THE ECOLOGY OF THE FREE-LIVING STAGES OF RABBIT TRICHOSTRONGYLES

by

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INTRODUCTION

The preparasitic, or free-living phase of the life-cycle of the Trichostongoylid nematodes parasitic in the rabbit includes the egg, the first and second stage preinfective larvae, and the ensheathed or infective larvae. The egg stage passes out in the faeces of the host and is deposited on the grass of the pasture. The first and second stage larvae normally remain in the faeces, and it is not until the third or infective stage is reached that migration from faeces to the grass blades occurs. Temperature, moisture and sunlight are generally considered to be the most important natural influences affecting the survival and development of the nematode eggs and larvae.

Studies conducted to investigate the effects of any one individual factor of the environmental complex either in the laboratory or outdoors, have often been criticised as academic and of doubtful practical significance, since in nature the various elements of the complex function simultaneously as determinants of the survival and development of eggs and larvae. Field studies in which the effect of the environment as a whole may be determined while undoubtedly of great value, invariably yield results which are valid under identical or very similar conditions only. Meterological conditions in any one locality are rarely or never duplicated from year to year

and such studies cannot demonstrate the actual role of each factor in causing the observed result. Hence both types of investigation apparently have their limitations. However, the greatest accuracy in the formulation of generalisations, interpretations and expressions of probability, so far as that is possible and advisable, can only be obtained from the information elicited by investigations of both types.

While numerous investigations have been conducted on the ecology of different Trichostongyle species in the laboratory and on pastures, the role of the actual time-temperature factors in the development of the preinfective and mature larvae of this species has been a confused subject. The necessity for accurate information on this subject is emphasized by the frequency with which the incidence of human Trichostongylosis has been reported in the last ten years.

Temperature was the principal element in the causation of the observed experimental results reported in this paper. The specific objectives of the investigation were to determine (1) the length of time it took the larvae to hatch, to become mature, to live in various culture media (clay, coarse sand, sterile rabbit faeces and loam) at 15°C, 20°C, 25°C, 30°C, 40°C and 47°C. (2) The survival time and death rate when exposed to all six temperatures. (3) The time and number of survival at the above temperatures after deep freezing, and the effect of alternate freezing and thawing. (4) The survival time when desiccated at freezing point and at the

above temperatures. (5) The difference in longevity between forms at continuous temperatures and forms removed each night from, and returned each morning to the above temperatures. (6) The effect of pH on hatching and longevity.

The rabbit Trichostrongyle species used in this work were (i) Trichostrongylus retortaeformis (Zeder, 1800) which in the adult or parasitic stage, feeds on the mucus of the small intestine of rabbits and hares, (ii) Graphidium strigosum (Dujardin, 1845), a larger species which feeds on blood in the stomach of these animals.

REVIEW OF LITERATURE

Work on the bionomics of Trichostrongyle nematodes has centred largely on species which are parasitic in the sheep and the literature on similar work with species to which the rabbit is a natural host, is very scanty. The tendency among most workers has been to assume that the results obtained with sheep Trichostrongyles ought to be very close to similar results with rabbit Trichostrongyles. This assumption appears justifiable in the light of an observation made by Leiper in 1947. From the experimental data he tabulated, it appears that wild rabbits can mechanically convey the larvae of gastro-intestinal nematodes of sheep to rested pastures from adjoining infected pastures, as well as reinfect them with Trichostrongyles from their own faeces.

On the basis of this observation by Leiper, it has been found necessary to include in this review, the results of bionomical work on sheep Trichostrongyles.

Probably the earliest observations on the bionomics of sheep nematodes in North America were those of Ransom (1906) indicating that the larvae of Haemonchus contortus showed little reduction in the number of food granules when kept out of doors from December 27 to March 22. He also stated that H. contortus infective larvae in faeces resisted 5 days exposure outside in the winter, being frozen and thawed 32 times and frozen for over 48 hours on those occasions.

Ransom (1908) also reported that infested pastures will not become free of stomach worm infection between October 25 and June 16 at Washington, D.C. Ransom (1910) later reported that stomach worm larvae having reached the infectious stage are able to withstand long periods of dryness and severe cold, though some of them succumb comparatively early. He also stated that if sheep, goats and cattle are kept out of a pasture for a year, it was safe to assume that practically all larval stomach worms would have died in that period.

Veglia (1915), under laboratory conditions, studied the effects of low temperatures on the eggs and mature larvae of H. contortus. Eggs stored for 48 hours at 0°C did not hatch. Mature larvae stored at 0°C for 6 months showed a 5-6% survival with the intestinal cells poor in granulations and with large vacuoles. Mature larvae did not survive 7 months at 0°C. Larvae exposed to a sudden decrease in temperature were able to resist the sudden decrease and survived although the first stage larvae seemed peculiarly susceptible and numbers died. Veglia also reported that he could not detect in any instance larvae that were killed as a result of exposure to cold weather in the veld if they were allowed to find shelter either in faeces or in the soil. He also concluded that in a moderate cold ambient the larvae remain in a better state of preservation and for a longer period than in warm weather. In

addition, he stated that during periods of drought in South Africa, the majority of eggs and larvae of the veld dies.

Cameron (1923) froze some larvae of Monodontus trigonocephalum in a glass capsule on an ice and salt mixture and although frozen for a very few minutes, none revived on returning to room temperature.

Lawson (1929) made tests in Connecticut to determine the overwinter survival of infective H. contortus larvae on pastures. He concluded that the larvae are able to survive the coldest season of the year and are present to infect sheep and lambs in the spring. He observed that they continued to remain active until affected by drying.

Mönnig (1930) in order to determine the possibilities for the development of Trichostrongylus spp. under the cold winter conditions of South Africa, made several experiments. As a result of his experiment #2, he concluded that Trichostrongylus larvae can only exceptionally reach the infective stage under winter conditions. He observed the effects of low temperatures on Trichostrongylus larvae by a series of three experiments in which infective larvae were exposed to a temperature of 0°C or lower for variable periods. In all instances, the larvae survived the exposure. Larvae frozen in water for 10 days were found to swim freely and prove infective. Larvae stored for 14 days in a culture jar at 0°C appeared to lose their migratory powers. He concluded that the preinfective stages of Trichostrongylus spp. are killed by freezing unless they

are sheltered. Low temperatures above freezing point cause slower development.

Rebrassier (1932) in Ohio reported that the larvae of Oesophagostomum columbianum will survive in the soil at least one winter season and still be infective for susceptible lambs. He did not, however, indicate soil temperatures during the survival period.

Dikmans and Andrews (1933) in an abstract, call attention to the fact that sheep they were using were infested with H. contortus Nematodirus spathiger and Ostertagia circumcincta, the infection having been acquired by grazing the parasite-free sheep on pastures vacant during the winter months, thus showing that whatever infection was present on the pasture in the fall had survived the winter.

Kausal (1933) states that heavy infestations (in New South Wales, Australia) may be encountered during the winter months in the southern winter-rainfall areas. Chabertia ovina from his survey showed remarkably little variation in seasonal fluctuation, being common at all seasons.

Gordon and Graham (1933) while conducting post mortems on four lambs observed a heavy infestation of Chabertia ovina in one lamb. They decided that this infestation was acquired during the cold winter months, indicating the resistance of the larvae of this species to climatic conditions.

Zawadowsky and Vorobiova (1934) reported that "Trichostrongylidae larvae, spending winter in the open under Moscow climatical conditions,

are practically deprived of any invasive significance towards spring."

Kammlade et al (1936) published data indicating the persistence of stomach worm larvae on pastures of blue grass in Illinois. In the data they presented, there is the suggestion that after a winter in central Illinois, such as that of 1935-36 (no temperatures given) the larvae of the stomach worm deposited the preceding season might not be nearly so important a source of infestation as the adult parasites carried through the winter in the host animal.

Broughton and Hardy (1936) in Texas, while making longevity studies under range conditions, observed a 22-month survival of Haemonchus contortus larvae as indicated by infections established in parasite-free lambs grazed on fenced plots. Subsequent observations (1937) indicated that after 31 months these plots were free from Haemonchus contortus infective larvae. Later (1938) they concluded that their studies showed that H. contortus larvae will remain viable for at least 22 months on the open range in West Texas. They did not submit temperature charts for the trial period and hence the effects of low temperatures cannot be estimated.

Griffiths (1937) showed that Ostertagia circumcincta, Nematodirus fillicolis and Trichostrongylus colubriformis were able to survive a Canadian winter in which the air temperatures ranged from -15°F to 52.5°F during a four-month period. H. contortus and Oesophagostomum columbianum apparently did not survive.

Schmid (1939) concludes from evidence presented that in New York the infective stages of the various species of small gastro-intestinal worms found in native sheep will remain viable for periods of at least 21 months. The parasites he refers to are presumably H. contortus, Ostertagia circumcincta, Cooperia curticei, Nematodirus spathiger, Bunostomum trigonocephalum, Moniezia expansa, Trichuris ovis, Oesophagostomum columbianum and Trichostrongylus spp.

Swales (1940), from the results of preliminary sheep surveys, hypothizes that if the free-living stages of a certain species do survive and are present in the pastures in the spring then one might expect these species to be present in reasonable numbers in lambs after they have grazed on the pasture for from six to eight weeks. His charts (assuming the above hypothesis) indicate that Moniezia expansa, Trichuris ovis, Ostertagia circumcincta, Nematodirus spp., and Haemonchus contortus survived the winter and accordingly the survival of Monodontus trigonocephalum, Chabertia ovina, Oesophagostonum columbianum, Trichostrongylus spp. and Cooperia spp. is low or non-existent. He further states that through an abnormality (very low numbers) due to anthelmintic treatment of several flocks in the survey, H. contortus cannot be considered in the above hypothesis.

Swales (1940) later reported from experimental data that H. contortus and O. columbianum do not appear to resist winter conditions on Canadian pastures. It was on these data together with

the preliminary survey work, that the basis for the control of internal parasites of sheep in Canada was established.

Shorb (1942) concluded that in the winter or late fall, there was no development to infectivity of preparasitic stages of ovine nematodes, but a small number of Ostertagia and Trichostrongylus in the egg or preinfective larval stage, the following sping. There was also evidence that the preinfective stages of Nematodirus and Ostertagia survived the cold weather of early spring.

Shorb (1943) found in Maryland that there was no survival on grass plots of H. contortus from December to April.

Swales (1943) reported results showing that on a pasture contaminated during the summer with small numbers of stomach worm eggs, the free-living stages died over winter. This over-winter loss occurred on a closely cropped pasture during a fairly normal winter, followed by conditions favourable to the acquisition of infection in the grazing season.

Kates (1943) reports that under conditions existing at Beltsville, Maryland over the fall, winter and spring of 1941-42 there was no evident survival on pasture of the preparasitic stages of O. columbianum, Cooperia curticei and B. trigonocephalum, a very low survival of H. contortus and Trichostrongylus spp. and a relatively high survival of Ostertagia spp. Nematodirus spp. and Trichuris ovis.

Shorb (1944) using experimentally infested grass plots, recovered infective H. contortus larvae that survived the winter but

these were sluggish, vacuolated and probably non-infective. He indicated that pastures kept free of sheep, from October until the middle of April, will contain only a few infective larvae of H. contortus.

Dinaburg (1944) by means of laboratory storage experiments, concluded that the preinfective larvae of Ostertagia circumcincta are more rapidly killed by freezing and by high temperatures than are eggs. Infective larvae are highly resistant to temperature extremes, but if frozen in ice at -6°C, they are killed within approximately two weeks. At a temperature just above freezing, they may survive over 271 days exposure when in water 5 mm deep. Above 0°C, the mortality increases with rising temperatures.

Hawkins et al (1944) indicated that under the climatic conditions at Michigan, H. contortus and O. columbianum are not perpetuated by the pastures but by the breeding flock. They stated that pastures infested with H. contortus are apparently freed of infestation in two months in late summer and early fall, with O. columbianum and Chabertia ovina in three and one half months. They found that Ostertagia circumcincta, Trichostrongylus colubriformis, Nematodirus spp. and Trichuris ovis larvae were still viable after four and one half months on pasture.

Seghetti and Marsh (1945) reported a carry-over of infective larvae of Ostertagia, Trichostrongylus and Nematodirus on a blue grass pasture in Montana which had not been pastured since November. They recovered the larvae from sod samples in April by means of the Baerman apparatus.

One of the most recent workers on the ecology of the larvae of Trichostrongylus retortaeformis of the rabbit is H.D. Crofton. In 1948, Crofton reported his findings on the rate of hatching, development and survival of the larvae of Trichostrongylus retortaeformis under dry and winter conditions. He also studied the daily fluctuations in the number of larvae on grass plots, and their rate of disappearance during successive months. When the maximum temperatures were below 50°F there was no hatching. He found that eggs passed in the autumn survived a cold winter but those passed during the coldest period died. In his experiments, there was little or no migration when the temperature did not exceed 55°F. A high death rate occurred in warm weather, in which the rate of evaporation was high, and the number of larvae on grass blades depended upon the climate at the time and on the effects of previous conditions on hatching and survival.

