THE LIFE HISTORY OF *PROSTHOGONIMUS OVATUS*

R. BODDEKE

THE LIFE HISTORY OF *PROSTHOGONIMUS OVATUS* RUDOLPHI, 1803

ACADEMISCH PROEFSCHRIFT

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I. EXPERIMENTS IN

INTRODUCTION

Flukes are non-segmented, flat worms, usually leaf-like in shape; their characteristic is the presence of suckers with which they attach themselves to the skin, mucosa, or other tissues of the host. Flukes of medical or veterinary importance have two suckers, an oral one around the mouth at the anterior end and a ventral sucker or acetabulum. The flukes in question are hermaphroditic; the reproductive organs are extremely complicated.

Flukes may be divided into two sub-classes:

1. *Monogenea*, in which the ovum produces a larva that matures without an intermediate host.
2. *Digenea*, in which the larva first becomes a parasite in a molluscan intermediate host and subsequently, after generations of larvae, reaches maturity in a vertebrate host either directly or after further evolution in a second intermediate host. The second host may be an arthropode, fish or plant. Within or outside this host the larva or cercaria develops into a metacercaria that usually encysts itself.

Apart from "blood flukes" (schistosomes), the trematodes may cause hepatic (*Clonorchis*), intestinal (*Fasciolopsis*) and pulmonary (*Paragonimus*) infections in man and domestic animals. Birds often harbour them in the female reproductive system. There still is considerable difference of opinion and confusion as regards the nomenclature and life cycle of some of these flukes.

Prosthogonimus ovatus Rud.*, the subject of this paper, belongs to the genus *Prosthogonimus* Lühe 1899, family of *Prosthogonimidae* Lahille 1922, and the subclass *Prosostomata* T. Odhner 1905[67], a sub-division of the class *Digenea* Van Beneden[4] 1858[4]. The mature worms are parasitic in hens and ducks; hence their economic importance. The genus became notorious because it gives rise to an infection in the oviduct of domestic fowl which may result in the presence of worms in the eggs of the hen and the duck. Ever since the 18th century the infestation of eggs by trematodes has caused great concern; it has been the source of a large number of publications[9, 21, 31, 32, 36, 37, 84, 89, 90, 116].

P. has been observed in many wild birds, mainly in the fabrician sac. Its life cycle has not yet been studied in sufficient detail. In all, 28 species have been described in Europe and Asia; this is a large number and the taxonomy of *Prosthogonimus* sp. is chaotic.

The purpose of the present study was threefold:

1. To present a complete description of the life cycle with as many ecological data as possible.

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* Indicated in this paper by P.

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2. To put order into the taxonomy by ascertaining through experiments in how far the difference between, and the characteristics of, the various species are real.
3. To study the economic aspects of *Prosthogonimus*.

DISTRIBUTION

Distribution of the genus Prosthogonimus

Africa: British East Africa[96], Egypt[54,108], the Transvaal[70]. *Australia:* North Queensland [65,66], Melbourne and Ballarat[41]. *Asia:* Burma[16]. China: Chekiang[135], Canton[135], Peking[134], Yang Chow[39], Nanking[44], Cheng Yang[68], Formosa[62]. India: Dharwar[26], Hyderabad[40], North India[27]. Indochina: Annam[79], Hanoi[43]. Japan[10,136]. Palestine[131]. Philippines[127]. Siberia: Lake Baikal[104], Transbaikal[105], Kasakstan[74], Kirghisia[115], Omsk[28], Turkestan[52,93], Ural[94]. *Europe:* Denmark[64], England[3], France[20,124], Bavaria[33], North-east Germany[81,87], Italy[75,107,113,114], the Netherlands[8], Poland[61,85,91], Sweden[78], Switzerland[25,38], East Prussia[34,35], Yugoslavia[22]. Russia: Galicia[47,48], Ukraine[60], Asoff-Don[104], Armenia[104], Moscow[102], Leningrad[14], Saratov[51]. *North America: U.S.A.:* Michigan[57], Minnesota[57], Mississippi[112], New Hampshire[63]. Canada: Manitoba[86], Ontario[58]. *South America:* Brazil: Mato Grosso[125,126,129], Minas Gerais[23]. The Argentine[132,133], Mexico[50], Puerto Rico[128], Venezuela[55].

Mature specimens of the P. family are found in all parts of the world; it is quite common in regions with moderate rainfall (see map). Its distribution is determined by the presence of first and second intermediate hosts, *i.e.*, snails and dragon-fly larvae; in areas where the intermediate hosts are lacking, the parasite is found only in birds of passage that were infected elsewhere. The extensive inventory compiled by SKRJABIN and his pupils[92–105,28] and by other investigators[1,5,6,14,24,42,80,110,111,117,118] proved that members of the P. family may occur in nearly every kind of bird; the family is extremely polyxene.

The ova of P. are voided with the faeces; this was first established by DE BLIECK and VAN HEELSBERGEN[8] in oviduct-infected birds and by ONO[68] in fabrician-sac infected birds.

SZIDAT[119], in Europe, and KOTLAN and CHANDLER[15,45], in North America, started the study of the life cycle of P. by the discovery of encysted metacercariae in the larvae and imagines of dragon-flies that were found to serve as second intermediate hosts. In Europe, the dragonflies were *Libellula quadrimaculata, Gom-*

phus sp.[119], *Cordulia aenea*[121], *Libellula brunnea*, and *Platycnemis pennipes*[33]; in North America they were *Tetragoneuria* sp., *Perithemis domitia*, *Pachydiplax longipennis*[112], *Leucorrhinia intacta*, *Tetrageneuria cynosura*, *T. spinigera*, *Epicordulia princeps*, *Mesothemis simplicollis*[57], *Ischnura verticalis*, *Anax junius*, *Sympetrum vicinum*, *Libellula pulchella*, *Plathemis lydia*, and *Gomphus spicatus*[30]. In Asia (Manchuria) the host was *Anax parthenope*[68-69].

MACY[57] described the first intermediate host of an American species, *Prosthogonimus macrorchis*. It is a small, prosobranchiate snail, *Amnicola limosa porata*, which harbours the sporocysts in the midgut; the cercariae develop inside the sporocysts. He succeeded in infecting dragon-fly larvae with the cercariae, although the cysts formed in the larvae were found to be non-infectious when fed to a bird. However, several attempts to infect snails by feeding them P. ova, as well as attempts to hatch the ova, were unsuccessful.

KRASNOLOBOVA[49] described miracidia of *Prosthogonimus cuneatus* but this report should be regarded as a doubtful testimony as almost certainly small infusoria were mistaken for miracidia.

BAUDET[2] succeeded in infecting ducks with encysted P. metacercariae—although the latter were unknown to him—by giving the birds access to a pond near which soft-shell disease was prevalent. SZIDAT[119], KOTLAN and CHANDLER[45,46], ONO[68], and later investigators who were acquainted with the metacercariae, infected birds by feeding them encysted metacercariae.

THE DISEASE IN BIRDS

In 1921 a situation of economic importance developed in the Dutch poultry industry, when Mr. Westerink, Poultry Consultant, of Willemsoord, Overijsel, asked the Institute for Parasitic and Infectious Diseases of the Veterinary Faculty, Utrecht University, to help him in fighting an outbreak of the disease known as *"laying soft-shelled eggs"*; no eggs had reached the egg market from the Willemsoord district since the middle of May, 1921.

The symptoms were as follows: The hens which ran free in the farmyards started laying eggs with a thin shell; later on the shell was lacking completely. At a further stage the membrane became thinner and finally only the white and the yolk were excreted. Eventually egg-laying stopped altogether although the hens continued to visit the nests regularly. The combs turned blue and some hens died. On the whole, the birds did not give the impression of being ill, but their movements were rather slow and sluggish. The abdomen was slightly swollen and tilted backwards so that the affected bird walked with the legs rather wide apart. The hens showed a strong urge to lay, but no eggs could be palpated; the anal opening of some birds revealed white encrustations.

Three hens were inspected; two looked healthy, one had a blueish comb. Trematodes found in the oviducts were identified by Professor IHLE as *Prosthogonimus pellucidus*. One of the two normal looking hens had a normal oviduct, in the other the mucous membrane was red and swollen in places and marked by haemorrhages. The hen with the blue comb suffered from an advanced stage of peritonitis.

This report on the dragon-fly disease[8] gave the complete story. Subsequent publications on the subject have added little information.

HIERONYMI and SZIDAT[35], reported on inflammation of the oviduct in a hen, the cause being a trematode, *Prosthogonimus intercalandus* n. sp.

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The first publications on the dragon-fly disease were followed by a flood of reports from different parts of the globe. SZIDAT[120] proved that even a few worms might cause grave disturbances and that 40 to 50 were fatal. He stated that the hens catch newly-hatched dragon-flies in the dawn when, still wet with the morning air, they cling to the shrubs.

Less attention was paid to the occurrence of P. in other domestic fowl, such as ducks, geese and turkeys. BAUDET[2] investigated the laying of soft-shelled eggs in ducks in a pool near the village of Olst (Netherlands). Seven ducks were allowed to enter the pool; three others were kept near the pond but were prevented from reaching the water. Prior to the experiment the faeces had been examined for P. ova. The result was that the seven ducks in the pool started laying soft-shelled eggs; they harboured large numbers of parasites in the oviduct. The control group continued to lay normal eggs and were free from parasites. In ducks, the symptoms of dragon-fly disease are restricted to the laying of soft-shelled eggs.

Another pathological, as well as economic, aspect is the presence of trematodes within the egg. MACY[58] recorded the case of a poultry-farmer in Ontario who was boycotted because the eggs he sold contained large numbers of worms.

As yet, no drugs are available to treat birds infected with P. Poultry farmers in areas where the parasite is endemic usually resort to the traditional preventive measure of keeping the hens cooped up as long as dragon-flies are on the wing.

MACY[58], who succeeded in infecting sparrows and crows with *P. macrorchis*, assumed that these birds are a "natural reservoir" of the parasite; he suggests that these species of birds be eradicated in regions where P. is endemic.

Distribution in the Netherlands

Steenwijk-Willemsoord, discovered by DE BLIECK and VAN HEELSBERGEN[8]. It seems that dragon-fly disease no longer occurs in this area; this was confirmed by inquiry among the veterinarians of the district.

Olst. BAUDET reported on a pool that was regularly infected with P. When re-visited in 1958 it was found that the disease had not occurred in this place for years; dragon-fly nymphs were not infected.

Island of Terschelling. Prior to 1940, the disease occurred regularly in the hens of poultry farms situated near the marshes "Dodemanskisten". Favourable winds carried huge swarms of dragon-flies from the Frisian mainland to the island. In 1958, the disease no longer occurred on the island.

North-east Polder. Several cases of Prosthogonimiasis were reported in 1946–1947, but no cases have been observed in the last decade.

Sloten (North Holland). According to VAN DER KROON, cases were regularly observed in and near this village in about 1935.

Staphorst. WILDERS, the local veterianarian, observed a few cases, but the date is unknown.

Hengelo (Overijssel). A small lake, Lonnekermeer, swarms with odonate larvae seriously infected with P.; dragon-fly disease is still endemic in the vicinity. All the experiments recorded in the present paper were carried out with metacercariae and sporocysts collected in this lake, which at present is the only habitat of P. in the Netherlands.

Lonnekermeer consists of two, separate, small lakes; both were dug in 1902 during the construction of Hengelo railway station. The larger lake covers nine hectares, the smaller seven; they are fed by well and rain water (Fig. 1 and 2). Owing to the isolated location the water is not polluted. Both lakes abound with fish; *Leuciscus rutilis* and *Esox lucius* are plentiful, *Perca fluviatilis*, *Tinca tinca*, *Cyprinus carpio*, and *Anguilla anguilla* also being present.

Notable aquatic plants are *Phragmites communis*, *Nymphaea alba*, *Utricularia minor*, *Fontinalis* sp., *Limnanthemum nymphaeoides*, *Iris pseudacorus*, and *Hottonia palustris*.

The population of insect larvae is enormous; apart from *Anisoptera* larvae—discussed extensively in this paper—there are numerous May-fly larvae, *Agrionides* larvae, and—in places with black mud—*Sialis* larvae.

Fig. 1. Lake "Lonnekermeer" (Overijssel), at present the only known habitat of *Prosthogonimus ovatus* in the Netherlands.

Fig. 2. Vegetation of Lonnekermeer with imago and hatching specimen of dragonfly *Cordulia aenea*.

The only snail present in appreciable numbers is *Bithynia tentaculata*. Some specimens of *Lymnaea stagnalis*, *Planorbis corneus*, and *Myxas glutinosa* were caught regularly.

There are many birds that breed in the lakes: wild ducks, coots, water hens, kingfishers and (tame) swans. The surrounding woods and reed fringes shelter a varied population of song-birds. Buzzards and hobbies represent the birds of prey.

EXPERIMENTAL TECHNIQUE

Methods and instruments. Large quantities of aquatic plants were dragged to the shore with a strong landing-net and a search was made for dragon-fly nymphs. Snails and nymphs were transported in plastic bags containing wet aquatic plants; by this means the animals were kept alive for several days.

The snails were examined microscopically for sporocysts by flatting the midgut between glass slides. Cercariae were obtained by keeping a large number of snails in a few litres of water for several days after which the water was centrifuged, the sediment being drawn up in a pipette. The results of five times centrifuging were examined under a binocular microscope; the selected cercariae were caught with a pipette and placed in a separate dish.

Cysts were collected from nymphs by decapitating the insects and severing them laterally, after which the cysts could easily be removed from the abdomen with handling needles. They were examined under a phase-contrast microscope, with polarized light, and under an ordinary microscope.

The cysts, together with tissue of the dragon-fly nymphs, were fed to the birds on a piece of bread or in other food.

The extraction of metacercariae from the cysts was carried out in 0.5 per cent trypsine solution, the correct temperature being kept up by a Reichert "Biotherm".

Bird faeces were examined for P. ova by carefully grinding small pieces of droppings with one part ether and one part half-diluted HCl. Centrifugation separated the faeces into two portions, the worm ova being found in the lower portion.

An Uhtermöhl microscope—a reversed microscope designed for the examination of plankton—was used to study the development of P. ova.

The birds were kept in a house with walls of transparent plastic; only the doors were covered with screening gauze; this construction had the great advantage that shy birds such as ducks and starlings could not harm themselves.

A polyethylene catheter with a 2 mm lumen, mark Neoplex, was used for the transplantation tests.

Fixation and staining. The mature live worms and the metacercariae obtained from cysts were flattened between two object glasses in 10 per cent aethanol. After about one hour, a 40 per cent formalin solution was sucked through and a few hours afterwards the worms were permanently preserved in 10 per cent formalin. The cysts were placed immediately in a 10 per cent formalin solution.

For permanent preparations, the worms were washed and stained in lactic acid carmine or alizarin cyanine; the latter generally gave the best results. After this we used the usual sequence of aethanol 70 per cent to xylol, and preservation in Canada balsam.

EXPERIMENTAL INFECTION OF BIRDS WITH *PROSTHOGONIMUS* CYSTS

General remarks

Our aim was to investigate whether the many species of *Prosthogonimus* described in Europe were true species or host modifications from one and the same species. In

the experiments we used only identical cysts from nymphs of one species of dragon fly, *Cordulia aenea*, caught in the Lonnekermeer lakes. The different birds are indicated in code; the code is explained below the *Table* that gives a survey of the experiments.

EXPERIMENTS IN BIRDS; REVIEW

	Code Number	*Date*	*Number cysts*	*Duration days*	*Number of worms*	*Notes*
Experiments with chickens						
(Rhode Island Red)	RO 11	27/8	?	14	64	Large worms; laying stopped 2nd day.
	RO 12	27/8	?	21	55	Large worms; laying stopped 2nd day.
	RO 21	26/1	25	18	7	
	RM 11	7/5	20	12	19	
	RM 12	7/5	20	34	12	
	RM 13	7/5	31	49	1	Size of worms increased with age.
	RM 14	7/5	9	5	2	
	RM 15	7/5	9	23	1	
	RM 16	7/5	9	6	8	
	RO 31	7/5	9	4	7	
	RO 32	7/5	40	22	33	Very large worms.
	RO 41	29/6	kath.	14	0	
	RO 42	29/6	kath.	14	1	Characteristics intermediate.
(White Leghorn)	WF 11	18/9	27	33	0	Small fabrician sac.
	WF 12	18/9	29	33	7	3 big and 4 small specimens.
	WM 11	22/6	22	5	0	Large fabrician sac.
	WM 12	23/6	19 + 20	6	0	Id.
	WM 13	23/6	22 + 20	6	6	Partly small, partly large specimens.
	WO 11	23/6	62	24	35	Very large worms; 5 soft shell eggs in peritoneal cavity.
	WO 12	23/6	77 + 18	24	3	Oviduct is reducing.
Experiments with ducks						
(Moschus duck)	BB 11	10/11	?	75	—	No eggs in droppings.
(Khaki Campbell)	KO 11	15/9	120			KO 11–13 stopped laying after one week.
	KO 12	15/9	56	35	0	
	KO 13	16/9	33			
	KO 21	6/5	45	25	0	Reducing oviduct.
	KO 22	6/5	51	27	22	Very large worms.
(Peking ducks)	PB 11	22/12	20	35	9	Very small worms.
	PB 12	22/12	40	53	5	Small worms.
	PB 13	22/12	10	67	3	Adult worms.
	PB 14	22/12	30	87	2	Smaller than in PB 13.
	PB 15	22/12	50	94	0	
Experiments with starlings	SB 11	15/5	5	5	0	
	SB 12	15/5	5	4	0	
	SB 13	15/5	7	4	0	
	SB 14	15/5	4	4	1	
Experiments with crows						
(Jackdaw)	MB 11	16/6	14		16	three infections.
	MB 12	16/6	27		3	
(Crow)	CB 11	16/6	27		7	

Second letter of code indicates whether the bird is: of unknown sex but possessing a fabrician sac (B); the bird is a young female with fabrician sac (F); a young male, with fabrician sac (M); or an adult female with an oviduct (O).

