 756.74** | 23.00** | 3.04 | 40.09** | 20.59** |
| SPEARMAN | | | | | | | |
| rank corr. | II | 5 | .96** | .86* | .25 | −.14 | .04 |
| coefficient | | | | | | | |
| measuring | V | 7 | .92** | .83** | .43 | −.55 | −.45 |
| dependence | VII | 16 | .84** | .75** | .06 | −.64** | −.53* |
| from variances on means | VI + VIII | 6 | .87** | .33 | −.12 | −.93** | −.90** |
| HARTLEY'S | II | 7; 18 | 12.64** | 3.54 | 2.09 | 1.88 | 1.94 |
| test for | V | 9; 14 | 55.18** | 7.04* | 2.42 | 6.96* | 5.41* |
| homogeneity | VII | 18; 21 | 2223.49** | 30.86** | 7.03** | 5.81* | 4.90* |
| of variances | VII[1]) | 18; 20 | (12.97)** | (2.72) | (2.38) | (5.81)* | (4.90)* |
| (values of | VI | 5; 41 | 11.13** | 3.06* | 1.57 | 2.47* | 2.17 |
| Fmax = $S^2$max/$S^2$min) | VIII | 3; 56 | 2.84** | 1.57 | 1.93* | 3.17** | 2.83** |

* 5% significance level
** 1% significance level
[1]) This gives the results of HARTLEY's test after excluding one outlying sampling date immediately after infection had become patent, but only in few animals. See also fig. 3.

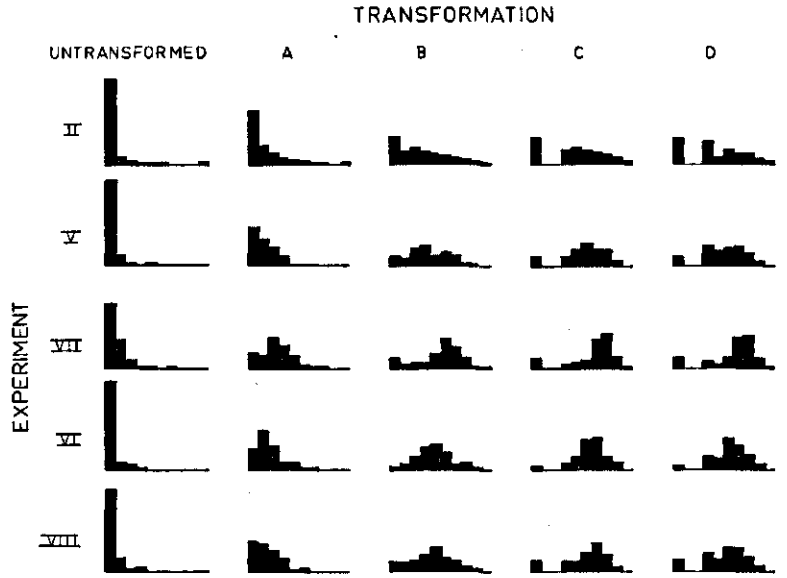



FIG. 2. The frequency distribution of egg-counts after various transformations, in several experiments.

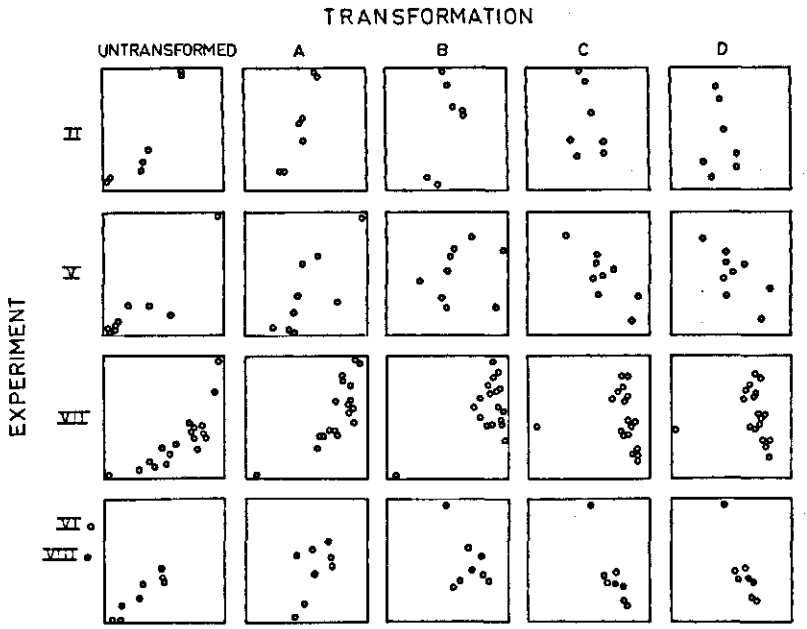



FIG. 3. The relation between mean and variance of egg-counts, per sampling date after various transformations (horizontal axes: means; vertical axes: variances).

Another remark concerns the distributions after the transformations C and D. The lowest class stands quite separated from the others. This is because no egg-counts exist between 0 and 10 e.p.g., due to the multiplication of the actual counts by 10. In the case where larval counts are performed, values between 0 and 10 occur, and a smooth figure is obtained.

Furthermore, from table 3 it can be seen that transformation B also yields the best results with respect to independence and homogeneity of variances. This is graphically illustrated in figure 3 which gives the relation between means (horizontal axis) and variances (vertical axis) from various experiments resulting from different transformations. The scales had to be adapted to the size of the figure; they are comparable between experiments within transformations, but not between transformations.

From the results above the conclusion was drawn that, before performing the classic statistical treatments, egg counts could best be transformed according to $y = 0.03^{-\frac{1}{2}} \operatorname{Sinh}^{-1} \{0.03\,(x + 0{,}5)\}^{\frac{1}{2}}$

This result was rather surprising as the value $k = 0.03$ had been derived from within sample-variances and -means. It should be stressed that this transformation is the best one only of the four investigated, and that there might well be another value of k which is still better.

3.1.2. *Pasture examinations*

The distribution of larval counts from pasture can be expected to be still more skew than that of faecal egg counts. Because every observation made consisted of counts from duplicate samples, k-values could be calculated within observations and between observations.

The k-values within observations were calculated for each of 15 series of observations. A series consisted of the counts from a field during a year (1965: 10 fields, 1966: 2 fields, 1967: 2 fields) and the samples taken in Experiment II during early spring on 43 practical farms. The 15k-values within observations obtained varied from 0.07 to 0.61 the weighted average being 0.15.

The k-values between observations were calculated in two ways. Firstly for each of the 15 series of counts mentioned already, the values of $\bar{x}$ and $s^2$ could be estimated. Secondly the counts were grouped into 11 groups according to season. The k-value calculated by the first method was 2.78 and that by the last mentioned method 2.15.

The normality of the distributions and the independence and homogeneity of variances was tested (according to the same methods used for faecal samples) for untransformed counts, for the square-root and logarithmic transformation and for BEALL'S transformation using the k-values 0.03, 0.15, 1.0 and 2.5. Of these six transformations the logarithmic transformation ($y = \ln (x + 0.5)$ appeared to give the best results, but its effect did not seem to differ much from that of BEALL'S transformation using $k = 1$ and $k = 2.5$.

Because the logarithmic-transformation is also used by other workers and is relatively easy to apply and to understand, we have chosen it for the analysis of larval counts from pasture samples.

## 3.2. Evaluation of techniques

### 3.2.1. *Examination of faecal samples*

#### The recovery rate of techniques

In contrast to many other workers we do not consider the recovery to be the most important quantitative attribute of a technique. In many cases the recovery rate can be increased only by a disproportionately large input of labour. We regard the variation of counts due to sampling and technical errors in relation to the total variation as a far more important criterion. Nevertheless some small experiments have been carried out to have at least an impression of the recovery-rates of various methods.

Experiment 1

The recovery of $E_2$ was determined by repeated centrifugation, after the sediment had been resuspended with a fine needle. The centrifugation was repeated until two successive slides yielded a zero count. Ten samples were examined. The number of eggs from the first coverslip, expressed as the percentage of the total number of eggs counted, is the percentage recovery.

Experiment 2

The recovery of $E_2$ was assessed by carrying out simultaneous counts by a dilution technique. On ten 100 cc faecal suspensions 4 $E_2$ counts were performed, and 4 aliquot samples of 1 cc were taken with the aid of a pipette and divided over a number of preparations (about 12). The recovery of $E_2$ was expressed as the percentage of the eggs counted by the dilution method.

Experiment 3

The recovery of $L_1$ was determined by adding an estimated number of larvae to 16 nematode-free faecal cultures. The recovery is expressed as the percentage of the larvae added.

Experiment 4

The recovery of $L_2$ was determined by adding an estimated number of larvae to 10 nematode-free cultures, as in experiment 3.

Experiment 5

The recovery of $L_2$ was determined by repeated extraction of 12 faecal cultures. Further extraction of cultures was stopped if on two successive occasions no larvae were found in the suspension after this had been concentrated by sedimentation. For some of the cultures the experiment extended over a long period (4–5 weeks).

Experiment 6

The recovery of $L_2$ was assessed by applying method $L_1$ to a culture after it had been extracted by method $L_2$. The non-extracted larvae were calculated as

3 × the number of larvae found by $L_1$, based on the results of experiment 3, the recovery was expressed as the percentage of the calculated total number.

Results from these experiments are summarized in table 4. They make clear that two fundamentally differing principles of recovery-testing can be distinguished, namely:

1. determination of the recovery by adding a known number of organisms to the substrate, or by counting them by an entirely different method, and
2. estimation of the recovery-rate by counting of the non-recovered organisms.

As far as recovery-rates of $E_2$ are concerned, LEVINE et al. (1960) obtained similar results, by repeated centrifugation as well as by comparing the D.C.F. counts with McMaster counts. The results indicate that determination of the non-recovered eggs or larvae (as practiced in experiment 1, 5 and 6) may lead to a false impression of the recovery, so experiments 2, 3 and 4 probably give the best estimates of the absolute recovery of $E_2$, $L_1$ and $L_2$ respectively.

In these experiments however, there is an indication of a positive relation between recovery and egg or larval concentrations, as indicated by the SPEARMAN rank correlation-coefficients (Rs); therefore results must be interpreted very cautiously.

One final remark should be made. Usually one expects a larger variation between recovery-percentages if the recovery has a low general level. The low level found for $L_1$ (experiment 3) however does not show a substantially larger variation than that found in other experiments. A possible explanation for this may be the fact that losses of larvae occur in several steps here: during the sieving-process, the centrifugation and the collection of larvae.

## The within sample variation

Table 5 and 6 present the results of experiment 7 and 8 respectively, which can briefly be described as follows:

### Experiment 7

From each of five faecal samples four 1 g subsamples were taken. In each subsample one $E_1$ count and one $E_2$ count was performed. The methods were applied alternately to the first and second 10 ml aliquot sample taken from the suspension. After resuspending the sediment in the centrifugetube, centrifugation was repeated and a second coverslip applied.

### Experiment 8

From a faecal sample 40 1 g subsamples were taken after thorough mixing for 5 minutes with a kitchen-mixer. The same observations were made on these subsamples as in experiment 7.

From the results it can be seen that method $E_2$ yields higher counts than method $E_1$. However this difference disappears, if two coverslips are examined. This suggests that the majority of eggs that are capable of reaching the coverslip (results from experiment 1 and 2 indicate that this is about 70%) have done so already after two coverslips.

TABLE 4. Recoveries of counting techniques

| Method | $E_2$ | | | | $L_1$ | | $L_2$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | |
| Sample | no. per gram | Rec. (%) | no. per gram | Rec. (%) | no. per gram | Rec. (%) | no. per gram | Rec. (%) | no. per gram | Rec. (%) | no. per gram | Rec. (%) |
| 1 | 4140 | 67.9 | 4050 | 63.0 | 1285 | 38.1 | 571 | 76.7 | 1527 | 81.2 | 397 | 89.4 |
| 2 | 3050 | 85.2 | 2875 | 50.0 | 839 | 44.1 | 383 | 67.2 | 417 | 85.6 | 212 | 83.0 |
| 3 | 1340 | 87.3 | 2225 | 71.9 | 513 | 33.1 | 247 | 58.2 | 280 | 78.6 | 209 | 88.5 |
| 4 | 1150 | 91.3 | 1525 | 54.1 | 380 | 42.0 | 119 | 68.1 | 241 | 80.1 | 174 | 96.6 |
| 5 | 610 | 60.7 | 1375 | 60.7 | 269 | 24.2 | 99 | 53.3 | 152 | 82.2 | 115 | 92.2 |
| 6 | 470 | 70.2 | 875 | 50.3 | 209 | 26.8 | 76 | 60.3 | 111 | 70.3 | 108 | 83.3 |
| 7 | 440 | 75.0 | 850 | 52.1 | 160 | 29.1 | 63 | 65.6 | 109 | 73.4 | 99 | 84.4 |
| 8 | 320 | 75.0 | 750 | 62.9 | 150 | 22.3 | 60 | 55.0 | 97 | 92.8 | 84 | 85.7 |
| 9 | 300 | 100.0 | 633 | 54.8 | 126 | 42.5 | 53 | 53.3 | 51 | 68.6 | 55 | 94.5 |
| 10 | 270 | 88.9 | 550 | 39.1 | 108 | 33.3 | 49 | 46.9 | 49 | 83.7 | 33 | 81.8 |
| 11 | | | | | 103 | 22.8 | | | 42 | 92.9 | 22 | 86.4 |
| 12 | | | | | 99 | 37.4 | | | 20 | 65.0 | | |
| 13 | | | | | 81 | 31.5 | | | | | | |
| 14 | | | | | 60 | 25.8 | | | | | | |
| 15 | | | | | 55 | 26.4 | | | | | | |
| 16 | | | | | 34 | 32.4 | | | | | | |
| Average | | 80.2 | | 55.9 | | 32.0 | | 60.5 | | 79.5 | | 87.8 |
| $R_s$ | | -0.35 | | 0.50 | | 0.37 | | 0.77** | | 0.10 | | 0.20 |

** = significant $P < 0.01$
* = significant $P < 0.05$

TABLE 5. Comparison of method $E_1$ and $E_2$, with application of 2 coverslips, in 4 subsamples from each of 5 samples.

| Sample | Method $E_1$ | | | | Method $E_2$ | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 coverslip | | 2 coverslips | | 1 coverslip | | 2 coverslips | |
| | Mean | Variance | Mean | Variance | Mean | Variance | Mean | Variance |
| 1 | 9.00 | 10.00 | 12.50 | 23.00 | 12.25 | 9.58 | 12.50 | 7.00 |
| 2 | 12.25 | 14.75 | 17.25 | 30.25 | 16.00 | 50.00 | 17.50 | 49.00 |
| 3 | 30.25 | 74.25 | 53.25 | 80.25 | 49.50 | 357.67 | 54.25 | 289.92 |
| 4 | 14.25 | 90.25 | 23.50 | 80.33 | 17.25 | 55.58 | 23.25 | 20.92 |
| 5 | 9.00 | 24.67 | 13.25 | 14.42 | 11.25 | 44.92 | 14.25 | 48.25 |

TABLE 6. Comparison of method $E_1$ and $E_2$, with application of 2 coverslips. in 40 subsamples from one well-mixed faecal sample.

| Method $E_1$ | | | | Method $E_2$ | | | |
|---|---|---|---|---|---|---|---|
| 1 coverslip | | 2 coverslips | | 1 coverslip | | 2 coverslips | |
| Mean | Variance | Mean | Variance | Mean | Variance | Mean | Variance |
| 17.90 | 30.58 | 26.50 | 25.28 | 22.90 | 38.48 | 26.70 | 39.34 |

Furthermore it can be seen that both methods have a within sample variance which is much larger than might be expected from a POISSON distribution of counts within samples. Two factors may be considered to be responsible for this deviation from the POISSON distribution namely;

1. the varying proportion of eggs that sticks to the first coverslip, and
2. the mixing of the sample before subsamples are taken, which clearly is inadequate in the case of experiment 7, where the usual method is applied.

A similar deviation from the POISSON-distribution of counts within samples could be concluded from experiments in which duplicate counts were carried out on duplicate subsamples, both for egg-counts and larval counts. Of these experiments only experiments 9 and 10 have to be briefly described yet.

Experiment 9

From 63 individual samples from calves two 1 g subsamples were taken and two $E_1$ counts were performed on each of them; only eggs of the trichostrongylid type were considered. Simultaneously, two $L_1$ counts were done in each of two 10 g subsamples taken from the same samples.

Experiment 10

The same was done with 30 individual samples according to methods $E_2$ and $L_2$.

Such four-fold counts were also carried out for method $L_1$ under routine conditions, in the experiments II through VI.

The within sample k-values, which give an impression of the deviation from

POISSON were given in table 1 and have been discussed above.

Whereas some indications were obtained of the causes of the deviations in the case of egg-counts (experiment 7 and 8, table 5 and 6), the reasons for the excess variance of larval counts within samples can only be the subject of speculation. Firstly the extraction-efficiency may vary between subsamples; secondly, due to slight differences in micro-climate or species of nematodes, the hatching-rate of eggs may differ from one culture to another, thirdly the original number of eggs may differ due to inefficient mixing. This factor, however, will probably be less important than in the case of egg counts, because of the relatively large weight (10 g) of subsamples.

It appears that the variation within samples in our counting methods is larger than the variance between the McMaster-counts in faecal samples from sheep found by other authors (see review of literature), although this discrepance is largely caused by our definition of within sample variance.

If, however, the within -sample variation is small relative to the variation between samples, the methods may still be very useful. Table 7 presents the analysis of variance from egg and larval counts in the various experiments. As F-values between samples are very high, all the methods are capable of showing quantitative differences between samples. It appears that differences between subsamples are also significant if larval counts are carried out, due to the three factors mentioned already. Differences between subsamples where eggs are counted, are not significant or significant only at the 5% level. This must be ascribed to the large variation between counts, rather than to small variation between subsamples.

The mean squares may be written as:

Mean square between counts $= \sigma_1^2$

Mean square between subsamples $= \sigma_1^2 + 2\sigma_2^2$

Mean square between samples $= \sigma_1^2 + 2\sigma_2^2 + 4\sigma_3^2$

The components of variance $\sigma_1^2$, $\sigma_2^2$ and $\sigma_3^2$, presented in the last column of table 7, may be suggestive of the most economic sampling scheme. In our experiments rather heterogeneous material was involved and it is quite obvious that taking more samples should in our case be preferred to taking more subsamples or performing more counts. Situations may exist however, where between-sample variation is much smaller, and as sampling of animals is very time consuming relative to subsampling or counting, in such cases increasing the number of subsamples or counts should possibly be preferred. Comparison of the withinsample variation of egg-counts with that of larval-counts (experiments 9 and 10) is suggestive of the latter being smaller than the former. Even the variance of larval counts from different subsamples is smaller than the variance between egg-counts within subsamples, in both experiments. The size of the experiments is rather small for drawing definite conclusions but a better reproducibility of larval counts was also indicated by a higher correlation between the larval counts by the two methods $L_1$ and $L_2$, compared to the correlation between $E_1$and $E_2$, $L_1$ and $E_1$ or $L_2$ and $E_2$ respectively, in an experiment where all four methods were applied simultaneously to 136 faecal samples (see table

TABLE 7. Analysis of variance within samples, of egg- and larval counts in faeces, after transformation of the counts according to BEALL's transformation, k = 0.03

| Material | Method | Source of variation | Degrees of freedom | Sum of Squares (S.S.) | Mean Square (M.S.) | F | Components of variance |
|---|---|---|---|---|---|---|---|
| Expt. 9 | $E_1$ | between samples | 62 | 1459.47 | 23.54 | 8.06** | 5.15 |
| | | between subsamples (within samples) | 63 | 184.23 | 2.92 | 1.52* | .50 |
| | | between counts (within subsamples) | 126 | 241.79 | 1.92 | | 1.92 |
| Expt. 10 | $E_2$ | samples | 29 | 845.44 | 29.15 | 40.49** | 7.11 |
| | | subsamples | 30 | 21.57 | .72 | 1.00 | .00 |
| | | counts | 60 | 43.11 | .72 | | .72 |
| Expt. 9 | $L_1$ | samples | 62 | 888.52 | 14.33 | 11.11** | 3.26 |
| | | subsamples | 63 | 81.51 | 1.29 | 7.58** | .56 |
| | | counts | 126 | 21.77 | .17 | | .17 |
| Expt. 10 | $L_2$ | samples | 29 | 644.51 | 22.22 | 36.43** | 5.40 |
| | | subsamples | 30 | 18.36 | .61 | 10.17** | .27 |
| | | counts | 60 | 3.76 | .06 | | .06 |
| Expt. V | $L_1$ | samples | 150 | 5922.90 | 39.49 | 77.43** | 9.74 |
| | | subsamples | 151 | 77.23 | .51 | 3.19** | .17 |
| | | counts | 302 | 49.07 | .16 | | .16 |
| Expt. VI | $L_1$ | samples | 213 | 5711.87 | 26.82 | 41.26** | 6.54 |
| | | subsamples | 214 | 139.63 | .65 | 3.61** | .24 |
| | | counts | 428 | 78.14 | .18 | | .18 |
| Expt. II, III, IV | $L_1$ | observations | 76 | 3500.49 | 46.06 | 36.27** | 5.60 |
| | | samples | 77 | 98.09 | 1.27 | 1.67** | .13 |
| | | subsamples | 154 | 117.53 | .76 | 4.22** | .29 |
| | | counts | 308 | 55.68 | .18 | | .18 |

** significant $P < 0.01$
* significant $P < 0.05$

TABLE 8. Relation between counts according to various methods in faecal samples.

| Experiment | Number of samples positive for both methods | Nature of samples | Method A | | | | Method B | | | | Coefficient of correlation R(a, b) | Ratio | Ratio of means after inversed transformation | Weighted average of ratio's |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Method | Number of counts per sample | Mean of transformed counts (ā) | Standard deviation S(a) | Method | Number of counts per sample | Mean of transformed counts (b) | Standard deviation S(b) | | | | |
| V | 131 | indiv. | $E_1$ | 1 | 7.008 | 2.844 | $L_1$ | 4 | 6.320 | 3.001 | 0.858 | $L_1/E_1$ | 0.75 | |
| VI | 207 | herd | $E_1$ | 1 | 7.482 | 1.479 | $L_1$ | 4 | 6.797 | 2.540 | 0.848 | $L_1/E_1$ | 0.75 | 0.76 |
| 9 | 60 | indiv. | $E_1$ | 4 | 5.370 | 2.265 | $L_1$ | 4 | 5.457 | 1.869 | 0.642 | $L_1/E_1$ | 1.05 | |
| 11 | 136 | indiv. | $E_1$ | 1 | 8.183 | 1.996 | $L_1$ | 1 | 7.094 | 1.803 | 0.596 | $L_1/E_1$ | 0.65 | |
| VII | 356 | indiv. | $E_2$ | 1 | 9.395 | 2.299 | $L_2$ | 1 | 9.074 | 2.409 | 0.819 | $L_2/E_2$ | 0.88 | |
| VIII | 165 | herd | $E_2$ | 1 | 7.349 | 2.742 | $L_2$ | 1 | 7.129 | 2.684 | 0.877 | $L_2/E_2$ | 0.91 | 0.88 |
| 10 | 30 | indiv. | $E_2$ | 4 | 8.813 | 2.699 | $L_2$ | 4 | 8.130 | 2.357 | 0.945 | $L_2/E_2$ | 0.77 | |
| 11 | 136 | indiv. | $E_2$ | 1 | 9.294 | 1.748 | $L_2$ | 1 | 8.949 | 1.804 | 0.676 | $L_2/E_2$ | 0.87 | |
| 11 | 137 | indiv. | $E_1$ | 1 | 8.159 | 2.008 | $E_2$ | 1 | 9.248 | 1.820 | 0.612 | $E_1/E_2$ | 0.66 | 0.66 |
| 11 | 136 | indiv. | $L_1$ | 1 | 7.094 | 1.803 | $L_2$ | 1 | 8.949 | 1.804 | 0.823 | $L_1/L_2$ | 0.48 | 0.48 |

8). If time-curves of egg output per animal or herd were drawn (exp. V, VI, VII), the curves of larval counts generally appeared to be oscillating less than the curves of egg counts, which also supports the better reproducibility of larval counts.

