STUDIES ON THE EPIDEMIOLOGY OF *DICTYOCAULUS VIVIPARUS* (BLOCH, 1782) INFECTION IN CATTLE

by

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Bovine Dictyocaulosis

Gupta

Dedicated to

my friend and colleague

the late

Dr. D. L. Archambault

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CLAIM OF ORIGINALITY

This represents the first extensive epidemiological study on *D. viviparus* to be undertaken in Canada in which factors responsible for the infection pattern and its persistence were examined.

A unified hypothesis, involving acquired resistance, seasonal fluctuations in pasture infestation and husbandry practices, was proposed to explain differences in age-incidence and seasonality of the infection.

The successful demonstration of the ability of *D. viviparus* larvae to overwinter on pasture, and the positive role of carrier animals in the persistence of the infection was described for the first time in Canada.

It was shown that *D. viviparus* larvae obtained from moose were not infective to calves.

The phenomenon of seasonal inhibition in *D. viviparus*, independent of the host's immune status, as demonstrated in this study has never before been described. A hypothesis was proposed suggesting that a change in the physiology of the host or larvae during late autumn, mediated through photoperiod and/or temperature, induces seasonal inhibition in *D. viviparus*. The maturation of inhibited larvae during spring was proposed as being either a built-in mechanism in the larvae or triggered by the host.

The morphology of inhibited *D. viviparus* larvae recovered from cattle has been described for the first time.

ABSTRACT

Ph.D. R. P. Gupta Animal Pathology

STUDIES ON THE EPIDEMIOLOGY OF DICTYOCAULUS VIVIPARUS (BLOCH, 1782) INFECTION IN CATTLE

Studies were conducted on the epidemiology of D. viviparus to examine the infection pattern and the reasons for the persistence of this parasite.

Infection appeared to be more frequent in young animals than adults, and showed a marked seasonality, with the highest incidence during the fall.

Persistence of this parasite appeared to be due partly to the ability of the larvae to overwinter on pasture, but more importantly to fresh contamination of pasture by carrier animals in the spring.

A phenomenon of seasonal inhibition in the development of D. viviparus during the late autumn was observed in all the calves studied. The reasons for this inhibited development during the late autumn and subsequent maturation during the spring were attributed to seasonal changes either in the physiology of the host, or larvae, induced by environmental conditions. The morphology of these inhibited stages was described with appropriate illustrations.

RESUME

Ph.D. R. P. Gupta Animal Pathology

ETUDES SUR L'ÉPIDÉMIOLOGIE DU DICTYOCAULUS VIVIPARUS CHEZ LE BÉTAIL

Des études furent menées sur l'épidémiologie du D. viviparus pour en examiner le mode d'infection et les raisons de la persistence de ce parasite.

L'infection semble être plus fréquente parmi les jeunes animaux que parmi les adultes, et est influencée d'une facon marquée par les saisons, avec une incidence plus élevée en autuomne.

La persistence de ce parasite s'explique partiellement par la capacité de la larve a survivre durant l'hiver dans les pâturages, mais surtout par la contamination des pâturages au printemps par des animaux porteurs.

Un phénomène d'inhibition saisonnière dans le développement du D. viviparus vers la fin de l'automne fut observé chez tous les veaux étudiés. Les raisons pour cette inhibition du développement vers la fin de l'automne at la maturation subséquente au printemps sont attribuées à des changements saisonniers dans la physiologie de l'hôte ou de la larve, provoqués par les conditions de l'environnement. La morphologie de ces stages inhibés est décrite accompagnée d'illustrations appropriées.

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INTRODUCTION

Epidemiological studies are made in an effort to elucidate the principles underlying the transmission patterns of a disease, with the object of formulating a basis for accurate prediction of outbreaks and effective control measures.

Until the middle of this century, a large number of parasitologists were engaged in taxonomic work but little was done in other fields of parasitology. However, from recent literature, it is evident that the emphasis is changing and valuable contributions have been made in the areas of epidemiology, immunology and host-parasite relationships in general. Epidemiological studies are often time-consuming, involving laborious efforts for a number of years before reaching a satisfactory conclusion, if any, and therefore are often neglected by researchers. Nevertheless, it is gratifying to learn that during the last two decades epidemiological work has gained its rightful place and is no longer looked upon as of mere academic concern. Results obtained by epidemiologists are now considered valuable assets by those who formulate control measures for parasitic diseases.

The role of epidemiology, as pointed out earlier, is of great value in eradicating or reducing the incidence of parasitic diseases in general. This seems to be even more important in the case of Dictyocaulus viviparus where even the most potent anthelmintics available have limited efficacy, and where animal husbandry practices

and pasture management, based on the sound knowledge of epidemiology, seem to be the only alternatives to curb the occurrence of this disease. The epidemiology of D. viviparus has always been a fascinating subject, particularly for parasitologists in the United Kingdom and Germany, where the disease is endemic. Consequently, extensive information on this aspect is available for these countries. Epidemiological studies are very complex in nature and the results are largely dictated by the local climate and related factors, such as husbandry practices, pasture management, soil type, drainage and herbage, none of which is constant. It is, therefore, probable that the results obtained in one part of the country may differ considerably from those obtained in another part of the same country; thus, epidemiological studies, to be of any value, should be carried out in an area where they are to be applied. Bovine parasitic bronchitis has been reported from almost every part of Canada. A recent study by Gupta and Gibbs (1969) showed that the lungworm parasite of cattle, D. viviparus, was widespread in Quebec. However, very little is known about the epidemiology of this parasite. It is with this aspect in mind that the present studies were undertaken to elucidate the factors responsible for the disease cycle as observed in this part of Canada.

In the present study, attempts were made to follow the disease patterns, under natural conditions, on lungworm infected farms for at least one annual cycle. In addition to this, a number of experiments were conducted under controlled conditions to substantiate and explain

the results obtained from field observations. The following aspects were studied:

a) Age incidence of D. viviparus infection on the farms.

b) Overwinter survival of infective larvae on pasture.

c) Role of carrier animals in reseeding pasture during the following grazing season.

d) Seasonal worm population fluctuations in calves.

e) The phenomenon of seasonal inhibition of D. viviparus in calves.

It is the results of these studies which form the substance of this thesis.

LITERATURE REVIEW

Historical Background

Allan and Johnson (1960) and Parker (1966) traced the history of bovine parasitic bronchitis or "husk" to Greek and Roman writings. According to these authors, Nicholls (1755) and Camper (1803) were the first to implicate lungworm as a cause of high mortality in cattle. It is remarkable that, although nothing was known about the life cycle and bionomics of lungworm, these authors related factors like age of the host, pasture and changing climate to the severity of husk. Cobbold (1875) and Raillet (1889) were among the first parasitologists who tried to work out the life cycle of D. viviparus, but they met with little success. It was not until Daubney's (1920) work that the entire life cycle of D. viviparus was described. From the available literature, it appears that during the first quarter of the Nineteenth Century, many people reported on outbreaks of lungworm disease and its chemotherapy. However, the most valuable work pertaining to the epidemiology of this disease was mainly carried out in Great Britain in the nineteen fifties.

Age Incidence

Most of the published information on the incidence of lungworm disease, excluding a few slaughter house studies, is the result of single visits, frequently based only on clinical and fecal examination, and rarely supported by postmortem findings. In addition to this, most of the reports do not include important information such as

percentage of animals examined, age of animals, time of the year when diagnosed, local animal husbandry practices and pasture management. Because of these limitations, the figures showing the incidence of lungworm disease can be misleading. Nonetheless, from the literature, one thing which emerges is that bovine parasitic bronchitis has no geographical boundaries. In fact, Taylor (1942) is of the opinion that bovine parasitic bronchitis is very likely to occur wherever cattle are kept, although it is not as ubiquitous as the worms responsible for parasitic gastroenteritis.

a. Incidence in Adult Animals

Lungworm disease has been encountered by practicing veterinarians for the last 200 years (Daubney, 1920) but it was not until the beginning of this century that reports of serious lungworm disease and its consequences were recorded. Western (1903) gave an account of an outbreak of acute lungworm disease in cows on a farm in England. The history of the outbreak suggests that yearlings and calves contaminated the pasture on which the cows were later grazed and consequently contracted the disease. Smythe (1937) and Taylor (1935;1942) recorded outbreaks of lungworm disease from different parts of the United Kingdom. Hudson (1951), in England, recorded 26 outbreaks of coughing in cows and in heifers between 1947 and 1951. Husk was confirmed in 15 of these outbreaks. He described the disease in adult cattle as often less acute but more persistent than in young stock.

Taylor (1952) observed numerous outbreaks of husk among milking cows in which every animal was more or less seriously affected. In the U.S.S.R., Penkov (1945) recorded nearly one-hundred percent infection in farms in his district and attributed fifty percent of the total mortality to this disease. Witter and Rountree (1953) and Rountree et al. (1954) reported outbreaks of acute bovine parasitic bronchitis from Maine, U.S.A. Jarrett et al. (1954), in a survey in Scotland, recorded 109 cases of parasitic bronchitis, of which, 46 cases were in adults with the peak incidence in September. However, during the spring months they examined 211 pairs of lungs from stirks (young bovines which have completed their first season at grass) and adults, and only five percent of these lungs contained adult worms. In Canada, Lafortune (1954) working in Quebec, Campbell and Wetherill (1957) in Ontario, and O'Donoghue (1958) in Alberta, all reported D. viviparus in lactating cows. Severe parasitic bronchitis among adult cattle has been reported from India by Malaki (1961), Awadhiya and Mehta (1962) and Gupta (1965).

Michel (1957) maintains that more and more outbreaks of parasitic bronchitis in adults are now recognised due to better diagnosis and understanding of the disease.

b. Incidence in Young Cattle

It is recognised that lungworm disease, where endemic, is more frequently a problem in younger animals. This view has been held by Allan (1959), Cunningham et al. (1956), Michel and Shand (1955) and Gupta and Gibbs (1969) among others.

In a survey conducted in England during January to May, Cunningham et al. (1956) found that 31 percent of the young stock, as compared to 3.8 percent of the older cattle, were infected with lungworm. Swanson et al. (1959) recorded the disease in Florida (U.S.A.) and found it to be more common in cattle six months to one-year old. Durham (1961) was of the opinion that husk is widespread throughout New Zealand and cattle of all ages could be infected but the disease was most frequent in calves. Wertejuk (1963) conducted a survey in Poland and examined some 23,000 cattle which revealed 122 centres of lungworm infection, spread over 13 districts. The infected animals were mostly calves between three to 12 months of age. Investigations into the occurrence of lungworm disease in calves were carried out by Popov et al. (1965) in Bulgaria. They examined 21,340 fecal samples from 217 farms, out of which, 104 farms were found infected with lungworm. On 30 of these 104 farms, calves suffered most from parasitic bronchitis.

Gupta and Gibbs (1969) studied the incidence of D. viviparus in cattle in the Province of Quebec. Postmortem examination of 9,766 pairs of lungs from young and adult animals, conducted over a period of two years, showed that 2.76 percent of these were infected with D. viviparus. There was a significantly higher incidence of infection in younger than in older animals, the average percentage infection rate being 4.25 in young animals and 1.29 in older animals.

In addition, the problem of D. viviparus infection in young cattle has been reported from many other countries, including Sweden (Petrelius, 1951), Belgium (Gregoire et al., 1957), England (Michel and Shand, 1955; Brown and Spedding, 1958), Ireland (Allan, 1959), Denmark (Larsen, 1960), Rumania (Olteanu et al., 1961), Egypt (Soliman and Zaki, 1962) the Democratic German Republic (Hiepe, 1964), Western Australia (Dunne, 1968) and Montana, U.S.A. (Jacobson and Worley, 1969).

Epidemiology

In studying the epidemiology of parasitic bronchitis, one must consider the following factors.

a. Life Cycle of D. viviparus

It appears that Cobbold (1875) first attempted to elucidate the life cycle of D. viviparus but it was not until the work of Daubney (1920) that this important aspect was worked out. However, it was Jarrett et al. (1960) whose classical work provided the finest details of the life cycle of D. viviparus. They divided the entire life cycle into four phases:

1. Penetration
2. Prepatent
3. Patent
4. Post-patent

1. Penetration Phase

During this phase the infective larvae exsheathed in the gut, penetrated the intestinal mucosa and made their way to the mesentric lymph nodes. This phase lasted from one to seven days. Infection with D. viviparus occurred by the oral route. However, there are reports where the possibility of an alternate source of infection has been envisaged. Kasparek (1900) reported prenatal infection of D. viviparus in calves of one to eight days old. Monnig (1950) suggested that if prenatal infection was possible, it could only occur when larvae, after reaching the lungs, accidently entered the circulation and were carried to the foetus. Subsequently, controlled experiments and field observations carried out by Porter and Cauthen (1942), Soliman (1953a), Enigk and Duwel (1962) and Poynter (1963) unequivocally refuted the validity of Kasparek's work and concluded that there was no evidence for prenatal infection with D. viviparus.

2. Prepatent Phase

This stage is associated with the migration of larvae from the mesentric lymph nodes to the lungs which takes place from seven to 25 days post-infection. There appears to be some controversy about the migratory route of D. viviparus from the gut to the lungs. However, Soliman (1953a), Jarrett et al. (1957) and Poynter et al. (1960) concluded from their observations that larvae reach the lungs via the lymphatics, a conclusion now widely accepted.

One feature which remains obscure in the life cycle of *D. viviparus* is the time required by the larvae to penetrate the gut wall, reach the mesenteric lymph nodes and finally, the lungs. It appears that the discrepancies between reports are due to differences in species of animal and larval dosage used. Soliman (1953a), working with guinea pigs, infected with 7,000 third-stage larvae each, recovered third-stage larvae from the mesenteric lymph nodes as early as 24 hours post-infection but it took between five to seven days for most of the larvae to migrate to the lungs. It was also demonstrated that larvae left the mesenteric lymph nodes after attaining the fourth-stage.

Jarrett *et al.* (1957) and Jarrett and Sharp (1963) were of the opinion that in a susceptible calf, larvae penetrated the gut and travelled to the mesenteric lymph nodes, where they underwent a moult to become fourth-stage larvae. Subsequently, the fourth-stage larvae reached the right side of the heart *via* the lymphatics and finally, the lungs, the whole process taking about five days. Contrary to this, Douvres and Lucker (1958), using guinea pigs, and Poynter *et al.* (1960), using guinea pigs and calves, obtained results suggesting that the larvae could reach the lungs as early as 24 hours post-infection; however, Jarrett and Sharp (1963) criticised these findings on the basis that the dosage levels used were too high and the number of larvae recovered from the lungs in the first few days was negligible.

During the prepatent phase, the host manifests the first clinical symptoms of the disease. The severity of the disease is proportional to the number of larvae ingested by the host over a given period of time. The worms in the lungs are still immature and fecal examination remains negative for lungworm larvae.

3. Patent Phase

During this period (25-55 days post-infection) the worms attain maturity and the infection becomes patent. The majority of the clinical symptoms, such as dyspnea, coughing, loss of appetite and decreased growth rate, are associated with this phase.

4. Post-patent Infection

This is a continuation of the patent phase and lasts from 55 to 70 days post-infection, during which time most of the animals recover. A large proportion of the worms are eliminated and larvae almost completely disappear from the feces.

b. Parasite-Environment Interrelationships

First-stage larvae of *D. viviparus* are passed out with the feces. Their subsequent development and survival is largely determined by the external environment, especially by temperature and humidity. It is obvious that knowledge of the interrelationships between the free-living stages and the environment is one of the important aspects in understanding the population dynamics of the pre-parasitic stages on pasture.

1. Development and Survival of Preparasitic Stages

(i) Effect of Temperature on Development:- Temperature, moisture, and oxygen are the three factors most important for the development and survival of the pre-parasitic stages. Daubney (1920) described the effects of temperature on the rate of development of these forms. It appeared that lungworm larvae were able to develop over a wide range of temperatures (5°C to 27°C), (Daubney, 1920; Rose, 1956), though Ershov (1956) maintained that when the temperature dropped to below 10°C or rose above 30°C development of the larvae was not possible. However, if one may judge by a conjoint consideration of the two most important factors, namely, the rapidity of development and the percentage of mortality during the process, then the optimum temperature falls within the range of 23°C to 27°C. This has been substantiated by the results of Monnig (1950), Rose (1956), Ershov (1956), Michel (1959) and Soulsby (1965).

Rose (1960), on the basis of his field experiments, found that during the summer in Weybridge, England, about 95 percent of the larvae would attain the infective form within three to four days. Whereas, during the winter only five percent of the larvae managed to reach the third-stage and, moreover, it took approximately 16 to 19 days. This led him to believe that the seasonal variations in the rate of development could therefore be attributed to seasonal differences in temperature. A somewhat similar observation has been made by Popov et al. (1965) in Bulgaria.

(ii) Effect of Temperature on Survival:- In comparing the resistance of different free-living stages of D. viviparus to unfavourable temperatures, Daubney (1920), Soliman (1953b), Ershov (1956) and Rose (1956) demonstrated that the first and second stage larvae were highly susceptible to low temperatures, whereas, the same temperature range seemed to favour the persistence of infective larvae. Rose (1956) recorded the survival of infective larvae in wet feces at different temperatures. The maximum survival of 22 weeks was obtained at 3-6^{o}C. From his results it is evident that, within limits, the survival time was inversely related to the temperature. He further demonstrated that the potential life-span of infective larvae, when stored at low temperature (3-6^{o}C), could be as long as 12 months. This has been substantiated by Gupta and Gibbs (1970).

There are reports (Hutyra et al., 1949; Vercruysse, 1952; Erhardova, 1962; and Popov et al., 1965) indicating the survival of infective D. viviparus larvae under extreme conditions of cold (0^{o}C to -30^{o}C) for a considerable period.

(iii) Effect of Moisture and Oxygen on Development and Survival:- Studies conducted by Daubney (1920), Taylor (1942;1951), Soliman (1953b), Rose (1956;1960) and Michel (1959) revealed that adequate moisture is essential for the development of the pre-parasitic stages of lungworm larvae.

Moisture is not only required for development but is also indispensible for larval survival. None of the free-living forms

are resistant to desiccation at any temperature (Rose, 1956; Michel, 1959; Parker, 1967), with the first and second stage larvae being particularly susceptible to dryness (Daubney, 1920; Ershov, 1956).

Badly drained damp pastures (Hudson, 1939; Taylor, 1942; Popov et al., 1965) dense herbage, particularly with broad leaves, and moist fecal material will hold adequate moisture for the development and survival of the pre-parasitic stages.

For the development of lungworm larvae, oxygen is essential and bacterial contamination or putrification of organic material, which consumes the oxygen, will hasten death (Daubney, 1920; Soliman, 1953b). In summer, a dry crust is formed over the surface of dung and the oxygen supply is cut off. Therefore, most of the larvae die (Taylor, 1952).

(iv) Overwinter Survival of Infective Larvae on Pasture:- There are numerous reports describing either the ability or inability of this parasite to overwinter on pasture. However, there is no clear picture and controversy still exists as to the capabilities of the larvae to survive and remain infective on pasture after the winter months.

Hudson (1939) claimed that in Southwest Scotland some pastures remained infected from one year to another and, in his opinion, even a hard frost was ineffective in destroying the larvae. Taylor (1942; 1951), on the basis of his field observations, produced evidence that larvae remained alive in the south of Scotland for approximately 12 months.

The most positive approach to clarify the question of overwinter survival of larvae on pasture was taken by some Glasgow workers (Jarrett et al., 1954;1955a;1957) and by Allan and Baxter (1957) in Ireland. The experimental procedure adopted was to infect paddocks and later to graze parasite-free calves on these paddocks after stipulated periods to determine the larval survival time. To support their field results they also contaminated grass grown in pots under controlled conditions. The grass was later fed to parasite-free calves after an interval of three, six, nine and 12 months. Under both conditions they demonstrated that larvae could survive and remain infective for as long as 12 months.

Reports from numerous other countries, including Swietlikowski (1959) in Poland, Erhardova (1962) in Czechoslovakia and Enigk and Duwel (1961;1962) in West Germany, indicated that lungworm larvae could overwinter on pasture. Lapage (1968), after critically reviewing the literature, concluded that infective larvae of D. viviparus are often, if not always, able to survive on pastures for six to 12 months.

Gupta and Gibbs (1970) demonstrated that lungworm larvae could overwinter on pasture in the Eastern part of Canada. However, they cautioned that many factors such as intensity of pasture contamination during the previous grazing season, stocking rate during the following year, and the annual fluctuations in the climate were involved, and that these may influence the survival of larvae on pasture. Since these factors can vary from year to year, it is quite likely that larval survival could be influenced one way or another.

There are many reports claiming the inability of lungworm larvae to persist on pastures over the winter months. Porter (1942), in Alabama, U.S.A., grazed parasite-free calves on a lungworm infected pasture after spelling it for stipulated periods. The test calves remained on pasture for one month, after which they were brought inside. Regular fecal followed by postmortem examination proved negative for evidence of lungworm infection. Wetzel (1952) used a technique similar to Porter (1942) but only carried out fecal examination on the test animals. He was unable to demonstrate over-winter survival. Similarly, Soliman (1952a,b), Stableforth (1953), Michel and Rose (1954), Michel and Parfitt (1955), Michel and Shand. (1955) and Parker (1967) concluded that larvae are unlikely to remain infective on pasture in the United Kingdom from late October to early February under their climatic conditions.

Results of other studies carried out in Holland (Vercruysse, 1952), in Canada (Choquette, 1954), in Bulgaria (Popov et al., 1965) and Belgium (Pouplard, 1968) also suggest the inability of D. viviparus larvae to survive and remain infective after winter months.

It is evident that the findings of one group conflict with those of other groups. Jarrett et al. (1955a) suggested that the differences in results could very well be due to the different criteria used to demonstrate the survival of infective larvae. These methods include:

1. Clinical and parasitological findings.
2. Demonstration of larvae on artificially infected plots, grass, soil or feces.
3. Allowing susceptible calves to graze on a naturally or artificially contaminated pasture after a given period and later to carry out clinical, parasitological and post-mortem examination of these calves.

The first two methods have certain disadvantages. It is very difficult to detect "silent" carriers by the first method, while by the second technique, demonstration of larvae is difficult and there is no proof that the surviving larvae are infective. The third method is the most efficient and positive approach to test the presence or absence of overwintered larvae. However, one must keep in mind that only a small percentage of larvae are likely to persist and remain infective after a long cold winter spell.

Another point which should be considered in the survival of the preparasitic stages is the difference in climate from one location to another. Thus, depending on climate, the larvae could survive in one place and not in another.

2. Translation

The term "translation" has been used to describe the process of movement of larvae from the feces to the pasture. The process of translation is known to be influenced by the factors mentioned below.

(i) Motility of the Infective Larvae:- Opinions on the ability of D. viviparus infective larvae to migrate actively from the fecal pat onto herbage are divided. Daubney (1920), working under laboratory conditions with optimum temperature, humidity and light intensity, demonstrated that larvae could migrate up to 1.6 inches perpendicularly (in culture jars) in about eight hours. Soliman (1953b), however, when conducting experiments under optimum conditions, found only slight vertical migration. Stableforth (1953) found that the migratory power of the larvae was very limited and that they could hardly travel more than an inch from the fecal pat under the best possible conditions and that they did not seem to climb onto herbage.

Rose (1956;1960), in an experiment to demonstrate the extent of vertical distribution of D. viviparus on grass blades, found that migration was not extensive and that almost all the larvae were confined to the lowermost inch of the grass blades. Michel (1959) observed that the number of larvae migrating from the fecal pat onto the surrounding herbage was less than 0.5 percent and that nearly all remained within two inches of the pat.

Contrary to these observations, there are reports (Orlov, 1946; Taylor, 1951;1952; Michel, 1957) suggesting that the active migration of D. viviparus is negligible and cannot be considered of any significance in the epidemiology of this disease.

Ershov (1956) summarized the findings of Russian workers and confessed that the question was still debatable and required

further experimental evidence to support one or the other conclusion.

(ii) Importance of the Consistency of Feces in the Process of Translation:- From the foregoing description, it seems likely that lungworm larvae are very feeble climbers and that most of them remain in the fecal pat. Therefore, rather than thinking of larvae on the herbage, one should consider them (larvae) in the feces (Michel, 1959). Experiments performed by Michel and Rose (1954), Rose and Michel (1957) and Spedding and Michel (1957) to clarify the mechanisms of the translation process demonstrated that, to a large extent, the success of infective larvae in reaching herbage depended upon the extent to which the infected feces contaminated the herbage.

The success of fecal material in reaching the herbage depends upon the interaction of two factors, namely, the prevailing environmental conditions and the consistency of the feces when passed. Liquid and semi-solid feces could easily be spread by either mechanical or biological agents. Environmental conditions such as rainfall, humidity and moderate temperature ($25^{o}C$ - $30^{o}C$) would keep the fecal material in a state facilitating its dispersal. On the other hand, solid feces and hot, dry climatic conditions would limit the dispersal of feces onto herbage. Consequently, the number of larvae reaching herbage would be modified by these two factors. This has been confirmed by Rose and Michel (1957) and Rose (1960) where they

recovered more larvae from herbage when semi-solid feces were spread onto the herbage. However, when solid feces were deposited in the form of pats, the larvae recovered from the surrounding herbage were small in number.

(iii) The Role of Herbage in the Process of Translation:- There appears to be a correlation between the rate of growth of herbage and the extent of translation (Michel and Parfitt, 1956). It is generally accepted that a rapidly growing lush sward, with deep, shade-producing cover, provides a warm and humid microclimate which favours the development and longevity of larvae. In addition, this micro-climate maintains fecal pats in a condition in which they can easily be spread. Soliman (1953b) suggested that when herbage is poor, most of the larvae will remain around the roots. When such grass grows, these larvae will be lifted with the growth and thus will be near the tips of the blades, readily available to the grazing animals.

(iv) Mechanical and Biological Agencies in the Process of Translation:- The translation of lungworm larvae by active migration seems negligible. How the lungworm larvae reach herbage still remains enigmatic. Some researchers have speculated that larvae can only reach the herbage when the feces do, thus, any mechanical or biological agencies which could spread infected feces onto herbage must be considered important in the process of translation. Taylor (1952), Michel and Rose (1954), Spedding and Michel (1957) and Rose and Michel (1957) believe that agricultural operations, for example,

chain harrowing, can substantially add to pasture infestation, particularly during wet weather. Biological agents which may be implicated in this process include animal feet and birds (Rose and Michel, 1957) and various coprophagic insects (Ershov, 1956). Of interest in this respect is the finding of Robinson (1962). He discovered, under laboratory conditions, that when larvae reached the third-stage they migrate to the surface of the fecal pat. At about the same time, there often occurs an abundant growth of sporangiophores of Pilobolus spp. (Phycomycetes:Mucorales) on bovine feces. A characteristic of this genus of mould is its robust discharge onto the surrounding herbage of the whole sporangium containing the ripe spores. It has been observed by this author that under suitable conditions, infective larvae actively migrate onto the sporangiophores and reach the upper surfaces of the sporangium. The larvae remain until the sporangium is discharged, when the spores, and the larvae, are thrown into the air and may be carried a distance of 10 feet. Robinson et al. (1962) found that 95 percent of the fecal samples examined in England were contaminated with Pilobolus spp. If this association between the mould and the larvae is widespread, it may shed some light on the process of translation.

(v) Rainfall and Translation:- Ershov (1956), Rose (1956), Enigk and Duwel (1962) and Popov et al. (1965) believe that, in order for the infective larvae to reach the host, they must free themselves from the fecal pat and reach the herbage. This could be facilitated by a suitable amount of rainfall which could wash the

larvae from the feces and scatter them on the herbage. Contrary to this, Rose (1956;1960) pointed out that, since the lungworm infective larvae assume an inactive, coiled form and cannot swim in water, the rain would wash them into the soil. Therefore, rain does not seem to favour the migration of larvae out of the feces. However, circumstantial evidence indicates that an adequate amount of rainfall, which could keep the feces wet, may support translation either directly or indirectly.

3. Transmission

The process whereby the larvae on the herbage are finally ingested by the host.

(i) Seasonal Transmission:- The concept of the availability of infective larvae to the grazing animal is complex. In order to be available to the grazing animal, larvae must develop to the infective stage and survive to be ingested. Transmission, to a great extent, is determined by the number of infective larvae reaching the herbage (translation) and their survival (Michel, 1959). The pasture infestation and, in turn the transmission, is governed by the environmental conditions (Rose, 1956;1960; Rose and Michel, 1957).

Herbage infestation seems to follow a seasonal pattern (at least in temperate regions) which depends more on climatic conditions than on the actual number of larvae passed onto the pasture.

During the winter, unfavourable environmental conditions lead to slow or completely suppressed larval development. This accounts for poor translation which is reflected in low transmission during this season.

During spring and early summer, translation is adequate but transmission is limited due to the high mortality of infective larvae as a result of warm and particularly dry climatic conditions. In addition, during winter, spring and early summer, the number of larvae per pound of herbage is relatively low. It has been suggested that it takes approximately three to four months (May to August) for the infection to build up on pasture. In summary, the poor transmission during the seasons mentioned above, could thus be explained in terms of unfavourable climatic conditions and the degree of pasture infestation.

In autumn, infection can rapidly increase on pasture (Jarrett et al., 1954). In addition, the cool and humid climate may provide optimum conditions for the persistence of these infective larvae on pasture.

It is the seasonal fluctuations in terms of translation and longevity of infective larvae which account for the differences in the rate of transmission from one season to another.

(ii) Herbage and Transmission:- Transmission to a certain extent is also affected by the rate of growth of herbage on pasture.

When the stocking rate is high, the herbage is short and the larvae are distributed on a relatively small quantity of herbage, this results in a high concentration of larvae per kg of herbage. However, the short herbage will not provide a favourable environment for the development and persistence of preparasitic stages as they are exposed to desiccation and heat which will decimate the larval population. On the other hand, luxuriant pasture will promote the longevity of infective larvae, but the concentration of larvae per kg of herbage will be diluted. Thus, the effect of herbage on transmission is complex, with the prevailing climatic conditions probably exerting an overiding influence (Michel and Rose, 1954; Michel, 1959).

(iii) Grazing Behaviour and Transmission:- As stated earlier, for translation to occur it is essential that contaminated feces should somehow get onto herbage. It is known that cattle will avoid grass that is grossly contaminated with manure and will also refrain from herbage growing around a fecal pat. This selective grazing by bovines has been experimentally confirmed by Michel (1955a) where it was demonstrated that cattle picked up fewer larvae in comparison to the number found on grass collected randomly from the same pasture. In a subsequent experiment, Michel and Rose (1954) concluded that, when the feces were thinly spread, the grazing bovine was unable to avoid the contaminated herbage, thus the transmission of infection was favoured only so long as the quantity of feces on the herbage did not render it unpalatable to the bovine.

4. Other Sources of Pasture Contamination

Cattle are not the only source for contamination of pasture with D. viviparus. A number of wild herbivores including bison, elk, fallow deer, moose, musk ox, reindeer, roedeer and white tail deer can become infected with D. viviparus and can infect pasture. The free-living stages, however, will be similarly influenced by the external environment.

It has been the consensus of many researchers (Petrelius, 1951; Rountree et al.,1954; Durrell and Bolton, 1957; Graesser, 1957; Gibbs and Tener, 1958; Swanson et al., 1959) that some of these wild herbivores, when infected with D. viviparus, could serve as lungworm reservoirs for domestic animals and, thus, play an important part in the epidemiology of lungworm disease. It should be pointed out that the bulk of the evidence implicating wild herbivores in the disease cycle is circumstantial. However, recently, some success has been reported in infecting calves with D. viviparus obtained from elk (Presidente and Worley, 1968) and in infecting Abyssinian gazelles and Indian antelope with D. viviparus obtained from cattle, (Enigk and Hilderbrandt, 1969).

According to Lapage (1968) and Lucker et al. (1964), D. filaria is host specific and cannot infect cattle. However, Parfitt (1963) experimentally infected six calves with D. filaria using a dosage level of 30,000 infective larvae per calf. All the calves revealed clinical symptoms of parasitic bronchitis during the prepatent period but the infection became patent only in three of the six calves. These results

are in agreement with the findings of Enigk and Hildebrandt (1969) who observed that D. filaria can cause clinical symptoms in calves similar to D. viviparus. Parfitt (1963) suggested that a closer look at the worms recovered from cases of parasitic bronchitis in cattle might show the presence of D. filaria more frequently than has been generally expected.

c. Parasite-Host Interrelationships

Once the infective larvae enter the final host, their subsequent behaviour (development and fecundity, particularly) depends upon the host's internal environment.

1. Infection Patterns in a Susceptible Host

The severity of lungworm disease in a susceptible host is determined by the number of larvae per kg of herbage to which the host is exposed for the first time (Michel and Parfitt, 1955;1956; Michel, 1959). If the mean level of herbage infestation is less than one-half larva per kg of herbage then the infection will become patent, but the calf will survive. On the other hand, when the count is between one-half to one and one-half larvae per kg of grass, then the host will die during the patent period. However, a higher pasture burden (more than one and one-half larvae per kg of herbage) will kill a calf during the prepatent period.

It has been found by Michel and Parfitt (1955;1956) and Michel and MacKenzie (1965) that resistance against D. viviparus infection is

acquired as early as nine days post-exposure. The degree of resistance has a direct bearing on the epidemiology of parasitic bronchitis. This led the above mentioned authors to postulate that where continuous grazing on infected pastures is practiced, cattle do not produce on the pasture an infestation dangerous to themselves, since they become refractory to infection by the time larvae they passed are infective and available on herbage. The salient point of their work revealed that "self augmentation," in the case of D. viviparus infection, is a remote possibility.

