FURTHER OBSERVATIONS ON THE LIFE HISTORY OF THE EYE WORM OF POULTRY

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Fielding (1926) has shown that the Surinam cockroach, Pycnocelus (Leucophaea) surinamensis L., is responsible for the transmission of the eye worm (Oxyspirura parvovum) of poultry in Queensland. He stated that the cockroach picked up the larvae, but on further experimental work he has been unable to provide a continuity of larval life under cultural conditions. He has shown that when the infected cockroach is eaten up by the fowl, it does not pass beyond the crop before the contained worms liberate themselves, and pass up through the oesophagus into the mouth, and thence through the infra-ocular sinus or naso-lachrymal duct into the eyes, reaching their destination in a few minutes.

Influence of heat in the liberation of larvae.

Owing to the fact that the larvae take such a short time to reach the eyes, it was thought that the influencing factor in their liberation could not be due to any digestion of the roach, as this was practically nil. It was considered that possibly temperature was the main factor in their release, by simply providing a stimulus. To test this a few experiments were conducted by placing intact cockroaches in water heated to about 35°C. to 37°C., and it was seen that almost immediately the larvae began to pass through the thinner portions of the body, i.e., around the limbs, etc.

Influence of Humidity on Egg Laying.

From a series of daily examinations of the faeces and the mucous of the mouth it was noted that although a few eggs could be found on most days, there was a definite increase in their numbers on the approach of or during rain.

Hatching of the Eggs under External Conditions.

A series of experiments was conducted with the object of noting whether the eggs would hatch and the larvae live under controlled conditions. The media TIME BEST

used were variable. It was found that in some cultures a fairly large percentage hatched, and that in some cases hatching was delayed up to a maximum time of 25 days from the start of the experiment. Soon after obtaining their freedom, however, the larvae became quiescent, and on keeping them under constant observation no movement was noted; it was consequently decided they were dead. This series was combined with observations on egg laying, longevity of the females, etc. The results are enumerated in Table 1.

TABLE 1.

s	eries. Cultural Solution.	Numb of adult female used.	t Longevity	Eggs passed on days.	Living larvae seen on days.	Remarks.
A	Water	. 14	Few hours			Adults burst. Dead larvae sees on 4th, 6th, 13th, and 17:1 day
В	o to the caronic	21	2-6 days	2nd-7th	5th-25th	In some experiments no larva- were seen
С	1.4% Sod. Chloride	4	3-8 days	2nd onward	8th-15th	In two experiments no larva-
D	0.9% Sod. Chloride + Eye Fluid	. 2	4 days	2nd-3rd	9th	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
E	0.9% Sod. Chloride + Eye Fluid I drop to each c.c.	11. · .	3-6 days	2nd onward	11th-17th	In two cultures the worms were teased. In three cul- tures no larvae were seen. In one culture no eggs were
F	1.0% Sod. Chloride 0.5% Sod. Phosphate 0.1% Sod. Dicarbonate in Aqua dist.	5	3-5 days	3rd onward	4th-11th	passed In two cultures no larvae were seen. In two cultures the worms were teased
G	1% Citrated fowl blood	3	3 days	2nd	$5 ext{th}$	In one culture no eggs were
H	Earth moistened with water	5	Few hours to 5 days	4th	3rd	In four cultures the adults burst, liberated eggs showed larvae on 3rd day. Other adult passed eggs on 4th day
Ι	Fowl faeces+Sand+Saline	12	Broken up	_	3rd-4th	but showed no development The cultures were made of egg
J	Sterile fowl faeces, Saline and Charcoal	10	Broken up		5th & 7th	free facces sterilized In three cultures no larve- were noted
K	Bread moistened with Sa- line	9	Broken up .	~_	3rd	Larvae in two experiments only, eggs seen in other
	and the second s					-

Free Larvae in the Oviduct of the Female.

The occurrence of larvae free in the oviduct was noted on a number of occasions while conducting the series tabulated below. It was interesting to note that although the females concerned were frequently laying eggs in the cultures, these larvae (in some cases) did not pass out, but remained alive in the oviduct for varying periods up to a maximum, in one female, of 5 days. In one case the larvae were alive on the death of the parent worm.

Experiments with Guinea-pigs.

In view of the finding that the development of larval nematodes on gaining access to an accidental host is generally arrested, and the third stage larvae remain in this stage, and proceed to re-encapsulate (Seurat), it was considered of interest to test this by introducing the eye worms to a guinea-pig, and at the same time to note whether they would find their way into the eye, and also, when deliberately introduced into the eye, whether development was arrested or not.