### The sensitivity of the techniques

The sensitivity of a technique is another factor that may be important, especially if low and zero counts occur frequently. It is defined as the minimum number of eggs or larvae per gram of faeces that can be demonstrated by the technique. It is clear that larval count techniques have a higher sensitivity than the egg count methods because by the former the larvae counted, are from 1g of faeces, whereas in the latter the eggs in 1/10 g of faeces are counted. Of course one should also take into account the recovery-rate of a method. In this way for the various methods the following sensitivities were calculated:

Sensitivity $E_1 = 10 \times 100/37 = 27$ e.p.g.
Sensitivity $E_2 = 10 \times 100/56 = 18$ e.p.g.
Sensitivity $L_1 = 1 \times 100/32 = 3.1$ l.p.g.
Sensitivity $L_2 = 1 \times 100/61 = 1.6$ l.p.g.

The recovery-rates used in the above calculations were those found in the experiments 2, 3 and 4 (see table 1) for $E_2$, $L_1$ and $L_2$ respectively. The recovery rate of $E_1$ (37%) was calculated from the recovery of $E_2$ and the ratio $E_1/E_2 = 0.66$ (see table 8).

### The relation between egg-counts and larval counts on faecal samples

Because of their high sensitivity, good reproducibility and the possibility of differentiation, larval counts may be very useful in epidemiological studies. One important condition has to be fullfilled however: there must be a strong, consistent relationship between the counts of eggs of the trichostrongylid type and larval counts. This relation depends largely on the hatching-rate of eggs under the prevailing conditions, and this hatching capacity may very considerably between season, animals, herds and nematode species.

Coefficients of correlation between egg and larval counts were calculated after the counts had been transformed according to transformation B. Only samples were considered where both egg counts and larval counts were positive, because samples in which both were negative would yield spuriously high correlation coefficients; and if only one of the counts was positive it appeared to be always a zero egg-count and a positive larval count, which is easily explained by the different sensitivity of the methods.

Coefficients of correlation are presented in table 8. One of the experiments mentioned in this table still needs a brief description.

#### Experiment 11

All four methods $E_1$, $E_2$, $L_1$ and $L_2$ were applied simultaneously to samples from 8 of the animals used in experiment VII. The eight animals selected were

divided equally between the different groups. For each method one subsample was taken and one count carried out.

From table 8 the conclusion may be drawn that, generally, there is a high correlation between egg and larval counts. In some cases however, the coefficients of correlation are somewhat lower (exp. 9, Exp. 11). This can be ascribed largely to the small standard deviation of the counts by both methods in these experiments.

This may be illustrated by the two correlations-coefficient for $E_2$ and $L_2$ that were found in experiment VII ($r = 0.819$) and experiment 11 ($r = 0.676$) respectively, which on first sight would not be expected to differ significantly from each other, as in fact $E_2$ and $L_2$ counts in experiment 11 form a part of the entire population of counts in experiment VII.

However, the latter also included the positive but very low egg and larval counts of the 3 control animals.

Analysis of covariance was applied to test whether the relation between egg and larval counts was influenced by the host-animal, by the herd, by the season and by the nematode species involved. As will be reported in another part of the study, the large majority of larvae belonged to the genera *Cooperia* and *Ostertagia*. Therefore, the influence of nematode species was determined as the effect of the percentage of *Cooperia* larvae on the relation between egg and larval counts. There was no indication that the relation was affected by any of the factors mentioned.

Figure 4 gives a graphical presentation of the relationship between egg and larval counts in the four larger experiments. On theoretical grounds it is not easy to decide which of the two regressionlines should be preferred for expressing the quantitative relation, that of y on x or that of x on y. As the presence of larvae is, in fact, the result of the (earlier) presence of eggs, the regression of larvae on eggs would seem the most logical one to take. On the other hand, as was seen in the within-sample variance tests, there is an indication that the number of larvae is the most accurately measured variate.

From figure 4 it can be seen that, for practical purposes, the ratio $\frac{\bar{x}}{\bar{y}}$ may serve very well for expressing quantitatively the relation between the number of eggs and the number of larvae. This ratio depends on several factors:

1. The recovery-rate of the egg- and larval counting techniques applied.
2. The hatching-rate of eggs.
3. The transformation applied.

Table 8 presents the ratio's of the counts by the various methods after the means of transformed counts had been reconverted to original counts (eggs or larvae per gram of faeces). Bearing in mind that such ratio's are subjected to very considerable variance, the values found are remarkably constant, at least in the four larger experiments (Exp. V and Exp. VI for $L_1/E_1$. Exp. VII and VIII for $L_2/E_2$). Furthermore, it can be seen that the ratio's $L_1/E_1$ and $L_2/E_2$ are different as a result of different recoveries.

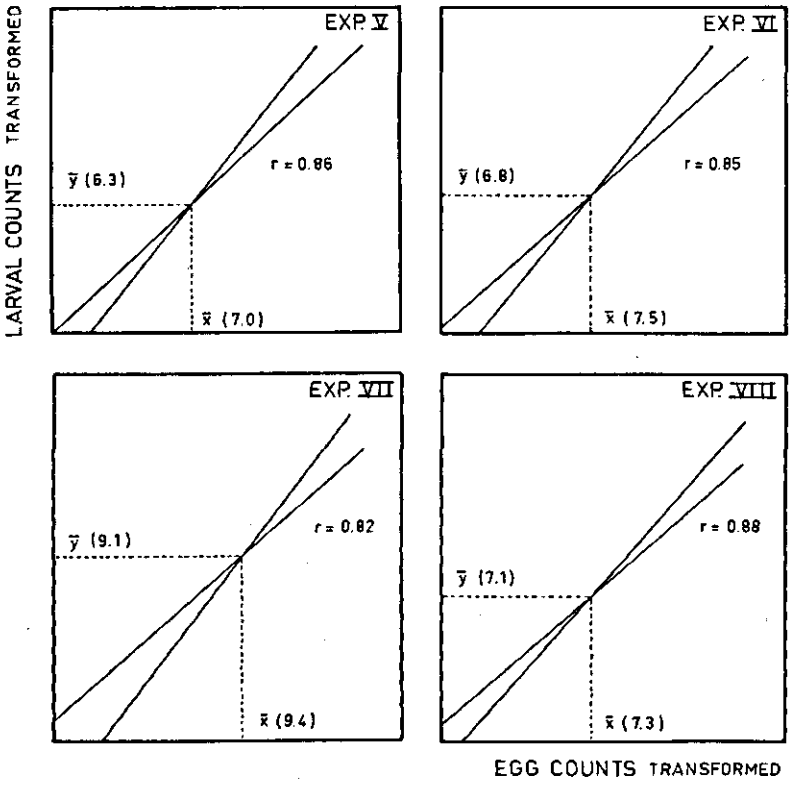



FIG. 4. The relation between egg-counts (x) and larval-counts (y) in faecal samples, in various experiments.

The ratio $L_1/L_2 = 0.48$ is in good agreement with the absolute recoveries found for $L_1$ and $L_2$, 32.0% and 60.5% respectively (table 4). The absolute recovery of $E_1$ has not been determined, it can be calculated from the ratio $E_1/E_2 = 0.66$ and the recovery of $E_2 = 55.9$ (table 4) as: $0.66 \times 55.9 = 36.9\%$. From the absolute recoveries and the ratio's $L_1/E_1$ and $L_2/E_2$ the proportion of eggs capable of producing a third stage larva may be estimated. Two estimates of this percentage are:

$P_1 =$ Recovery $E_1$/Recovery $L_1 \times L_1/E_1 = 36.9 \times 0.76/32.0 = 87.6\%$

and

$P_2 =$ Recovery $E_2$/Recovery $L_2 \times L_2/E_2 = 55.9 \times 0.88/60.5 = 81.3\%$

It should be stressed that these percentages have to be seen as only a rough estimate, because of the limited number of samples used for the recovery tests. Moreover, the results of these tests were suggestive of a positive relation between recovery and egg-concentration.

## Comparison of the results from individual samples to those from herd samples

If one is interested in studying the course of egg-output of a large number of herds it is almost impossible, or at least very difficult, to do this by collecting individual, rectal samples.

Various objections may be raised against the use of herd-samples taken from fresh faecal pats on the pasture.

Firstly, no impression is obtained from the dispersion of counts throughout a herd. This may be serious if large differences exist between herds with respect to uniformity (in age and breed) and consanguinity. Furthermore, as is often argued, eggs may have developed into pre-infective larvae if four -days-old faecal pats are taken, and this might lead to spuriously low egg-counts. Finally nematodes other than those parasitizing cattle might be attracted by faeces and might disturb egg and larval counts.

The results given in table 8 and figure 4, show that the relationship between egg and larval counts is almost exactly identical in individual samples and in herd samples.

In experiments V and VII the egg-output of the herds has been determined both as the mean of individual samples and from herd-samples taken simultaneously. Figure 5 shows the course of egg output from both herds in each of

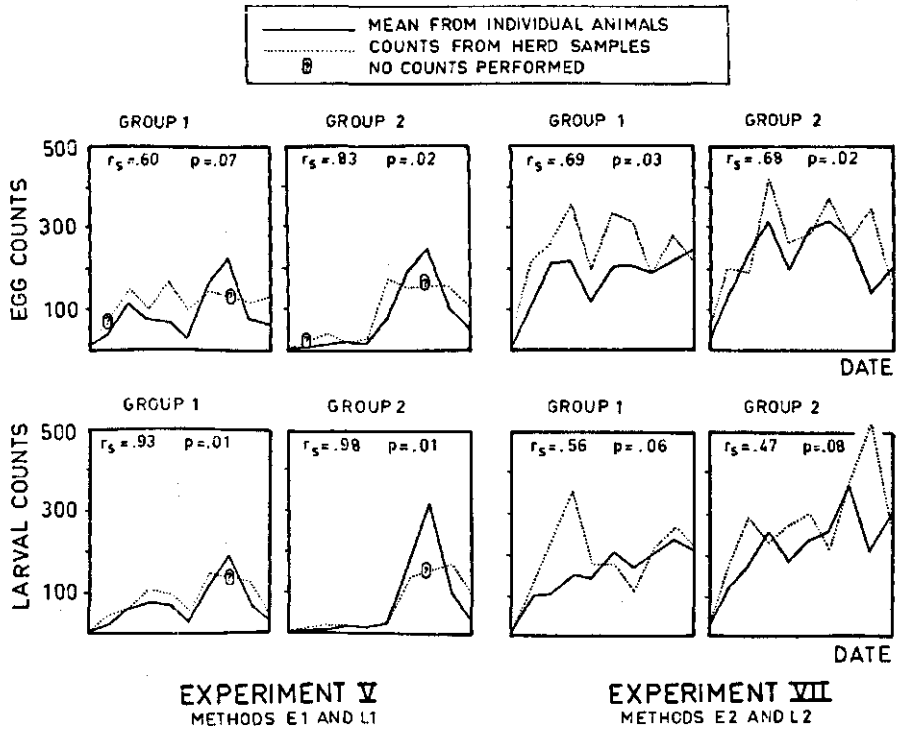



FIG. 5. Egg-output, as measured by egg-counts and by larval-counts, in individual samples and herd-samples of faeces.

the experiments, as measured by mean egg and larval counts from individual samples and from herd samples.

The mean count from individual samples was calculated from the mean of transformed counts. The relationship between these means and the counts from herd samples can be characterized by the SPEARMAN coefficient of rank-correlation (see figure 5). The probability for these coefficients are also presented. It can be concluded that there is as strong a relationship between the counts as reasonably may be expected from the errors involved in both of the methods.

As far as the levels of counts are concerned, the figure is suggestive of a higher level of the counts from herd samples, both in the case of egg counts and larval counts. This difference was significant when tested by the signed-ranks test. If, however, the means of untransformed individual counts were compared to the herd-sample counts, the difference was no longer significant. This may be explained as follows:

If the sampling procedure of herd samples is recognized, it is clear that a count from such a sample is in fact the arithmetic mean of counts that would have been obtained from separate defaecations. Given the distribution of counts between defaecations, arithmetic means will generally be higher than the mean calculated from the transformed counts (which is close to the geometric mean of counts).

If, finally, the errors of the two methods of sampling are to be evaluated, a comparison can be made between:

*a.* the variance between $L_1$ counts from individual calves within dates of sampling from each of the two groups of experiment V and

*b.* the variance between $L_1$ counts from herd samples within observations in experiments II, III and IV. (see table 7)

Concerning *a*, the two calves that were introduced later into the two herds were not included in the analysis. The variance between counts within sampling dates, within groups (7 animals) was calculated as $S_1^2 = 4.18$ after transformation of counts

Therefore, the variance of the mean from 7 animals
$S^2_2 = 4.18/7 = 0.60 \quad S_2 = \sqrt{0.60} = 0.77.$

The variance mentioned under b can be read directly from table 7: $S^2_3 = 1.27 \quad S_3 = 1{,}13.$

It may be concluded that the variation of a single herd sample is larger than the variation of the mean of individual samples from 7 calves. However, if one realizes that the variation between observations is very large when compared to the within-variation, the difference becomes less important and more weight should be given to the time needed for each of the sampling and counting procedures. The two systems can only be compared directly for $L_1$ counts in the above mentioned experiments.

3.2.2. *The examination of pasture samples*

The recovery rate of the technique

Experiment 12.

In order to get an impression of the efficiency of the grass-washing-process 10 grass-samples of variable weight were washed five times in succession in 4 l of water, after they had been soaked (as usual). For 3 hours prior to the first washing. Results are presented in table 9. It can be concluded that after 4 washings the large majority of larvae had been washed off, and that the recovery after the first three washings is reasonably constant. In this small experiment there is no indication that the recovery after 3 washings depends on sample-weights or numbers of larvae present.

TABLE 9. The recovery of infective larvae from successive washings of grass samples.

| Weight of sample (g) | Total number of larvae recovered | Cumulative percentage recovered from 5 successive washings | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 607 | 366 | 57.9 | 69.9 | 87.9 | 94.7 | 99.9 |
| 500 | 209 | 50.7 | 74.6 | 89.4 | 92.3 | 100.0 |
| 718 | 198 | 39.4 | 69.2 | 90.4 | 91.9 | 100.0 |
| 500 | 157 | 45.9 | 70.1 | 87.9 | 100.0 | 100.0 |
| 517 | 147 | 63.9 | 76.8 | 91.8 | 100.0 | 100.0 |
| 436 | 134 | 76.9 | 95.6 | 100.1 | 100.1 | 100.1 |
| 507 | 94 | 53.2 | 76.6 | 90.4 | 100.0 | 100.0 |
| 489 | 94 | 46.8 | 60.6 | 84.0 | 96.8 | 100.0 |
| 428 | 62 | 50.0 | 85.5 | 95.2 | 100.0 | 100.0 |
| 522 | 50 | 44.0 | 82.0 | 100.0 | 100.0 | 100.0 |
| Average | | 52.9 | 76.1 | 91.7 | 97.6 | 100.0 |

Experiment 13.

In order to assess the efficiency of the counting technique a known number of larvae (25, 50 or 100) was introduced into centrifuge tubes containing suspensions of grass-washings which were free from gastrointestinal nematode larvae. The results are presented in table 10. There was no significant relation between the number of larvae introduced and the recovery rate.

Experiment 14.

The results of the experiments 12 and 13 were checked by a small experiment in which the recovery was determined by adding known numbers of larvae to ten grass-samples, which had been taken from a field that at least during the last 10 years had never been grazed by animals, and from which control-samples showed to be free of gastrointestinal nematode larvae.

The results of this experiment are presented in table 11.

It can be seen that there is an average recovery of 60.4% which is very high in comparison with the results of experiment 12 and 13 which show a recovery of 90% of the larvae after washing and about 55% of the larvae from the washings,

TABLE 10. The recovery of larvae previously added to suspensions in centrifuge-tubes.

| Number of larvae added | Number of suspensions | Number of larvae recovered | | Percentage recovery |
|---|---|---|---|---|
| | | average | range | |
| 25 | 14 | 14.5 | 10–19 | 58.0 |
| 50 | 31 | 28.1 | 20–39 | 56.2 |
| 100 | 30 | 53.3 | 40–79 | 53.3 |
| | | Average | | 55.4% |

TABLE 11. The recovery of infective larvae previously introduced into grass-samples.

| Weight of sample (g) | Number of larvae introduced | Number of larvae recovered | Recovery (%) |
|---|---|---|---|
| 185 | 650 | 597.5 | 91.9 |
| 190 | 300 | 245.0 | 81.7 |
| 300 | 200 | 165.0 | 82.5 |
| 225 | 150 | 61.5 | 41.0 |
| 255 | 100 | 67.5 | 67.5 |
| 185 | 75 | 50.0 | 66.7 |
| 210 | 60 | 40.0 | 66.7 |
| 210 | 50 | 17.5 | 35.0 |
| 160 | 40 | 15.0 | 37.5 |
| 165 | 30 | 10.0 | 33.3 |
| | | Average | 60.4 |

thus suggesting a total recovery of about 50%. Furthermore there is a very large variation between recovery rates; from 33.3% to 91.9%, and finally there is a significant positive relation between the number of larvae added and the recovery-rate, the SPEARMAN rank-correlation coefficient being $R_s = 0.97$.

It is difficult to draw general conclusions from these experiments, concerning the recovery of larvae from grass samples. Several objections may be raised against each of the experiments. It is likely for instance that the position of the larvae in the grass samples in experiment 14 was quite different from the natural conditions in experiment 12. This is probably one of the factors causing the high recovery (60.4%) in experiment 14. Furthermore the percentages larger than 80% in this experiment are exceptionally high and must be looked upon with reserve. Finally, if the results from faecal samples are considered the results of experiment 13, where no positive relation existed between the recovery and the number of larvae introduced, as in the experiments 2, 3, 4 and 14, are also open to some suspicion.

### The influence of sample-weight and water-content on larval counts

As has already been discussed in chapter I (4.4.) expressing the pasture infec-

tion as numbers of larvae per unit of weight of grass has its pros and cons.

In our material the weight of pasture samples varied from 50 to 990 g. These sample-weights may directly influence the larval counts because of differences in extraction efficiency. Apart from this there may be an overall positive relation between the weight and the number of larvae. The sampling scheme followed permits us to estimate this relationship within and between sampling occasions (or: observations).

The relationship within observations can be assumed to be positive because the heaviest of the two samples will generally contain a larger proportion of big pinches of grass, which are likely to come from the taller, dense growing grass around faecal pats. This positive relation may remain, even after correcting the number of larvae for sample weight, as the number of larvae per kg grass will undoubtly be the highest on the grass around faecal pats.

The relationship between observations is subject to a variety of factors which may either strengthen or neutralize each other:

*a.* Seasonal differences probably work in the direction of a negative relationship, because during spring and early summer the most abundant grass growth take splace whereas larval numbers are generally low.

*b.* The size of fields may also cause a negative relation between sample weights and larval numbers as on the smaller fields the smaller sample weights are found, whereas high larval numbers may be present due to crowding effects.

*c.* The weather may cause a positive relationship; during cloudy and rainy weather the weights tend to be higher but so also may the numbers of larvae.

As stated in the description of the sampling procedure the weight variation caused by *a* and *b* has been decreased artificially by adaptation of the size and number of grass pinches to the area and growth stage of the grass. Due to this factors it is impossible to predict how the relation will be between observations. Table 12 presents the coefficients of correlation between sample weights and larval numbers, and between sample weights and numbers of larvae/kg grass, both within and between observations.

TABLE 12. Coefficients of correlation between larval counts and weights of pasture samples.

| Experiments | Correlation between numbers of larvae per sample and sample weight | | Correlation between numbers of larvae per kg of grass and sample weight | | Number of observations |
|---|---|---|---|---|---|
| | Between observations | Within observations | Between observations | Within observations | |
| II III IV | 0.29** | 0.05 | 0.02 | –0.06 | 136 |
| V | 0.00 | 0.33** | –0.13 | 0.18 | 40 |
| VI | 0.22* | –0.11 | 0.01 | –0.18 | 84 |
| VII | 0.23* | 0.32** | 0.09 | 0.23* | 110 |

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

The results show that before correction for sample weight the number of larvae is slightly but significantly correlated with the sample weight but generally this is not the case after the counts have been expressed as numbers/kg grass. Only in experiment VII there is a correlation between the number of larvae/kg and sample weight, which is significant at the 5% level.