This argument conflicts with the opinions of Wetzel (1948) and Jarrett et al. (1954) who maintained that outbreaks of parasitic bronchitis in cattle are the outcome of a build-up of infection between the animals and herbage. It was hypothesized that animals would continue to reinfect themselves by ingesting larvae which they themselves passed in ever increasing numbers, until their worm burden reached a point where the symptoms of disease appeared.

In general, D. viviparus infection in a susceptible host follows the pattern outlined under parasitic factors in the section dealing with the life cycle.

2. Infection Pattern in a Resistant Host

An acquired resistance against D. viviparus infection is known to develop and cattle generally become refractory to a subsequent infection. This has been demonstrated by many workers, including

Porter and Cauthen (1942), Wetzel (1948), Taylor (1951), Jarrett et al. (1955b;1959a,b), Rubin and Lucker (1956), and Michel (1955b;1959;1962). However, there are a few reports where a patent infection has been reported in a resistant host, but under such circumstances the prepatent period is long the patent period short, and the number of worms recovered during postmortem examination from the lungs is very small.

The acquired resistance is complex in nature and can be manifested in a number of ways (Rubin and Lucker, 1956; Michel and MacKenzie, 1965). Among these are:

1. It can lead to "self-cure" of the existing worm population.
2. The fresh larvae may fail to become established in the lungs.
3. The development of those larvae that do manage to reach the lungs may be inhibited.
4. The larvae may be eliminated before attaining maturity.

The precise fate of larvae ingested by a resistant animal has been investigated by Michel (1956) and Rubin and Lucker (1956). They speculated that larvae in such a host may be trapped or destroyed either in the intestine or lymph nodes during migration. Similar observations have been made by Wilson (1970) while working with D. filaria. However, Poynter et al. (1960) argued that it is in the lungs and not the mesenteric lymph nodes where larval suppression occurs.

Smythe (1937) reported that in certain temperate regions, where the winter was not sufficiently cold to destroy larvae and where cattle were seldom housed, a mild type of parasitic bronchitis could be continuous throughout the year. However, this does not agree with the work of Rubin and Lucker (1956) and Michel (1968), where a strong immunity against D. viviparus has been shown to develop and the cattle become refractory to reinfection. Under these conditions it would be difficult to visualize how cattle could continue to reinfect themselves without building up any resistance.

The importance of carrier animals as a source of lungworm infection was recognized by Taylor (1935) and Smythe (1937) while investigating outbreaks of bovine parasitic bronchitis on farms in England. Since Wetzel (1948), in Germany, was not convinced that overwinter survival occurred, he investigated the role of carrier animals under field and experimental conditions, which led him to believe that the disease was carried over by subclinically infected animals which continuously contaminated the environment. It appears that Taylor and Michel (1952;1953) were the first to demonstrate the presence of immature worms in the lungs of the experimentally infected animals and they tied in this faculty of dormancy to the survival of the parasite and disease cycle.

The evidence incriminating carriers as a potential source of infection was obtained by Jarrett et al. (1954;1955a;1957) in several different ways, both under field and experimental conditions. They purchased known lungworm infected animals and housed them under conditions which precluded reinfection. From their results, it is evident that some of the animals started reshedding lungworm larvae after having been negative for extended periods. They also observed a similar phenomenon of reshedding under normal husbandry practice in housed cattle. To substantiate their findings, the authors studied the incidence of carrier animals in abbatoirs during January to May. The results showed that 30 percent of the yearlings and four percent of the adults harboured a small number of worms. Cunningham et al. (1956) recorded approximately a similar incidence of carrier animals in Britain and concluded that carrier animals are a major factor in the persistence of the disease. Michel and Shand (1955) carried out a most extensive survey of lungworm disease outbreaks and stated that the ultimate source of infection is in the small number of larvae disseminated in the feces of carrier animals.

There are numerous other reports from different parts of the world incriminating carriers as the main source for the persistence of the disease. Some of these reports include: Choquette (1954) in Canada, Ershov (1956) in the U.S.S.R., Enigk and Duwel (1962) and Frick (1964a,b) in Germany, Parker (1967) in England, Poupplard (1968) in Belgium, and Gupta and Gibbs (1970) in Canada.

3. Longevity, Generation Interval and Population Control of D. viviparus

From the literature it appears that, at times, a long patent period of more than a year has been described in D. viviparus by Wetzel (1948), Jarrett et al. (1955a), Ershov (1956), Groves (1957) and Swietlikowski (1959). However, Hudson (1939), Michel (1955b; 1959), Rubin and Lucker (1956), Jarrett et al. (1960), and Frick (1964a,b) believe that both experimental and natural infections do not last for more than 50 to 90 days.

The generation interval of D. viviparus under optimum conditions is about 30 to 35 days (Jarrett et al., 1960; Soulsby, 1965). The parasite has to live under two entirely different environments, namely, in the host and on pasture, thus the phrase "optimum conditions" must refer to both of these habitats. A suitable internal environment will mean a fully susceptible host exposed to infection for the first time, whereas, optimum external conditions will encompass a variety of factors, including temperature, moisture and oxygen. Any divergence from the optimum conditions, either internally or externally, will lengthen the generation interval. Factors such as resistance of the host and unfavourable environmental conditions will directly or indirectly extend the generation interval, which may then be up to six months or so.

D. viviparus cannot multiply in the final host, thus, the population in the host is dependent on the number of infective larvae

available on pasture. It is evident therefore that the degree of pasture infestation, to a certain extent, would be reflected in the number of worms in the host. Again, pasture infestation is dependent on two different criteria; the number of larvae deposited on pasture and the prevailing environmental conditions. If, by chance, both these conditions are favourable, a rapid build-up of infection on pasture can be expected (Michel, 1959) and a fully susceptible host on such a pasture will pick up large numbers of infective larvae and consequently harbour a larger worm burden (Michel and Parfitt, 1955; 1956). On the other hand, if the source of pasture contamination is limited and the environmental conditions are unfavourable, then these circumstances will limit the number of infective larvae per kg of herbage. In this case, even a fully susceptible host will obtain only a few worms. This amply illustrates that environment could either restrict or favour the worm population in the host.

In population control another factor is the host itself. Taylor (1951) suggested that the development of specific resistance is one of the most important of the numerous interrelated factors governing the rise and fall of parasite populations. A strong acquired resistance against D. viviparus is known to develop and cattle can become refractory to the infection (Jarrett et al., 1955b; Michel, 1959; Michel and MacKenzie, 1965; Poynter, 1970). All these authors maintain that when a resistant animal is re-infected, only a few worms succeed in reaching the lungs. Furthermore, most of them are quickly eliminated and only a small residue

persists and they fail to develop at the normal rate. The final outcome is that a resistant host will have fewer worms, if any, even though it is constantly exposed to infection.

There is yet another facet called "self-cure" which plays a significant part in controlling the parasite population in the host. Wetzel (1948) has described a "self-cure" phenomenon in D. viviparus which is analogous to the one reported by Gordon (1967) in Haemonchosis in sheep.

d. The Phenomenon of Inhibited Development

A number of different terms are currently being used by workers to denote the condition in a parasite's growth in which the development is delayed. Some of the terms include: arrested, inhibited, retarded or interrupted development, and physiological or seasonal inhibition. These terms are often interchangeably used to describe very similar kinds of development. In this thesis, wherever these terms are used, they qualify a state of development (in the case of D. viviparus, mostly at the early fifth-stage), particularly during late autumn and the winter months. It should be pointed out here that inhibited development should not be confused with the temporary burrowing into the gut mucosa or other organs by some parasites as these events are usually associated with migratory behaviour.

Taylor and Michel (1953) envisaged the importance of dormant stages in the epidemiology of parasitic diseases and stated, "it is an essential part of the ecological adaptations of almost all

parasitic nematodes that they should be able to survive for a protracted period outside the final host, either as a larva enclosed in an egg shell, as a free-living larva, or as a free-living larva encysted within an intermediate host. It is possible that larvae which have succeeded in gaining access to the final host should show a tendency to become dormant once again if any reaction of the host operates against their development." A somewhat similar opinion was expressed by Gordon (1957) who suggested that, although the reasons for inhibited development remain obscure, the object for this dormancy, as far as the parasite is concerned, is to surmount, in a quiescent phase, the unsuitable environmental conditions prevailing either internally or externally.

Kotlan (1949) first coined the term "histotropic phase" and ascribed this to the migratory phenomenon characteristic of nematode larvae which, upon introduction into the alimentary tract and reaching their normal habitat, invade the mucosa in order to pass the third and, perhaps, also the fourth moult in that situation. He described the histotropic phase under two categories; firstly the "regular," in which the larvae remain in the mucosa for a certain period and then return to the lumen of the gut; secondly the "irregular," in which the larva remains in the mucosa for an extended period and "exhibits chronic processes." Since the work of Kotlan, the attitude towards the histrotropic phase has changed dramatically despite the wide differences in the opinions concerning

the underlying mechanism(s) of this faculty. It is now unequivocally accepted that a large number of parasites undergo retarded development for an extended period at a precise point in development (Madsen, 1962; Dunsmore, 1965; Gibbs, 1967; Michel, 1968; Armour, 1970a).

1. Acquired Resistance

Most of the authors agree, in general, that the host acquires the ability to inhibit the parasites' development only as the result of prolonged exposure to infection. Some of the researchers have expressed their view very strongly in favour of acquired resistance as the only cause for the induction of inhibition. Soulsby (1957; 1958;1960;1966) has repeatedly emphasized that arrested development undoubtedly is a basic manifestation of immunity. He maintains "there can be little doubt that retardation of development is a basic function of immunity and, furthermore, almost all effects detrimental to the parasite are essentially an interference with its metabolism. It should be realised that, not only are immune processes responsible for retardation, but also their constant presence is required if inhibition is to be maintained. It would appear that a kinetic system is being inhibited which, in the absence of its antagonist, will function normally." Madsen (1962) postulated that "the expression of retarded development does not represent a phase in the sense of behavioural pattern innate to the parasite but rather a phenomenon dependent upon the degree of resistance in the host."

Another proponent (Michel, 1968) is confident that acquired resistance plays a major role in causing retarded development.

The role of acquired resistance in the phenomenon of inhibited development is very clearly demonstrated in the case of D. viviparus by Michel (1956;1957;1959;1968), Jarrett et al. (1959a,b), Michel and Cornwell (1959), Michel and MacKenzie (1965) and Poynter (1970). Michel (1968) claimed that animals grazing on infected pasture acquired sufficient immunity within nine to 11 days to protect themselves from a moderate exposure. He further emphasized that immunity against D. viviparus is multifaceted and that one of the factors involved causes inhibition. Taylor (1951) and Taylor and Michel (1952;1953) provided evidence, both from field studies and experimental work, that D. viviparus had the ability to undergo retarded development, particularly in resistant animals. Their examination revealed that the majority of larvae inhibited at the early fifth-stage and that they can remain in this stage for months before resuming development to attain maturity.

The mechanism(s) by which host immunity retards the development is not precisely understood. Soulsby (1957) and Dunsmore (1961) suggested that, on many occasions where delayed development occurs, it is most probably due to the inhibition of the moulting period by an immune response not sufficient to kill the parasite. Other suggested factors involved in this complex phenomenon of inhibition include the role of cellular, humoral or

anti-enzyme antibodies which may interfere physically or biochemically with the developmental process (Stewart, 1958; Soulsby, 1963; Crandall and Arean, 1964).

There is other direct evidence which lends support to the argument that immunity plays a part in the phenomenon of arrested development. It has been shown by a number of workers with parasites (Chandler, (1936) and Sarles and Taliaferro (1936) both using Nippostrongylus muris; Roberts, (1957) using Haemonchus placei; Roberts et al. (1963) using Oesophagostomum radiatum; Wilson (1970) using Dictyocaulus filaria) that on transplanting inhibited stages into a non-immune host, the larvae resume growth immediately and attain maturity within a stipulated time. Immuno-suppressive drugs have been used by some researchers to show that a decline in the host's resistance enhances parasitic development. This has been substantiated by the results of Parker (1961) working with Nippostrongylus brasiliensis in guinea pigs, Soulsby and Owen (1965) with mixed infections in sheep and James and Johnstone (1967b) with Haemonchus contortus in sheep. Both these approaches demonstrate that whenever the specific resistance declines, the larvae are no longer suppressed by the immune mechanism of the host and subsequently continue development. This is confirmed by Soulsby (1957), Crofton (1958) and Field et al. (1960), where the decline in the host immunity has been alleged to be the cause of the "spring rise" phenomenon in sheep. However, there are conflicting reports on the

earlier results of the effect of transplantation and immuno-suppressive drugs on subsequent maturation of arrested larvae. Blitz and Gibbs (1971) demonstrated that after transplantation in mid-winter of inhibited larvae of Haemonchus contortus into non-immune ewes, the maturation of the larvae did not occur until approximately 10 weeks later. Similarly, Dunsmore (1965), Brunsdon (1966), Soulsby (1966) and Gibbs (1967) were unable to stimulate dormant larvae to resume development by injecting the host with immuno-suppressive drugs. Thus, there is enough evidence to claim that inhibited development, in certain host-parasite relationships cannot be ascribed to the conventionally understood immune mechanisms (Jennings et al., 1967; Michel, 1968; Muller, 1968; Anderson et al., 1969; Armour, et al., 1969a,b).

2. Age per se of the Host

Gibson (1959) and Brunsdon (1962a)revealed that parasite-free sheep, when exposed to Nematodirus spp. were unable to resist the establishment of infection, but were quite efficient in inhibiting the development of infective larvae. Silverman and Patterson (1960) demonstrated that normal development of Haemonchus contortus in susceptible mature sheep is slow in comparison to the same infection in susceptible lambs. According to Michel and MacKenzie (1965) acquired resistance against D. viviparus appears to develop more rapidly in older animals; the worms initially established may be more rapidly lost, or there may be greater interference with

their development to maturity. Comparable results were obtained by Smith and Archibald (1968) who noticed poor development of Cooperia spp. in susceptible yearlings, when compared with susceptible calves, and postulated that retarded development might be influenced by the age of the host.

Contrary to these findings, Herlich (1960), working with mixed infections of gastrointestinal parasites in cattle, and Dineen and Wagland (1966b), working with Haemonchus contortus infection in sheep, were unable to demonstrate any effects of age on the development of parasites.

In view of these conflicting reports, it is difficult to draw any general conclusion as to the effect of age per se on the phenomenon of retarded development.

3. Adult Worms

It has been demonstrated that the presence of a "critical" number of mature worms in certain nematode infections (Trichonemiasis in horses, Gibson, (1953); Haemonchosis in calves, Roberts and Keith, (1959); Ostertagiasis in sheep, Dunsmore, (1963); Nematodiriasis in sheep, Donald et al., (1964)) might be responsible for inhibiting development of larvae of their kind. Indirect evidence concerning the role of mature worms has been provided by Roberts and Keith (1959) and Dunsmore (1963) where differential removal of adult worms, either actively (self-cure) or passively (anthelmintic), provoked the maturation of concurrently inhibited stages. Michel (1963) working with

Ostertagia ostertagi in calves, maintained that somehow the presence of adult worms facilitated inhibited development. The mechanisms by which the presence of adult worms induces dormancy are not properly understood. Dineen (1963) and Donald et al. (1964) speculated that any particular host may allow a certain number (threshold) of parasites to mature but prevent the maturation of any further developing larvae. This could be due to lack of"living room" (Taylor and Michel, 1953), crowding (Russell et al., 1966) or to the resistance of the host as a result of the presence of adult worms (Michel, 1968).

Yet another observation strongly suggests that resumption of development of inhibited stages is not influenced by the presence of adult worms. Michel (1963) was unable to demonstrate any quantitative relationship between the number of mature worms present and the magnitude of inhibition. In fact, Gibbs (1967), James and Johnstone (1967a), Muller (1968) and Wilson (1970) have reported that animals in which larval development was greatly inhibited had fewer mature worms than those with few inhibited stages.

One cannot escape the conclusion that the role of adult worms in inducing dormancy remains ambiguous.

4. Crowding

A possible correlation has been demonstrated in some parasitic infections between the size of a single infective dose

and the percent inhibition. One of the explanations forwarded by Martin et al. (1957) for the massive inhibition of Ostertagia ostertagi in calves was that it was due to the ingestion of a large number of larvae on pasture over a short period of time. A similar relationship has been reported by Dunsmore (1960) working with Ostertagia circumcincta in sheep, Donald et al. (1964) working with Nematodirus spathiger in sheep, Russell et al. (1966) working with Obeliscoides cuniculi in rabbits and Ikeme (1970), working with Ascaridia galli in poultry, and on all occasions, the authors experimentally infected the test animals with a wide dosage range of infective larvae. Their results suggest that in heavy infections, a proportion of the larvae do not develop to maturity at a normal pace and remained inhibited for considerable periods.

No precise reason has been given for the inhibition in the instances where its magnitude is dose dependent, but it has been speculated to be due to lack of "living room" or the non-availability of specific nutrients required for the growth of whole populations. Russell et al. (1966) suggested that excess larvae remained in a state of arrested development awaiting either a decline in the host's resistance or the elimination of adult worms.

The results of Ross (1963), Anderson et al. (1966), Dineen and Wagland (1966a), Ritchie et al. (1966), Herlich (1967), Muller

(1968) and Wilson (1970) do not suggest any relationship between the dosage level and percent inhibition. In fact, Anderson et al. (1966) and Armour et al. (1967b) concluded that, "it was unlikely that massive inhibition under their experimental conditions was solely a function of high level of infection over a short period."

5. Strain Differences

Many researchers have recognised the occurrence of strains of a number of parasites which, although morphologically indistinguishable, differ in their host range, pathogenecity, or host-parasite relationships. Crofton and Whitlock (1965a,b) have demonstrated physiological differences (with reference to the optimum temperature requirement for hatching) between different strains of Haemonchus contortus and Ostertagia circumcincta in different geographical regions. Armour et al. (1967a,b;1969b) have revealed significant differences in inhibited development between various strains of Ostertagia ostertagi in parasite-free calves. The so-called "field strain," which was isolated from naturally infected calves, had the capability of arrested development, particularly during late autumn and winter. Their second "strain," the "laboratory strain," was repeatedly passaged in calves inside the laboratory for 10 to 12 years. This strain showed only a limited tendency for arrested development when compared under identical conditions with the field strain. The authors postulated that both strains could exist independently

under natural conditions or that following the original isolation of the culture from the field, the strain responsible for inhibition was eliminated due to selection pressure, giving rise to the laboratory strain. Armour (1970a) concluded that, in addition to environmental factors, an innate susceptibility of a particular strain of parasites was of paramount importance in inducing dormancy. The theory of strain differences in Ostertagia ostertagi, as the cause of inhibited development, has not been whole-heartedly endorsed by Michel (1967) and Sollod (1967) who warned that the concept of strain differences does not take into account the different aspects of inhibition observed under natural conditions. However, from the results published by Armour et al. (1967a) and Armour (1970a), it appears probable there could be two strains of Ostertagia ostertagi, one which had the faculty to survive in the host during winter in a dormant phase, while the other had the capacity to overwinter on pasture as infective larvae.

Michel and MacKenzie (1965), and Parfitt and Sinclair (1967) have noticed inhibited development in D. viviparus in calves infected experimentally with a single dose. They attributed this observation to the differences in cultures in the D. viviparus used, which had varying proportions of larvae relatively unable to complete the whole process of development. Somewhat similar observations were made by Connan (1968) working with Ostertagia circumcincta in sheep. He postulated there may in effect be two populations of infective larvae available, those which are

potentially able to become dormant and those which develop normally in the host.

6. Age of the Infective Larvae

There are reports implicating age of the infective larvae as a factor in delaying their development. Madsen (1962) realized that fresh and aged eggs of Ascaridia galli were more likely to undergo their partial migration in the gut mucosa of the chicken when compared with the eggs of optimum viability and infectivity. Michel and Parfitt (1955), Swietlikowski (1969) and Cornwell and Jones (1970) have noticed a fall in the infectivity of aged larvae of D. viviparus and fewer larvae were able to attain maturity. Stockdale et al. (1970) observed that infections produced with infective Obeliscoides cuniculi larvae stored at 4°C for several weeks contained approximately 30 percent more arrested larvae than those produced with larvae freshly recovered. These results conflict with those reported by Connan (1968) who was unable to induce dormancy by ageing larvae of Ostertagia circumcincta for six months in the refrigerator at 4°C. Similarly, the results of Armour et al. (1969a,b) working with Ostertagia ostertagi in calves, Ayalew (1969) working with Ostertagia circumcincta in sheep, Gupta and Gibbs (1970) working with D. viviparus and Michel et al. (1970) working with Cooperia oncophora in calves, are not in agreement with those of the above mentioned workers. From their work with these species, it is evident that of the infective larvae ingested by sheep or calves grazing in the late autumn, a large

proportion of those which established were inhibited. However, on the same pasture in the following May, only a small proportion of larvae which established were inhibited in their development, although these larvae were much older at this time.

It appears, due to these contraversial results, that more work is needed before attempting to establish any correlation between the age of the infective larvae and inhibition.

7. Physiology of the Host

From the published work on the phenomenon of inhibited development, it is axiomatic that some researchers, without minimizing the role of acquired resistance, have conceded that, at times, factors other than host immunity appear to play an important part in inducing dormancy. Dunsmore (1965) has reviewed the literature, giving examples of a number of parasites whose development is influenced by the host's physiology. He envisaged that seasonal physiological fluctuations in the endocrine hormones of a host could influence, either directly or indirectly, the behaviour (growth pattern) of parasites living in the host. James and Johnstone (1967a), working with Ostertagia circumcincta in sheep, suggested that, "the disappearance of inhibited larvae in the autumn in penned sheep and the persistence of inhibited forms until the end of summer are more in keeping with seasonal physiological changes in the animal." Anderson (1965) and Armour et al. (1967a,b) working with Ostertagia ostertagi in calves, Gibbs (1967) working

with Haemonchus contortus in sheep, and O'Sullivan and Donald (1970) working with mixed gastrointestinal parasites in sheep, visualized the influence of the host's physiology on the growth pattern of their parasites. This approach has been critisized by some workers (see below) who speculated that there is a seasonal change in the physiology of the infective larvae rather than the host.

8. Physiology of the Infective Larvae

Inhibition of development in Ostertagia circumcincta (Connan, 1968), Ostertagia ostertagi (Anderson et al., 1969), Haemonchus contortus (Gibbs, 1967; Blitz, 1970) and D. viviparus (Gupta and Gibbs, 1970) under field and experimental conditions shows a distinct seasonal pattern. This has been termed "physiological inhibition" by Anderson et al. (1965) and has been attributed to the seasonal changes in the physiology of the infective larvae on pasture (Armour et al., 1967a,b). It has been hypothesized that, akin to diapause in insects, a change may be involved in the physiology of the infective larvae on pasture due to seasonal fluctuations in the environment (particularly light/dark ratio and temperature). Jennings et al. (1967) were the first to attribute massive inhibition of Ostertagia ostertagi in parasite-free calves during late autumn to a change in the physiology of the infective larvae, and related it to a diapause-like phenomenon observed in many insects. Blitz (1970) working with Haemonchus contortus has observed 70-90 percent inhibition in parasite-free

lambs during late autumn. He speculated that there may be an autumn photoperiod-induced change in the physiology of the infective larvae similar to insect diapause.

9. Change in Diet

Parasites depend upon the host for nourishment for their continued growth. Since the phenomenon of inhibited development in some parasites is seasonal, it is logical to attempt to implicate seasonal differences in components of the host's diet.

Although there are numerous reports (Lucker and Neumayer, 1947; Gordon, 1950; Brunsdon, 1962b) in which the diet of the host has been correlated with the establishment and after-effects of the parasitic infections, information is limited on the role of nutrition in the development of the various stages of a parasite. Poeschel and Todd (1969) fed semi-purified and natural diets to lambs (infected experimentally with Haemonchus contortus) and demonstrated significant differences in the percent inhibition. Their results revealed that fewer parasites were established, and more were inhibited in lambs fed natural diets when compared with lambs which were fed a semipurified diet. The authors were unable to pinpoint whether the differences in the percent inhibition on the two different rations were due to the physical properties of the diet or to the absence in the ration of factor(s) required for continued growth. Connan (1969) was unable to demonstrate any significant differences in the percent inhibition of Ostertagia circumcincta

infection in lambs which were fed different rations (mature grass, hay and early grass).

The subject requires further investigation to clarify whether a relationship exists between diet and the phenomenon of seasonal inhibition.

e. Factors Responsible for Triggering of Inhibited Larvae to Attain Maturity

From the preceding discussion, it appears that a multitude of factors are involved in the etiology of inhibited development. It is, therefore, likely that factors which influence the resumption of growth of the arrested larvae are equally complex in nature. Accordingly, Michel (1968) believes that the factors which are the cause of inhibited development in a parasite population need not be the same as those which control its subsequent development.

The bulk of the evidence implicates the immune status of the host as a key factor in inducing dormancy (Madsen, 1962; Soulsby, 1966; Michel, 1968) and the reversal of the immune status could lead to parasite maturation (Soulsby, 1957; Crofton, 1958; Connan, 1968; O'Sullivan and Donald, 1970). The importance of immunity in affecting parasite maturation has been further supported by the use of chemical agents which lower the resistance of the host. Such treatment is alleged to provoke a marked rise in the number of eggs per gram of feces, suggesting renewed development of the arrested

larvae (Soulsby and Owen, 1965; James and Johnstone, 1967b). Similarly, in some cases where inhibited stages were transplanted into a non-immune host they developed normally in the new environment (Chandler, 1936; Roberts, 1957; Wilson, 1970).

One of the explanations offered for the "spring rise" phenomenon in sheep is that stress per se on the host influences the number of eggs per gram of feces, an increase in which is thought to be due to the maturation of arrested larvae. The stress could be physiological (Crofton, 1954; Field et al., 1960); environmental and/or nutritional (Paver et al., 1955). However, Dunsmore (1965) has not endorsed these findings.

Recently, the endocrine status of the sexually mature host has been suggested as playing a role in triggering the development of arrested larvae to maturity. This has been well authenticated in the "spring rise" phenomenon in bred, lactating ewes. Dunsmore (1965), Gibbs (1967) and Arundel and Ford (1969) have postulated that hormones associated with late pregnancy and lactation (prolactin) might have a stimulatory effect on the arrested larvae. These results are in agreement with those of Oshima (1961), Olsen and Lyons (1965) and Batte and Moncol (1967) in which a similar stimulatory mechanism by the host hormones has been implicated in the phenomenon of renewed development of arrested larvae. However, Connan (1968), and O'Sullivan and Donald (1970) do not inplicate host hormones as having a direct triggering action on the inhibited

stages. It is suggested that the endocrine status of the lactating ewe brings about a generalised loss of resistance which in turn initiates the development of arrested larvae. This host-hormone-parasite relationship may be true for sexually mature animals, but the factors causing the maturation of arrested larvae (during spring) in sexually immature animals remains unexplained.

A relatively new approach to explain the mechanism whereby the inhibited stages resume growth en masse has led some researchers (Armour et al., 1967a,b; Jennings et al., 1967) to suspect a diapause development similar to that seen in insects. According to this hypothesis the arrested larvae do not depend on any stimulus from the host to resume development, but do so because of an innate mechanism associated with the diapause. Blitz (1970), working with Haemonchus contortus, has visualized a similar mechanism. He has postulated that, "the arrested larvae underwent a period of diapause development over winter and developed spontaneously when this process was complete."

Thus, the suggested reasons for the reactivation of arrested larvae to attain maturity can be placed in either of two categories; one, where the loss of immunity is the principal reason, and two, where the factors which operate are independent of the host's immune status. However, it should be made clear that none of the hypotheses, independently or together, explain satisfactorily all the facets of inhibited development; the factors responsible for the reactivation of arrested larvae to maturity therefore remain largely conjectural.

MATERIALS AND METHODS

Field Studies on Farms

A. Selection of Farms and Animals

From earlier work done at Macdonald College, (Gibbs, unpublished data) a number of farms in Quebec and Ontario were known where lungworm disease had been a problem in past years. Additional information on parasitic disease (particularly on parasitic bronchitis) on these farms was obtained from the attending veterinarians before launching the present studies. Some of these farms were then selected for epidemiological investigations on *D. viviparus* infection based on the following criteria:

1. *D. viviparus* infection was prevalent on these farms.
2. The pasture management and animal husbandry practices were broadly similar to those generally followed in this part of Canada.
3. They had medium-sized herds, with animals of all age groups, that is, cows, yearlings and calves.
4. The farmers were willing to co-operate in the programme.

(a) General History of the Farms Surveyed

Five farms, A. B. C, D and E in Eastern Ontario, and one, F, in the Eastern Townships of Quebec were selected for study.

The farms in Eastern Ontario were situated in a low-lying area with poorly-drained pastureland, an area which was often inundated

in spring with flood water from an adjacent river. All the farms had permanent calf pastures close to the barn on which the winter and spring calves were pastured during the grazing season (May to November). The pastures for the adult animals were often ploughed and reseeded. The farms had medium-sized herds, of approximately 75 to 150 animals each, which were maintained under semi-intensive conditions with poor to good management. The herds were largely self-contained and purchase of animals from outside was rare.

On the farm in the Eastern Townships of Quebec (F), about 400 Jersey cattle were maintained. The owner had separate barns and permanent pastures for calves, yearlings and cows. Mixing of animals of different age groups was avoided. The grazing land which was used for calves and yearlings was very poorly drained and had a pond as a source of drinking water.

Winter conditions in both these areas are severe and the animals were housed generally from late November to the following mid-May. While inside, the adult animals were stanchioned. However, there was no uniform practice for housing of the young animals which varied from tying them individually to putting them into box-stalls singly, or in groups.

On all these properties, gastrointestinal parasitism was prevalent in the calves often leading to severe helminthosis which necessitated treatment. On the other hand, the adult stock appeared

to thrive and did not show evidence of parasitic problems other than low fecal nematode egg counts. According to the owners, they had not experienced a serious lungworm problem for a few years prior to the visit, although occasionally, during the fall, they had noticed some calves and yearlings coughing while on pasture. These animals usually improved after a few weeks and the coughing ceased. At the time of inspection, it was noticed that many calves were weak, emaciated, stunted and a few showed clinical signs suggestive of lungworm disease. On one of these farms, the loss of calves due to this disease was reported to have been as high as 50 percent. However, the adult stock appeared to be free from the disease.

(b) Selection of Animals

Approximately 15 percent of the total cattle population, on each of the farms, covering animals of different age groups, were selected for the study. Some of these animals already had lungworm infection (diagnosed by fecal examination) and the rest had clinical symptoms suggesting that they were also suffering from parasitic disease. The animals included in this study were identified by plastic necktags.

B. Techniques Used for Detecting D. viviparus Infection

(a) Collection of Fecal Samples

It was planned to sample the animals at approximately monthly intervals for one annual cycle (October, 1967, to October, 1968) with

the object of studying the pattern of D. viviparus infection under normal husbandry practices. Fecal samples were collected from individual animals per rectum, care being taken between samples to remove any residual fecal material from the hand in order to prevent intermixing of samples. The samples, roughly 25 gms, were kept in individually labelled, screw-capped bottles and refrigerated until examined for evidence of D. viviparus infection.

(b) Fecal Examination

Fecal examinations were carried out based on the technique of Parfitt (1955). The procedure was:

1. Ten gms of feces were weighed and placed in a four-ounce wide-mouth bottle with 75 ml of water. The fecal material was finally broken down by shaking the bottle.
2. The fecal mixture was washed through a 60 mesh screen (Fisher's U.S. Standard Sieve Series No. 60) using a strong water jet. The total suspension was then made up to 500 ml which was stored at $4^{o}C$ overnight.
3. The supernatant was decanted and the residue was resuspended in saturated sugar solution to make it to 60 ml. This was poured into four centrifugation tubes of 15 ml capacity each, until the meniscus was convex. A cover slip was placed on each tube.
4. The sample was centrifuged at 1,000 r.p.m. (International Centrifuge, Universal Model, UV. International Equipment Co., Boston, U.S.A.) for three minutes.

5. The coverslips were gently removed and washed in a petri dish in 10 ml of water.
6. The washings were examined under a compound microscope for D. viviparus larvae.

(c) Postmortem Examination

Lungs were examined by the techniques of Jarrett and Sharp (1963) and Michel and MacKenzie (1965) for the presence of mature and immature D. viviparus. The procedure used was as follows:

1. The lungs were cut into their seven lobes and each lobe was opened down to the finest bronchioles with scissors.
2. All the visible D. viviparus were picked up with a pair of entomological forceps and were preserved in 70 percent alcohol.
3. The lobes were then immersed with open bronchi and bronchioles facing downwards into a pail containing four litres of warm (37°C) salt water (1.0 percent NaCl) to prevent bursting of the worms. The lobes were shaken and massaged and left overnight in an incubator (37°C) to facilitate migration of larvae out of the lungs.
4. Next day, the lungs were removed from the pail and the fluid was passed through a stack of three sieves (Fisher's U.S. Standard Sieve Series Nos. 60, 100 and 200). The residue on the sieves was washed, placed in separate bottles and allowed to stand until the supernatant fluid had cleared. This was gently decanted off and the residue was examined under the stereoscopic microscope.