Experiment 1. A large number of eye worms (40 to 50) in the third stage were placed in the mouth of a guinea-pig, which was kept under observation for six weeks, but no evidence of worms in the eyes or re-encapsulation was found.

Experiment 2. Owing to the smallness of the eye cavity, it was decided to use only a limited number of the worms (about 12), which were placed in the left eye; thereafter the worms were extracted at varying intervals for the purpose of noting whether development kept pace with worms in their normal habitat. It was found that the parasites in this situation developed normally (so far as the experiment went), but apparently they could not find their way from one eye to the other, as none were seen except in the original locution. The measurements, which compared favourably with those from the chicks and ducklings, are given in Table 2.

TABLE 2.

Day.	No. of Worms.	Measurements in mm.	Remarks.
$6 \mathrm{th}$	3	6.55 , $6.65 \& 7.37 \times 0.19$ to 0.24	Worms moulted.
8th	2	$6.18 \& 6.37 \times 0.19$	
12th	3	5.28 , $6.8 \& 7.0 \times 0.18$ to 0.22	
16th	2	$9.24 \& 8.84 \times 0.24$	Apparently moulting.
26 th	1	8.22×0.24	Development advanced.

Preliminary to Experimental Infection of Cockroaches.

Since the majority of local poultry yards are infected with eye worms, and in consequence of a very large percentage of the cockroaches being infected, it became necessary to find an uninfected yard with plenty of cockroaches. This was difficult, since yards uninfected with eye worms were usually almost barren

of cockroaches. About 800 roaches were examined from different parts of the town, but batch after batch had to be discarded owing to infection. After some weeks of patient work examining the body cavity for the presence of capsules and larvae, and the alimentary tract microscopically for the presence of eggs or first stage larvae, a batch was obtained which showed no infection in about 100 cockroaches examined, and it was considered that this lot could be used for the purpose of experimental infection.

Experimental Observations on Infection.

Experiment 3 was carried out with the object of ascertaining the time taken from the ingestion of the eggs of the worm to the time the larvae are infective. For this purpose a large batch of cockroaches were starved for three days. They were segregated in a large museum jar containing sterilized earth and a few dried leaves for hiding-places; bread soaked with physiological salt solution containing a large number of eggs, which had been taken from teased female worms, was then introduced into the jar. The bread was noted on the third day to contain a few living larvae, and was all eaten up by the fourth day. The results of examinations are tabulated below.

Experiment	Started.
D	

10th.

13th.

Day.		
1st.	Nymph and female examined.	Fully and under-developed eggs seen in
		gut.
	Bread examined.	Only eggs observed.
2nd.	Bread examined.	Only eggs observed.
3rd.	Nymphs and males examined.	Eggs and larvae measuring 235-260μ in
		length and $12-13\mu$ in diameter seen in gut.
	Bread examined.	Eggs and larvae similar to above.
4th.	Bread consumed.	
6th.	Nymphs examined.	Eggs, uncapped eggs, and larvae seen in gut, one larva just emerging from egg

shell, which took 3 hours to get clear; this larvae measured 250 x 13μ . No larvae seen in body cavity. Nymph and female examined. The nymph showed larvae in gut measuring $235-250\mu$ by $12-14\mu$. The female showed larvae free in body cavity.

Experiment Started.

Day.			•
17th.	Male, female, and examined.	nymphs	Capsules measuring 273 by 364μ in diameter; the contained larvae, measuring from 485 to 600μ by 26 to 28μ , were quiescent.
18th.	Nymphs examined.		Capsules and worms similar to above. Larvae quiescent.
25th.	Nymphs examined.	· -	Capsules found measuring 295 to 309μ by 360 to 436μ ; the contained worms measured 0.63 mm. to 1.01 mm. long by 0.028 mm. to 0.035 mm. in diameter.
32nd.	Nymphs examined.		Capsules found containing larvae measuring 1.9 mm. to 2 mm. long by 0.07 mm. to 0.08 mm. in diameter.
39th.	Nymphs examined.		Capsules found containing worms measuring 3·4 mm. to 3·8 mm. long by 0·118 mm. to 0·127 mm. in diameter. (See Experiment 4.)
46th.	Nymphs examined.		Capsules found containing worms measuring 4.6 mm. to 4.9 mm. long by 0.127 mm. to 0.145 mm. in diameter. (See Experiment 5.)
52nd.	Nymphs examined.		Capsules found, worms measured 4 mm. to 5·3 mm. long by 0·127 mm. to 0·145 mm. in diameter. (See Experiments 6, 7, and 8.)