In the experiments II, III and VII the pasture samples were classified either as 'dry' or 'wet' samples. Because of the hour at which samples were collected, most of them were wet.

Occasionally, however, one or two 'dry' observations were made. Such an observation (or the mean of two successive ones) was compared to the mean of the observations done before and after it, with respect to three attributes: sample weight, number of larvae and number of larvae/kg grass.

If this comparison was made over all samples by the signed-rank-test it appeared that 'dry' samples differed significantly from 'wet' samples in weight, but not in numbers of larvae recovered or larvae per kg grass.

Concluding it may be stated that differences in weight or water content of grass samples do not contribute significantly to the variation of the number of larvae/kg grass, because this variation depends almost exclusively on the larval counts proper.

## The variation of larval counts on pasture

An analysis of variance was carried out after logarithmic transformation ($y = \ln (X + 0.5)$) of the counts. Because the same technique was used in all experiments, it was possible to pool all observations made. The results are presented in table 13.

TABLE 13. Analysis of variance of larval counts from pasture samples.

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F | Components of variance |
|---|---|---|---|---|---|
| Between observations | 369 | 4754.615 | 12.89 | 8.70** | 2.851 |
| Between samples (within observations) | 370 | 547.859 | 1.48 | 3.08** | 0.500 |
| Between counts (within samples) | 740 | 355.456 | 0.48 | | 0.480 |

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

The results of experiment I (where samples from the tussocks around faecal pats were investigated) are not included.

From this table it may be concluded that the method of measuring pasture infection is capable of demonstrating differences between observations and between samples within observations. The latter variation might be decreased by a more intensive sampling process, whether such an intensification is worthwhile depends on what differences should be considered important.

## 3.3. The relative abundance of species

### 3.3.1. *General considerations*

It should be pointed out that the relative abundance of species in the host animal may differ widely from that in the faeces, due to different egg producing capacities of the worms. The eggs were differentiated to generic level. *Nematodirus spp.* and *Trichuris spp* eggs could be directly differentiated, eggs belonging to the 'Trichostrongylid-group' were differentiated by the cultivation of third stage larvae. Larvae classified as *Oesophagostomum spp* may in some cases have been *Charbertia spp*, although these are considered to be typical sheep-parasites.

A further question to be considered here, is the fact that egg-differentiation and larval differentiation have different 'sensitivities'. Egg differentiation takes place on the basis of the routine egg counts, so, referring to the sensitivity of the egg count methods, it may be said that a sample has to contain 10–20 *Nematodirus* or *Trichuris* eggs per gram in order to have a reasonable chance of being found positive. With larval differentiations, on the other hand, one has a reasonable chance of obtaining a positive sample if the faeces contain 1 or 2 eggs per gram of any species or genus, at least if a 20–30 g subsample is used. It should be realized, however, that the sensitivity for a species also depends partly on the abundance of other species in the sample, because of the constant number of larvae which is examined, irrespective of the egg content of the sample. If for instance a sample contains 1000 larvae per gram of species *a*, and 1 larvae per gram of species *b*, the probability of finding the sample positive for *b*, if 100 larvae are examined, is approximately 9,5%, if we assume that no selection occurs and the larvae are distributed according to the Poisson law.

The particular time and temperature of incubation chosen (9 days at 27°C) may have discriminated against certain genera or species. In section 3.2.1. no influence could be shown of the percentage *Cooperia spp* upon the relation between egg counts and larval counts, which is an indication that at this time and temperature the hatching rate of eggs of *Cooperia spp* and *Ostertagia spp* (the genus next in abundance) does not differ significantly. In a limited number of faecal samples which also contained eggs of the more rarely occuring genera namely *Trichostrongylus spp*, *Oesophagostomum spp*, *Bunostomum spp* and *Strongyloides spp*, different temperatures (27 and 20°C) and times (6 to 17 days) were studied in order to determine whether some of these genera would be favoured, but no indication in that direction was found, except perhaps for *Bunostomum spp* whose optimal time at 27°C may be somewhat shorter than 9 days.

Finally, if the relative abundance of species has to be expressed in quantitative terms, this can be done in two different ways. Firstly it can be expressed as the percentage of samples that is positive for any species. Secondly it can be expressed as numbers of eggs per gram of faeces or percentages, for separate species per faecal sample. Both methods have their merits.

Whereas the percentage of positive samples is a useful measure to express the incidence of various genera, particularly the relatively rare ones, the number of

eggs or larvae per gram of faeces is of much more value for genera such as *Cooperia* and *Ostertagia* which are found in almost all the faecal samples from calves.

In this section the abundance of species will be reported in terms of percentage of positive samples. In section 3.4, which deals with the results of egg counts, the egg output of the very common genera *Cooperia*, *Ostertagia* and *Nematodirus* will be separately reported, together with the 'total egg count'

### 3.3.2. The abundance of species in the faeces of various age groups of cattle

Table 14 presents the results of larval differentiations in faeces from cattle of all age categories. The samples were taken from animals on the University Farm during January 1967 and January 1968. None of the samples taken was positive for *Nematodirus spp* and *Trichuris spp.*, and egg counts for the 'trichostrongylid group' were on a very low general level.

TABLE 14. Numbers and percentages of samples positive for various genera, taken from the dairy herd of the University Farm during the stall-period (January 1967 and January 1968).

| Age of cattle (years) | Number of positive samples | Number and percentage of samples positive for: | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Cooperia | Ostertagia | Tricho-strongylus | Haem-onchus | Buno-stomum | Oesophago stomum | Strongy-loïdes |
| < 1 | 12 | 12 (100) | 12 (100) | 9 (75) | 0 (0) | 2 (17) | 2 (17) | 2 (17) |
| 1–2 | 37 | 27 ( 73) | 35 ( 95) | 31 (84) | 2 (5) | 15 (41) | 17 (46) | 1 ( 3) |
| 2–3 | 34 | 20 ( 59) | 32 ( 94) | 27 (79) | 3 (9) | 10 (29) | 15 (44) | 0 ( 0) |
| 3–4 | 19 | 8 ( 42) | 18 ( 95) | 9 (47) | 0 (0) | 1 ( 5) | 11 (58) | 0 ( 0) |
| 4–6 | 25 | 4 ( 16) | 24 ( 96) | 9 (36) | 2 (8) | 1 ( 4) | 11 (44) | 0 ( 0) |
| >6 | 24 | 8 ( 33) | 23 ( 96) | 7 (29) | 1 (4) | 1 ( 4) | 10 (42) | 0 ( 0) |

It can be seen that the abundance of the eggs of the genera normally occuring in calf faeces, namely *Cooperia*, *Ostertagia* and *Trichostrongylus*, is similar in the age category < 1 year and in calves during their first grazing period (see table 15). Clearly *Cooperia spp* occur less frequently as animals grow older and, although a sharp distinction between *Cooperia oncophora* and *Cooperia spp.* as made by KEITH (1953) could not be made by us under all circumstances, in this particular material the *Cooperia* specimens found in older cattle belonged undoubtedly to the *Cooperia spp.* group, whereas in the animals less than 2 years old, *Cooperia oncophora* was the predominant species of this genus. *Ostertagia spp* were consistently present in all age categories. *Trichostrongylus spp.*, not very frequently found in faeces of calves during their first grazing period, appear to be present in a large proportion of the samples from animals less than 3 years old. Thereafter there is some indication of decreasing frequency. *Bunostomum spp* which are only rarely seen in faeces of young calves show the same pattern. *Oesophagostomum spp*,, also very rarely seen in faeces of young calves show a relatively high frequency in all age categories. The number of samples

found positive for *Strongyloides spp* was too small to draw any conclusion.

The most important conclusion that can be drawn from this table is, that, except for *Nematodirus spp.*, all the genera commonly occuring in cattle which produce an infective, third stage larva can be found in faeces after 9 days' culturing at 27 °C.

3.3.3. *The abundance of eggs of different species in faeces of calves*

Table 15 presents the results of egg and larval differentiations from experiments II, V, VI, VII and VIII, in terms of numbers and percentages of positive samples. It can be seen that results from individual samples and herd samples are very similar if the totals for the various genera are compared. It appears that the frequency of positive samples for *Nematodirus spp* and *Trichuris spp* is higher in the case of individual samples than for herd samples, and lower for *Trichostrongylus spp.*

Evidently, experiments II, V and VII, which were all carried out on the same pasture, can not be considered representative for the situation in the field with respect to *Nematodirus ssp.*, *Trichuris spp.* and *Trichostrongylus spp.*

Comparisons between experiments and between seasons as made below, have all been tested by the $\chi^2$-test.

If the results of the various *experiments* are compared a few remarks can be made concerning the genera *Nematodirus*, *Trichuris* and *Haemonchus.*

It appears, see table 15, that *Nematodirus* were found less frequently in experiment V (49% positive) than in experiment II and VII (72% and 67% respectively). This difference was significant. There is also an apparent difference between experiments VI and VIII, but this is not significant.

As both experiments V and VI were conducted during 1966, one might consider a climatological factor. In the case of experiment VI however, the low average percentage is obviously brought about by the low percentage found in November/December, which is a sampling occasion very late in the season, that does not occur in the other experiments.

In experiment V, there was a sudden drop of *Nematodirus* egg excretion about 14 weeks after introduction onto pasture. This phenomenon was not seen in two older bull calves which were temporarily stalled, and also not in experiment II, where the heavily infected group had already been treated with an anthelmintic by that time, and not in experiment VII where the calves were stalled 3, 6, 9, 12 or 15 weeks after introduction onto pasture.

This problem is further considered on in the sections that deal with the seasonal influence on abundance of species and the course of egg excretion.

Turning to *Trichuris ovis*, it may be seen that in the course of the experiments II, V and VII, conducted during successive years at the University Farm, there is an increase in the percentage of positive samples (see table 15). This increase was significant from experiment V to Experiment VII but not from experiment II to experiment V. A significant difference also exists between experiment VI and VIII. Again climatological conditions before and during the 1966 and 1967 seasons may have been responsible. On the other hand, however, a gradual

TABLE 15. Numbers and percentages of samples positive for various genera. Samples taken from calve

| Nature of samples | Experiment | Month | Differentiation of eggs: Number of positive samples | Number and percentage of samples positive for: Nematodirus | Trichuris | Number of positive samples |
|---|---|---|---|---|---|---|
| Individual samples, taken from rectum | II | June | 5 | 0 ( 0) | 1 (20) | |
| | | July | 30 | 22 (73) | 0 (0) | |
| | | Aug. | 36 | 28 (78) | 7 (19) | |
| | | Sept.* | 28 | 19 (68) | 10 (36) | |
| | | Oct.* | 10 | 9 (90) | 1 (10) | |
| | | Total | 109 | 78 (72) | 19 (17) | |
| | V | May/June | 36 | 32 (89) | 0 ( 0) | 41 |
| | | July | 30 | 21 (70) | 6 (20) | 32 |
| | | Aug. | 46 | 14 (30) | 22 (48) | 47 |
| | | Sept. | 29 | 2 ( 7) | 11 (38) | 31 |
| | | Total | 141 | 69 (49) | 39 (28) | 151 |
| | VII | June | 52 | 43 (83) | 1 ( 2) | 26 |
| | | July | 81 | 73 (90) | 9 (11) | 40 |
| | | Aug. | 108 | 69 (64) | 81 (75) | 67 |
| | | Sept. | 86 | 47 (55) | 61 (71) | 44 |
| | | Oct. | 43 | 17 (40) | 18 (42) | 22 |
| | | Total | 370 | 249 (67) | 170 (46) | 199 |
| | TOTAL | | 620 | 396 (64) | 228 (37) | 350 |
| Herd samples, taken from pasture | VI | June | 40 | 18 (45) | 0 ( 0) | 40 |
| | | July | 43 | 23 (53) | 7 (16) | 43 |
| | | Aug. | 43 | 21 (49) | 15 (35) | 43 |
| | | Sept.* | 30 | 8 (27) | 11 (37) | 30 |
| | | Nov./Dec. | 40 | 1 ( 3) | 4 (10) | 43 |
| | | Total | 196 | 71 (36) | 37 (19) | 199 |
| | VIII | June | 15 | 5 (33) | 3 (20) | 21 |
| | | July/Aug. | 56 | 32 (57) | 2 ( 4) | 51 |
| | | Sept. | 72 | 35 (49) | 36 (50) | 72 |
| | | Oct. | 69 | 23 (33) | 37 (54) | 69 |
| | | Total | 212 | 95 (45) | 78 (37) | 213 |
| | TOTAL | | 408 | 166 (41) | 115 (28) | 412 |
| TOTAL | | | 1028 | 562 (55) | 343 (33) | 762 |

* Anthelmintic-treated animals or herds excluded.

ılfherds during or shortly after their first grazing season.

| Differentiation of larvae | | | | | | |
|---|---|---|---|---|---|---|
| Number and percentage of samples positive for: | | | | | | |
| Cooperia | Ostertagia | Tricho-strongylus | Haemonchus | Buno-stomum | Oesophago-stomum | Strongy-loides |
| No larval differentiations performed | | | | | | |
| 40 ( 98) | 27 ( 66) | 12 (29) | 6 (15) | 0 ( 0) | 0 (0) | 1 (2) |
| 32 (100) | 30 ( 94) | 4 (13) | 0 ( 0) | 0 ( 0) | 0 (0) | 0 (0) |
| 47 (100) | 46 ( 98) | 11 (23) | 0 ( 0) | 0 ( 0) | 0 (0) | 0 (0) |
| 31 (100) | 29 ( 94) | 11 (35) | 2 ( 6) | 0 ( 0) | 0 (0) | 2 (6) |
| 150 ( 99) | 132 ( 87) | 38 (25) | 8 ( 5) | 0 ( 0) | 0 (0) | 3 (2) |
| 25 ( 96) | 21 ( 81) | 1 ( 4) | 1 ( 4) | 3 (12) | 0 (0) | 0 (0) |
| 40 (100) | 40 (100) | 4 (10) | 1 ( 3) | 1 ( 3) | 1 (3) | 0 (0) |
| 62 ( 93) | 60 ( 90) | 9 (13) | 46 (69) | 1 ( 1) | 0 (0) | 0 (0) |
| 42 ( 95) | 42 ( 95) | 7 (16) | 19 (43) | 0 ( 0) | 0 (0) | 0 (0) |
| 22 (100) | 21 ( 95) | 4 (18) | 6 (27) | 1 ( 5) | 0 (0) | 0 (0) |
| 191 ( 96) | 184 ( 92) | 25 (13) | 73 (37) | 6 ( 3) | 1 (1) | 0 (0) |
| 341 ( 97) | 316 ( 90) | 63 (18) | 81 (23) | 6 ( 1) | 1 (0) | 3 (1) |
| 40 (100) | 38 ( 95) | 7 (18) | 3 ( 8) | 0 ( 0) | 1 (3) | 0 (0) |
| 43 (100) | 43 (100) | 7 (16) | 5 (12) | 0 ( 0) | 1 (2) | 0 (0) |
| 43 (100) | 43 (100) | 9 (21) | 2 ( 5) | 1 ( 2) | 1 (2) | 0 (0) |
| 30 (100) | 29 ( 97) | 14 (47) | 3 (10) | 1 ( 3) | 1 (3) | 0 (0) |
| 43 (100) | 43 (100) | 27 (63) | 1 ( 2) | 0 ( 0) | 0 (0) | 0 (0) |
| 199 (100) | 196 ( 98) | 64 (32) | 14 ( 7) | 2 ( 1) | 4 (2) | 0 (0) |
| 18 ( 86) | 16 ( 76) | 6 (29) | 0 ( 0) | 0 ( 0) | 1 (5) | 1 (5) |
| 51 (100) | 47 ( 92) | 9 (18) | 4 ( 8) | 2 ( 4) | 0 (0) | 0 (0) |
| 72 (100) | 72 (100) | 27 (38) | 20 (28) | 5 ( 7) | 1 (1) | 3 (4) |
| 68 ( 99) | 68 ( 99) | 38 (55) | 15 (22) | 2 ( 3) | 0 (0) | 2 (3) |
| 209 ( 98) | 203 ( 95) | 80 (38) | 39 (18) | 9 ( 4) | 2 (1) | 6 (3) |
| 408 ( 99) | 399 ( 97) | 144 (35) | 53 (13) | 11 (3) | 6 (1) | 6 (1) |
| 749 ( 98) | 715 ( 94) | 207 (27) | 134 (18) | 17 ( 2) | 7 (1) | 9 (1) |

build-up of *Trichuris* infection may have taken place on the pasture used at the University Farm. The difference between experiment VI and VIII might be ascribed to the way in which the farms were selected: in experiment VI also 'healthy' farms were included, whereas in experiment VIII the prevention of trichostrongylosis by grazing on aftermath was tested on farms which had experienced troubles in the preceeding years. Probably *Trichuris ovis* eggs are not as effectively removed from the pasture by mowing as are larvae of trichostrongylids.

Concerning *Haemonchus spp* large differences between years are suggested by table 15, experiments VII and VIII (1967) both showing a significant higher percentage of positive samples than experiment V and VI (1966) respectively. The percentages in experiment VII are particularly high.

Here, the majority of these larvae were found in faeces of three 'control' calves which had not been brought out into pasture. These calves showed very low egg-counts but the majority of larvae cultured from their faeces belonged to a species that could not be properly classified by the key of KEITH (1953). The source of this infection was probably the fresh-made hay from a pasture that had been grazed by sheep. The measurements of the larvae were in good agreement with those of *H. contortus* found by ECKERT (1963). Most of the larvae that could not be classified in the earlier experiments (V and VI), but whose measurement data were still available could afterwards be classified as *Haemonchus* larvae. The problem is that in faeces of normally grazed calves these *Haemonchus* larvae, if they occur, do so in only very limited numbers as compared to *Cooperia* and *Ostertagia* larvae.

According to ECKERT (1963) the larvae of *H. contortus* may be confused with those of *O. ostertagi* and this danger exists particularly if very low numbers of *Haemonchus* are present. In our experience from experiment VII, however, the *Haemonchus* larvae could be distinguished readily from those of *Ostertagia*, by the fine, sharp filamentous ending of the sheath tail of *Haemonchus* of course this need not to be true in other faecal material.

The differences in the frequency of samples positive for *Haemonchus spp* between experiments VI and VIII can be attributed to differences in weather conditions (the 1967 season being relatively warm after a mild winter) and secondly to the different selection procedure of farms for these experiments, but our feeling is that confusion of *Haemonchus* and *Ostertagia* is the earlier experiments has also played a role. No clear indication that *Haemonchus* infections of calves are associated with the presence of sheep could be obtained from experiments VI and VIII (see table 16). The relation was not significant, possibly due to the low numbers of farms that carried sheep or had done so during the preceding year.

If the incidence of various genera is compared *from season to season* within each of the experiments, it appears that there are clear differences for some genera. Such a 'seasonal' effect, may in fact be the result of a complex of various factors such as development of host resistance, presence of immature and mature worms, and chances of reinfection.

TABLE 16. The relation between the presence of sheep and positiveness of calves for *Haemonchus spp.* in experiment VI and VIII (numbers of farms).

| | Sheep present | | | Sheep absent | | |
|---|---|---|---|---|---|---|
| | Expt. VI | Expt. VIII | Total | Expt. VI | Expt. VIII | Total |
| Positive for Haemonchus | 3 | 5 | 8 | 11 | 20 | 31 |
| Negative for Haemonchus | 4 | 1 | 5 | 25 | 31 | 56 |

A clear seasonal influence on the presence of *Nematodirus* eggs was found in the experiments V, VI and VII, whereas the effect was not significant in experiments II and VIII (see table 15). This lack of significance may be explained as follows: in experiments II the heavily infected group was treated with an anthelmintic and excluded from the analysis from September onwards. The low infected group started late with the excretion of eggs as a result of the treatment of the field they were grazing, and a large proportion of the animals from this group remained positive. The same applies to experiment VIII: as a result of grazing on aftermath the egg excretion remained at a low level on most farms throughout the season. The phenomenon of suppression of egg-output that occurs in some species if animals are heavily infected (see section: 3.4 of this chapter) is not shown here.

It can be seen that the excretion of *Trichuris* eggs shows a peak during August or September. This effect of season was significant in all experiments. For *Trichostrongylus spp.* a significant influence of season was only found in experiments VI and VIII but not in experiments V and VII. Again this is considered to be suggestive of a particular situation existing on the University Farm where exp. V and VII were carried out, which is not entirely representative for field conditions.

As pointed out earlier, the sensitivity of the procedure of differentiation is not constant for a certain species, but depends on the numbers of other species that are present. For *Trichostrongylus* in experiment VI for instance, it can be seen that, between seasons, there is a negative relationship between the general level of egg output and the number of samples found positive for this genus, which is suggestive of the above mentioned dependency. Careful examination showed, however, that within seasons the relation did not exist, not even after excluding the farms that had been negative at all five times of sampling. This, together with the comparison of the two variates in experiment VIII where both show a seasonal increase, led us to the conclusion that this possible source of misinterpretation has played no role.

3.4.1. *General remarks*

Because a mutual relationship exists between larval infection of pasture and egg-output of animals these variates will be presented in one figure, together with the presence and number of animals involved. This presentation has been done on the untransformed scales of larvae/kg grass and eggs/g faeces because these are familiar to most readers. Figures are also comparable between experiments because, generally, also the absolute size of the scales is the same. The reader should realize that differences for example between two pastures, may seem small in the figure but may, statistically, be highly significant. In experiment V, for instance, there was a significant difference between larval infections of pastures; from fig. 13 it seems that the first half of the period has contributed less to this difference than the second half, but the differences were, on the transformed scale, of the same magnitude during the entire period.

Larval counts from pasture have, after the appropriate transformation, been examined by means of analysis of variance. In tables 17, 19, 20, 21, 23 and 27 it can be seen that in the majority of cases a significant interaction between pasture and date of sampling is found. This might suggest that the larval counts in two pastures follow a different course in time. We feel that interpretation of such an interaction should be very cautions as it can include a part of the technical variation that should have been incorporated in the between-sample-variances, but has not been so included because of unknown factors which affect counting and sampling procedures from date to date. Therefore the main effects of 'Pasture' and 'Date of sampling' have in all cases also been tested against residual variance + interaction and this did not alter our conclusions.