C. Detection of Potential Carrier Animals

(a) Fecal Examination

Approximately monthly fecal examinations from October, 1967, to October, 1968, were carried out on selected animals on all six farms to detect D. viviparus larvae in the feces. Animals which started reshedding the following spring, after being negative during the winter, were classified as carriers.

(b) Pasturing Potential Carriers and Parasite-Free Calves Together

On farm A, lungworm carrier animals were detected during the fall of 1967 and spring of 1968, therefore, four parasite-free calves were grazed from June 20 to August 20, 1968, along with the carriers on a large rough pasture of unknown lungworm status (about 50 acres) to determine whether transfer of infection to the parasite-free calves would occur. The test calves were held on this pasture for two months. In mid-August, 1968, following removal of the four parasite-free calves, another pair of parasite-free calves were grazed with this group of animals (carriers) for a similar period for the same reason. The levels of infection acquired by these two groups of calves were also used to indicate the degree of pasture infestation during the early and late grazing season. After removal of the test calves from the pasture they were held inside, under parasite-free conditions, for four weeks and examined (fecal and postmortem) for D. viviparus infection.

On farm D, the owner grazed his lungworm-free calves with six potential carriers on a previously contaminated overwintered pasture. Fecal examinations were done on these calves to detect D. viviparus infection.

D. Overwinter Survival of D. viviparus Larvae

(a) On Pastures

Two farms, one in Eastern Ontario (A) and the other in the Eastern Townships of Quebec (F) were selected to test the possible overwinter survival of D. viviparus larvae. The pastures, approximately four acres in size, had stands of mixed timothy and clover hay and were poorly drained.

Both the pastures were heavily contaminated with D. viviparus larvae by naturally infected calves during the summer and fall of 1967. They were not grazed from November, 1967, until April, 1968. In May, 1968, two parasite-free calves ("overwintering tracers") were put onto each pasture before they had been grazed by any of the farmer's cattle. The tracers were allowed to graze for three weeks then brought inside and held under parasite-free conditions for four weeks. They were then examined (fecal and postmortem) for D. viviparus infection.

(b) Inside the Barn

On farm C, over the summer of 1968, six calves were kept in a barn which had held lungworm infected calves during the fall of

1967. Fecal examinations were carried out on these calves to determine whether there had been any acquisition of lungworm larvae.

E. Seasonal Worm Population Fluctuations in Permanent and Tracer Calves

Following the removal of the "overwintering tracers" on farm A, a "permanent" group of eight parasite-free calves along with the farmer's eight spring calves (lungworm-free) were put onto the same previously infected, overwintered pasture. At approximately monthly intervals (Table I), except in September, 1968, two of the "permanent" calves were removed and held inside for four weeks, under conditions which precluded accidental parasitic infection. Fecal examinations and autopsy were the criteria used to detect lungworm infection.

Two additional parasite-free calves ("tracers") were put onto the same pasture at monthly intervals, except in September, 1968, removed after one month, held for a further month inside under parasite-free conditions, killed and examined to study the availability of lungworm larvae on pasture during different months.

Monthly fecal examinations were carried out on the farmer's eight calves from June to November, 1968, for evidence of lungworm infection.

Table I. Experimental design for a study of seasonal worm population fluctuations in permanent and tracer calves on Farm A.

	May 1968	June 1968	July 1968	Aug. 1968	Sept. 1968	Oct. 1968	Nov. 1968	Dec. 1968
Permanent Calves								
Farmer's calves	0	8	8	8	8	8	8	0
Exp. Permanent calves out onto pasture	0	8	0	0	0	0	0	0
Brought in	0	0	2	2	0	2	2	0
Necropsied	0	0	0	2	2	0	2	2
Tracer Calves								
Out onto pasture	2	2	2	2	0	2	0	0
Brought in	2	0	2	2	0	2	2	0
Necropsied	0	2	0	2	2	0	2	2

Experimental Studies at Macdonald College

A. The Animals

For both the field and experimental work, parasite-free calves two to three months of age were used. The procedure used to raise parasite-free calves was as follows: young calves (approximately one week old) of any breed or sex were purchased and then housed under conditions designed to prevent accidental parasitic infection. The calves were fed milk replacer (Bovo Milk Replacer, Co-op Federee, Montreal, Quebec) until weaning. They were gradually switched onto calf starter (16 percent Protein Grower, Robin Hood Flour Mills, Quebec) with good quality hay fed ad lib. The "tracer" calves were raised in batches so that they were of a comparable age when used. If any of the calves, while inside or on pasture, became sick (other than prasitic disease) they were treated accordingly. The calves were regularly examined using Parfitt's (1955) technique for evidence of parasitic infection before using them for experimental work.

B. Culture of Larvae

(a) Culture of Larvae from Infected Feces

1. Feces from infected source calves were collected per rectum.
2. About 200 gms of feces was wrapped in a double layer of cheesecloth and then placed in a Baermann apparatus (Noble and Noble, 1962) containing lukewarm water. The

apparatus was held at room temperature for at least 12 hours to allow the larvae to migrate out of the fecal material.

3. About 30 ml of fluid was drawn off, washed and centrifuged repeatedly until the supernatant was clear.
4. The sediment was resuspended in about 40 ml of distilled water in a conical test tube and aerated continuously (by an aquarium pump) at room temperature for seven days.
5. At the end of this period, most of the larvae had reached the infective stage (Plates I and II).

(b) Culture of Larvae from Gravid Female

1. Fresh gravid *D. viviparus* females were collected at the abbatoir and brought to the laboratory as soon as possible.
2. The females were crushed gently through a 400 mesh screen (Canadian Standard Sieve Series, Aperture .037 mm) over a petri dish containing distilled water.
3. The eggs so obtained were washed two or three times in distilled water and the sediment was resuspended in about 40 ml of distilled water.
4. The eggs were then aerated for eight to nine days at room temperature ($25^{\circ}C$), after which, the infective larvae were harvested and stored.

C. Storage of Larvae

1. The infective larvae were washed and centrifuged until the supernatant fluid had cleared.

Plate I. Third-stage larvae of D. viviparus. Clear and translucent larvae are alive (1), whereas, those which are granular and opaque are dead (2).

Plate II. Third-stage larva of D. viviparus with the characteristic two sheaths (1 & 2).

2. The sediment was resuspended in distilled water so that the final concentration of larvae was approximately 500 to 1,000 larvae/ml.
3. About 10 to 15 ml of larval suspension was poured into flat bottles so that the depth of the culture suspension in the bottles did not exceed more than 2 to 3 mm.
4. The bottles were loosely sealed with cotton wool to allow some aeration and stored at 4°C until used.

D. Overwinter Survival of D. viviparus Larvae

(a) Plot Contaminated Experimentally by Spreading Infected Feces

On the Macdonald College farm, a small pasture plot 12' x 20' in size, which had been free of cattle for many years, was selected. From November 14, 1967, to December 1, 1967, feces were regularly collected from lungworm infected calves, thinly spread in shallow trays and kept moist at room temperature to facilitate the development of lungworm larvae to the infective stage. Subsequently, the feces were thoroughly mixed and a few samples of 10 gms each were taken to estimate the total number of infective larvae. The total infected material weighed 20 kg and contained approximately 162,000 infective D. viviparus larvae. In addition, about 68,000 third-stage lungworm larvae cultured in the laboratory were also mixed with the fecal material. On December 1, 1967, the infected feces were evenly spread (in the form of small fecal pats) onto this pasture plot. The paddock remained unoccupied throughout the winter

and spring. In the first week of June, 1968, a parasite-free calf (approximately two months of age) was put onto this plot to determine whether overwinter survival of lungworm larvae had occurred.

(b) Paddocks Contaminated by Animals Infected with D. viviparus

A pasture of about two acres in size, well fenced, adequately drained and with mixed timothy and clover as herbage was selected as the experimental plot. It had not been grazed by cattle for at least 20 years previously. From June to early November, 1968, calves heavily infected with D. viviparus were grazed on it.

The severity of pasture infestation was tested by grazing a pair of parasite-free calves on this pasture during September, 1968. Both calves died within a month due to clinical lungworm disease. The pasture was closed from late November, 1968, until the following May, 1969, at which time, a further two parasite-free calves were placed on it to check the possibility of overwintering of lungworm larvae. The test calves were kept on the pasture for 15 days, brought into the barn and held for an additional 21 days under conditions which prevented accidental parasitic infection. Fecal examination and autopsy were the criteria used to detect D. viviparus infection.

E. Detection of Potential Carrier Animals

(a) Fecal Examination

For this experiment, a group of four calves were selected

which, on the basis of fecal larval yield, were heavily infected with D. viviparus during the grazing season of 1968. These calves were brought inside on November 12, 1968, and were housed under parasite-free conditions. They were fed a commercial feed (16 percent Protein Calf Starter Grower, Robin Hood Flour Mills, Quebec) while inside. Regular weekly fecal examinations were conducted from November 12, 1968, to July 30, 1969, and later postmortem examinations were done on July 30, 1969, for evidence of persisting lungworm infection.

(b) Pasturing Potential Carriers and Parasite-Free Calves Together

A paddock of approximately one-half acre which had not been grazed by cattle within the last 10 years was selected. The animals used in this study were experimentally infected in October, 1967, with D. viviparus and were held inside on concrete floors. In two of them, Nos. 81 and 67, the infection became patent and they continued to shed larvae until November 14, 1967, and January 14, 1968, respectively. Thereafter, both these calves remained negative throughout the period of the experiment. The infection never became patent in the third animal (No. 61). On June 10, 1968, these animals, along with two parasite-free calves, were put out onto the above mentioned pasture. Regular weekly fecal examinations followed by postmortem examinations, were done on the test calves for evidence of lungworm infection.

F. Seasonal Worm Population Fluctuations in Permanent and Tracer Calves

Experiments I and II were designed to varify the results of the seasonal worm population fluctuations observed under field conditions. The experimental design is presented in Table II.

Experiment I: The pasture used in this experiment was the same as that used to demonstrate overwinter survival of lungworm larvae. During May, 1969, a group of 12 parasite-free calves of two to three months of age, designated as the "permanent group," along with four potential lungworm carrier animals, were put onto this pasture plot. Two of the permanent calves were removed each month, from June to November, and held inside under parasite-free conditions for four weeks. Regular weekly fecal, followed by postmortem worm counts,were done to determine the number of mature and immature D. viviparus in the lungs.

Experiment II: In addition, two parasite-free calves, designated as the "tracer group," were grazed along with the permanent group calves for two weeks each month (May to November) and were then removed and held inside for four weeks under conditions which precluded parasitic infection. Regular fecal and later postmortem worm burdens were estimated as described earlier for the permanent calves. The object of putting two tracer calves each month with the permanent calves was two-fold. First, to determine the degree of pasture infestation, and thus the availability of infective larvae to the calves on

Table II. Experimental design for a study of seasonal worm population fluctuations in permanent and tracer calves, at Macdonald College.

Expt. Calves	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Perm. calves put out	12	0	0	0	0	0	0	0
Perm. calves brought in	0	2	2	2	2	2	2	0
Month killed	0	0	2	2	2	2	2	2
Tracer calves put out	2	2	2	2	2	2	2	0
Tracer calves brought in	2	2	2	2	2	2	2	0
Month killed	0	2	2	2	2	2	2	2

pasture during the different months, and second, to study the behaviour of D. viviparus in so-called resistant calves (permanent group) and susceptible calves (tracer group) during the entire grazing season.

G. Seasonal Worm Population Fluctuations in Calves Infected Experimentally

It has been suggested by Armour et al. (1969a,b) that the seasonal inhibition observed in O. ostertagi in parasite-free calves could be due to variations in the larvae on pasture. It is conceivable that if the source of larvae was "immune" calves (calves that had received repeated exposure to larvae) then the larvae might not develop normally and show a high percentage of inhibited forms. The idea of these two experiments (III and IV) was to see if larvae derived from "high immunity animals" (the permanent calves) would behave differently from those obtained from "low immunity animals" (the tracer calves) when given to susceptible calves.

Source of Infective Larvae: Sufficient larvae for the whole experiment were cultured within one week from the permanent calves in August, 1969, by the technique described earlier. These infective larvae were stored in shallow water at 4 ^{o}C to be used over the experimental period (August, 1969, to April, 1970).

D. viviparus larvae were also cultured in sufficient numbers for the whole experiment from the tracer calves in October , 1969,

and stored in shallow water at 4°C to be used in the experiment (November, 1969, to April, 1970).

The experimental design for Experiments III and IV is given in Table III.

Procedure for Experiment III: Two parasite-free calves, the contemporaries of those used as "tracers" in the field trial, were infected each month from August, 1969, to April, 1970, with larvae from the "permanent" calves. The total infective dose (approximately 5,600 larvae) was divided into eight equal parts containing about 700 larvae each. The calves were dosed orally with a metal syringe on alternate days over a period of 15 days. The calves were held for 28 days post-infection under conditions designed to prevent accidental parasitic infection. Regular fecal examinations, followed by necropsy, were the criteria used to estimate D. viviparus infection.

Procedure for Experiment IV: Two parasite-free calves were infected each month from November, 1969, to April, 1970, with larvae cultured from the "tracer" calves. The technique for infecting these calves and their subsequent fecal and postmortem examination was the same as described in Experiment III.

Experiment V: In order to determine whether the age of the infective larvae had any effect on the subsequent development of the parasitic stages, larvae of two different ages were used in Experiment V.

Table III. Experimental design for a study of the phenomenon of inhibited development of *D. viviparus* in parasite-free calves.

a. Calves infected with larvae cultured from "permanent" calves. Experiment III.

	Aug. 1969	Sept. 1969	Oct. 1969	Nov. 1969	Dec. 1969	Jan. 1970	Feb. 1970	Mar. 1970	Apr. 1970	May 1970
No. of calves infected	2	2	2	2	2	2	2	2	2	0
Month necropsied	0	2	2	2	2	2	2	2	2	2

b. Calves infected with larvae cultured from "tracer" calves. Experiment IV.

No. of calves infected	0	0	0	2	2	2	2	2	2	0
Month necropsied	0	0	0	0	2	2	2	2	2	2

In 1969, D. viviparus larvae were cultured from naturally infected calves during the third week of September and stored at 4°C in the refrigerator until used. Five parasite-free calves were infected orally with a single dose of approximately 7,500 larvae per calf on November 11, 1969, when the larvae were approximately six to eight weeks old.

In 1970, D. viviparus larvae were cultured from naturally infected calves during October (15 to 22) and refrigerated at 4°C until November 6. Five parasite-free calves were infected per os with a single dose of approximately 12,000 larvae per calf when the larvae were roughly 15 days old.

The calves were held under parasite-free conditions for four weeks to allow maturation of the larvae. Fecal examinations and autopsy were the criteria used to detect D. viviparus infection.

H. Identification and Description of Inhibited Stages of D. viviparus

Calves naturally or experimentally infected with D. viviparus were held under parasite-free conditions for at least four weeks after they were removed from the source of infection. At the end of this period, it was assumed that those larvae which had not attained maturity were inhibited in their development. The calves were necropsied and all the worms from the lungs were recovered using the technique of Michel and MacKenzie (1965). Female worms with eggs in their uteri and male worms with well formed bursae, bursal rays and other accessory organs were classified as adults while the rest were regarded as inhibited stages.

Procedure for the Examination of Immature Stages: Immature worms were collected from the lungs in normal saline. They were then transferred to 70 percent alcohol for 24 hours and later cleared in lacto-phenol. A representative number of larvae were then mounted on microscopic slides and examined under a phase-contrast microscope. The following criteria were used in identification: Male larvae - sheath, cephalic dilatation, mouth parts, genital primordium, length, bursa and accessory parts. Female larvae - sheath, cephalic dilatation, mouth parts, genital primordium, position of vulva, ovary and ovejector, tail and length.

Photography: Appropriate photomicrographs were taken to illustrate the characteristics of late fourth and early fifth immature stages of D. viviparus.

I. Experiment to Test Infectivity to Calves of D. viviparus Obtained from Moose

Live adult D. viviparus were collected from a moose (Alces americanis) killed in Northern Quebec. The adult female worms were kept in physiological saline and refrigerated until brought to Macdonald College. They were then broken up by pressing through a fine wire screen (Canadian Standard Sieve Series, Aperature .037 mm), the eggs collected by centrifugation and washing and then cultured to the infective larval stage by the technique described earlier (Culture of Larvae from Gravid Females).

Two parasite-free calves six weeks of age were respectively given an oral dose of 6,000 and 12,000 of these larvae. These dosage levels would normally have resulted in clinical infections. The calves were held under parasite-free conditions for five weeks before being killed. Repeated fecal examinations were carried out during this period. The calves were then necropsied and their lungs examined for the presence of D. viviparus (Michel and MacKenzie, 1965).

J. Weather Data

Weather data was obtained from the Macdonald College observatory which is located within two miles of the experimental pasture plot.

RESULTS

Field Studies on Farms

1. Age Incidence

(a) Farm A

In order to study the pattern of lungworm infection eight young and 12 adult animals were selected from a herd of approximately 120 for regular fecal examination.

From the results (Table IV) it is evident that a high proportion of young animals examined were positive for lungworm larvae in their feces, furthermore, the number of larvae recovered per 10 gms of feces indicates that the animals were heavily infected. None of the adult animals included in the study were positive for *D. viviparus* infection.

(b) Farm B

Twelve young and two adult animals were selected from a herd of roughly 75 animals to study the pattern of lungworm infection. Fecal and clinical examinations during November, 1967, revealed that eight out of 12 young animals were suffering from severe lungworm disease. It was rather unfortunate that the animals could not be examined during the following two months, thus the pattern of disease was not clear-cut. However, it can be seen in Table V that the subsequent fecal examinations remained consistently negative for any evidence of lungworm infection. Adult animals remained negative for lungworm infection throughout the period of observation.

Table IV. Fecal examinations of young and adult animals on Farm A.

Month & Year	Young		Av. larvae/10gm of feces	Adult		Av. larvae/10gm of feces
	Total Exam.	Total Pos.		Total Exam.	Total Pos.	
Oct/67	4	1	31	0	0	0
Nov/67	8	4	18	12	0	0
Jan/68	8	2	3	12	0	0
Feb/68	8	1	1	12	0	0
Mar/68	8	0	0	11	0	0
May/68	8	3	5	11	0	0
June/68	8	3	3	9	0	0
July/68	8	2	5	12	0	0
Aug/68	8	3	3	9	0	0
Oct/68	8	2	5	12	0	0

Table V. Fecal examinations of young and adult animals on Farm B.

Month & Year	Young		Av. larvae/10gm of feces	Adult		Av. larvae/10gm of feces
	Total Exam.	Total Pos.		Total Exam.	Total Pos.	
Nov/67	12	8	409	2	0	0
Feb/68	7	0	0	2	0	0
Mar/68	7	0	0	2	0	0
May/68	7	0	0	2	0	0
June/68	7	0	0	2	0	0
July/68	7	0	0	2	0	0
Aug/68	6	0	0	1	0	0
Oct/68	7	0	0	2	0	0

(c) Farm C

A screening test in October, 1967, revealed a high incidence of D. viviparus infection in young animals. Therefore, the following month, nine young and eight adult animals were selected from a herd of about 85 animals for regular fecal examination. It is clear (Table VI) that lungworm larvae were not detected in the feces of the young animals during the winter and early spring. It was interesting to note that two out of the nine young animals started reshedding larvae during May, 1968, while still inside the barn. Fecal examinations after May, 1968, remained consistently negative for lungworm larvae. All the adult animals examined on this farm showed no evidence of lungworm infection.

(d) Farm D

A screening test for D. viviparus infection during October, 1967, indicated the possibility of a high incidence of the disease. Therefore, during November, 1967, 18 young and 10 adult animals were selected from a herd of about 150 animals for the study. From the results (Table VII) it is evident that the young animals remained negative for D. viviparus larvae in their feces throughout the period of observation except during July and October, 1968, when only one animal started reshedding. This suggests that the disease had almost disappeared from this farm. Adult animals once again showed no evidence of lungworm infection.

Table VI. Fecal examinations of young and adult animals on Farm C.

Month & Year	Young		Av. larvae/10gm of feces	Adult		Av. larvae/10gm of feces
	Total Exam.	Total Pos.		Total Exam.	Total Pos.	
Oct/67	3	3	7	0	0	0
Nov/67	9	4	25	8	0	0
Feb/68	9	0	0	7	0	0
Mar/68	9	0	0	7	0	0
May/68	9	2	7	7	0	0
June/68	0	0	0	4	0	0
July/68	9	0	0	5	0	0
Aug/68	8	0	0	5	0	0
Oct/68	8	0	0	5	0	0

Table VII. Fecal examinations of young and adult animals on Farm D.

Month & Year	Young		Av. larvae/10 gm of feces	Adult		Av. larvae/10gm of feces
	Total Exam.	Total Pos.		Total Exam.	Total Pos.	
Oct/67	2	2	143	0	0	0
Nov/67	18	0	0	10	0	0
Feb/68	17	0	0	5	0	0
Mar/68	17	0	0	5	0	0
May/68	10	0	0	8	0	0
June/68	7	0	0	8	0	0
July/68	10	1	3	10	0	0
Aug/68	4	0	0	7	0	0
Oct/68	12	1	9	10	0	0

(e) Farm E

During October, 1967, fecal samples were collected from five young and one adult animal which had clinical symptoms similar to parasitic bronchitis. Fecal examination revealed that four out of the five young animals were suffering from lungworm infection. Therefore, in November, 1967, 10 young and 10 adult animals out of a herd of 75 animals were selected for regular fecal examination to study the pattern of D. viviparus infection.

From the results (Table VIII) it is clear that the general pattern of lungworm disease was almost the same as observed on other farms, that is, a high incidence during fall followed by almost negligible incidence during winter and early spring. Once again adult animals remained negative for lungworm infection throughout the period of observation.

(f) Farm F

Thirty-three young and 25 adult animals were selected out of a herd of about 400 animals to study the pattern of lungworm infection. The results (Table IX) show that young animals were positive for the infection during November and December, 1967, and July, 1968, only, which is similar to the pattern observed on other farms. A significant deviation was that one adult animal was found positive for D. viviparus infection during May, 1968. However, the rest of the adult animals remained negative for evidence of lungworm infection.

Table VIII. Fecal examinations of young and adult animals on Farm E.

Month & Year	Young		Av. larvae/10gm of feces	Adult		Av. larvae/10gm of feces
	Total Exam.	Total Pos.		Total Exam.	Total Pos.	
Oct/67	5	4	59	1	0	0
Nov/67	10	1	12	10	0	0
Feb/68	9	0	0	9	0	0
Mar/68	9	0	0	9	0	0
May/68	9	1	3	9	0	0
June/68	9	0	0	8	0	0
July/68	8	0	0	9	0	0
Aug/68	7	2	4	6	0	0
Oct/68	8	0	0	7	0	0

Table IX. Fecal examinations of young and adult animals on Farm F.

Month & Year	Young		Av. larvae/10gm of feces	Adult		Av. larvae/10gm of feces
	Total Exam.	Total Pos.		Total Exam.	Total Pos.	
Nov/67	33	7	27	25	0	0
Dec/67	31	3	5	23	0	0
Jan/68	30	0	0	19	0	0
Apr/68	32	0	0	19	0	0
May/68	16	0	0	17	1	8
July/68	19	1	3	12	0	0

When the results of all the farms (A, B, C, D, E and F) are pooled, a clear pattern of D. viviparus infection under natural conditions is evident. The results (Table X) obtained are summarized as follows:

(a) The incidence of lungworm infection was much higher in young than in adult animals.

(b) The young animals were usually only lightly infected.

(c) From the fecal examinations it can also be deduced that in most of the young animals the infection cleared up after they were brought inside.

(d) A number of young animals which were positive for lungworm infection during the fall started reshedding D. viviparus larvae in spring on one or more occasions after having been negative for extended periods.

(e) In the case of the adults, only once (May, 1968) was a positive result obtained.

(f) D. viviparus infection was distinctly seasonal with the highest incidence during the fall.

2. Overwinter Survival of D. viviparus Larvae on Farm Pastures and Inside Barns

Repeated fecal examinations of the "overwintering tracer" calves on Farm A and F were negative for larvae in the feces, and no mature or immature D. viviparus were detected in the lungs during postmortem examination (Table XI). In August, 1967, the attending veterinarian on Farm A diagnosed a fatal lungworm infection on the

Table X. Fecal examinations on all farms (pooled data).

Month & Year	Young		Percent Positive	Adult		Percent Positive
	Total Exam.	Total Pos.		Total Exam.	Total Pos.	
Oct/67	14	10	71	1	0	0
Nov/67	90	24	27	67	0	0
Dec/67	31	3	10	25	0	0
Jan/68	38	2	5	31	0	0
Feb/68	50	1	2	35	0	0
Mar/68	50	0	0	34	0	0
Apr/68	32	0	0	19	0	0
May/68	59	6	10	54	1	2
June/68	31	3	10	31	0	0
July/68	61	3	5	50	0	0
Aug/68	33	5	15	28	0	0
Oct/68	43	3	7	36	0	0

Table XI. Overwinter survival of lungworm larvae on farm pastures and inside barns.

Location	No. of Calves	Date when Pastured	Date brought Inside	Criteria	Results
Eastern Ontario					
Farm A	2	15/5/68	30/5/68	Fecal exam. Necropsy	Negative
Farm E	8	June/68	Nov/68	Fecal exam.	Negative
Farm B	11	June/68	Nov/68	Fecal exam.	Positive
Farm C	8	Lungworm-free calves were housed in a barn which had had *D. viviparus* infected animals previously.		Fecal.exam.	Negative
Eastern Townships					
Farm F	2	15/5/68	30/5/68	Fecal exam. Necropsy	Negative

basis of postmortem examination of a month-old calf which had never been out from the stable. However, on Farm C, where calves were overwintered in a barn that had previously held infected calves, the test calves remained negative for lungworm larvae in the feces. Similarly, on Farm E, where only fecal examinations were conducted on the "overwintering tracer" calves grazed on previously contaminated, overwintered pasture, no evidence of lungworm infection was noted, indicating once again that the larvae did not overwinter.

On Farm B, the results were somewhat contradictory. On this farm, seven out of 11 lungworm-free calves picked up D. viviparus infection while grazing on a previously contaminated overwintered pasture. However, there was a chance of contamination from cattle in an adjacent pasture.

3. Detection of Potential Carrier Animals

(a) Fecal Examination

Repeated fecal examinations over a period of one annual cycle on animals (Farms A, B, C, D, E and F) showed that some of the animals whose feces contained larvae during the late fall and early winter and had stopped shedding for the rest of the winter, started reshedding during spring while still inside the barn (Table XII). Of particular interest was the observation that larval reshedding by most of the animals was well synchronised (occurred in May). It is also obvious that the number of larvae excreted by these animals was very few.

Table XII. Fecal examinations of carrier animals. Number of larvae/10gm of feces.

No. of Animal	Oct 1967	Nov* 1967	Dec 1967	Jan 1968	Feb 1968	Mar 1968	Apr 1968	May 1968	June 1968	July 1968	Aug 1968	Sept 1968	Oct 1968
68	31	13	x	0	0	0	x	0	0	2	1	x	9
72	0	36	x	0	0	0	x	8	2	0	0	x	0
70	0	0	x	3	0	0	x	0	1	0	0	x	0
71	0	0	x	0	1	0	x	2	6	0	0	x	0
46	1	0	x	0	0	0	x	3	0	0	0	x	0
42	2	0	x	0	0	0	x	10	0	0	0	x	0
20	134	0	x	0	0	0	x	0	3	0	0	x	0
16	9	0	x	0	0	0	x	3	0	0	0	x	0
2	16	0	x	0	0	0	x	0	0	2	0	x	0

x - not examined.

* - all stabled from Nov/67 to May/68 inclusive.

(b) Pasturing Potential Carrier Animals and Parasite-Free Calves Together

Results on Farm A revealed (Table XIII) that on both occasions, namely, in June and August, 1968, parasite-free calves contracted lungworm infection when pastured with carrier animals. It is interesting to note that although only a few of the carriers started reshedding and then only low numbers of larvae per 10 gm of feces, nevertheless, the test calves picked up severe lungworm infection.

The results (Table XIII) on Farm D, in which lungworm-free calves contracted infection while grazing with carriers, were not conclusive as there was a possibility of overwinter survival of larvae on pasture.

Experimental Studies at Macdonald College

1. Overwinter Survival of D. viviparus Larvae on Pasture

(a) Survival on Experimentally Contaminated Pasture

A young, parasite-free, Holstein calf was put onto the experimentally infected, overwintered pasture on June 6, 1968. Unfortunately, the calf died on June 15, 1968, due to reasons other than parasitic bronchitis. Postmortem examination of the lungs for mature or immature worms proved negative for lungworm infection.

(b) Survival on Paddocks Contaminated by Animals Infected with D. viviparus

The results (Table XIV) of the experiment conducted during

Table XIII. Results of pasturing potential carrier animals and parasite-free calves on transfer of *D. viviparus* infection.

Location	No. of Calves	No. of (Potential)* Carrier Animals	Date when Calves Pastured with Carriers	Criteria	Results
Farm A	4	20	20/6/68	Fecal exam.	Positive
Farm A	2	20	20/8/68	Fecal exam.	Positive
Farm D	9	6	15/6/68	Fecal exam.	Positive

* Based on previous history.

Table XIV. Overwinter survival of *D. viviparus* larvae on paddocks contaminated by animals infected with *D. viviparus*.

Calf Number	Date put out onto overwintered infected pasture	Date Removed	Date Positive for Lungworm	Postmortem Worm Count
73	12/5/69	26/5/69	13/6/69	17
75	12/5/69	26/5/69	13/6/69	28

1968-69 showed that lungworm larvae had overwintered on this pasture and were infective. Repeated fecal examinations and later postmortem examinations were carried out on the calves and revealed patent lungworm infections in both animals.

2. Detection of Potential Carrier Animals

(a) Regular Fecal and Autopsy Examination

Regular fecal examinations (Figure 1) showed that all the animals were suffering from lungworm disease during the summer and fall of 1968. However, during the winter, the fecal examinations failed to reveal any patent lungworm infections. It was, however, interesting to note that two out of four animals started reshedding small numbers of larvae during the spring of 1969. Postmortem examination (Table XV) of the animals on July 30, 1969, was not in keeping with the fecal examination results obtained during the spring. One out of four animals had two mature worms but the remaining calves were negative for adult worms. The discrepancies between the fecal examination and postmortem findings could be due to the fact that the postmortems were performed at a time when the fecal examinations were negative for D. viviparus larvae.

(b) Pasturing Potential Carriers and Parasite-Free Calves Together

Regular (twice-weekly) fecal examinations of the two test calves revealed patent lungworm infections in both of them after about 60 days with the carriers. Subsequently, at postmortem

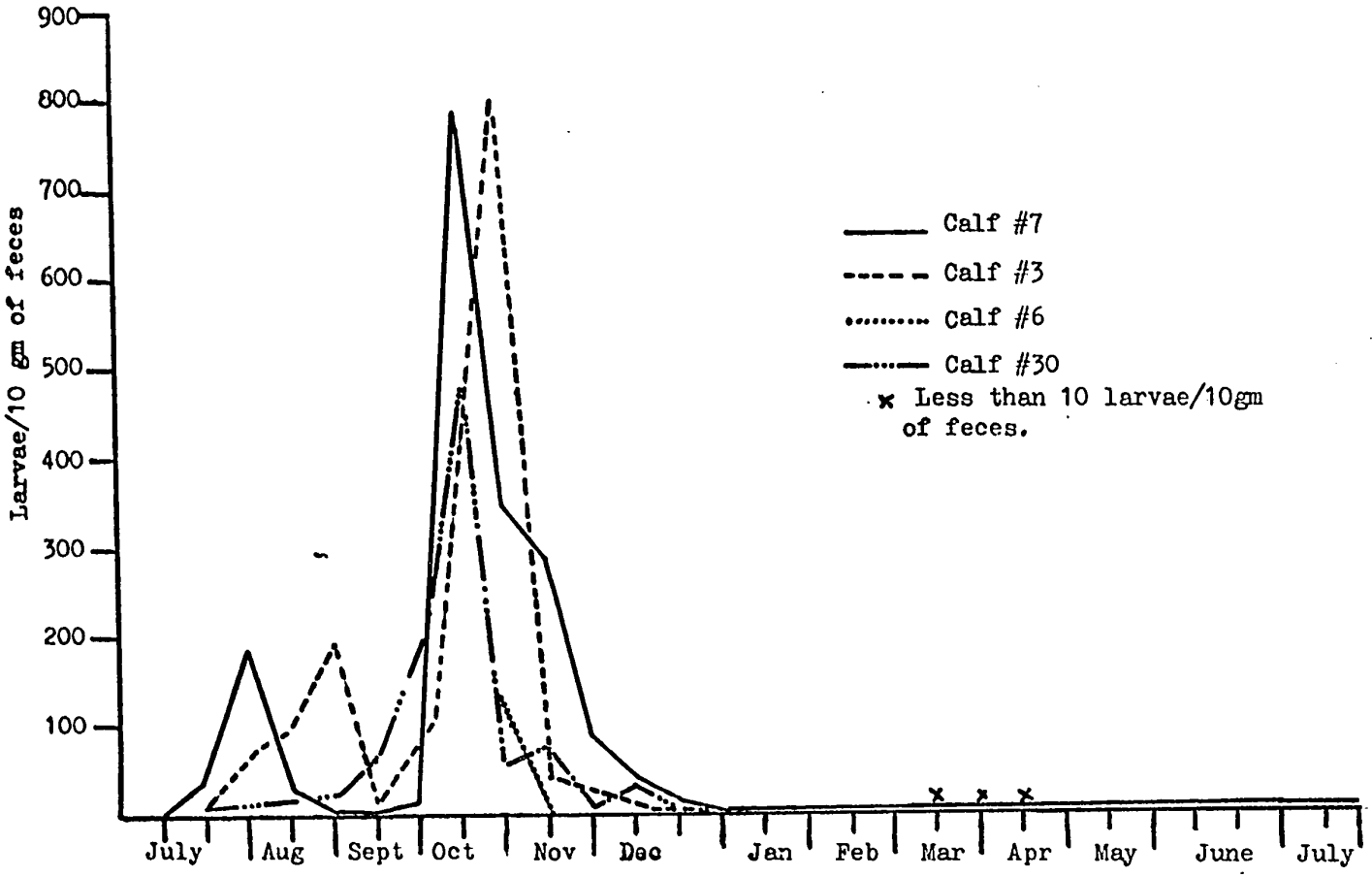

Figure 1. Fortnightly Fecal Larval Counts of Potential Carrier Animals at Macdonald College Farm During 1968 and 1969.