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According to BRAUN[11], the main characteristics of *P. ovatus* Rudolphi, 1803 and *P. cuneatus* Rudolphi, 1809 are as follows:

P. ovatus	*P. cuneatus*
Ovary dorsal, at the level of the ventral sucker	Ovary distal of the ventral sucker
Uterus convolutions oral of the ventral sucker	Uterus convolutions distal of the ventral sucker only
Rozette-shaped uterus at the distal end of the body.	Convoluted uterus at the distal end of the body

Other authors usually refer to BRAUN's description; they mention the hosts in which the species were found, without describing the specimen obtained. SKRJABIN, however, recorded the dimensions of the suckers of a specimen of *P. cuneatus* found in *Ardea cinerea* as they differed from those in typical specimens.

MACY[58] and DOLLFUS[20] listed a large number of hosts for both species; in some cases an author mentioned that two species inhabited the same host, sometimes even the same specimen. The latter was recorded in the case of *Corvus frugilegus*, *Gallus domesticus*, and *Corvus cornix*. At first glance this might seem to indicate that two different species were involved.

However: (1) the size and habitus of the type specimen of *P. ovatus* strongly suggested that we were dealing with a very young form. The relatively large testis in Prosthogonimidae, with pronounced protandry, is a significant criterion; so is the mediocre development of the uterus. When judged according to the criteria the type specimen of *P. cuneatus* should be considered considerably older.

(2) The numbers in which the two species were present in individual wild birds differed markedly; BYCHOVSKAJA-PAVLOVSKAJA[14] recorded that the number of *P. ovatus* specimens found in a single bird varied from one to 70; in *P. cuneatus* from one to 28. Our investigation revealed that the number of parasites in the fabrician sac depended chiefly on the age of the worms (see the experiment in young ducks, page 277). The ratio of the figures suggests that *P. ovatus* was younger than *P. cuneatus*.

The following experiments were carried out to establish whether two separate species were really present:

A. *Experiment with starlings (Sturnus vulgaris)*

As several authors record the presence of *P. ovatus* in starlings this bird was chosen for host experiments.

Five young starlings were obtained from the Amsterdam Zoological Garden; natural infection with P. could be excluded. The birds were fledglings; one died after two days and dissection showed that it had not been infected. The other four birds (SB 11, 12, 13, and 14)—still at an age they had to be fed—were given five, five, seven, and four cysts, respectively, along with the food on May 15th, 1959. The number of cysts was small so as to ensure rapid development of the worms. The four birds were examined four days after infection; SB 11, 12 and 13 were not infected, but SB 14 yielded one specimen which had all the characteristics of *P. ovatus* when compared with BRAUN's description and illustration (*Fig.* 3).

The results clearly indicated that typical *P. ovatus* may be bred from cysts in *Cordulia aenea*. In view of the brief period of development it was shown that *P. ovatus* (Rudolphi) is a young form of the parasite.

As we found later on that young starlings easily regurgitate food pushed into their beak we think that the low yield in this experiment may be connected with the manner of feeding.

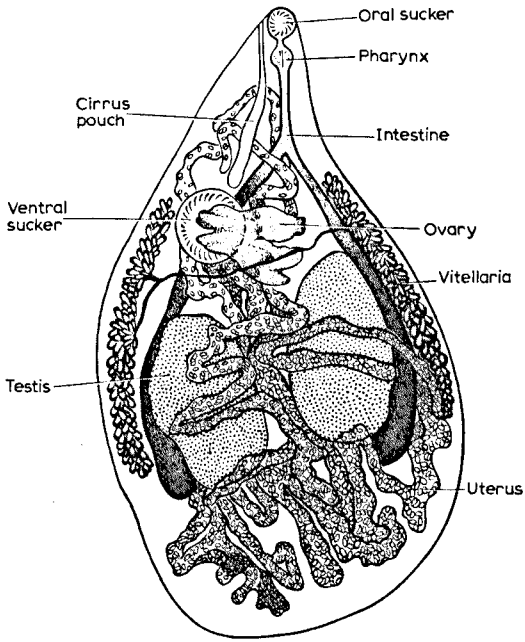

Fig 3. Prosthogonimus ovatus from fabrician sac of starling SB 14, age 4 days, mounted in canada balsam.

Specimen from SB 14	*P. ovatus according to* BRAUN
Ovary dorsal, at the level of ventral sucker	Idem
Rozette-shaped uterus at distal end of the body	Idem
Uterus convolutions also orally of ventral sucker	Idem
Pharynx length 0.099 mm, width 0.113 mm	Pharynx 0.1–0.16 mm
Diameter oral sucker 0.165 mm	Diameter oral sucker 0.146–0.167 mm
Diameter ventral sucker 0.338 mm	Diameter ventral sucker 0.354–0.447 mm
Testes length 0.747, width 0.592 mm	Testes length ? width ?
,, 0.804, ,, 0.522 mm	
Length of body 2.98 mm	Length of body 3–6 mm
Width of body 1.61 mm	Width of body 1–2 mm

B. *Experiments with jackdaws* (*Corvus monedula*)

The experiments served to establish whether *P. ovatus* and *P. cuneatus* are different species or only specimens of different age belonging to the same species; both species were found in several kinds of Corvides, sometimes in one and the same bird.

For this reason a jackdaw (MB 11) was repeatedly infected with cysts stuffed into the beak together with food. (The jackdaws were obtained from the village of Vogelenzang where there is no P. infection.) The experiment lasted from 16th to 30th June, 1959.

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First	infection,	June 16th	27	cysts yielded	4	specimens
Second	,,	June 23rd	8	,, ,,	5	,,
Third	,,	June 26th	10	,, ,,	7	,,

Dissection of the bird on June 30th, 1959, revealed 16 worms belonging to three distinct categories of size. They are correlated with the three infections in *Table I.* The decreasing yield in worms was quite remarkable. As the oldest worms were only two weeks old there could hardly be a question of death through age. It is more likely that the older worms were expelled by a subsequent infection. The youngest specimens, four days old, were very small; they were of the same type as the metacercariae that may be liberated from the cysts.

First group:	*Dimensions in mm*			
Length of body	0.888	1.255	0.931	1.184
Width of body	0.677	0.649	0.663	0.465
Ventral sucker, diameter	0.224	0.240	0.221	0.226
Oral sucker, diameter	0.197	0.176	0.169	0.169
Ratio Vs/Os	1.140	1.308	1.250	1.333
Testis length and width	0.183 and 0.127	0.141 and 0.140	0.155 and 0.141	
length and width	0.197 and 0.141	0.169 and 0.141	0.155 and 0.141	

Infection experiments using chicks (see page. 276) showed that the number of parasites markedly influenced their growth; the presence of old worms retards the development of the younger ones. This conclusion is drawn on the strength of the marked difference in development between the specimens in the jackdaw and the single specimen of the same age from the starling's fabrician sac.

The seven-day-old second group of worms showed characteristics intermediate between those of *P. cuneatus* and *P. ovatus* (*Fig. 8*). The shape of the yolk glands and the light-brown colour corresponded well with those in the specimen from the Fabrician sac of the starling (SB 14). However, there were practically no uterus convolutions in front of the ventral sucker. The position of the ovary was variable; when the worms were not flattened it lay entirely or partly at the level of the ventral sucker. When the worm was pressed flat—as in microscopical inspection—the ovary shifted towards the distal end.

Second group	*Dimensions in mm*			
Length of body	2.737	2.33	1.804	3.102
Width of body	1.833	1.38	1.184	1.847
Ventral sucker	0.494	0.495	0.437	0.486
Oral sucker	0.254	0.219	0.240	0.261
Ratio Vs/Os	1.94	1.87	1.82	1.87
Testis length and width	0.846 and 0.778	0.564 and 0.423	0.536 and 0.423	0.663 and 0.522
length and width	— —	0.564 and 0.437	— —	0.677 and 0.494

Larger specimens of P. had to be flatted and fixed between two slides for microscopical examination; this was unnecessary in smaller specimens as they are flatter

Third group (Fig. 9)	*Dimensions in mm*			
Length of body	5.22	4.54	5.7	4.9
Width of body	2.61	2.96	2.8	2.4
Ventral sucker	0.733	0.733	0.677	0.620
Oral sucker	0.324	0.352	0.254	0.282
Ratio Vs/Os	2.26	2.17	2.66	2.20
Testis length and width	0.987 and 0.804	0.973 and 0.818	0.917 and 0.790	1.015 and 0.620
length and width	0.846 and 0.804	0.917 and 0.832	1.029 and 0.635	— —

and the covering slide did not affect the shape. The flattening of worms by squeezing creates an artificial difference between P. "*ovatus*" and "*cuneatus*" by shifting the ovary! In the mounted material collected by other investigators, *e.g.* BRAUN[11], it is difficult to evaluate to what extent the differences in the position of the ovary are real. TIMON DAVID[124] described *P. cuneatus* from the fabrician sac of a magpie (*Pica pica*) in which the ovary lay partially dorsally to the ventral sucker.

The experiments demonstrated clearly that the differences between *P. ovatus* and *P. cuneatus* are not real; they are due to differences in age and perhaps also to differences between the hosts. We are of the opinion that on grounds of priority the name of *Prosthogonimus ovatus* should be maintained.

Another jackdaw (MB 12) was infected simultaneously with infection in a crow (CB 11); this served the double purpose of ascertaining possible differences between

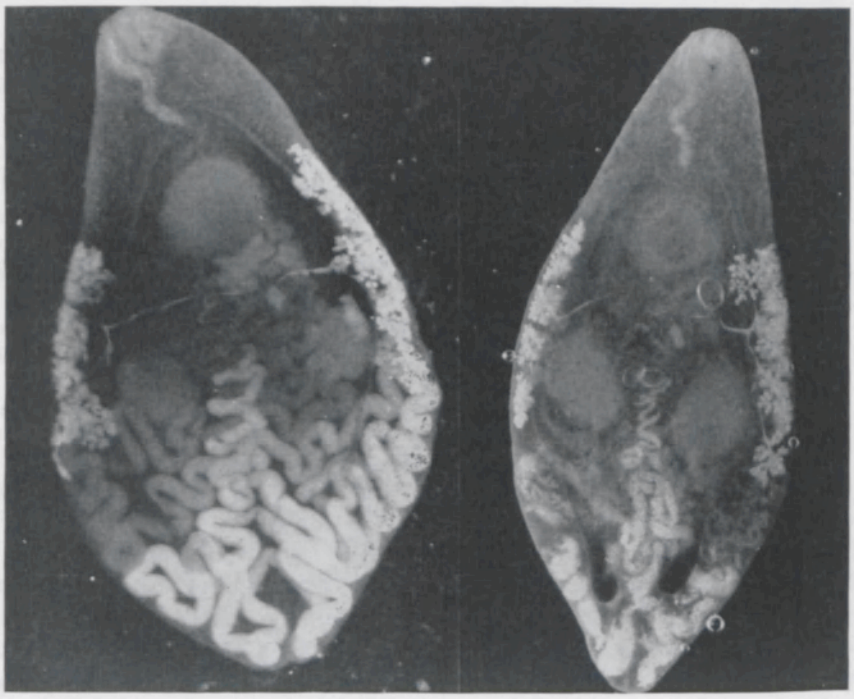

Fig. 4. (13.5 x). *Fig. 5.* (11.2 x).

Fig. 4. Specimen from fabrician sac of jackdaw MB 12. (Photo W. L. van Utrecht).
Fig. 5. Id. from fabrician sac of crow CB 11. (Photo W. L. van Utrecht).

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the specimens bred from the jackdaw and the crow, the former being older than those obtained in jackdaw MB 11; they consequently presented another link in the evolution cycle in this host.

The experiment lasted for 27 days; it was started on June 16th, 1959, 22 cysts being administered with the food. Examination of the fabrician sac revealed three specimens of P. (*Fig. 4*); the dimensions were compared with those from the fabrician sac of the crow.

C. *Experiment with a carrion crow* (*Corvus corone*)

The experiment had a double purpose:

1. To compare the specimens of the same age from the fabrician sac of the crow and of related birds such as the jackdaw.
2. To establish whether *P. fülleborni* is a proper species or a host modification of *P. ovatus*. *P. fülleborni* SKRJABIN AND MASSINO[102] was found in the fabrician sac of a hooded crow (*Corvus cornix*) shot down in the vicinity of Moscow. According to modern ornithological views, *Corvus cornix* and *Corvus corone* are one and the same species.

As *Corvus cornix* does not breed in the Netherlands it was impossible to obtain young birds of this sub-species; the test was therefore made in a young *Corvus corone* (CB 11) caught in Amsterdam, which precluded natural infection with P. (see page 265); 41 cysts obtained from *Cordulia aenea* larvae were stuffed into its beak with the food. The experiment was begun on June 16th, 1959. The bird was killed after 27 days; its fabrician sac contained seven specimens of P. (*Fig. 5*). This experiment was run simultaneously in a *Corvus monedula* (MB 12). Comparison with the typical specimens of *P. fülleborni* described and depicted by SKRJABIN AND MASSINO[102] reveals a striking resemblance.

MEASUREMENTS OF SPECIMENS DESCRIBED BY SKRJABIN AND MASSINO[102] COMPARED WITH THOSE OF CROW CB 11 AND JACKDAW MB 12

	Body length	*Body width*	*Oral sucker*	*Ventral sucker*	*Testis length*	*Testis width*	*Pharynx length*
Skr. and Mass.							
(1)	8.152	3.45	0.287	0.895	0.902	0.789	0.317
(2)	6.945	2.65	0.247	0.828	1.199	0.677	0.297
CB 11							
(1)	6.9	3.2	0.282	0.805	1.043	0.733	0.211
(2)	7.8	3.4	0.345	0.923	1.220	0.789	0.226
MB 12							
(1)	7.0	3.1	0.366	1.306	1.058	0.620	0.226
(2)	6.5	4.0	0.331	0.945	0.846	0.564	0.226

There was a significant similarity between specimens from the fabrician sac of CB 11 and those described by Skrjabin and Massino as *P. fülleborni*.

Tests preceding this experiment had given an idea of the rate of growth of *P. ovatus* under various conditions; on the strength of these observations, the age of the specimens described by Skrjabin and Massino was estimated at four weeks.

The results of the experiment made it clear that *P. fülleborni* is not a proper species but a host modification of *P. ovatus*.

D. *Experiments with chicks*

Several experiments were made with chicks. The latter have many advantages as test animals as they are available throughout the year, they do not demand special food or housing, and—together with ducklings—they are the only birds of which adult specimens with a functioning oviduct are easily obtained.

Experiments with leghorn chicks (female) WF 11 and WF 12. The experiment lasted from September 18th to October 21st, 1958. The young hens were about three months old and had been hatched and raised in the Amsterdam Zoo.

27 and 19 cysts, respectively, were administered to them on pieces of bread. No change occurred in the condition of the birds during the experiment. They were killed and examined after 33 days.

MEASUREMENTS OF LARGE SPECIMENS FROM THE FABRICIAN SAC OF LEGHORN CHICK WF 12

Body length	*Body width*	*Ventral sucker*	*Oral sucker*	*Testis length*	*Testis width*
10.0	6.0	0.973	0.452	1.13	0.656
9.1	5.2	0.987	0.352	1.03	0.695

The fabrician sac of WF 11 was hypotrofic; no P. specimens were found. WF 12 had a well-developed sac which contained three large and four small specimens. This was a remarkable result as in all other tests the specimens resulting from a single infection were practically equal in size; this will be discussed later on.