Crofton (1949) showed that controlled grazing on hill pastures reduced the number of trichostrongyle parasites on the grazed portions by limiting the extent of faecal contamination. He observed that where there was uneven grazing, a local concentration of infective larvae occurred on the grazed portions. The number of larvae on grass blades was lowest when dew was formed and highest between midday and 5 pm. A maximum number of larvae on a pasture was found in August and he stated that the larval population could be reduced by 55% as a result of resting a pasture for 3 weeks, while a week after the return of the

sheep, the reduction was 99%. Even a rest of two weeks reduced the number of larvae considerably.

Wetzel and Enigk (1937) have investigated the life history of Graphidium strigosum in detail. They find that both the 4th stage larva and the adult worm are equipped with ventral glands of which the function is unknown. Up to the 9th day after infection the 4th stage larva inhabits the excretory ducts of the fundic glands of the stomach. The adult worm lives in the mucus and does not attach itself to the mucus membrane. Contrary to previous opinion, the authors found no cases of haemorrhagic gastritis in their heavily infested experimental rabbits. They attribute the resulting anemia and emaciation of the hosts to disturbances of the digestive system. Infective larvae are found to have very little resistance to desiccation so that Graphidium strigosum is restricted to areas having a damp sub-soil or to rainy seasons.

One of the most important points overlooked by most workers in the literature reviewed above, relates to the actual soil temperature of the pasture during the winter period. Many of the articles reviewed contain data on the air temperatures, during the periods of observation and thus infer that soil temperature corresponds to the recorded air temperature, but such does not appear to actually occur.

Franklin (1919) cited by Keen (1930) recorded the effect of a 3-inch snow layer on soil temperature at Edinburgh. This is shown in the accompanying table.

Effect of a snow layer 3" deep on soil temperature at Edinburgh (Franklin)

Date 1919	Time	Air Temp. over snow °C	Soil Surface Temp. under snow °C	Soil Temp. 4" depth °C	Soil Temp. 8" depth °C
Nov.14	6 am	-15.0	0.0	1.0	2.1
"	noon	- 8.6	0.0	1.0	2.1
"	6 pm	- 9.6	0.0	1.0	2.1
"	midnight	- 7.7	0.0	1.0	2.1
Nov.15	6 am	-13.7	0.0	0.9	2.0
"	noon	- 4.6	0.0	0.9	1.8
"	6 pm	- 6.5	0.0	0.9	1.8
"	midnight	- 9.5	0.0	0.9	1.8

This table shows that temperatures of -15°C may occur at the surface of 3 inches of snow without producing any detectable change in the soil temperatures.

Mail (1930) briefly reviewed early work on soil temperatures and conducted studies on the winter soil temperatures and their relation to subterranean insect survival. He showed that under depths of from 3 to 13 inches of snow in December in Minnesota, the temperature of the soil, 2 inches below the soil surface, remained between ±1°C

(34° and 30°F) even though the air temperature varied during the month between -1.5°C and -23.5°C (29°F and -10°F). In a parallel observation on bare ground the 2-inch level was recorded at -16°C (3°F) on a day when the air temperature followed the atmospheric trend. The soil at the 8-inch, 16-inch and 24-inch levels under snow remained at temperatures constantly above the freezing point, while even the 24-inch level under the bare ground fell to only -3.5°C (26°F). Thus these observations show that during the winter months, providing there is a covering of snow, the soil temperatures remain constantly near the freezing point, but in the event of a thaw which removes all the snow, followed by a severe frost, the soil temperatures rapidly become subzero.

Keen (1930) states that the effect of snow on the soil surface is to produce a layer of low conductivity, further stating that the fairly constant temperature of the soil under a layer of snow is not solely due to low conductivity but to the latent heat of the ice-crystals. He explained that at the bottom of the frozen soil, the temperature is 0°C and apart from temporary super-cooling, it cannot fall below this value. If, owing to an intensification of the frost spell, the temperature at the bottom of the frozen layers begins to fall below 0°C, more ice is formed and the temperature returns to 0°C, owing to the evolution of latent heat. The result is therefore a slow increase in the depth of frozen soil. Hence during the whole process of freezing and the converse process of thawing, surface

variations of temperature have practically no effect on the temperature of the soil below the frozen layer. Keen also included a graph showing the fairly constant soil temperatures during a cold winter with fluctuations in air temperature between -5.0°C and 10°C.

Baver (1940) cites the investigations of Petit (1898) which show that frost penetration is more rapid and its disappearance slower under bare conditions than under grass or surface mulches. From Petit's chart on frost penetration on bare and on grass-covered soils, it may be seen that the grass cover decreased the rate and depth of penetration of frost when compared with bare soil. When thawing occurred, frost disappeared from the protected soils sooner than from the bare, owing to the fact that the latter soils were frozen to a greater depth. Baver also states that overgrazed pastures freeze deeper than those under controlled grazing because the better growth of grass on the latter gives more protection to the soil.

Swales (1940) reported on soil temperatures in the middle St.Lawrence River area, as recorded at Macdonald College. His examination of the records showed that at the 4-inch and 8-inch soil levels, the temperature remains constant during the cold weather until a thaw removes the snow, at which time the 4-inch level shows the greater variation. On all days on which the temperature of the soil fell below 28°F, the snow covering was absent or sparse due to a previous thaw. He states that the air temperature itself has little effect

upon the soil temperature in the presence of snow and further that snow and not soil is the efficient insulating agent protecting organisms against very low temperatures. His records of soil temperatures show the relative constant temperatures to which the free-living stages of nematodes would be exposed. From these observations, it is apparent that even on the soil surface, animal organisms are not exposed to very low temperatures during the winter months in Eastern Canada. One other point of importance in this work is the suggestion that if the organisms were covered with faecal material or soil, it is probable that there would be slightly less variation in temperature, but there are no data to show that larvae are able to migrate downwards into the soil to an appreciable depth.

MATERIALS, METHODS AND APPARATUS.

(a) Infection and Infective Larvae.

Eggs, preinfective, and mature larvae of Trichostrongylus retortaeformis were used in the first part of these experiments. Toward the end, similar stages of Graphidium strigosum were substituted. The faeces containing the eggs was obtained from laboratory rabbits in which pure infestations of Trichostrongylus retortaeformis and Graphidium strigosum had been established. The two lots of rabbits were confined in two cages separated by a distance which made a mixed infection impossible.

Each rabbit was infected with about 500 mature larvae, by syringing the larvae down a rubber tubing into the pharynx. Before infection, the faeces of each rabbit was examined to exclude the possibility of a previous infection with any nematode worm. No such case was found, since all the rabbits used were carefully bred in the laboratory. The eggs of T. retortaeformis were demonstrated in the faeces of five-month old rabbits thirteen days after infection (November 10 - November 23) and as early as five days in rabbits seven to eight weeks old. The exact relationship between the age of the rabbit and the period in which infection was observed, was not investigated.

(b) Methods used in reovering Larvae.

In all cases in which the determination of actual time was required, faecal pellets were collected five to ten minutes after they

were discharged by the infected rabbits. To avoid the collection of free-living larvae with the faeces, the pan suspended below the rabbit cage was lined with paper so that the faeces fell directly on the paper. Where mature larvae were needed, advantage was taken of their migration through moist filter paper lining a petri-dish which was used to compress the concentrated faecal contents of a smaller petri-dish. After seven days concentration, the filter paper was removed and the larvae adhering to the wall of the petri-dish were recovered with water in a fairly clean condition. The concentrated faeces was kept moist throughout the development of the eggs. No such migration through wet filter paper was observed, however, in the case of Graphidium strigosum at the temperature range used in this study, and collection of these larvae was mainly by the Baerman technique.

(c) The "constant-temperature" incubator.

The experiments were largely conducted in a giant constant-temperature incubator. It was compartmented into six chambers maintained at the constant temperatures of 15°C, 20°C, 25°C, 30°C, 40°C and 47°C by means of electric coils. The chambers were insulated with cork which was reinforced with cardboard to make the chamber airtight. Each chamber was provided with a tight-fitting door through which a bi-metallic thermometer was passed to register the temperature on a scale engraved on the outer surface of each door. Additional source of heat was obtained from a diesel motor electrically operated to generate

heat at fixed intervals. The interior of each chamber was evenly illuminated with a lamp of 15 Watts. An enamel pan which was constantly filled with water kept the atmosphere of each chamber humid. Where freezing temperatures were required, a household refrigerator was used.

(d) Media used in this study.

Except where the effect of desiccation was being studied, larvae were ordinarily stored in small petri-dishes containing tap water to a depth of 5 mm. The percentage survival after exposure to each of the factors considered, was usually computed from about 1,000 larvae isolated in a small petri-dish, and left to stand at each of the six incubator temperatures for the specified length of time.

Other media used in these experiments were coarse sand, black loam, New Brunswick red clay, and sterile rabbit faeces. Before use, each of these was put in a large beaker and placed in the auto-clave. Steam under a maximum boiler pressure of 15 pounds was admitted for 3 hours, after which time each medium was considered helminthologically sterile.

The apparatus used for all soil experiments, was assembled as shown in fig.2. It consists of a cylindrical paper box container with a maximum volume of 250 cc resting on a large petri-dish of 10 cm diameter. A smaller petri-dish 9 cm in diameter, was used as a lid for the paper box. A 1" depth of soil was placed in the large petri-dish of the apparatus. Freshly passed rabbit faeces containing Trichostrongyle eggs was broken up, moistoned and mixed with an equal

volume of the appropriate soil. The soil-faeces mixture was evenly spread over the soil in the large petri-dish to a depth of 1/4". A preliminary egg count was done on each sample of faeces used. The paper box was then completely filled with the required type of soil so as to bury the soil-faeces mixture in about 2 1/2" of soil. The smaller petri-dish was used to cover the paper box to minimize evaporation. In the experiment on the longevity of larvae in these media, the culture was kept moist by adding about 25 cc of water into the contents of the larger petri-dish whenever signs of dryness were noted.

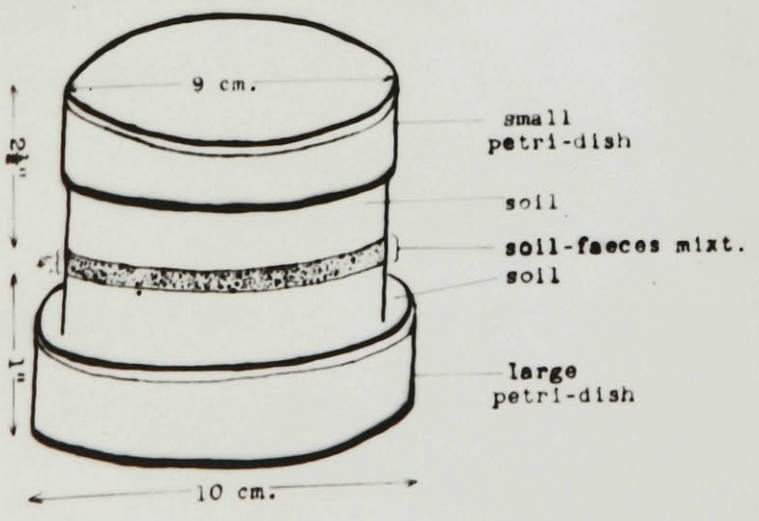

Fig. 2. Apparatus for culturing Trichostrongyle eggs in different media.

Fig. II. Apparatus for culturing Trichostrongyle eggs in different media.

EXPERIMENTAL

A. Experiments with Eggs.

In the following experiments, it was considered unnecessary to isolate eggs from the faeces since in nature, Trichostrongyle eggs are rarely isolated from the faeces of the host and such operation tended to increase the time in which recorded results were observed. In order to expose most of the eggs to the factors which were investigated, however, the faeces containing them was broken up and ground to a powdery consistency. The faeces was then exposed to the factor under consideration for the specified length of time; removed, moistened and left at 25°C or 20°C for one week. The larvae recovered from it by the Baerman technique, were counted on a ruled slab of glass under the binocular microscope. Experiments with eggs were limited to (i) the effect of desiccation at different temperatures (ii) the effect of deep freezing (iii) viability under water (iv) time of hatching in rabbit faeces,loam, sand and clay at 15°C, 20°C, 25°C, 30°C, 40°C and 47°C.