Table XV. Regular fecal followed by postmortem examinations of potential carrier animals for the persistence of *D. viviparus* infection.

Calf No.	Results of Fecal Examinations				Postmortem Examination	
	Summer	Fall	Winter	Spring	Mature	Immature
7	Pos.	Pos.	Neg.	Neg.	2	7
30	Pos.	Pos.	Neg.	Neg.	0	0
6	Pos.	Pos.	Neg.	Pos.	0	4
3	Pos.	Pos.	Neg.	Pos.	0	0

examination, 21 and 17 mature D. viviparus respectively were recovered from them. It is evident (Table XVI) that the carrier animals were able to transfer the disease to susceptible calves when they were pastured together.

3. Seasonal Worm Population Fluctuations in Permanent and Tracer Calves on Pasture (Macdonald College)

(a) Permanent Calves (Expt. I)

(i) Fluctuations in Larval Counts:- The results (Figures 2 and 3, Table XVII) of regular weekly fecal examinations of the permanent calves revealed an increasing number of larvae per 10 gm of feces from July until the first half of September, 1969, (except on August 29, 1969). The highest mean number of larvae, 435 per 10 gm of feces, was recorded on September 5, 1969. From October to December, 1969, a progressive reduction in the larval yield was evident in the groups concerned and the count became almost negative from October 17 to December 26, 1969. The calves suffered clinically from D. viviparus infection for a relatively short period (August and September). A sudden sharp decline in the larval yield occurred on September 26, 1969, which might be analogous to the "self-cure" phenomenon observed in other host-parasite relationships. When the monthly fecal larval counts of the individual pairs of calves were plotted (Figure 2) they showed the same two peaks which although they varied in magnitude from one pair to another, were well synchronised in timing. This indicates

Table XVI. Results of pasturing potential carrier animals and parasite-free calves on transfer of *D. viviparus* infection at Macdonald College.

Location	No. of Carrier Animals	Pasture Size	No. of Test Calves	Date when Pastured with Carriers	Date Pos. for *D. viviparus* in feces	Post-Mortem Worm Count
Macdonald College	3	one-half acre	2	10/6/68	8/8/68	21 & 17

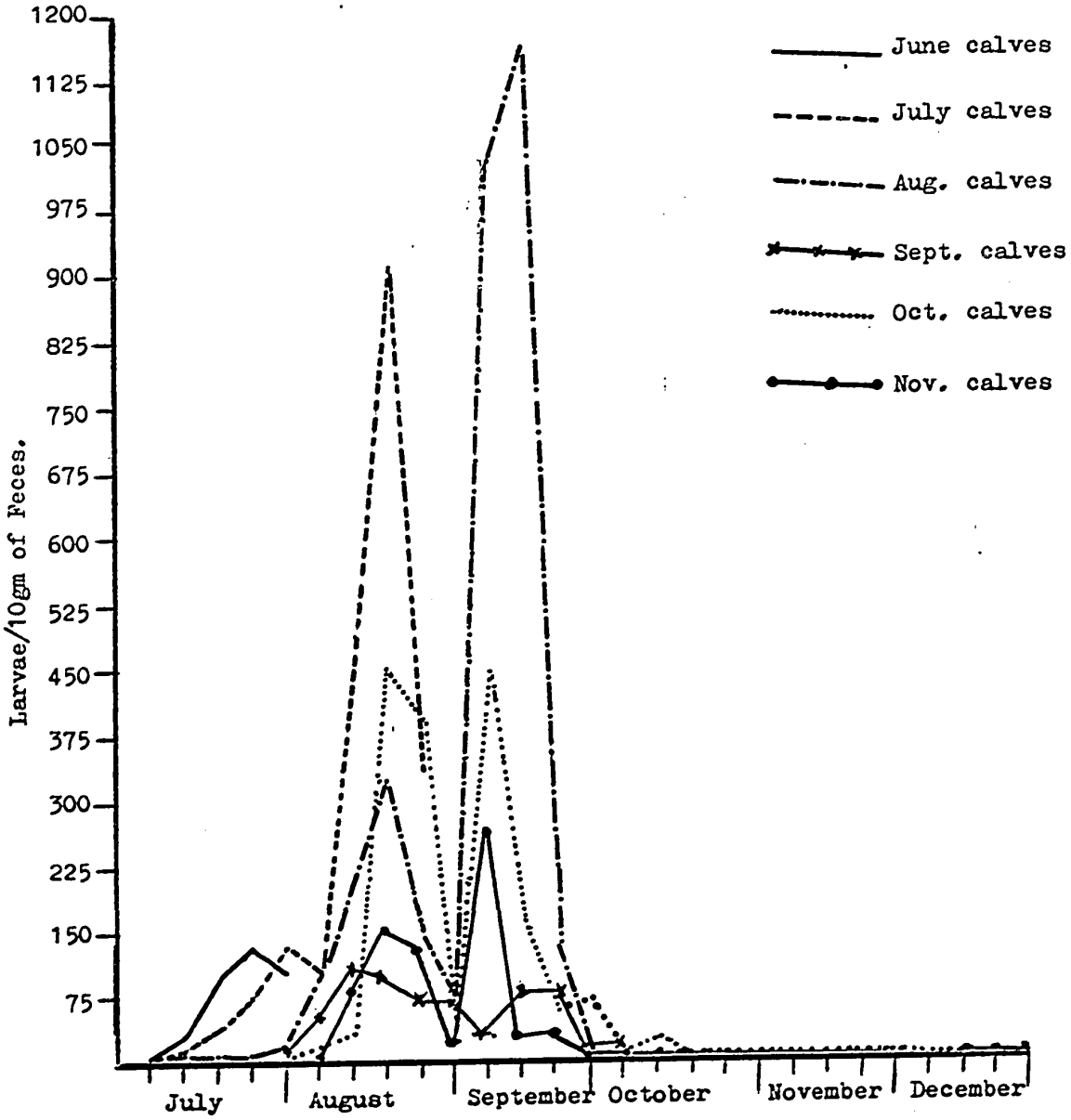

Figure 2. Average Weekly Fecal Larval Counts Per Pair of Permanent Calves During 1969. (Expt. I).

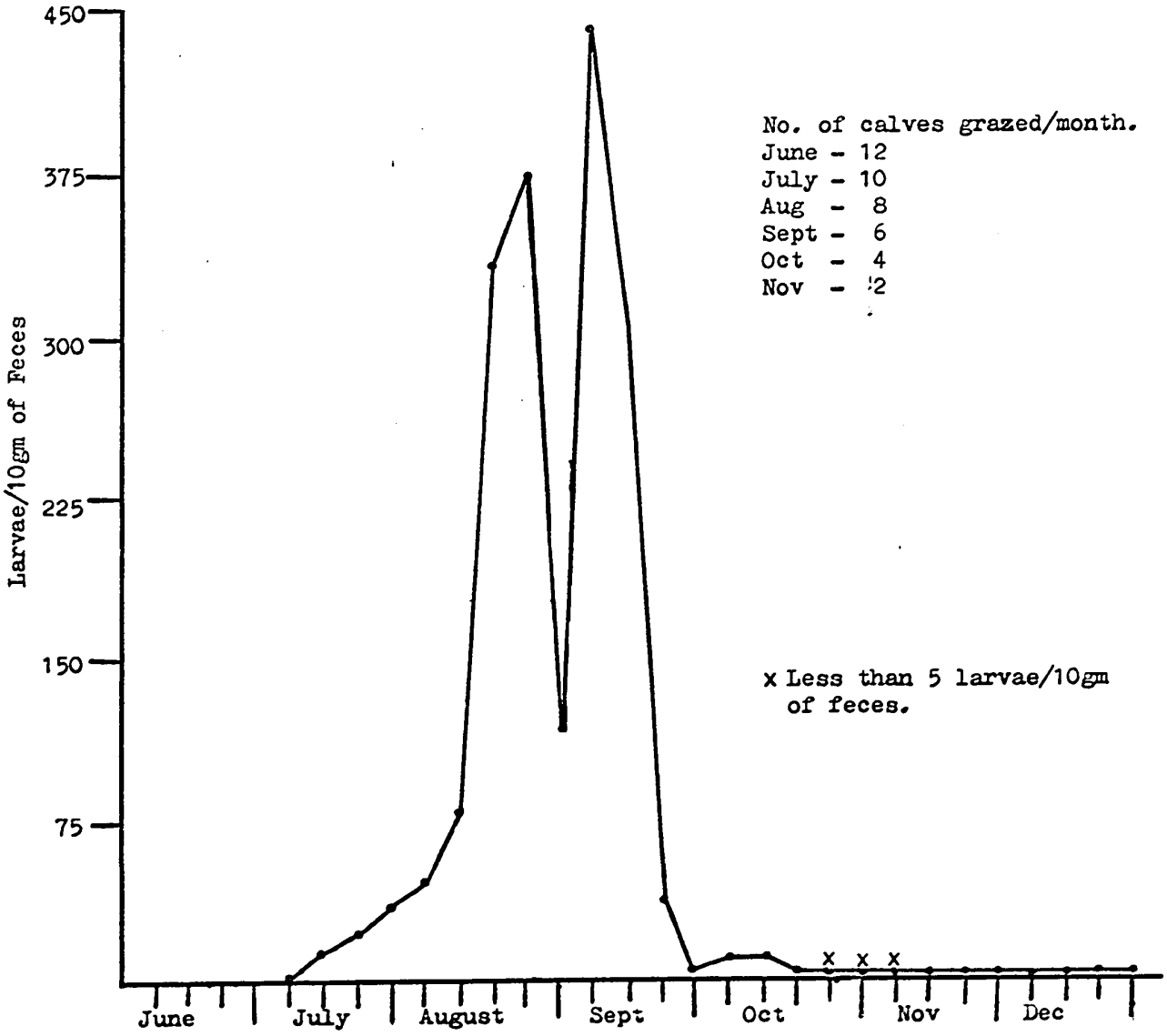

Figure 3. Weekly Fecal Larval Counts on Permanent Calves During 1969 (Pooled Data). (Expt. I).

Table XVII. Weekly faecal larval count of permanent calves (larvae per 10 gm of faeces). Expt. I.

Calf No.	Put Out	Brought In	Killed	July				August					September			
				1	2	3	4	5	6	7	8	9	10	11	12	13
80	26/5/69	2/7/69	30/7/69	3	26	45	165									
93	26/5/69	2/7/69	30/7/69	4	32	63	91									
70	26/5/69	1/8/69	29/9/69	0	0	7	26	38	103	378	400	270				
88	26/5/69	1/8/69	29/9/69	2	23	58	118	218	96	490	1520	400				
78	26/5/69	1/9/69	29/9/69	1	6	2	1	8	68	181	127	21	1520	735	29	7
87	26/5/69	1/9/69	29/9/69	0	0	0	2	15	129	458	252	128	533	1540	225	7
81	26/5/69	1/10/69	29/10/69	2	4	3	3	6	51	232	150	8	7	1	3	0
86*	26/5/69	1/10/69	15/10/69	3	33	26	23	121	132	56	150	125	22	4	13	0
79	26/5/69	31/10/69	28/11/69	0	0	0	0	23	58	900	765	98	856	19	3	2
82	26/5/69	31/10/69	28/11/69	4	5	6	8	6	38	286	96	163	26	55	10	3
77	26/5/69	28/11/69	29/12/69	0	0	0	0	11	59	115	88	11	7	2	0	0
92	26/5/69	28/11/69	29/12/69	2	6	3	4	5	42	216	180	22	511	46	58	1

*Died 15/10/69.

Contd.

Table XVII. Contd.

Calf No.	Put Out	Brought In	Killed	October					November				December			
				14	15	16	17	18	19	20	21	22	23	24	25	26
80	26/5/69	2/7/69	30/7/69													
93	26/5/69	2/7/69	30/7/69													
70	26/5/69	1/8/69	29/9/69													
89	26/5/69	1/8/69	29/9/69													
	26/5/69	1/9/69	29/9/69													
87	26/5/69	1/9/69	29/9/69													
81	26/5/69	1/10/69	29/10/69	32	0	0	0									
86	26/5/69	1/10/69	29/10/69	6	40											
79	26/5/69	31/10/69	28/11/69	0	0	0	0	0	0	0	0	0				
82	26/5/69	31/10/69	28/11/69	0	0	0	0	0	1	0	0	0				
77	26/5/69	28/11/69	29/12/69	0	0	0	0	0	0	0	0	0	0	0	0	0
92	26/5/69	28/11/69	29/12/69	8	1	1	1	0	0	0	0	0	0	0	0	0

that the trend in the rise and fall in fecal larval output among the different pairs followed a stereotyped pattern.

(ii) Fluctuations in Mature and Immature Worm Populations:- The numbers of mature and immature worms recovered during autopsy from the permanent calves for the different months is given in Table XVIII. The mean minimum (61) and mean maximum (268) number of adult D. viviparus were recorded from the calves autopsied in July and August, 1969, respectively. It appears that the average worm burden of the calves killed during August, September and October, 1969, were comparable. Autopsy examination of the calves slaughtered during November and December were negative for mature worms.

The results also show that all the worms recovered from the calves from June to August, 1969, were mature. The first sign of arrested development in D. viviparus was noticed in the permanent calves in September when 23 percent of the total worm population was inhibited. However, 100 percent inhibited D. viviparus were recorded in the calves in October and November, 1969 (Figure 4).

A comparison (Plate III) of the size of adult worms collected from the permanent calves of different months (June to September) revealed some interesting differences in size and fecundity. D. viviparus collected from the permanent calves in August and September, 1969, were distinctly stunted when compared with their counterparts in June and July. Although an average of 148 and 163 mature lungworms were recovered from the calves in August and

Table XVIII. Fluctuations in mature and immature worm populations in permanent calves. Expt. I.

Calf No.	Put Out	Brought In	Fecal Examination	Killed	Worms at Necropsy		Group Average		Group Ratio	Percent Inhibition
					Mature	Immature	Mature	Immature	Mature:Immature	
90	26/5/69	2/7/69	Positive	30/7/69	60	0	61	0	100:0	0
93	26/5/69	2/7/69	Positive	30/7/69	55	0				
70	26/5/69	1/8/69	Positive	29/8/69	59	0	268	0	100:0	0
88	26/5/69	1/8/69	Positive	29/8/69	476	0				
78	26/5/69	2/9/69	Positive	29/9/69	184	0	148	0	100:0	0
87	26/5/69	2/9/69	Positive	29/9/69	111	0				
81	26/5/69	1/10/69	Positive	29/10/69	0	11	163	37	77:23	23
86*	26/5/69	1/10/69	Positive	15/10/69	326	63▼				
79	26/5/69	31/10/69	Positive	28/11/69	0	14	0	16	0:100	100
82	26/5/69	31/10/69	Positive	28/11/69	0	18				
77	26/5/69	28/11/69	Positive	29/12/69	0	15	0	18	0:100	100
92	26/5/69	28/11/69	Positive	29/12/69	0	21				

* Died 15/10/69.

▼ A proportion of larvae classified as immatures might represent normally developing stages.

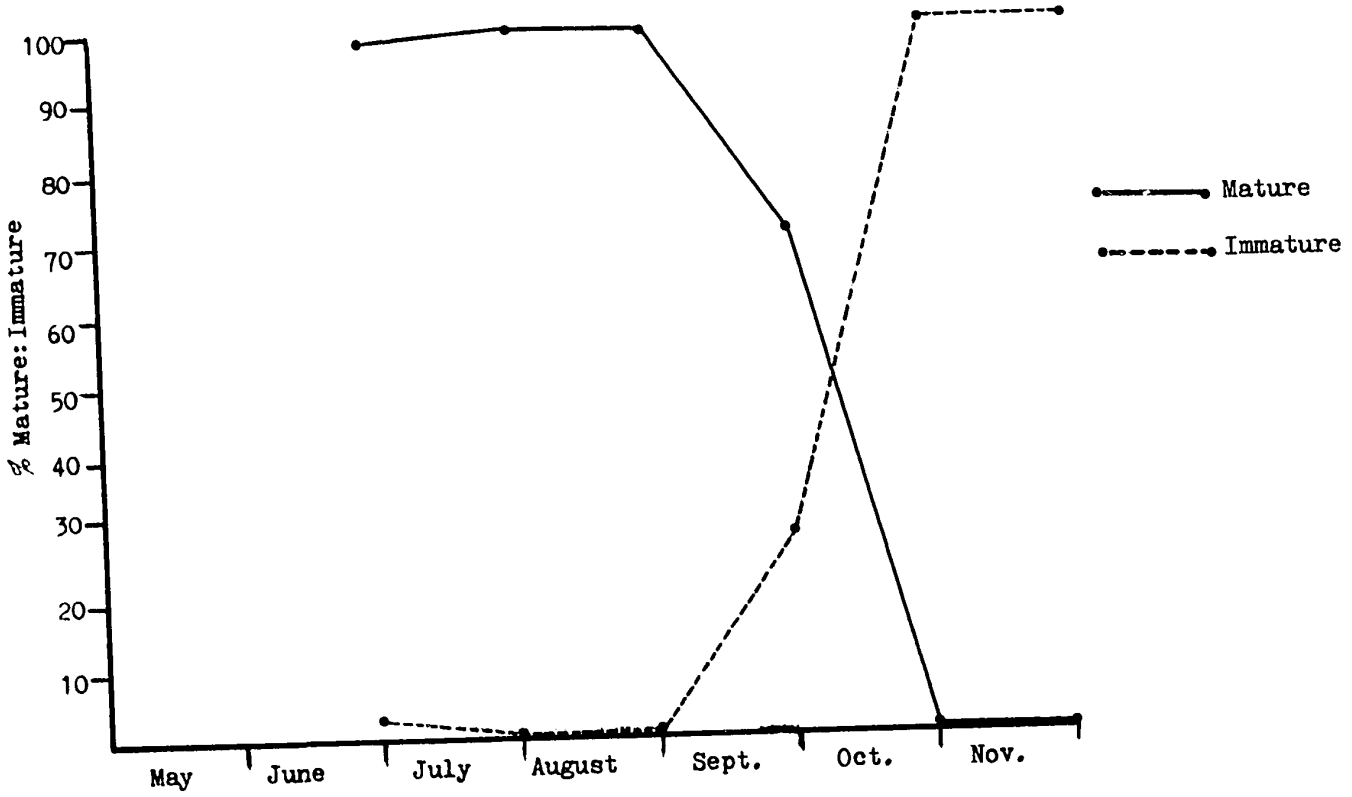

Figure 4. Fluctuations in the Ratio of Mature: Immature D. viviparus in the Permanent Calves During 1969. (Expt. I).

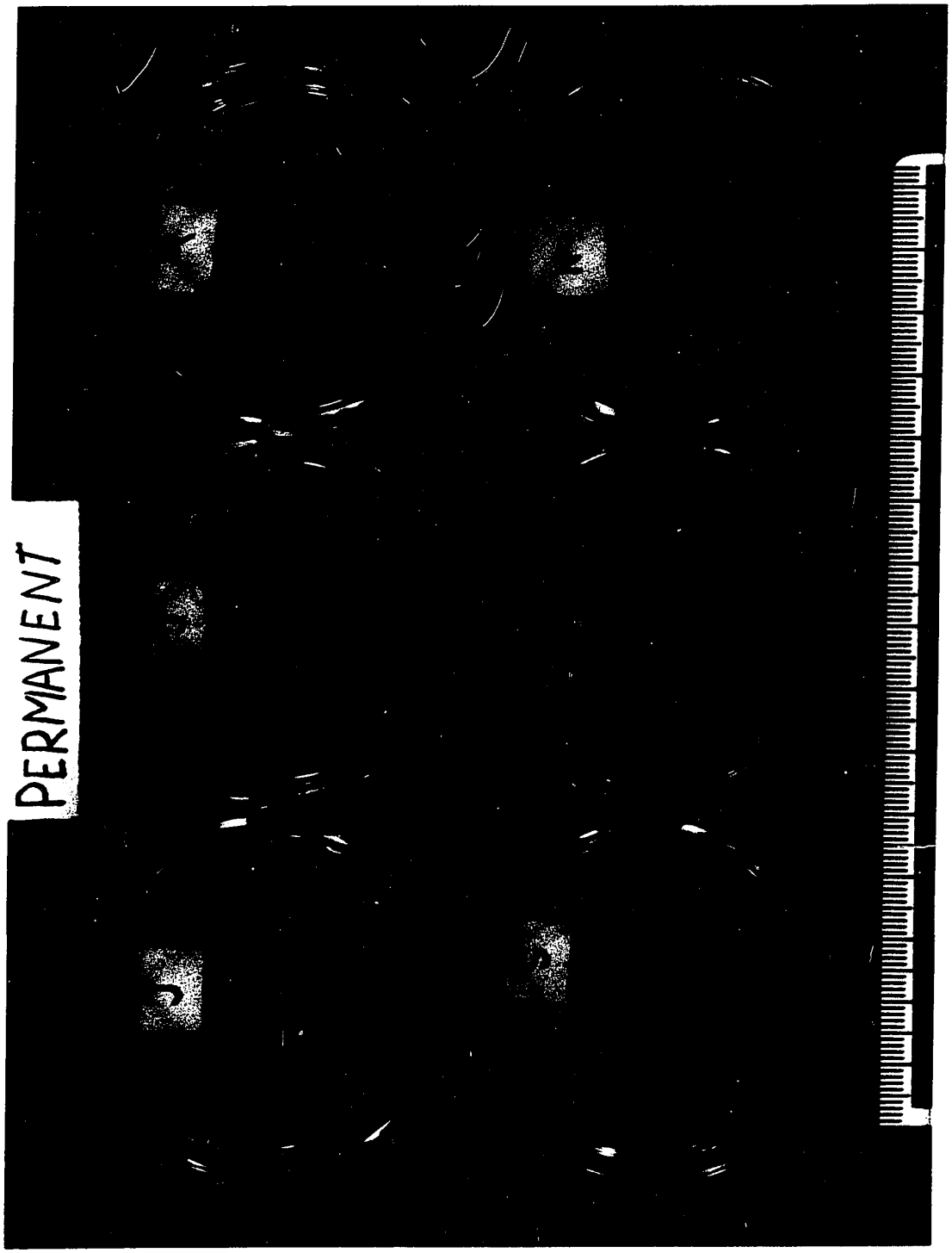

Plate III. Differences in sizes of D. viviparus recovered from permanent calves in different months. J-June, J-July, A-August, S-September, O-October, N-November. (25 mature D. viviparus in each petri dish except in October and November when no adults were recovered.

September respectively their final mean fecal larval count was seven (August) and 20 (September) per 10 gm of feces as compared to fecal larval counts of 128 and 335 from comparable numbers of worms in June and July respectively. This definitely indicates lowered fecundity in the later months.

(b) Tracer Calves (Expt. II)

(i) Fluctuations in Fecal Larval Counts:- Wide variations were noticed in fecal larval counts from the tracer calves during the various months (Table XIX). In general, fecal larval yields increased from May to October, 1969, from an average of 17 larvae per 10 gm to 1,700 larvae per 10 gm of feces respectively. However, repeated fecal examinations of the tracer calves in June and November, 1969, proved negative for lungworm larvae.

(ii) Fluctuations in Mature and Immature Worm Populations:- The number of adult worms recovered from the tracer calves also showed considerable variation (Table XX). In May, the tracer calves had an average of 23 adult worms whereas those in June were negative for D. viviparus. An average of 94 and 565 mature lungworms were recovered from the tracer calves in July and August respectively. The tracer calves in September, 1969, succumbed to lungworm infection before the scheduled date of killing. However, it is obvious that this group had the highest number of adult worms when compared with the worm burden of the tracer calves in

Table III. Weekly fecal larval counts of tracer calves (larvae/10gm of feces). Expt. II.

Month & Year	Calf No.	Put Out	Brought In	Sampling Dates June 13th	15th	18th							
May 1969	73*	12/5/69	26/5/69	6	4								
	75	12/5/69	26/5/69	28	31	79							
June 1969	83	26/5/69	9/6/69	Both calves negative									
	90	26/5/69	9/6/69										
				Sampling Dates July 25th	27th	29th	31st	Aug 2nd	4th	6th	8th	10th	
July 1969	91	2/7/69	16/7/69	2	11	14	18	28	78	48	680	375	
	76	2/7/69	16/7/69	1	13	25	88	138	208	265	195	495	
				Sampling Dates Aug. 31th	27th	29th	31st	Sept.2nd	4th	6th	8th	10th	
Aug. 1969	85	1/8/69	15/8/69	29	55	2625	770	1060	450	775	820	980	
	95	1/8/69	15/8/69	35	35	12	13	1434	875	650	450	670	
				Sampling Dates Sept.26th	28th								
Sept. 1969	147*	2/9/69	16/9/69										
	96*	2/9/69	16/9/69	300	450								
				Sampling Dates Oct. 24th	26th	28th	30th	Nov. 1st	3rd	5th	7th	9th	11th
Oct. 1969	97	1/10/69	15/10/69	1	216	250	375	3400	490	850	1700	1350	2335
	156	1/10/69	15/10/69	0	3	35	235	1000	2950	780	1540	890	1065
Nov. 1969	152	31/10/69	14/11/69	Both calves negative									
	157	31/10/69	14/11/69										

* Calf #73 died on 17/6/69
Calf #147 died on 15/9/69
Calf #96 died on 30/9/69

Table XI. Fluctuations in mature and immature worm populations in tracer calves. Expt. II.

Calf No.	Put Out	Brought In	Fecal Examination	Killed	Worms at Necropsy		Group Average		Group Ratio	Percent Inhibition
					Mature	Immature	Mature	Immature	Mature:Immature	
73*	12/5/69	26/5/69	Positive	17/6/69**	17	0	23	0	100:0	0
75	12/5/69	26/5/69	Positive	19/6/69	28	0				
83	26/5/69	9/6/69	Negative	7/7/69	0	0	0	0	0	0
90	26/5/69	9/6/69	Negative	7/7/69	0	0				
91	2/7/69	16/7/69	Positive	13/8/69	82	0	94	0	100:0	0
76	2/7/69	16/7/69	Positive	13/8/69	106	0				
85	1/8/69	15/8/69	Positive	11/9/69	540	0	565	0	100:0	0
95	1/8/69	15/8/69	Positive	11/9/69	590	0				
147*	2/9/69	15/9/69**	Positive			644♦				
96*	2/9/69	17/9/69	Positive	30/9/69**	8250					
97	1/10/69	15/10/69	Positive	12/11/69	3310	375	2635	300	89:11	11
156	1/10/69	15/10/69	Positive	12/11/69	1960	225				
152	31/10/69	14/11/69	Negative	12/12/69	0	54		36	0:100	100
157	31/10/69	14/11/69	Negative	12/12/69	0	18				

* These animals died on dates marked with **

♦ A proportion of larvae classified as immatures might represent normally developing stages.

other months. The tracers of October, 1969, had an average of 2,635 mature worms. A careful search for adult D. viviparus during necropsy of the tracer calves in November, 1969, proved futile.

All the worms recovered from the tracer calves from May to August, 1969, were mature.Since the calves in September died prematurely, the ratio of mature to immature worms recovered from these calves could not be included in these results on inhibited development. The first sign of inhibited development was noticed in the tracers in October, 1969, which showed a ratio of 89:11 mature to immature worms respectively. An interesting observation was that in November, the tracer calves had 100 percent immature D. viviparus (Figure 5).

4. Worm Population Fluctuations in Calves Experimentally Infected with Larvae Obtained from "Permanent" Calves (Expt.III)

(a) Fluctuations in Larval Counts- The results (Table XXI) show that the final average larval counts of the test calves in August, September and October, 1969, were 1,695, 284 and three larvae per 10 gm of feces respectively. It is interesting to note that the number of larvae per 10 gm of feces per mature worm recovered declined dramatically from August to October (August, 5; September, 2; October, 0.1). Repeated fecal examinations of the calves from November, 1969, to April, 1970, remained negative for any evidence of lungworm infection.

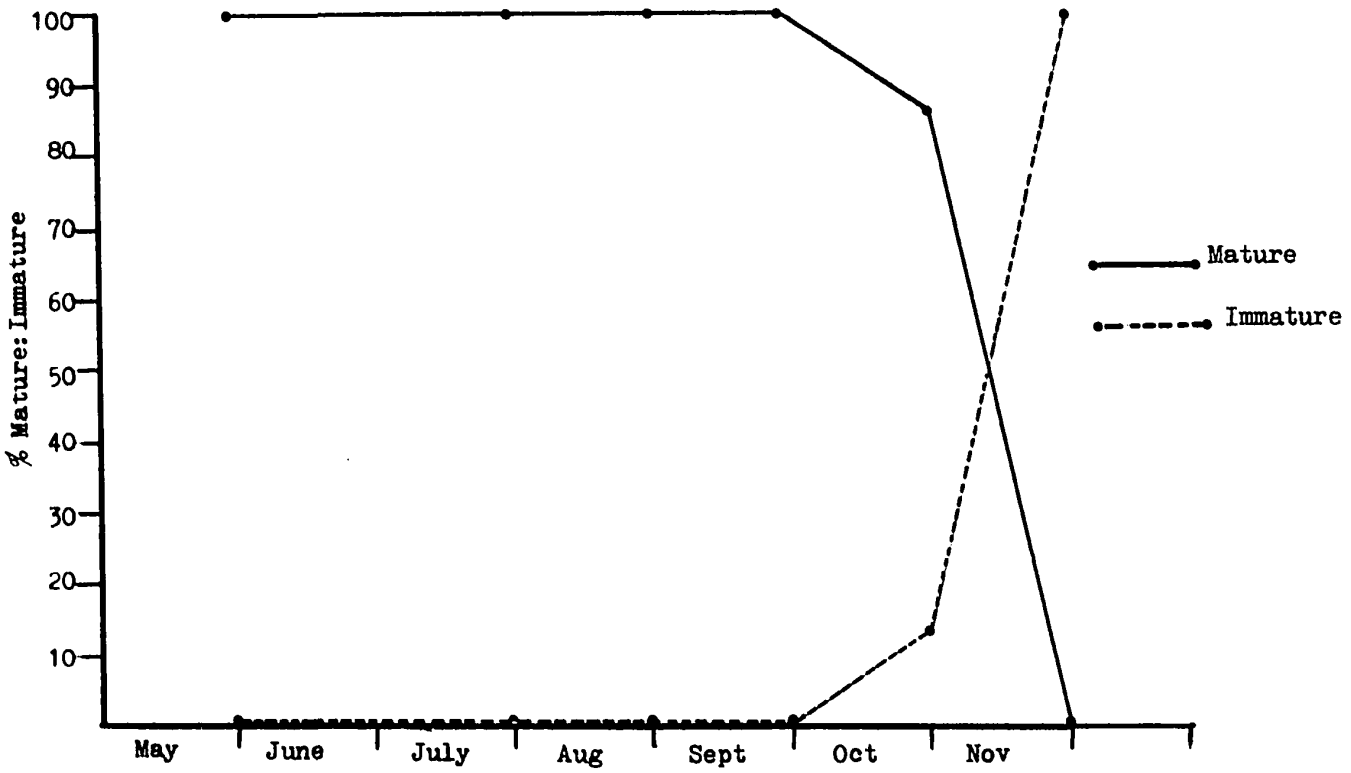

Figure 5. Fluctuations in the Ratio of Mature: Immature D. viviparus in the Tracer Calves During 1969. (Expt. II).

Table XII. Fluctuations in mature and immature worm populations in calves infected with larvae obtained from permanent calves. Expt. III.