The four small specimens were 3–4 mm in length and about 2 mm in width; exact measurements cannot be given as the specimens were lost through a mishap in the mounting procedure.

Comparing the dimensions of the two large specimens with those of *P. brauni* from the fabrician sac of the hen, we find a striking similarity which is enhanced by the fact that the specimens were mounted in about the same flat shape; the ventral sucker lies approximately against the branching intestine.

MEASUREMENTS OF P. BRAUNI FROM THE FABRICIAN SAC OF GALLUS DOMESTICUS (SKRJABIN, 1919

Body length	*Body width*	*Ventral sucker*	*Oral sucker*	*Testis length*	*Testis width*
6.8	5.46	0.89	0.53	1.0	0.53

REVIEW OF EXPERIMENTS MADE IN RHODE ISLAND RED COCKERELS

The experiments with Rhode Islands Red cockerels (RM 11–16), started on May 7th, 1959, was one of the two largest experiments in our investigation. Like the experiments with Peking ducks, they had the purpose of yielding as many data as possible on growth, yield, the influence of the number of worms on the growth, maximal age, development of different organ, *etc.* RM 14 and RM 16 were used for a yield experiment. The test with RO 31 ran parallel with this experiment (see page 279).

Although the specimens that grew in the fabrician sac of the cockerels also showed protandry, it was less pronounced than in specimens from the oviduct that grow in summer. Growth also was much slower than in specimens from the oviduct

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EXPERIMENTS IN R.I.R. COCKERELS

	RM 14	*RM 16*	*RM 11*	*RM 15*	*RM 12*	*RM 13*
Duration (days)	5	6	12	23	34	49
No. of cysts	9	9	20	9	20	31
No. of worms	2	8	19	1	12	1
Yield (percentage)	22	88	95	11	60	3.2
Body length (mm)	1.81	2.04	3.07	4.51	5.24	8.10
Body width (mm)	1.10	1.27	1.68	3.0	3.13	4.9
Ventral sucker (mm)	0.226	0.420	0.372	0.624	0.719	0.917
Oral sucker (mm)	0.141	0.240	0.216	0.288	0.338	0.324
Testis length (mm)	—	0.327	0.346	0.438	0.694	0.712
Testis width (mm)	—	0.315	0.282	0.348	0.520	0.606

COURSE OF THE EXPERIMENT: The about three-week-old cockerels grew well during the test and remained in excellent condition. The cysts of *Cordulia aenea* were administered on pieces of bread, usually together with the investigated larva.

The yield of the infection was remarkably low in RM14 and RM15; this could not be explained. If it is not necessary—for instance in a yield test —it is inadvisable to work with less than 20 cysts.

A third test with low yield, RM13, concerned an infection of 49 days; this may have been a case of dying off through age, as it was also clearly observed in the experiment in PB11–PB15.

at the same time of the year. Generally speaking it may be stated that there is a correlation between growth and protandry: the faster the growth the stronger the tendency to produce transient complete male characteristics.

The specimens obtained in this experiment (*Fig. 7*) showed the type of *P. cuneatus;* it should be kept in mind that the proportion of the suckers is not constant but changes during the development of the worm; this is discussed on p. 283.

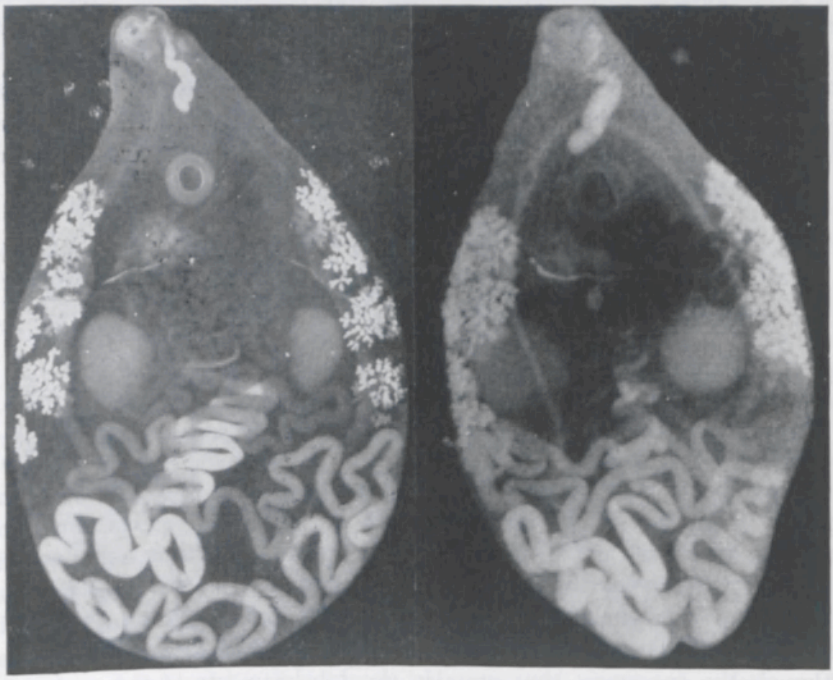

Fig. 6. (7.6 x). *Fig. 7.* (12.6 x).

Fig. 6. Specimen from oviduct of chicken RO 32. (Photo W. L. van Utrecht).
Fig. 7. Id. from transplantation experiment. (Photo W. L. van Utrecht).

The mature specimens showed differences with specimens from the fabrician sac of the White Leghorn hen; they are attributable to racial differences between the hosts.

Although it is not mentioned, at may be assumed that the type specimen of *P. brauni* was obtained in a White Leghorn, as at that time (1919) this was the most common laying-hen in Europe.

Another test was made in White Leghorn cockerels WM 11–13, in order to cultivate six-day old specimens from the fabrician sac and to transfer them to the oviduct by means of a catheter (see p. 281).

E. *Experiment with ducklings*

An experiment with three-week-old Peking ducklings was started in December, 1958. The principal problem investigated were the many species of the Posthogonimus family found in the fabrician sac of different kinds of ducks. These species *P. anatinus*, *P. skrjabini*, *P. rudolphii*, *P. karausiaki*, *P. querquedulae*, *P. horiuchii*, *P. orientalis*, and *P. penni* have a distinct common characteristic, viz. a poorly developed uterus. The term intercoecal uterus is used in the description of a few species; it means an uterus that lies entirely within the intestinal branches.

The differences between these species are slight and not distinct, so the authors restricted themselves to the description of one particular specimen. The question was whether this is a group of closely related species or one species that occurs specifically in ducks, or whether it is a number of host modifications of one polyxenic species. As in this case the hosts are closely related the modifications may be very slight.

Cysts from larvae of *Cordulia aenea* were used for infections. As we wanted to study the yield of the infection, 10, 20, 30, 40, and 51 cysts, respectively, were administered to five birds. The ducklings were infected on December 22nd, 1958, with cysts on pieces of bread. The infection did not affect the condition or appetite of the birds; they grew rapidly. For five weeks the droppings were searched for P. ova but without success.

PB 11 (20 cysts) was killed and examined after 35 days; there were nine very small specimens, the worms being at an early stage of development. This was most unexpected as earlier experiments (as well as the literature) had made it apparent that the worms are mature and full-grown in 35 days; however, all these experiments and data from the literature dealt with infections made in the spring or the summer.

The influence of the *seasonal factor* was checked by infecting an adult hen, as there are many data on the growth of P. in the oviduct of this fowl; the result of the test (RO 21) proved that this was a case of seasonal influence (see p. 284).

PB 12 was examined after 53 days; five specimens had developed from 40 cysts; they were only slightly larger than those from PB 11 (*Fig. 11*). PB 13 (10 cysts), after 67 days, yielded three specimens that were mature (*Fig. 12*). This wide difference might be due to the dates on which the two birds were examined, February 13th and February 27th; if around February 13th the rate of growth had speeded up to the spring and summer rhythm, this might offer an explanation for the marked difference in size.

PB 14 (30 cysts), after 87 days on March 19th, yielded two specimens that were considerably smaller than those from PB 13. This might indicate the growth inhibition of Prosthogonimus in winter varies in different hosts, even if the latter are as similar as in this case. A difference in the growth rate due to the difference in the number of administered cysts does not play a part in prolonged experiments.

PB 15 (50 cysts) was examined after 94 days; it was not infected.

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REVIEW OF RESULTS OBTAINED IN EXPERIMENTS WITH DUCKLINGS

	PB 11	*PB 12*	*PB 13*	*PB 14*	*PB 15*
Number of cysts	20	40	10	30	50
Experiment started	Dec. 22	Dec. 22	Dec. 22	Dec. 22	Dec. 22
Experiment ended	Jan. 26	Feb. 13	Feb. 27	Mrch. 19	Mrch 26
Duration in days	35	53	67	87	94
No. of Prosthogonimus	9	5	3	2	0
Yield (percentage)	45	12.5	33	6.6	0
Lengths of worms (*mm*)	1.62	2.22	7.4	4.6	—
Diameter ventral sucker (μ)	437.1 (av.)	528.8 (av.)	733.2 / 916.5 2 ex.	676.8 / 465.3 2 ex.	—
Diameter oral sucker (μ)	211.5 ,,	267.9 ,,	408.9 / 352.5 ,,	296.1 / 246.8 ,,	—
Proportion ventral/oral s.	1.7–2.2	1.97	1.8–2.6	2.3–1.9	—

As regards their characteristics, the specimens of P. obtained in this experiment belonged entirely to the group of "species" that have been recorded in the fabrician sac of ducks. It was clear that they were host modifications of *P. ovatus*.

F. *Experiments with adult chickens*

Two full-grown, two-year-old Rhode Island Red hens (RO 11 and RO 12) were obtained from the Amsterdam Zoo where they had been housed in a coop of fine-mesh wire netting which precluded infection with P. The hens were laying well.

The infection was made on August 27th, 1957, with cysts that were still in the larvae of *Cordulia aenea* from Lonnekermeer Lake. The number of cysts was quite sufficient to cause a severe infection.

The behaviour of the hens was checked daily; the day after the infection both laid an egg, but after this, laying stopped completely. The droppings were examined regularly for worm ova.

(RO 11) After eleven days a large number of P. ova were found in the droppings of RO 11; in the droppings of RO 12 the ova appeared 14 days after the infection. RO 11 was killed 13 days after infection; the oviduct yielded 63 specimens of P. that were identical with *P. pellucidus* as described in the literature.

The worms were about 7 mm in length. The largest specimens were found higher in the oviduct than in the case of the smaller ones. Apart from the worms there were a few irregularly shaped albuminoid concrements in the oviduct. The inner wall of the oviduct was undamaged, with no sign of lesion.

(RO 12) This bird was killed after 21 days; the oviduct contained 55 very large specimens of P. identical with *P. pellucidus*. The largest worms measured 11 mm. They were concentrated in the upper part of the oviduct; there were no albumin clots. A black substance—possibly the faeces of the worms—was spread like a film over the inner surface of the oviduct.

The condition of the hens was carefully checked during the experiment; apart from the abrupt cessation of laying there was no change whatever. The combs did not turn blue, the birds were lively, and the weight did not change appreciably; RO 11 weighed 1,795 g before infection and 1,730 g 13 days later; RO 12 was 2,092 g before infection, and 2,110 g 21 days later. The appetite was excellent, although RO 12 ate nearly twice as much as RO 11. Ova of Trichuris and Ascaris were found regularly in the droppings of RO 12; dissection revealed large numbers of these parasites in the intestine. RO 11 was free from these parasites.

The marked difference in the quantities of food ingested by the two hens might be attributable to the infection with intestinal parasites in RO 12.

In order to ascertain whether the observed retardation in the development of the worms in the Peking ducklings PB 11–PB 15 was due to *seasonal influences or to*

the type of host, a Rhode Island Red hen (RO 21), two years old and laying well was infected with 25 cysts on January 26th, 1959. Tests in RO 11 and 12 had already shown that in summer the development in this host is rapid.

(RO 21) The droppings were searched for P. ova for 17 days, but without success. On the 18th day seven very small specimens were found which supported the assumption that this was question of seasonal influence.

MEASUREMENTS OF TWO SPECIMENS FROM THE OVIDUCT OF R.I.R. HEN RO 21 MOUNTED IN CANADA BALSAM

Body length	*Body width*	*Ventral sucker*	*Oral sucker*	*Testis length*	*Testis width*
1.98	0.648	0.252	0.228	0.168–0.168	0.132–0.120
2.33	0.780	0.264	0.252	0.204–0.252	0.156–0.132

Another experiment in hens was carried out as a parallel test with the infection of Rhode Island Red cockerels (RM 11–16). Simultaneously with these cockerels two Rhode Island Red hens (RO 31 and 32) were infected in order to ascertain whether the differences between specimens from the oviduct and from the fabrician sac are due to *modifications within the organ* or to a *selection of the cysts* among various species by the harbouring organ itself. In the first case we would be dealing with cysts of a single species which may develop in both organs. In favourable circumstances, therefore a very high yield (100 per cent) might be expected in both tests.

In the second case—with cysts of various species that are selected inside the organ —the optimal yield would be 50 per cent if it were assumed that the numerical ratio of cysts of various species was the same in the two birds.

(*RO 31 and RO32*)

RO 31 was infected with nine cysts, and RO 32 with 40 cysts on May 7th, 1959. RO 31 was examined after four days; the oviduct contained seven small specimens.

AVERAGE MEASUREMENTS OF SIX SPECIMENS FROM THE OVIDUCT OF RO 31 MOUNTED IN CANADA BALSAM

Body length	*Body width*	*Ventral sucker*	*Oral sucker*	*Testis length*	*Testis width*
1.86 mm	0.93	0.26	0.24	0.51–0.53	0.39–0.40

Comparison with specimens of the size from RO 21 shows about the same measurements for the suckers, but there is a very marked difference in the development of the testes. The fast-growing young specimens from RO 31 had proportionally very large testes; apparently the latter develop more rapidly than the other organs. This emphasizes the protandy of Prosthogonimus.

When growth is slow, as in winter, a specimen develops in which all organs are proportioned as in a specimen of the same age that has developed in summer and which, for that reason, is many times as large. The size of the worm is not a criterion for its age. Large testes in proportion to the body length, combined with a moderately too poorly developed uterus, means a very young specimen.

RO 32 was examined after 22 days; the oviduct contained 33 very big specimens (*Fig. 6*), the organ showing no signs of damage.

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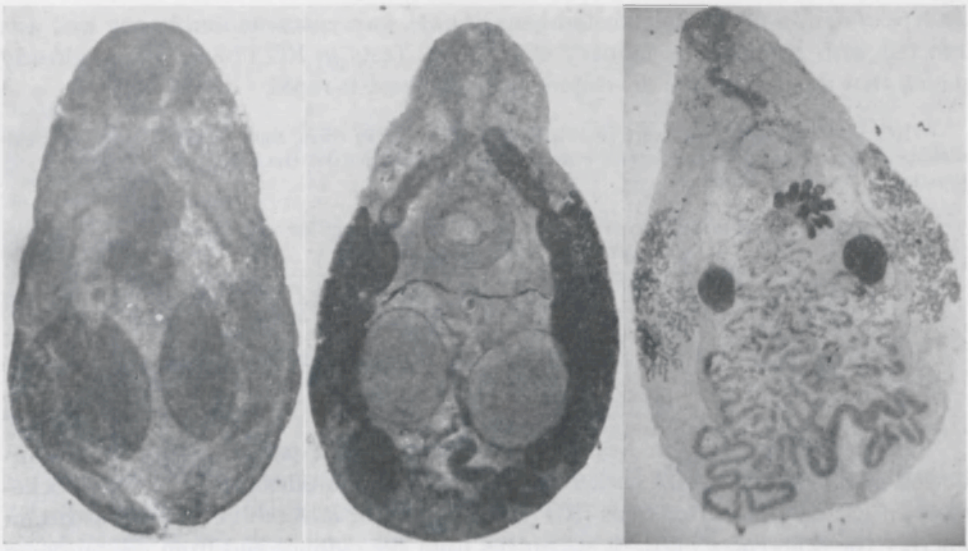

Fig. 8. (28.6 x). *Fig. 9.* (15.0 x). *Fig. 10.* (8.4 x).

Fig. 8 and *fig. 9.* Specimens from fabrician sac of jackdaw MB 11. (Photo J. J. Willemse).
Fig. 10. Id. from fabrician sac of chicken RM 13. (Photo J. J. Willemse).

The other Rhode Island Red hens were used in the transplantation test with young specimens; *vide sub H.*

As none of the hens showed signs of dragon-fly disease, except for a single soft-shelled egg and an abrupt cessation of laying, an experiment was made with two White Leghorn hens (WO 11 and 12) to ascertain whether this was due to a better tolerance to the infection in Rhode Island Reds; this experiment is discussed later on.