Egg-output has, for every individual animal or herd, been characterized by:

1. level of egg-output, this is the mean over the entire period and
2. trend of egg-output, this is the mean level of egg-output > 10 weeks after first exposure to infection, minus the mean level of egg-output from patency until 9 weeks after first exposure.

This procedure has been followed for: total egg-counts *Nematodirus spp.* egg-counts, *Cooperia spp.*-egg-output and *Ostertagia spp.*-egg-output. The last two were calculated from the egg count of trichostrongylid-type eggs and the results of larval differentiation. In this way 8 different egg-output-variates were calculated and analyzed namely: $L_T$ (= level of total egg-counts), $C_T$ (trend of total egg-counts) $L_N$ (= level of *Nematodirus spp.*) and so on: $C_N$, $L_C$, $C_C$, $L_O$ and $C_O$.

In the analyses below these egg-count characteristics have been investigated in relation to various other factors such as age, breed, sex, size, pasture grazed, and so on for individual animals, and in relation to mean age, grazing intensity and so on for herds of animals. The growth performance of animals has been examined in relation to pasture grazed, egg-output, breed, etc. The relations are presented as correlation – matrices.

In these correlation-matrices the following symbols are used.

| | | |
|---|---|---|
| $L_T$ | = level of total egg-output | (II, V, VI, VII) |
| $C_T$ | = trend (course) of total egg-output | (II, V, VI, VII) |
| $L_C$ | = level of *Cooperia spp.* egg-output | (V, VI, VII) |
| $C_C$ | = trend of *Cooperia spp.* egg-output | (V, VI, VII) |
| $L_N$ | = level of *Nematodirus spp.* egg-output | (II, V, VI, VII) |
| $C_N$ | = trend of *Nematodirus spp.* egg-output | (II, V, VI, VII) |
| $L_O$ | = level of *Ostertagia spp.* egg-output | (V, VI, VII) |
| $C_O$ | = trend of *Ostertagia spp.* egg-output | (V, VI, VII) |
| W | = weight of animals when brought on pasture | (II, V, VII) |
| G | = growth of animals during experiment | (II, V, VI, VII) |
| A | = age of animals | (II, V, VI, VII) |
| B | = breed of animals | (II, V) |
| S | = sex of animals | (II, V) |
| P | = pasture grazed by the animals | (II, V, VII) |
| P.I. | = overwintered pasture infection | (VI) |
| A.D. | = age distribution of calves within herds | (VI) |
| F | = level of supplemental feeding | (VI) |
| G.I. | = grazing intensity | (VI) |
| R | = number of rotations to 'clean' pasture | (VI) |
| Si | = sire of calves | (VII) |
| G.P. | = length of grazing period | (VII) |

The correlations were further analyzed by the multiple-regression-technique to find out whether a given correlation could or could not be the result of a third factor, influencing both variates.

3.4.2. *Results from Experiment I.*

Figure 6 presents the curve of larval counts in pasture samples, taken from 100 marked tussocks around faecal pats laid down by grazing heifers in August-September 1964.

It is clear that the larval concentration reached a peak in November and declined gradually until the following spring. This is in good agreement with the results of other workers, although MICHEL (1968) concluded from numerous experimental series that the concentration may remain at a high level during the winter season. Possibly the decrease is somewhat overestimated in this experiment due to migration of larvae away from the faecal pat. In the figure, however, the results of a few observations on 'random' samples have also been plotted, and these seem to parallel the course found in 'tussock' samples. Therefore we are inclined to conclude that in this experiment there was indeed a gradual decrease during winter, related to the time of contamination and the weather conditions.

The 95% confidence intervals of the means are also presented in figure 6. As every observations consisted of 8 samples, $S\bar{x}$ could be estimated over all 14 sampling occasions, and values of $\bar{x} \pm 1{,}96\ S\bar{x}$ for each date were reconverted into larvae/kg grass.

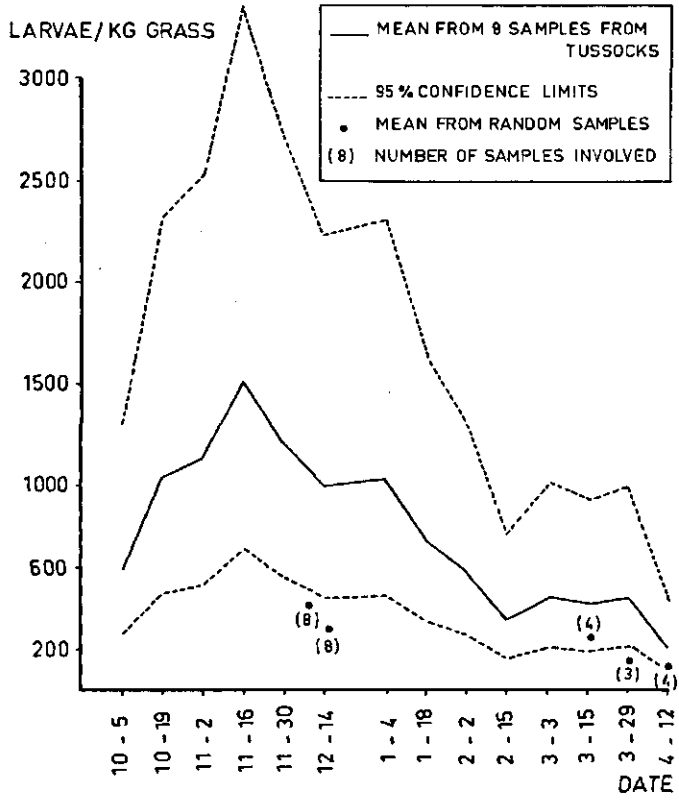



FIG. 6. The course of larval counts in experiment I.

### 3.4.3. *Results of Experiment II*

#### Larval counts on pasture

Figure 7 presents the course of larval counts. Analysis of variance (table 17) showed that both factors 'Pasture' and 'Date of sampling' had a significant influence on these counts ($P < 0.005$). The figure suggests that it takes several weeks before contamination of pasture is followed by a clear increase of larval infestation, which is in good agreement with the findings of workers in England

TABLE 17. Analysis of variance of larval counts on pasture, experiment II.

| Source of variance | degrees of freedom (d.f.) | sum of squares (S.S.) | Mean square (M.S.) | F | P |
|---|---|---|---|---|---|
| Pasture (P) | 1 | 118.58 | 118.58 | 105.87 | $< 0.005$ |
| Date of sampling (D) | 13 | 101.48 | 7.81 | 6.97 | $< 0.005$ |
| Interaction P × D | 13 | 47.30 | 3.64 | 3.25 | $< 0.005$ |
| Error | 28 | 31.29 | 1.12 | - | |
| Total | 55 | 298.65 | - | | |

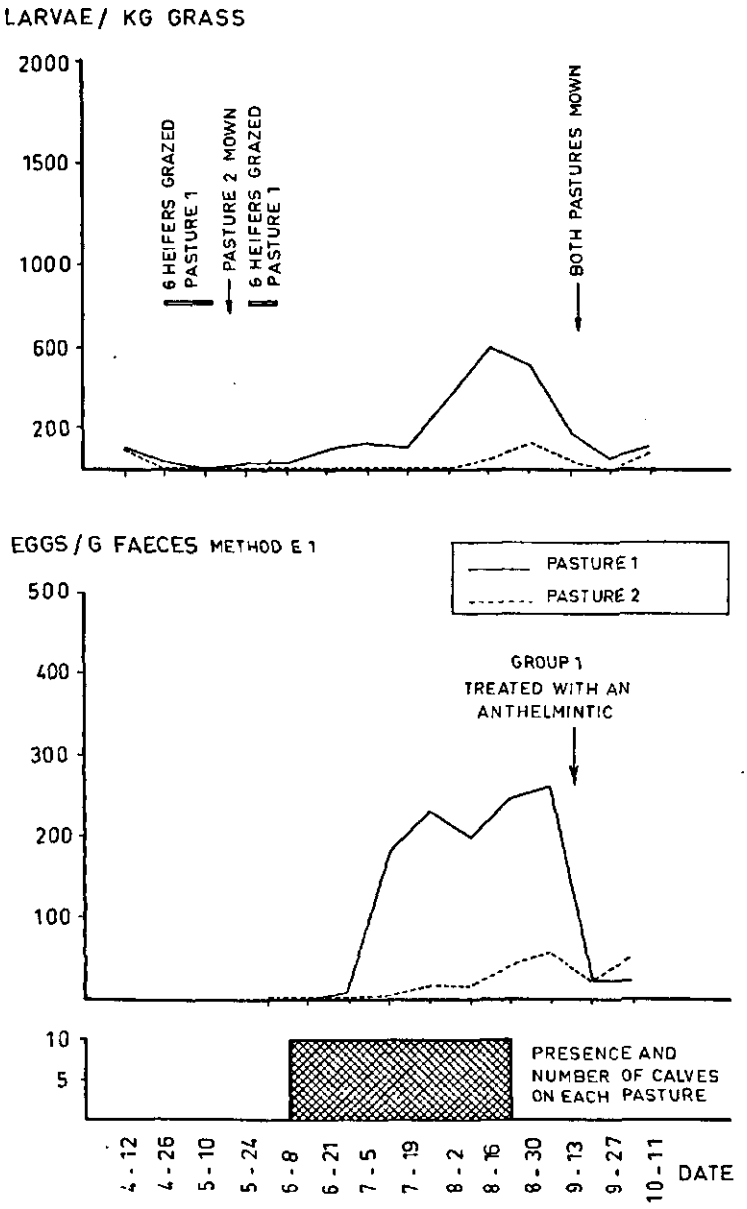



FIG. 7. The herbage infestation and egg excretion in experiment II.

(MICHEL, 1969d). Such an increase probably is the combined effect of the translation of eggs in faeces to 3rd-stage larvae on pasture and the selective grazing of animals by which relatively clean herbage is removed and heavily contaminated grass is left behind.

### Egg counts in faeces

The egg-output of the two groups are presented in figure 7. Egg-count characteristics $L_T$, $C_T$, $L_N$ and $C_N$ were calculated over the period from patency until anthelminthic treatment of group 1. Table 18 presents the correlation of these factors to others. After analyzing these correlations by multiple regression, pasture appeared to be the only factor that exerted a significant effect on level of total egg-counts (the mean level was 219 e.p.g. and 19 e.p.g. for animals on pasture 1 and 2 respectively). The excretion of *Nematodirus spp.*-eggs was not different between groups, being very low (12 and 10 e.p.g. respectively) in both groups.

This might be explained by the low *Nematodirus* egg-output of the contaminating heifers and by the possibility that *Nematodirus*, has not as effectively been removed by mowing as larvae of other trichostrongylids.

Table 18 suggests that trend of egg-output is related to breed and sex of animals, but it can also be seen from the same table that these two factors were interrelated. After regression-analysis none of these factors remained significant. Results indicated that influence of sex would be the largest if it was significant, and results from experiment V (table 22) were in the same direction. This would imply that egg-counts of female calves show a less positive trend (or a more negative one) than those from males. Further studies on this point would be of interest.

### The growth performance of animals

During the period from introduction into pasture until treatment of group 1, the mean live weight gain of group 1 animals was 740 g/day with a standard deviation from the mean of 300 g/day and of group 2:1010 g/day, with a standard deviation from the mean of 190 g/day. This difference is significant ($P < 0.05$). Table 18 shows that the growth of animals is related to the factors: level of total egg-output, initial weight, age, and pasture. After regression analysis none of these factors remained significant. This means that the difference between pasture groups can be partly ascribed to differences between pastures in quality and palatability of the herbage and that this influence strictly, cannot be separated from that of the higher infection-level. Also the better growth of older animals that also have a higher initial weight cannot be attributed to one of these factors alone, because they are interrelated. If level of egg-output and initial weight were the only factors considered, both were significant ($P < 0.01$).

### The effect of anthelmintic treatment.

After stalling, the animals were given a daily ration of hay *ad libitum* and 2 kg of concentrates (S.E. 63, d.c.p. 18%). The treatment had a clear effect on egg-output (figure 7) and on hay intake of the animals (figure 8).

TABLE 18. Correlations between various characteristics in experiment II. For the key of the symbols, see page 63

| | $L_T$ | $C_T$ | $L_N$ | $C_N$ | W | G | A | B | S | P |
|---|---|---|---|---|---|---|---|---|---|---|
| $L_T$ | 1.00 | +0.17 | +0.36 | -0.11 | −0.15 | −0.61** | −0.09 | −0.01 | −0.00 | +0.88** |
| $C_T$ | | 1.00 | +0.32 | +0.66** | +0.01 | +0.12 | −0.04 | −0.45* | +0.52* | +0.00 |
| $L_N$ | | | 1.00 | +0.42 | −0.16 | −0.33 | −0.21 | −0.22 | +0.31 | +0.09 |
| $C_N$ | | | | 1.00 | −0.04 | +0.15 | −0.16 | −0.23 | +0.21 | −0.39 |
| W | | | | | 1.00 | +0.56* | +0.88** | −0.46* | −0.01 | −0.11 |
| G | | | | | | 1.00 | +0.46* | −0.35 | +0.31 | −0.53* |
| A | | | | | | | 1.00 | −0.33 | −0.16 | −0.01 |
| B | | | | | | | | 1.00 | −0.50* | 0 |
| S | | | | | | | | | 1.00 | 0 |
| P | | | | | | | | | | 1.00 |

** $P < 0{,}01$
* $P < 0.05$

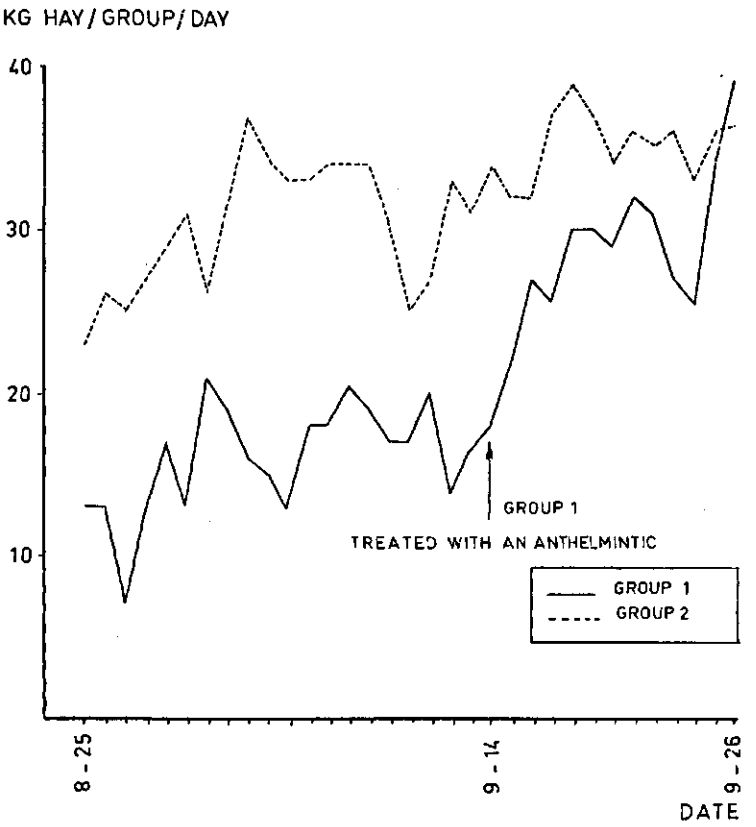



FIG. 8. Hay consumption of the groups after stalling.

## Conclusions

Two pastures which were grazed with egg-excreting yearlings and mown in spring respectively, showed a different level of larval contamination. This difference was reflected by two groups of 10 calves each, that were grazed on them, both with respect to the egg-output and the growth performance of the animals.

### 3.4.4. *Results of experiment III*

### Larval counts on pasture

Level and course for the various pastures are presented in figure 9. The analysis of variance is given in table 19. Further analysis revealed that pasture 1 (control) differed significantly from pasture 2 (phenothiazine) and this differed significantly from pastures 3 and 4 (grazing on aftermath). The latter two did not differ significantly from each other and for that reason they have been presented as one curve in figure 9.

The high level of pasture 1 corresponds with the higher level of egg-output of group 1. It can be seen that again, there is an interval of at least 4 weeks before

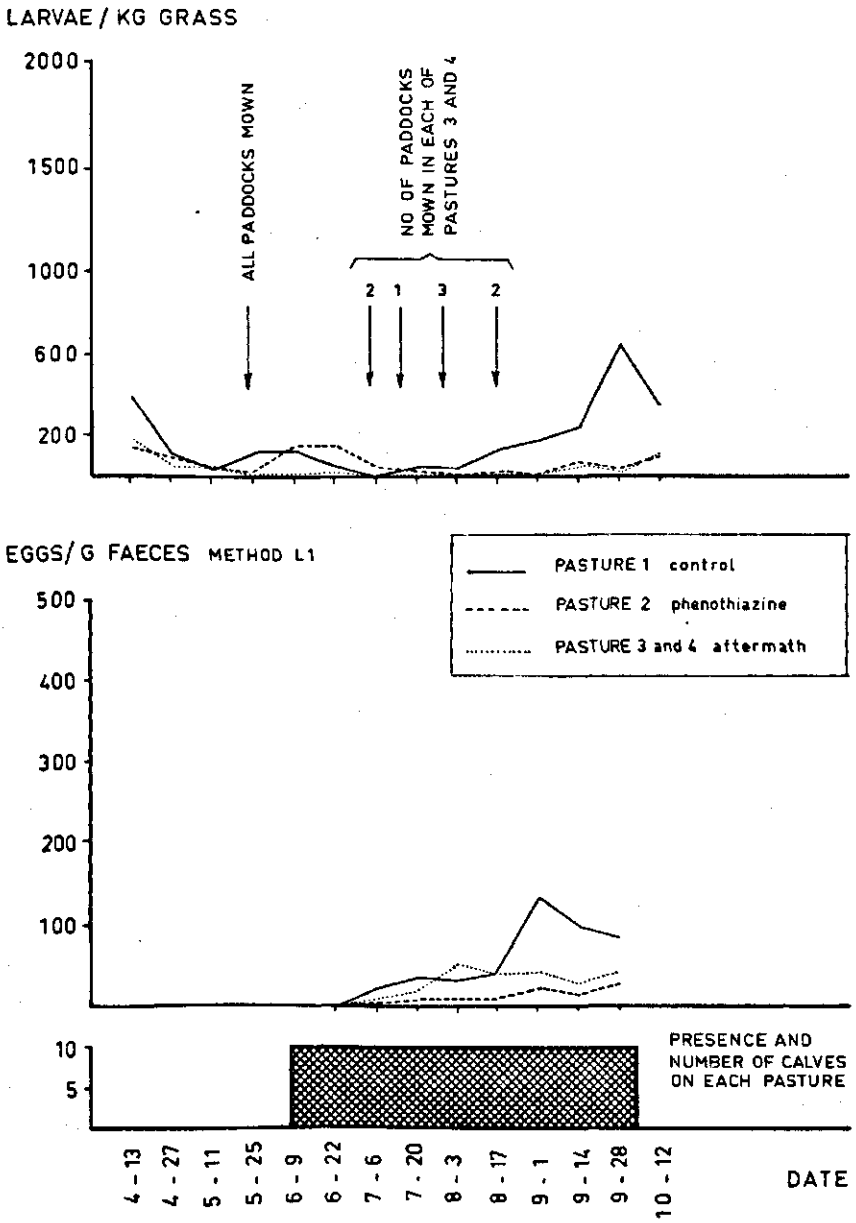



FIG. 9. The herbage infestation and egg-output in experiment III.

TABLE 19. Analysis of variance of larval counts on pastures, experiment III.

| Source of variance | d.f. | S.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Pasture (P) | 3 | 22.48 | 7.49 | 10.86 | < 0,005 |
| Date of sampling (D) | 13 | 74.79 | 5.75 | 8.33 | < 0,005 |
| Interaction P × D | 39 | 87.11 | 2.23 | 3.23 | < 0,005 |
| Error | 56 | 38.40 | 0.69 | - | |
| Total | 111 | 222.78 | | | |

deposited eggs result in increasing larval counts from pasture-samples.

The high level of pasture 1 in late summer, if compared to pasture 2 is probably the result of higher egg-output of group 1. The higher level of egg-output of groups 3 and 4, if compared to group 2 does not result in higher herbage infestation, which is easily explained by the fact that the contamination is spread on twice as large an area. In addition it might be assumed that by the intermediate mowing of the pastures 2 and 4 some larvae are removed from the pasture and a relative bad environment for the larvae is left behind.

The course of herbage-infestation in the early part of the season is more difficult to explain. The 1965-experiment at 'de Vlierd' differed from those in other years, with respect to the first crop of grass. Whereas in other years the pasture for the controlgroup and the anthelmintic-treated group was not mown, it was decided this year to mow all the paddocks because calves were late and grass-growth was relatively early. The decrease of larval counts that occurs usually in the early months of the grazing season did not occur on pastures 1 and 2. The difference between pasture 2 on one hand and pastures 3 and 4 on the other, which was significant, occurred during June and July (see figure 9). The difference might be partly explained by the different grazing-pressure, on 6th of July calves had been grazing the total area of pasture 1 and 2, whereas only half of the total area of pasture 3 and 4 had been grazed. However, it is clear that a direct effect of mowing on larval infestation is not seen on pastures 1 and 2 in this period.

### The egg-output of animals

The egg-output (see figure 9) was determined by larval counts according to method $L_1$ in cultured herd-samples. When comparing the level of egg-output with that of other experiments, the reader should take into account the low relative recovery of this method mentioned earlier in this chapter. Analysis of the transformed counts using the between-sample variation within sampling occasions, estimated as $S^2 = 1.27$ (see table 7) showed that: groups 3 and 4 did not differ significantly at any sampling date. Group 1 and 2 differed significantly on every occasion. Group 1 differed from group 3 and 4 on the last 3 sampling occasions and from group 4 also on 6th July. Group 2 differed from groups 3 and 4 on 3rd and 17th August.