(i) The Effects of Desiccation on the Eggs

Fresh rabbit faeces containing the eggs of T. retortaeformis were broken up, ground and left exposed in petri-dishes at 8°C, 15°C, 25°C, 30°C, 40°C and 47°C. While no attempt was made to desiccate the eggs with a dehydrating agent, contact with water was avoided

and the faeces remained dry throughout. Five gram samples were moistoned and incubated at 25°C at varying intervals of time. Faeces (containing eggs) which were stored between 10°C and 29°C showed little or no signs of deterioration with time. Infective larvae were recovered in large numbers on incubating samples stored from December 5, 1949 to July 5, 1950 (8 months) at the temperatures of 10°C, 20°C, 25°C, and 28°C. The deterioration of these eggs above 30°C and below 10°C made it necessary to investigate the time-temperature factor involved in their viability. Five gram samples of fresh broken faeces from rabbits infected with T. retortaeformis were therefore exposed to 40°C, 47°C and 8°C. A sample from each culture was frequently examined and after a number of tests the following results were obtained.

Eggs in rabbit faeces exposed to 47°C did not hatch after three days, eggs at 40°C remained viable for 10 days and those at 30°C were still viable after 2 months though less than 5% (on the basis of a preliminary egg-count) actually hatched on incubation. The eggs stored in faeces at 8°C, hatched when incubated at 25°C, 4 months but not after 6 months.

Similar tests were also made on the eggs of Graphidium strigosum, special attention being given to fully embryonated eggs. The results tabulated below show the number of larvae recovered from 5 gm samples of the faeces stored at the temperatures indicated.

Time Temp	30°C	40°C	47°C	8°C
4 days	45	10	7	115
7 days	13	3	-	89
10 days	7	-	-	41
15 days	-	-	-	19

These results indicate that the eggs of T. retortaeformis are more resistant to desiccation at any temperature than those of Graphidium strigosum. A possible explanation of this difference in the resistance of the eggs, it was felt, may lie in the thickness of the egg shell. An examination of both eggs under the high dry power of the microscope, shows that the egg of T. retortaeformis though smaller (75-80 μ by 40-45 μ) than that of G. strigosum (98-106 μ by 50-58 μ), has a decidedly thicker wall.

In order to determine whether any particular stage of the egg was more resistant to dryness than the other stages, fresh rabbit faeces containing the eggs of T. retortaeformis, was crushed and incubated in the ordinary way, while the development of the eggs was watched by examination of samples taken at frequent intervals, and left to dry in an exposed paper box at room temperature (roughly 26°C). The results obtained after 8 months storing, December 5,1949 to July 5, 1950 showed the following:

Eggs of T. retortaeformis which were isolated with faeces from the culture after they had reached the tadpole stage and then stored at room temperature, were all killed after eight months drying. Most of the eggs isolated in a similar way in the morula stage had gradually developed to the tadpole and embryonated stages, the moisture in the faeces and the humidity of the room being adequate to make this possible. Signs of deterioration were noted in some eggs that had attained the different stages of development characteristic of the eggs of this species.

Morphology of the desiccated egg.

T. retortaeformis eggs.

A microscopic examination of the eggs (fig.1) showed that in eggs stored at room temperatures below 10°C, development had progressed to the tadpole stage. The tadpole larva had died, however, and the regular contour of the egg-wall was distorted. An examination of the faecal pellets stored at 30°C under the binoculars showed that most of the eggs had actually hatched, this temperature and the water content of the faeces were optimum to make this possible. Most of the larvae had died, however, as a result of the dryness. Above 40°C, most of the eggs were still in the 64 cell stage but some of these cells had begun to degenerate (fig.1b). Most of the eggs stored between 10°C and 28°C, had developed to the embryo stage, but dead larvae were strangely absent in the faeces at these temperatures, suggesting that hatching had not yet occurred. Hatching occurred

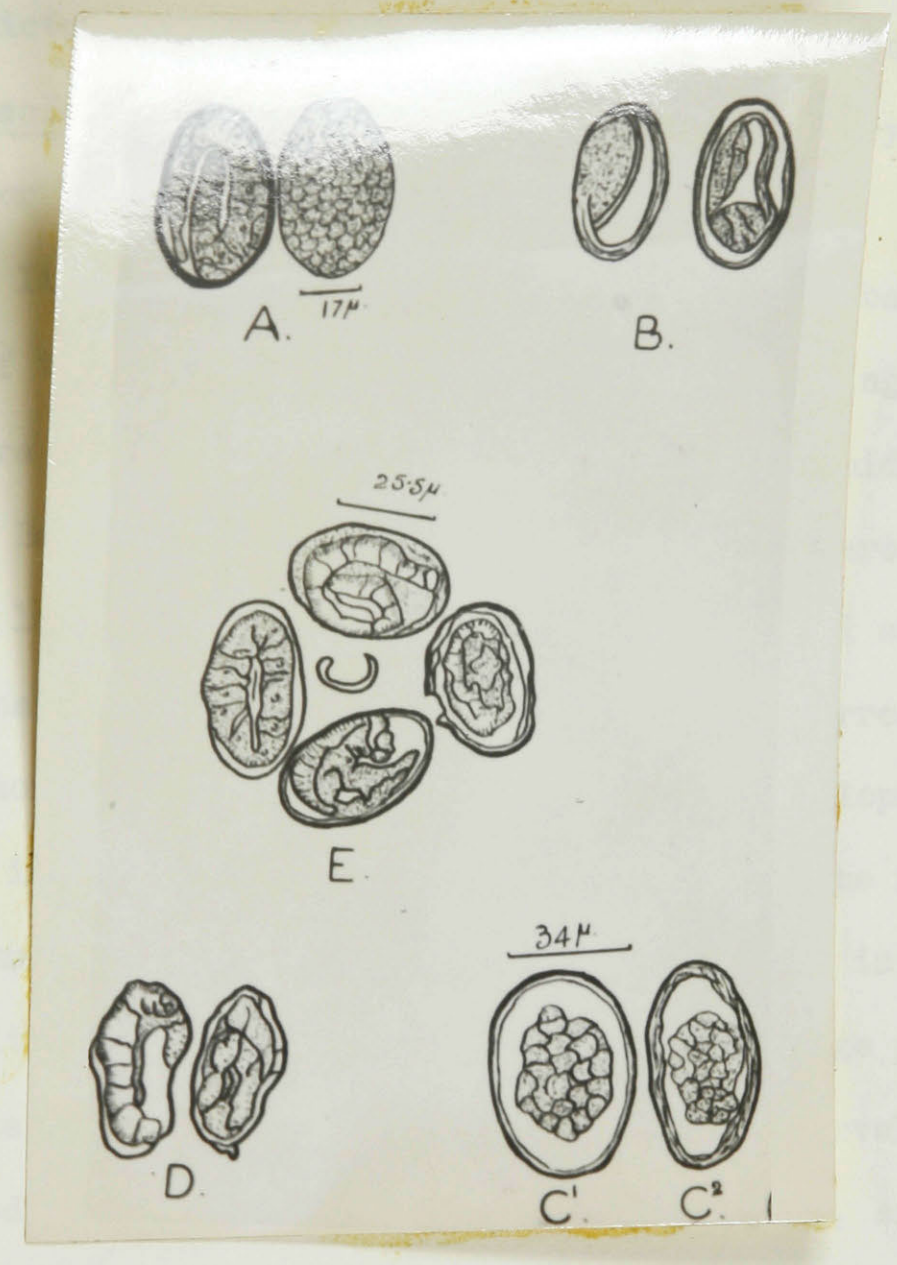

Fig.I. The effect of dryness and freezing on the morphology of rabbit Trichostrongyle eggs (camera lucida drawings).

A. Normal eggs - tadpole and morula stages.
B. Eggs dried at 47°C.
C¹. G. strigosum eggs after 10 weeks freezing.
C². T. retortaeformis eggs after 10 weeks freezing.
D. Eggs dried for 6 months at 8°C.
E. Eggs dried at room temperature (26°C approx.) for over 8 months.

in two days, however, when the faeces containing these eggs was moistoned and left at 25°C; and 300 of the mature larvae obtained from this culture infected a 3-month old rabbit after ten days. The microscopic picture of the eggs of G. strigosum were as described above for T. retortaeformis eggs, but no eggs hatched after drying at room temperature for 15 days.

Discussion: It can therefore be concluded that the subsequent development of the eggs of T. retortaeformis after drying, depends on the temperature at which they were dried and on the humidity of the environment. Between 10°C and 28°C in a humid atmosphere, such as prevails in a laboratory, eggs in the morula stage will slowly develop to the embryonated stage when further development is arrested till the eggs are moistoned and incubated. Since this development of the egg commences immediately the faeces is evacuated by the rabbit and usually continues till the embryonated (tadpole) stage is reached, it is difficult to determine the resistance of the stage preceeding the tadpole stage. Above 40°C, and at or below 0°C, development to the embryonated egg stage cannot proceed during drying, since the cells of the morula stage have already begun to degenerate. Embyronated eggs do not survive long drying above 47°C, though they may resist desiccation indefinitely at room temperature.

Contrary to these results, Zawadowsky (1929) states that Trichostrongylus eggs do not resist desiccation; Mönnig (1930) states that Trichostrongylus egg stages preceeding those with complete

embryos do not resist dryness.

Other workers investigating this phenomenon include Looss (1911), who reported in the case of Strongylus spp. of the horse that completely embryonated eggs resist desiccation for 6 but not for 9 months. Theiler and Robertson (1915) working with Trichostrongylus douglassi reported that faeces, containing eggs and first stage larvae kept dry for 2 years gave rise to mature larvae but not for 36 months. None of these authors, however, has mentioned the temperature at which the desiccation was carried out.

The maximum period beyond 8 months, to which the eggs of T. retortaeformis may resist drying without losing their viability within the temperature range of 10°C to 30°C, was not determined in this work.

(ii) Low temperature studies

Among the Strongylid nematodes, it has generally been found that temperatures near freezing point arrest development and that degeneration may occur (Schwartz 1925, and Sprehn 1925 on Bunostomum phlebotomum, De Blieck and Baudet 1926 on strongyles of the horse, etc.) or that freezing may cause death of the eggs or larvae in a short time (Boulenger 1915 on Nematodirus filicollis, Ransom 1906 and Veglia 1916 on H. contortus, etc.). Mönnig (1930) on the Bionomics of free-living stages of Trichostrongylus spp. in sheep, observed that the preinfective stages are fairly rapidly killed by actual freezing and that resistance to cold may persist for a fair period where there is a certain amount of protection.

The effects of continuous freezing and of alternate freezing and thawing of the eggs were studied. The temperature of the household refrigerator used for freezing was constant at 0°C. Fresh rabbit faeces containing Trichostrongylid eggs in the 64-cell morula stage and samples containing embryonated eggs were broken up and ground to a fine powdery consistency. A 700 gm sample for alternate freezing and thawing was put in a petri-dish, covered and stored away in the refrigerator for continuous freezing. At frequent intervals, the faeces for alternate thawing and freezing was removed from the refrigerator and left in the laboratory for 3 days during which period it regained its normal consistency. Five gram samples from it were cultured at 25°C after each removal. Five gram samples were also cultured from the specimen exposed to continuous freezing. The larvae were recovered from each sample by the Baerman technique. When it was found that the eggs in these cultures hatched at room temperature over a period of seven to eight days, it became necessary to repeat the Baerman recovery method on every culture each day till no more larvae were recovered from the sample. This was done by pouring off the Baerman water after it had stood for 2 hours, leaving the faeces in a cheese-cloth wrapping in a Baerman funnel, and counting the larvae recovered. The process was repeated the following day. The number of larvae recovered from 5 gm faecal samples containing the eggs of T. retortaeformis and G. strigosum respectively are shown in graphic form in table 3.