Attention is here drawn to the occurrence of two frequently, and sometimes three, larvae in the one capsule.

Experiment 4. Chick one week old given a large number of capsules from roaches which had been fed with bread containing eggs 39 days previously. No eye worms were discovered in the eyes of the chick. Result negative.

Experiment 5. Broken roaches (infected 46 days previously), containing large number of capsules, given to a two weeks' old chick. Result negative.

Experiment 6. Chick three weeks old given 2 nymphs (infected 52 days previously), containing a large number of capsules. Worms observed on the tongue, roof and floor of mouth 4 minutes afterwards, but were not seen in the eyes until 45 minutes had elapsed. Result positive.

Experiment 7. Similar to above, with 3 nymphs. Worms in the mouth 4 minutes after introduction and inside of the eyes after 6 minutes. Result positive.

Experiment 8. Chick three weeks old given four unbroken roaches after anaesthetizing (infected 52 days previously). Worms were not observed in the mouth, but were seen passing through the naso-lachrymal duct into the eyes 20 minutes after introduction. Result positive.

Control chick to above experiments remained negative throughout.

Observations on Infection under Natural Conditions.

A young infected rooster which had been used for a previous experiment was introduced into a pen in which no infected fowls had been kept and no infected cockroaches had been found after a long series of prior examinations. Roaches were then examined from this pen at frequent intervals, and after 61 days capsules and worms from these roaches were given to a chick (Experiment 9). Worms were found in the eyes 15 minutes afterwards. Result positive after 61 days.

Experiment 10 to note whether the Worms are Infective to Pigeons.

A full-grown pigeon was given two cockroaches, one of which was dead, but contained a large number of capsules and free worms; the other roach was killed and opened to ascertain the presence of capsules. Eight minutes after introduction the worms were in the eyes. Result positive.

Table 3.

Observations on the Worms in the Cockroaches.

•		o our backets.					
Days after Feeding. 3rd-10th	No. of Worms. Many	Location. Free in gut	$^{ m Measur}$ Leugth. 245–260 μ	ements. Diameter. 12–14µ	Remarks. 1st stage		
10th-14th	Many	Free in body	320360μ	18 - 20μ	larvae. Ist stage		
17th-18th	Few	In capsules	$485-600\mu$	$26-28\mu$	Jarvae. Ist stage		
25th 32nd	Few 2	In capsules In capsules	0.63-1.01 mm. $1.91-2.09$ mm.	0·028-0·035mm, 0·073-0·082mm,	larvae. Moulting 2nd stage larvae.		
39th	4	In capsules	(1) 3·5mm.	$0.127\mathrm{mm}$.	2nd stage		
46th	1	In capsules	(2) 3·8mm. (3) 3·7mm. (4) 3·4mm. (1) 4·9mm. (2) 4·9mm. (3) 4·8mm. (4) 4·6mm.	0·118mm. 0·118mm. 0·118mm. 0·145mm. 0·145mm. 0·136mm. 0·127mm.	larvae.		
			(1) 1 0mm.	O.144HIM.			

Days after	No. of		Measurem	ents.	
Feeding.	Worms.	Location.	Length.	Diameter.	Remarks.
52nd	4	In capsules	$(1) \ 5 \cdot 3 \text{mm}.$	0.09mm.	3rd stage
e e e €.			$(2) \ 4 \ 0 \text{mm}.$	0.1mm.	larvae.
			(3) $5 \cdot 1 \text{mm}$.	0.09mm.	
			(4) 5.09 mm.	$0.1 \mathrm{mm}$.	

Observations on the Worms from Introduction to Fowl to Egg Laying of Female.

This series was intended to supply information regarding the measurements at the different stages in continuation of the previous series; time taken from introduction of larvae to definitive host, to maturity, and egg-laying of females. For this purpose the experimental chicks and ducklings were used as reservoirs, from which the worms were withdrawn at varying intervals. The results are tabulated in Table 4.

TABLE 4.