The egg-output of group 5, which was kept indoors and fed freshly mown grass was very low, but was positive on all dates after 6th July.

## The weight gain of animals

The daily weight gain during the grazing period was 706 g, 683 g, 841 g, 713 g and 806 g for groups 1, 2, 3, 4 and 5 respectively. This is illustrated by figure 10. The differences in growth performance between groups were smaller than in comparable experiments at 'de Vlierd' in other years (OOSTENDORP and HARMSEN, 1968). This is ascribed to mowing of the first grass-crop for the control group (2). No deaths occurred this year, as was the case in other years. The differences between groups in growth performance were significant as was revealed by analysis of variance and TUKEY's test for differences among means. Group 1 differed from group 3 ($P < 0.05$), group 2 differed from group 3 ($P < 0.01$) and from group 5 ($P < 0.05$) and group 3 differed from group 4 ($P < 0.05$). It was also investigated whether there was any difference in the course of growth, between groups. It appeared that group IV differed significantly from each of the other groups ($P < 0.01$), showing a more concave curve. This can be readily explained by the treatment of this group, which received less concentrates than the other groups and evidently could not utilize the pasture fodder as efficiently early in the season, as it could later.

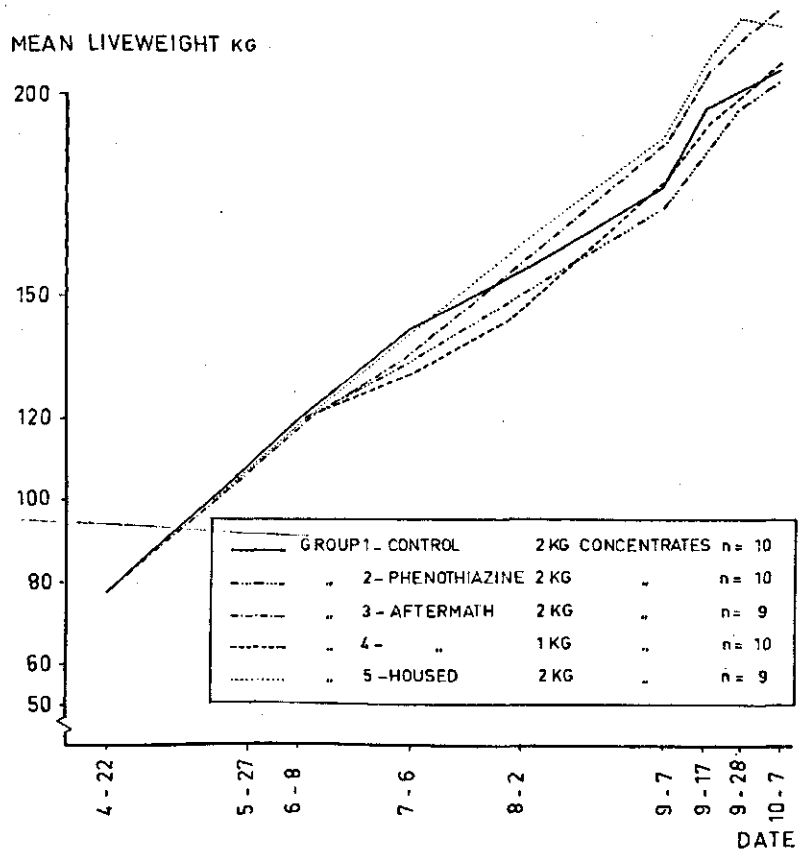



FIG. 10. The live weight gain of the various groups in experiment III.

It is very difficult to interpret whether the difference between the control group and phenothiazine group on one hand and group 3 and 5 on the other has partly been the result of different worm-infections or that a difference between pastures with respect to herbage quality, and palatibility has entirely been responsible. Experiments in other years were more convincing regarding this question.

### Conclusions

Although the experiment cannot be seen as representative for the grazing experiments carried out at 'de Vlierd' during several years some conclusions may be drawn. Firstly, the egg-output was suppressed by continuous phenothiazine-treatment and this prevented the usual occurrence of the mid-summer increase of larval infestation of herbage. This was not reflected by the growth of the animals, however.

Possibly the difference in growth between groups 1 and 2 on one side, and groups 3 and 5 on the other, is caused mainly by better quality and palatibility of the herbage. If it has been caused partly by differences in worm-infection, as in other years, this might be ascribed to differences in residual pasture-infection, overwintered from the previous year, when comparable treatments were carried out on the same areas.

The results in this experiment suggest that the direct influence of mowing on larval infestation is doubtful, but that its effect is working indirectly, by increasing the time intervals between successive grazings and by enlarging the area where a certain contamination is spread on.

### 3.4.5. *Results from experiment IV*

### Larval counts on pasture

These are presented in figure 11. The histories of the pastures (in terms of the mowing of pasture or the grazing of it by a certain age-group of animals) are included in the figures. Analysis of variance (table 20) showed that pasture 1 differed significantly from the other three pastures and between these latter no significant difference could be demonstrated. This means that in a pasture grazed periodically by cattle and not mown, a larval infection may build up during the grazing season, even if the level of egg-excretion of the grazing animals is very low (see figure 12). On the other three pastures, where various age-groups were successively grazed and the grass was mown off once during the

TABLE 20. Analysis of variance of larval counts on pastures, experiment IV.

| Source of variance | d.f. | S.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Pasture (P) | 3 | 74.05 | 24.68 | 13.34 | $< 0{,}005$ |
| Date of sampling (D) | 12 | 91.50 | 7.63 | 4.12 | $< 0{,}005$ |
| Interaction P × D | 36 | 189.66 | 5.27 | 2.85 | $< 0{,}005$ |
| Error | 52 | 96.08 | 1.85 | – | |
| Total | 103 | 451.29 | | | |

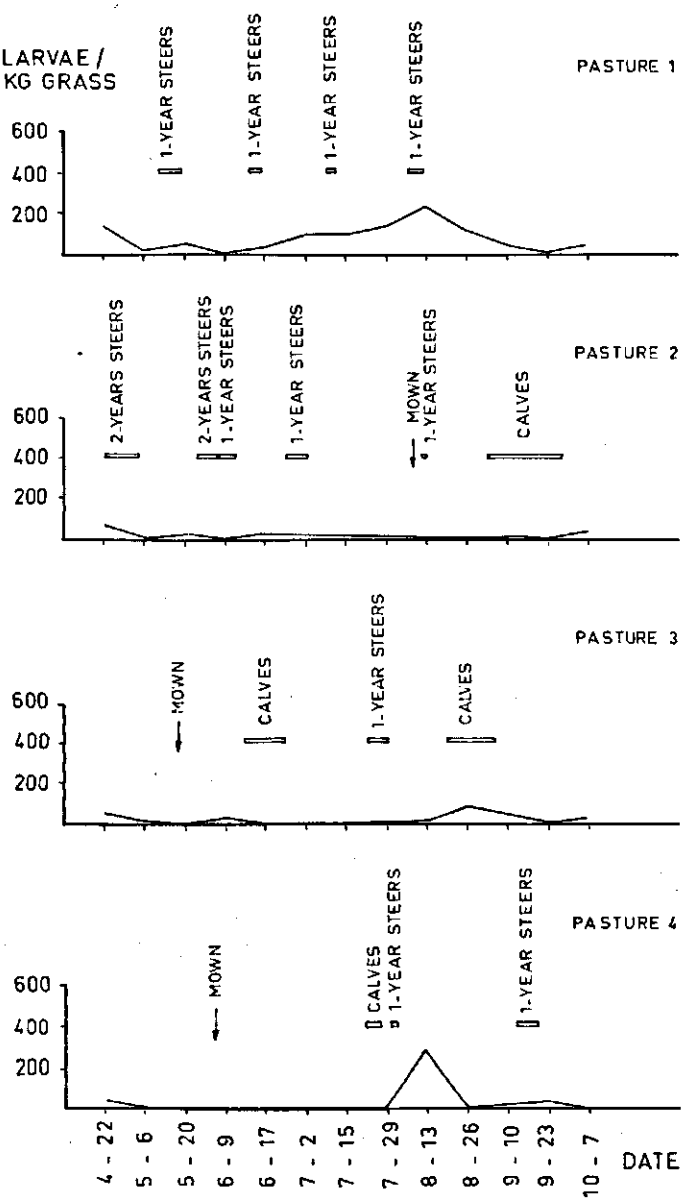



FIG. 11. The herbage infestation on four pastures in experiment IV.

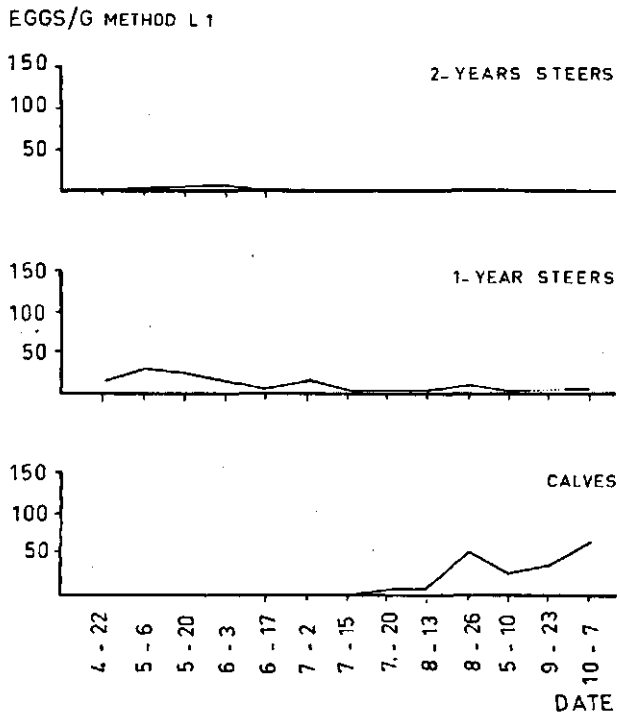



FIG. 12. The egg-output of various age-groups at experimental farm 'C. R. Waiboerhoeve', experiment IV.

season, larval counts did not reach a high level. The observation on 13th of August in pasture 4 seems to constitute an exception, but it was the result of only one extremely high sample, the *duplo* sample being negative, as were many other samples on that pasture during that period. Therefore no great value should be attached to such an isolated observation.

If levels of pasture-infections in this experiment are, in general, compared to those of other experiments (see figures 7, 9, 13 and 17), then it is clear that high larval infections are only found in pastures where, during an extended period, egg-excreting animals are grazed without intermediate mowing of pastures.

## The egg-output of animals

This has been estimated as in the preceeding experiment (III), by $L_1$-counts in cultured herd-samples, and is presented for the 3 age-groups in figure 12. The results are in good agreement with the experience of many workers that egg-output decreases with age. The level and course of egg-output in the calves is comparable to that of the aftermath groups in experiment III. This has been achieved by grazing the calves on pasture like pastures 2, 3 and 4, preferably when the grass has tregrown after mowing. It illustrates that the system of prevention by grazing on aftermath is practicable on a farm-scale, even if relatively large numbers of young, susceptible and egg-excreting animals are present.

### The weight-gain of animals

The daily weight-gain of the calves during the grazing period was 890 g. This favourable performance can be ascribed to a low level of infection together with the good quality of the pasture which is available when such a grazing system is practised.

### Conclusions

From this experiment it can be concluded that the increase of larval concentrations on pastures, as it occurs on continuously grazed calf-paddocks, can also be observed on pasture grazed periodically by yearling-cattle with a rather low egg-output. It did not occur on 3 pastures where grazing of calves was alternatively applied with the grazing of older animals and mowing of the pasture.

### 3.4.6. *Results of experiment V*

### Larval counts on pasture

The pasture infestations are presented graphically in figure 13. Analysis of variance (see table 21) revealed that there were significant differences between pastures and between dates of sampling. In this experiment the increase of larval counts occurs 4–6 weeks after the period that eggs are excreted in significant numbers.

TABLE 21. Analysis of variance of larval counts on pasture, experiment V.

| Source of variance | d.f. | S.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Pasture (P) | 1 | 26.57 | 26.57 | 48.31 | < 0,005 |
| Date of sampling (D) | 19 | 248.65 | 13.09 | 23.80 | < 0,005 |
| Interaction P × D | 19 | 59.08 | 3.11 | 5.65 | < 0,005 |
| Error | 40 | 21.93 | 0.55 | – | |
| Total | 79 | 356.23 | | | |

### Egg counts in faeces

The level and course of egg-output which are presented in figure 13, are the results from 6 animals from each of the groups. 2 Animals from each group were excluded from both the figure and the statistical examinations, namely the two young calves (one in each group) that were introduced into pasture 18 days later and the oldest bull-calves from each group that were stalled for 5 weeks during the experiment (see also the description of the experiment, chapter 2). If these 4 animals are included, egg-count curves and results from the statistical analysis do not change significantly. It seemed sound, however, to leave them out.

As can be seen in table 22, the mean level of total egg-output was negatively related to the initial weight of the animals but did not show any relationship with pasture grazed, age, sex and breed. The trend of total egg-output, however, differed significantly between pastures ($P < 0.01$) and was also related to the initial weight ($P < 0.05$) of the animals. The difference between pastures is clearly visible in figure 13. The effect of initial weight could imply that heavier

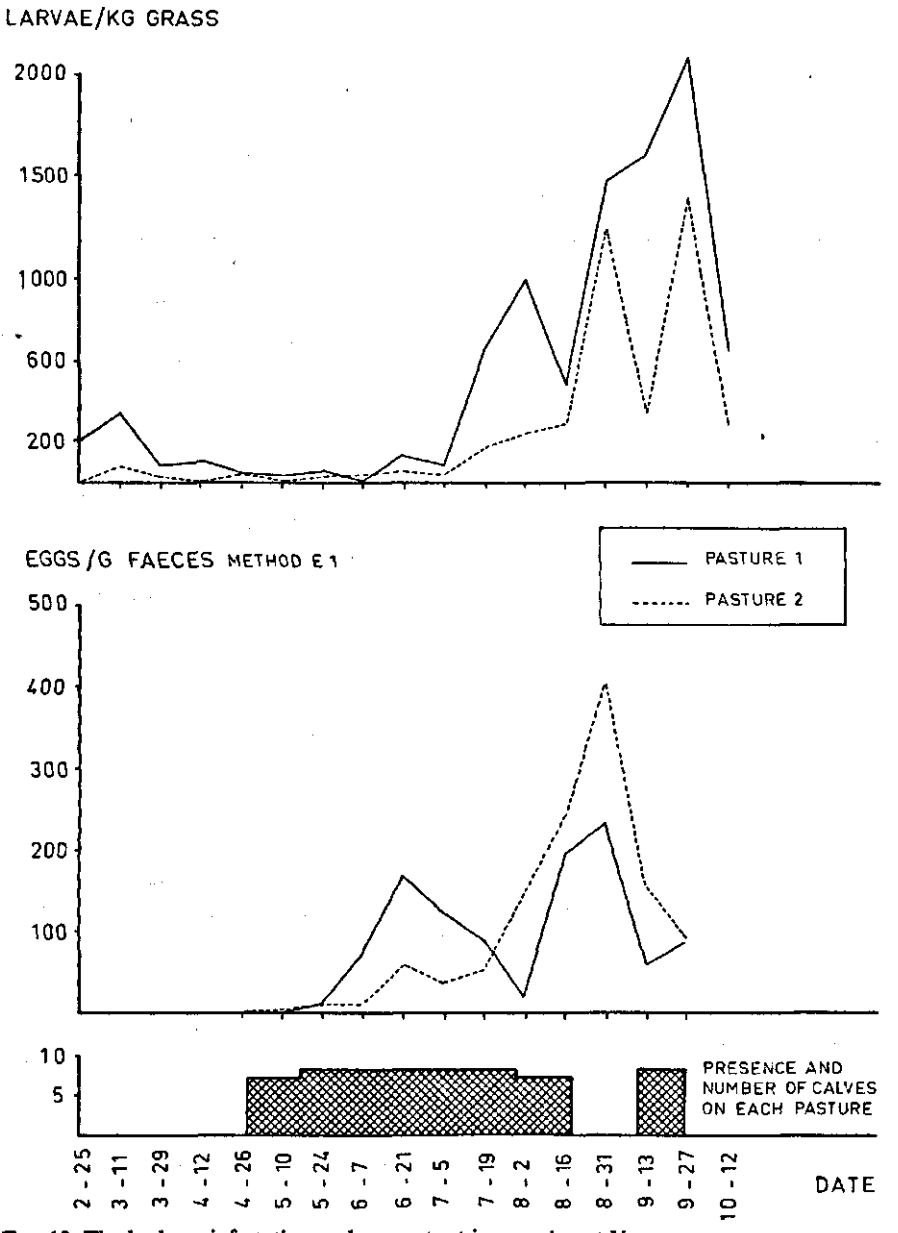



FIG. 13. The herbage infestation and egg-output in experiment V.

TABLE 22. Correlation between several characteristics in experiment V. For the key of the symbols, see page 63.

| | $L_T$ | $C_T$ | $L_N$ | $C_N$ | $L_C$ | $C_C$ | $L_O$ | $C_O$ | W | G | A | B | S | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $L_T$ | 1.00 | +0.16 | +0.91** | −0.89** | +0.90** | +0.38 | +0.77** | +0.40 | −0.58* | −0.44 | −0.49 | −0.40 | +0.36 | +0.08 |
| $C_T$ | | 1.00 | −0.01 | +0.02 | −0.06 | +0.94** | −0.10 | +0.69** | −0.65** | −0.19 | −0.60* | −0.37 | +0.45 | −0.76** |
| $L_N$ | | | 1.00 | −0.90** | +0.73** | +0.26 | +0.70* | +0.16 | −0.51 | −0.36 | −0.40 | −0.28 | +0.31 | +0.09 |
| $C_N$ | | | | 1.00 | −0.74** | −0.24 | −0.83** | −0.38 | +0.58* | +0.54 | +0.51 | +0.37 | −0.34 | −0.23 |
| $L_C$ | | | | | 1.00 | +0.09 | +0.71** | +0.22 | −0.34 | −0.27 | −0.31 | −0.34 | +0.22 | +0.34 |
| $C_C$ | | | | | | 1.00 | −0.02 | +0.67* | −0.80** | −0.30 | −0.62* | −0.36 | +0.43 | −0.73** |
| $L_O$ | | | | | | | 1.00 | +0.46 | −0.29 | −0.46 | −0.43 | −0.30 | +0.31 | +0.35 |
| $C_O$ | | | | | | | | 1.00 | −0.52 | −0.49 | −0.65* | −0.42 | +0.51 | −0.34 |
| W | | | | | | | | | 1.00 | +0.27 | +0.80** | +0.36 | −0.33 | +0.38 |
| G | | | | | | | | | | 1.00 | +0.31 | +0.72** | −0.51 | −0.12 |
| A | | | | | | | | | | | 1.00 | +0.61* | −0.61* | +0.11 |
| B | | | | | | | | | | | | 1.00 | −0.84** | −0.17 |
| S | | | | | | | | | | | | | 1.00 | +0.00 |
| P | | | | | | | | | | | | | | 1.00 |

** $P < 0.01$

* $P < 0.05$

(older) animals show a more decreasing trend or a less increasing trend than the younger animals which accords with the opinion of several authors that egg-counts tend to decrease or are suppressed by the immune-response of the host and that the immune-response is more marked in older animals.

From figure 14, where egg-output for various genera is presented, and from table 22 it can be seen that the difference in trend between pastures is attributable entirely to *Cooperia spp.* For this genus the difference is highly significant ($P < 0.002$) and no difference for *Nematodirus spp.* or *Ostertagia spp.* could be demonstrated.

In figure 14 it is also clear that *Nematodirus spp.* egg-counts became negative rather early. This was seen almost simultaneously in all animals. However the two older bull-calves, that were stalled continued their *Nematodirus* egg. excretion until 14 days after they had been put on pasture again. This suggests that intake of larvae plays some role in this suppression of egg-output. From group 2, one animal died from parasitic enteritis just before the experiment ended. Examination of the gastro-intestinal tract for worms revealed large numbers of *Nematodirus helvetianus* adults, among them females whose uteri contained large numbers of eggs. This animal had been negative for *Nematodirus* eggs in the faeces for 6 sampling-dates preceeding its death, that is a period of 12 weeks.

Although conclusions cannot be drawn from these fragmentary observations, they were too interesting to leave them unreported.

## Live-weight gain of animals

The growth performance of the two groups (6 animals) did not differ significantly: group 1 animals gained 600 g/day (st. dev. from the mean = 100g/day and group 2 animals 640 g/day (st. dev. from the mean = 270 g/day. Lack of difference was clearly caused by the animal from group 2 that died from parasitism. Its growth was only 130 g/day. If this animal is excluded and the 4 animals that had been left out (2 in each group) are taken into consideration quite a different picture is seen: group 1 (8 animals) gained 593 g/day (st. dev. from mean = 118 g/day) and group 2 (7 animals) 759 g/day (st. dev. from mean = 158 g/day). This difference is significant at the 5% level.

It is hard to draw any conclusions. On purely statistical grounds the calf that died can be considered as an outlier and it is reasonable or even necessary to exclude it from further calculations. On the other hand, on clinical grounds and from autopsy it was concluded that parasitic gastro-enteritis was the cause of death and all workers familiar with the subject know that 'outliers' within a host population are a common phenomenon.

From work in progress, two experimentally infected groups of calves reacted in striking similarity with the groups in this experiment. Here a significant growth retarding effect was seen in the group that received the higher larval doses, and this group showed the same depression of *Cooperia spp.* egg-counts. (KLOOSTERMAN et al., in prep.). For that reason we are inclined to conclude that there was some growth depression in group 1, which grazed the pasture with the largest larval infection.