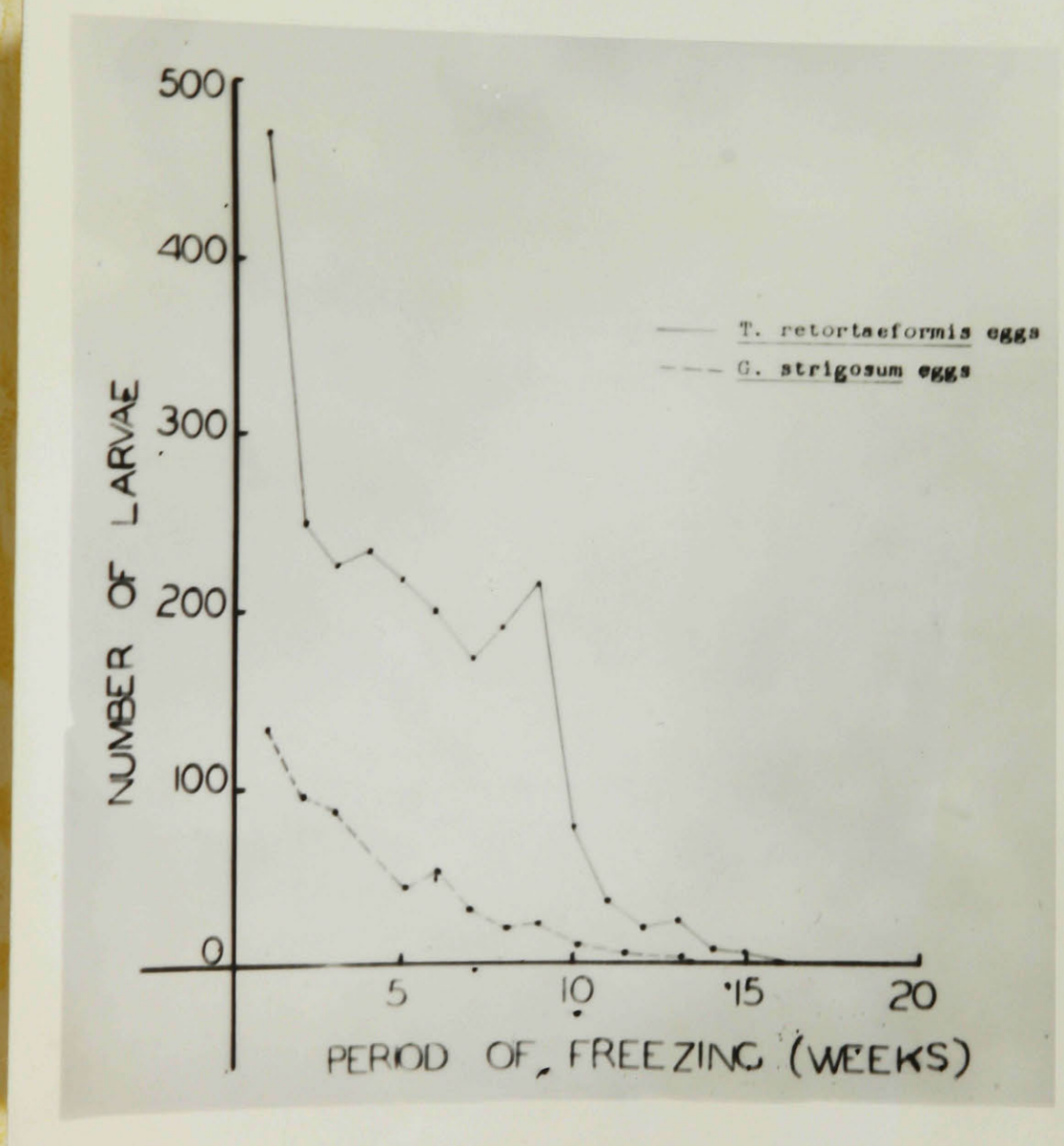

Fig. IIIA. The influence of continuous freezing on the hatchability of Trichostrongyle eggs.

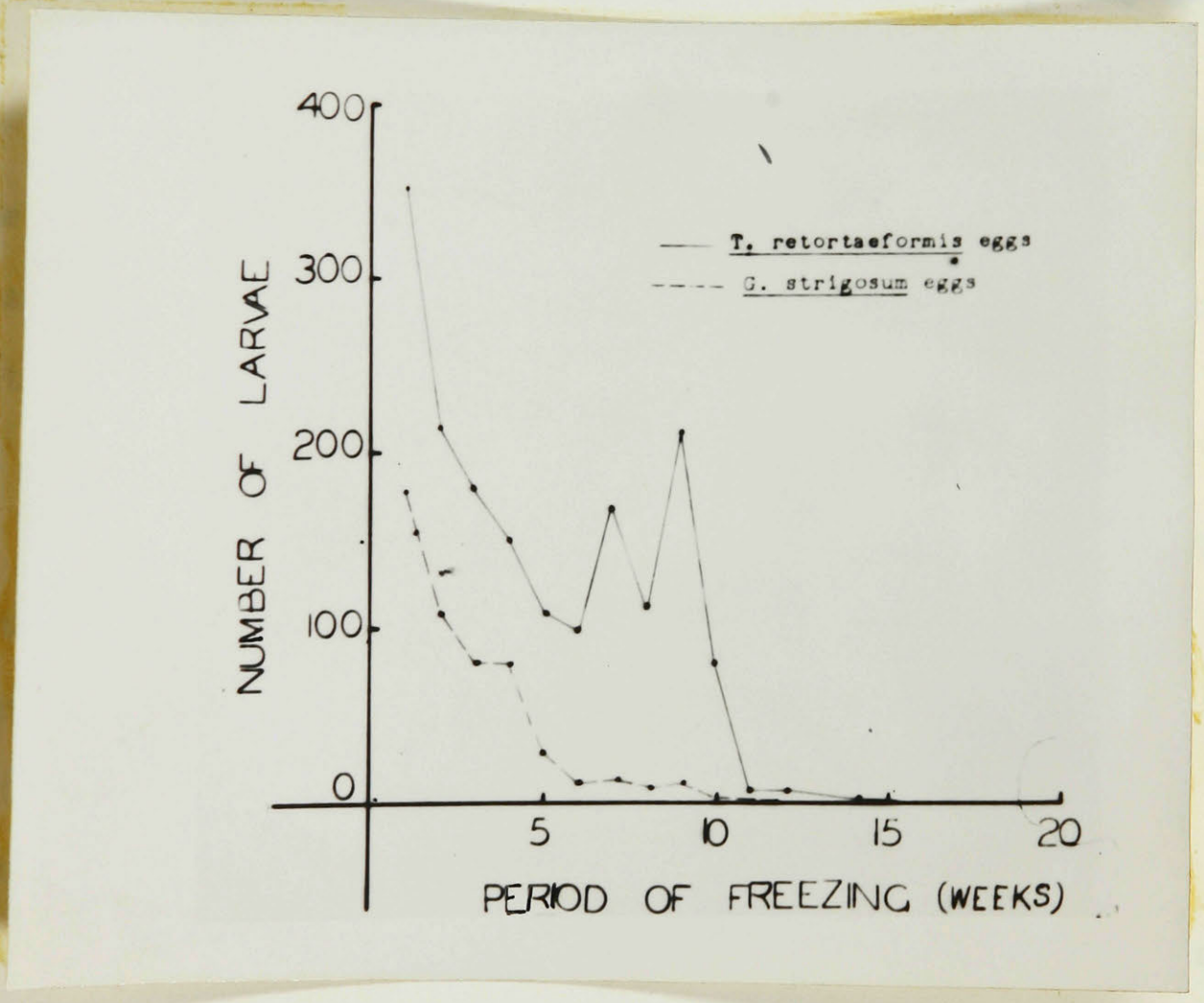

Fig. IIIB. The influence of alternate freezing and thawing on the hatchability of Trichostrongyle eggs.

Discussion: From the results of this experiment it can be seen that the eggs of T. retortaeformis amd G. strigosum, when alternately frozen and thawed are killed much more rapidly than the eggs at continuous freezing temperatures. T. retortaeformis eggs continuously frozen for 106 days may give rise to first stage larvae but not for 113 days. G. strigosum eggs continuously frozen for 84 days may give rise to first stage larvae but not for 90 days.

The microscopic appearance of a long-frozen egg (fig.1c) shows that the cells of the egg had begun to degenerate. The cells closest to the egg wall disintegrate first and degeneration proceeds toward the center. The egg-wall appears to remain intact during this process and is possibly the last part of the egg to disintegrate.

In contrast to these results, Veglia (1915) studied the effects of low temperatures on the eggs of H. contortus in the laboratory. He reported that eggs stored for 48 hours at 0°C did not hatch. Mönnig (1930) placed crushed fresh faeces of sheep infected with a Trichostrongylus spp. in the shade of a tree in winter. The night temperatures were very frequently below freezing point, yet he observed that development proceeded slowly up to a time when the larvae died, not having reached the infective stage.

Since the present work was done in the laboratory, the exact role of shelter in reducing the mortality of eggs exposed to freezing temperatures, as postulated by Mönnig (1930), cannot be assessed.

(iii) Viability of Eggs under Water.

It is a well known fact that most nematode eggs require Oxygen for their development and that they do not usually develop in water deeper than 3 mm. This was not the case with Trichostronglus and Graphidium spp. used in these experiments. Theiler and Robertson (1915) state that the eggs of Trichostrongylus douglassi remain viable under water for two months. Veglia (1916) found that "in liquid medium kept immobile and deeper than 0.5 cm,a rather small percentage of the eggs of Haemonchus contortus hatch after four days, but the larvae made very little progress, and within 12 to 15 days death occurs, presumably owing to lack of air." After repeated tests, the longest periods that the eggs remained viable, as determined by Mönnig (1930), were 13 days for Oesophagostomum columbianum and 45 days for Haemonchus contortus and sheep Trichostrongyles.

In the present author's experiment, the faeces of rabbits with pure infections of T. retortaeformis and Graphidium strigosum respectively were broken up in water, strained through cheese-cloth and the fine faecal particles and eggs which passed through were placed in beakers containing water about 5 cm deep. At frequent intervals, samples of the sediment were removed, mixed with sterile rabbit faeces and incubated, a control sample was incubated at the beginning of the experiment. After repeated tests the following results were obtained. The eggs of T. retortaeformis remained viable for 37 days and those of G. strigosum were still viable after 15 days under water.

A further test was made to investigate more closely the hatching of Trichostrongyle eggs in water. This was done by submerging eggs in water contained in 1/2 pint milk bottles. The depths of water ranged from 2 mm to 50 mm. They were left to stand in the incubator at 25°C and 20°C for T. retortaeformis and G. strigosum eggs respectively. At varying intervals, the content of each bottle was examined under the binocular microscope. Hatching occurred after 4 days in all bottles containing water to a depth of 2 to 5 mm. After 9 days (July 7,1950 - July 16,1950) the eggs of T. retortaeformis submerged in 20 mm of water which was turbid with faecal matter, also hatched. The eggs of G. strigosum did not hatch in water deeper than 5 mm.

The progress of the larvae after hatching under water was followed. In determining their longevity in this medium, it was considered necessary to study this in the light of the experience already gained from the lengevity of corresponding larval stages which hatched in faeces before they were placed in water. About 50% of the G.strigosum larvae that hatched under 3 mm of water died 2 days later. A preliminary experiment showed that immature stages of G. strigosum under similar conditions survived in water from 2 to 5 days. Those that survived developed to the mature stage in water containing a suspension of faecal matter.

The larvae of T. retortaeformis that hatched under water survived in this medium for five weeks without any noticeable decrease in their numbers, and developed to the mature stage during this period.

Trichostrongyle eggs, which were isolated from rabbit faces and placed in clean tap water varying in depth from 2 to 5 mm, did not hatch. The contents of the eggs which did not hatch were seen under the microscope to have decomposed and numerous bacteria and infusonia had developed in the specimens.

Discussion: The results of this experiment stand in sharp contrast to those of Mönnig (1930) who observed no hatching of the eggs of Oesophagostomum columbianum and sheep Trichostrongyles under water. It would appear that the presence of faecal material (containing vegetable matter, rotifers and infusoria) in the water exerts some influence on the hatching of the eggs. It is the present author's opinion that the tadpole larva in the fully embryonated egg which continues to push against the egg-wall as it revolves in the egg, easily succeeds in rupturing the wall when the latter comes into contact with some hard vegetable material in the water. The absence of faecal matter could be the explanation also for the inability of eggs placed in clean tap water to hatch. The young larvae were found to be pushing constantly against the faecal matter in the water, and the suggestion has been made by some workers, that it is this pushing movement that enables development to the mature stage in water.

(iv) Hatching of eggs in different soils and in Faeces.

The aim of this experiment was to determine (1) the time of hatching of Trichostrongylid eggs in the soils used and in sterile

rabbit faeces (ii) the time of maturity and the longevity of larvae in these media. The results on the time of maturity and longevity of larvae are reported in the section on mature larvae.

Loam, New Brunswick red clay, coarse sand and sterile rabbit faeces were used as the culture media in this experiment. The apparatus was assembled as shown in fig.II and as described in the section of this paper on materials, methods and apparatus. This experiment was conducted at 15°C, 20°C, 25°C, 30°C and 40°C. After repeated trials, no hatching occurred at 47°C and further experiments at this temperature were discontinued.

(a) Time of Hatching in different Media.

The results tabulated below, were obtained after a series of trial experiments in which the eggs had either hatched and larval growth proceeded to a second stage or in which no eggs hatched at all. The statistics on such trial experiments have not been included. The time of hatching was held to coincide approximately with the time when less than 50 first stage larvae were recovered from the culture. The results are shown in table II.