Month & Year	Calf No.	Dates Infected	Fecal Examination	Date Killed	Necropsy Worm Count		Group Average		Group Ratio		Percent Infection Estab.	No. of Larvae/10gm of Feces/Adult Worm at Necropsy	No. of Larvae/10gm of Feces at Necropsy
					Mat.	Imm.	Mat.	Imm.	Mat.	Imm.			
Aug/69	119	Aug 11-25/69	Positive	22/9/69	298	0	330	0	100	0	6	5	1360
Aug/69	145	Aug 11-25/69	Positive	22/9/69	361	0							2030
Sept/69	176	Sept 13-27/69	Positive	24/10/69	100	0	144	0	100	0	2.6	2	68
Sept/69	177	Sept 13-27/69	Positive	24/10/69	188	0							500
Oct/69	178	Oct 1-15/69	Positive	12/11/69	59	11	78	11	85	15	1.6	0.1	6
Oct/69	187*	Oct 1-15/69	Negative	24/10/69**	97	11♦							0
Nov/69	181	Oct 31-Nov 14/69	Negative	12/12/69	0	24	0	22	0	100	0.4	0	0
Nov/69	183*	Oct 31-Nov 14/69	Negative	19/11/69**	0	19♦							0
Dec/69	224	Dec 1-15/69	Negative	12/1/70	0	13	0	13	0	100	0.2	0	0
Dec/69	186	Dec 1-15/69	Negative	12/1/70	0	12							
Jan/70	231	Dec 31-Jan 14/70	Negative	12/2/70	0	18	0	14	0	100	0.2	0	0
Jan/70	229	Dec 31-Jan 14/70	Negative	12/2/70	0	9							0
Feb/70	234	Feb 2-16/70	Negative	16/3/70	0	16	0	10	0	100	0.9	0	0
Feb/70	237	Feb 2-16/70	Negative	16/3/70	0	3							0
Mar/70	238	Mar 2-17/70	Negative	15/4/70	2	7	2	9	19	81	0.2	0	0
Mar/70	233	Mar 2-17/70	Negative	15/4/70	2	10							0
Apr/70	251	Apr 1-15/70	Negative	13/5/70	0	0	0	0	0	0	0	0	0
Apr/70	254	Apr 1-15/70	Negative	13/5/70	0	0							0

* These animals died on dates marked with **

♦ A proportion of larvae classified as immatures might represent normally developing stages.

Each calf was dosed with 5,600 D. viviparus infective larvae.

(b) Fluctuations in Mature and Immature Worm Populations

Postmortem worm recoveries (Table XXI) revealed considerable differences in calves from one group to another. During August, September and October, 1969, an average of 330, 144 and 78 adult worms were recovered respectively, per calf. However, the calves from November, 1969, to February, 1970, were negative during necropsy for mature D. viviparus in their lungs but in March, 1970, two adult worms were recovered from each calf. A careful post-mortem examination of the experimental calves in April, 1970, was negative for the presence of adult worms. There appears to have been a progressive fall in the infectivity of larvae from August, 1969, to March, 1970. By April, 1970, the larvae appeared to have lost their infectivity completely.

The ratio of mature to immature worms recovered from the calves necropsied during the different months (August, 1969, to March, 1970) are presented in Table XXI and Figure 6. In August and September there were no immature worms. In the test calves during October 15 percent of the total worm burden was immature. It should be noted that one calf of this group died prematurely (ninth day from the last dose of larvae) therefore it is possible that some of the worms classified as immatures might have been normally developing parasites. The experimental calves in November and December, 1969, and in January and February, 1970, had 100 percent immature worms. Once again, one calf in the

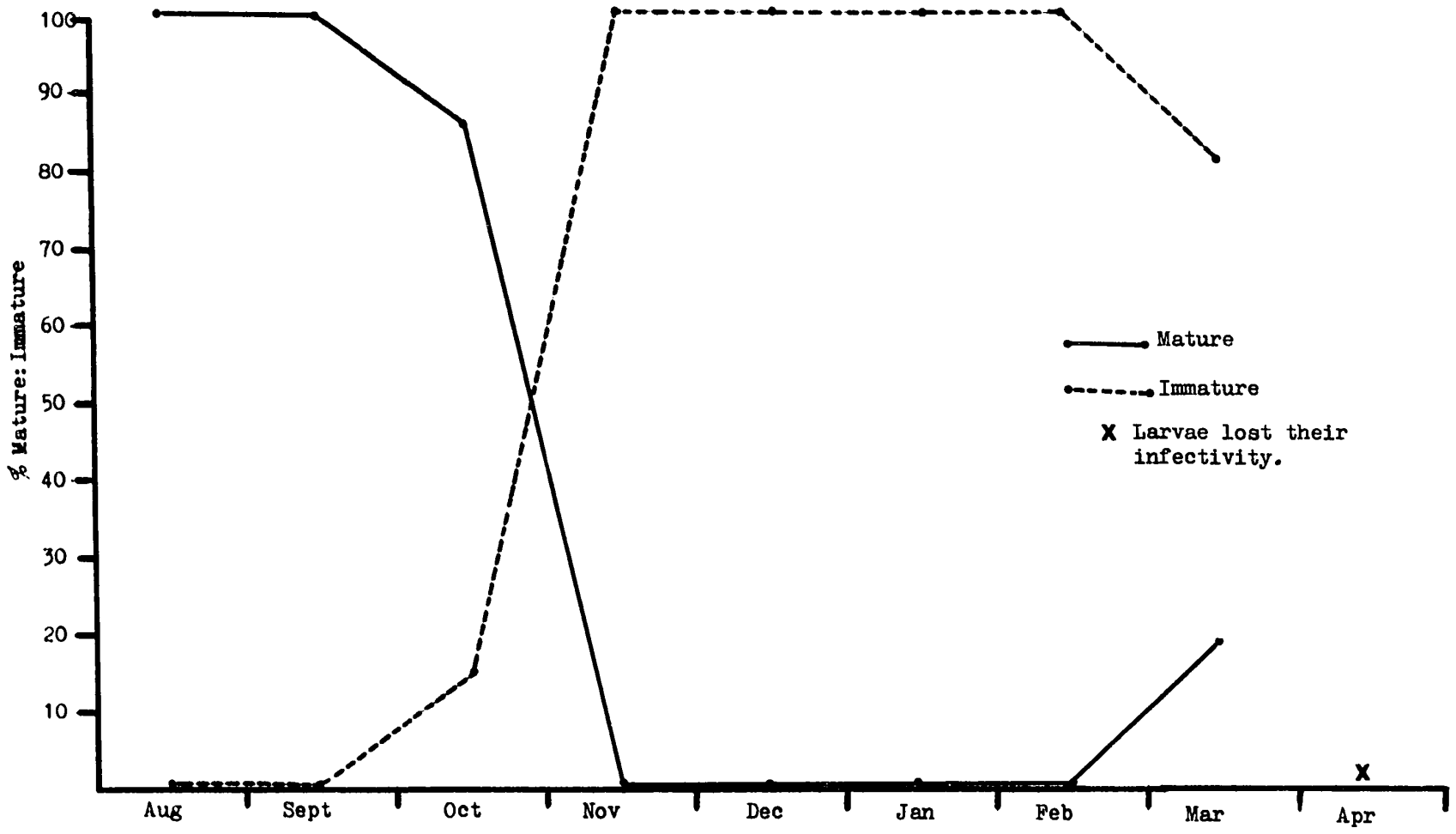

Figure 6. Fluctuations in the Ratio of Mature: Immature D. viviparus in Calves Infected with Larvae Obtained from Permanent Calves During 1969-70. (Expt. III).

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November, 1969, group died before the scheduled date and it is possible that a proportion of worms classified as immatures might represent normally developing stages. An interesting observation was that during March, 1970, 19 percent of the worms recovered were mature and the rest were immatures. As previously mentioned, the experimental calves in April, 1970, were negative for both mature and immature worms.

5. Worm Population Fluctuations in Calves Experimentally Infected with Larvae Obtained from "Tracer" Calves (Expt. IV)

(a) Fluctuations in Larval Counts

Repeated fecal examinations revealed (Table XXII) patent lungworm infections in the test calves in November, 1969, and March, 1970, only. It is interesting to note that the number of larvae produced per 10 gm of feces per mature worm during November and March was 0.25 and 0.84 respectively. This indicates a possible difference in fecundity in the worms during the two different months. The test calves from December, 1969, to February, 1970, and April, 1970, were negative for evidence of larvae in their feces.

(b) Fluctuations in Mature and Immature Worm Populations

An average of 71 and 14 mature worms per calf were recovered during postmortem examination of the calves in November, 1969, and March, 1970, respectively. The mature worms recovered from these calves were stunted and thin when compared with the worms collected

Table XIII. Fluctuations in mature and immature worm populations in calves infected with larvae obtained from tracer calves. Expt. IV.

Month & Year	Calf No.	Dates Infected	Fecal Examination	Date Killed	Necropsy Worm Count		Group Average		Group Ratio		Percent Infection Estab.	No. of Larvae/10gm of Feces/Adult Worm at Necropsy	No. of Larvae/10gm of Feces at Necropsy
					Mat.	Imm.	Mat.	Imm.	Mat.	Imm.			
Nov/69	183	Oct 31-Nov 14/69	Positive	12/12/69	70	2	71	2	97	3	2.6	0.3	22
Nov/69	184	Oct 31-Nov 14/69	Positive	12/12/69	72	2							14
Dec/69	223	Dec 1-15/69	Negative	12/1/70	0	27	0	23	0	100	0.8	0	0
Dec/69	180	Dec 1-15/69	Negative	12/1/70	0	19							0
Jan/70	190	Jan 2-16/70	Negative	12/2/70	0	23	0	21	0	100	0.8	0	0
Jan/70	227	Jan 2-16/70	Negative	12/2/70	0	19							0
Feb/70	230	Feb 2-16/70	Negative	16/3/70	0	8	0	10	0	100	0.4	0	0
Feb/70	235	Feb 2-16/70	Negative	16/3/70	0	12							0
Mar/70	236	Mar 2-16/70	Negative	15/4/70	8	7	14	8	68	32	0.8	0.8	0
Mar/70	270	Mar 2-16/70	Positive	15/4/70	19	8							16
Apr/70	245	Apr 1-15/70	Negative	13/5/70	0	0	0	0	0	0	0	0	0
Apr/70	248	Apr 1-15/70	Negative	13/5/70	0	0							0

Each calf was dosed with 5,600 D. viviparus infective larvae.

from the tracer calves on pasture during August and September, 1969. From the number of worms established in these calves from a constant infective dose (5,600 larvae) it is evident that the infectivity of the larvae progressively declined from November, 1969, to February, 1970. However, there was a slight rise in the infectivity during March, 1970. Postmortem examinations of the calves from December, 1969, to February, 1970, and April, 1970, were negative for evidence of mature worms.

From these results (Figure 7, Table XXII) it is also evident that during November only three percent of the total worm burden in the calves was immature; whereas in the calves during the months of December, 1969, to February, 1970, 100 percent inhibition was observed. It was interesting to find only 32 percent immature worms from the calves in March, 1970. A careful postmortem examination of the calves in April, 1970, proved negative for mature or immature D. viviparus in their lungs.

6. Results of the Effect of Age of Infective Larvae in the Phenomenon of Inhibited Development (Expt. V)

When calves were infected with larvae aged for 42-56 days, fecal examinations from the 21st to the 28th day post-infection were negative for lungworm larvae. Postmortem examination on December 8 revealed that all the worms were inhibited (Table XXIII). However, fecal examinations of calves (28th day post-infection) infected with 14-day old larvae, revealed patent lungworm infections in all

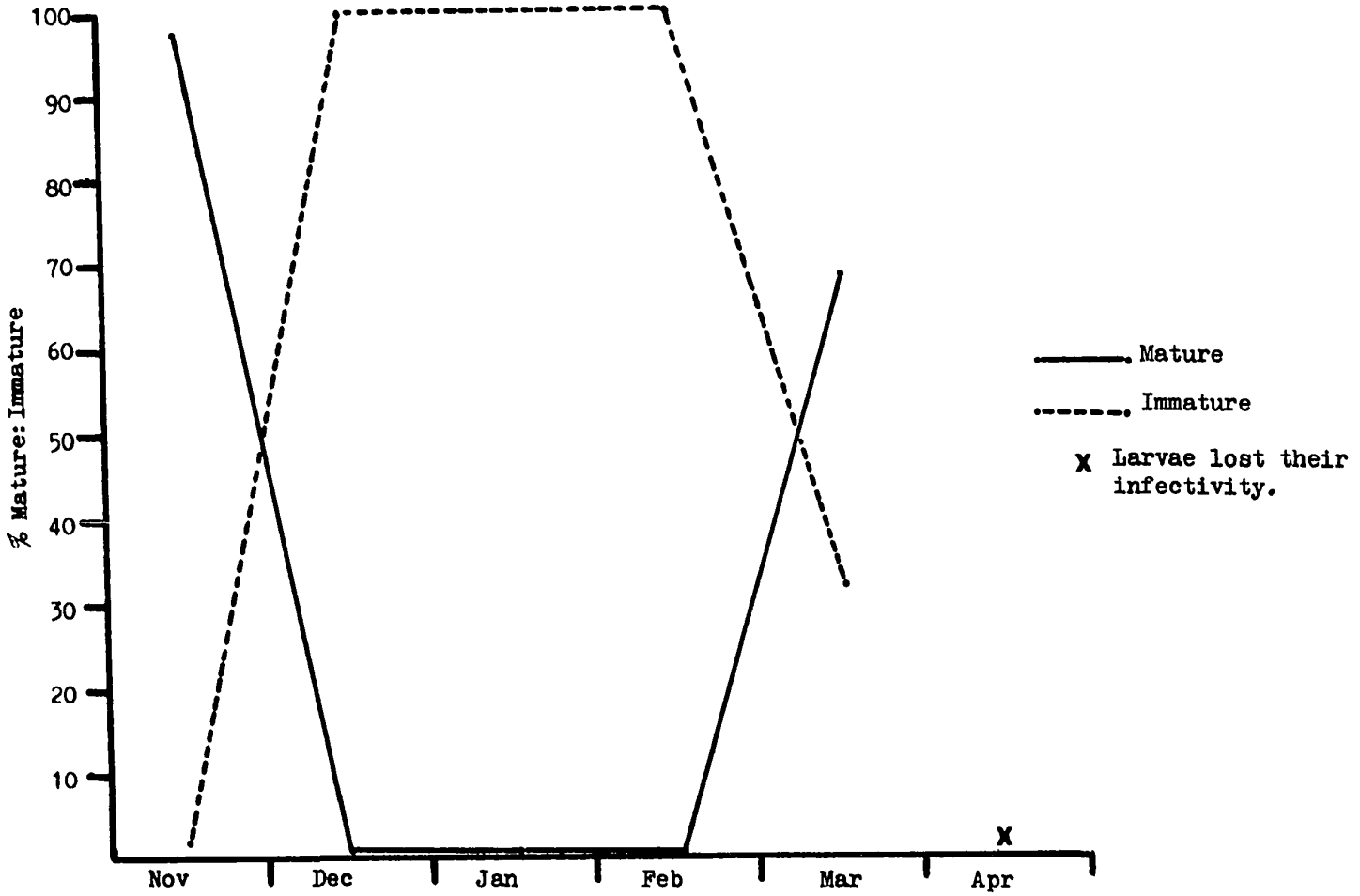


Figure 7. Fluctuations in the Ratio of Mature: Immature D. viviparus in Calves Infected with Larvae Obtained from Tracer Calves During 1969-70. (Expt. IV).

Table XXIII. The effect of age of infective larvae in the phenomenon of inhibited development. Expt. V.

Calf No.	Calves Infected with Aged Larvae *D. viviparus* Recovery at Autopsy		Calves Infected with Fresh Larvae *D. viviparus* Recovery at Autopsy	
	Mature	Immature	Mature	Immature
1	0	48	53	5
2	0	72	13	17
3	0	64	299	57
4	0	76	176	30
5	0	26	240	75

the calves. Subsequent postmortem examinations of these calves showed that 87 percent of the total worm population recovered were mature (Table XXIII).

7. Results of the Identification and Description of Inhibited Stages of D. viviparus

Females:- Twenty-six female inhibited stages were randomly collected from a pooled sample and measured. Their length ranged from 0.9 to 2.5 mm with an average of 1.72 mm (Figure 8). The anterior end had a small cephalic inflation, the buccal capsule was shallow and inconspicuous and the oesophagus was well demarcated from the surrounding tissue (Plate IV). The tail end was sharp and pointed and the anus was patent (Plate V). The ovaries were clearly differentiated with a prominent ovejector (Plate VI). In approximately 80 percent of the female larvae examined the vagina was patent (Plate VII).

Males:- Twenty-two male, inhibited stages were randomly collected and measured. Their length ranged from 0.9 to 2.7 mm with an average of 1.71 mm (Figure 8). The anterior end showed a cephalic inflation, buccal capsule and oesophagus similar to the female inhibited larvae. The testes were well developed (with spermatids seen on a few occasions) and distinctly differentiated from the surrounding tissue (Plate VIII). The bursa was rudimentary and the bursal rays were small (Plate IX). However, the spicules and gubernaculum appeared differentiated but not keratinized (Plate X). Approximately 20 percent of the total immature male

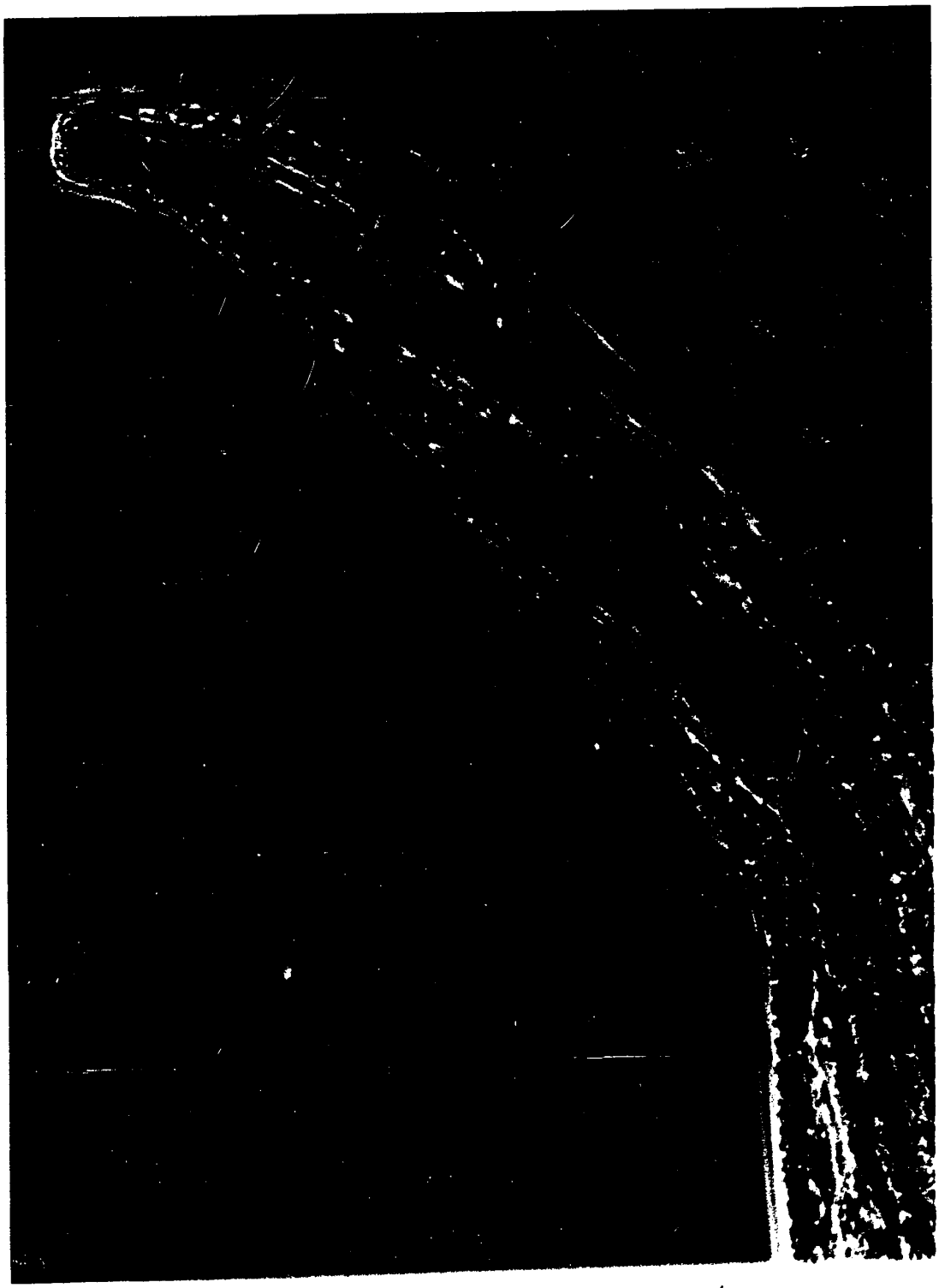
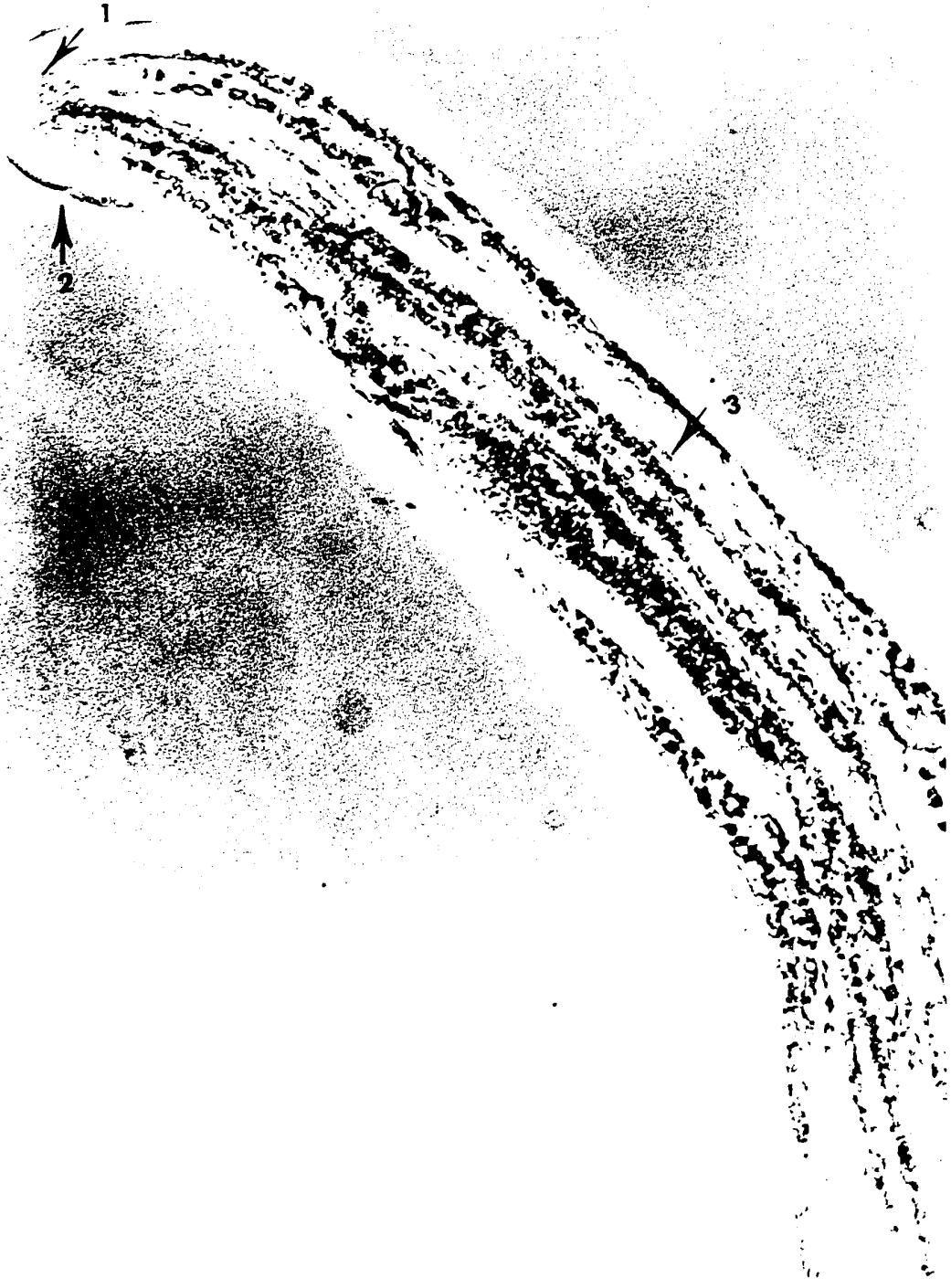


Plate IV. Anterior end of a female D. viviparus larva (early fifth-stage). Shallow buccal cavity (1), cephalic inflation (2), oesophagus (3).
(x 725).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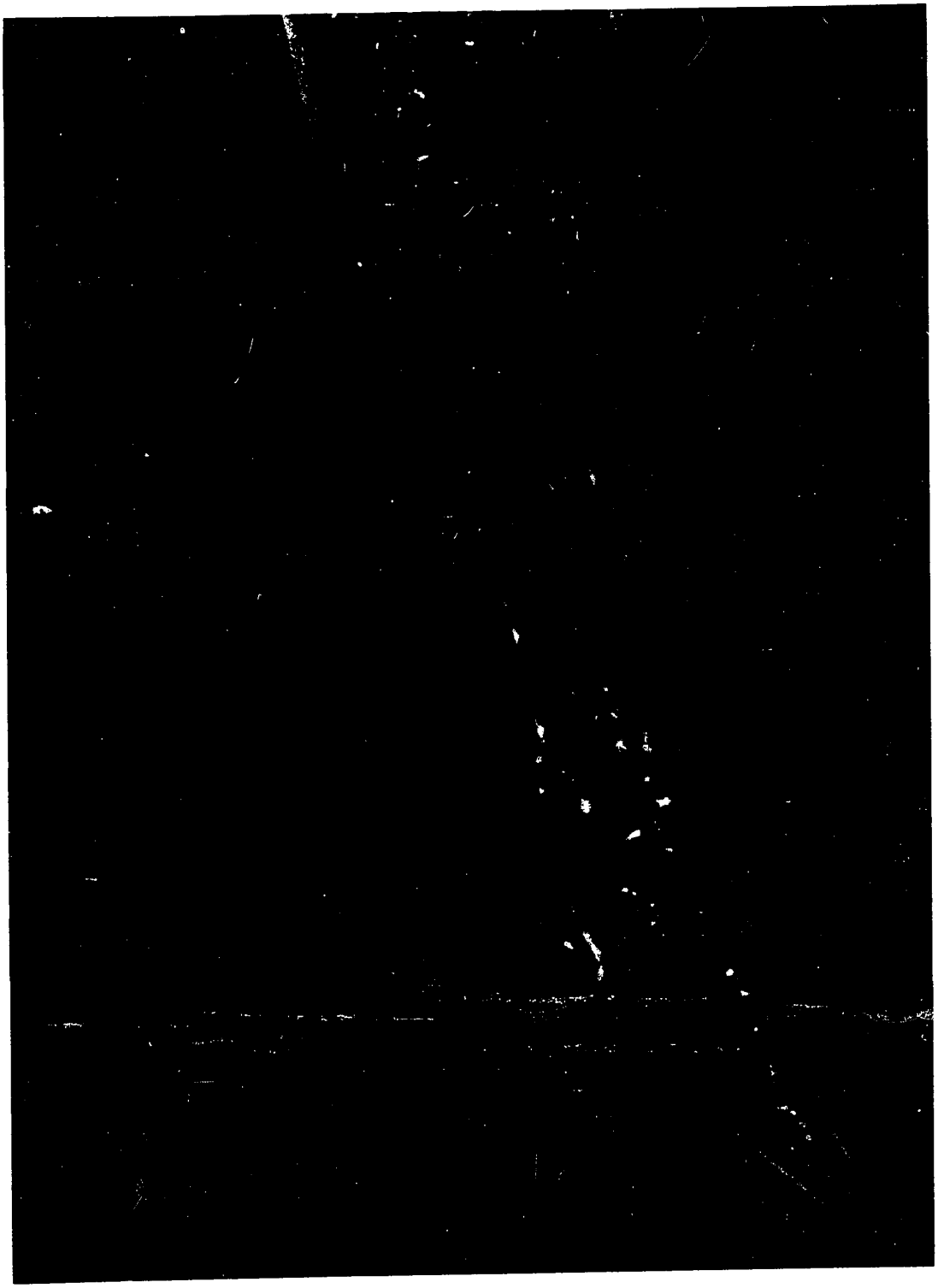

Plate V. Posterior end of a female D. viviparus larva (early fifth-stage). Patent anus (1), pointed tail end (2). (x 796).


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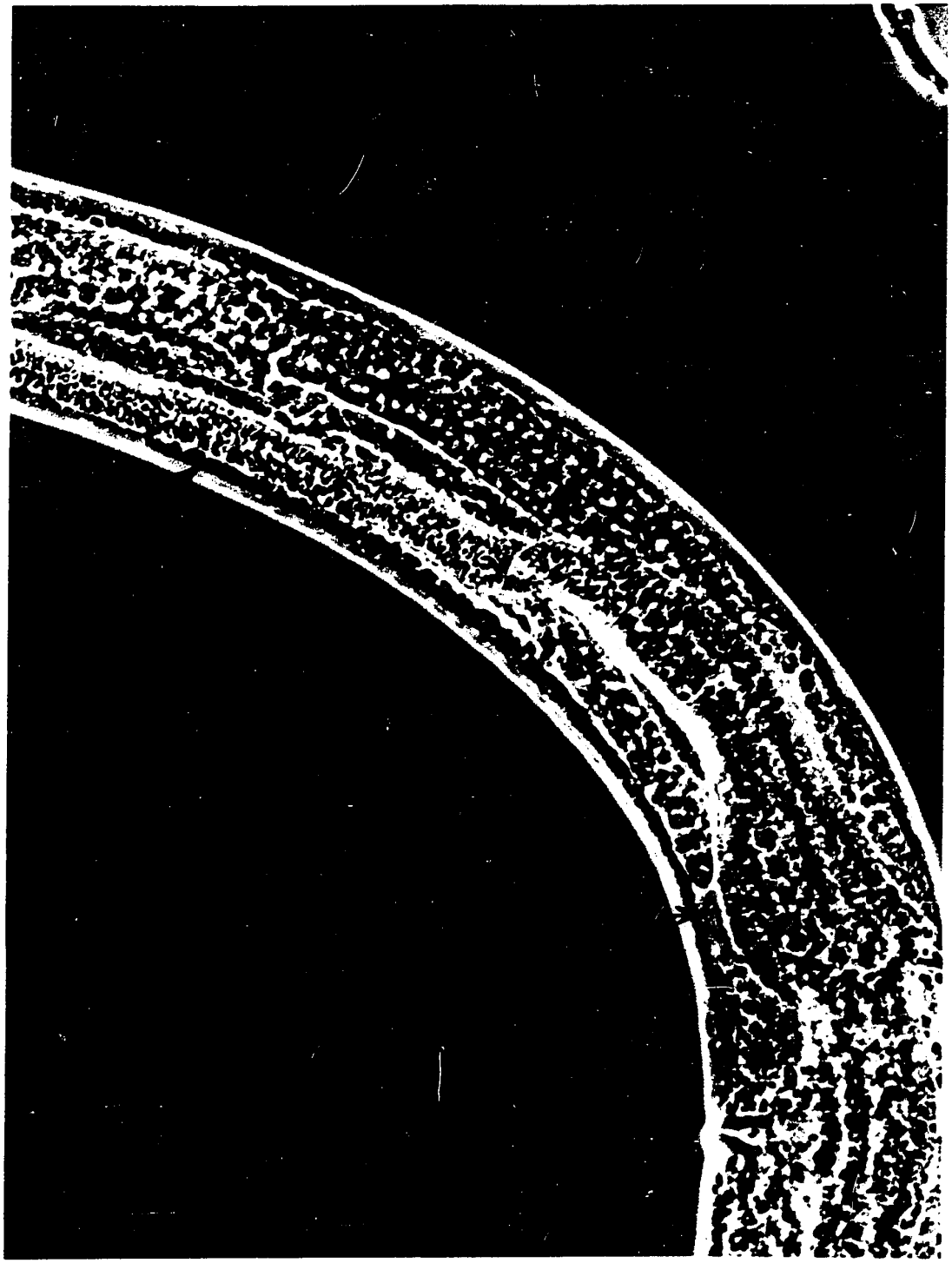
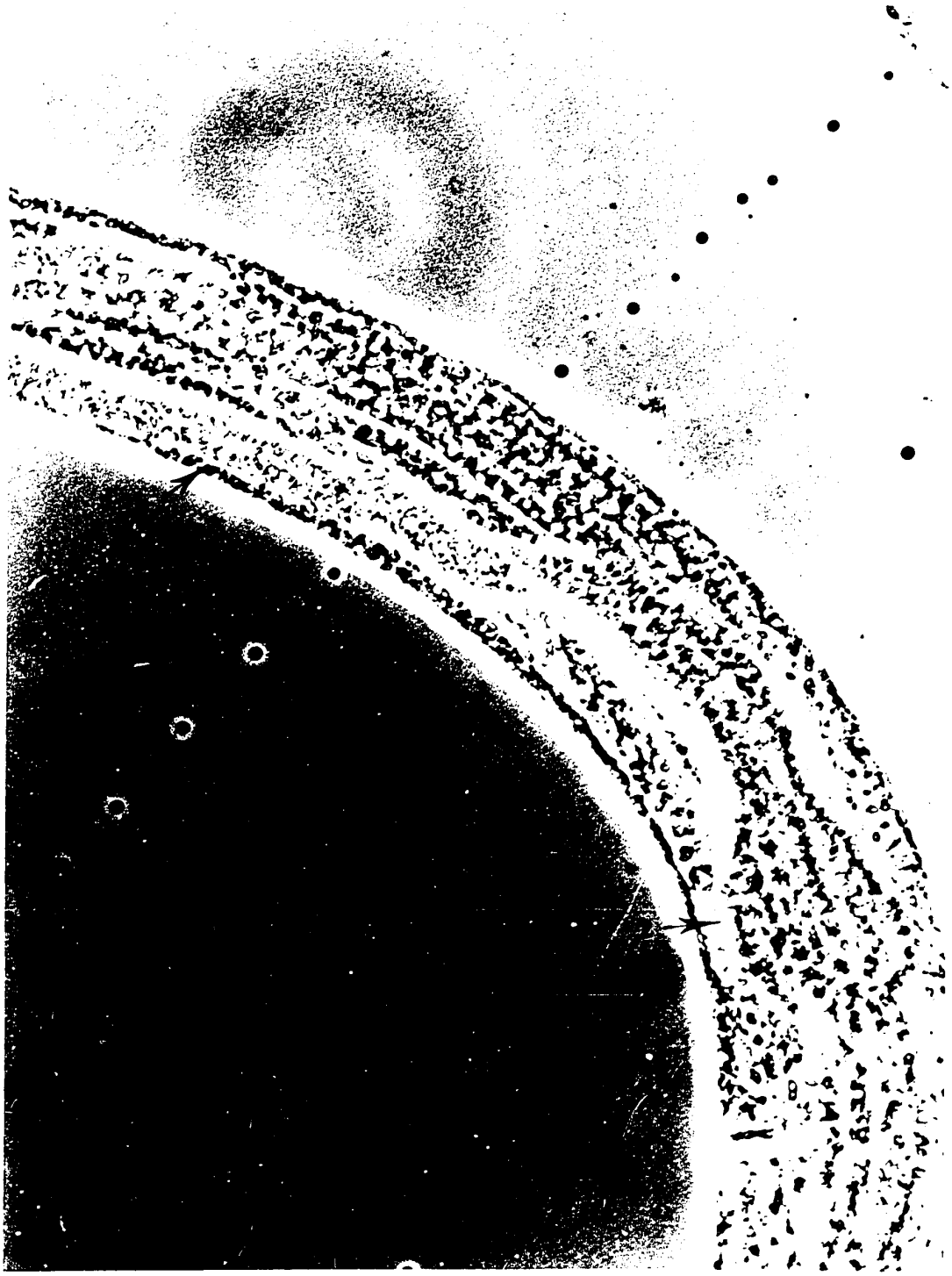


Plate VI. **Genitalia** of a female D. viviparus larva (early fifth-stage). Ovary (1), ovejector (2). (x 495).