The more-or-less mature specimens obtained in these and the foregoing experiments from the oviducts of hens and ducks after an infection period of at least two weeks in a summer season of six months are compared in the *Table.*

SURVEY OF EXPERIMENTS WITH ADULT HENS AND DUCKS HARBOURING MATURE SPECIMENS OF P. OVATUS AS COMPARED WITH TWO FORMS FROM LITERATURE

						Forms	
	27/8/57	*27/8/57*	*7/5/59*	*23/6/59*	*6/5/59*	*P. pellucidus*	*P. longus*
Duration (days)	14	21	22	24	27		
No. of cysts	unknown	unknown	40	62	51		
No. of worms	64	55	22	35	22		
Yield (percentage)	—	—	55	56.5	43.1		
Body length (mm)	10.25	12.73	11.5	8.6	11.04	9	14–16
Body width (mm)	4.35	6.93	8.0	5.2	5.11	4–5	5–7
Diam. ventral sucker	0.71	0.79	0.94	0.83	0.86	1–1.3	1.16
Diam. oral sucker	0.61	0.78	0.83	0.69	0.78	0.9	1.06
Ratio suckers	1.16	1.0	1.13	1.21	1.11	1.28	1.09
Testis length	1.23	1 52	1.56	1.25	1.62	—	2
Testis width	0.89	1.26	1.26	0.86	0.99	—	—

G. *Experiments with adult ducks.*

The aim was to obtain specimens of *P. ovatus* grown in the oviduct of ducks, as we wished to ascertain whether there existed differences between these specimens and those from hens' oviducts.

It was also interesting to establish the influence of the infection on the egg production of the duck.

Three Khaki Campbell ducks (KO 11, 12 and 13) were infected on September 15th and 16th, 1958; KO 11 was fed 108 cysts on a piece of bread and 12 in a larva, KO 12 56 cysts in larvae, and KO 13 33 cysts in larvae. The ducks were housed in a single coop which made it impossible to examine the droppings of the individual birds for P. ova; this disadvantage was partly overcome by regular checking and sampling of different clots of faeces. P. ova were found on October 11th, 25 days after infection. This was the sign for isolating the ducks in order to find out which bird was infected. It was KO 13; the other two apparently were not infected. After a few days the production of P. ova ceased, obviously due to the onset of moulting; moulting caused the oviduct to shrink. P. cannot invade or maintain itself in a resting oviduct.

KO 12 was killed 35 days after infection; its oviduct had shrunk to a thin strand.

A second experiment with adult ducks (KO 21 and 22) was started on May 6th, 1958; 45 cysts in larvae were administered to KO 21, 51 cysts in larvae to KO 22. The general condition and the appetite of the birds did not change during the experiment.

KO 21 was examined 25 days after infection; the oviduct had shrunk and no specimens of P. were found. KO 22 was killed after 27 days; the oviduct was in active condition and 22 large specimens of *P. ovatus*, their characteristics similar to those of specimens from a hen's oviduct, were found (See *Table*). The size of the worms was about the same as in 21-day-old specimens from the hen's oviduct if infection had taken place in summer.

Development in the duck's oviduct is probably slower than in the hen, as discussed on p. 286; the effect of P. infection on the production of eggs is to be discussed later on.

Conclusion. The experiments showed that infected larvae of *Cordulia aenea* from Lonnekermeer lake—which in starlings, jackdaws, carrion crows and ducklings developed into *P. ovatus*—produced the pellucidus type in hens; therefore, *P. pellucidus* should be regarded as a host modification of *P. ovatus*.

No exact measurements of the suckers of *P. pellucidus* can be given. BRAUN[11] measured the specimens of v. LINSTOW; he concluded that the latter recorded the diameter of the inner part of the sucker. In "typical" flattened specimens BRAUN found a diameter of 0.9 for the oral, of 1.0–1.3 mm for the ventral sucker. In two likewise flattened specimens he recorded: length of oral sucker 0.766–0.833, width 0.666–0.733 mm; ventral sucker 0.833–1.0 mm.

Two non-flattened specimens of v. LINSTOW had the following diameters of suckers: oral 0.666 mm, ventral 1.0 mm.

When the worms are flattened before mounting reliable and comparable figures are obtained as regards the diameter of the suckers; when the worms are not flattened various organs may shrink, thereby making the figures unreliable.

H. *Transplantation of young specimens from the fabrician sac into the oviduct*

Does a change of environment during the growth affect the development of the worm?

It has already been stated that there exists a correlation between the body length and the size of the ventral sucker in worms in the fabrician sac. In the sac of hens and ducks the growth of the oral sucker soon comes to a halt, the proportion of the diameters of the oral and ventral suckers shifting from 1 : 1.08 to 1 : 3. In the oviduct

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the original proportion between the suckers persists during the growth of the worm.

When young specimens are transferred from the fabrician sac to the oviduct there might be three possibilities of further development provided the worms survive transplantation:

1. Development continues as it did within the sac, the ventral sucker continuing to grow whereas the oral sucker comes to a halt.
2. After transplantation the worm develops in the "oviductal" manner, *i.e.*, the proportion between the suckers remains as it was at the moment of transplantation; in the oviduct the suckers grow at the same rate.
3. The influence of the new environment is so strong that after a time the initial development is completely undone; the relative size of the suckers becomes similar to that of specimens that have developed entirely in the oviduct.

Experiment. Three eight-week-old white leghorn cockerels (WM 11, 12, 13) were infected on June 22nd (WM 11) and June 23rd, 1959 (WM 12, 13) with 22, 19, and 22 cysts, respectively. WM 11 was killed on June 27th; no P. was found, the fabrician sac was hypotrophied.

WM 12 and 13 were both infected with 20 additional cysts on June 27th. Eventual examination showed that WM 12 harboured no P., its sac being hypotrophied. WM 13 harboured seven specimens, three large and four small, in accordance with the infection and superinfection. They were transferred to the oviduct of two Rhode Island Red hens (RO 41 and 42) by means of a polyaethylene Porges Neoplex 21/10 catheter with a 2 mm lumen. The worms were sucked into the catheter with some egg-white of a somewhat stale hen's egg. The catheter was inserted 12 cm into the oviduct via the cloaca after the hen had been laid on its back. RO 41 was infected with three small specimens (three days old), RO 42 with four larger, six-day-old specimens. The hens were killed after 14 days.

RO 41 harboured no P.; the oviduct was poorly developed. RO 42's oviduct was well developed; it contained one specimen of P. (*Fig.* 7) with the following measurements:

Body length 6.8 mm; body width 3.7 mm; oral sucker 423–479, average 451.2 μ; ventral sucker 690.9–733.2, average 712 μ; ratio suckers 1.58; length ovary 1240.8 μ, width 930.6 μ; testes length 1043.4 μ, width 930.6 μ, and 1128 μ length, 1071.6 μ width.

The proportion between the oral and ventral sucker was the same as at the moment of transplantation into the oviduct, although the worm grew rapidly, much more rapidly than if it had developed entirely in the fabrician sac; its size was similar to that of a five-week-old specimen from the fabrician sac of a hen. The development of the testes was very rapid; they were markedly larger than those in a specimen of equal size from the fabrician sac of a hen.

Comparison of the specimen with those from the oviduct of a hen showed that its size lay between that of two-week and three-week-old specimens, provided the trematodes grew in summer. The testes were larger than in 14-day-old, smaller than in 21-day-old specimens.

The results showed that from the moment of transplantation in the oviduct the worms developed in the way a worm develops in the oviduct, but that the initial development in the fabrician sac was not obliterated by the influence of the new environment. This result, as well as the fact that trematodes adapted to the fabrician sac are able to maintain themselves in the oviduct, proves that these differences are not a matter of vital importance to *P. ovatus*.

I. *Yield experiments*

Were the differences obtained in worms by various infection experiments conditioned by the organ or the host, or did the cysts belong to different species all of which had *Cordulia aenea* as the second intermediate host?

In the yield experiment, hen RO 31 with a functioning oviduct and a cockerel RM 16 were both infected with nine cysts from a single dragon-fly nymph that contained 21 cysts; cockerel RM 11 was infected with 20 cysts from another nymph.

If *P. pellucidus* and *P. cuneatus* were different species one of which could only maintain itself in the oviduct, the other only in the fabrician sac, the organs might make a selection of the administered cysts. If it were a question of a mixture of these and other species that could only develop in a special organ and in specific final hosts, a low yield of the infection might be expected. A high yield would indicate that we were dealing with a single species which, however, shows many host and organ modifications

The experiment covered only a brief period of time as the yield of the infection decreases rapidly with the age of the worms.

RO 31 was killed after four days; it harboured seven young specimens of P. which had all characteristics of *P. pellucidus*, *i.a.*, suckers of approximately the same size.

RM 16 was killed after six days; eight specimens had developed from the nine cysts; the suckers differed markedly in size, the proportion being 1 : 1.6.

The 20 cysts administered to RM 11 had produced 19 specimens of P. after 12 days, the proportion between the suckers being about 1 : 1.8.

The entire series RM 11–16, as well as the experiment in jackdaws MB 11 and 12, showed that the proportion between the suckers during the growth of the worm changes constantly in favour of the ventral sucker when the parasite develops in the fabrician sac.

The result of the yield experiment demonstrated clearly that the Prosthogonimus cysts found in *Cordulia aenea* belong to one and the same species which is very polyxenic as regards the final host, with modifications conditioned by the type of host and organ.

COMMENTS

Differences in ratio diameter ventral sucker/diameter oral sucker

Graph plotting of the ratio of the diameter of the two suckers, and of the diameter of the suckers against the corrected body length shows the differences in development between suckers of specimens from the oviduct and the fabrician sac. In the oviduct, the ratio of the diameters of the suckers remains nearly constant during growth of the worm, fluctuating between 1.0 and 1.3; it is the same as in metacercariae inside cysts. In the fabrician sac, the ratio changes gradually until the diameter of the ventral sucker is about three times that of the oral sucker.

When plotting the diameter of the suckers against the body length it becomes clear that the changes in the ratio of the diameters in specimens from the fabrician sac is caused by arrest in growth at an early stage of the oral sucker, the growth of the ventral sucker keeping pace with the growth of the entire animal. The correlation between body length and diameter of the suckers is less distinct in the specimens from the oviduct.

In specimens from the oviduct and the fabrician sac of the hen, the suckers of specimens from the oviduct are of equal size; they are smaller than the ventral sucker of a specimen of the same size from the sac, and larger than the oral sucker of the latter.

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In seeking an explanation of the differences, attention should be paid to the great difference between the lumen of an oviduct and of a fabrician sac. The latter is very narrow compared with the former. There are even marked differences in this respect, *e.g.* between the sacs of the Peking duck and the starling.

Inside the sac the worms can scarcely creep; under these conditions the ventral sucker serves as a means of clinging rather than for movement. The expulsive force exerted by peristaltic movements and the outflow of mucus increases as the trematode grows larger; this might explain the linear correlation between body length and the diameter of the ventral sucker.

OSHMARIN[71] found strongly developed suckers and copulation organs in trematodes from the cloaca; the position of the organs made it possible for copulation to take place while the worms remained stuck; apparently all is arranged to withstand the expulsive forces in the cloaca.

The oral sucker serves to suck blood; the benzidine test of the intestinal contents proves that the worms suck blood. The reaction is specific for haemoglobin which stains intense blue. Apparently a certain size of oral sucker is sufficient to supply the growing worm with abundant blood meals. The fact that the oral sucker in specimens from the oviduct is considerably larger than those from the sac cannot therefore be explained by a greater need of food; this was confirmed by the finding that the specimen transferred from sac to oviduct by means of a catheter had a much smaller oral sucker after 14 days in the oviduct than the specimens of the same size that had developed in the oviduct from the start. This also proved that the large oral sucker is not a matter of life and death to the specimens in the oviduct.

P. macrorchis, in the oviduct, has suckers the diameters of which have a ratio 1 : 1.7. Suckers of about equal size enable *P. ovatus* to creep relatively rapidly in the oviduct; this was observed—and filmed—when the oviduct was dissected. The activity of specimens in the fabrician sac was slight; the worms were never seen to creep.

Rate of growth of Prosthogonimus ovatus

Little is known of the differences in the rate of growth; our investigation revealed five factors which affect this rate:

(*a*) *The season.* The season in which infection took place greatly affected the growth; experiments with the Peking ducks PB 11–15 and the hen RO 21 proved that growth is retarded in winter. In PB 11 the worms were very small (*Table*, p. 270) at an age of 35 days; after 53 days they had scarcely increased in size. However, in PB 13 adult specimens were found after 67 days; it might be concluded that development suddenly speeded up between February 13th and 29th, 1959. A control test was made with a hen as it was conceivable that the very slow growth was not due to the season but to the type of host. In Rhode-Island Red chickens the worms develop very fast in summer; ova are found in the faeces as early as 11 and 14 days after infection. However, when a very productive laying-hen was infected with 20 cysts on January 26th, 1959, only seven very small specimens were found after 19 days (*Fig. 13*); this proved the inhibition of the growth rate.

The moment at which the inhibition starts in the autumn was suggested by the test with WF 11 and 12 which was carried out between September 18th and October 21st, 1958. Seven specimens were found in the fabrician sac of WF 12, three of which

were very large and mature, the other four being quite small. The specimens found after a single infection with cysts of *P. ovatus* in winter, spring or summer were always of about equal size. The test suggests strongly that three worms were in summer condition whereas the four others were in winter condition. It appears that the winter inhibition starts suddenly and that in spring it is abolished with equal abruptness.

Fig. 11. (40.1 x). *Fig. 12.* (12.3 x). *Fig. 13.* (46.4 x).

Fig. 11. Specimen from fabrician sac of Peking duck PB 12, mounted in canada balsam. (Photo J. J. Willemse).

Fig. 12. Id. from fabrician sac of Peking duck PB 13, mounted in canada balsam. (Photo J. J. Willemse).

Fig. 13. Id. from oviduct of chicken RO 21, mounted in canada balsam. (Photo J. J. Willemse).

The available data do not indicate the influences that govern the inhibition. Probably it is a question of an internal factor in the metacercaria as the oviduct of the hen functions normally in winter. Moreover, all experiments with birds were carried out in a centrally heated hall. The inhibition of growth in the worms in winter is an excellent adaptation to the seasonal cycle. Many birds, especially aquatic birds (coot, moorhen, mallard, lesser grebe) may breed as late as August and even September, so that in winter there are young birds with a fabrician sac. On the other hand, other water birds breed very early (February) (wild duck, cormorant, blue heron)[7]; consequently there are also birds with a functioning oviduct in winter. Both groups may become infected by gobbling dragon-fly larvae.

However, the production of P. ova in winter serves no purpose as the low temperature of the water precludes all activity of the snails that have to eat the ova.

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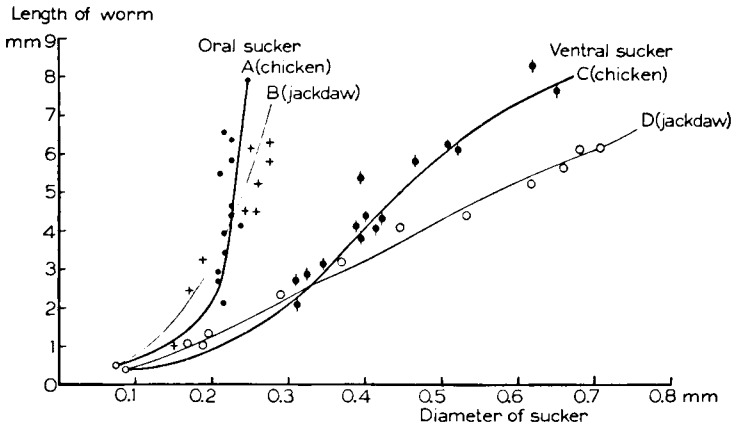
The life-span of P. ova is probably not more than two months. Thus, the inhibition in the development of the worms in winter counters the wastage of ova of *P. ovatus*.

(*b*) *Influence of the organ in which the parasite is living.* There is a marked difference in the rate of growth of worms in the fabrician sac and in the oviduct when infection takes place in summer. The worms in the sac are sexually mature after four weeks, and fully grown after five to seven weeks. In the hen's oviduct these figures are 10–12 and 21 days, respectively. As regards the domesticated duck, the figures are probably slightly higher, but the difference cannot be more than one week; even in this case the figures stay below those for the fabrician sac.



Graph 1. Differences in development of oral and ventral suckers in specimens from the fabrician sac. A. Oral sucker (chicken) B. Oral sucker (jackdaw) C. Ventral sucker (chicken) D. Ventral sucker (jackdaw).

Evaluation of the rate of growth, the moment at which the worms become fully grown and sexually mature should take into account that *P. ovatus* becomes much larger in an oviduct than in a fabrician sac.