Table II - Time of hatching in different Culture Media in hours

A. Trichostrongylus retortaeformis

Media Temp.	15°C	20°C	25°C	30°C	40°C	47°C
1. Rabbit fæces	36	25	19	19.5	17.5	no hatching
2. Clay	40.5	35	24	26	29	" "
3. Loam	48	39	20	23	27	" "
4. Sand	54	42	22	24	31	" "

B. Graphidium strigosum

Media Temp.	15°C	20°C	25°C	30°C	40°C	47°C
1. Rabbit faeces	7 dys	51 hrs	47 hrs	56 hrs	no hatching	no hatching
2. Clay	4 "	33 "	30 "	32 "	"	"
3. Loam	6 "	40 "	35 "	29 "	"	"
4. Sand	7 "	56 "	43 "	65 "	"	"

The results show that the eggs of G. strigosum take a slightly longer time to hatch at any of the temperatures considered than do the eggs of T. retortaeformis.

They also show that temperatures above the optimum which is about 25°C for T. retortaeformis, do not necessarily cause a more rapid development. No eggs hatched at 47°C, and the few larvae that were recovered at 40°C, made little progress and were killed by the heat of the incubator two to three hours later. They survived, however, when transferred to 25°C immediately after they had hatched at 40°C, and development to the mature stage proceeded normally. In sterile rabbit faeces, the hatching of T. retortaeformis eggs occurred faster than at any other temperature, but this result was obtained in only a few of the many experiments conducted on hatching at this temperature.

Contrary to these observations, Mönnig (1930) states that temperatures above the optimum (about 26°C for sheep Trichostrongyles) cause a more rapid development but degeneration follows soon after. In his experiment with sheep Trichostrongyles, rarely an egg hatched at 37°C. Theiler and Robertson (1915) on Trichostrongylus douglassi, Boulenger 1915 on Nematodirus filicollis and Daubney 1920 on Dictyocaulus filaria and D. vivipara seem to agree with the views expressed by Mönnig.

The time of hatching was increasingly prolonged below the optimum temperature. No hatching occurred in moist rabbit faeces containing the eggs of T. retortaeformis at 0°C, but faeces which was moistened and stored in a commercial refrigerator on November 28,1949 at a temperature of 2°C, contained active third stage larvae when examined on February 8,1950 (after 72 days). The eggs of both

species, in a separate experiment, were found to hatch in the four media used in this investigation at 2°C, when the media were kept moist. On no occasion, however, was hatching observed at 0°C. Hatching at 2°C, in the experiment referred to, occurred from three to four weeks. The exact time was not determined for each culture medium at this temperature.

It may be stated, therefore, that at subfreezing temperatures, drying is lethal to Trichostrongyle eggs contained in rabbit faeces. When supplied with an adequate amount of moisture, however, these eggs hatch and the immature larvae develop to the infective stage even at subfreezing temperatures.

(iv) Hatching and the pH of the media.

A series of experiments was conducted to find out if the eggs had any preferences for any of the media used. This was done by weighing out 50 gm of each medium and culturing 10 gm of rabbit faeces containing the eggs of T. retortaeformis in it. Since the moisture, weight and numbers of eggs of all the media were approximately equal, an attempt was made to correlate the differences observed in the number of eggs hatching in these media with the pHs and the temperature of the media. The apparatus used is shown in fig.II. Each culture was left to stand at the appropriate temperature for 14 days. This period of time had been previously established as that in which a maximum number of larvae was recovered from each culture. The pHs of the media which did not vary much from the values given below, (in this

series of experiments) were determined with a Beckman's Electrode pH meter. The relationship between the number of larvae recovered from each medium, and its pH is given in figures IV (a) and (b). This study was conducted on the eggs of T. retortaeformis.

Table III - The pHs of the Media

Media	pH
Clay	5.1
Sand	5.9
Loam	5.6
Rabbit faeces	6.5

From these graphs, it can be seen that at temperatures near the optimum (between 20°C and 25°C for T. retortaeformis) a maximum number of eggs hatch at about pH 5.1. At temperatures removed from this, the pH of maximum hatching tends to be slightly increased. A maximum number of larvae were recovered at 15°C and 30°C between the pHs of 5.6 and 5.9. A negligible number of eggs hatched at the high pH of 6.5.

Discussion: This study was not conducted on G. strigosum eggs. The observations of Buhr 1934 (cited by Blanchais 1945) who stated, after examining 100 hares in different regions of Germany and Hungary, that Graphidium is not found on sandy soils where Trichostrongylus would otherwise be found, strongly suggests that the difference might be due, apart from other things, to the pH of this medium. In the present author's experiment, sand had the highest pH but yielded the smallest

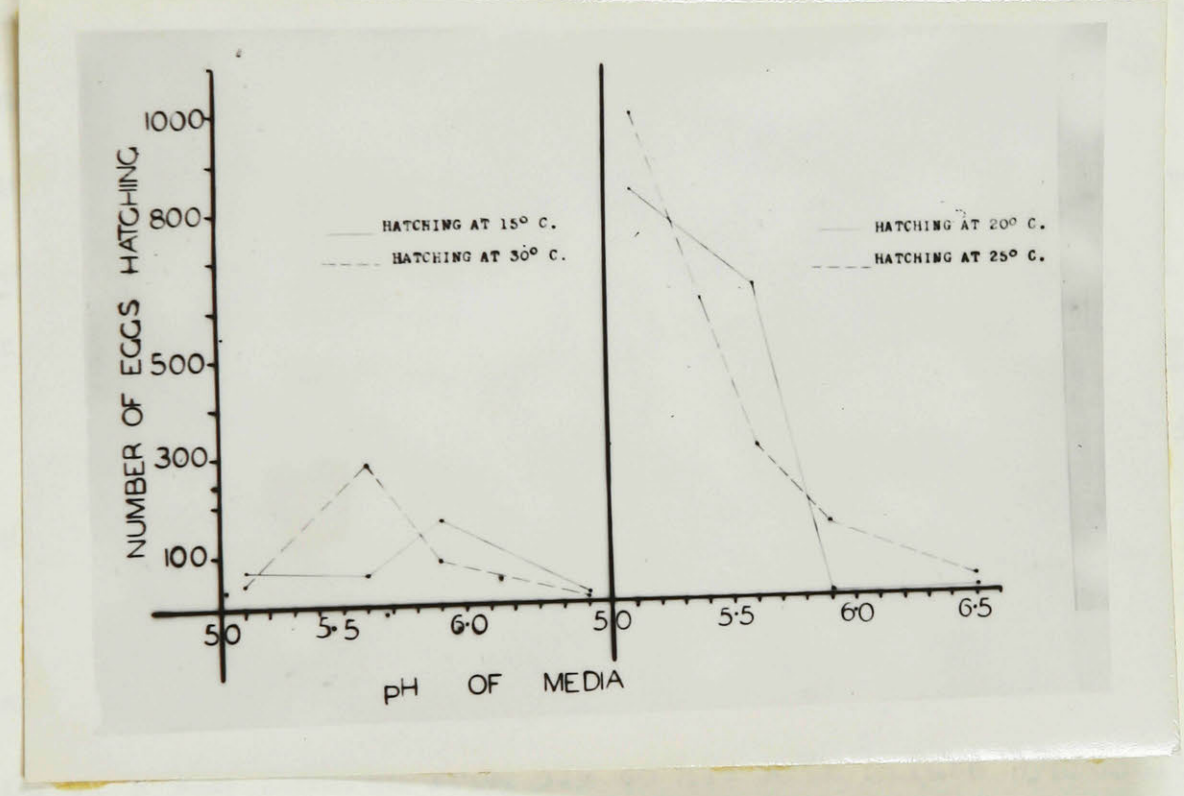

Fig.IV. The relationship between the pH of media and the hatchability of eggs in them.

number of larvae of all the natural soils used in culturing.

A few workers have studied the effects of pH on parasitic nematodes. Enigk (1938) found that the exsheathed third-stage larvae of Graphidium strigosum lived longest in a medium of pH 3.0-4.0; at pH 8.0 or above they died in 24 hr; at pH 1.0, they were very active but died in 2 days. Crofton (1947) showed that pH 4-5 was the most favourable pH for ecdysis and survival of T. retortaeformis infective larvae. This pH is within the range quoted for the stomach contents of rabbits. The effect of pH on the hatching of the eggs of these two species, is lacking in the literature. If relatively few eggs of T. retortaeformis with an optimum pH range of 4-5 hatched in sand (pH 5.9), it is easy to understand why the eggs of Graphidium strigosum with an optimum pH range of 3.0-4.0, may not hatch in sandy districts, though very few eggs do so under laboratory conditions.

In another experiment, 2 sand cultures, A and B, were set up each containing 10 gm of infected rabbit faeces in 50 gm of sand. The pH of A was lowered from 5.9 to 4.9 with dilute Hydrochloric acid and the pH of B remained at 5.9. Both cultures were left at 25°C for two weeks. Three hundred and fifty-six T. retortaeformis larvae were recovered from A while only 73 larvae were recovered from B at the end of this period. At a pH of 3.7 however, no eggs hatched and very few eggs hatched at a pH of 4. This experiment therefore establishes the optimum pH range for the hatching of T. retortaeformis eggs at pH 4-6.

B. Experiments with Immature and Mature Larvae.

As is the case with other strongyles, the infective larvae of Trichostrongylus spp. differ very much in their habits from and are more resistant to various adverse factors than the immature stages. Consequently, the experimental results obtained with immature larval stages have shown little or no resemblance to the results of similar experiments with mature infective larvae. In most of the experiments reported below, mature larvae have been used since they are more resistant than the preinfective stages to the factors considered. In some cases, the results obtained with the immature stages have been cited for the purpose of comparison.

Mature Larvae

An accurate knowledge of the structure and dimensions of the mature larvae of the two species used in these experiments is necessary in order not to confuse them with the immature larval forms. The dimensions of the three larvae of Graphidium strigosum are 310 µ - 380 µ for the first stage larva, 480 µ - 530 µ for the second stage larva and 680 µ - 740 µ for the third stage larva. The corresponding stages of T. retortaeformis measure 250 µ - 300 µ, 380 µ - 470 µ, and 540 µ - 600 µ. The mature stages of both species are characterised by their slender form and their more numerous and more darkly pigmented intestinal cells. The immature stages have less darkly pigmented intestinal cells and are thicker and move about more sluggishly in

water. Perhaps the easiest way of recovering only mature larvae of T. retortaeformis is the "moist filter-paper" technique described in the section of this paper on methods, materials and apparatus. Very little migration was noted in Graphidium strigosum at the temperatures used in this study, and recovery was mainly by the Baerman technique.

(i) Low Temperature Studies

Ransom (1906) states that H. contortus infective larvae in faeces resisted 85 days exposure outside in the winter, being frozen for over 48 hours on three occasions. Veglia (1916) reports that Haemonchus contortus infective larvae on blotting paper resisted freezing for 6 months when 5-6 per cent recovered. Other investigators who have reported the resistance of other species of larvae to freezing are Ortlepp (1925) on Triodontophorus tennicollis, Schwartz (1925) on Bunostomum phlebotomum, Theiler and Robertson (1915) on Trichostrongylus douglassi, De Blieck and Baudet (1926) on strongyles of the horse. Cameron (1923) reported that low temperatures kill the larvae of Bunostomum trigonocephalum.

(a) Alternate Freezing and Thawing.

The effects of alternate freezing and thawing were studied over a period of 3 months during which time most of the larvae died. One thousand mature T. retortaeformis larvae were deep frozen for 4 months and the rate of recovery studied. The chambers of a commercial refrigerator with a constant temperature of $0^{o}C$ were used. For alternate freezing and thawing, 1000 larvae were stored in the refrigerator

in each of six petri-dishes marked 15°C, 20°C, 25°C, 30°C, 40°C and 47°C. After the required period of freezing, these larvae were returned to the incubator chambers of the above temperatures and the ice left for 3 hours to thaw. On removal from the incubator, the number of larvae surviving was found by counting the larvae under the binoculars on a slab of glass which was graduated for this purpose. To determine which had survived, each larva was touched with the point of a mounted pin. Those that survived were seen to wriggle on being thus stimulated. The results are shown in table IV.

Table IV - Percentage Survival of Larvae after Alternate Freezing and Thawing

A. T. retortaeformis mature larvae

% Survival

Period of freezing (in days) / Temp.	15°C	20°C	25°C	30°C	40°C	47°C
1 day	100%	100	100	100	100	48
3 days	100	100	100	100	100	21
7 "	100	95	100	100	92	0
17 "	100	95	100	93	80	0
45 "	86	82	77	74	9	0
59 "	80	61	54	51	0	0
87 "	62	15	3	0	0	0

B. G. strigosum mature larvae

Period of freezing (in days) / Temp.	15°C	20°C	25°C	30°C	40°C	47°C
1 day	6	6	4	6	2	1
3 days	1	2	4	6	0	0
7 days	1	2	1	4	0	0
9 days	1	1	0	1	0	0

For the purpose of comparison, a study of the resistance of first and second larval stages of T. retortaeformis to alternate freezing and thawing was also made. The results showed that of 1000 larvae in the first and second stages which were frozen and thawed three times in a period of 7 days, only seven survived at 15°C, four at 20°C and one at 25°C. It was therefore concluded that the resistance of immature larvae to alternate thawing and freezing is much less than that of the mature larvae.