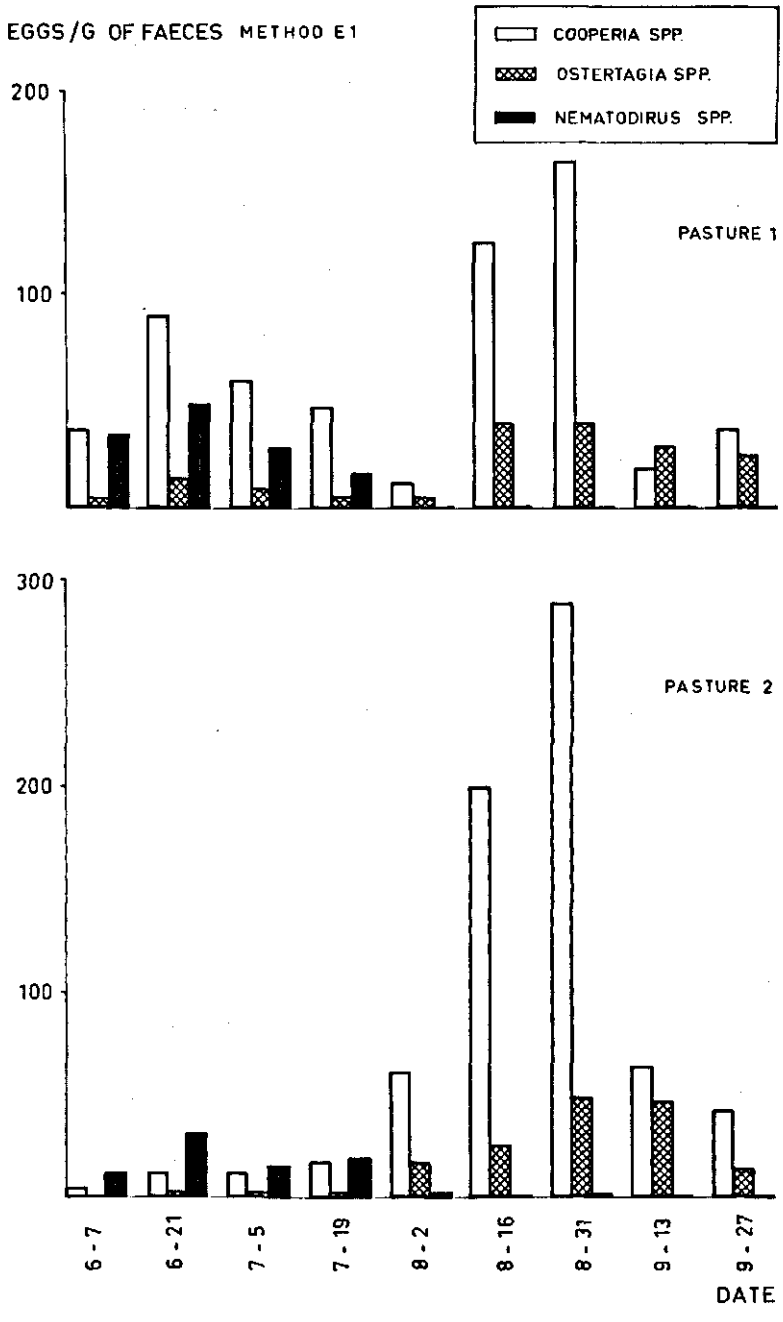



FIG. 14. The egg-output of genera in experiment V.

### Conclusions

From this experiment it was concluded that the differences between pastures in the previous year (expt. II) were more or less continued in the following year. The level of pasture infestation was higher and rose earlier on pasture 1. This difference was reflected by egg counts during the first weeks after patency. However, over the entire grazing period no difference in level of egg-output between group 1 and group 2 was seen. This was caused by a suppression of egg-counts occurring in group 1. The growth of calves generally corresponded with the level of herbage infestation, but also in group 2 serious clinical parasitism was observed.

### 3.4.7. *Results from experiment VI*

### Larval counts on pasture

Analysis of variance revealed that significant differences existed between farms, but not between the two periods of sampling in March and April respectively (table 23).

TABLE 23. Analysis of variance of larval counts from pastures, experiment VI.

| Source of variance | d.f. | S.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Pasture (P) | 40 | 498.55 | 12.46 | 7.46 | < 0,005 |
| Period of sampling (D) | 1 | 2.40 | 2.40 | 1.44 | n.s. |
| Interaction P × D | 40 | 79.31 | 1.98 | 1.19 | n.s. |
| Error | 82 | 137.26 | 1.67 | – | – |
| Total | 163 | – | – | – | – |

The number of larvae/kg grass varied per sample from zero to more than 4000, and the mean number from 4 samples per farm from 1 to 1560, the latter numbers being calculated by reconverting means of transformed values into larvae/kg grass. See also figure 15.

From samples with a reasonably large number of larvae, differentiations were carried out. In this way 2299 larvae were determined with the following result: (table 24).

TABLE 24. Differentiations of larvae from grass-samples, experiment VI.

| | March | April | Total | |
|---|---|---|---|---|
| Cooperia spp. | 731 | 650 | 1381 | |
| Ostertagia spp. | 320 | 168 | 488 | |
| Nematodirus spp. | 107 | 306 | 413 | |
| Trichostrongylus spp. | 9 | 3 | 12 | |
| Others | 2 | 3 | 5 | |
| Total | 1169 | 1130 | 2299 | $P < 0.001$ ($\chi^2$-test) |

The conclusion can be drawn that from March to April the abundance of *Nematodirus spp.* larvae has increased whereas other genera, especially *Ostertagia* decreased. This is in good agreement with the results in experiments V and VII (see figure 18). The relative increase of *Nematodirus spp.* may be the result of overwintering of 3rd stage larvae within the eggs, which are released by the increasing temperatures in spring.

## Egg counts in faeces

From figure 15 which presents the relation between grassland-infection and the mean of egg-counts per herd, the reader may obtain an impression of the variation between farms. Figure 16 presents the course of egg-counts for various genera from the group of farms (13) where anthelmintic treatment took place in early September, and the remaining farms (30) further referred to as 'untreated' farms.

This treatment took place on the basis of egg-excretion and the clinical appearance of animals. From figure 16 it appears that the trend of egg-counts is

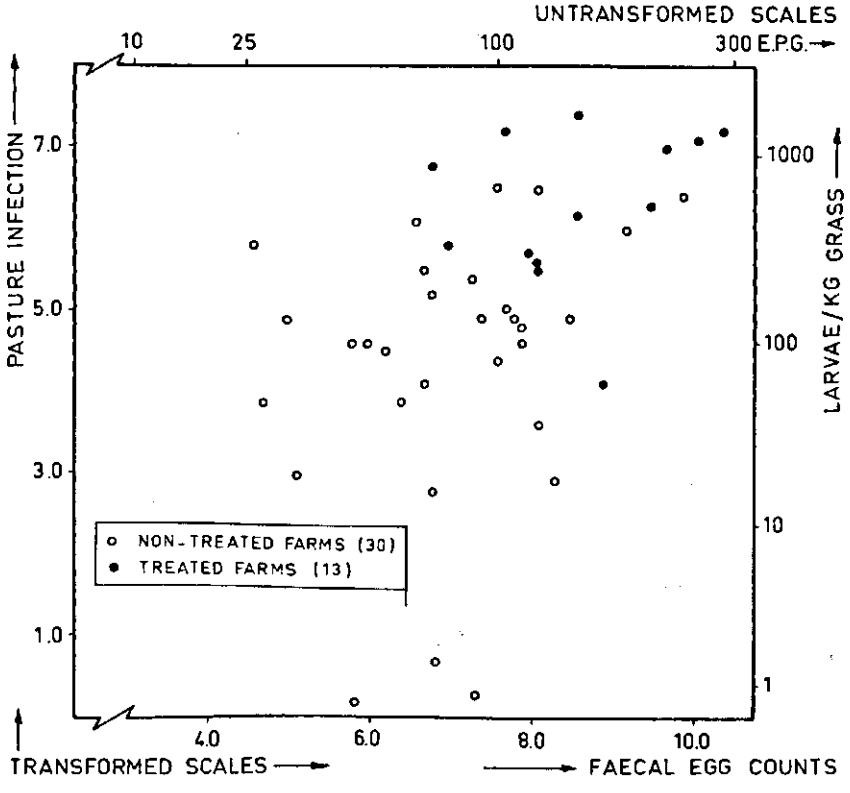



FIG. 15. The relation between residual pasture infection and the mean level of egg-output during the grazing season, experiment VI.

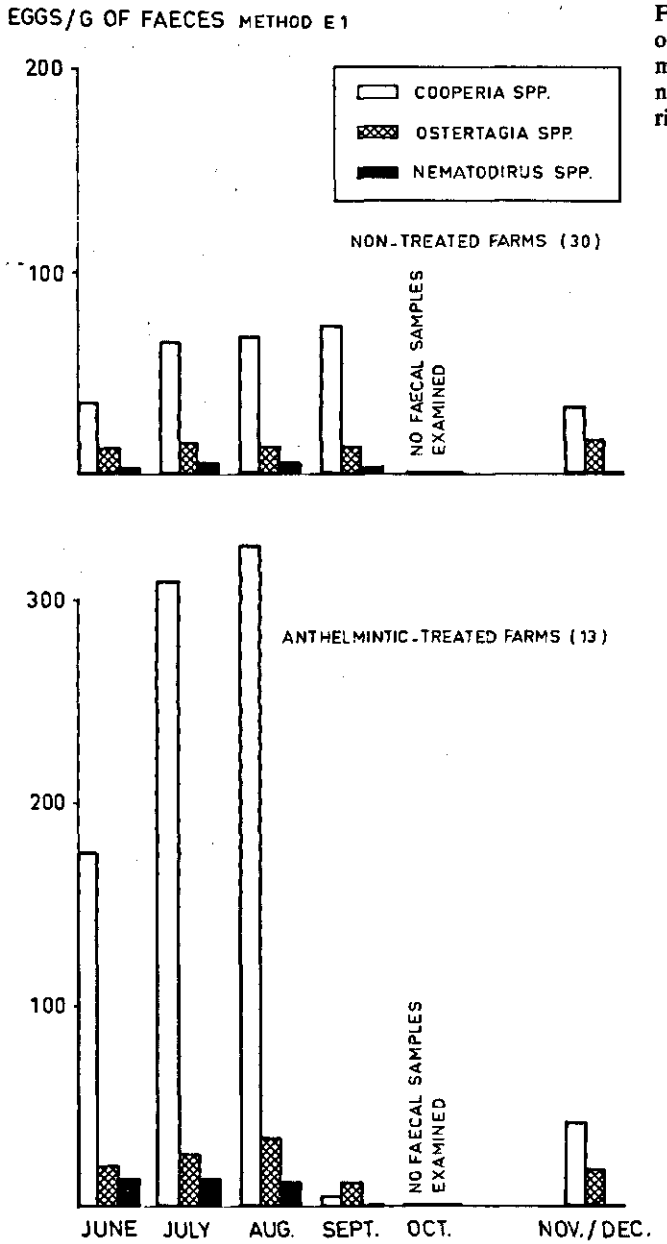



FIG. 16. The egg-output of three genera in anthelmintic-treated farms and non-treated farms, experiment VI.

profoundly influenced by the treatment. This trend (expressed as usual, see introductory remarks of this section) was as follows:

| | 95% confidence interval | |
|---|---|---|
| | untreated farms | treated farms |
| Total eggs | $+ 0.03 \pm 0.39$ | $- 1.49 \pm 0.55$ |
| Nematodirus spp. | $- 0.24 \pm 0.22$ | $- 0.92 \pm 0.61$ |
| Cooperia spp. | $+ 0.13 \pm 0.41$ | $- 1.75 \pm 0.62$ |
| Ostertagia spp. | $+ 0.01 \pm 0.29$ | $- 0.07 \pm 0.51$ |

It can be concluded that treatment clearly decreased the egg-output of *Cooperia spp.*, but seemed less effective against *Ostertagia spp.* As far as *Nematodirus spp.* is concerned, we are inclined to suppose that most of the egg-counts would have become negative or low spontaneously (see also experiment V).

In table 25 the correlation coefficients between egg-output characteristics and other factors are presented. Information on such factors as: feeding during the raising period, soil-type, liverfluke infection, mowing percentage and the ratio $\frac{\text{calves + yearlings}}{\text{older animals}}$ was also available but none of them could contribute significantly to the explanation of egg-output and/or growth performance. Of the factors in table 25 some need further explanation.

Age and age distribution.

For each of the five faecal sampling occasions the age and age distribution has been characterized by giving the animals born before January the value 3, those born from January 1 to March 31 the value 2, and those born later the value 1. On most farms the situation did not change during the grazing period, but on some of them older calves were removed and eventually replaced by younger ones. Therefore this procedure has been followed. In this way 5 frequency distributions were obtained for each of the farms.
For each of the farms the mean and standard deviation was calculated which are referred to as age and age distribution respectively.

The growth performance

This was characterized by the mean deviation from the regression of heart-girth on age. As all animals were from the F. H. -breed and practically all were females (except two young bull calves which were excluded) the deviation from the ideal curve for F. H. females could be calculated for each of the animals, because on the day of measuring, the exact age of the animals was recorded from the identification-cards. The above mentioned regression and the computer-programme for calculation of deviations was kindly provided by Vos (1969) whose help and interest is gratefully acknowledged here.

### The supplementary feeding

This was given on all farms, but the form in which it was given varied. Use was made of cheese-whey, skimmed milk, milk replacer and concentrates. It has been expressed in Starch Equivalents/animal/day.

### The grazing intensity

This was calculated by the formula:

$$\text{G.I.} = \frac{\text{Number of calf-days until 28th of August}}{\text{Total area (ha) occupied by the herd until 28th of August}}$$

The reader will probably be puzzled by the date mentioned in the formula. This was chosen because:

1. The grazing intensity over the entire grazing period would be determined largely by the very large areas the calves often get if in September the harvesting of hay and silage has stopped. The variation in grazing intensity is therefore larger during the first months than during the entire period, and with respect to gastro-intestinal parasitism the first 4 months can be considered as most important.
2. On this date on all but 2 farms the calves had not been rotated during the preceeding two weeks. After it, the rotations occurred again with higher frequency. Moreover, on the treated farms (13) advice was given to move the calves after treatment, which took place in the first week of September.

### The number of rotations to clean pasture

By clean pasture is meant a pasture that has been mown previously, or has not carried young stock earlier in the season.

From table 25 it appears that the level of total egg-counts is related positively to the pasture infection and negatively to the number of rotations. Also age and grazing intensity seem to bear some relation to it, although these are not significant. When analyzed by multiple regression, it appeared that two of these four factors were significant: Pasture infection ($P < 0.001$) and age ($P < 0.05$). The relation with pasture infection is presented graphically in figure 15.

From table 25 it can also be seen that the relation of total egg-counts to various factors is practically identical to that of *Cooperia spp.* egg-counts. This is easily understood if figure 16 is examined.

The trend of egg-counts is very difficult to interpret from table 25, as it is so profoundly influenced by the anthelmintic treatment. For the trend of *Ostertagia spp.* which was hardly influenced by treatment, it may, however, be concluded from table 25 that it is negatively related to age, and this is confirmed by multiple regression analysis together with other factors ($P < 0.05$). This might suggest that in older animals *Ostertagia* egg-counts show a lower increase or a stronger decrease than in younger ones.

The same analysis of egg-counts has been carried out for the 30 untreated farms separately (table 26). The same conclusion could be drawn about the level of total egg-counts and those of the genera, as from table 25. None of the factors had a significant effect on trend of total egg-counts.

TABLE 25. Correlations between various characteristics, experiment VI. (Treated + untreatedfarms). For the key of the symbols, see page 63.

| | $L_T$ | $C_T$ | $L_N$ | $C_N$ | $L_C$ | $C_C$ | $L_O$ | $C_O$ | P.I. | A | A.D. | G | F | G.I. | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $L_T$ | 1,00 | −0,47** | +0,55** | −0,47** | +0,95** | −0,52** | +0,61** | −0,08 | +0,67** | −0,27 | −0,20 | −0,10 | +0,12 | +0,29 | −0,35* |
| $C_T$ | | 1.00 | −0,10 | +0.29 | −0.50** | +0.94** | −0.32* | +0.53** | -0.50** | −0.09 | +0.13 | +0.16 | +0.01 | −0.48** | +0.22 |
| $L_N$ | | | 1.00 | −0.61** | +0.45** | −0.07 | +0.26 | +0.07 | +0.45** | −0.16 | −0.24 | −0.28 | −0.18 | +0.14 | −0.04 |
| $C_N$ | | | | 1.00 | −0.44 ** | +0.22 | −0.24 | +0.06 | −0.45** | +0.07 | +0.09 | +0.38* | +0.09 | −0.35* | +0.04 |
| $L_C$ | | | | | 1.00 | −0.58** | +0.42** | −0.07 | +0.66** | −0.13 | −0.16 | −0.04 | +0.17 | +0.32* | −0.38* |
| $C_C$ | | | | | | 1.00 | −0.26 | +0.33* | −0.52** | −0.05 | +0.10 | +0.12 | +0.00 | −0.47** | +0.21 |
| $L_O$ | | | | | | | 1.00 | −0.42** | +0.29 | −0.17 | −0.16 | −0.10 | −0.01 | +0.11 | −0.11 |
| $C_O$ | | | | | | | | 1.00 | −0.00 | −0.34* | +0.10 | +0.07 | −0.05 | −0.19 | −0.09 |
| P.I. | | | | | | | | | 1.00 | −0.01 | −0.15 | −0.07 | +0.09 | +0.30* | −0.24 |
| A | | | | | | | | | | 1.00 | +0.11 | +0.20 | +0.00 | −0.08 | +0.19 |
| A.D. | | | | | | | | | | | 1.00 | −0.11 | +0.07 | +0.07 | +0.01 |
| G | | | | | | | | | | | | 1.00 | +0.12 | −0.25 | +0.03 |
| F | | | | | | | | | | | | | 1.00 | −0.30 | −0.05 |
| G.I. | | | | | | | | | | | | | | 1.00 | −0.16 |
| R | | | | | | | | | | | | | | | 1.00 |

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

TABLE 26. Correlations between various characteristics in 30 untreated farms, experiment VI. For the key of the symbols, see page 63.

| | $L_T$ | $C_T$ | $L_N$ | $C_N$ | $L_C$ | $C_C$ | $L_O$ | $C_O$ | P.I. | A | A.D. | G | F | G.I. | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $L_T$ | 1.00 | −0.16 | +0.53** | −0.36* | +0.91** | −0.16 | +0.44** | +0.04 | +0.52** | −0.33 | −0.28 | +0.15 | +0.07 | −0.05 | −0.27 |
| $C_T$ | | 1.00 | +0.05 | +0.14 | −0.22 | +0.93** | −0.07 | +0.59** | −0.26 | −0.23 | +0.23 | −0.05 | +0.07 | −0.23 | +0.01 |
| $L_N$ | | | 1.00 | −0.37* | +0.36* | +0.10 | +0.17 | +0.18 | +0.47** | −0.20 | −0.33 | −0.30 | −0.12 | −0.07 | +0.02 |
| $C_N$ | | | | 1.00 | −0.33 | +0.09 | −0.09 | −0.12 | −0.37* | +0.10 | +0.04 | +0.33 | −0.08 | −0.39* | −0.12 |
| $L_C$ | | | | | 1.00 | −0.31 | +0.13 | +0.10 | +0.49** | −0.13 | −0.22 | +0.19 | +0.14 | −0.01 | −0.32 |
| $C_C$ | | | | | | 1.00 | +0.14 | +0.36* | −0.25 | −0.24 | +0.21 | −0.18 | +0.16 | −0.24 | +0.01 |
| $L_O$ | | | | | | | 1.00 | −0.39** | +0.08 | −0.24 | −0.27 | +0.15 | −0.03 | −0.21 | +0.01 |
| $C_O$ | | | | | | | | 1.00 | +0.04 | −0.33 | +0.22 | +0.05 | −0.16 | +0.08 | −0.02 |
| P.I. | | | | | | | | | 1.00 | +0.09 | −0.18 | +0.07 | +0.05 | +0.06 | −0.13 |
| A | | | | | | | | | | 1.00 | +0.10 | +0.06 | −0.04 | −0.08 | +0.22 |
| A.D. | | | | | | | | | | | 1.00 | −0.18 | +0.18 | +0.06 | +0.01 |
| G | | | | | | | | | | | | 1.00 | −0.06 | −0.17 | −0.13 |
| F | | | | | | | | | | | | | 1.00 | −0.32 | −0.11 |
| G.I. | | | | | | | | | | | | | | 1.00 | +0.25 |
| R | | | | | | | | | | | | | | | 1.00 |

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

The trend of *Nematodirus* egg counts was significantly influenced by pasture infection and grazing intensity, and after regression analysis these factors remained significant ($P < 0.05$).

Finally one important remark can be made. In table 26 it can be seen that the pasture infection, which greatly influences the egg- output of animals, has a different influence on the trend of *Nematodirus spp.* and that of *Ostertagia spp.* egg-counts, while the trend of *Cooperia spp.* (and as a consequence, also that of the total egg-counts) occupies a place between these two genera.

## The growth performance of animals

The mean of the deviations per herd, over all 43 herds, was + 0.99 cm heart-girth. At this age 1 cm heart-girth corresponds to approximately 4 kg live-weight. The standard deviation from this mean was 4.91 cm, and this is suggestive of a considerable variation in growth-performance of calves under field-conditions; the selection of farms certainly has played a role here.

If the relation of growth to egg-output characteristics and other factors is examined in table 25, the *Nematodirus spp.* egg-count trend is the only factor showing a significant correlation. In non-treated farms (table 26) the correlation is not significant. After analysis by the multiple regression technique the reverse was true: in the non-treated farms a significant positive relationship existed ($P < 0.05$), whereas in all farms the relation was not significant ($P < 0.10$). On 13 of the 43 farms no *Nematodirus spp.* eggs were found at any sampling occasion.

Such a relation might suggest either that *Nematodirus spp.* worms are particularly important in causing growth-depression, which is not supported by the literature, or that the presence of *Nematodirus* on any farm is indicative of a farm management which is sub-optimal with respect to growth of calves.

It has nevertheless, become evident from this experiment that growth of calves under field conditions cannot easily be related to the level of egg-counts in faecal samples, not even if other growth-affecting factors are taken into account.

## Conclusions

Between pastures, where calves were to start grazing early May, significant differences in overwintered infection were seen in March and April. These differences were correlated with the level of total egg-output during the following season, and to the trend of *Nematodirus* egg-output. The age of the calves was another factor that was significantly correlated to egg-output. Of other factors, such as level of supplemental feeding, the grazing intensity and the number of rotations, no significant influence could be seen on total egg-output. The trend of *Nematodirus spp.* egg-output was significantly influenced by the grazing intensity.