(*c*) *Type of host.* As stated sub (*b*), the growth of the parasite in the oviduct of the duck is slower by a week than in the oviduct of the hen. As regards the fabrician sac, no difference existed between specimens from different hosts. The conclusions drawn from experiments on the rate of growth in the fabrician sac in chicks held good for experiments with crows, jackdaws and starlings.

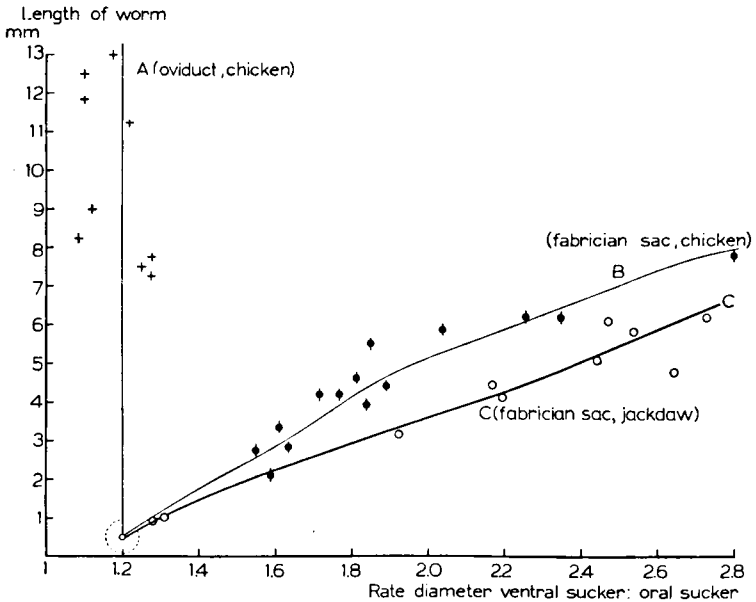
(*d*) *Number of worms.* The number of specimens in the fabrician sac is of influence on the growth; the larger the number, the slower the growth of P. This phenomenon was not observed in the oviduct of the hen; probably the lumen of this organ is so much wider than that of the fabrician sac that there was no question of crowding, at least not in the number of worms cultivated in our tests.

An evaluation of the influence of the number on the growth of *P. ovatus* is greatly hampered by the fact that the number decreases steadily with increased age.

(*e*) *Number of infections.* When a single host is repeatedly infected within a brief period of time the younger specimens are inhibited in their development, as is clearly evidenced by the experiment with MB 11 in which the youngest, four-day-old

specimens were practically identical with metacercariae at the moment the latter leave the cysts.

After a single infection, four-day-old specimens are easily distinguishable from metacercariae, even if the stage of development depends on the number.



Graph 2. Differences in rate of diameters of ventral and oral sucker during development of *P. ovatus* in different organs.

CONCLUSIONS

The growth of *P. ovatus* is a complicated process in which at least five factors play a part, although their influence is not equally strong; the relatively small number of experiments was not sufficient completely to solve this complicated problem. The chief difficulty in the experiments was that factor (*d*) (number) was never constant as the output of infections may differ markedly.

Other points which hampered the investigation were the regular decrease in the number of worms with increased age and the expulsion of old generations of worms by a new infection (experiment with jackdaws, page 271).

INFECTION OF BIRDS IN NATURE

Infection in nature may occur in different developmental stages of the dragon-fly. Aquatic birds may infect themselves by the ingestion of larvae the whole year round provided they have a functioning oviduct or fabrician sac.

Especially divers, such as the coot, red-crested pochard, pochard, *etc.*, have no difficulty in catching dragon-fly najads. In shallow water swimming ducks, such as the mallard, teal or ruddy shellduck, as well as wading birds, such as the spoon-bill, curlew or black-winged stilt, are well able to catch najads.

The metamorphosis of *Cordulia aenea* and *Libellula quadrimaculata* present an

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important possibility for mass infection; in the Netherlands it takes place in the course of about one week, in the period between April 20th and May 7th. The exact date varies from year to year, depending on whether spring is early or late; in 1959 the *Corduliae* in Lonnekermeer lake came on the wing in the last week of April.

The behaviour of the aquatic birds was observed for a few days during this period. The many young ducks caught insects all day long, family by family. The chicks swam side by side and systematically explored the reed fringe, constantly gobbling insects. That many *Corduliae* fell victim to the greedy chicks was illustrated by the fact that a dragon-fly, the metamorphosis of which we were filming, was snatched up by a duckling in an unguarded moment!

Other water-birds were also busily catching insects the whole day long; we observed it in a water-hen with chicks and in two adult coots. There were remarkably large numbers of starlings, blackbirds and mistlethrushes in the reeds, mostly young birds. The metamorphosis of *Cordulia aenea* does not take long and we followed the process from minute to minute; it took only 2½ hours from the moment the skin of the larva burst open until the dragon-fly flew away. However, the larvae creep from the water at dawn a long time before the metamorphosis starts, so that we may reckon with a period of not less than six hours. In localities where dragon-flies occur in large numbers and are as heavily infected, as in Lonnekermeer lake, there is little doubt that water-birds may become heavily infected as soon as the flies take wing.

In connection with the occurrence of dragon-fly disease among domestic fowl the literature devotes considerable attention to the infection of birds by mature dragon-flies; the appearance of this disease was nearly always the motive for investigating Prosthogonimus.

However, as regards the life cycle of the parasite, the infection through mature dragon-flies is much less important than the infection caused by the ingestion of larvae or of dragonflies during metamorphosis. The possibility that the droppings of land-birds fall into waters suited to P. is small, especially in the case of young birds that are fed with dragon-flies in the nest, typical meadow-birds such as the quail, the great bustard or the corncrake, and domestic fowls such as the chicken or the turkey.

Several land-birds which might not be suspected of eating dragonflies were mentioned as P. hosts, *e.g.*, the nuthatch and great and lesser spotted woodpecker[14]. In this connection it is interesting that at the Lonnekermeer lake the metamorphosis of *Cordulia aenea* not only occurred on the reeds but also in trees along the shore; in one of the trees a great spotted woodpecker was nesting. The greater part of mature dragonflies fall victim to the birds in the early morning when they are not yet on the wing but cling motionless to grasses, shrubs, etc. Only a few types of birds are able to catch dragonflies on the wing, for instance the hobby [123], the swift or the swallow[78]. All birds that are infected solely by eating mature dragonflies may be considered dead end hosts.

SUMMARY

For some decades the fluke *Prosthogonimus ovatus* has been known to cause enzootics in chickens and ducks in the Netherlands; this was of some importance to the poultry industry. At present only one site is known to harbour the parasites. There are a number of obscure points regarding the development, ecology and taxonomy of this worm, which the author approached by animal experiments.

Several species of birds (starling, jackdaw, crow) and specially of poultry (Peking duck and Khaki Campbell duck; white Leghorn and Rhode Island Red chickens) have been infected with cysts of *Prosthogonimus ovatus* from the dragon fly Cordulia aenea. As a result, all species of Prosthogonimus described from Europe and Asia proved to belong to one species: *Prosthogonimus ovatus* Rudolphi em Bodekke, 1960.

Transplantation experiments proved that while the worm inhabits the fabrician sac, its oral sucker remains small; while in the oviduct it increases in size. The parasites move in the oviduct, using both suckers; in the fabrician sac the oral sucker has only a sucking function.

If growth is rapid in young specimens, the male stage tends to dominate more so than during slow growth. The rate of growth of *Prosthogonimus ovatus* depends on: (a) *season*; during winter growth is very slow; (b) the *number* of animals; a small number enhances growth; (c) *age* in a population of worms; the presence of adult specimens bars the growth of juvenile, from a repeated infection; (d) the affected *organ;* growth is rapid in the oviduct, it is slow in the fabrician sac; (e) the *host*, but only moderately so; development is quicker in the oviduct of the hen than in that of the duck.

RESUMEN

II. Experimentos en pájaros. Durante las decadas recientemente passadas el tremátodo, Prosthogonimus ovatus en los Países Bajos ha causado enzootias de gallinàs y palomas que son de importancia para la granja avícola. Ahora solo uno lugar está conocido donde el parásito aún se encuentra. El desarollo, la taxonomía y la ecología de este gusano muestran numerosos problemas aún no resultos. Se ha tratado resolverles con la ayuda del experimento efectuado con animales.

Numerosas especies de pájaros (estornino, chova, corneja) y, sobre todo, variedades de gallinas (leghorn blanca y Rhode Island roja) y de patos (pato Pekin y Khaki Campbell) se infectaban con metacercarias de Prosthogonimus ovatus arran cadas dela libélula Cordulia aenea. El examen de los gusanos cultivados de esta mancra ha provado que todas las especies del género Prosthogonimus descriptas en Europa y Asia apartienen a una sola especie, Prosthogonimus ovatus Rudolphi, em. Boddeke.

Experimentos teniendo por objeto la transplantación de los parásitos del bolsillo de Fabricius al oviducto han probado que la ventosa adhesiva oral deja pequeño en el bolsillo, y vuelva mas grande en el oviducto porque en el oviducto el tremátodo se mueve usando alternativamente, la ventosa adhesiva oral y el acetabulo, mientras que en el bolsillo de Fabricius la ventosa adhesiva oral ne siros otro objecto que el de apretar el parásito al tejédo del bolsillo.

Quando los indivíduos jovenes crecen rápidamente la fase masculina del desarrollo prevalecer mas que quando el crecimiento está retrasado. La velocidad del crecimiento depende (1) de la sazón; muy retardado en el invierno; (2) del número de parásitos ocupando un cierto sitio; mas pequeño el número, mas rápido el crecimiento; (3) de la presencia de parásitos jovenes y adultos en la misma población como resultado de infecciones repetidas; los adultos empiden el desarrollo de los jovenes; (4) del sitio de los parásitos; en el oviducto el crecimiento es mas rápido que en el bolsillo de Fabricius; (5) de la especie del huésped; en el oviducto de la gallina los parásitos se desarrollan mas rapidamente que en el del pato.

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II. THE INTERMEDIATE HOSTS

A. THE SECOND INTERMEDIATE HOST

Experiments with dragonflies

According to LIEFTINCK[1], *Cordulia aenea* occurs very frequently in stagnant water, especially in peat bogs, from the first days of May until the first half of July. This, however, was not confirmed during my investigation. Apart from Lonnekermeer Lake, where the species abounds, I was able to collect only a few larvae in the wider environment. In my experience, this dragonfly is very scarce nowadays in the western provinces of the Netherlands; a few non-infested specimens were caught in small sheets of water in the dunes of Meyendel, near The Hague. Diligent search over a period of 15 years has failed to produce a single larva of the Cordulia type in the vicinity of Amsterdam.

During the present investigation, several unsuccessful attempts were made to catch larvae of *Anisoptera* in the Ankeveen Lakes (mentioned by LIEFTINCK[1]). On the other hand, many mature *Anisoptera* were observed in the region of the Vecht Lakes in the summers of 1957 to 1959; they were specimens of the genera *Aeshna* and *Sympetrum*, and of the species *Brachytron pratense* and *Libellula depressa*. Nonetheless, it is not at all certain that we are confronted with a lasting decline in *C. aenea* and *L. quadrimaculata*. It is quite possible that the density of these dragonflies varies markedly from year to year!

The larvae of *Cordulia aenea* (*Fig. 1*) live between twigs and fallen leaves in shadowy places and between aquatic plants in sunny spots. The larvae are very sluggish. Measurements of larvae caught on September 10th, 1957, revealed two distinct groups. The first was formed by larvae about 20 mm in length and 9 mm in breadth; the variation was slight. These larvae were at least 14 months old. They were practically full grown and they will be on the wing in the following spring; this was confirmed in the laboratory. The other group consisted of larvae about 12 mm in length and 6 mm in breadth; the variations were more marked. They were immature larvae born in the spring of 1958. A difference in age of a month is not uncommon in connection with the flying period of the dragonflies; at the age of about four months this causes an appreciable difference in size.

Distribution of cysts in Cordulia larvae collected at different sites

A large number of larvae was caught in the autumn of 1957 on the same day at three different sites in the Lonnekermeer Lake; these sites are indicated in the map as *a*, *b*, and *c* (*Fig. 2*).

Site *a:* A large number of larvae were caught, on the average four per scoop, with the dip-net. The brook was closed on the side of the lake by a small dam which permitted only a scanty over-

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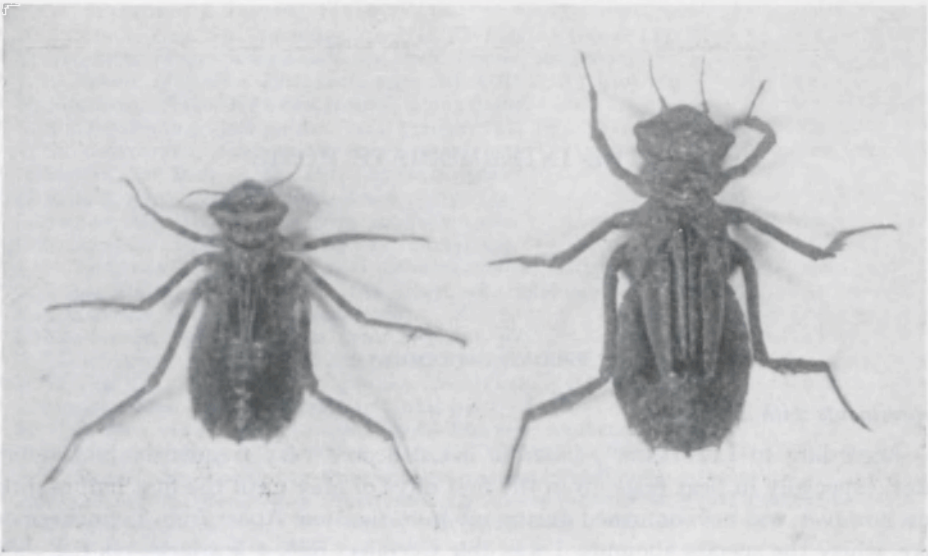

Fig. 1. (2.1 ×) *Cordulia aenea* larvae; on the right about 16 months old, on the left about four months old. (*Photo J. Bakker*).

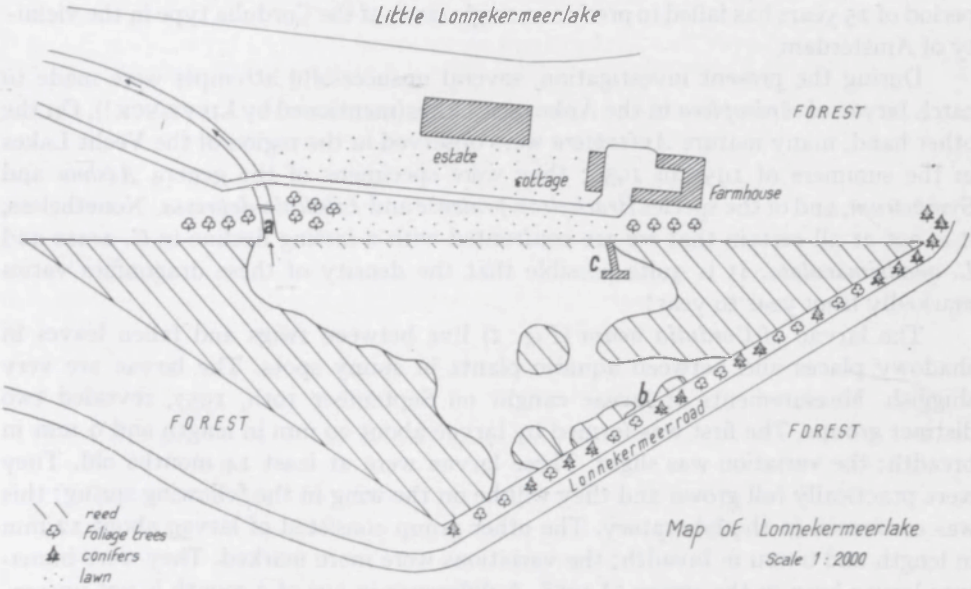

Fig. 2.

flow; this dam kept the water in the lake at a constant level. The bottom of the brook on both sides of the dam was covered with twigs and leaves. The shores were sheltered by trees and shrubs; this made the spot very shaded. There were few snails.

The number of cysts per larva varied markedly; in larvae more than a year old we found 6, 3, 45, 2, 84, averaging 28 cysts per larva (c/l); in younger larvae 0, 1, 5, 6, 0, 0, 0, averaging 1.7 c/l.

Site *b:* Shallow water between reeds, densely shadowed by trees on the shore. Average number

of larvae per scoop, two. Few snails. In the larvae of more than one year old the number of cysts found were 6 × 0, 9 × 1, 1 × 2, average 0.66 c/l; in younger larvae no cysts were found (average 0 c/l).