(b) Continuous Freezing.

Many workers have reported their findings on the resistance of nematode larvae to continuous freezing, and are unanimous that for any period of time, continuous freezing is relatively less harmful to nematode larvae than alternate freezing and thawing. Parnell (1934) stated that frequent alternate freezing and thawing can be relied on to kill the free-living stages of horse strongyles. He found that the continued low temperatures of a severe Canadian winter were insufficient to kill "sclerostome eggs." Zawadowsky and Vorobiova (1934) concluded that between 97 and 99 per cent of Trichostrongyle larvae failed to survive 4 months of winter conditions as found at Moscow. The few that survived were probably too weak to be infective.

In the present author's experiment, 1000 mature larvae of T. retortaeformis were deep frozen from December 29,1949 to April 30, 1950 (4 months) in water 10 cm deep. The temperature of the refrigerator was 0°C throughout the experiment and no thawing occurred. At the end

of the period the ice was allowed to thaw slowly at laboratory temperature (26°C). The revival of the larvae which were still alive occurred over a period of eight days. The results are as follows:

Day	1st	2nd	3rd	4th	5th	6th	7th	8th
No. of larvae reviving	5	17	21	28	31	33	33	34

This result shows that 3.4% of the mature larvae survived continuous freezing in the laboratory for 4 months. When those surviving were left in tap-water at room temperature, none survived after two weeks. Five hundred mature larvae frozen for 14 days successfully infected a 7-month old rabbit.

In a similar experiment, 1000 mature larvae of G. strigosum were deep frozen at 0°C from May 9, 1950 to July 9, 1950 (2 months) and none recovered.

Discussion: The results of both continuous freezing and alternate freezing and thawing show that T. retortaeformis is much more resistant than G. strigosum to freezing temperatures. Wetzel and Enigk (1938), cited by Blanchais 1945, found that in ice at 3°C, 20% of the larvae of G. strigosum are killed in 14 days after 3 thawings. Wetzel and Enigk (1938) also found that 40% were killed in 24 hours at continuous freezing temperatures. Their conclusions that the resistance of G. strigosum is as good as that of other Trichostrongyles cannot be

accepted by the present author. T. retortaeformis larvae, as shown on table IV, were frozen seven times and thawed six times in a period of 12 weeks and 62% survived at 15°C, 15% at 20°C and 3% at 25°C. Not more than 1% of the mature larvae of G. strigosum survived four freezes and three thawings in a period of 10 days at any of the temperatures used in this investigation. Three point 4 per cent of T. retortaeformis mature larvae survived continuous freezing for 4 months but similar stages of G. strigosum failed to survive 2 months continuous freezing.

This experiment has also demonstrated the importance in their revival of the temperature to which T. retortaeformis larvae are exposed after freezing. A greater percentage of larvae revived when exposed to lower temperatures, after freezing than when exposed to higher temperatures. This would tend to suggest that death is related to freezing and also to the degree of change in temperature during the thaw. If after freezing, mature larvae were allowed to remain in the incubator chambers for less than 15 minutes -- that is before the water in which they were frozen had attained the temperature of the incubator -- the percentage of survival is found to be about equal in every specimen. It should be expected, therefore, that a sudden onset of Spring with moderate temperatures following a very cold winter should be as lethal to T. retortaeformis larvae as a mild winter with frequent freezing and thawing.

This influence of temperature on revival after freezing has not been noted in the case of G. strigosum; for example there was no difference in the percentage revival of larvae at 30°C and at 15°C

after 24 hours freezing. This strongly suggests that death in G. strigosum larvae was more a result of actual freezing than a result of the degree of change in temperature.

Resistance to Desiccation

Conflicting results have been reported by many authors on the resistance of infective Trichostrongyle larvae to desiccation. Theiler and Robertson (1915) found that Trichostrongylus douglassi larvae resisted desiccation for 9 months. Zawadowsky (1929) states that a small percentage of the larvae of the Trichostrongyles of sheep revive after two months of dryness. Ransom (1906) found that H. contortus larvae dried in faeces for 35 days could recover. Veglia (1916) placed Haemonchus cntortus larvae in faeces under a tree where they dried out; after 3 months 50 per cent revived. Concerning Oesophagostonum columbianum Veglia (1923) states that this species closely resembles Haemonchus contortus in its tropisms and resistance. Daubney (1928) states, concerning Haemonchus contortus larvae, that they do not possess any very great powers of resistance against desiccation, although in this respect they are not quite as defenceless as the hookworm larvae; also that Trichostrongyle larvae are similar to Haemonchus contortus in their resistance and desiccation.

Explaining the reason for these inconsistent results, Mönnig and a few others have stressed the importance of such factors as the presence or absence of debris around the larvae, the relative humidity

of the atmosphere and therefore also the temperature and the time of the year. All these are factors which determine the "actual degree of dryness"of the larvae, and this degree of dryness appears to be the most important factor on which the result depends. It would therefore be necessary,in all experiments of this nature, to work in an atmosphere of known or controlled humidity and temperature.

The constant temperature incubator described in the section on materials, methods and apparatus was used for most of the experiments on desiccation. Two methods of desiccation were employed. In the one, larvae free from debris were removed from the culture and placed in a few drops of clean water in small petri-dishes and the water was allowed to dry away. In the second method, larvae were desiccated in a desiccator containing anhydrous calcium chloride at 20°C. At the expiration of the desiccation period, 5 cc of water was added to the contents of the petri-dish and the larvae were hydrated for about 12 hours. It was found that this period of hydration was sufficient to enable larvae that were still alive to revive. The experiment on free drying was conducted at the six temperatures of this study. The results on the percentage survival of these larvae (table V) were obtained from 1000 larvae treated in each temperature.

Table V - Effect of Desiccation on Mature Larvae

A. Free Drying Method.

(1) T. retortaeformis

% Survival

Period of Desiccation \ Temp.	0°C	15°C	20°C	25°C	30°C	40°C	47°C
1 hour	100	100	100	100	100	100	0
12 hours	100	100	100	100	95	90	0
46 hours	100	100	100	98	93	75	0
7 days	100	100	100	93	60	30	0
14 days	100	100	94	80	53	0	0
21 days	100	100	94	76	15	0	0
28 days	100	95	70	35	9	0	0
35 days	100	81	50	21	3.0	0	0

(2) G. strigosum

% Survival

Period of Desiccation \ Temp.	0°C	15°C	20°C	25°C	30°C	40°C	47°C
1 hour	100	100	100	100	100	85.7	0
2 hours	100	94	88.3	10	0	0	0
3 hours	100	57	42	0	0	0	0
7 hours	73	10	12	0	0	0	0
24 hours	47	2	0	0	0.	0	0
30 hours	0	0	0	0	0	0	0

B. $CaCl_2$ Drying Method at 20°C

% Survival

Species \ Time	1/2 hr.	1 hr.	2 hr.	6 hr.	12 hr.	1 day	2 days	3 days
1.T.retortaeformis	100	93.2	80.0	80.0	73.4	50	17.9	1.0
2. G.strigosum	51.4	22.6	0	0	0	0	0	0

Discussion: The results shown in table V prove conclusively that T.retortaeformis is more resistant to desiccation than G.strigosum. The feeble resistance of G. strigosum to desiccation has been emphasized by some writers. Blanchais (1945) observed that the mature larvae of this species when placed in a shade in a dry atmosphere for 24 hours, only 30% survived, (no temperatures were mentioned). All were destroyed after 48 hours. In another experiment, Blanchais (1945) placed one rabbit dropping containing numerous larvae in an open petri-dish in the laboratory at a temperature less than 15°C, three days afterwards, the dropping was dry, five days later no living larvae were recovered from the dropping.

The practical importance of the actual degree of dryness in the desiccation and survival of G.Strigosum larvae is illustrated by the observation of Blanchais (1945). He observed that whereas the small water content of rabbit faeces exerted an unfavourable effect on the development of larvae, the water content of the faeces of bovines was sufficient to guarantee development to the infective stage even in a dry atmosphere. For a similar reason, only on grounds containing vegetation which retains moisture, is the development of the eggs of

G. strigosum contained in rabbit faeces possible. Since the vitality of the larvae is very relatively feeble, it could be predicted therefore, that infestation by G. strigosum is dependent, among other things, on the humidity of the subsoil, on atmospheric precipitation, and on the season more strictly so than in the case of T. retortaeformis.

The observations of Wetzel and Enigk coincide with those of Hundt 1928 (made during a period of 15 years) which reveal that the parasitic infestation of hares is a function of the rainfall curve. The period of maximum infestation coincides consequently, in the Central European countries, with the three humid months in the Spring, thus creating for young hares a source of infestation of much importance.

Retaking this question in 1938, Enigk estimated that it is possible to suppose that after a short desiccation, only an inferior number of G. strigosum larvae could develop in the host against the number that develop in normal conditions. The author subjected to desiccation for 40 minutes, 300 larvae which he subsequently placed in a humid atmosphere after the appearance of lively contractions. He then injected these larvae by means of a syringe into the digestive organ of the rabbit. The host succumbed at the end of 28 days. On autopsy, 98 worms (47 males, 51 females) were recovered from the rabbit stomach being 32.6% of the larvae. The intensity of infestation was therefore similar to that observed normally.

The eggs of T. retortaeformis were observed by the present writer on the fifteenth day in the faeces of a rabbit infected with

300 larvae which were dried for seven days at 20°C. A similar number, dried at 30°C were too weak to infect an experimental rabbit. It may therefore be concluded that the lethal effect of desiccation on the larvae of Trichostrongyle nematodes depends on the actual degree of dryness.

Viability under water at different Temperatures.

In pastures and grass districts inhabited by rabbits and hares, pools of water often constitute the only source of water available to these animals. The presence of infective Trichostrongyle larvae in such a medium will depend upon (i) their viability in water, (ii) the temperature of the water and to a large extent also (iii) the presence or absence of organisms or other agents destructive to these larvae such as bacteria. To evaluate the significance of water as a source of infection of these animals with Trichostrongyles, it was considered necessary, therefore, to combine the first two factors.

Most of the tests of this nature hitherto made on larvae of different species have been carried out with larvae in clean water and very often at room temperatures only. The importance of slight differences in temperature in the longevity of these larvae in water, is illustrated by the results obtained in this experiment. Some of the results of other authors are tabulated here for comparison.

Table VI - Longevity of Larvae in Water

Species	Viability in water	Author
Trich. douglassi	13 months	Theiler and Robertson (1915)
Nematodirus filicollis	11 months	Boulenger (1915)
Strongyles of Horse	4 months	De Bliek and Baudet (1926)
Ostertagia circumcinta	3 months (at least)	Morgan (1928)
Bunostomum trigonocephalum	several months	Cameron (1923)
Haemonchus contortus	5 1/2 months	Veglia (1916)
Strongyloides stercoralis	5 days	Fülleborn (1914)
Sheep Trichostrongyles	7 months	Mönnig (1930)

Most of the above are the results of laboratory experiments performed at room temperature.

Infective larvae of T. retortaeformis were isolated in tap water and about 5000 were placed in each of six petri-dishes on March 25,1950. The depth of water in each petri-dish was 5 cm and each dish was covered so as to allow access of air to the bottom, and placed in the incubator at the appropriate temperature. The temperatures of this investigation were, as usual, 47°C, 40°C, 30°C, 25°C, 20°C and 15°C. Fresh tap water was added weekly to restore the water level which had dropped owing to evaporation. The number of larvae surviving was determined by counting.

To simulate the fluctuations in atmospheric temperatures, 1000 larvae were placed in water in each of six petri-dishes as described above and exposed to the incubator temperatures. They were removed each night and left on the laboratory bench (room temperature was about 26°C), and returned each morning to the incubator temperatures. The results are shown on tables (VII) and (VIII). A similar experiment with G. strigosum was conducted during a period of 12 weeks and the results show that there is no appreciable difference in the longevity of the mature larvae of this species and those of T. retortaeformis in water. Bacteria were found to develop at the bottom of each vessel and the larvae that died decomposed and disappeared. The general effect of simulating might and day temperatures was an increase in their longevity in water of the larvae which were thus treated, over those exposed to continuous incubator temperatures. Hence, of the T. retortaeformis larvae treated as described in this experiment at 30°C, while none survived among the forms left constantly at this temperature for 3 months, 8.5 per cent of the forms in which day and night temperatures were alternated survived after this period. The reverse is true, however, of the larvae exposed to 15°C. Ninety nine point 0 per cent of the larvae left constantly at this temperature survived after three months while only 8 per cent survived among the forms in which day and night temperatures were alternated.