The growth of calves, which was only measured at the end of the grazing season was not significantly related to initial pasture infection or level of total egg-output. This may have partly been caused by the anthelmintic-treatment.

It was clear that as early as June and July some herds were ill-thriving and at that period a significant relation between growth and pasture infection or faecal egg-counts may well have existed.

The growth performance was significantly related to the trend of *Nematodirus spp.* egg-output. As this egg-output character was also related to pasture infection and to grazing intensity the possibility is considered that high *Nematodirus* egg-counts may be indicative for a management which is sub-optimal with respect to growth of calves.

### 3.4.8. *Results from experiment VII*

#### Larval counts on pasture

The course of larval counts is presented in figure 17. The analysis of variance (table 27) shows that no significant differences existed between pastures.

TABLE 27. Analysis of variance of larval counts on pastures, experiment VII.

| Source of variance | d.f. | S.S. | M.S. | F | P |
|---|---|---|---|---|---|
| Pasture (P) | 1 | 0.02 | 0.02 | 0.01 | n.s. |
| Date of sampling (D) | 54 | 733.00 | 13.57 | 8.70 | < 0,005 |
| Interaction P × D | 54 | 165.82 | 3.07 | 1.97 | < 0,005 |
| Error | 110 | 171.31 | 1.56 | | |
| Total | 219 | | | | |

Evidently the grazing of animals, early in the season, with different levels of egg-output had not resulted in different pasture-infections. This may be ascribed to several factors. Firstly the level of egg-output of contaminating animals was low (see description of experimental design) and may have been too low in relation to the high number of overwintered larvae. Secondly the pasture that carried the contaminating animals with the lowest level of egg-excretion, may have offered a significantly better microclimate to the larvae because it was shaded partly by tall trees until about 10 o'clock a.m. This had not been realized at the beginning of the experiment. Thirdly, the general weather conditions may have been unfavourable for the translation process.

In this experiment a longer period elapsed before excreted eggs from the experimental animals could be demonstrated to result in higher pasture-infestation, when a comparison with earlier experiments (II, III and V) is made. The peak was very high, however, and was observed after successive days of rainy weather. Before that time, in late June and July, the season had been exceptionally dry. This phenomenon of delayed migration of larvae out of the faeces has also been observed by other workers (see review of literature).

In figure 18 the relative abundance of *Cooperia spp.*, *Ostertagia spp.* and *Nematodirus spp.* is presented. From the pasture samples taken in experiment V and VII, the larvae were differentiated if reasonably large numbers were available. It can be seen that the frequency of *Nematodirus spp.* is high during the spring months and falls in July when the new generation of larvae develops. In

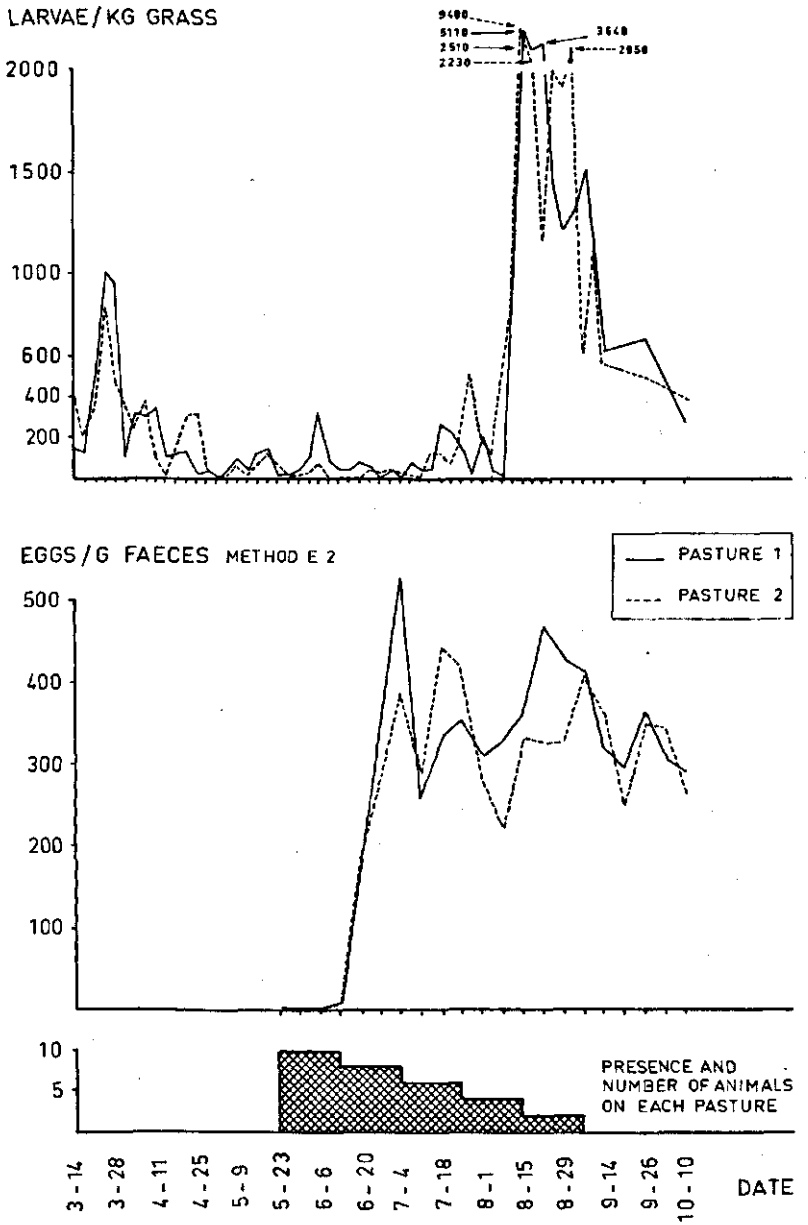



FIG. 17. The herbage infestation and egg-output in experiment VII.

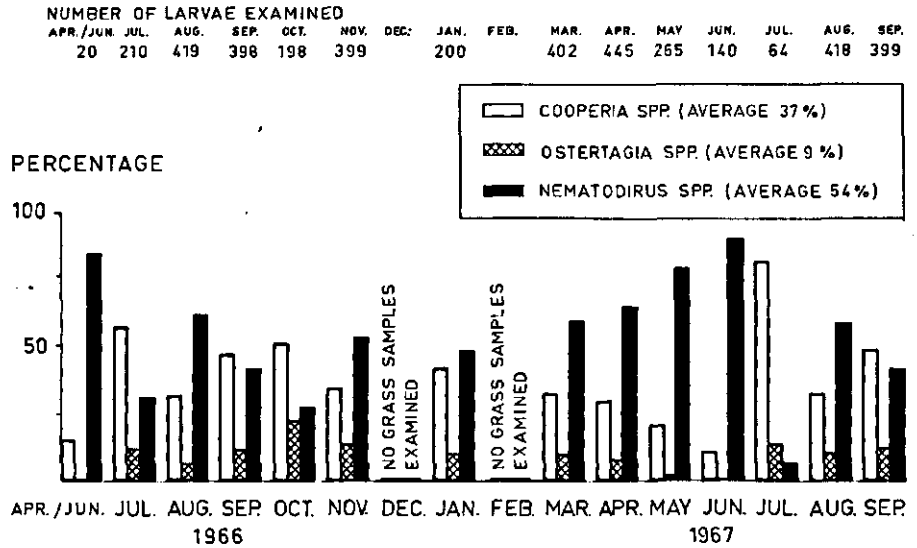



FIG. 18. The relative abundance of three genera in herbage samples, experiment V and VII.

both years August shows a relatively high frequency of *Nematodirus spp.* which is an affirmation of the finding by others that this genus develops more slowly than *Cooperia spp.* and *Ostertagia spp.* (see review of literature).

## Egg-counts in faeces

The course of egg-output of the two pasture groups is presented in figure 17. Table 28 presents the correlation between egg-output characteristics and various factors. It can be seen that the pasture did not influence the egg-output which is in good agreement with the results of larval counts on pasture. The trend of *Nematodirus* egg-counts differed significantly between pastures, however, ($P < 0.05$). No explanation of this result could be found in the larval differentiations that were carried out on pasture samples.

It was very surprising to find a positive relation between age and level of egg-counts, because the calves did not differ more than 7 days in age, and were nearly 3 months old when exposed to pasture infection. Moreover, the relation with age (or: initial weight) was found to be negative in earlier experiments (II, V and VI) where variation in age was much larger. The only explanation we see, is that this positive relation has arisen by chance.

An indication was found that egg-output level differed between sire-groups ($P < 0.10$). Calves from 'Bernhard' and 'Adema 653' had a mean transformed egg-count of $9.85 \pm 0.95$ and $10.68 \pm 0.90$ respectively. These values correspond with 236 and 320 e.p.g. The course of egg-output for the two sire-groups is presented in figure 19.

It is interesting to note that the difference in egg-output between sire-groups during the first weeks after patency (4 weekly observations) was significant

TABLE 28. Correlations between various characteristics in experiment VII. For the key of the symbols, see page 63.

| | $L_T$ | $C_T$ | $L_N$ | $C_N$ | $L_C$ | $C_C$ | $L_O$ | $C_O$ | W | G | A | P | Si | G.P. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $L_T$ | 1.00 | +0.42 | +0.80** | +0.25 | +0.72** | +0.16 | +0.28 | +0.38 | −0.06 | −0.14 | +0.60** | +0.10 | −0.50* | −0.08 |
| $C_T$ | | 1.00 | +0.18 | +0.60** | +0.47* | +0.59** | +0.05 | +0.23 | −0.04 | +0.38 | +0.16 | +0.06 | +0.03 | +0.48* |
| $L_N$ | | | 1.00 | +0.28 | +0.48* | −0.02 | +0.36 | +0.38 | +0.19 | −0.21 | +0.49* | +0.17 | −0.18 | −0.51* |
| $C_N$ | | | | 1.00 | +0.46* | −0.07 | +0.30 | +0.36 | +0.23 | +0.39 | +0.28 | −0.48* | +0.26 | +0.03 |
| $L_C$ | | | | | 1.00 | +0.05 | +0.11 | +0.07 | −0.15 | −0.02 | +0.33 | −0.24 | −0.37 | −0.14 |
| $C_C$ | | | | | | 1.00 | −0.22 | +0.08 | −0.06 | +0.32 | −0.07 | +0.41 | −0.23 | +0.31 |
| $L_O$ | | | | | | | 1.00 | +0.49* | +0.49* | −0.09 | +0.46* | +0.33 | +0.04 | −0.16 |
| $C_O$ | | | | | | | | 1.00 | +0.14 | +0.16 | +0.55* | +0.06 | −0.08 | −0.05 |
| W | | | | | | | | | 1.00 | +0.42 | +0.40 | +0.27 | +0.17 | −0.20 |
| G | | | | | | | | | | 1.00 | +0.36 | −0.18 | +0.18 | +0.28 |
| A | | | | | | | | | | | 1.00 | +0.07 | −0.09 | +0.05 |
| P | | | | | | | | | | | | 1.00 | −0.06 | +0.05 |
| Si | | | | | | | | | | | | | 1.00 | +0.05 |
| G.P. | | | | | | | | | | | | | | 1.00 |

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

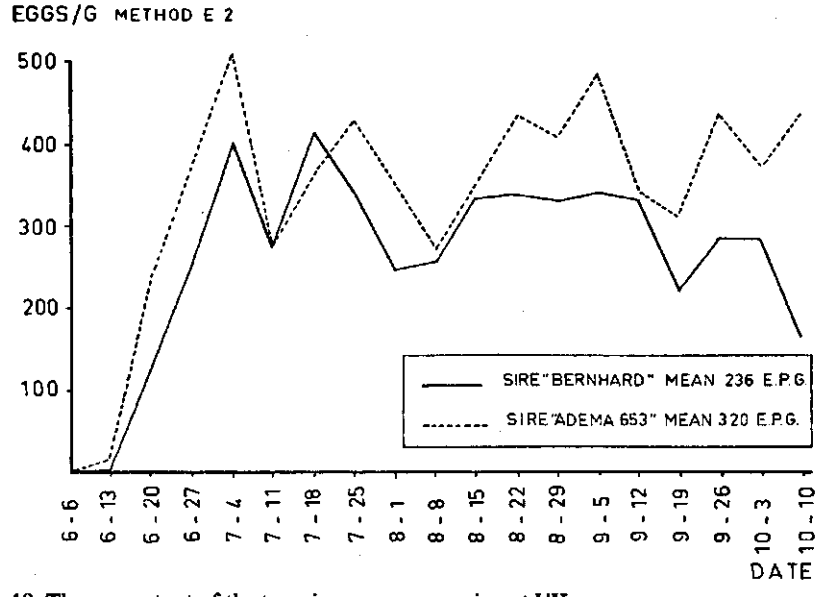



FIG. 19. The egg-output of the two sire-groups, experiment VII.

($P < 0.01$). This suggests that there was some difference, before, by immunity response, egg-counts were suppressed in both groups.

Figure 20 presents the egg-output of calves grouped according to length of grazing period. The dotted line presents the 5th power regression of egg-counts on time, estimated from all the available egg-counts. As can be seen in table 28, there was no relation between length of grazing period and mean level of total egg-counts, but the trend of egg-counts was significantly related to this factor, although this effect was mainly caused by the first group to be stalled. An explanation may be that in this group the egg-output had decreased as a result of aging of the worm-population harboured by the animals. For the separate genera it appeared that the level of *Nematodirus spp.* was negatively related to the length of grazing period. From figure 20 it can also be seen that approximately 3 weeks after stalling (which is marked by arrows), there is some peak in egg-output. This effect was not significant, but the FRIEDMAN-test on total egg-counts of 4 observations after stalling, yielded a probability of $P < 0.10$. This might suggest that the interruption of the larval intake serves as a release on egg-output, which is suppressed by an immunological response of the host. This hypothesis would fit well in the experiences of other authors (MICHEL, 1966; DONALD et al., 1965).

It could also be an explanation of the fact that in experiment VI no influence of rotations to clean pasture was found.

## The liveweight gain of animals

As can be seen in table 28, none of the factors pasture, initial weight, age, sire and length of grazing was significantly related to the weight gain of animals.

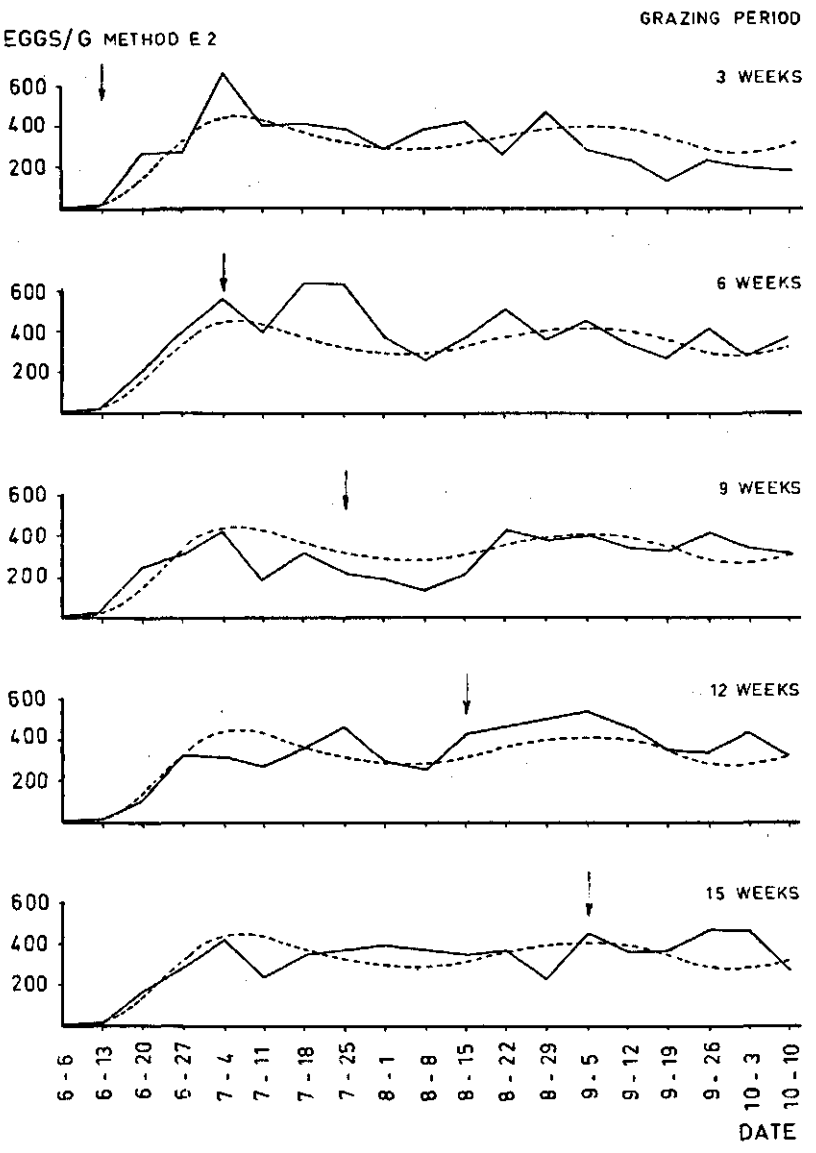



FIG. 20. The egg-output of the animals in experiment VII, grouped according to length of grazing period.

In spite of the rather high level of residual herbage infestation and the substantial egg-output of the animals, the growth-performance was satisfactory: 890 g/day with a standard deviation from the mean of 100 g/day. This growth-performance did not differ significantly from that of 3 calves (1 from 'Adema', 2 from 'Bernhard') that have been kept indoors all the time. The results of the P.M. examinations on 4 animals (see nest paragraph) suggest also that parasitism has hardly played a role in this experiment except for one calf (see description of experiment) that after 6 weeks grazing showed severe signs of gastro-enteritis. The reason of the satisfactory growth might be sought in the fact that all animals have been stalled sooner or later and received a good ration then. There was no evidence, however, of a difference in growth between calves grazed for different periods.

The reason that no parasitism is observed might be that pasture infection reached a high level rather late in the season, and at that time the majority of the calves had been removed from the pasture, so that grazing intensity and, by this, the degree to which the 4 remaining animals were forced to eat heavily contaminated herbage, was greatly reduced. Another possibility is that the genetically determined resistance of the calves to the parasites was exceptionally good.

### Worm counts Post Mortem

By the P. M. examination no immature worms were found, and only low numbers of adults. These belonged to *O. ostertagi, C. oncophora, N. helvetianus, T. axei* and *C. surnabada* in this order of abundance. In the first stalled group worm-numbers were lower (322 and 455) than in the group stalled on 5th September (760 and 1530). This difference is quite conceivable but no conclusions can be drawn from this very limited number of animals.

### Conclusions

Five groups of calves exposed for different periods to a natural infection showed no significant difference in mean level of egg-output. This finding is supporting the conclusion of experiment VI that the level of total egg-counts is not significantly influenced by the number of rotations to 'clean' pasture.

An indication was found that between the two sire-groups a different reaction to trichostrongylids occurred.

### 3.4.9. *Results from experiment VIII*

### The egg-output of animals

Results have not been analyzed in the usual way. As this experiment was in fact a test on a farm-scale of the prevention method of 'grazing on aftermath' and egg-counts appeared to be low in the early months of the season, it made little sense to distinguish between level and trend of egg-output. The egg-output for the various genera is presented in figure 21. The reader should recognize that the method used for egg-counting ($E_2$) has a higher recovery than that used in experiment VI. It can be seen that egg-counts continued to rise throughout the season.

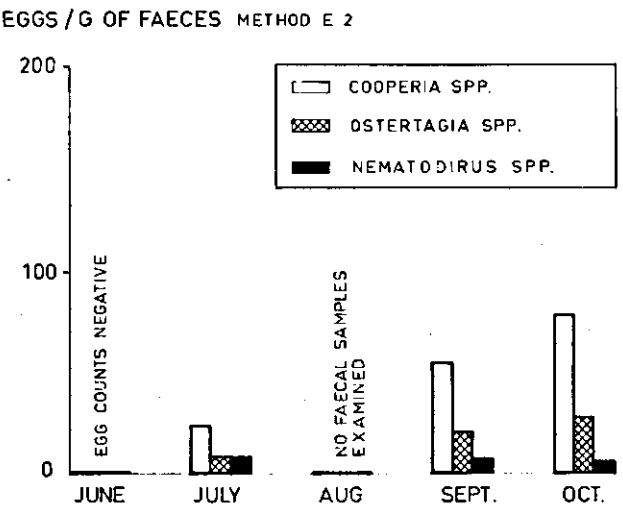



FIG. 21. The egg-output of three genera, experiment VIII.

## The growth performance of animals

From 55 of the herds the mean daily live-weight gain was available: 787 g/day with a standard deviation from the mean of 97 g/day. If it is realized that on the majority of these farms troubles with gastro-intestinal worms had been experienced in the preceeding year(s), this growth performance is very convincing with respect to the beneficial effect of grazing on aftermath.

The growth per herd has been correlated to the mean level of egg-counts, 52 farms being available for this analysis. For total egg-counts, *Ostertagia spp.* and *Nematodirus spp.*, the coefficients of correlation with growth were −0.12, −0.12 and−0.32 respectively. As in earlier experiments, egg-output of *Nematodirus spp.* showed the closest relationship, the correlation being significant ($P < 0.05$).

# 4. DISCUSSION

## 4.1. Evaluation of techniques for faecal egg counts and larval counts on herbage

In this study some attention has been paid to recovery, sensitivity and causes of technical variation of various methods. Emphasis has been put, however, on variability of counts under routine conditions in a fairly large number of samples of varying origin.

In spite of the rather low recovery percentages of several methods, it could be shown that all methods are capable of demonstrating between-sample differences. By the application of nested sampling schemes, information was also obtained on the relative efficiency of separate steps in the entire sampling and counting procedures. It is clear from the results that efficiency can only be increased by intensification of subsampling or by raising the recovery -rate with a disproportionately large input of extra labour.

