

SOME STUDIES ON THE VIABILITY AND DEVELOPMENT OF THE OVA
OF ASCARIDIA LINEATA (SCHNEIDER)

by

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A. B., Austin College, 1928

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1931

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INTRODUCTION

Parasitic nematodes, though only a small part of the phylum Nemathelminthes in comparison with the large number of free-living species, are nevertheless of prime importance due to their pathogenicity and ubiquity.

The members of the class Vertebrata are hosts for the greater portion of these parasites which may be found in practically all of the organs of the body. The digestive,

circulatory, and respiratory systems, with their accessory ducts and glands, and the skeletal muscles, are the most commonly infested parts.

The frequency of their occurrence and the pathological conditions produced by these nematode parasites has resulted in their recognition as being of major importance in the field of medicine. Those in the alimentary tract of sheep, horses, hogs, cattle and chickens have long been an important problem to the stockman and poultryman. The economic loss sustained from these parasites has been a considerable inducement for the development of substantial and effective means of control. The development of adequate anthelmintics has furnished a fertile field of investigation, but other measures are essential for complete control of these parasites. Since the eggs play a very important role in the transmission of the parasite from one host to another, the study of the factors influencing their viability and development has been given much consideration by workers in this field.

Ackert (1923) found the large roundworm of poultry, Ascaridia lineata (Schneider), which is commonly parasitic in the intestines of chickens, to have an incidence of 51 per cent in chickens examined at Manhattan, Kansas. This high percentage of infestation shows the economic importance of this parasite. The losses among young chickens, that are

obviously due to parasitism with this nematode, have been stated by poultry specialists at this Station to be one of the most important that poultry raisers must face.

Ackert and Herrick (1928) and Graham, (1930), K.S.C. Master's Theses, have presented considerable evidence that young growing chickens develop a resistance to this parasite as they grow older. This age resistance develops gradually and is highly effective in keeping infestations to a very low level by the time the birds are twelve weeks old. Thus, it is obvious that this parasite does its main damage to young chickens and unless extreme care is taken during the brooding period they will become infested from egg-polluted soil in poultry yards or houses. Infestations in older birds, although harmless to them, will result in spreading these eggs. Unless means are taken to check it, this vicious cycle will be repeated over and over again under the present type of poultry sanitation.

The brooding of chickens on hail screen runs is recommended by the poultry specialists of this Station. In view of the fact that the age resistance develops to a point where it is highly effective by the time the chickens are twelve weeks old, this method will materially reduce the infestation among a flock. However, most poultry raisers find it difficult to keep chickens under these conditions for over eight weeks, and so it must be recognized as being

no more than a valuable aid in the reduction of Ascaridia infestations.

The experiments and observations reported in this thesis were made for the purpose of studying some of the factors that influence the viability and the development of the ova of Ascaridia lineata with the hope that the results obtained would have a bearing upon the control of the parasite. The principal problems undertaken involved (1) a study of the effect of climatic conditions upon the development and viability of the ova at Manhattan, Kansas, and (2) a study of the development and viability under controlled laboratory conditions.

HISTORICAL REVIEW

The ability of the ova of some of the Ascarids to remain viable for long periods of time has been observed by several investigators. Davaine (1863) kept the ova of Ascaris lumbricoides alive for five years. Morris (1911) found the embryos motile in A. lumbricoides ova that had been kept two years in 2 per cent formalin. Martin (1926) reported that eggs of Ascaris suum stored for four years in a moist condition at -5° to $+10^{\circ}$ C. developed to the infective stage when placed at an optimum temperature.

Various investigators have studied the effect of chemicals upon the viability of the ova of some of the

parasitic nematodes. Galli-Valerio (1914) succeeded in developing the ova of A. lumbricoides to the fully developed embryo stage in solutions of sulphuric, hydrochloric, nitric and acetic acids, and antiformin, 50 per cent or less in strength, and in saturated solutions of copper sulphate, iron sulphate and copper acetate. Wigdor (1918) placed embryonated ova of Toxascaris limbata in 10 per cent hydrochloric acid, 50 per cent oxalic acid, 20 per cent acetic acid, 50 per cent ammonium hydroxide, 1 per cent mercury dichloride and oil of turpentine and three days later found them still alive.