Site *c:* Landing-stage, depth of water 1 m., bottom covered by a carpet of waterplants (*Batrachium aquatile, Fontinalis* sp.). There were large numbers of larvae between the plants, ten per scoop with the dip-net; also many snails, for the most part *Bithynia tentaculata*. Number of cysts found in larvae of more than one year old: 11, 8, 2, 9, 0, 3, 1, 23, 4, 7, 1, 6, 0, 4, 16, 0, 1, 15, 6, 0, 1; average 5.62 c/l. The younger larvae contained an average of 0.43 c/l.

These figures show the wide difference in the number of cysts between larvae of more than one year old and younger specimens; on site *a* the proportion was 13 : 1, on site *c* 16 : 1. This difference cannot be explained solely by the difference in age. The more vigorous respiratory movements of older and larger larvae and the much larger quantity of water they use undoubtedly also play a part; still, this is only the case when the cercaria does not actively seek the larva but is passively borne along with the current. During the infection experiments, under the binocular microscope, there was no evidence of a purposeful movement of the cercariae in a certain direction.

Sites *a* and *b* were very similar but for the current present on site *a*. In view of the small number of snails on both sites, infection of any importance would not be possible unless cercariae are carried along, *i.e.*, when there is a current. The effect of the latter will vary from place to place as the current is not uniformly strong. In my opinion this was the cause of the marked variation in catches on site *a*. In October, 1958, a larva caught at this point contained 115 cysts; this is the largest number of *Prosthogonimus* cysts ever found in a single larva.

On site *b*, on the other side of the lake, there were also few snails, but no current, or perhaps a weak current towards the opposite shore. Thus, the conditions under which the larvae existed were more uniform than on site *a*. The number of cercariae was small. The result was a slight infestation, spread regularly, without peaks.

On site *c* we found a large number of snails among the larvae; the cercariae are formed locally and in large numbers. There will be differences in infection ratio as the sluggish snails and dragonfly larvae are not mobile. As only a small percentage of the snails (about eight) were infected with *Prosthogonimus*, it is probably of great consequence whether a larva lies close to an infected snail or relatively far removed from it; this was evidenced by the gradual increase in the number of cysts found in the larvae in a relatively small area of variation (0 to 23). The foregoing strongly suggests that the larva is not actively sought out by the cercaria.

Statistical considerations on the spread of cysts among the larvae

It can be established statistically whether the cercaria actively search for the larva or whether the cercaria is conveyed quite passively into the larvae with the currents.

We used Poisson's method for this purpose; in the so-called Poisson distribution the registered facts occur independently of each other. If this be the case, there is no significant difference between variance and average. If the variance is significantly greater than the average, the occurrence of the registered facts is not independent. In other words, if a certain fact occurs once, the chance of a repetition increases.

As regards the spread of the cysts in the larvae from a site the question arises whether the infection occurs independently, or whether the chance of the formation of a second cyst is greater when one or several cysts are already present?

If the cercaria actively seeks the larva, the slight differences in distance between dragonfly larvae and an infested snail would be negligible in the case of a dense snail and larva population as on site *c*. In that case the formation of cysts may be expected to occur more or less independently.

If the cercaria is conducted passively towards the larva by the circulating ventilation current of the latter, even the smallest difference in distance between infested snail and larva would be of importance. In such an event the chance that a second cercaria penetrates the dragonfly larva would be greater after a previous one has succeeded in causing an infestation.

In these tests we used larvae caught on site *c* on August 24th, 1957 (*i.e.*, speci-

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mens more than one year old); the average number of cysts was 5.62. The variance was 39.1. The standard deviation of the average was 1.37; the standard deviation of the standard deviation was 0.97. These data show that the variance differed significantly from the average; the distribution of the cysts over the larvae is clearly not a Poisson distribution. This is in agreement with the conclusions drawn from the behaviour of the cercariae, the mode of life of the snails and the larvae, and the low percentage of infested snails.

OTHER SECOND INTERMEDIATE HOSTS OF *PROSTHOGONIMUS OVATUS*

Although in Lonnekermeer Lake *Cordulia aenea* was by far the most important second intermediate host, *Prosthogonimus* cysts were also found in other Anisoptera larvae, viz.:

Libellula quadrimaculata, known as second intermediate host since 1927[2], but uncommon in Lonnekermeer Lake. One larva containing a single cyst was caught in the autumn of 1958; two specimens containing three and four cysts, respectively, in the spring of 1959.

Orthetrum cancellatum; four mature cysts were found in a larva caught in Lake Lonnekermeer in December, 1957. In the pool near Olst, where BAUDET[3] established dragonfly disease in tame ducks in 1925, the only Anisoptera larva present in 1958 was *Orthetrum cancellatum*.

Leucorrhinia caudalis and *Leucorrhinia pectoralis:* four *L. caudalis* larvae and 13 *L. pectoralis* larvae were caught on site *c* in April, 1959. One *L. caudalis* specimen harboured two mature cysts; all others were uninfested. This was the first find in Europa of *Prosthogonimus* cysts in larvae of this genus. Nonetheless, the infestation was slighter than in *L. intacta* in the United States, in which there was an average of 10.4 cysts per dragonfly in Glenwood Lake[4].

The *Leucorrhinia* genus has a one-year cycle; the larvae could be caught only in spring. They are probably non-existent in early summer and in the autumn and winter they are so small that determination is impossible. This implies that the strongest growth of these larvae takes place in the spring; *L. pectoralis* goes on the wing about May 20th[1]. The production of cercariae is profuse in the summer months until well into October. The infestation of the larva depends greatly on its size. All these factors collaborate in making small the chances of infestation of the larvae of this genus in Europe.

Matters are different in Minnesota in the United States. Thaw does not set in until the end of March and the dragonflies go on the wing in the last days of May; consequently, the spring period of larval growth is much shorter than in Western Europe. It is possible that the larvae in the United States grow more quickly in summer; this would heighten the chance of infestation.

In addition, *L. intacta* is undoubtedly an exceptionally favourable host for *P. macrorchis*, as evidenced by the average infestation and the maximum of 90 cysts per dragonfly; the average is nearly as high as in *Epicordulia* spec. which has a two-year life cycle.

Aeshna cyanea: two mature *Prosthogonimus* cysts were found in a larva of this species in May, 1959; up till then *A. cyanea* was unknown as a host to *Prosthogonimus*. In July, 1959, a young larva was caught with 12 mature P. cysts and two not yet encysted metacercariae. This find was of taxonomic value as *Aeshna* has a larva of a type other than the other mentioned hosts of *P. ovatus*.

ONO[5] found *Anax parthenope* as a second intermediate host of *P. putschkowskii; Anax parthenope* does not occur in the Netherlands. The *Anax* genus has a larva of the same type as *Aeshna*.

Gomphus spec. is recorded as a host to *P. pellucidus*[6]. MACY[7, 8] mentioned *Gomphus spicatus* as a host to *P. macrorchis*.

Libellula brunnea was stated to be an important second intermediate host to *Prosthogonimus* spec. in Bavaria[9]; the number of cysts varied from one to 60. Another species, *Platycnemis pennipes* harboured only one or two cysts.

This survey of second intermediate hosts of *P. ovatus*, as well as the American species mentioned in the introduction, warrant the conclusion that nearly all Anisoptera larvae may be infested with *Prosthogonimus* spec.

Environment and density of the dragonfly larvae greatly influence the chances of infestation. Specialisation of a certain *Prosthogonimus* species as regards one or

more types of dragonfly larvae in a particular part of the world may be a consequence thereof.

As regards the environment of the larvae, the pH of the water is especially of major importance. Dragonfly larvae in acid water (pools on the heath or in the woods) are hardly ever infested with *Prosthogonimus* as *Bithynia tentaculata* and other snails do not occur in acid water.

Infestation experiments with Prosthogonimus cercariae from Bithynia tentaculata

Bithynia tentaculata caught during our first trip to Lonneker harboured cercariae which closely resembled those of *P. macrorchis* described by MACY[4]; however, there were only four of them, too few for an infestation experiment.

The many snails collected during the next trip contained this one as well as another type of xiphidocercaria which could be differentiated without difficulty due to a larger size, a more vigorous whip of the tail and a very large stylet; we used only the former, although it was difficult to obtain a sufficient number of these cercariae from a small quantity of water which is necessary in an infestation experiment.

Bithynia in small vessels of water are apt to die soon unless the water is changed regularly; however, the slow production of cercariae makes it necessary not to change the water. In addition, the most heavily parasitized snails—of vital importance in the experiment—are the first to die if conditions are unfavourable. The cercariae, also, are not visible with the naked eye which made catching with a pipette impossible.

Concentration method. The following method was used to concentrate the cercariae. The water in the aquarium was centrifuged with a hand centrifuge for about 40 seconds. The precipitate was quickly sucked up with a pipette and deposited in a cup; the results of centrifuging five times were sorted out under the binocular microscope. The *Prosthogonimus* cercariae were caught with a pipette and placed in a separate vessel; the number thus collected was about four a time.

Experiments. When about 25 cercariae had been collected, a larva of *Cordulia aenea* was added to them for 20 to 24 hours. The larvae were obtained in a pool in the dunes where *Bithynia* did not occur. Other dragonfly larvae used in infestation experiments were *Libellula depressa* that had previously been infested with *Plagiorchis* spec.

A number of larvae of *Cordulia aenea*, which were less than a year old, were caught on site *b* in Lonnekermeer Lake and were placed in a tank with snails.

After nine days a larva from the snail tank was killed; one metacercaria was found, not encysted, but enveloped by an elastic membrane (*Fig. 3*); it measured 120 μ in length and 60 μ in breadth. The stylet was 22 μ which agreed with that in the original cercaria. MACY's experiments had shown that *Prosthogonimus* cercariae do not immediately start to encyst but surround themselves with a membrane which allows the animal to attain a length of 500–600 μ in 60 days. Only then is the capsule formed, when the cercaria is fullgrown. Our finding was therefore a strong indication.

A second larva died in the snail-tank after 14 days; it also harboured a metacercaria, 128 μ in length, 80 μ in breadth, the stylet being 22 μ.

After 28 days all remaining larvae but two were killed and examined. Two *L. depressa* larvae harboured only old cysts of *Plagiorchis* spec. Whether this old infestation prevented a new one, or whether *L. depressa* was unsuitable as a host to *Prosthogonimus* is uncertain.

One of the two *Cordulia* larvae was exposed to only three cercariae; it was not infested. The other larva had had better chances of infestation, the result being four large metacercariae similar to those which may be liberated from very young *Prosthogonimus* cysts by cautiously breaking the cystic wall. They measured 400–296 μ (*Fig. 4*), 258–224 μ (coiled specimen difficult to measure), and 320–256 μ; the fourth broke. The metacercariae were still within the elastic membrane; the dark excretion bladder, the suckers and the pharynx were clearly discernible.

The two *Cordulia aenea* larvae that remained in the tank with *Bithynia* for 36 days yielded three full-grown cysts with a nicely striated wall, and one mature cyst plus a young metacercaria (200–100 μ), respectively. The metacercaria was enveloped by a membrane inside which the stylet floated loose in the fluid; the stylet measured 22 μ.

It appears most improbable that these cysts were present in the larvae before the onset of the test.

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No cyst was found in any of the one-year-old larvae from site *b* in Lonnekermeer Lake. The slight chances of infestation for the *Cordulia* larvae on that site was also evidenced by the low average (0.66 cyst per larva) in the two-year-old animals.

Liberation of metacercariae from the cysts

We used a 0.5 per cent trypsin solution in a cylinder-shaped cuvette. The cysts were placed in the latter, after which it was put on a heatable Reichert object table

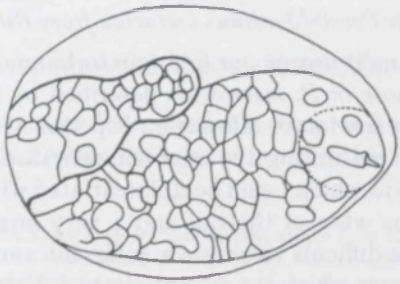

Fig. 3. (350 ×) Young metacercaria in elastic membrane, still with stylet.

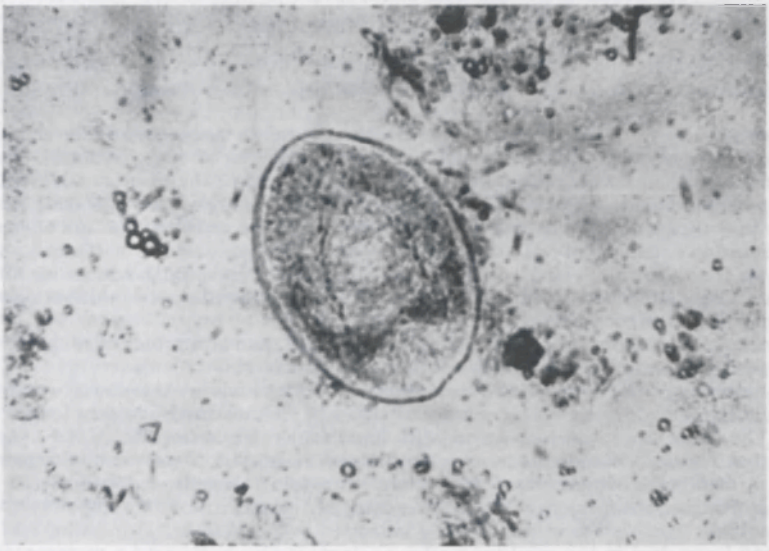

Fig. 4. (92 ×) Metacercaria nearly mature, but not yet encysted. (*Photo J. Bakker*).

(Biotherm) with which temperatures up to 45° C may be attained. The entire process could be followed under the microscope.

After about 15 minutes the metacercaria started to move violently inside the cyst. Probably the cystic wall is softened by the action of trypsin and the wildly moving metacercaria planes off the wall, so that the lumen widens constantly[11]. In some cases the entire cystic wall eventually disappeared, the metacercaria being enveloped only by the membrane. No metacercaria has ever been observed to break through this membrane, although little force is needed to remove it from the outside. It may be assumed that inside the intestine it is broken by friction with the intestinal contents.

At a temperature of 45° C the total disappearance of the cystic wall took at least 2.5 hours. The temperature distinctly affected the rapidity of the test; at lower temperature the process evolved more slowly. The thickness of the wall was also of importance: the thicker the wall, the longer the time needed by the metacercaria to remove the wall. We experimented a few times with a pre-treatment with 0.5 per cent HCl[4]; it did not change the course of the test.

There were doubts as regards the vitality of the dark-brown cysts which were found regularly, although in small numbers, between the normal transparent specimens. However, in the trypsin solution these cysts yielded metacercariae which did not in any way differ from those of the transparent cysts.

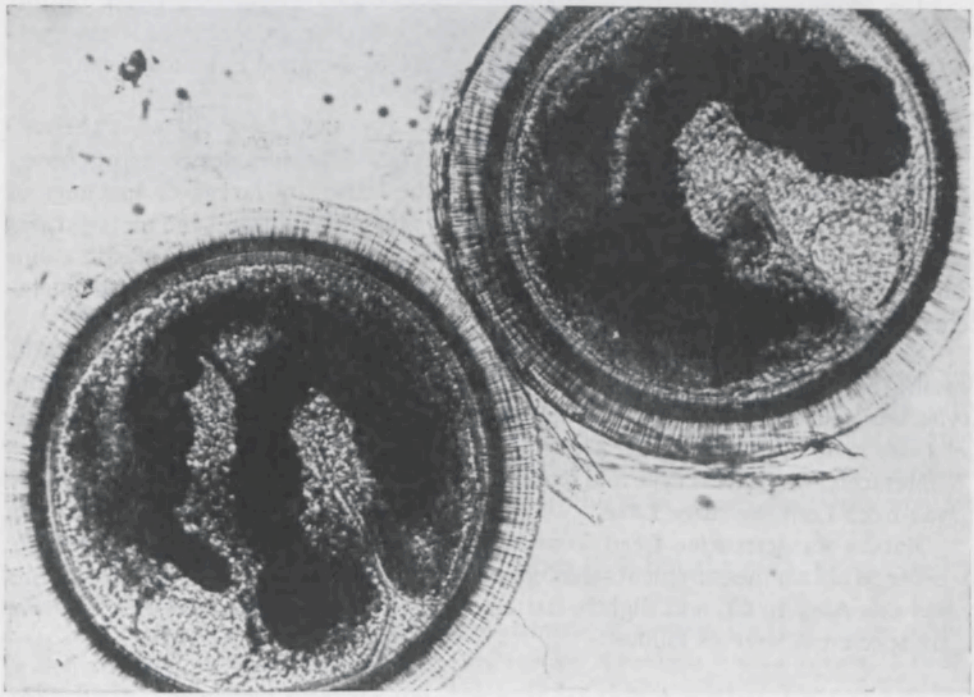

Fig. 5. (120 ×) Encysted metacercariae. (*Photo J. Bakker*).

Cysts (Fig. 5).