The counting of larvae in cultured faecal samples has some advantages if extremely low egg-counts are expected, e.g. shortly after infections have become patent, in cases where anthelmintic treatment has taken place, or as a check on the 'wormfreeness' of animals. Although there were indications that larval counts in faeces had a smaller technical variation than direct egg-counts with the methods used here, this advantage would be of doubtful value because between-sample variation is so high. The extra labour involved in the culturing process is a clear disadvantage. Futhermore the 9 days interval one has to wait before results become available, is a serious drawback for those workers who believe in the diagnostic value of worm-egg-counts in faeces. Finally, the genera *Nematodirus* and *Trichuris* are not present in faeces cultured for 9 days.

If the purpose of any study is to compare egg-output in a large number of herds, collection of socalled herd samples from a number of fresh defaecations in pasture has great advantages over the collection of individual rectal samples, because time is saved and the organisation required for sampling a large number of herds is much easier. Samples can be taken at any time of the day and, if necessary even two samples can be taken simultaneously. Some drawback may be seen in the fact that a herd sample reflects the arithmetic mean of a number of defaecations, whereas such a mean, in view of the skew distribution of egg-counts, is not the most realistic one. If, however, this distribution possesses a skewness which is approximately the same from herd to herd, as Hunter and Quenouille (1952) suggest, this disadvantage is probably not too serious.

### 4.2. Larval counts on pasture

Much of the information obtained on this point confirms the results of Michel (1969d) in England. The seasonal pattern on calf paddocks is the same: minimal concentration during April, May and June, when grass-growth is abundant, overwintered larvae continue to die out and no new, free migrating larvae (except perhaps *Nematodirus spp.*) develop. During July-August, partly depending on rainfall, a marked increase takes place, resulting from the newly deposited eggs. That we do deal here with a new generation of larvae can also be seen from the change in relative frequency of various genera (figure 18).

During the winter a more or less gradual decrease of larval concentration is observed. This decrease seemes to be less pronounced for *Nematodirus spp.* while results suggest that larvae of *Ostertagia spp.* are least capable of overwintering. These results are in good agreement with those of Rose and Kutzer (see review of literature).

From the results of experiments III and IV, it can be seen that the above mentioned seasonal pattern can only be observed where more or less continuous grazing of egg excreting animals takes place, without intermediate mowing. This, of course, has very important consequences for the development of prevention -methods by grassland management.

What we regard as more or less new information is the large variation that exists in overwintered infection between fields (experiment VI). The differences between fields were significant. An effect of soil-type and water-level could not be demonstrated and also wheather conditions cannot have played a role in this geographically restricted area. Although no data were systematically collected on this point we tend to conclude that these differences have been brought about by different grazing history, particularly during the preceeding year, more particularly during the late summer and autumn. The mean number over all farms was 320 larvae/kg (wet) grass, which is high if compared to the results found in England by that time of the year.

### 4.3. The level and course of egg-counts from the epidemiological point of view

If the results of the various experiments are compared a few points become clear.

Firstly, in all experiments egg-counts show an increase after the infection has become patent; this rise continues for a shorter or longer period, but lasts in these experiments at least until approximately 6–7 weeks after first exposure to infection. There-after the more heavily infected herds remain on a certain level (expt. II, III and VII) or even fall suddenly to a lower level (expt. V). Animals brought on pasture with low larval infestation continue to rise for a longer time or even for the entire season (expt. III, IV, V and VII). This was also observed by Michel, et al., 1970, in field observations in England. In experi-

ment VI the same tendency could be seen, although the relation between trend of egg-counts and overwintered pasture infection, which could be examined, of course, only in non-treated herds, was not significant (table 26). If, however, these 30 farms were divided into 2 groups on the basis of level of pasture infestation, the mean egg-counts were:

| | June | July | Aug. | Sept. | Nov./Dec. |
|---|---|---|---|---|---|
| on farms with low pasture infection | 26 | 48 | 57 | 89 | 26 |
| on farms with high pasture infection | 46 | 87 | 77 | 57 | 36 |

It should be realized that these farms were already a selection out of the total population of 43 farms. No clear distinction existed between the two above mentioned groups; there is a gradual transition over all farms, from one extreme curve to the other extreme. This is the main reason for the lack of significance and we, therefore, cannot speak of 'typical patterns'.

The second point of importance is that the different genera show different courses of egg-output. This is best seen in figures 14 and 16 which can be assumed to give the most accurate reflection of field conditions. It can be seen that the picture of total egg counts is, for the most part, dependent on *Cooperia spp.* egg-output. Futhermore suppression of egg-counts occurs relatively early for *Nematodirus spp.* and later for *Ostertagia spp.*, while *Cooperia spp.* occupies some place between these two. Clearly there is no constancy in relative abundance from season to season. This result conforms to those of BÜRGER et al., (1966) in N.W. Germany and those of ROBERTS (1957) and PETERSON (1957) in Australia; MICHEL et al. (1970) on the other hand, reported a fifty-fifty ratio between *Cooperia spp.* and *Ostertagia spp.* from field observations in England, without a consistent tendency for one of these genera to predominate at particular times of the year.

A third point worth mentioning is that in all experiments, the egg-output during the first weeks after patency was related to the pasture infection to which the calves were exposed.

Egg-output, from the epidemiological point of view, measures the contamination of pasture. MICHEL et al. (1970) suggested that the minimum-time required was longer for eggs when deposited in early spring, and became shorter as the season proceeded, being minimal at about July and August. whereafter it grew longer again. MICHEL (1969d) furtheron concluded that eggs deposited during autumn were relatively unsuccesful in completing their development. In an experiment to be published elsewhere (PACENOVSKY et al., in prep.) it appeared that eggs deposited after mid-August, proved to be the main source of larvae found in March, April and May of the following year. The most important reason of the difference between our findings and those of MICHEL is probably the relatively greater abundance of *Nematodirus spp.* and *Cooperia spp.* compared to *Ostertagia spp.* It is considered that calves, put on pasture in the spring may be exposed to different levels of infection, which has also strongly been suggested by the re-

sults of experiment VI. SMITH and ARCHIBALD (1969) have shown that, with the same type of infection as in our country (*Cooperia spp.*, *Nematodirus spp.* and *Ostertagia spp.*), overwintered infections can be dangerous for calves even if animals are put on pasture in late June.

This information, together with the existing correlation between initial infection level and egg-output during the first weeks after patency, suggests that under suitable weather conditions, under set-stocking, the rate of the increase in larval concentration which occurs in July, might well partly depend on initial infection taken up by the calves. In experiment II, III and V there was a clear relationship between eggs excreted and subsequent larval infestation of herbage.

Therefore, faecal egg-counts will remain indispensable in epidemiological studies.

### 4.4. THE LEVEL AND COURSE OF EGG-COUNTS FROM THE DIAGNOSTICAL POINT OF VIEW

As was pointed out in the review of literature, little agreement exists between authors on the value of faecal egg-counts made for diagnostic purposes. Our best measure for disease is the growth performance of animals. In the field growth of animals is influenced by many factors, such as age, nutrition, genetic capacity for growth, sex, breed, and presence of other pathogens. Therefore, in field material correlations between growth and egg-output can be expected to be rather low, whereas in controlled experiments under certain conditions, some degree of correlation may be seen. Table 29 summarizes the relation between growth and some egg-output characteristics. Among these the level and course of *Cooperia spp.* is not given, as the results for this genus are almost identical to those for total egg-counts. Of all the characteristics, *Nematodirus spp.* egg-output (level and course) seems to be best related to growth, particularly in the field-experiments (VI and VIII) where many herds were involved. As stated

TABLE 29. Growth related to several egg-count characteristics, compiled from the tables 18, 22, 25, 26 and 28.

| Experiment | II | V | VI (All farms) | VI (Non-treated farms) | VII | VIII |
|---|---|---|---|---|---|---|
| LT | −0.61 ($P<0.01$) | −0.44 | −0.10 | +0.15 | −0.14 | −0.12 |
| CT | +0.12 | −0.19 | +0.16 | −0.05 | +0.38 | - |
| LN | −0.33 | −0.36 | −0.28 ($P<0.10$) | −0.30 ($P=0.10$) | −0.21 | −0.32 ($P<0.05$) |
| CN | +0.15 | +0.54 ($P<0.10$) | +0.38 ($P<0.02$) | +0.33 ($P<0.10$) | +0.39 ($P<0.10$) | - |
| LO | - | −0.46 | −0.10 | +0.15 | −0.09 | −0.12 |
| CO | - | −0.49 | +0.07 | +0.05 | +0.16 | - |

earlier we do not consider this as a causal relationship, but prefer to suppose that both phenomena (the high egg. output of *Nematodirus spp.*and the depressed growth performance) are caused by some common factors. These might be use of calf paddocks accompanied by a high grazing intensity, whereby an accumulation of pasture infection may occur through several years. Because of the good capability of overwintering of *Nematodirus spp.* this genus might serve as an indicator of such conditions, while the growth depression can be caused by other worms, e.g. *O. ostertagi.*

General agreement exists between various authors, that differences in rate of infection (i.e. the numbers of larvae taken in) are related to the growth of animals. The results of experiments II, III and V confirm this opinion. Because the egg-counts during the first weeks of patency give a certain reflection of the larval intake such counts might be usefull, particularly under conditions of set-stocking. Two restrictions should be made, however.

Firstly as experiment VII indicated, these counts may depend partly on hereditary factors. Secondly, the time at which depression of egg-counts occurs may depend partly on the level of larval intake. This depression may occur early, even after 1 week of patency. Further research is necessary to answer the question whether these factors are of importance under field-conditions.

In this way egg-counts might be useful for accompaniment of large scale prevention-experiments. As an aid for diagnosis, however, the egg-counts have little value or may even be misleading. This is illustrated by experiment V. At the time that calves show signs of unthriftiness egg-counts can be suppressed even to a level which is lower than that of comparable calves that have experienced a smaller infection.

The information from larval differentiation in faeces, which means in fact a differentiation between *Cooperia spp.* and *Ostertagia spp.* cannot add considerably to the explanation of the relation between egg-counts and growth, as can be seen in the tables 18, 22, 25, 26 and 28. From table 30 it can be seen that the results of larval differentiation can to a certain extent be predicted if the egg-count of a sample is known. If egg-counts are high ($\geqslant$ 200 e.p.g.) one can be

TABLE 30. The relation between level of egg count and type of infection.

| Egg count level | Total number of samples | Number of samples of various infection types | | | |
|---|---|---|---|---|---|
| | | <60% Cooperia | 61–80% | 81–90% | 91–100% |
| 1 (0 or 1 eggs) | 83 | 41 | 17 | 11 | 14 |
| 2 (2–4 eggs) | 112 | 42 | 29 | 17 | 24 |
| 3 (5–9 eggs) | 142 | 32 | 45 | 33 | 32 |
| 4 (10–19 eggs) | 147 | 27 | 51 | 41 | 28 |
| 5 (20–39 eggs) | 143 | 5 | 38 | 53 | 47 |
| 6 (40 or more eggs) | 86 | 2 | 9 | 34 | 41 |
| | 713 | 149 | 189 | 189 | 186 |

almost sure that the majority of larvae (> 60%) will belong to *Cooperia spp.* The reverse is not always true: an egg-count may be low and nevertheless more than 90% *Cooperia spp.* larvae may be found; this is particularly so in the early part of the season.

Larval differentiation alone or combined with egg-count level might be a better measure from the diagnostic point of view than the egg-count characteristics used so far in this study, because *Ostertagia spp.* counts are completely overshadowed by the counts of *Cooperia spp.* Several attempts were made to find a 'score', based on egg-counts and larval differentiation, which could improve the explanation of growth retarding effects but we did not succeed.

We agree with many authors that faecal examinations are of doubtful value for diagnostic purposes.

### 4.5. Farm management in relation to the epidemiology

Prevention of parasitic gastro-enteritis by farm-management has been the objective of many studies. Such prevention may be achieved either by measures that increase the resistance of calves against the effects of parasitism, or by measures that keep the intake of larvae as low as possible. The discussion here will be confirmed to the latter.

Rotational grazing has in this country, as in many others, proven to yield unsatisfactory results. The control-group (group 1) in experiment III provides an example, and results of this experiment were consistent with many others carried out on the same farm (Oostendorp et al., 1965; Oostendorp and Harmsen, 1968).

All the above mentioned experiments were convincing with respect to the value of rotational grazing on aftermath as a prevention method. Testing of this method on a practical scale over 2 years confirmed the experimental results. (See experiment VIII, and Oostendorp and Harmsen, 1968). A few remarks can be made.

Firstly, the growth improvement produced by grazing on aftermath has to be seen as the combined result of a smaller infection and a better availability of grass of good quality and palatability. Further studies to separate these effects will be of interest.

Secondly the lay-out of experiments on 'De Vlierd' has been such that the various treatments have been run on the same areas in successive years , in order to get an impression of the long-term effects of the methods. However it is quite possible that the growth-retarding effects accompanied by relatively high egg-counts in the control groups have partly been the result of high levels of over-wintered infections. In several of the experiments on 'De Vlierd', as well as in experiment VI, it was not exceptional to find growth depression as early as June. It would be interesting to study on 'De Vlierd' the effect of aftermath grazing on a field previously used for control groups and the reverse. Finally, it remains to be studied whether the system of grazing on aftermath can be sim-

plified, because it is rather complicated and this surely forms a barrier for many farmers.

This leads us automatically to the system developed by MICHEL (1966) which is based on the seasonal course of larval counts on pasture. This system advocates the grazing of calves on a clean pasture until the midseason increase of larval infection can be expected. At this time, the calves should be brought to a new clean pasture after an effective anthelmintic treatment. The system, which is indeed as simple as it should be, also fits in extremely well with general farm practice in the Netherlands. One remark should be made, however. The results of this study indicate that in this country overwintered infection plays a significant role in the epidemiology of the disease, so that it is of vital importance that the first pasture should be clean indeed. Therefore, we cannot agree that the statement of MICHEL et al. (1970) 'what could be considered a dangerous pasture infection has not been seen after April' would apply to the situation in the Netherlands. What should be considered a dangerous pasture infection depends on several factors. Not only the larval concentration, but also the infectivity of the larvae, the resistance of animals and, last but not least, the degree to which animals are forced to take up the grass around faecal pats, will determine whether a pasture is dangerous or not. It cannot be said that a larval concentration is without danger simply because, later in the season, concentrations are 100 or even 1000 fold higher. Firstly, it is not the absolute intake of larvae but rather the logarithm of the doses which seems to be related to the number of worms acquired (ANDERSON et al., 1966). Secondly calves can be considered as worm-free when they are turned out in spring, and for that reason they are likely to be more susceptible than later in the season, when they are older and have had some experience with the parasites.

At present we see the ideal prevention method as a combination of the system of OOSTENDORP and that of MICHEL. As the farmer likes very much to have the calves near the farm-house in order to feed milk and milk products when they are young, and to keep a close eye on the animals, it should be possible to reserve such a paddock, which in the preceeding year has preferably not carried calves, and has been mown two successive times during summer and autumn of the previous season. The larval infection on this paddock undoubtedly will be low in the spring. This field can be grazed safely until July. After that the calves should be rotated monthly on aftermath which is then available. The first paddock can be mown twice and is safe for use again next year. Under this system we expect that anthelmintic treatment will not be necessary or economic. Many of these matters need further investigation, however.

# SUMMARY

In this study an attempt has been made to make a contribution to the epidemiology of gastro-intestinal parasitism in calves under Dutch conditions. Methods of prevention by grassland management have recently been developed in England and in the Netherlands. The work in our country had an empirical approach and the need for some epidemiological basis was felt. For this purpose investigations were carried out by means of a few grazing experiments with calves, as well as by survey work on practical farms. Observations included larval infestations of pasture, egg-output in faeces and growth of animals.

Concerning the methods used for larval counts in pasture samples and egg-counts in faeces, emphasis was put on the reproducibility of counts under routine conditions. It was concluded that:

1. Due to very large between-sample variation, intensification of sampling is of much more importance than refining laboratory techniques. It may be stated that those techniques are the best that are least time-consuming.
2. Also from the view point of labour-input, collection of 'herd samples' taken from fresh faecal pats on the pasture, should be preferred to rectal sampling of individual animals, in case that investigation of a large number of herds is necessary.

With respect to the larval infestation of grassland the following conclusions could be drawn:

1. The seasonal pattern which can be observed on calf paddocks was very similar to that found by MICHEL in England in studies extending over several years.
2. This course was only found on fields where during the entire season egg-excreting animals were more or less continuously grazed, without intermediate cutting.
3. Of the genera *Nematodirus*, *Cooperia* and *Ostertagia* the first mentioned succeeded best of all in overwintering, whereas the last mentioned genus was least succesful.
4. In the field, significant differences in larval infection exist between fields that are destined to be grazed by calves when turned out in spring. This variation is reflected by the level of egg-counts of calves later in the season.

From egg-counts in faecal samples it was concluded that:

1. They have no value as an aid for diagnosis.
2. This is due primarily to the phenomenon of suppression of egg-output. During the first weeks after patency egg-counts reflect larval intake fairly accurately, but later this relation is disturbed so that egg-output is either maintained at a constant level, or even falls below the level of egg-counts seen in calves that have experienced a smaller infection.
3. The phenomenon of egg-count suppression is seen in different degrees among the various genera and there are also indications that it occurs for genera in a certain order:

first and strongest in *Nematodirus spp.*, later and less clearly in *Ostertagia spp.* while *Cooperia spp.* occupies a position between these two.

4. Egg-counts may be of value for monitoring large scale experiments by which new methods of prevention are tested, and where small infections of pasture and animals are present.

From the observations on growth performance of animals the following conclusions were drawn:

1. Gastro-intestinal parasites can, under experimental conditions, be demonstrated to cause growth-inhibition.
2. The growth performance of calves in the field during their first grazing season shows considerable variations.
3. No relation can be demonstrated between egg-output of calf-herds and growth, not even if other growth affecting factors are taken into account.

The above mentioned conclusions are discussed in chapter 4, particularly with reference to work recently published in the Netherlands and in England. Good methods of prevention, by grazing management have been developed. For finding the most economic system for various situations in the field, further investigations will be necessary.

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# SAMENVATTING


Voor de beperking van schade door maagdarmwormen bij kalveren staan de boer verschillende middelen ten dienste. De laatste 10 jaar zijn publikaties verschenen, waaruit blijkt dat bepaalde vormen van grasland-gebruik tot die middelen behoren.

In deze studie is getracht, om, voor Nederlandse omstandigheden, een bijdrage te leveren aan de epidemiologische basis die aan dergelijke preventie methoden ten grondslag moet liggen. Daartoe werden in enkele beweidingsexperimenten enerzijds en op praktijkbedrijven anderzijds waarnemingen gedaan aan de infektie van het grasland met infektieuze larven, de eiuitscheiding van de dieren en de groei van de dieren.

Wat betreft de methodiek van larventellingen in grasmonsters en eitellingen in faeces monsters werd in het bijzonder aandacht besteed aan de reproduceerbaarheid onder de omstandigheden van routine onderzoek. Daarbij werden de volgende konklusies getrokken:

1. Gezien de grote variatie tussen monsters, is het duidelijk dat intensivering van de bemonstering van veel groter belang is voor betrouwbare resultaten dan verbetering van techniek. Gesteld mag worden dat die techniek de beste is die de minste tijd kost.
2. Bij faeces onderzoek op een groot aantal praktijkbedrijven verdient, om reden van tijdsbesparing, het nemen van een koppelmonster uit verse mestflatten op de weide de voorkeur boven het rectaal bemonsteren van dieren.

Omtrent de larven-infektie van grasland konden de volgende konklusies worden getrokken:

1. Eenzelfde seizoenspatroon kan worden waargenomen als dat wat door MICHEL in Engeland gedurende een reeks van jaren is vastgesteld.
2. Dit patroon wordt alleen gevonden op weiden waar gedurende het gehele seizoen eiuitscheidende dieren min of meer kontinu geweid worden, doch niet op weiden die éénmaal of meer malen per seizoen gemaaid worden.
3. Van de geslachten *Cooperia*, *Ostertagia* en *Nematodirus* overwintert het laatstgenoemde het best, en *Ostertagia* het minst goed.
4. Er is in de praktijk een aanzienlijke variatie in besmetting tussen percelen welke als eerste weide voor kalveren worden gebruikt. Deze variatie wordt weerspiegeld door het later waargenomen niveau van eiuitscheiding der dieren.

Ten aanzien van de eitellingen in faeces monsters kon worden vastgesteld dat

1. zij geen waarde hebben als hulpmiddel bij de diagnose;
2. dit in het bijzonder veroorzaakt wordt door het verschijnsel dat eitellingen, die direkt na het patent worden van een infektie een goed beeld kunnen geven van de opgenomen hoeveelheid larven, op een zeker moment, o.a. afhankelijk van de hoeveelheid opgenomen larven, worden verlaagd of althans op een bepaald niveau worden gehandhaafd.
3. het waarschijnlijk is dat bovengenoemd verschijnsel bij de verschillende gene-

ra van wormen in verschillende mate en in een bepaalde volgorde optreedt, het eerste en het sterkst voor *Nematodirus* en het laatst en minst duidelijk voor *Ostertagia*, terwijl *Cooperia* wat dit betreft een tussenpositie inneemt.

4. het niet uitgesloten is dat eitellingen van waarde kunnen zijn bij de begeleiding van experimenten waar bepaalde preventie methoden worden getest en lichte besmettingen bij de dieren aanwezig zijn.

Uit de waarnemingen omtrent de groei van kalveren werd gekonkludeerd dat:

1. maagdarmwormziekte in gekontroleerde experimenten als oorzaak van groeivertraging kan worden aangewezen.
2. de groei van kalveren gedurende het eerste weide-seizoen op praktijkbedrijven een aanzienlijke variatie vertoont
3. op praktijkbedrijven geen verband kan worden aangetoond tussen groei en eiuitscheiding van de dieren, ook niet als zo goed mogelijk rekening wordt gehouden met een groot aantal andere faktoren, die de groei beïnvloeden.

Bovengenoemde konklusies worden in het hoofdstuk 'diskussie' besproken, in het bijzonder in het licht van recentelijk in ons land en in Engeland gepubliceerd onderzoek. Daarbij wordt vastgesteld, dat nader onderzoek op verschillende punten gewenst is.

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