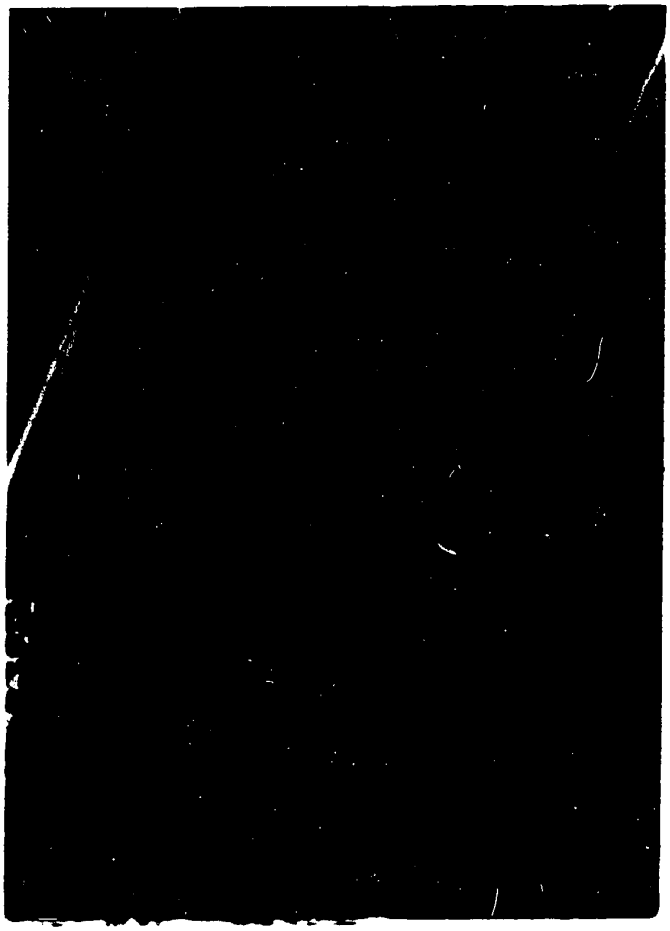
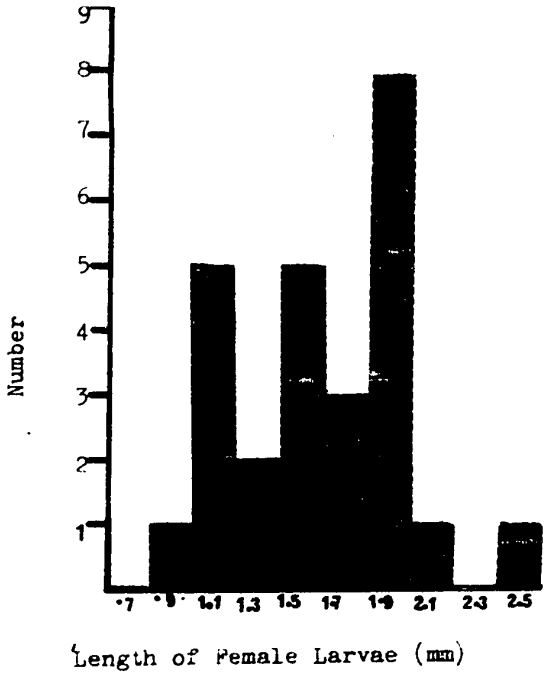


Plate VII. Genitalia of a female *D. viviparus* larva (early fifth-stage showing patent vagina). (x 785).



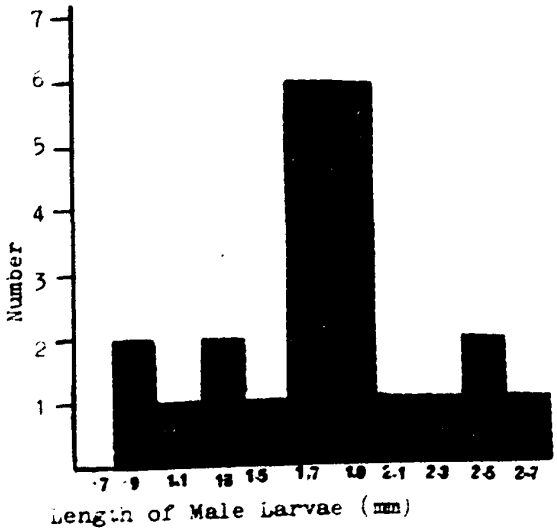


Figure 8. Length Distribution in mm of Incubated D. viviparus Larvae. Male and Female.

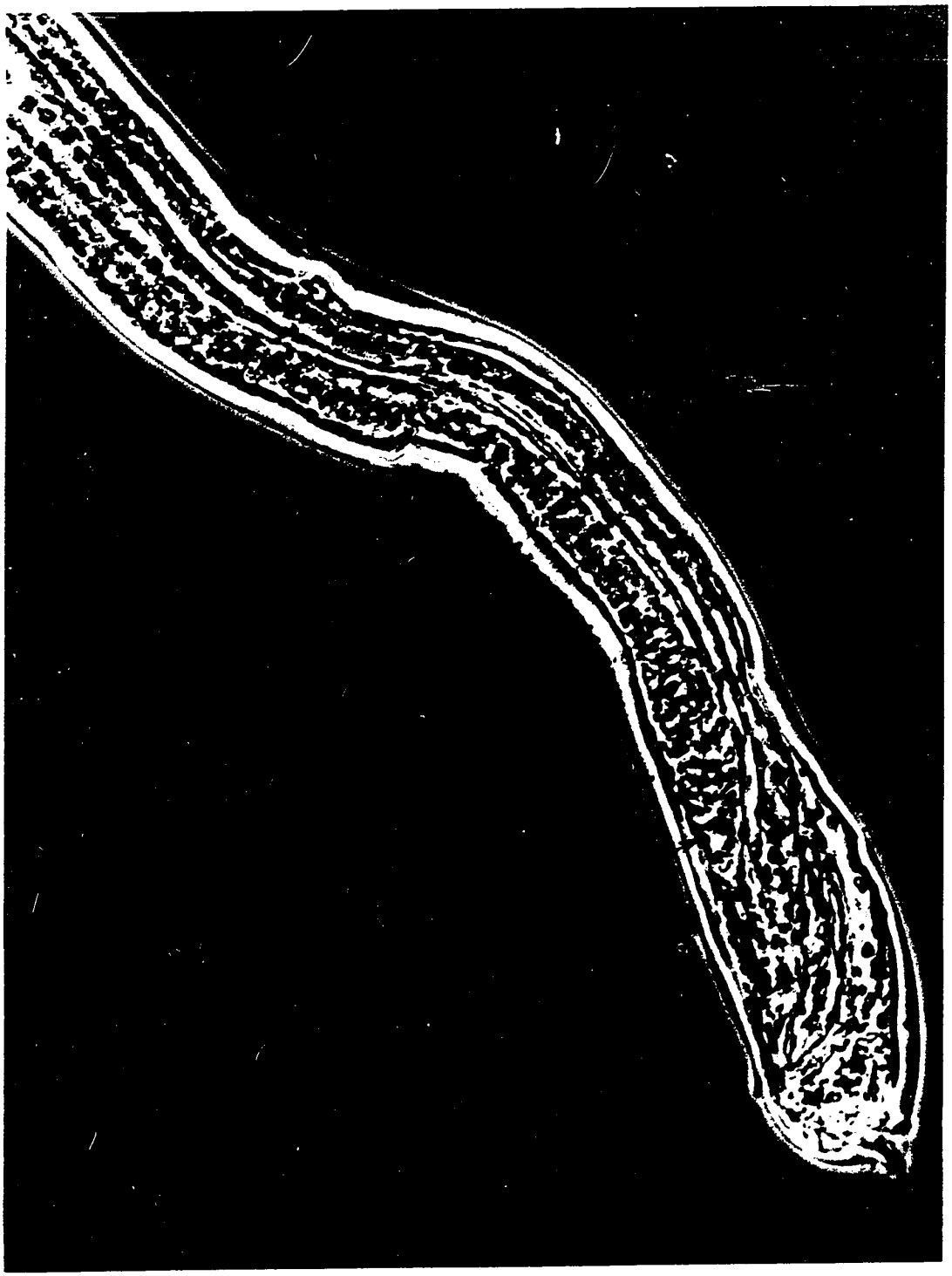
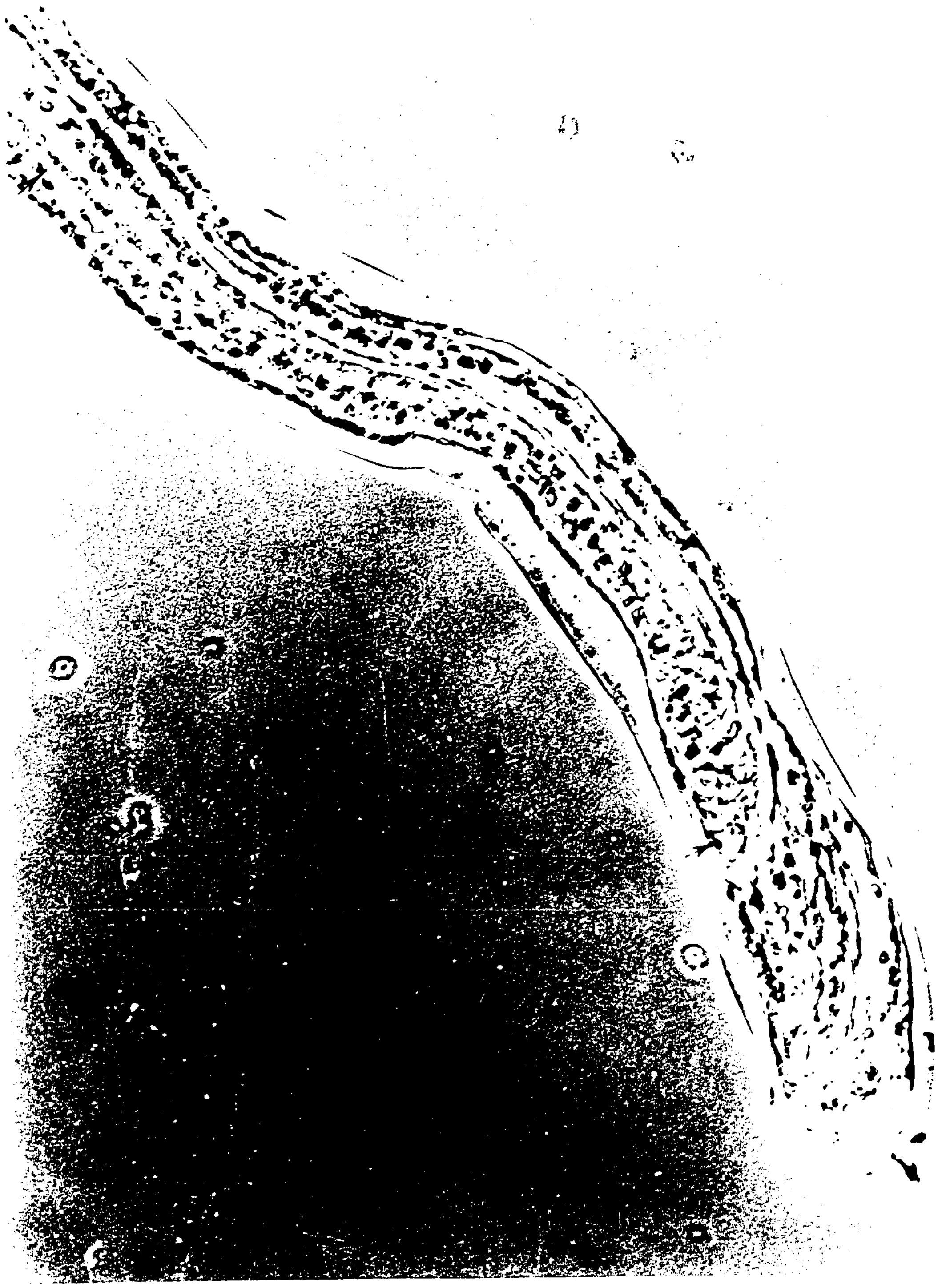


Plate VIII. Posterior end of a male *D. viviparus* larva (late fourth-stage showing genitalia).
(x 480).



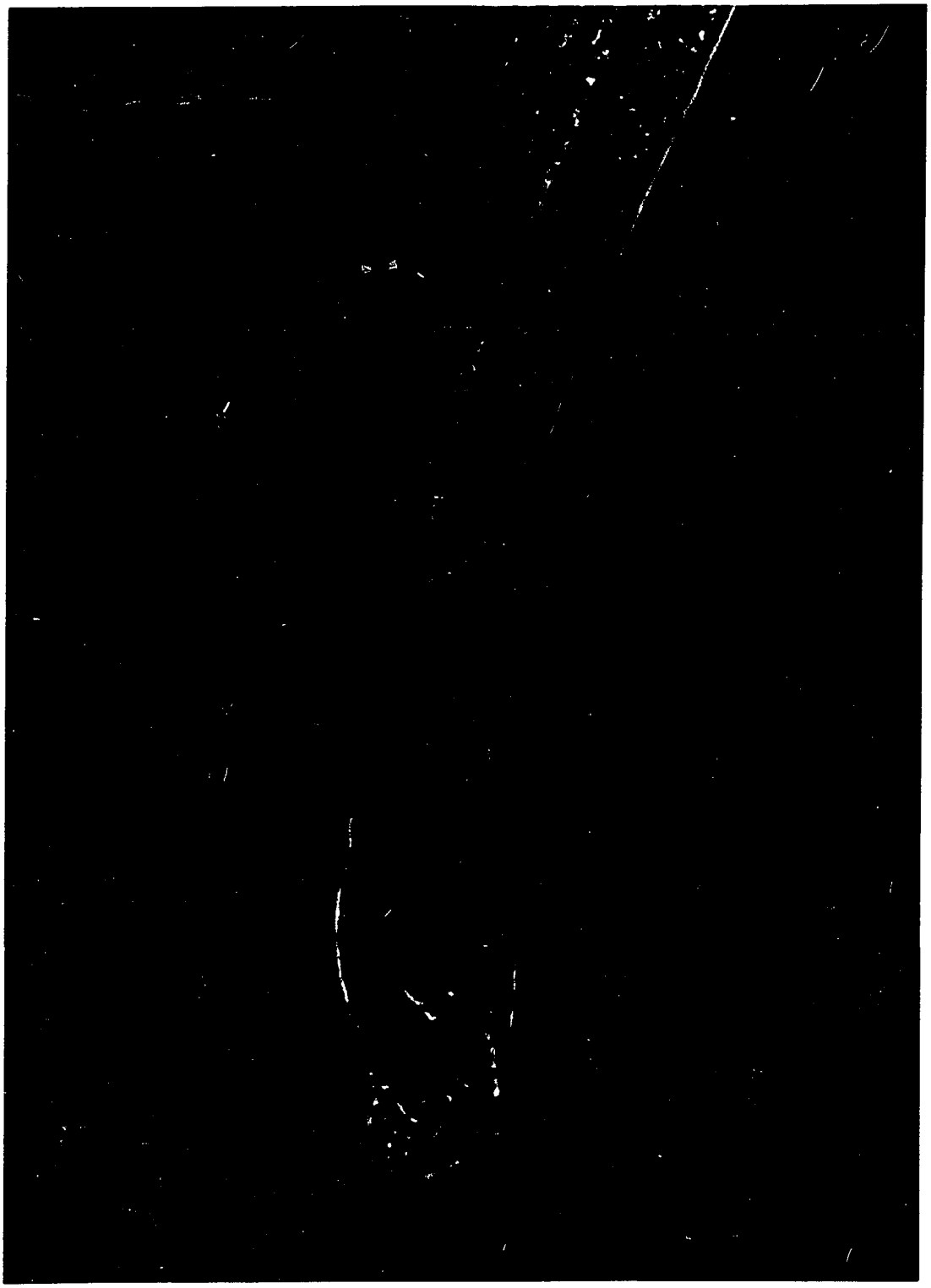
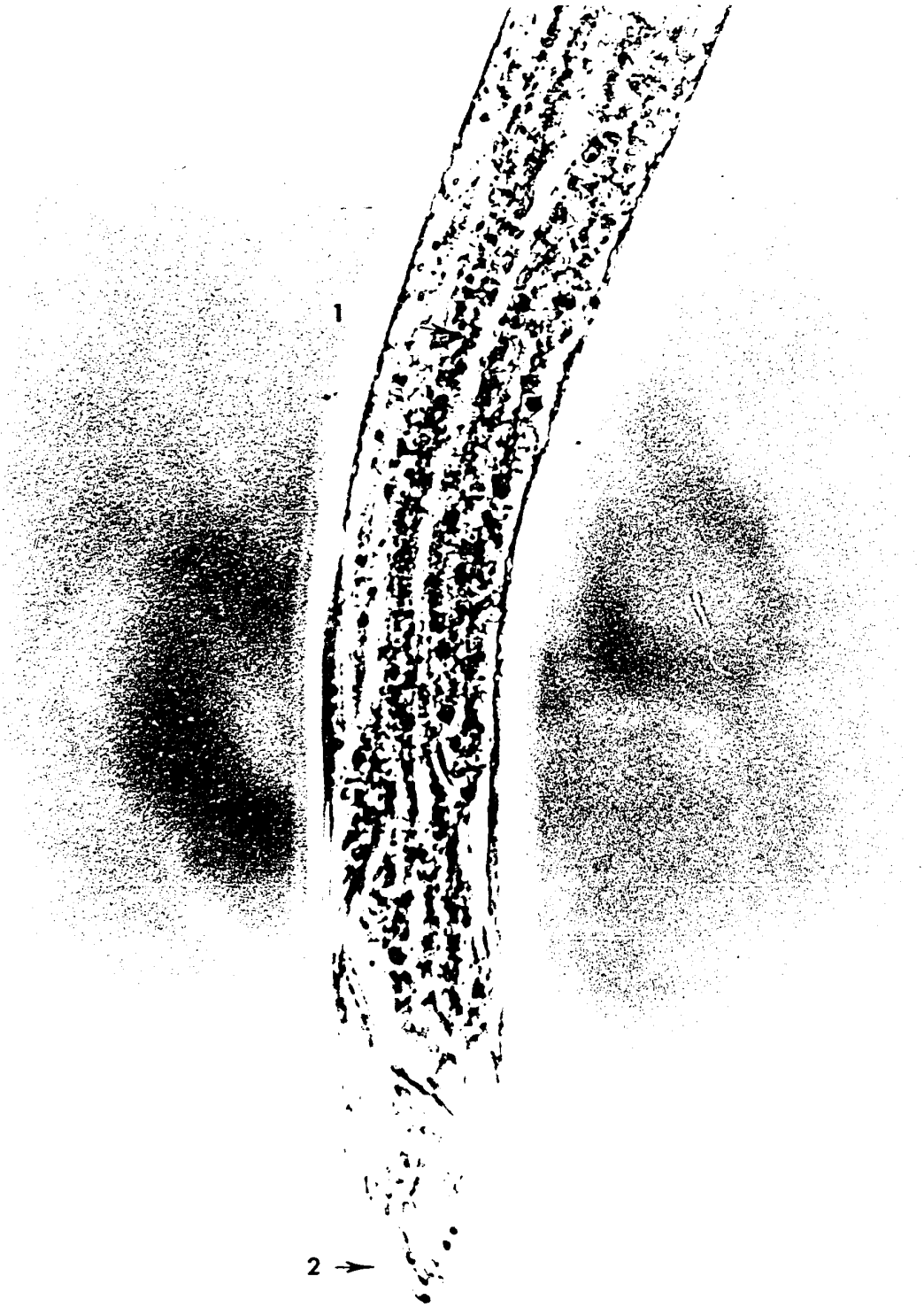


Plate IX. Posterior end of a male D. viviparus larva (early fifth-stage). Intestine (1), bursa (2). (x 640).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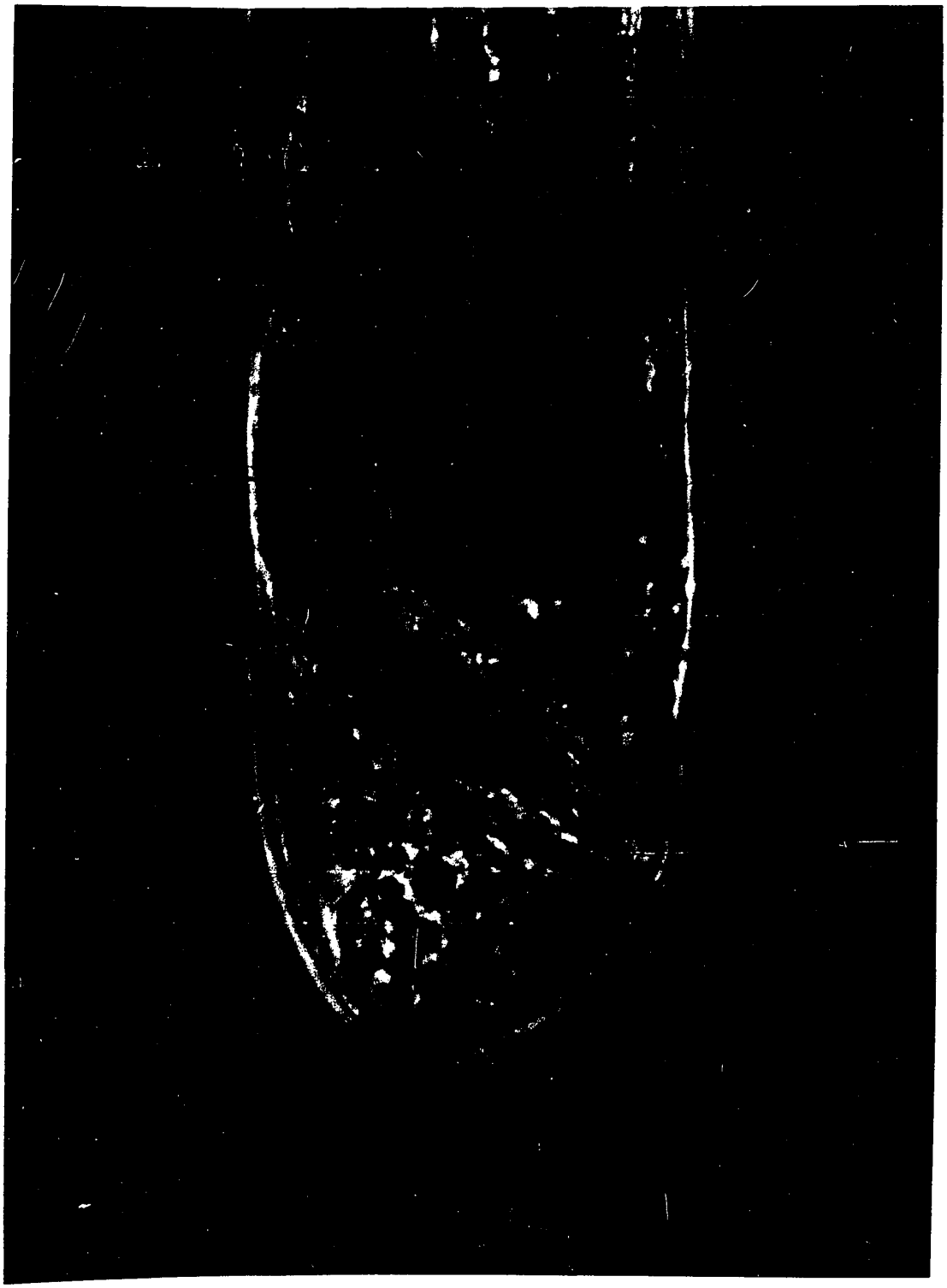

Plate V. Posterior end of a male D. viviparus larva (early fifth-stage). Spicules (1), gubernaculum (2), bursa (3). (x 660).


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larvae were in the late fourth-stage with a sheath around them (Plate XI). The bursa and bursal rays were relatively less well developed when compared with the early fifth-stage larvae (Plate X). For comparison Plate XII shows the bursa, bursal rays, spicules and gubernaculum of a mature D. viviparus.

8. The Infectivity to Calves of D. viviparus Obtained from Moose

Clinical examination (respiratory rates, signs of pneumonia, coughing and body temperature) of the two calves during the 35 days after infection with 6,000 and 12,000 larvae respectively, did not reveal any symptoms which could be related to parasitic bronchitis. Repeated fecal examinations (22nd to 35th day post-infection) were negative for patent lungworm infection. The calves were then killed (35th day post-infection) and their lungs carefully examined for the presence of lungworms, with negative results.

9. Weather Data

Mean minimum and mean maximum temperatures and precipitation were recorded weekly for one annual cycle (March, 1968, to February, 1969) and are presented in Figure 9. Precipitation in this part of Canada from May to October is generally as rain which is followed by snow from November to April. The snow stays on the ground, sometimes to a depth three to four feet, until April.

A hypothetical line has been drawn on Figure 9 to show the duration of the optimum temperature for the development of pre-

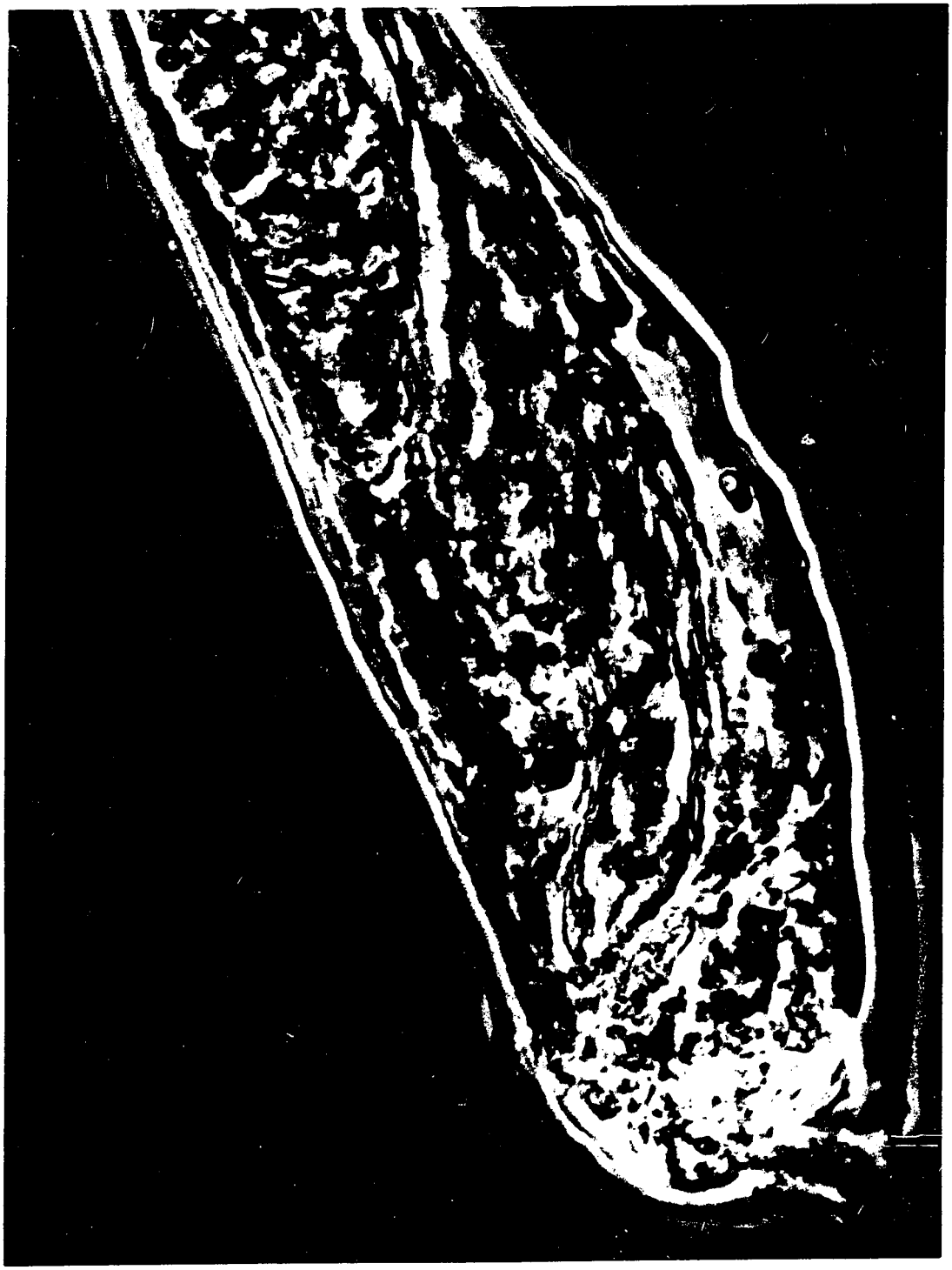
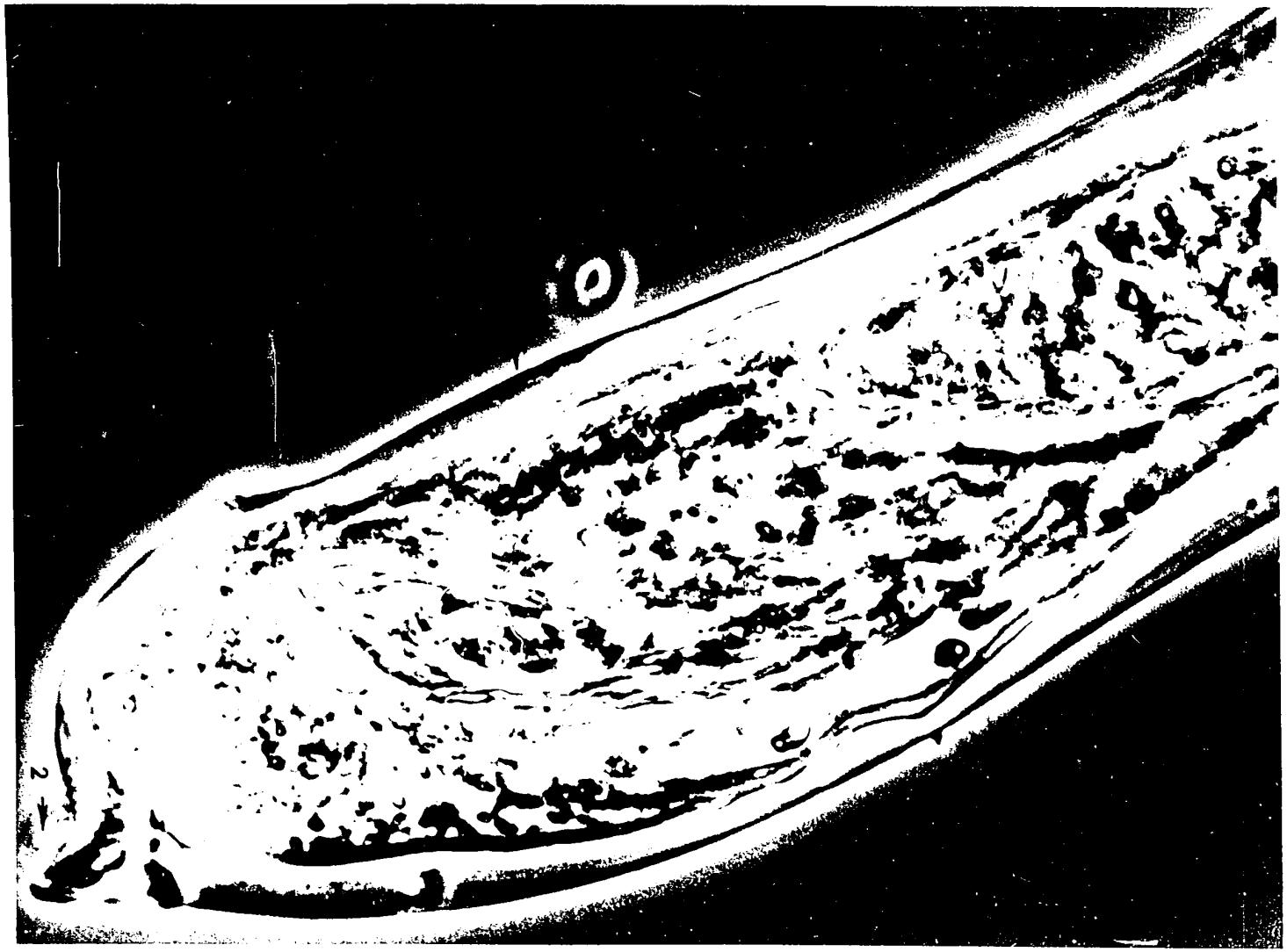


Plate XI. Posterior end of a male D. viviparus larva (late fourth-stage). Sheath (1), rudimentary bursal rays (2). (x 1360).