Kobayashi (1919) reported that *Ascaris* ova developed and lived in a 10 per cent formalin solution, and that ova in feces in a formalin solution were in a more favorable environment for development because the formalin stopped putrefaction and thus gave a more plentiful supply of oxygen. Ransom and Foster (1920) stated that a 10 per cent solution of potassium bichromate made a good medium for the development of *Ascaris* eggs. They also found that ova containing fully developed embryos were still active in ova kept five weeks in crude petroleum and petrolatum, and remained alive as long as five days in full strength antiformin even though all except the inner membrane of the shell was dissolved away. Vajda (1922) demonstrated that antiformin would dissolve the outer capsule but the inner

membrane remained intact until after the death of the embryo. Yoshida (1920) reported A. lumbricoides ova as able to live at least 40 days after the fecal mass containing the ova had been for at least four hours in one of the following: 0.5 per cent potassium permanganate, 1 per cent corrosive sublimate, 1.5 per cent nitric acid, 10 per cent hydrochloric acid, 7 per cent sulphuric acid, 7 per cent glacial acetic acid and 10 per cent formalin. Yoshida also quoted his student K. Hotta, as having found that these ova develop in 14 per cent hydrochloric acid, 9 per cent sulphuric acid, 8 per cent glacial acetic acid, 0.3 per cent phenol and 12 per cent formalin, but that ova failed to reach full development in slightly higher concentrations, although they lived for several days. Kobayashi (1921) reported that the ova of A. lumbricoides lived more than a month in urine and for several days in 1 to 4 per cent ammonia at low temperature; that they developed in a strong aqueous solution of sodium chloride, and that the ova of both A. lumbricoides and Trichocephalus trichiurus withstood putrefaction of the media. Cram (1924) found that A. lumbricoides ova would develop after having been immersed two hours in solutions of 5 per cent phenol and 3 per cent cresol, and that embryonated ova immersed in these solutions for two hours remained active. It has been clearly shown by the results of these investigators that

the lethal concentrations of these various chemicals are much higher for these parasitic nematode ova than for most living tissue; therefore, except under unusual circumstances, it would not be practical to use chemicals in the treatment of soil that had been polluted, and often it would not be feasible to treat fecal material.

The study of the viability of nematode ova under controlled laboratory conditions gave some presumptive evidence relative to their viability under natural field conditions. Ackert (1920) reported that ova of A. lineata endured 15 hours of freezing at temperatures between -11.6° and -8° C. Riley and James (1921) found that unsegmented ova of Heterakis papillosa would develop normally after having been frozen a month at about zero Fahrenheit. Cram (1924) found that developed embryos in A. lumbricoides ova were not killed by a ten-day exposure to a temperature of -6° to -17° F., but were killed by a twenty-day exposure at the same temperature. Unpublished investigations carried out by Hartman in 1923 and 1924 at this Station showed that unsegmented ova of A. lineata developed after exposure to a temperature of -12° to -14° F. for one and one-half hours, but embryonated ova were killed when exposed to the same temperature for the same length of time.

For the lethal high temperature, Ogata (1925) found that eggs of A. lumbricoides lost their power of development after exposure to a temperature of 122° F. for 45 minutes. Itagaki (1927) found that ova of A. lineata exposed to temperatures from 50° to 53° C. for five minutes were still viable, but at a temperature of 54° C. for five minutes, they were killed.

Desiccation is one of the most lethal factors. Hartman (ibid) found that only a few embryonated ova of A. lineata were alive after being exposed 105 hours in air with a relative humidity of 29 to 43 at temperatures of 57° to 80° F., but that all were killed at a relative humidity of 24 to 30 at the same temperatures. Owen (1929) found that one week of exposure of embryonated Toxocara canis ova in air which had a relative humidity of 31 was lethal to 75 per cent of the ova.

In the study of *Ascaris* eggs under natural field conditions, Baillet (1866) found that embryonated eggs of A. suum remained viable after 12 months exposure to the heat of summer and the cold in winter. Ross (1916) reported that eggs were still viable after having been exposed to the hot sun of India for six weeks. Ransom (1923) in a report of the Zoological Division, incorporated in the Report of the Chief of the Bureau of Animal Industry, stated that live embryos were found in *Ascarid* eggs buried