Days					
after Number Intro- of			Measur	ements. Diameter	
duction.	Worms.	Source.	Length in mm.	in m.m.	Remarks.
1	1	Duckling	$5 \cdot 3$	0.182	Moulting.
$1\frac{1}{2}$	1	Duckling	$5 \cdot 1$	0.16	Moulting.
$1\frac{1}{2}$	1	Duckling	$5 \cdot 5$	0.136	Moulting.
3	1	Duckling	$5 \cdot 64$	0.109	Moulted = 4th stage larva.
$4\frac{1}{2}$	1	Duckling	$5 \cdot 9$	0.14	Buccal cavity increasing in
. 6	3	Duckling	$5 \cdot 22$	0.14	size. Muscular oesophagus dis-
. 0	D	rydekning		0.14	
			4.55	0.127	tinct.
			$5 \cdot 1$	0.11	
7	1	$\mathbf{Duckling}$	$5 \cdot 46$	0.145	
. 8	3 -	Duckling	$5 \cdot 27$	0.127 -	•
			5.49	0.145	
			$5 \cdot 46$	0.127	
9	. 2	Duckling	$5 \cdot 5$	$0 \cdot 127$	
			5.3	0.118	
12	5	Ducklings (2)	5.5	0.145	Length of oesophagus,
		Chicks (3)	$6 \cdot 18$	0.182	0.819 mm.
			7.09	0.182	Distance to nerve ring,
			$8 \cdot 3$	0.23	0·182 mm.
			$8 \cdot 46$	0.218	

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Days after	Number		Measu	rements.		
Intro- duction.	of Worms.	Source.	Length in mm.	Diameter in mm.		Remarks.
. 1 5	4	Duckling (1)	8.5	0.25	•	
		Chieks (3)	$9 \cdot 0$	0.25		
			9.19	0.24	Moulting.	
in a second			$7 \cdot 18$	$0 \cdot 24$		
19	1	Chiek	7.8	0.27		
26	2	Chick	5.8	$0 \cdot 1$		
e e e e e e e e e e e e e e e e e e e			$9 \cdot 0$	0.24		
28	1	Chick	$8 \cdot 2$	0.218		
38 -	2	Chick	$4 \cdot 9$	0.127		
			7.6	0.25	Egg layin	g noted.

Attention is directed to the size of No. 1 worm in each case on the 26th and 38th days. These worms were obviously under-developed.

Distribution of Oxyspirura parvovum, O. mansoni, and Pycnocelus (Leucophaea) surinamensis.

That there is an intimate association between the known distribution of the eye worm, Oxyspirura parvovum, and the cockroach, Pycnocelus (Leucophaea) surinamensis in Australia has recently been pointed out by Fielding. Both vector and parasite are known to occur at Townsville, Cairns, Ayr, and Hughenden. Messrs. Conigrave and Pugh informed the writer that the worm occurs at Darwin, the cockroach having been recorded previously from that locality.

It would appear also that the association between *O. mansoni* and the Surinam cockroach holds good, since both occur in China, Java, Brazil, and Honolulu. In Rabaul the eye worm occurs; and in New Ireland, which is closely adjacent, the cockroach has been recorded. This association is further accentuated by the fact that workers in Florida have recently found a cockroach implicated in the transmission of *O. mansoni*, which is regarded as the same species as the Australian vector.

SUMMARY OF CONCLUSIONS.

Although living larvae have been detected in the oviduct of the female worms (in which position they can live for some days), and notwithstanding the fact that living larvae have been noted up to a maximum of 25 days in culture, it would appear that there is no continuity of larval life under experimental conditions. It is now considered that normally the eggs hatch in the gut of the cockroaches. One larva was observed emerging from the egg two days after

the last possible ingestion of eggs by the insect; larvae were noted in the gut six days after the last possible ingestion of eggs, and were found in the body cavity ten to thirteen days after the initial feed, or six to nine days after the last ingestion of eggs. Seventeen days after the initial feed, encapsulated larvae were found, which were approximately twice the size of newly-hatched larvae. It was found that the larvae were not infective 46 days after experimental feeding of the insect, but are so 52 days after, and that the female begins laying eggs 38 days after entering the definitive host, thus making the cycle of the parasite complete in approximately 13 weeks, though under more favourable conditions this time may probably be somewhat reduced. It was found also that on introducing infected cockroaches to pigeons the latter became infected, and that worms introduced into the eye of a guinea-pig were capable of continuing their development, but when introduced into the mouth of this animal they were incapable of reaching the eye.

Lastly, from the known distribution of the eye worms, O. parvovum, O. mansoni, and the cockroach Pycnocelus (Leucophaea) surinamensis, it would appear that there is a direct association between them.

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REFERENCE.

 Fielding, J. W.: Aust. Journ. Exp. Biol. and Med. Science, 3 (1926), pp. 225-32.