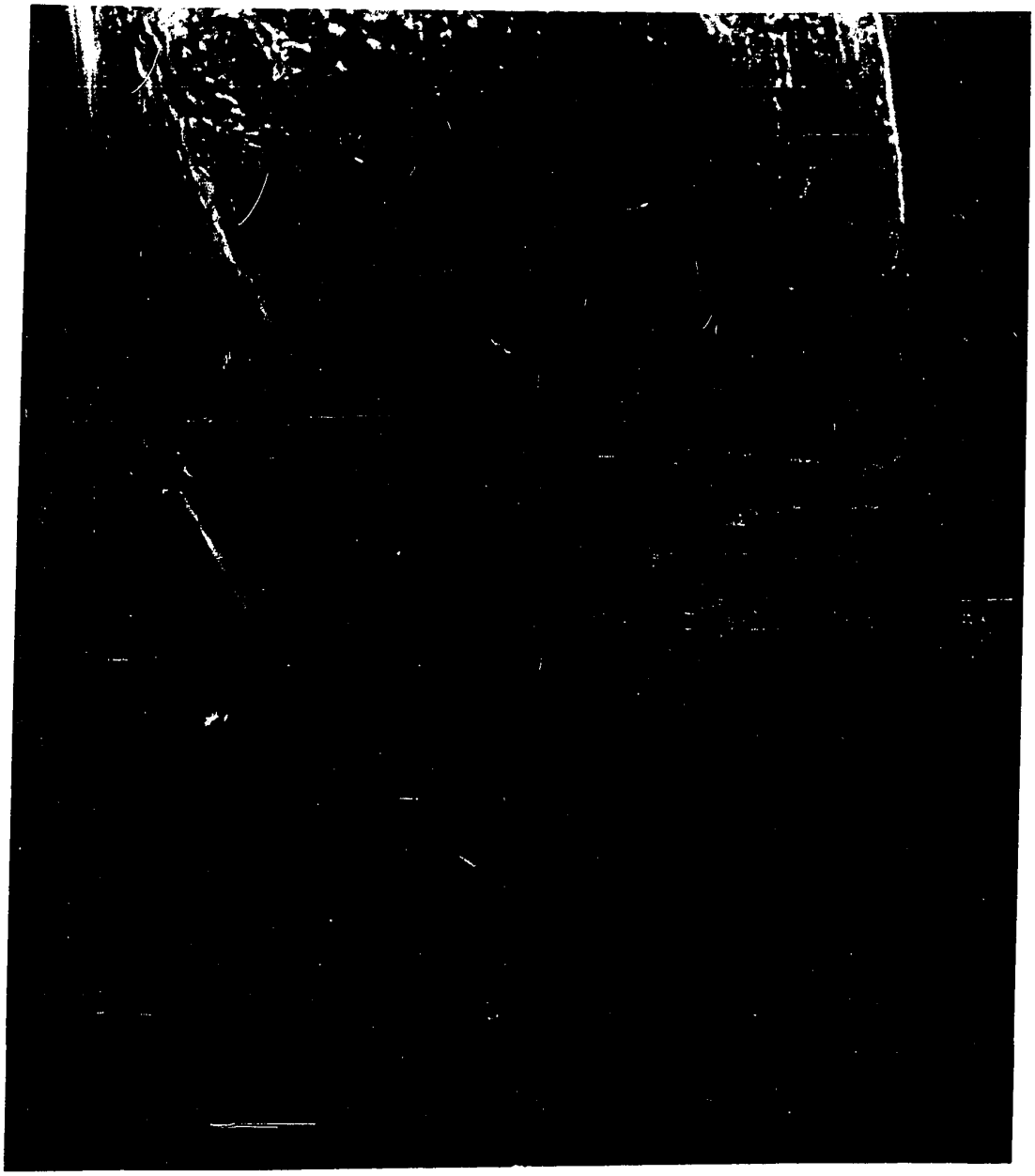
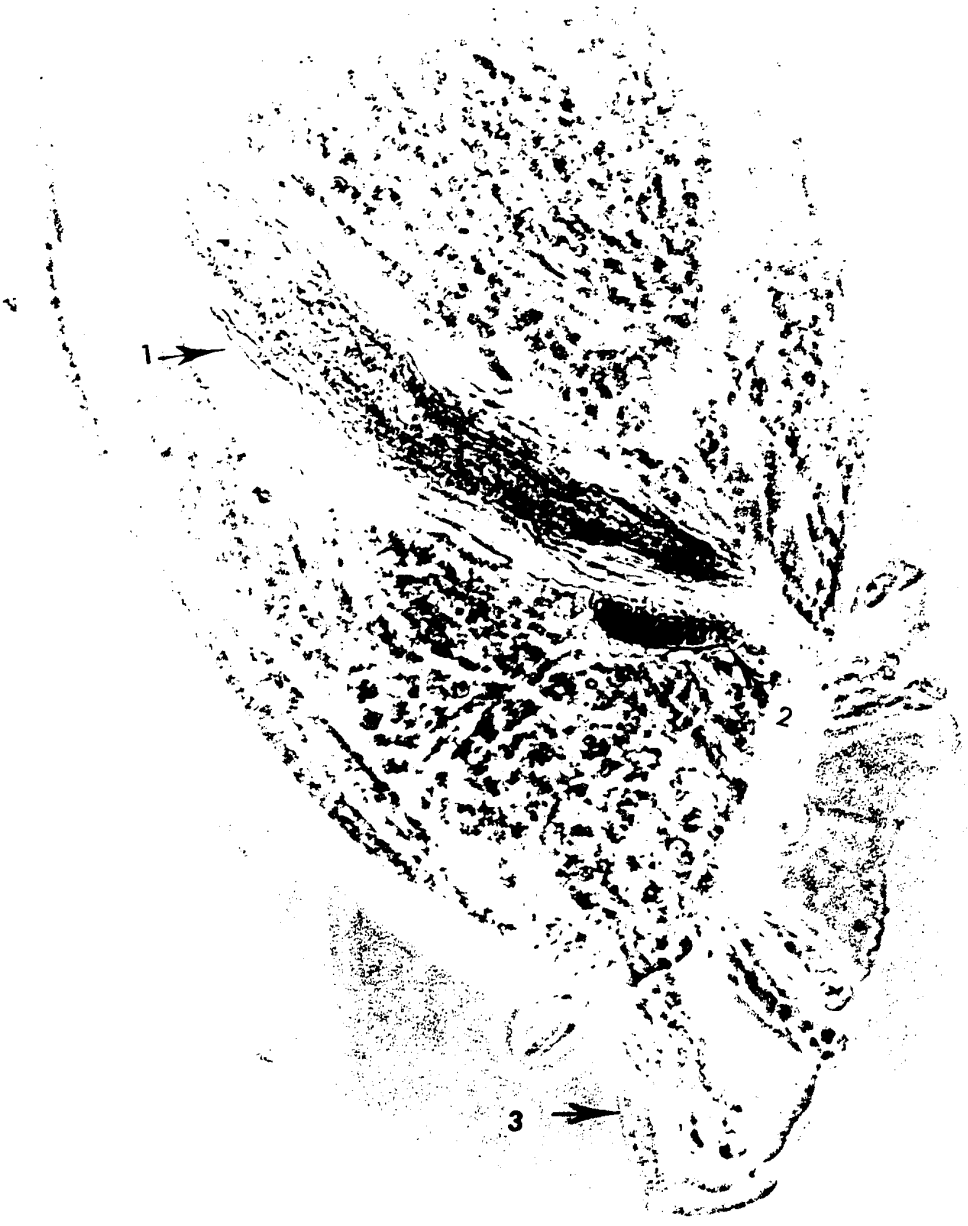


Plate XII. Posterior end of a mature male D. viviparus. Spicules (1), guberneculum (2), bursal rays (3). (x 525).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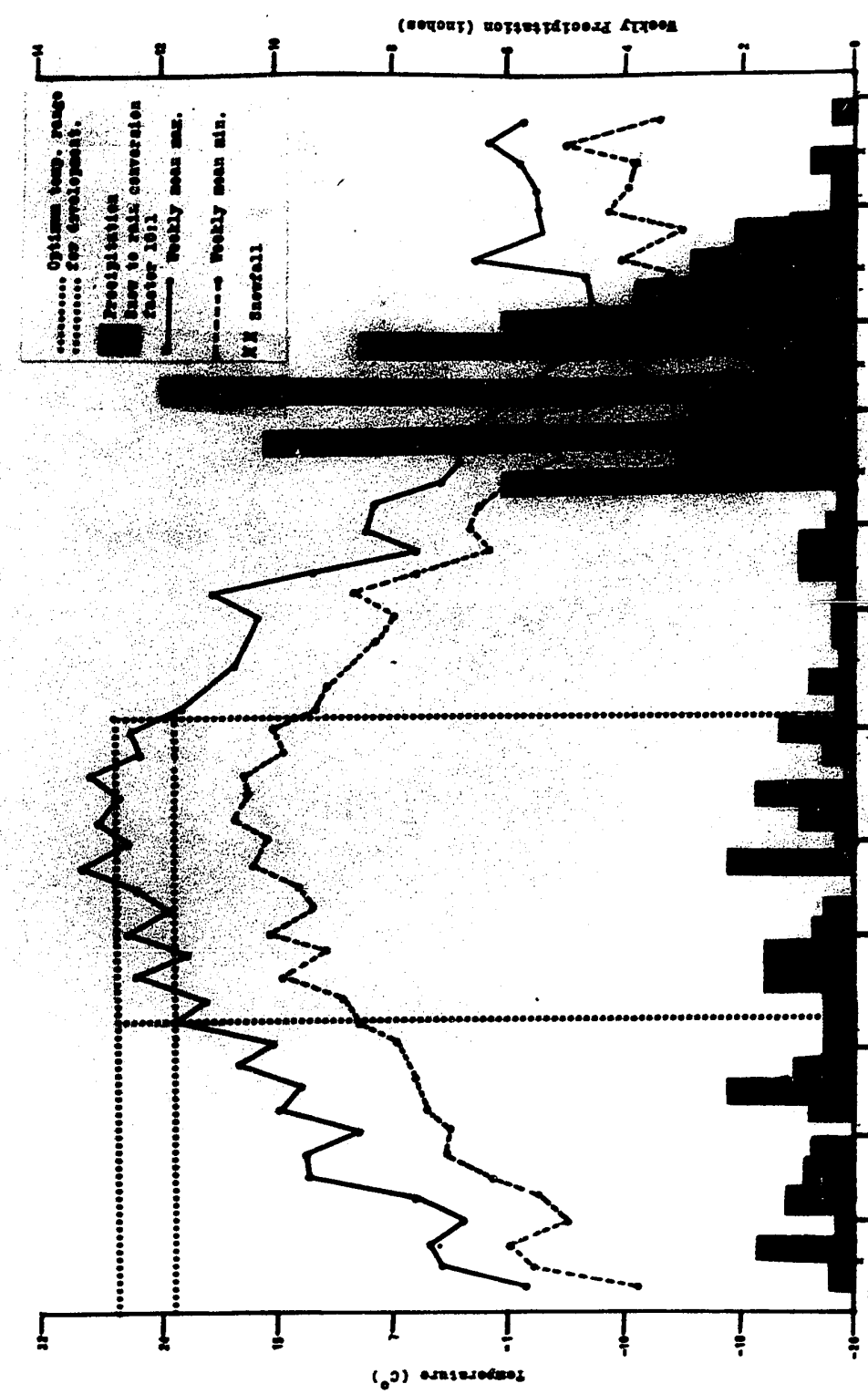

Figure 9. Temperature and Precipitation (March, 1968, to February, 1969) Recorded at Macdonald College Observatory.

parasitic stages to the infective stage (middle of June to middle of September). From the second half of September to the middle of November the temperature may be suitable for the survival of infective larvae on pasture. Later, the snow cover protects the larvae from extreme cold during the second half of November to the following April. It appears that the precipitation may be just adequate for the development of lungworm larvae. Therefore, the limiting factor for the development of free-living stages of lungworm to the infective stage on pasture might be the temperature in this region of Canada.

DISCUSSION

A. Age Incidence

The survey on the age incidence of lungworm infection was carried out on a selected and limited number of animals for one annual cycle, mainly to elucidate the pattern of disease under natural conditions. The results (Tables IV to X) clearly demonstrate that the disease is more of a problem in young than in adult animals. Parallel results, of a high incidence of disease in young animals, have been reported from different countries, particularly where lungworm infection is endemic. Some of these countries are: Canada (Choquette, 1954; Gupta and Gibbs, 1969), England (Michel and Shand, 1955; Cunningham *et al.*, 1956), Germany (Enigk and Duwel, 1962), Ireland (Allan, 1959), Poland (Wertejuk, 1963) and the U.S.A. (Lanier, 1970). There can be exceptions, however, since Jarrett *et al.* (1954) and Campbell and Wetherill (1957) at times found the disease to be more prevalent in adult animals. This unusual situation could be explained by the observations of Michel and Coates (1958) and Michel (1962) who found that in the absence of infection, immunity against *D. viviparus* declines and the animals become susceptible to reinfection. In the cases reported by Jarrett *et al.* (1954) and Campbell and Wetherill (1957) the adult animals may have been removed from the source of infection for some time and could have lost their immunity. Subsequently, the animals may have been exposed to heavy infection which resulted in lungworm disease.

Michel (1959) stated that there was no evidence of true age resistance against D. viviparus infection. In the absence of age resistance, he suggested two possible reasons to explain the differences in the incidence of this disease. One, that the infection in older animals seldom reached patency and, if it did, that the patent period was short when compared with that in young animals. Secondly, older animals in endemic areas had the opportunity of acquiring immunity when they were young and were thereafter resistant to subsequent infection in later life.

In addition, there are limitations to the techniques used for detecting infections. This is particularly true in diagnosing the disease in adult animals where the infection is generally mild and there are relatively few worms for the size of the animal. Fecal examination in such cases can be misleading. The results of the present study were based on fecal examination only, therefore, the picture of the incidence of lungworm infection in older animals should be treated with caution.

B. Seasonality

It is evident from the results (Table X) that lungworm disease had a definite seasonal pattern in this region of Canada with the highest incidence being seen during the fall. This is in agreement with the findings of Gupta and Gibbs (1969) in Canada and also those reported by Jarrett et al. (1954), Boev and Ivershina (1954),

Sudachenkov (1955), Gregoire et al. (1957), Groves (1957), Swietlikowski (1959), Bohn (1961) and Dmitriev (1964) in different parts of the world.

Two main reasons have been put forward to explain the seasonality of the disease; 1) local climate and 2) husbandry practices.

1. Effect of Local Climate on the Seasonality of D. viviparus Infection

The development and survival of the preparasitic stages of D. viviparus depend mainly on the prevailing climatic conditions, particularly with respect to the temperature and relative humidity. According to Soulsby (1965) the optimum temperature required for the development of free-living stages of D. viviparus is in the range of 22.5 to 27.0°C. This is in agreement with the findings of Rose (1956;1960) and Rose and Michel (1957). It has been suggested that the population of the infective stages of D. viviparus on pasture fluctuates due to the seasonal changes in the climate (Michel, 1959).

In the temperate zone it appears that environmental conditions during late summer and fall are likely to be optimal for a build-up of D. viviparus infection on pasture, consequently, a high incidence of lungworm infection in susceptible animals seems a likely event during these seasons.

In this region of Canada, favourable temperatures for D. viviparus free-living stages to develop to the infective stage

occur from the second week of June to the second week of September (Figure 9). During the rest of the year the temperature is too low to expect any substantial addition in pasture infestation. However, it is possible that from the second half of September to November, infective larvae on pasture may persist and might at times give rise to mild infection in animals. It can thus be envisaged that sufficient numbers of infective larvae on pasture to start new infections could only accumulate from July onwards. This would account for the seasonality of the disease in this area.

2. Effect of Husbandry Practices on the Seasonality of *D. viviparus* Infection

The overwinter survival of lungworm larvae on pasture and the epidemiological significance of such larvae in the disease process is questionable. However, the role of carrier animals in reseeding pasture during the next grazing season is well authenticated (Michel and Shand, 1955; Gupta and Gibbs, 1970). Since the carrier animals are only lightly infected, pasture contamination by them will be limited. The susceptible animals, therefore, when pastured with the carriers will generally acquire a low-grade infection during the early part of the grazing season (May to July). However, in turn, these animals will contaminate the pasture within a relatively short period and, coupled with this, favourable weather conditions during August to October will

create a high degree of pasture infection. It can thus be visualized that the incidence of lungworm infection would be highest during these months. This hypothesis is substantiated by the results of the present study where severe lungworm infection was observed during late summer and early fall. A similar explanation has been forwarded by Taylor (1953) and Jarrett et al. (1954) who see the outbreak of lungworm disease as a result of build-up of infection between calf and pasture.

The marked seasonal incidence of the disease could also be due to the fact that the occurrence of the disease depends on the availability of the infective larvae to the host. In Eastern Canada animals are housed from November to May under conditions which prevent parasitic infections. Thus, the incidence of lungworm infection would be lowest during this period. The animals are pastured from June to October and this is the time when they come into contact with infective material.

C. The Importance of Overwinter Survival of D. viviparus Larvae on Pasture and in Barns in the Epidemiology of D. viviparus Infection

On Farms A and F, the parasite-free calves when grazed on a previously contaminated, overwintered pasture, remained free from lungworm infection which suggested that infective larvae of D. viviparus were unable to overwinter on these properties during 1968-69. Additional observations on Farm A (Table XI) throughout

the grazing season proved that lungworm larvae did not overwinter on this farm. Similarly, repeated fecal examinations on the lungworm-free calves on Farm E, which were grazed from June to November, 1968, on a previously contaminated, overwintered pasture, gave no indication of lungworm infection. On Farm C, the results were again negative for D. viviparus infection in lungworm-free calves although they were housed from May to November, 1968, in a barn which had held positive lungworm animals the previous winter (November, 1967, to April, 1968). There could be two possible reasons for this, one that these calves were tied up individually, thus reducing their chances of contact with a potential source of infection and two, that the D. viviparus larvae were unable to develop or survive long enough to be picked up by the calves inside the barn.

A somewhat contradictory situation was encountered on Farm A where a young calf contracted lungworm disease and died without ever having been on pasture. Enigk and Duwel (1962) observed a similar incident and suggested that housing carriers and lungworm-free calves loose together in a box-stall could lead to such infection. It is suspected that mechanical transportation of infected feces into the calf pen was the reason for this unusual case.

Results (Table XI) on this issue from Farm B contradicted those recorded on Farms A, C, E and F, as lungworm-free calves,

when pastured on a previously contaminated, overwintered pasture, contracted heavy D. viviparus infections. The most likely explanation, in the absence of overwinter survival, could be the situation of the calf-pasture on this farm which was such that mechanical transportation of infected material could have occurred either from the owner's lungworm carrier animals or from the neighbours' infected animals.

On Farm D, where the owner grazed his lungworm-free calves and lungworm carrier animals together, on a previously infected, overwintered pasture, the source of infection for the calves could have been either due to overwinter survival or by way of the carriers.

The field observations on the ability of lungworm larvae to overwinter were inconclusive. Consequently, an experiment was carried out on the Macdonald College farm during 1968-69 to settle this question. From the results (Table XIV) it was evident that larvae did overwinter at this time.

From the literature (Poynter, 1963; Poynter and Selway, 1966) it appears that opinions regarding the ability of the preparasitic stages of D. viviparus to overwinter on pasture are equivocal. One group of researchers (Jarrett et al., 1955a; Allan and Baxter, 1957; Enigk and Duwel, 1961; Poynter, 1963) have demonstrated that the infective larvae of D. viviparus can overwinter on pasture.

On the other hand, there are reports (Porter, 1942; Michel and Shand, 1955; Popov *et al.*, 1965; Pouplard, 1968) which claim that lungworm larvae cannot overwinter on pasture. Jarrett *et al.* (1955a) have reviewed the issue and suggested that much of the controversy could be due to the different criteria used by workers to demonstrate the survival on pasture. According to them the most reliable method is to allow susceptible calves to graze on an infected pasture after a stipulated period and later to carry out clinical, parasitological and postmortem examinations of the animals to determine the presence or absence of parasites. However, it should be realized that only a small percentage of larvae are likely to persist and remain infective after winter. Apart from the suggestion of Jarrett *et al.* (1955a), it is important to realize that the effect of climate on the overwinter survival of lungworm larvae on pasture should not be overlooked. It is quite likely that the conflicting results reported above could be due to the differences in local climate. For example, one cannot equate the results on this issue obtained in Southeast England with those from Eastern Canada as there are tremendous differences in climate. I am inclined to believe that local climate is a determining factor on the ability of free-living stages of *D. viviparus* to overwinter on pasture.

The results reported in this study are based on grazing susceptible calves on contaminated pastures under husbandry

practices applicable to this region of Canada. Regular fecal, followed by postmortem examinations were the criteria used for evidence of lungworm infection in the calves.

It is difficult to comprehend why larvae apparently did not overwinter during 1967-68 whereas, during 1968-69, they did. Possible explanations for these contradictory results are differences in:

a) The intensity of pasture contamination during the previous grazing season.
b) Pasture conditions with respect to herbage and drainage.
c) The stocking rate per acre.
d) The annual fluctuations in the climate.

On Farms A, C, E and F larvae were unable to overwinter during 1967-68, this could have been due to low pasture contamination from fewer calves per acre than on Farm B. Also, the winter of 1967-68 was severe with sub-zero temperatures before the snowfall. It is possible that severe frost before the snowfall could have decimated the larval populations and those larvae that survived were present in insufficient numbers to be of epidemiological importance. On Farm B, if the infection came from the overwintered pasture and not from fresh contamination, it could have been due to heavy pasture contamination during the former grazing season and a very high stocking rate (approximately 50

calves per acre) during the following spring. The positive results on overwinter survival on the Macdonald College farm during the winter of 1968-69 were considered to be due mainly to the heavy pasture contamination which occurred during the preceding grazing season and also to the early snowfall which took place on November 12, 1968, and stayed until the following May. This presumably could have protected the larvae from adverse atmospheric temperatures. It has been amply proven that good snow cover is favourable for the survival of nematode larvae (Swales, 1940). It has been observed by the author that larvae will remain infective for at least eight months when held in the refrigerator, in shallow water, at a temperature of $4^{o}C$.

This is the first time that overwinter survival of lungworm larvae has been demonstrated in this region of Canada. The implication of this in the persistence of the disease and in formulating control measures have to be investigated in detail. The number of D. viviparus which were recovered from the calves grazed on a previously contaminated, overwintered pasture (Table XIV) on the Macdonald College farm were small and it is difficult to assess the importance of this degree of survival of lungworm larvae relative to the carrier animal in the epidemiology of lungworm infection. However, it should be realized that the initial light infection at the beginning of the grazing season could well be responsible for a dangerous pasture infestation during the following grazing months.

D. The Importance of Carrier Animals in the Epidemiology of D. viviparus Infection

1. Reshedding Phenomenon in Carrier Animals

It was seen (Table XII) that a number of yearlings on the farms studied started reshedding lungworm larvae on one or more occasions after being negative for extended periods. Similarly, fecal examinations (Table XV, Figure 1) of the carrier animals at Macdonald College showed reshedding during spring after being negative during the preceding fall and winter. In both these cases, the animals were housed under conditions which precluded reinfection. This suggested that reshedding of larvae in the carriers might have been due to the maturation of larvae in spring which were arrested in their development during the winter. Comparable results have been reported by Jarrett et al. (1954) and Swietlikowski (1959) who demonstrated that some animals which were consistently negative for lungworm larvae during winter, on several occasions during April and May, started reshedding. From the results of this experiment as well as those quoted above, it appears that the situation is probably analogous to the "spring rise" phenomenon seen in Haemonchus contortus infection in sheep (Gibbs, 1967) and Type II ostertagiasis in cattle (Armour, 1970a).

On the other hand, Wetzel (1950) and Swietlikowski (1959) have noticed uninterrupted shedding where animals continued to shed larvae throughout the winter and into the next season.

From their experiments it was not clear whether the animals were housed during winter or grazed outside throughout the year. From the life cycle of D. viviparus it is clear that the patent period does not last for more than 55 days. Thus, lungworm infected animals cannot shed larvae throughout the year in the absence of reinfection and it appears that the animals in question were exposed to the infection continuously.

2. Grazing Potential Carriers and Susceptible Calves Together

Results on this aspect from Farm A, where four and two parasite-free calves were pastured from June to August and from August to October respectively with lungworm carrier animals (potential) showed that patent D. viviparus infection developed in the test calves (Table XIII). It is reasoned that the carriers contaminated the pasture and in turn, the test calves picked up infection. However, in this case, the possibility of overwinter survival of larvae could not be completely ruled out because this pasture had been grazed the previous year by adult cattle of unknown lungworm status.

Studies on Farm D, where lungworm-free calves were grazed together with carrier animals, on a previously contaminated, overwintered pasture, revealed that seven out of eight calves became heavily infected with D. viviparus. Once again, the source of infection of the calves in question could have been either

overwintered larvae or fresh contamination of pasture by carrier animals. Indirect evidence on the role of carrier animals was obtained on Farms A and E where the owners grazed their lungworm-free calves on a previously contaminated, overwintered pasture, but kept their carrier animals away from the calves. Fecal examinations of these calves throughout the grazing season (June to November) remained negative for evidence of *D. viviparus* infection. It can, therefore, be assumed that carrier animals, rather than the overwintered larvae, are more certain sources of infection.

The data gathered on the different farms (A, D and E) suggested that carrier animals could play an important part in the persistence of lungworm disease from year to year. However, on all the farms there was the possibility of an alternate source of infection. Thus the results were circumstantial and further evidence under controlled conditions was deemed essential to substantiate field observations.

From the experiments conducted at Macdonald College it is apparent that the parasite-free calves, when pastured together with carrier animals (potential), contracted lungworm infection (Table XVI). It is interesting to note that none of the carriers ever showed any larvae in their feces while grazing with the test calves. However, during postmortem examination of the carriers, 35 immature

and three mature females were recovered from the three carrier animals. Thus it appears that the carriers were shedding too few larvae to be detected by fecal examinations. The pasture used in this study had been free from cattle for at least 10 years. Moreover the situation of the pasture was such that the chances of any accidental contamination with lungworm was highly unlikely. Taking everything into consideration the evidence appeared conclusive that the only possible source of infection of the calves was the carrier animals.

The importance of lungworm carrier animals in the contamination of pastures the following spring has been reported by several workers from different countries. However, opinions vary regarding the relative importance of carriers versus other sources of infection, such as overwinter survival of larvae on pasture, continued reinfection where animals are outwintered and contamination of pasture by wild herbivores. One group of researchers, Wetzel (1948), Michel and Parfitt (1955), Michel and Shand (1955), Groves (1957), Parker (1967) and Pouplard (1968) are convinced that pastures in all possibility lose lungworm infection after winter spelling, and the only likely source of fresh contamination of pastures the following grazing season is by carrier animals. Briefly, the opinions of the authors cited above could be adequately summarized by quoting Michel and Shand (1955) who stated "the ultimate source of infection is in the form of larvae passed by carrier animals

and not larvae that have persisted on the ground through the winter."

The second group of investigators who, although recognizing the part played by carrier animals in the epidemiology of lungworm disease, emphasize other sources of infection as mentioned earlier, in the perpetuation of the disease. Among the principal supporters of this concept are Jarrett et al. (1954;1955a;1957), Cunningham et al. (1956), Swietlikowski (1959) and Enigk and Duwel (1962). However, in none of these reports have the authors tried to evaluate the relative importance of these factors in the persistence of lungworm infection. An objection to the theory of carrier animals as the only source in contaminating pasture the following grazing season is posed by the question, how could a small number of larvae possibly give rise to a severe pasture infestation? Michel and Shand (1955) suggested two possible ways by which this could be accomplished. Firstly, the infection could be augmented by susceptible animals, and secondly, lush herbage could provide a microclimate conducive for larval development and persistence. A somewhat similar opinion was expressed by Jarrett et al. (1954) to explain the reason(s) for severe pasture infestation during late summer and early fall. From the results of this study it appears that both sources, namely, the overwinter survival of lungworm larvae on pasture (Table XIV) and carrier animals (Table XIII and XVI) may serve to start a fresh infection. The evidence

suggested that the carrier animals, however, are the more certain and important means for the persistence of lungworm infection in this region of Canada.

It must be pointed out here that the importance of carrier animals versus overwinter survival of lungworm larvae on pasture in the perpetuation of lungworm disease will vary from one country to another or from one location to another. It is quite likely that in places where the winter is mild the residual pasture infection would be a problem, however, in countries like Canada where the winter is severe, possibly the carrier animals are the ones which keep the disease going from year to year.

E. Seasonal Worm Population Fluctuations in the Permanent and Tracer Calves on Pasture and in Calves Experimentally Infected Inside

1. Fluctuations in Larval Count

Experiment I:- The larval count in the early grazing season (May 26, 1969, to August 8, 1969) was low (Table XVII), however, during the next four weeks it rose to the highest peak. Thereafter, a sudden dramatic fall in the larval count (September 26, 1969) was noticed. From this point onwards a progressive decline in the larval count continued until October 17, 1969, following which, all the remaining calves became almost negative for evidence of patent lungworm infection.

The initial low fecal larval count reflects low levels of infection in the calves which could be due to a small number of

infective larvae available to them on pasture early in the grazing season. This might have been due to the severe cold during the winter which decimated the larval population on pasture, or to the fact that the larvae shed by the carrier animals during the spring might not have been able to reach the infective stage due to unfavourable climatic conditions, particularly temperature, as is evident from the weather data (Figure 9).

The sudden rise in larval yield during the next few weeks appears to be a consequence of heavy pasture infection (reasons will be discussed later). Another possibility for this upsurge in fecal larval count could be a slow but steady increase in the number of adult worms in the calves as a result of continuous acquisition from grazing.

The sudden dramatic fall in the fecal larval count could be interpreted as a "self-cure-like" phenomenon reported by Wetzel (1948) in D. viviparus infection which is similar to that observed in other host-parasite relationships (Gordon, 1967).

Experiment II:- Tracer calves were pastured each month with the permanent calves with the object of determining the fluctuations in pasture infestation, and the availability of infective larvae to the permanent calves from month to month during the entire grazing season. Fecal examinations (Table XIX) of the tracer calves during different months showed a continuous increase in the

number of larvae excreted per 10 gm of feces (except June) which indicates that the tracer calves continued to acquire more and more worms as the season progressed. This indicates that the number of larvae/kg of herbage available to the calves continued to increase. The pasture infestation during September was so high that both the tracers of that month died from clinical lungworm disease.

Low fecal larval yields in the tracer calves of May suggested that the larvae which survived overwinter on pasture were few and therefore the pick-up was low. The negative results during June could have been due to any one or a combination of the following possibilities. Firstly, that the overwintered larvae on pasture rapidly died off, secondly, that the lush growth of herbage during the early part of the spring led to the dilution of the larvae to such an extent that they became epidemiologically insignificant and thirdly, that the fresh contamination was unable to attain the infective stage due to unfavourable climatic conditions as explained earlier. The severe infection in the tracer calves from July to October suggested that the pasture was heavily contaminated during this time with infective larvae. Studies on the bionomics of D. viviparus preparasitic stages in the United Kingdom by Rose (1956), Rose and Michel (1957) and Rose (1960) showed that the environmental conditions during summer and fall are optimal for the development and survival of infective lungworm

larvae on pasture. Climatic conditions (Figure 9, see hypothetical line for optimal temperature) in this region of Canada appeared conducive for free-living stages to reach the infective stage only during the summer months. However, during fall the temperature was favourable for the survival the infective larvae. This leads to a severe pasture infestation during late summer and early fall which explains precisely the occurrence of heavy lungworm infections in the tracer calves during this time of the year.