The diameter of the cysts found in larvae of *Cordulia aenea* varied from 0.36 to 0.64 mm, the differences being caused chiefly by a variation in the thickness of the wall; the size of the lumen was more constant. *Prosthogonimus* cysts from *Aeshna cyanea* larvae were much smaller and of a slightly oval shape. This finding was important as it shed a new light on the question of species differentiation.

The only other Eurasian species the cyst of which is known is *P. putschkowskii*. ONO[5] found it in *Anax parthenope* in the vicinity of Mukden. PANIN[10] examined 1,800 specimens of *Prosthogonimus* spec. from different hosts and on the strength of this classified *P. putschkowskii* under *P. cuneatus*. Mature specimens of *P. putschkowskii* from the fabrician sac of the hen cannot be differentiated from *P. ovatus* from the fabrician sac of the same host. The marked difference in measurements of

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the cysts of the two species, as well as the differences as regards the second intermediate hosts, might be considered objections against this identification. The table shows the measurements of the cysts from *Anax parthenope*[5] and other hosts.

MEASUREMENTS OF CYSTS IN MM

Host	*Average diameter*	*Large specimen*	*Small specimen*
Cordulia aenea	0.508	0.65	0.36
Aeshna cyanea	0.389–0.376	0.427–0.407	0.332–0.330
Anax parthenope	0.36 –0.33	?	?

The finding of the cysts in *Aeshna cyanea* throws a bridge between *P. ovatus (forma cuneatus)* and *P. putschkowskii*, both as regards the measurements of the cysts and the second intermediate host; the species may be assumed to be identical.

Metacercariae

The metacercaria is the stage in the life cycle of Trematodes occurring in the second intermediate host; in *Prosthogonimus* the latter are larvae or imagines of dragonflies. The tail of the cercaria has been lost; this differentiates the metacercaria from the cercaria. The immature metacercariae are enveloped by an elastic membrane; the youngest specimens have a stylet which later on is shed. When the metacercariae grow older they become encysted.

Measurements: 24 hours after infestation, length 120 μ, breadth 60 μ, stylet still present *(Fig. 3)*. After 28 days the length is 300–400 μ, the breadth 250–300 μ. The stylet has gone and the intestinal branches are clearly discernible in this stage; as yet there is no cystic wall *(Fig. 4)*.

Metacercariae of this type and of varying size have often been found in dragonfly larvae from Lonnekermeer Lake.

Mature metacercariae freed from the cysts were very mobile and contractile. In order to obtain measurements that could be compared with those of mature worms it was necessary to fix, and slightly flatten, them (*Fig. 6*). The measurements in mm in six specimens were as follows:

Length of body	0.987	0.980	1.114	1.043	1.021	1.088
Breadth of body	0.324	0.494	0.381	0.366	0.422	0.300
Diameter oral sucker	0.125	0.141	0.142	0.133	0.122	0.122
Diameter ventral sucker	0.141	0.179	0.166	0.155	0.144	0.133

B. THE FIRST INTERMEDIATE HOST

Experiments in snails

Infection of Bithynia tentaculata. The snail *Bithynia tentaculata* belongs to the Prosobranchiata; it is very common in the Netherlands. Prior to the closing of the Zuyder Sea the species did not occur in North-Holland north of the river IJ but as the water gradually became less salty *Bithynia tentaculata* appeared in the IJssel Lake; today it is present in the entire province of North-Holland.

In the Frisian Islands it has been found only near Formerum (Terschelling). The species occurs especially in water with many water plants[14], also on the undersurface of floating clumps of *Iris, Carex* and *Conium* spp. Experiments confirmed the reports[15] that it is a true detritus eater.

Bithynia tentaculata occurs in Europe, Asia Minor, Siberia and North Africa.

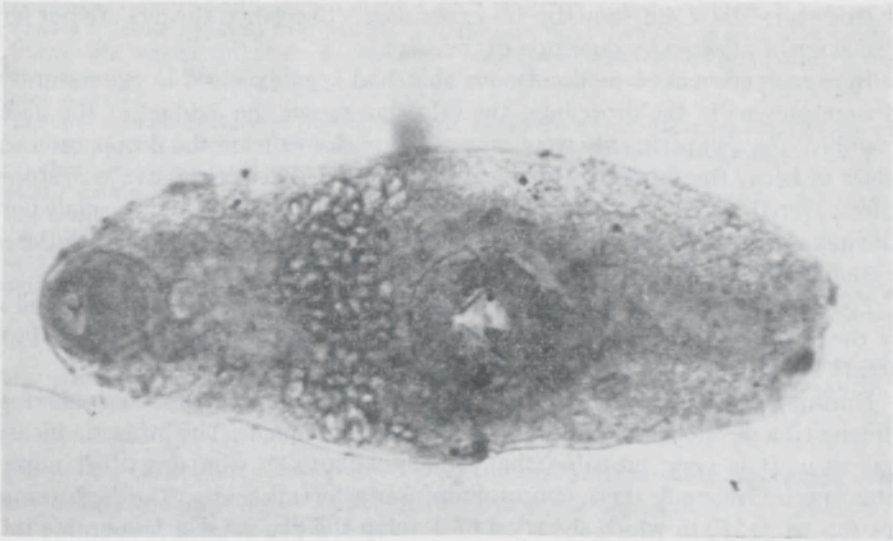

Fig. 6. (140 ×) Mature metacercaria freed from the cyst.

Generally speaking, infestation of the first intermediate host of a trematode may come about in three ways, *viz.*,

(1) A free-swimming larva (miracidium) develops from the ovum after a few days or weeks and bores into the body of the snail; Fasciola, Paragonimus, Echinostoma, Paramphistomides are examples of this. When laid the ovum is not embryonated.

(2) The ovum that is already embryonated when laid is swallowed whole by the snail; the larva emerges from the ovum inside the snail (examples: Opistorchides, Heterophyides, Dicrocoeliides).

(3) The ova are already embryonated when laid, but the miracidium gets free (Schistosomides).

MACY[4] fed eggs of *P. macrorchis* to specimens of various kinds of snails, among which the first intermediate host of this species *Amnicola limosa porata*, a prosobranchiate. The genera *Bithynia* and *Amnicola* are closely related. The ova had been laid by worms from the oviduct of a hen; they had been obtained according to the method developed by KRULL[16] for collecting ova of lung trematodes of frogs; the trematodes are placed in cold water, after which they yield masses of ova. MACY's experiments had negative results; only operculated eggs were found in the faeces of the snails. The latter were not infected and no cercariae were found. When placed in water, the ova showed no signs of development.

This failure may be attributed to a faulty method in collecting the ova; KRULL's method was meant for trematodes in cold-blooded animals. If the ova embryonate before they leave the uterus or the body of the host, this will happen at the temperature of the water. However, in trematodes in warm-blooded animals, such as *Prosthogonimus*, embryonation takes place at the host's body temperature. It could clearly be observed that the P. ova underwent a change during their passage through the uterus; the ova in the first convolutions are violet in colour, the colour gradually changing to brown; the ripe ova that are excreted are completely brown.

However, MACY did show by his experiments that when the ova are not fertile, the shell is not affected by digestion of the snail.

In my experiments I used only ova that had been excreted in the natural way and were present in the droppings, the fabrician sac or the oviduct of the host. In September, 1957, experiments were carried out with ova from the droppings and the oviduct of hens; the droppings were carefully rinsed. Part of the ova was stored in bottles covered by black paper; only the neck was left uncovered. A small portion of the ova was placed in a container under the Utermöhl microscope so that the same ova could be examined every day.

For a few months no development in the eggs was observed; after this period they gave the impression of being empty. The operculum was usually still in place, but the contents had apparently "flown away".

During the experiment large masses of bacteria and small pear-shaped infusoria with long cilia developed in the rinsed-out faeces and mucus. The infusoria measured about 25 μ. It is very probable that KRASNOLOBOVA[17], who described miracidia of this species, mistook these coprozoic infusoria for miracidia. The high temperatures (up to 34° C) in which she tried to develop the ova is most favourable for the development of the infusoria. On the other hand, the danger that the ova perish if they stay long in these high temperatures after being excreted from the host's body is certainly not imaginary.

In November, 1958, the experiment was repeated in the same way with ova from the oviduct and faeces of a Khaki Campbell duck and with ova from the fabrician sac of a young hen. No development of the ova was observed during a period of six weeks; no miracidia emerged from the eggs.

We then tested the possibility that the eggs are swallowed whole by the snail. The litterature shows that this possibility is much more probable than the foregoing.

SLUITER *et al.*[18] recorded a large number of trematode cycles, showing that in cases in which there was a question of swallowing of the eggs by the first intermediate host, the ova had measurements which resemble those of the *Prosthogonimus* ova, and that they were embryonated. If there was a question of a free-swimming larva, the ova were markedly larger. This is confirmed by Oshmarin[19].

Experiment

The ova, in droppings or mucus that had rotted for a few weeks, were spread on the bottom of a petri dish. Checking the detritus for the content in ova hardly ever revealed an empty shell.

As tapwater often killed the snails, the petri dishes were filled up with water from the aquarium in which the snails were kept. The latter were kept for 24 hours in the petri dishes where they crept about actively; the quantity of detritus decreased considerably during this period. Subsequently the snails were placed in dishes with clean water and left there for 48 hours. The faeces produced in these dishes were examined under the microscope, the findings being as follows:

Date	*Host*	*Organ*	*Full ova*	*Empty shells*
Nov. 1958	KO 13	oviduct	1	8
,, 1958	WF 12	fabrician sac	0	9
June 1959	RO 32	oviduct	1	8
,, 1959	,, 32	,,	1	12
,, 1959	,, 32	,,	8	9
,, 1959	KO 22	,,	2	9
,, 1959	,, 22	,,	0	8

The large number of full eggs in the third count of RO 32 were due to the fact that six eggs clung closely together; possibly they had stuck to the outside of the snail, in spite of precautions.

Our results suggested that the infestation of snails takes place in this way; MACY's results gave corroboration. If this had been a case of simple digestion, the ova in MACY's test would also have been digested.

The twelve snails infected in November, 1958, were kept alive until the spring of 1959, when six specimens died. In three of the latter the central intestinal gland could be examined; it was nearly entirely replaced by a mass of unripe sporocysts. The structure of the latter strongly suggested *Prosthogonimus*; however, many sporocysts bear a close resemblance to each other, so that this point cannot be considered proved.

On July 18th, 1959, a *Bithynia* was examined; its central intestinal glands contained a sporocyst that was nearly mature; three cercariae were already so. The latter measured 142.6 μ in bodylength, 53 μ in body-breadth, 114.7 μ length of tail, 21 μ breadth of tail, 20.5 μ length of stylet, 4.4 μ breadth at base of stylet, 35.7 μ oral sucker, and 18.6 μ ventral sucker These measurements agree with those found of *Prosthogonimus* cercariae.

The snails used in this experiment were collected in the vicinity of Amsterdam where *P. ovatus* does not occur, on the bank of a much used ship canal where birds seldom alight; 20 of these snails were examined for cercariae, without result.

These and earlier tests have proved that the first intermediate host of *P. ovatus* infects itself by the ingestion of embryonated ova of the parasite.

Behaviour of the cercaria

The *P. ovatus* cercaria behaved differently from all other xiphidocercariae observed during our investigation; the latter were all distinctly creeping cercariae which did not swim much. They were very difficult to loosen from the substrate by means of a waterstream from a pipette. They often floated with a curled tail.

The *P. ovatus* cercaria scarcely crept; it sat quietly with an upturned tail and continuous action of the anterior sucker could be observed. It reacted immediately to movements in the water by releasing its hold on the substrate and rolling with the movement. However, even without movement in the water the cercaria swam a great deal compared with related *Plagiorchis* cercariae; on the whole the latter also swam more slowly. Still, no directed search for a host could be established.

The cercaria was weakly negative-phototropic; this is evidenced under the microscope by the markedly uncommon appearance in the lighted part of the surface; when the entire surface was uniformly lighted the animals were equally distributed.

The reaction of the cercaria to a slight movement of the water by starting to swim is quite suitable for directing it towards the dragonfly larvae which serve as second intermediate hosts and which by their respiration cause circulating movements in the water. However, there is no question of an active search of the larva, as was proved also by other data such as the distribution of the cysts over the larvae of a population, the proportion in infestation in first and second-year larvae of a single site, and the difference in infestation between larvae in different localisations.

This makes it impossible for *P. ovatus* cercariae to enter an insect larva if there are no respiratory currents to draw them into the anus.

Development of Prosthogonimus in the first intermediate host. Ova (Fig. 7)

The ova are oval in shape, one pole being provided with a small lid, the other with a small spine. The colour is light brown and transparent. The measurement 24–32 μ in length, 15–20 μ in breadth.

At the moment of excretion the eggs contain an unmistakable embryo with a

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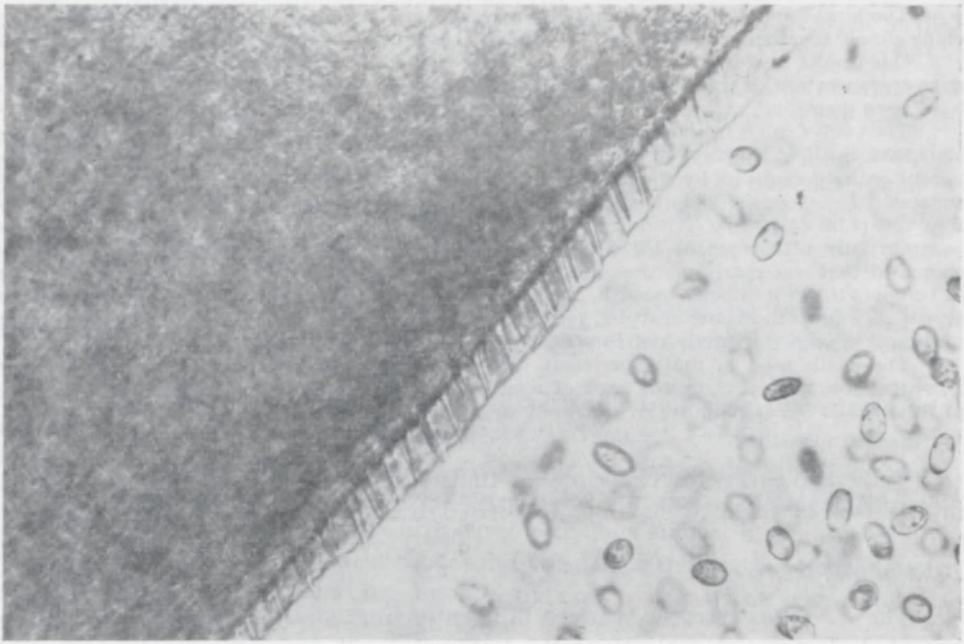

Fig. 7. (200 ×) Cuticula spines of *P. ovatus*. Many ova visible on the right. (*Photo J. Bakker*).

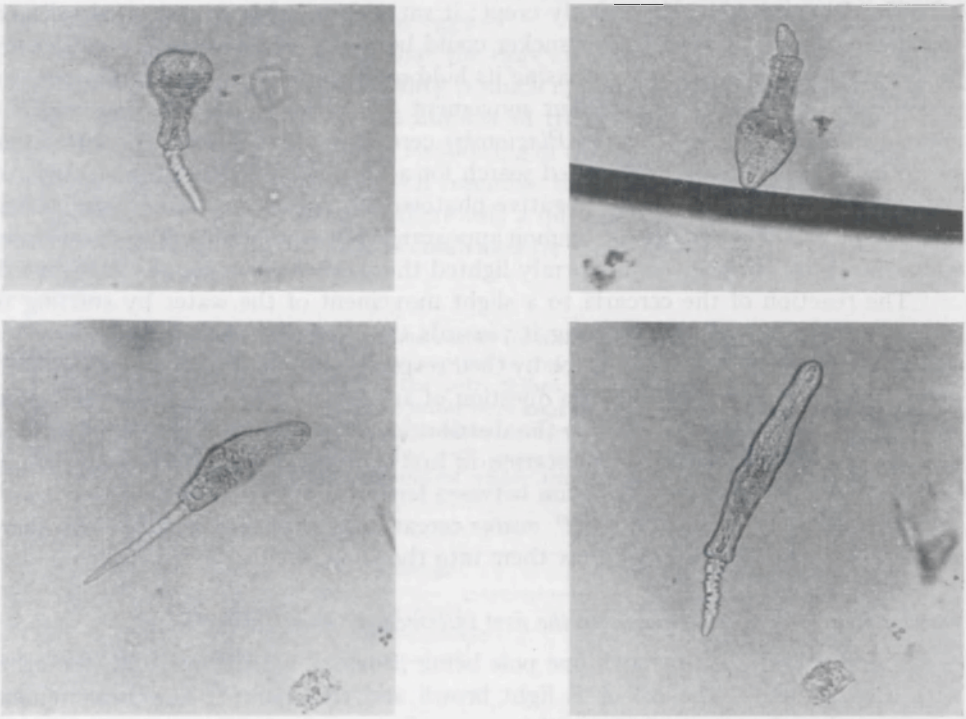

Fig. 8. P. ovatus cercaria; four photographs of the live animals taken with Leitz microscope with Visoflex and Leica. (microflash). (*Photos W. L. v. Utrecht*).