375 days at Chicago, Illinois. Hartman (ibid) found that undeveloped ova of A. lineata exposed to natural field conditions at Manhattan, Kansas, in the months of July and August, 1923, died within 28 days when in an unshaded location and as far as six inches under the surface of the soil, but when in a shaded location some lived at even one and two inch depths. Martin (1926) reported that eggs of A. suum were viable after exposure to winter weather in Nebraska. Brown (1927) found that 94 per cent of A. lumbricoides ova in feces on the surface of shaded humus soil in the region of Penonome, Republic of Panama, during July, August and September, remained viable 56 days, but on sandy soil in the sun all were dead within 21 days. Raffensperger (1927) found that eggs of A. suum placed on the surface of the soil at Chicago, Illinois, in September, 1923, and recovered 366 days later contained motile embryos. Brown (1928) observed that A. suum ova in sandy soil out of doors at Baltimore, Maryland, remained viable during the winter of 1926-27, but were killed during the warm weather of the spring months.

Caldwell and Caldwell (1928), in making observations on the development of the ova of the pig and human *Ascaris* under natural conditions in southern Alabama, found that these eggs in feces exposed to sunlight on the surface of the soil for 15 hours (the actual number of days exposed was three) where the temperature of the feces was 130° F.,

were all killed. Whereas, the eggs in feces exposed to the same conditions except in shade (temperature of feces not given), the death of the eggs was not apparent within ten days. Owen (1929) found that undeveloped ova of Toxocara canis planted in soil cultures near Minneapolis, Minnesota, on July 5, and exposed to full sunlight died of desiccation while still in the morula stage. Otto (1929) found that ova of the human Ascaris developed and remained alive during the summer of 1928 on the surface of sand, loam and clay soils in the shade in southern Virginia, but eggs on these soils in sunlight died rapidly.

The lethal effect of sunlight is doubtful other than indirectly; i.e., its effect on temperature and moisture. Ackert (1920) observed continuous development of A. lineata eggs exposed seven days to the sun in June and July at temperatures from 23° to 30° C. Schwartz (1922) carried out experiments in the Philippine islands and reported that embryonated eggs of Ascaris vitulorum in painted and unpainted vials exposed to the sunlight for one hour were killed. Manalang (1927) found that dried ascaris ova on a glass slide exposed to direct sunlight in Zamboanga, Philippine Islands, for one-half hour were not killed, but one and one-half hours exposure completely prevented larval development.

These results would lead one to believe that the high temperature produced by the sun, rather than any effect of the sun's rays, was the lethal factor.

MATERIALS AND METHODS

The eggs of the nematode, Ascaridia lineata (Schneider) were used in these experiments. They were obtained by cutting the anterior end from mature female worms and pressing the internal organs out into clean Petri dishes. The uteri were separated and the proximal portions examined for mature eggs, and if mature, were macerated in from one-half to one cc. of water in another Petri dish. All the eggs to be used at any one time, or part of an experiment, were collected in one Petri dish. This insured uniformity of all of the cultures. A portion of the original culture was always incubated as a control for development.

The egg shell of A. lineata is smooth and somewhat translucent. The various stages of segmentation, curved and coiled embryo (embryonated, motile embryo or infective stage), can easily be observed with the low power of the ordinary compound microscope.

In determining the viability of all stages of development below the coiled embryo, the ability to develop to the coiled embryo stage when placed in water at temperatures from 28° to 34° C. was considered as the criterion. The

motility of the coiled embryo stage was considered as sufficient evidence of the viability of those in that stage. In a few cases in which it was difficult to make accurate observations, the cultures were fed to small chickens, which were killed from six to eight days later, and the intestinal contents removed by the technic developed by Ackert and Nolf (1929) and examined with a binocular dissecting microscope for larvae.

In placing eggs in the soil (soil cultures) under natural conditions, various methods were tried in an effort to find one by which the eggs could be recovered in sufficient quantities and condition that accurate observations could be made of the stage of development and the degree of viability with the minimum of equipment, labor and time. The methods employed were (1) eggs were placed in the center of flower pots immediately above a very thin layer of kaolin at a depth of soil stated in experiments, (2) eggs were placed in three-eighths by three inch glass tubes above a thin layer of kaolin at soil depths stated in experiments and (3) the method used at the present time, in which about half a drop of water containing eggs from the main egg culture was placed on a microscope cover glass and the water allowed to evaporate. In drying, the eggs were cemented to the coverglass by their viscous coating. A little soil was

placed on the side containing the eggs and another cover glass added. In this condition, the eggs were placed in soil in flower pots.