Morphological changes

Most of the larvae studied in this experiment, were observed to cast their sheaths in water just before they died. It may possibly

be that on casting their sheaths, they then became susceptible to bacterial attack which destroys them. Veglia (1916) also observed that from the first month onwards many H. contortus larvae cast their sheaths. In a similar experiment with sheep Trichostrongyles, Mönnig (1930) observed that larvae did not live as long in water containing mud and small invertebrates as they did in tap water and that they died off sooner in water exposed to sunlight than in water in the shade. Contrary to the results of the present author's experiment, Mönnig did not consider the rise in temperature which was the effect of the sun, of great practical consequence.

Table VII - Viability in 5 mm Water at different Temperatures

A. Larvae constantly exposed to incubator Temperatures

(i) T. retortaeformis

% Survival

Incubator Temp. (°C) \ Time	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks	14 wks	16 wks
47	0	0	0	0	0	0	0	0
40	47	1.1	0	0	0	0	0	0
30	100	82.9	41.5	23	10.7	4.7	0	0
25	100	99.6	91.3	73.5	25.5	5.2	0.63	0
20	100	86.7	83.3	78.5	75.3	63.8	59.3	46.7
15	100	100	100	100	99.8	99.5	99.2	66.1

(ii) G. strigosum

% Survival

Incubator Temp. (°C) \ Time	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks
47	0	0	0	0	0	0
40	75.3	55.2	46.7	26.7	12.5	0
30	91.0	70.5	54.0	50.2	43.2	12.0
25	100	100	100	100	94.0	90.0
20	100	100	100	100	100	100
15	100	100	100	100	100	100

Table VIII - Viability in 5 mm Water at different Temperatures

B. Larvae exposed to alternated Day and Night Temperatures

(i) T. retortaeformis

% Survival

Incubator Temp. (°C) \ Time	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks	14 wks	16 wks
47	0	0	0	0	0	0	0	0
40	92.6	14.1	4.3	0	0	0	0	0
30	100	98.0	95.7	57.0	41.5	11.3	10.0	8.5
25	100	99.3	97.3	76.9	60.0	7.9	7.9	4.1
20	100	100	100	100	81	44.1	37.8	17.3
15	100	100	100	100	95	10	8.2	2.3

(ii) G. strigosum

% Survival

Incubator Temp. (°C) \ Time	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks
47	0	0	0	0	0	0
40	76.5	70.6	64.7	29.4	17.7	0
30	78.5	78.5	78.5	76.3	71.4	71.4
25	100	100	93.0	93.0	88.9	77.8
20	100	100	95.7	90.9	90.9	27.3
15	100	100	100	100	100	67.0

Maturity and Longevity of Larvae in Different Culture Media.

This experiment is a continuation of that on the hatching of eggs in different media reported earlier in this paper. The apparatus which is exactly the same, was assembled as shown in fig.2. The aim was to determine the time it took the larvae to mature and to live in sand, clay, loam and rabbit-faeces cultures in which have been buried the eggs of T. retortaeformis and G. strigosum respectively. Six cultures of each medium were exposed to the six incubator temperatures used in this study, and were constantly moistened by adding about 25 cc of water every day to each culture.

The Baerman isolation technique was employed in recovering the larvae from the media and, as in a previous experiment, the time of maturity was determined after a series of trial experiments, the statistics on which have not been included in this paper. The criteria for maturity were based on the descriptions of the mature larvae given on page43 of this paper. The time limit for the submission of this work has not enabled the observations on the longevity of larvae at the lower temperatures to be pushed to a conclusion and for the same reason, the longevity of G. strigosum in the different media, has not been studied. The times of maturity after hatching at different temperatures are shown in table IX.

Table IX - Time of maturity after Hatching (in hours)

A. Trichostrongylus retortaeformis

Media Temp.	15°C	20°C	25°C	30°C	40°C	47°C
1. Rabbit fæces	83	58	42	38	no mature larvae	no hatching
2. Clay	91	80	56	40	"	"
3. Loam	102	89	46	48	"	"
4. Sand	117	95	46	89	"	"

B. Graphidium strigosum

Media Temp.	15°C	20°C	25°C	30°C	40°C	47°C
1. Rabbit faeces	7-8 days	4 days	2-3 days	5 days	no mature larvae	no mature larvae
2. Clay	8-10 days	1-2 "	2 "	1-2 "	"	"
3. Loam	8-10 "	2 "	3 "	1-2 "	"	"
4. Sand	10 "	2-3 "	4 "	no mature larvae	"	"

Discussion: The information available on the hatching and maturity of sheep Trichostrongyles in the literature, was that given by Mönnig in 1930. He outlined the periods of development of the free stages of T. instabilis and T. rugatus under optimal conditions as follows: "Eggs hatch 19 hours after having been passed by the sheep, the first ecdysis takes place 25 hours later, the second ecdysis 19 hours after the first, the infective stage being reached under optimal conditions in 63 hours." His medium was sterile sheep faeces and he worked at a temperature of 26°C. Concerning the development of G. strigosum, Blanchais (1945) stated that an embryo is formed in the egg in 24 hours at 20°C and that hatching occurs 8-10 hours afterwards, that is 32-34 hours after the egg is laid. He further observed that the first moult occurs 20-30 hours after the hatching of the egg and the second moult 2 or 3 days later without the larva shedding its second sheath.

Reference has already been made in the section of this paper on the hatching of eggs, to the tendency of T. retortaeformis eggs to develop faster than those of G. strigosum. The same appears to be true of the development of the free-living larval stages. Very few eggs of G. strigosum hatched at 30°C and no hatching occurred above this temperature. While the free-living larvae of T.retortaeformis attain maturity 58 hours after hatching in faeces at 20°C, corresponding stages of G. strigosum do so in 3 to 4 days. The times of maturity after hatching at 15°C, are 3-4 days for T. retortaeformis and 7-8

days for G. strigosum. The optimum temperatures for the development of T. retortaeformis lie between 25°C and 30°C while those for G. strigosum lie between 15°C amd 20°C.

Longevity in different Culture Media.

This experiment was set up as shown in fig.2, in February 1950 and was allowed to stand at the six temperatures of this study through July 1950. The media used were clay, coarse sand, loam and sterile rabbit faeces. At frequent intervals samples from the cultures were examined for larvae and whenever it was suspected that all the larvae in any culture had died, a thorough examination by the faecal sampling method was carried out. Each culture was moistened whenever signs of dryness were noted. The results of this work, which was done with the larvae of T. retortaeformis, are expressed in days on table X.

Table X - Longevity of T. retortaeformis larvae in different Media

Time in days

Media	Temp.	15°C	20°C	25°C	30°C	40°C	47°C
Clay		over 180	over 180	over 180	40-42	4-6	no hatching
Sand		" 180	" 180	47-50	12-14	1-2	" "
Loam		" 180	" 180	over 180	30-35	3-4	" "
Rabbit faeces		" 180	" 180	87-91	21-23	2-3	" "

This experiment was continued for 180 days. At the end of this period, the larvae which were still living in each culture medium were isolated by the Baerman technique and counted. The results are shown on table XI.

Table XI - *T. retortaeformis* larvae surviving 180 days in different Media

Culture Medium / Temp.	15°C	20°C	25°C	30°C	40°C	47°C	pH
Clay	7	114	16	0	0	0	5.1
Sand	13	34	0	0	0	0	5.9
Loam	25	59	270	0	0	0	5.6
Rabbit faeces	3	10	0	0	0	0	6.5

pH and Longevity in different Media.

Reference has already been made to the relationship of the pH and the number of eggs found to hatch in each medium. A correlation of the number of larvae surviving for over 180 days in each medium to the pH of the medium, proves conclusively that apart from the temperature, moisture, presence or absence of bacteria in the soil, the pH of the soil plays an important part in the longevity of larvae in it. Hence, the greatest number of larvae lived for over 180 days in clay (pH 5.1), next to clay in favouring the longevity of larvae was loam (pH 5.6), then sand (pH 5.9) and lastly sterile faeces (pH 6.5).

Crofton (1947) showed that pH 4.5 was the most favourable pH for the survival of T. retortaeformis infective larvae. Mönnig (1930) who investigated the longevity of sheep Trichostrongyles in different soils stated that they die off sooner in sandy soil, and attributed this to their migrating more easily in this medium than in any other soil. In the present author's experiments, however, the number of larvae migrating to the filter papers with which the cultures were covered was largest in clay, then loam, faeces, and lastly sand.

The largest number of larvae, as shown on table XI, lived in each medium at 20°C and the graph obtained by plotting the number of larvae recovered at this temperature against the pH of the medium is shown in fig.V. The larvae recovered from each culture had undergone the two preliminary moults and were in the infective stage.

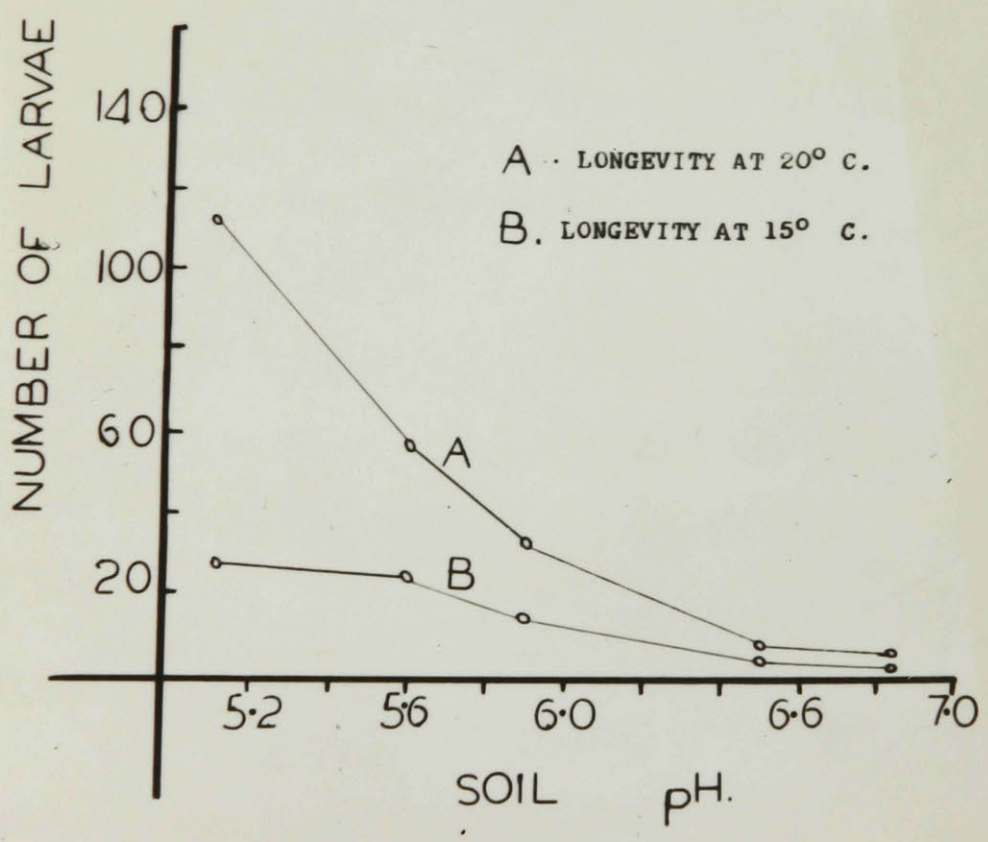

Fig.V. The effect of soil pH on the longevity of mature Trichostrongyle larvae in it.

Notes on Migration, Skin Penetration and Thermotropism.

(1) Migration from Cultures.

This subject has received considerable attention in the last few years and the literature on it contains not only work on Trichostrongyle larvae but also similar work on the free-living larvae of other nematodes. It is now fully well established, that the migratory activity of the free-living larvae of any nematode is governed by the consistency of the medium (nematode larvae cannot migrate out of water or a wet medium) and by temperature, light intensity and moisture. Crofton (1948) in a series of ecological studies on the immature phases of T. retortaeformis, reported his findings on the effect of climatic factors on the availability of the infective larvae of this species to the host. He studied the rate of hatching, development and survival of the larvae of T.retortaeformis under dry and winter conditions, the daily fluctuations in the number of larvae on grass plots and their rate of disappearance during successive months. When the maximum temperatures were below 50°F, there was no hatching. Eggs passed in the autumn survived a cold winter but those passed during the coldest period died. There was little or no migration when the temperature did not exceed 55°F. A high death rate occurred in warm weather, in which the rate of evaporation was high. The number of larvae on grass blades depended upon the climate at the time and on the effect of previous conditions on hatching and survival.