Experiment III:- From the results (Table XXI) it is evident that lungworm infections became patent in the experimental calves from August to October. Fecal larval count per mature worm differed tremendously from one month to another (August 5.0; September 2.0; and October 0.1). This appears due to lowered fecundity, the reasons for which will be discussed later.

Experiment IV:- D. viviparus larvae were detected only in the feces of the calves in November and March (Table XXII). Once again the fecundity of the mature worms differed during November (0.25/worm) and March (0.84/worm).

2. Fluctuations in Mature and Immature Worm Population

The behaviour of D. viviparus in susceptible animals exposed to infection continuously, is debatable. Michel and Parfitt (1956), Michel and MacKenzie (1956) and Michel (1959;1962;1968) have suggested that a calf grazing on a moderately contaminated pasture

for nine to 11 days will develop resistance which will prevent the maturation of the majority of larvae acquired after that time, provided the pasture infestation does not fluctuate too much. This means that immunity against D. viviparus develops quickly and the worm burden, under natural conditions, is regulated by the number of larvae acquired by a calf during the first few days on pasture. Contrary to this, Wetzel (1948) and Jarrett et al. (1954) emphasized that immunity against D. viviparus does not develop before 16 to 22 weeks after exposure. This fits well into the results of Jarrett et al. (1954) who observed that it takes about 10 to 15 weeks for the calves on infected pasture to become clinically sick. It was reasoned that the calves probably contracted an initial light infection during the early part of the grazing season and it was the larvae of these lungworms or even the second generation which might be responsible for clinical disease.

The results of this study conflict with those reported by the former group and are in agreement with those of the latter group. It is evident from Experiment I that the worm burdens remained high in the calves until September (average worm burden, June 61, July 268, August 148, and September 163). This implies that immunity to D. viviparus did not develop within a short period. However, the results (Table XVIII) suggest that normally lungworm disease is the outcome of a build-up of infection between the calves and pasture and that it takes approximately two to three months before

the calves accumulate sufficient numbers of worms to make them clinically sick. This contention derives support from the findings of Gupta and Gibbs (1969;1970) who have noticed a significantly high incidence of lungworm infection in Quebec cattle during the fall. The evidence of acquired immunity was clear-cut in the permanent calves from August onwards. Mature worms recovered from these calves in August and September were stunted (Plate III) and the fecundity was greatly reduced when compared with the worms recovered from the calves in June and July. In addition, the calves in October and November had only immature worms although they were grazing on a heavily contaminated pasture. The influence of acquired immunity in the phenomenon of inhibited development will be discussed later.

The results of Experiment II (Table XX) revealed an ever increasing number of adult worms recovered during postmortem from the tracer calves from May to October (except June) although the calves were exposed to infection for a constant period (14 days) during each month. This suggests that the number of larvae/kg of herbage available to the calves constantly increased from May to October. The negative results for June were explained in the previous section. The tracer calves in November had a few immature worms. Possible reasons for this inhibited development in a fully susceptible host are quite interesting and will be discussed later.

The numbers of mature worms recovered from the calves of Experiment III (Table XXI) declined from August to October (August, 330, September, 144, and October, 78). From November to February all the worms recovered were inhibited. However, during March, 19 percent of the worms recovered were mature.

The lungworm larvae that were used had been cultured during August, 1969, stored at 4°C and used throughout the experiment as required. It is obvious that these larvae aged as the season progressed (August to April). A progressive fall in the recovery of mature worms during the early part of this experiment (August to October) and later (November to February), the complete absence of mature worms in the calves, could among other things be attributed to the age of the infective larvae. Parallel results of the reduced infectivity of old larvae of D. viviparus have been reported by Michel and Parfitt (1955), Swietlikowski (1969), Cornwell and Jones (1970), Michel (1969;1970) and Poynter (1970). However, the recovery of 19 percent mature worms from the calves in March when the larvae were much older (seven months) suggested that age of larvae is not responsible for the decline in infectivity of the D. viviparus larvae.

However, the results of Experiment IV (Table XXII) indicate that ageing of larvae could be involved in the lowering of the infectivity. In none of the calves in Experiments I, II and III during November was there a patent infection but in Experiment IV

the infection reached patency. This could only be explained on the basis that the larvae were fresh and thus were relatively more vigorous and infective. Once again the results from December to February and March followed the same pattern as in Experiment III. Possible explanations for the inhibited development during December to February and partial maturation during March will be given in the following section.

F. The Phenomenon of Inhibited Development in D. viviparus

From the results of Experiments I to IV (Tables XVIII, XX, XXI and XXII) it is evident that D. viviparus undergoes retarded development during autumn. As is evident from the literature review, numerous suggestions have been made to explain the mechanism(s) responsible for inducing inhibited development. In this section, I plan to evaluate critically the different hypotheses in the light of the results obtained in these studies.

Acquired Immunity:- It has been well authenticated in many host-parasite relationships that hosts acquire resistance to reinfection with parasitic diseases (Soulsby, 1965; Michel, 1968). This is particularly true for D. viviparus where experimental or natural reinfection seldom becomes patent (Jarrett et al., 1955b; Rubin and Lucker, 1956; Michel and MacKenzie, 1965). This is further supported by the results of vaccination against D. viviparus which provoked strong immunity, as pointed out by Poynter et al. (1970). Michel (1959) suggested that immunity to D. viviparus is manifested in several ways, one of which is to induce inhibition.

The results of the present study do not suggest that acquired immunity is the cause of the inhibited development seen in the fall. In Experiment I (Table XVIII) all the worms recovered from the permanent calves in June, July and August were mature. Retarded development started in the calves in September and rose to 100 percent in the calves in October and November. According to Michel (1959) and Michel and Cornwell (1959) calves acquire sufficient immunity within nine to 11 days, after initial exposure, to prevent the maturation of the majority of larvae acquired thereafter. If this is true, one would expect to have seen some degree of retarded development in the permanent calves during July and August. However, this was not the case, the inhibited development was only found in the calves from September onwards. This implies that immunity to D. viviparus does not develop rapidly, as suggested by the above mentioned workers. However, the inhibited development observed in the permanent calves from September to November could still be ascribed to the acquired resistance of the hosts. In this case, if acquired resistance is the cause of inhibition, it would appear that immunity to D. viviparus develops slowly. On the other hand, it is quite likely that inhibition in D. viviparus could be independent of the host's immunological status. That this appears to be so can be seen in the results of Experiments II to IV. In Experiment II, the tracer calves used during the different months (May to November) all had opportunities to acquire resistance. However,

from the results (Table XX) it can be seen that up to September there was no inhibition. Thereafter, the percent inhibition in the calves of the following months (October, 11 percent and November, 100 percent) is of particular interest. A logical explanation of these results would be that factors other than the host's immunity were involved in inducing inhibition. This contention was supported by the results of the next two experiments (III and IV). The calves of these experiments were infected with a constant number of infective larvae over a period of 14 days. However, the results (Table XXI and XXII), with respect to the percent inhibited larvae recovered from the calves in different months, showed great seasonal variation. Once again it appears that acquired resistance has nothing to do with the inhibited development. If immunity had some influence, then under the conditions of Experiments III and IV, one would expect to observe approximately uniform degrees of inhibition in the experimental calves from the different months. However, inhibition in the calves of Experiment I (permanent calves) was noticed much earlier than in the calves of any of the other three experiments. This could, among other possibilities, be due to the acquired resistance in these calves.

Of interest in this context are the findings of Michel (1955b), Michel and MacKenzie (1965) and Parfitt and Sinclair (1967) who reported inhibited development of D. viviparus in calves infected for the first time with a single dose. The results

of these authors as well as those reported here cast further doubts on the validity of the assumption that acquired resistance is the only cause of arrested development in D. viviparus. This does not, however, rule out the role of acquired immunity in the phenomenon of inhibited development in other host-parasite relationships. The essential point of these observations is that inhibition in D. viviparus could occur under conditions which exclude the operation of acquired immunity.

Presence of Adult Worms:- The presence of a "hypothetical" number of adult worms in the host has been alleged to retard the development of their kind as suggested by Gibson (1953), Dunsmore (1963) and Michel (1963) among others. The results of the present studies (Experiments I to IV) negate this theory of the role of adult worms in inducing inhibited development. In fact, it is clear (Tables XVIII, XX, XXI and XXII) that the highest numbers of mature worms were associated with zero percent inhibition and vice-versa. These findings are in agreement with those reported by Dunsmore (1965) and James and Johnstone (1967a) working with Ostertagia circumcincta; Gibbs (1967) working with Haemonchus contortus; and Jennings et al. (1967) working with Ostertagia ostertagi, where a lack of correlation between the number of adult and immature worms has been reported.

Age of the Host:- In some host-parasite relationships, the age of the host at the time of infection is thought to be of

significance in inducing dormancy (Gibson, 1959; Silverman and Patterson, 1960; Herlich, 1960; Brunsdon, 1962a). However, Dineen and Wagland (1966b) were unable to demonstrate any relationship between the age of the host and its ability to inhibit the development of its parasites. In the present studies all the animals used were approximately of the same age except in Experiment I where the animals were older at the time of necropsy as can be seen from the Experimental Design (Table II). In the light of this fact, one could associate age of the calves removed during September onwards as a contributory factor in the etiology of inhibited development. In the rest of the experiments (II to IV) where the experimental calves were approximately of the same age, no direct conclusion on the influence of age in the phenomenon of inhibited development could be drawn. However, as is evident in the results (Tables XVIII, XX, XXI, XXII and XXIII) tremendous differences in the degree of inhibited development of *D. viviparus* in calves (of approximately the same age) were noticed during different months. These results could only lead to the conclusion that at least in this case the age of the calves was not responsible in influencing inhibited development.

Age of the Infective Larvae:- Another explanation for the phenomenon of inhibited development is centered on the age of the infective larvae as argued by Michel and Parfitt (1955), Madsen (1962), Armour (1970a,b), Michel (1970) and Poynter (1970). It is

reasoned that the older larvae are unable to complete their development within the normal time since they lack vigor and infectivity.

In this study inhibited development in Experiments I and II started from September and October respectively. Since the pasture was continuously infected from May to September the age of the infective larvae available to the calves of different months while on pasture could not be ascertained accurately. However, from the studies on the bionomics of D. viviparus (Rose, 1956; Rose and Michel, 1957) one can deduce that D. viviparus larvae will continue to develop to the infective stage upto the middle of September in this region of Canada (Figure 9), whereas, during October and November, it is unlikely that fresh contamination would reach the infective stage. It is reasonable to assume that a great number of infective larvae available to the calves of Experiments I and II upto September were fresh, whereas, the larvae available to the calves in October and November were relatively older. Thus, among other possibilities, the arrested development noticed in October and November could have been due to the age of the infective larvae.

In experiments III and IV the age of the larvae dosed to the calves in different months was accurately calculated. The results (Tables XXI and XXII) show that the degree of inhibition increased with the increasing age of larvae except during March. In

Experiment III during September and October when the larvae were approximately 30 and 60 days old, zero and 11 percent inhibition was noticed in the calves, respectively. However, in November when the larvae were 90 days old, 100 percent inhibition was noticed in the calves. The results of Experiment IV (Table XXII) showed a remarkable deviation from the first three experiments, as during November only two percent inhibition was noticed. This difference in inhibition rate could only be ascribed to the fact that the larvae used in this experiment during November were fresh and could be relatively more viable and infective.

Parallel results (Table XXIII) were obtained in Experiment V conducted specifically to determine the importance of the age of infective larvae. In this experiment, all the variables were comparable except the age of infective larvae which were 14 and 42 to 56 days. The ratio of mature to immature worms recovered from the calves in this experiment was of particular interest. In the infection using aged larvae all the worms recovered at autopsy were inhibited whereas, when fresh larvae were used, the ratio of mature to immature worms was 87:13 respectively. The differences observed in the worm population structure in this experiment could only be attributed to the differences in the larvae associated with ageing. It has been demonstrated by Stockdale *et al.* (1970) in the case of *Obeliscoides cuniculi* that storing larvae at 4°C in the refrigerator for several weeks

induces changes in the larvae and as a result, the majority of them were subsequently inhibited. The present results strongly suggest that age of the larvae might be involved in the phenomenon of inhibited development.

However, if age of the infective larvae is responsible for inducing inhibition it is difficult to explain why there was maturation of larvae during March, as these were the oldest larvae used (7 months). Similarly, under field conditions with Ostertagia ostertagi (Armour et al., 1969a,b) with Ostertagia circumcincta (Ayalew, 1969) and with D. viviparus (Gupta and Gibbs, 1970), a high percentage of inhibited development was noticed in the animals grazing on infected pasture during late autumn. However, on the same pasture during the following spring, only a small percentage of inhibition was noticed although the larvae on pasture were much older by the time of these observations. Coupled with the results of Experiments III and IV this invalidates the role of the age of infective larvae as the only factor in the etiology of inhibited development.

Strain Differences:- It has been postulated by Anderson et al. (1965), Connan (1968) and Armour et al. 1967a,b;1969b) that a parasite may have different strains with differences in their capabilities to undergo retarded development. It was envisaged that the population of these two strains fluctuates on pasture, and that during late autumn, the number of larvae destined for inhibited development increases tremendously.

The results (Tables XVIII and XX) of Experiments I and II revealed a high percentage of inhibited larvae in the calves of October and November which could be explained by seasonal changes in the populations of different strains of D. viviparus, if one assumes that the populations of larvae which are destined for inhibited development build up during the fall. However, the results of Experiments III and IV (Tables XXI and XXII) showed wide variations in the ability of larvae to undergo inhibited development from one month to another. It is important to mention that the number of immature worms recovered from the calves of Experiments III and IV from December to February varied from 0.2 to 0.8 percent of the total infective dose. It could thus be interpreted that the infective larvae used in these experiments contained approximately one percent of the larvae capable of retarded development. Armour (1970a) has speculated that a similar situation might exist with Ostertagia ostertagi where he suspects that the percent of "inhibition prone" larvae under natural conditions is fixed, but the availability of such larvae increases during autumn since these larvae are resistant to autumn environmental conditions. However, the interpretation of "two strains in one population" does not hold good in the light of the results obtained in Experiments III and IV. From these results it is evident that, using larvae from the same population, the ratio of mature to immature worms varied from 100:0 during August and September, to 0:100 during December to February and approximately 44:56 during

March. This reversal in the ratios of mature to immature worms cannot be satisfactorily explained on the basis of the two-strain theory. Thus, it appears that factors other than strain differences were responsible for the phenomenon of inhibited development at least in this instance.

Physiology of the Host:- A change in the physiology of the host, other than its immune status, has been proposed as a factor in the phenomenon of inhibited development (Dunsmore, 1965; James and Johnstone, 1967a; Gibbs, 1967). It has been satisfactorily documented that the physiology of living organisms is influenced by photoperiod. Therefore, a seasonal change in the metabolism in general and neurosecretion in particular, with seasonal changes in the light:dark ratio can be envisaged. For the continued growth of a parasite, the host must provide all the required nutrients, failing which the parasite may die or may cease development. It is possible that during autumn, at the time of maximum inhibition, the essential growth factor(s) required by the parasite are not made available and consequently the parasite is unable to grow to maturity. These experiments (I to IV) were not designed to study the effect of photoperiod on the host and its worm population structure in relation to development of the parasites. However, the results showed that the larvae had a strong tendency for retarded development during late autumn. It is quite feasible to tie in the seasonal inhibition with a change in the physiology of the host. It is clear from the results (Experiments III and IV)

that some of the larvae attained maturity during the spring (March, 1970). Similarly, under field conditions, Jarrett et al. (1955a) and Gupta and Gibbs (1970) have noticed reshedding during spring in a proportion of lungworm carrier animals. From these observations one could implicate a change in the physiology of the host during spring that triggers directly or indirectly, the inhibited larvae to resume development. However, this has not been substantiated by the results of Blitz (1970) working with Haemonchus contortus who was unable to change the ratio of mature:immature worms by exposing the host to different photoperiodicity.

Physiology of the Larvae:- In many parasites, Haemonchus contortus, Ostertagia circumcincta, Ostertagia ostertagi and Cooperia oncophora (among others) a high percentage of inhibition occurs during late autumn. This seasonality led some researchers to speculate that seasonal changes in the physiology of the infective larvae on pasture (Armour et al., 1967a,b; Jennings et al., 1967; Blitz, 1970) mediated through photoperiodicity and temperature might be responsible for inducing inhibition. Malczewski (1970) considers "inhibition of growth of larvae in autumn and increased elimination of eggs in spring are closely related phenomena and are manifestations of a remarkable adaptability of the abomasal parasites to climatic conditions and the way of the host's life, assuring the continuance of parasitic species."

The results of Experiment II are of particular interest in this context. It will be seen that during November in parasite-free (non-immune) calves there was 100 percent inhibition which could not be attributed to the acquired resistance of the host. Thus, among other possibilities, it is quite likely that the inhibited development observed in these calves was due to a change in the physiology of the infective larvae on pasture. This was further substantiated by the results of Experiments III and IV where 100 percent inhibition was found in the calves during December to February infected experimentally with the larvae cultured in the laboratory and stored at $4^{o}C$ in the refrigerator. It has been suggested by Armour (1970a,b) that storing larvae at low temperatures (under autumn environmental conditions) for more than six weeks could initiate a change in the physiology of the infective larvae. If the change in the physiology of the larvae while on pasture or in the laboratory is similar to the one observed in insects (diapause) then it is to be expected that the larvae will undergo diapause development in all hosts whether immune or non-immune.

Maturation of Arrested Larvae:- Numerous hypotheses have been put forward to explain the maturation of arrested larvae during the spring. The results of this study invalidate the role of acquired resistance, the presence of adult worms, crowding, age of the host, age of the infective larvae and strain differences in causing

retardation. However, it appears that a change in the physiology of the host or larvae might be instrumental in the resumed growth of the arrested larvae. A similar mechanism has been visualized by Anderson et al. (1965), Dunsmore (1965), Gibbs (1967), Jennings et al. (1967), Armour et al. (1969a,b), Michel et al. (1970) and Blitz and Gibbs (1971) in other host-parasite relationships. If one assumes that the larvae underwent diapause-like development during autumn, then their subsequent maturation during the spring could be due to the termination of the same by this time. It is also possible that a change in the host's physiology during the spring could have provided the needed stimulus for inhibited larvae to mature. However the first possibility appears to be a more satisfactory approach to explain these results.

It is pertinent to mention that these studies were primarily undertaken to elucidate some of the mechanisms underlying inhibited development of D. viviparus. They were not designed to determine the reason(s) for their subsequent growth. In the light of this approach, the evidence gathered from these studies implicating physiology of the host or larvae in the phenomenon of renewed growth, is circumstantial.

G. Morphogenesis of Inhibited Stages of D. viviparus

Immature stages of D. viviparus have been associated with the disease cycle by many researchers (Taylor, 1935: Smythe, 1937).

However, it appears that Taylor and Michel (1952;1953) were the first to recover immature stages of D. viviparus (early fifth-stage) from cattle which had been removed from the source of infection for some weeks. About 80 percent of the immature worms recovered by these authors were in the early fifth-stage and the rest were in the late fourth-stage. Douvres and Lucker (1958) described the morphogenesis of the parasitic stages of D. viviparus recovered from experimentally infected guinea pigs which were killed at different time intervals (18, 23, 43, 144 and 154 hours post-infection). The growth pattern and morphological characteristics observed in experimentally infected guinea pigs may be different from those observed in arrested development of D. viviparus in its definitive host. From the results in these experiments, it was seen that approximately 80 percent of the inhibited stages were in the early fifth-stage and the rest in the late fourth-stage. The length of male and female larvae varied from 0.9 to 2.7 mm. The genital system of both male and female (early fifth-stage) was well developed. One interesting observation was that although the larvae varied in length their genital system was always developed to the same extent. It appears that the larvae continue to grow, although at a slow pace, throughout their stay in the lungs but instead of attaining maturity within 25-30 days, it takes about 180 days.

H. The Importance of Wild Herbivores in the Epidemiology of D. viviparus Infection

Many authors have alleged that wild herbivores (Rountree et al., 1954; Durrell and Bolton, 1957; Swanson et al., 1959) as well as sheep (Parfitt, 1963) can serve as lungworm reservoirs for cattle, although in most of the reports, the evidence is circumstantial.

Recently, some success has been reported in infecting calves with D. viviparus from wild herbivores and infecting elk with D. viviparus from cattle (Presidente and Worley, 1968). Similarly, Parfitt (1963) has successfully infected calves with Dictyocaulus filaria. However, Lapage (1968) maintained that Dictyocaulus filaria is host specific and cannot infect cattle. Taylor (1951) speculated that deer are the normal host for D. viviparus since the parasite and the host live in complete harmony without bothering one another (usually accepted as a sign of long association). If this hypothesis of cross-infection between the wild ruminants and cattle is true, then under a system of ranching where wild herbivores and cattle share the same pasture, lungworm disease will be difficult to control.

From the present results, it is evident that D. viviparus obtained from moose failed to infect calves under experimental conditions. This could be due to the fact that the D. viviparus obtained from the moose, although morphologically indistinguishable

from D. viviparus of cattle, might be physiologically a different strain which is host specific for moose.

From these findings it appears that more research under field and laboratory conditions is essential before drawing any conclusions on the role of wild herbivores and other mammals in the persistence of lungworm infection.

CONCLUSIONS

In any consideration of the epidemiology of a parasitic disease where there is an external phase, the effects of climate are of major importance. This is particularly true when there is great contrast in climatic factors from one season to another such as occurs in temperate regions. In this region of Canada, the climate can broadly be divided into two periods. One is a warm, wet and humid period which extends from June to September. The mean maximum and mean minimum temperatures during this period range from 26° to 30°C and 7.5° to 15°C respectively, with a relative humidity of 60 to 90 percent. The second period is cold and dry and lasts from October to May. In addition, however, there are wide variations in the temperatures within these periods. Generally, October and November, and April and May, are mild with mean maximum temperatures in the range of 0° to 10°C. However, December to March is very cold, characterized by sub-zero temperatures. Precipitation from May to October is generally rain followed by snow from November to April. The snow stays on the ground sometimes to a depth of three to four feet until April.

Due to severe winter conditions, the grazing season extends only from May to November, for the rest of the year the animals are housed.

Generally, milking cows are grazed separately, preferably on good pasture. Dry and pregnant cows are put out onto relatively poorer pasture and are left there throughout the grazing season. Yearlings (animals in their second grazing season) and calves (animals in their first grazing season) are normally pastured together on less desirable pasture. The stocking rate is usually high, and the pastures are poor and overgrazed. At times, the calves are put out onto pasture in batches, as the season progresses, which appears to be a dangerous practice from an epidemiological point of view.

In late spring (May), the yearlings and calves are turned out onto pasture. Under conditions of endemicity, the calves will come into contact with infective larvae from two potential sources. The first of these is larvae that have overwintered on pasture, the other is from carrier animals infected the previous year. From the results, it appears that the ability of lungworm larvae to overwinter in this region is inconsistent and dependent on a number of factors. The most important of these is undoubtedly annual fluctuations in the climate. In this region where the winter season is extremely cold, a large proportion of larvae on pasture will perish during this season. However, under certain conditions, it is possible that a small number of larvae could persist under a thick blanket of snow or might be protected in the warmth generated in a manure pile. Under such circumstances, it has been shown that calves will acquire D. viviparus larvae and will develop patent

infections. The progeny of these lungworms could subsequently give rise to a heavy pasture infestation which, in turn, could prove dangerous to the calves later in the season. It should also be pointed out that overwintered larvae, to be of epidemiological significance, must survive in sufficient numbers to start a fresh infection. This, therefore, implies that as a secondary condition, the level of pasture contamination the previous year must have been high. As a general rule, however, it seems that overwinter larval survival on pasture, as a source of infection in this region, is not common. The parasite must, therefore, depend heavily on an alternate method for persistence over the winter period. D. viviparus has accordingly developed, in addition, the faculty to overwinter successfully in the host. Larvae acquired by animals during the late fall do not mature but remain inhibited, mostly in the early fifth-stage, throughout the winter and mature during the following spring.

Such animals in which lungworm larvae overwinter act as carriers. In the majority of cases, the carriers are lightly infected and shed very low numbers of larvae on pasture. Since environmental conditions during May to July are suboptimal for the development and survival of the preparasitic stages of D. viviparus, pasture infestation remains low. Calves grazing with the carriers will acquire relatively light infections during this time. However, as the grazing season progresses, the rate of pasture contamination will increase, since by this time, in addition to the carriers, the

newly infected calves will also add to pasture contamination. Coupled with this are the favourable climatic conditions during August and September for the development and survival of the preparasitic stages. Inevitably, this will give rise to a severe pasture infestation in late summer and early fall which may persist until October. This combination of factors leading to high pasture infestation accounts for the severity and high incidence of the disease seen in calves during this period (August to October). This is especially true if young or susceptible calves are introduced as was demonstrated in the tracer calves which died at this time from heavy lungworm infections. However, yearlings may show little or no symptoms of lungworm disease, although grazing on the same heavily contaminated pasture, because of their size and acquired immunity.

It, therefore, appears that the disease in calves is largely due to self-augmentation of the infestation on pasture. The calves pick up light infections in June. The first generation of these worms reach pasture by the middle of August and these are the larvae which are responsible for the heavy infections in calves at this time. By the time the second generation of these worms is produced (October), the temperature is too cold for further larval development. It can be seen that under average conditions, only one generation of D. viviparus per season is possible in this region of Canada. From the results, therefore, it is quite clear that carrier animals form a very important and efficient link in the disease cycle in this region.

There is also the possibility of wild ruminants serving as lungworm reservoirs for cattle. However, the evidence incriminating wild herbivores in the persistence of disease is mostly circumstantial. In the course of this work, D. viviparus obtained from a moose proved non-infective to calves. This does not of course rule out the possibilities of cross-infection from other wild ruminants.

As mentioned earlier, lungworm larvae acquired by animals during late autumn remain inhibited in their development throughout the winter. This phenomenon was noticed in all the animals studied and from November on, 100 percent inhibition was observed in the larvae acquired. The total number of larvae inhibited in the animals was generally very low although the pasture was heavily contaminated with D. viviparus larvae at this time. It is possible that only a small proportion of the larvae available to the animals during late autumn are capable of inhibited development or that a large proportion of those that are ingested fail to establish.

The phenomenon of seasonal inhibition in D. viviparus observed in these studies is very interesting both from an epidemiological and from a biological point of view. A number of probable reasons were considered concerning the mechanism(s) responsible for this phenomenon.

From the results, it appeared that factors which naturally suggest themselves, such as acquired resistance, presence of adult

worms, crowding, strain differences, age of the host and age of the larvae, in fact, had little influence on the inhibition observed.

However, the available evidence strongly suggests that some form of seasonal change, either in the physiology of the host or in the larvae, during late autumn, might be responsible for this inhibited development. None of the experiments was specifically designed to prove or disprove the likelihood of one or the other of these alternatives. However, since in Experiments III, IV and V larvae apparently only inhibited after six to eight weeks of holding in the refrigerator at $4^{o}C$, it appears that a change in the physiology of the larvae might be the more likely alternative.

As suggested by Armour _et al._ (1969b) and Armour (1970b), with _Ostertagia ostertagi_, it is believed that autumn conditions (photoperiod and/or temperature) initiate a change in the physiology of the larvae on pasture somewhat similar to that observed in diapause in insects. This opinion appears to be justified since larvae underwent inhibition during autumn in all hosts irrespective of considerations such as immunity or age. Since a proportion of these inhibited larvae later matured during the spring, it is further suggested that this could be a form of diapause development which is terminated at this time either by some intrinsic mechanism in the larvae or mediated _via_ a host stimulus. This maturation occurs at an appropriate time for larval development and survival

on pasture as well as coinciding with the mixing of the carrier animals with the new and susceptible crop of calves. It would appear that in this region of Canada, the ability to survive over winter as inhibited larvae in carriers represents the most reliable way for D. viviparus to persist from one generation of calves to another.

The epidemiology of lungworm disease in this region is thus complicated both by the phenomenon of inhibited development and the possibility of overwinter survival of larvae on pasture. Any measures to control the disease should take into consideration both these eventualities. Since young animals are most susceptible, attention should be primarily directed at preventing disease in them.

To this end, it appears important that only fresh pastures, uncontaminated by adult cattle for at least one previous grazing season, be used for grazing the young stock. In the absence of such pastures, calves should be raised inside until they are one year old.

It is further strongly recommended that young and adult animals should not be pastured together.

All potential carriers should be treated, before they are put onto pasture, with an anthelmintic with good efficacy on both mature and immature worms.

Since D. viviparus has a strong tendency to undergo inhibited development during late fall, it is also suggested that animals be treated and moved to clean pastures or brought in at this time. This will prevent animals from becoming carriers.

In spite of the fact that it is not as widespread a problem as it appears to be in some other parts of the world, D. viviparus still continues to plague the Quebec farmer. It is believed that the present work will give a clearer understanding of the epidemiology of this condition so that a more rational approach to its control may be undertaken.

SUMMARY

A. Age Incidence:- The incidence of lungworm disease was much higher in young animals than adults. This difference was primarily due to resistance to D. viviparus acquired in early life by the adult animals.

B. Seasonality:- A marked seasonal variation was observed in the incidence of D. viviparus infection, with the highest number of cases occurring during late summer and early fall. This seasonality appeared to be due to a number of factors. Initial low levels of pasture infestation from May to July due to low levels of parasitism in carrier animals, unsuitable environmental conditions for preparasitic larval development on pasture, and inability of larvae to overwinter on pasture in large numbers, accounted for a low incidence in the calves during these months. This situation then changed as environmental conditions became more favourable for larval development and in addition, there was self-augmentation of pasture infestation from newly infected calves. The result was a build-up of pasture infestation during late summer and early fall which was responsible for the severity and high incidence of infection during this time of the year. This period was, in turn, followed by one of decreasing pasture infestation due to unsuitable climatic conditions, a build-up of acquired resistance in the host, and the housing of cattle during the winter, resulting in a low incidence during December to April.

The seasonality of lungworm infection in this region of Canada is, in consequence, mainly due to a combination of seasonal fluctuations in pasture infestation, acquired resistance in the host and husbandry practices.

C. Overwinter Survival:- The ability of lungworm larvae to overwinter on pasture in this region appeared to be erratic. Larvae did not overwinter during 1967-68, whereas during 1968-69 they did. It was postulated that a number of factors, such as the intensity of pasture infestation during the previous grazing season, pasture conditions with respect to herbage and drainage, stocking rate during the following grazing season and annual fluctuations in climate played important parts in influencing the overwinter survival of larvae.

From the present studies, climate appeared to be the most important single factor determining the ability of larvae to overwinter in this region of Canada. A mild winter, particularly with early, heavy snowfall, facilitated survival of lungworm. In contrast, sub-zero temperatures before snowfall, could destroy larvae on pasture.

D. Carrier Animals:- The importance of carrier animals in the persistence of lungworm infection from one year to another was clearly demonstrated in these studies by more than one method.

A proportion of potential carrier animals started reshedding larvae during the spring, after being found negative for extended periods during the previous fall and winter. It was, therefore, reasoned that carriers, when turned out to graze, would contaminate pastures for susceptible animals. This was subsequently demonstrated repeatedly under field and experimental conditions. Wherever potential carriers and susceptible calves were grazed together the infection was successfully transferred to the calves.

Additional indirect evidence on this issue was obtained on a number of farms. It was observed that when susceptible calves were grazed separately from the carrier animals on a previously contaminated, overwintered pasture, these calves remained free from lungworm infection.

E. Wild Herbivores:- When parasite-free calves were dosed with infective larvae of D. viviparus obtained from a moose they did not become infected. However, this did not rule out the possibility that D. viviparus from other wild ruminants would not be infective to cattle.

F. Development of D. viviparus in Calves of Experiments I to IV:- The results indicated that the infective larvae acquired by the "permanent" calves from June to August reached maturity. However, from September onwards, most of the larvae picked up by these calves were inhibited. A progressive fall in the fecundity of the mature worms, and stunting

of growth, was noticed in worms recovered from calves in August and September. A somewhat similar trend of mature to immature worms, lowered fecundity and stunting was observed in the calves in Experiments II to IV, except that the occurrence of these events was delayed by one or two months.

G. Seasonal Inhibited Development:- From these studies it was evident that D. viviparus had a strong tendency to undergo inhibited development during late autumn.

A number of different possibilities were examined as reasons for this inhibition. It was found that acquired resistance, presence of adult worms, crowding effects, strains of parasite and age of the host or larvae were not responsible for inducing inhibition.

The evidence obtained from these studies implicated a seasonal change in the physiology of the host or larvae during late autumn, induced by prevailing environmental conditions, as being responsible for inhibition. However, the results of Experiments IV and V could not be explained satisfactorily on the basis of a change in the host's physiology. It, therefore, appears more likely that a change in the physiology of the larvae on pasture due either to autumn environmental conditions on pasture or storage of larvae for six to eight weeks at this appropriate time of the year in vitro was responsible for inducing the state of arrested development. The change in the larvae was suggested as being similar to that observed

in diapause in insects. On the basis of this hypothesis, the sudden and complete inhibition of larval development during late autumn and their subsequent maturation during spring could be visualized as the onset and termination of a diapause-like phenomenon.

H. Morphology of Arrested Larvae:- Eighty-percent of the inhibited larvae recovered from the calves during autumn were in the early fifth-stage and the rest were in the late fourth-stage. A representative sample of the arrested larvae was collected and examined. The morphology of these arrested larvae and particularly the development of the genetalia in both sexes, was described and illustrated.

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