In other experiments where the eggs were placed under controlled conditions, they were placed in hanging drop slides, imbedding dishes, and on ordinary microscope slides and allowed to dry in order to cement the eggs to the glass containers. The experiment, or culture, date, stage of development and whether dry or in water were written on the container.

In recovering the eggs from the soil when in glass tubes, the soil and eggs were removed down to the kaolin and placed in a Petri dish. If the eggs were to be examined for development, 30 per cent antiformin was added and allowed to remain about an hour in order to free the eggs from the soil particles. Then, the culture was examined with the low power of the compound microscope. If the viability of the eggs was to be determined, water and a few drops of 10 per cent formalin were added and the soil culture incubated at a temperature of from 28° to 34° C. from 10 to 14 days.

When the eggs were between cover glasses, the glasses containing the soil and eggs between them were placed in a Petri dish and treated with antiformin as previously

described. When accurate observations could not be made, the culture was washed with water, additional soil containing eggs was placed with it, and all fed to a chicken. The chicken was killed from six to eight days later and the intestinal contents examined for larvae.

EXPERIMENTAL RESULTS

Experiment I. Due to the common opinion of poultrymen in this locality, that eggs of A. lineata remain viable over winter months and afford a source of infection for young chickens during late spring and early summer, this experiment was undertaken to determine whether or not undeveloped A. lineata eggs placed near the surface of the soil under climatic conditions would remain viable during the winter months.

On December 15, 1929, undeveloped eggs from the uteri of A. lineata were placed in soil in a flower pot at a depth of one-fourth to one-half inch. Undeveloped ova in feces from chickens infested with A. lineata were also placed in soil in a flower pot at the same depth. The eggs were recovered 19 days later and were found to be dead. The temperature during the time the eggs were exposed ranged from 40° to 0° F.

Experiment Ia. In October, 1930, the decision was made to again expose A. lineata ova to climatic conditions

during the winter. Four separate plantings of undeveloped ova of A. lineata were made; viz., October 12 and 18, November 29 and January 11. In each case plantings were made at five separate depths: one-half, two, three, four and six inches. The temperature during this experiment ranged from 3° to 71° F. A sample was recovered from each culture of the plantings on January 26 and incubated. All of the cultures were viable except the culture at the half inch depth of October 12. On March 19, another sample from each culture was removed except the half-inch depth culture of October 12. This culture had been destroyed. All of the egg cultures removed were viable.

Experiment II. If eggs did not remain viable over winter months, it would be advantageous to know the approximate date that eggs dropped on the soil would remain viable until the warm days of spring would cause them to develop to the infective stage. Undeveloped eggs from uteri and in feces from infested chickens were placed in the soil as in Experiment I, on February 27, 1930. Eggs from the soil culture containing eggs from uteri were recovered 15 days later and found to be viable. The temperature, during this period, ranged from 43° to 12° F. After 22 days of exposure, eggs from both uteri and fecal cultures were recovered and found to be viable. The temperature during the period of exposure ranged from 54° to 12° F.

Experiment IIa. On February 23, 1931, undeveloped ova were placed in the soil at depths from one-eighth to three-eighths inch. Eggs were removed from the cultures on April 14 and found to be viable.

Experiment III. It seemed desirable to know something of the viability of eggs under natural field conditions during the spring months. Eggs from uteri were placed in soil in glass tubes at a depth of one-fourth to one-half inch on March 15, 22, and 30, and remained until May 30, 1930, after having been exposed 76, 69 and 61 days respectively. Motile embryos were found in all cultures.

Experiment IV. From these various experiments and the results obtained by other investigators studying the development and viability of parasitic nematode eggs, it seemed logical to conclude that the development and viability of these eggs under natural conditions during the warmer months of the year depended almost entirely upon the moisture content of the soil, the air, or both, as well as upon temperature. A study of the development and viability of the eggs under conditions favorable for development and which were very similar to those found around the watering pans in the average poultry yard, i.e., moist and shaded, was undertaken.

On May 3 and 24, June 9, July 9, and September 13, 1930, eggs were placed in the soil at depths from one-eighth to one-half inch. A piece of tar paper roofing was placed over

the cultures to keep out the sunlight. These cultures were moistened every two or three days. Eggs were removed about every two weeks and examined for development and viability. Eggs from the culture of May 3 required from three to four weeks to develop to the infective stage; those from the culture of May 24 required about three weeks; those of June 9 required from two to three weeks, and those of July 9 and September 13, two weeks. Eggs from the culture of May 3 recovered 77 days later were found to be viable; those of May 24 were viable after 56 days exposure, and those of June 9 and July 9 were found viable after 42 and 114 days exposure, respectively. The examinations for the viability of the cultures just recorded were the last examinations made of these various cultures. Eggs under these conditions might remain viable much longer.