In another study Crofton (1949) showed that the number of T. retortaeformis infective larvae fluctuated on grass blades throughout the day, being lowest when dew was formed and highest between midday and 5 pm. The number of larvae on a pasture reached its maximum in August. He suggested that the larval population was reduced by 55% as a result of resting a pasture for 3 weeks while a week after the return of the sheep, the reduction went up to 90%.

The only mention found in the literature on the migratory activity of the infective larvae of G. strigosum is by Blanchais (1945). He stated that they reach the first part of the herbage in the morning when the humidity is adequate, and that the infestation of the host occurs passively by mouth.

In the present work, an attempt was made to compare the migratory activity of T. retortaeformis and G. strigosum. The method used was the "faecal concentration" method described previously. The faecal culture containing the eggs of either species was kept moist and migratory activity was measured by the number of larvae that migrated through the wet filter-paper used in covering the concentrated faeces. This study was conducted at the optimum temperatures of 25°C - 30°C for T. retortaeformis and 15°C - 20°C for G. strigosum. After three weeks, 215 infective larvae of T. retortaeformis had migrated from 25 gm of faeces through the moist filter paper covering it, to the wall of the large petri-dish used in covering the culture. In this period also, only 28 infective

G. strigosum larvae had performed a similar migration, while many more larvae of this species were still on the faecal surface of the filter paper. After a series of similar experiments in which different proportions of the larvae of these two species were found to migrate as described above, it was decided that a better ratio of their migratory activities could be obtained on grass pastures. It was concluded, however, that the mature larvae of these two species, do migrate from faecal cultures under optimum conditions.

(II) Skin Penetration.

Certain authors, have asked themselves if the infective larvae of G. strigosum could not reach its definite habitat (that is, the stomach of the rabbit) per cutaneous penetration. Goodey (1922) obtained a negative result on rats and mice. Wetzel and Enigk (1937) experimented on two rabbits 10-12 weeks old. They shaved the skin at the level of the abdomen, placed infective larvae on this spot and the larvae showed no tendency to pierce the shaven skin of the host. A subsequent autopsy of these animals did not reveal the presence of any nematode in the stomach. Mönnig (1930), using the method of Goodey on Trichostrongylus instabilis and Trichostrongylus rugatus of sheep, obtained negative results.

A modification of the method used by Goodey (1922) was tried on several occasions by the present author. A small specimen-bottle which was filled with water was covered with part of the shaven abdominal skin of the mouse, stretched taut over the mouth of the

bottle and held in place with a rubber band, such that the water in the bottle was in contact with the skin. A drop of water containing a large number of larvae was placed on the external surface of the skin and allowed to evaporate. After two days, the water content of the specimen-bottle was examined for larvae. The results were negative for G. strigosum and T. retortaeformis and the observations of previous workers that these two species are non-skin penetrators, was thus confirmed.

(III) Thermotropism.

To test for thermotropism, a piece of glass tubing 10 cm long and 5 mm in diameter was filled with water containing a large number of T. retortaeformis larvae and corked at both ends. A thin copper wire was wound around the middle of the tube and one end was left to project at right angles and horizontally from the tube, which was supported at the ends. The projecting wire was now warmed, but the temperature was not raised as high as to cause visible convection currents in the water. This was controlled by the movements of small particles of matter in the water. A similar experiment was performed with the larvae of G. strigosum. The results are as follows:
The larvae of T. retortaeformis which were near the middle of the tube soon became very active but no definite migration towards the warmest region was observed. The larvae of G. strigosum were quite indifferent to the difference in temperature.

C. MORPHOLOGICAL CHANGES.

1. Morphology after death from desiccation.

Veglia (1915) observed the appearance of dead and living larvae of H. Contortus under the process of desiccation, noting the following to be constant: In the dead larvae, the edges of the intestine are rather indistinct, the granulations are very fine and slightly yellowish. Dead desiccated larvæ immersed in water had nearly lost the internal structure and the intestinal cells contained numerous large vacuoles. It appeared that the larvae were killed by desiccation as soon as the granulation stores in the cells of the intestine were exhausted. In his discussion of the mechanism of desiccation, he stated that the desiccation of the intestinal cells seemed to be the most fatal process, stating also that fat present in the protoplasm of the intestinal cells evidently retarded the desiccation process. Mönnig (1930) observed that the larvae of sheep Trichostrongyles recovered after a prolonged period of dryness, showed considerable changes in the intestinal cells. The number of reserve food granules had decreased and gas bubbles had accumulated. In spite of these changes, these larvae were very infective.

The microscopic picture of the desiccated larvae of the two species used in this study was very similar. There was a marked decrease in the number of food granules in dead desiccated larvae and the few that were still left were constantly observed to reflect the yellow ray of the illumination light. A dead larva was therefore

distinguished from one that was still living after desiccation, by this characteristic yellowish colouration. Some of the intestinal cells of larvae that had died from desiccation were vacuolated and the readiness with which smaller vacuoles coalesced to form larger ones strongly suggests that they contained fat which must have been present in the protoplasm of the intestinal cells. The mechanism of death from desiccation would therefore appear to be a process in which the water and fat contents of the intestinal cells are liberated by evaporation and melting respectively, with a consequent collapse of these cells.

2. Morphology after death from freezing.

Very little is known at present about the mechanism of death by cold in nematode larvae. There doesnot appear, from the literature reviewed, to have been an attempt by many authors to explain the cause of death of larvae at low temperatures, the exception being Furman (1944) who observed the rupture of the cuticle and suggested it was the cause of death. This temperature was -6°C. Barker (1945), hypothesized that the death mechanism in nematode larvae, with a quick freeze was as follows: "Under the action of the sudden lowering of the temperature the protoplasmic sol changes to a gel. This gel then undergoes a syneretic change with the formation of the globule-like structures observed in most dead larvae, thus constituting the death process. The globule like structures during the process of formation produce changes in intracellular osmotic pressure so the cell

membrane eventually ruptures, producing the characteristic intestinal disruption. On returning to a higher temperature, the globule-like structures, which may be composed of fats or possibly proteins, undergo further changes to produce the characteristic coalescing feature observed in larvae exposed to the heat of the illumination lamp of the microscope."

Observations on slow-freezing carried out by the present author, have failed to show rupture of the sheath or cuticle as the cause of death. The degenerative changes occurring in the intestinal cells after freezing, were constant and strongly suggestive of the cause of death. Larvae that were dead from over two months freezing showed an extreme disruption of the intestinal tract, part of which had protruded from the excretory pore. No constant morphological changes were noted in larvae that died from a short exposure to freezing temperatures.

GENERAL DISCUSSION AND SUMMARY

In this thesis, an attempt has been made to examine more closely under laboratory conditions the influence of the main environmental factors which determine the development and survival of the free-living stages of rabbit Trichostrongyles. The two species studied in this work were (i) Trichostrongylus retortaeformis (Zeder, 1800) which in the adult stage feeds on the mucus of the small intestine of rabbits and hares. Severe infestations may cause disease and not infrequently death. (ii) Graphidium strigosum (Dujardin, 1845) a larger species which occurs in the stomach and occasionally the small intestines of rabbits and hares where it feeds on blood. Accordingly, a fewer number of G. strigosum adult nematodes is more harmful to the host than a corresponding number of T. retortaeformis worms.

The experiments here reported show that the subsequent development of Trichostrongyle eggs after drying, depends on the temperature at which they were dried. The eggs of T. retortaeformis kept dry between 10°C and 28°C will still be viable after 8 months. Under similar conditions, the eggs of G. strigosum will remain viable for just over 2 months. Eggs dried at temperatures above 40°C are killed in 3 to 4 days, while eggs kept dry at temperatures below 8°C will deteriorate very slowly over a period of 6 months. The

reports of previous investigators do not afford a suitable basis for comparison with the results of the writer's experiments on the resistance of embryonated eggs to desiccation. While no appreciable difference was observed in the resistance to desiccation of embryonated and unembryonated eggs of the two species considered here, Loess(1911), Theiler and Robertson (1915) and Mönnig (1930) state that the embryonated egg is the resistant stage.

T. retortaeformis eggs continuously frozen at 0°C for 106 days may give rise to first stage larvae but not for 113 days. Similarly, G. strigosum eggs under the same conditions may give rise to first stage larvae after 84 days, but not after 92 days. Alternate freezing and thawing, however, is more lethal to Trichostrongyle eggs than continuous freezing and it reduces their viability to 98 days for T. retortaeformis and 70 days for G. strigosum. After a series of experiments, the eggs of T. retortaeformis remained viable under water for 37 days as compared with 15 days for the eggs of G. strigosum.

From the results on the quantitative hatching of eggs in different culture media, it would appear that the pH of the soil influences the hatching of eggs just as much as its temperature, moisture and air-content. Many more eggs hatched in clay (pH 5.1) than in any other medium. Next in favourableness to hatching was loam (pH 5.6), then sand (pH 5.9) and lastly sterile rabbit faeces (pH 6.5). Generally, Trichostrongyle eggs hatch more readily in a slightly acid medium than in a neutral or an alkaline one. In

the four media used in this study, hatching occurred earliest at 25°C. Higher temperatures did not necessarily cause a more rapid development, and the time of hatching was considerably prolonged at lower temperatures.

It is well known that the Baerman apparatus is of limited efficiency for the recovery of larvae from faeces. Despite its imperfection, however, it was found practicable for the comparative nature of this work. Another method of recovering infective larvae is the faecal concentration method previously described and which was found to be very successful with the larvae of T. retortaeformis.

The resistance of infective Trichostrongyle larvae to adverse factors was greater than that of first and second larval stages; and, accordingly, most of the experiments reported have been conducted with the former. The result on the survival of mature T. retortaeformis larvae after four months continuous freezing is in line with the statement of Zavadovsky and Vorobiova (1937) who reported that after 4 months freezing under winter conditions in Moscow, only about 3-4 per cent of the frozen Trichostrongylid larvae survived, but the survivors were too weak to be infective. The infective larvae of G. strigosum did not survive two months continuous freezing and the present writer cannot agree with the statement of Wetzel and Enigk (1938) that the larvae of this species resist freezing temperatures to the same extent as most other Trichonstrongyle larvae. As was the case with the eggs of these two species, alternate

freezing and thawing is more lethal to mature Trichonstrongyle larvae than continuous freezing. Even when alternately frozen and thawed, the larvae of G. strigosum were less resistant to freezing temperatures than those of T. retortaeformis. Significant in the experiment on alternate freezing and thawing, is the greater percentage of larvae that revive after freezing when thawing is allowed to proceed at lower temperatures. The practical importance of this, is that a sudden onset of Spring with moderate temperatures following a very cold winter could be expected to be as lethal to Trichostrongyle larvae as a mild winter with frequent freezing and thawing.

The two methods employed in the drying of infective larvae demonstrate that death from desiccation depends on "the actual degree of dryness" of the environment surrounding the larvae. T. retortaeformis larvae exposed to a humidity of 0 per cent (in chloride) died after three days while 100 per cent survived desiccation in a humid atmosphere at 25°C for over 30 days. A review of the literature shows that contradictory results on the resistance of many Trichostrongyle larvae have been reported by many authors mainly because they failed to recognise the importance of humidity and temperature in the desiccation of larvae. The extremely feeble resistance of G.strigosum infective larvae to desiccation stands in sharp contrast to that of T. retortaeformis. On no occasion did the larvae of G. strigosum survive 48 hours desiccation in a humid atmosphere at any of the temperatures of this study. The seemingly small difference in the

water content of the faeces of rabbits and bovines makes a considerable difference in the development and survival of the free-living stages of G. strigosum. While development proceeds normally in bovine faeces exposed to a humid atmosphere, rabbit faeces exerts an unfavourable influence on it under the same conditions. It has been suggested by Hundt (1928) and confirmed by Wetzel and Enigk (1938) that the parasitic infestation of hares with this nematode, is a function of the rainfall curve. It would appear that the explanation for the striking absence of this parasite on the American continent and its maximum occurrence during the three humid months of Spring in Central Europe, may be found in the rainfall curves of these places.

The longevity of infective Trichostrongyle larvae in water over a period of 4 months suggests that stagnant pools found in pastures cannot be ruled out as a source of infection for rabbits and hares. The effect of simulating day and night temperatures in the laboratory experiments here reported, was an increase in the longevity of larvae stored in 5 mm of water over those stored at constant temperatures. The most favourable range of temperature for the development and longevity of T. retortaeformis larvae was 25°C - 30°C, and 15°C - 20°C for G. strigosum. A dilute acid medium was found to be more favourable than a neutral or an alkaline one for the longevity of infective Trichonstrongyle larvae. The two species considered in this study, migrate from faecal cultures, show no response to thermotropism and are non-skin penetrators.

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