Experiment V. The approximate date in the fall months when eggs dropped on the soil under favorable conditions will cease to develop on account of cold weather, is of importance to the poultryman in order that he may know when chickens can be placed on clean ground (unpolluted) and not be in danger of becoming infected by eggs from any parasitized chickens that may be in the group.

Eggs placed in the soil on October 3, 1930, and kept moist were in the 2-8-cell stage eight days later. When examined 22 days after being placed in the soil they were

still in the 2-8-cell stage. In Experiment IV, eggs placed in the soil under favorable conditions on September 13, reached the infective stage in two weeks.

Experiment VI. In addition to studying the viability of eggs in the soil, a very limited study was made on the viability of eggs in one of the poultry houses on the poultry farm of this Station during December, 1929, and January, 1930. The house was about 14 by 28 feet, with a partition dividing it into two sections. The floor was of concrete, and straw was placed on wire netting overhead about seven feet from the floor in order to make the house warmer. Large windows opening to the south were kept open except in real cold weather. Chickens were kept in the house during the experiments.

Undeveloped eggs were placed in dry Petri dishes in the corner by the dividing partition. A hygrothermograph was placed beside them to record the temperature and relative humidity. The temperature during the experiment, which ran for 60 days, ranged from 8° to 55° F. The average relative humidity was 60 to 70. Eggs exposed 30 days were viable, but those exposed 60 days were not.

Experiment VII. It seemed desirable to study the viability of A. lineata ova at temperatures sufficiently low to prevent development and yet not low enough to destroy life in the egg. Zavadovsky and Sidarov (1928) found that the

eggs of the human and the pig Ascarids would not develop below 7° to 8° C., and that eggs of the dog and horse Ascarids would not develop below 6° to 7° C. The cool room of the Dairy Department of this Station was found to be the most suitable place for this experiment. The temperature range was from 32° to 44° F. There was usually sufficient water in the room to insure an atmosphere of high relative humidity.

In this experiment, the results of which are given in Table I, the different letters represent separate egg cultures, i.e., egg cultures made at different times. The egg cultures of the various series were placed in hanging drop slides, imbedding dishes and on microscope slides. The culture, date and whether in water or dry were written on these containers.

The results show that the eggs of the series containing eggs in the undeveloped stage remained viable from 126 to 196 days. The cultures of series A and B that were examined after 149 and 196 days exposure, respectively, were the last cultures of those series; therefore, the eggs might have remained viable longer. At the time of this writing, observations were still being made on the cultures of series D and G.

Table I. Ascaridia lineata Ova at Temperatures of 32°- 44° F.

Series	Stage of Development	Days Exposed	Condition During Exposure	Results
A*	Undeveloped	149	In water	Viable
A*	Undeveloped	149	Dry	Viable
B*	Undeveloped	196	In water	Viable
B*	Undeveloped	196	Dry	Viable
C	Undeveloped	126	In water	Viable
C	Undeveloped	146	In water	Not viable
D	Undeveloped	191	In water	Viable
G	Undeveloped	157	In water	Viable

E	4-32 Cell	62	Dry	Not viable
E	4-32 Cell	62	In water	Not viable
G	4-32 Cell	68	In water	Viable (few)
G	4-32 Cell	29	In water	Not viable
G	4-32 Cell	29	Dry	Viable (few)
H	4-32 Cell	19	In water	Not viable
H	4-32 Cell	19	Dry	Not viable

D	Light end**	73	Dry	Not viable
E	Light end**	59	In water	Not viable
F	Light end**	36	In water	Viable (few)
F	Light end**	36	Dry	Not viable
G	Light end**	28	In water	Viable
G	Light end**	28	Dry	Viable (few)
J	Light end**	12	In water	Not viable
J	Light end**	12	Dry	Not viable
J	Light end**	12	In water	Viable
J	Light end**	12	In water	Viable
J	Light end**	18	In water	Viable (few)
J	Light end**	18	Dry	Viable (few)
J	Light end**	25	In water	Not viable
J	Light end**	25	Dry	Not viable
J	Light end**	48	In water	Not viable
J	Light end**	48	Dry	Not viable

D	Motile embryo	45	In water	Viable
D	Motile embryo	45	Dry	Viable
D	Motile embryo	65	In water	Viable
D	Motile embryo	65	In water	Viable
D	Motile embryo	101	Dry	Not viable
E	Motile embryo	42	In water	Viable
E	Motile embryo	42	Dry	Viable
J	Motile embryo	63	In water	Not viable
J	Motile embryo	63	Dry	Not viable
J	Motile embryo	87	In water	Viable (few)
J	Motile embryo	87	Dry	Viable (few)
J	Motile embryo	97	In water	Not viable
J	Motile embryo	97	Dry	Not viable
K	Motile embryo	35	In water	Not viable
K	Motile embryo	35	Dry	Not viable
M	Motile embryo	37	In water	Not viable
M	Motile embryo	37	Dry	Not viable

*Last culture of that series.

**Stage of development between late morula and curved embryo.

One culture of the series in the 4-32-cell stage of development remained viable 68 days, but the results of those in the other series indicate a much shorter period of viability.

In the series containing eggs in the light end stage of development, the longest period that any culture remained viable was 36 days, but the cultures of the other series indicate that the average number of days is less than that.

Eggs in the motile embryo stage of development remained viable from 42 to 87 days.

Experiment VIII. A limited study was made of the viability of eggs at temperatures at which the eggs would develop and at a high relative humidity. Undeveloped eggs on microscope slides were placed over two Petri dishes containing a little water and a few drops of formalin in a six liter glass specimen jar, and the jar cover sealed with vaseline. The temperature ranged from 22° to 23° C. Eggs removed from three to four weeks later had developed to the coiled embryo stage. Eggs removed 204 days after being placed in the jar contained motile embryos but they were very sluggish.

DISCUSSION OF DATA

The results of the study of the viability of A. lineata eggs in the soil from December 15, 1929, to January 3, 1930,

at a depth less than one-half inch indicates that eggs deposited on the soil will not remain viable over the winter months. Death was doubtless due to the intermittent cold weather, since the time of exposure and the condition of the soil would not permit the eggs to dry out sufficiently to be killed by desiccation.

The soil culture of October 12, 1930, at the one-half inch depth was not viable when recovered January 29, 1931, whereas, all cultures at other depths were viable. Also, cultures at the one-half inch depth of October 18, November 29 and January 11, as well as those at greater depths, were viable. The failure of the one-half inch culture of October 12 to remain viable might have been due to the soil at that depth and at that time, being sufficiently warm to allow development to begin, and as other experiments show, eggs in the partially developed stage are less resistant than undeveloped eggs.

The conflicting results as shown by the experiment during the winter of 1929-30 and that of 1930-31 may be accounted for by a study of the temperatures of the two winters. During the exposure of the eggs in December, 1929, the minimum temperatures were 6°, 1°, 1°, 2°, 0° and 3° F., December 18 to 23, respectively. Whereas, the minimum temperatures during the winter of 1930-31 were 11°, 13° and 3°F.

on December 30, January 13 and 14, respectively. Thus, the minimum temperature was lower and extended over a longer period during the exposure of the eggs in the winter of 1929-30 than in the winter of 1930-31.

The mean minimum temperature was also lower during the winter of 1929-30 than during that of 1930-31. Therefore, it appears that eggs on the surface of the soil during a real cold winter will be killed, whereas, eggs may remain viable during a mild winter such as that of 1930-31. But eggs three inches or more below the surface of the soil will remain viable during even the more severe winters.

If eggs will not remain viable during the more severe winters, the earliest date that eggs deposited on the soil in late winter months will remain viable depends upon the temperature. The results of Experiment II show that eggs deposited the latter part of February will remain viable. The date that eggs reached the infective stage in the spring months is given by Hartman (*ibid*) as the first week in May. This is about the time that the second brood of chicks is getting out on the ground, and it is the experience of poultrymen that these chicks suffer most from this parasite.

Just how long eggs under natural field conditions will remain viable during the spring and early summer months depends entirely upon the moisture content of the surrounding media. The results of Experiment III indicate that eggs

will remain viable 60 to 70 days. If there is very much rainfall or the humidity is high during these months, there will be a relatively high percentage of infestation of young chickens with A. lineata. The reports of the poultry specialists of this Station support this statement.

Danheim (1925) found that embryonated eggs of Ascaris equi dried from one to four days at about 30° C. would hatch out when remoistened. This process of hatching is believed by some to take place in nature and result in the destruction of many eggs of parasitic nematodes. In addition to the belief just stated, it appears that the stages of development between the undeveloped and the embryonated stages are the least resistant, as indicated by the results of Experiment VI, and in these stages many are destroyed by desiccation and the high and low temperatures to which they are subjected during the year.

The results of Experiment IV show that eggs under conditions similar to those found around the watering pans in the average poultry yard will remain viable and a source of infection from July to December. There are indications that eggs might remain viable even from May to December.

Chickens will not become infected by eggs from parasitized chickens during colder months of the year, i.e., eggs deposited during months in which the temperature is below that required for development. If poultry pens have been

allowed to remain idle during summer months in order to rid them of viable A. lineata eggs, it is of value to the poulman to know the approximate date that chickens placed in these pens will not be in danger of becoming infested from eggs from parasitized birds that may be present in the flock. The temperature of the weather being the main factor under consideration in this case, the date after which eggs will not develop to the infective stage will vary. But from the results in Experiment V, October 1 may be considered the approximate date after which eggs will not reach the infective stage.

Just how long A. lineata eggs will remain viable in a poultry house will depend on several factors, e.g., stage of development, temperature and humidity. The results of Experiment VI show that undeveloped eggs in a poultry house during December and January, were viable the first 30 days, but were not viable at the end of 60 days. The exact lethal factor or factors are not known in this case, but are believed to be the combination of all three mentioned above. The temperature at the beginning of the experiment was high enough for the eggs to start development. In these stages of development, indicated by Experiment VII to be the least resistant stage, the prolonged period of intermittent temperatures, sometimes below freezing, combined with the drying effect of the air resulted in the death of the eggs.

In studying the viability of eggs at temperatures at which development would not take place, i.e., 32° to 44° F., it was found that eggs in the undeveloped stage would remain viable longer than those in any other stage. Undeveloped eggs remained viable 196 days and might have remained viable longer. The longest period that eggs in stages of development between the undeveloped and embryonated stages was 68 days. This was about twice as long as results of other cultures indicate (the longest in other cultures was 36 days, and in this one only a few were viable). The fact that a few remained viable 68 days might be explained in that sometimes some eggs do not start developing until one or two days after being placed in the incubator. In this case they would be grouped as undeveloped eggs. The results indicate that eggs in stages of development between the undeveloped and embryonated remain viable from 30 to 40 days. Eggs in the embryonated stage remain viable 87 days.

The exact per cent of water required in the air in order that A. lineata ova will develop is not known. But eggs placed in air of relatively high humidity at temperature of 22° - 24° C. will develop to the infective stage and remain viable for at least 175 days.

SUMMARY

1. Undeveloped eggs of Ascaridia lineata (Schneider), exposed to climatic conditions from December 15, 1929, to January 4, 1930, in the soil at one-half inch or less in depth, were killed. But eggs at one-half inch, two, three, four and six inch depths from November 29, 1930, to March 19, 1931, were not killed.

2. Undeveloped eggs, placed under natural field conditions in the soil at depth of one-half inch or less as early as February 27, 1930, and February 23, 1931, remained viable until spring.

3. Undeveloped eggs, placed in the soil at depths of one-half inch or less on March 15, 22, and 30, contained motile embryos on May 30, 76, 69, and 61 days later, respectively.

4. Eggs, exposed to conditions similar to those around watering pans in the average poultry yard during the summer and fall months, remained viable as long as 114 days, and might have remained viable longer.

5. The temperature, as early as the first week in October, is not high enough for the development of the eggs to the infective stage.

6. Undeveloped eggs, placed on the floor of an unheated poultry house during December and January, remained viable 30 days, but were dead at 60 days.

7. Undeveloped eggs at a temperature of 32° to 44° F. remained viable longer than those in other stages, while embryonated eggs remain viable longer than those in stages of development between undeveloped and embryonated. Undeveloped eggs remained viable 196 days and might have remained viable longer. Embryonated eggs remained viable 87 days.

8. Eggs will develop in air of high relative humidity at temperature of 22° to 24° C., and remain viable for at least 175 days.

ACKNOWLEDGMENTS

The writer wishes to express his appreciation of the aid of Dr. James E. Ackert in the planning and consummation of these experiments, and to Mr. George L. Graham for assistance in the preparation of the manuscript.

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