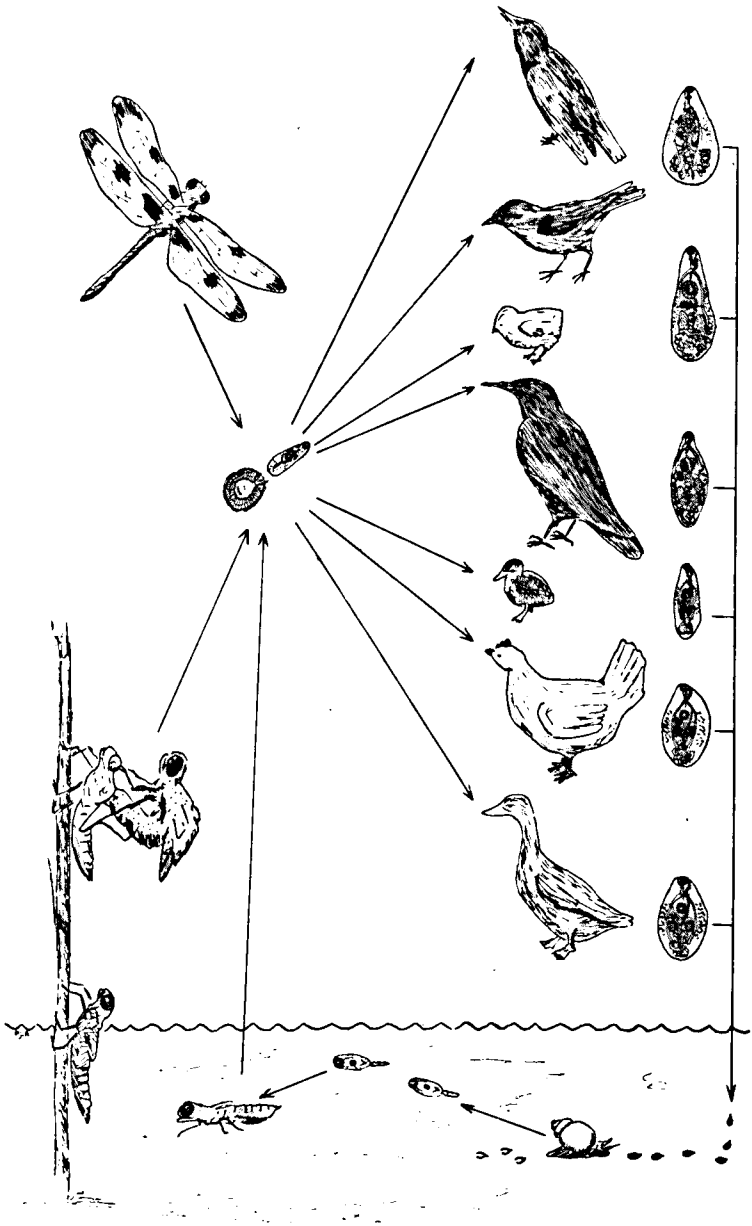

Fig. 9. Diagram of life cycle of *Prosthogonimus ovatus*. (*Drawing P. J. Roos*).

point which lies below the lid. Against the wall of the ovum there is a layer of cells which surround the embryo.

The eggs are excreted with the droppings of the host; in our experiments ova were detected several times in the faeces of hosts which had an infestated oviduct. In no instance did we find a P. ovum in faeces of hosts with a fabrician sac. This

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was due not only to the often more massive infestation of the oviduct but also to the much larger production of ova of specimens from the oviduct.

Sporocyst

The sporocyst of *P. ovatus* is lodged in the central intestinal gland of *Bithynia tentaculata*, often in large numbers. They are nearly round globules, yellowish white in colour; when mature, the sporocysts are transparent. The measurements of mature specimens vary in size; if the shape is nearly round, the diameter is 150–100 μ. When it is more oval, the length varies from 130–220 μ, the breadth from 95–155 μ.

This sporocyst bears a very close resemblance to that of *Cercaria helvetica* XII[12]; the latter measures 120–170 μ in length and 90–145 μ in breadth.

In young sporocysts little or no structure is discernible. The mature specimen contains one to three cercariae and an undifferentiated mass.

Cercaria

It is a xiphidocercaria bearing a stylet in the anterior sucker *(Fig. 8)*. The body-length and breadth in moving animals is variable; in resting conditions the length is 120–135 μ, the breadth 60–65 μ. Tail in rest 70 μ in length, 19 μ in breadth. The anterior sucker is distinct, 32–37 μ in diameter. The ventral sucker is less clearly discernable, 17–19 μ in diameter. Stylet 20–23 μ long, breadth at the base 4–5 μ. The point is distinctly winged. Penetration cells three on each side. No flame cells were distinguished with certainty.

The cercaria bears a very close resemblance to *Cercaria helvetica* XII[12], although the stylet of the latter is smaller. The measurements of *Cercariae helvetica* XII are body-length 160–180 μ (stretched) and 70–95 μ (contracted); body-breadth 30–35 μ (stretched) and 70–80 μ (contracted); length of tail 120–150 μ (stretched) and 70 μ (contracted); breadth of tail 12–16 μ (stretched) and 20–28 μ (contracted); oral sucker 26–31 μ, average 17 μ, in diameter; three penetration cells on each side; stylet 16–17 μ.

At any rate, the *Prosthogonimus* cercaria belongs to the *Cercariae microcotylae* (Lühe) and among those to the sub-group *helvetica*[12]. Other points of resemblance between the *Prosthogonimus* cercaria and *Cercaria helvetica* XII are the host, *Bithynia tentaculata*, and the shape and measurements of the sporocyst.

SUMMARY

Several species of Anisoptera function as second intermediate host to *Prosthogonimus*. Infestation takes place in the larval stage of the dragonfly. In the course of the present investigation three new second intermediate hosts were found: *Orthetrum cancellatum*, *Leucorrhinia caudalis*, and *Aeshna cyanea*. The factors determining the infection of the dragonfly larvae are discussed in detail, as are the differences in environment and climate between the European species *P. ovatus* and the American species *P. macrorchis*.

Statistical analysis of the distribution of the cysts among the larvae proved that infestation takes place accidentally.

Experimental infestation of dragonfly larvae yielded nearly mature metacercariae.

The snail *Bithynia tentaculata*, which serves as the first intermediate host,

infests itself by ingesting the ova of the parasite; experimental infestation of the snails with *Prosthogonimus* yielded sporocysts and cercariae.

The behaviour of the cercaria is described in detail, attention being directed towards the correlation between the behaviour of the cercaria and the mode of infestation of the dragonfly larvae.

A description is presented of the ovum, sporocyst and cercaria of *Prosthogonimus ovatus*.

RESUMEN

II. Los huéspedes intermediarios. Experimentos con la libélula Cordulia aenea. Varias especies de *Anisoptera* funcionan como huéspedes intermediarios del *Prosthogonimus*. La infección se realiza en el estado larval de la libélula. En el curso de la investigación se han descubierto tres nuevos huéspedes intermediarios secundarios, a saber: *Orthetrum cancellatus, Leucorrhinia caudalis* y *Aeshna cyanea*. Se discuten los factores que determinan la infección de las larvas de *Cordulia aenea*, así como las diferencias en el ambiente y el clima entre la especie europea *P. ovatus* y la americana *P. macrorchis*.

Se comprobó mediante un análisis estadístico de la distribución de los quistes en las larvas, que la infección se efectúa accidentalmente. La infección experimental en las larvas de la libélula produjo metacercarias apenas maduras.

El caracol *Bithynia tentaculato* que sirve como primer huésped intermediario, se infecta a sí mismo por la ingestión de los huevos del parásito; la infección experimental de los caracoles con *Prosthogonimus* produjo esporoquistes y cercarias.

Se describe detalladamente el hábito de las cercarias, dando especial atención a la relación entre el comportamiento de las cercarias y el modo de infección de las larvas de la libélula.

Se ofrece una descripción del huevo, del esporoquiste y de la cercaria de *Prosthogonimus ovatus*.

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III. TAXONOMY AND ECONOMICAL ASPECTS

DIFFERENT FORMS AND CHARACTERISTICS OF THE SPECIES

The results of the infection tests in birds emphasize first and foremost the marked difference between specimens from the fabrician sac and from the oviduct. The *development of the suckers* is a most important point, as well as the proportion in size between the ventral and the oral sucker. The literature holds that the diameter of the suckers and the proportion between the diameters of the two suckers are important characteristics for determination. Our investigations established that these diameters, as well as the proportions between them, depend on the organ in which the parasite grows and on the age of the worm.

The proportion is of taxonomic value only if specimens of approximately the same age, size, host and organ are compared; for instance, when comparing MACY's[1] data on *Prosthogonimus macrorchis* with those on *P. ovatus*, distinct differences may be observed.

The position of the ovary is another point to which taxonomic importance is attached: whether it is at the level of the ventral sucker or caudally of the ventral sucker. The position depends on the host, the organ and the age. In large specimens from the fabrician sac of the jackdaw, the position of the ovary may markedly be altered by flattening out the worm.

Intercoecal uterus is a characteristic mentioned for a large number of species from the fabrician sac of several kinds of ducks. However, this characteristic is purely artificial. PANIN[2] substituted a differentiation into "uterus between intestinal branches" and "uterus slightly protruding from between the intestinal branches". All the same, no matter how this characteristic is formulated, it lacks all taxonomic value. It is a typical host modification of specimens from the fabrician sac of ducks. *P. macrorchis* from the duck's fabrician sac also has an intercoecal uterus, as had *Schistogonimus rarus*.

The position of the *vitelline glands* depends grearly on the degree of contraction in the worm. If in fixation the degree of contraction on the left and right sides differs, marked differences in the position and the distance over which the vitelline glands extend may occur in one and the same animal.

Length of the *cirrus pouch*. This organ is very contractile, the length depending on the degree of contraction. When a score of specimens are obtained in a single infestation test and are completely uniform in a number of characteristics, it is easy to prepare a series of fixed specimens with cirrus pouches in all stages of contraction, from "reaching to the intestinal fork" to "reaching to the ventral sucker".

The distance between the ventral sucker and the intestinal fork (difference between *P. brauni* and *P. cuneatus*) depends on the degree of contraction of the worm. In a regularly contracting live specimen, the distance becomes smaller when the worm contracts and larger when it stretches. The length of the *cuticula spines* depends on the size of the specimen; the larger the worm, the longer the spines.

Size of eggs. The eggs in the uterus of young, immature specimens are smaller than those excreted by mature specimens. Measuring the eggs in the uterus is rejectable.

Conclusion. None of the characteristics on the strength of which the species of the *Prosthogonimus* genus used to be differentiated are by themselves suitable; in our opinion they are quite useless except one, the proportion between the diameters of the suckers. This means a total subversion of the taxonomy of this genus. In addition, it is difficult to substitute other criteria.

There are significant differences in the development of the suckers of *P. ovatus* and *P. macrorchis* in comparable phases of development. This might also hold good for other species, such as *P. vitellatus* from Australia, an isolated continent as regards its fauna.

Characteristics in other phases of the cycle

There is such a variety of second intermediate hosts of *P. ovatus* and *P. macrorchis* that a differentiation between *Prosthogonimus* species on the strength of this point is quite impossible.

The thickness of the cystic wall and the diameter of the cysts, are also variable in different second intermediate hosts of *P. ovatus*. Although only a single first intermediate host is known for *P. ovatus* and for *P. macrorchis, viz. Bithynia tentaculata* and *Amnicola limosa porata*, respectively, this does not mean that only these snails may act as first intermediate hosts to these species. *Bithynia tentaculata* occurs in large parts of Eurasia. Its oecological substitute in regions where the snail does not occur might also take its place as a first intermediate host of *P. ovatus*. In this connection it is important that the ways of life of *Amnicola limosa porata* and *Bithynia tentaculata* are identical in many respects.

It is obvious that another first intermediate host may also cause differences in sporocysts and cercariae; this is suggested by wide variations in other phases of *P. ovatus*.

Really reliable species differentiation in the *Prosthogonimus* genus is difficult to obtain by the old methods, with the exception of the differentiation between *P. macrorchis* and *P. ovatus*.

Perhaps a chromosome-count in specimens of *Prosthogonimus* from different parts of the world might facilitate the species differentiation; young, rapidly growing specimens from the oviduct offer an excellent opportunity for this.

TAXONOMY

Thirty-one species of the *Prosthogonimus* genus have been described; 26 of these are mentioned and for the most part depicted by DOLLFUS[3], the remaining five are described by JAISWAL[4]. WITENBERG AND ECKMAN[5] attempted drastically to reduce the number of species on taxonomic grounds; they included only 21 species in their considerations.

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Nomenclature proposed by WITENBERG AND ECKMAN[5]

Prosthogonimus karausiaki Layman 1926[6] *P. anatinus* Markov 1902[7] *P. furcifer* Railliet 1924[8]	Species inquirendae
P. brauni Skrjabin 1919[9] *P. folliculus* Reid and Freeman 1936[10] *P. fülleborni* Skrjabin and Massino 1925[11] *P. horiüchii* Morishita and Tsuchimochi 1925[12] *P. macrorchis* Macy 1934[14] *P. querquedulae* Yamaguti 1933[15] *P. putschkowskii* Skrjabin 1913[13]	*P. putschkowskii* Skrjabin 1913[13], probably identical with *P. cuneatus* Rudolphi 1809[16]
P. orientalis Yamaguti 1933[15] *P. skrjabini* Zakharov 1920[18] *P. rudolphii* Skrjabin 1919[9]	*P. rudolphii* Skrjabin 1919[9]
	P. ovatus Rudolphi 1803[17] *P. dogieli* Skrjabin 1916[19]
P. japonicus Braun 1901[20] *P. leei* Hsü 1935[21] *P. longus morbificans* Seifried 1923[23] *P. pellucidus* v. Linstow 1873[22]	*P. pellucidus* v. Linstow 1873[22]
	P. vitellatus Nicoll 1915[24, 25]

This simplification takes no account of the geographical distribution of the species. Out of the seven maintained species, five have been recorded in Eurasia, *P. dogieli* (British East Africa) having been found in a European migratory bird (*Hirundo rustica*). *P. vitellatus* (Australia) is the only species outside the region of occurrence of the other six species.

PANIN[2] examined 1,800 specimens of *Prosthogonimus* spec. and compared the characteristics; he also included five species described since WITENBERG AND ECKMAN[5], namely, *P. indicus* SRIVASTAVA[26], *P. macroacetabulus* CHAUHAN[28], *P. penni* KU[27], *P. sinensis* KU[27], and *P. pseudopellucidus* TUBANGUI AND MASILUNGAN[29].

Nomenclature proposed by PANIN[2]

I. Prosthogonimus subgenus	*P. ovatus* *P. dogieli*	
II. Macrogenotremata	*P. longus morbificans* *P. macrorchis* *P. cuneatus* *P. vitellatus*	*P. pellucidus* *P. japonicus* *P. putschkowskii* *P. brauni* *P. furcifer* *P. fülleborni* *P. leei* *P. folliculus* *P. sinensis* *P. penni* *P. pseudopellucidus* *P. macroacetabulus* *P. indicus* *P. cuneatus*
III. Mediogenotremata = *P. anatinus*		*P. skrjabini* *P. rudolphii* *P. horiuchii* *P. orientalis* *P. querquedulae* *P. karausiaki* *P. anatinus*

PANIN took more account of the geographical distribution than did WITENBERG AND ECKMAN; he maintained the American species *P. macrorchis*. That PANIN maintains *P. anatinus*, and WITENBERG AND ECKMAN *P. rudolphii* is a difference of minor importance as WITENBERG AND ECKMAN classified *P. anatinus* among the species inquirendae, thereby excluding it from the group in which it is classified by PANIN.

Two other obviously identical species in the two classifications are *P. pellucidus* and *P. longus morbificans*. VON LINSTOW described *P. pellucidus* as a species in which the proportion of the size of the oral to that of the ventral sucker was 1 : 1.3; BRAUN[31] recorded the proportion as being 1 : 1.22, HIERONYMI AND SZIDAT[30] as 1 : 1.08. These figures all lie within the limits of the proportions found in specimens from the oviducts of the hen and the duck. SEIFRIED[23] recorded the following measurements for *P. longus morbificans:* oral sucker 1.12 mm in length, 1 mm in breadth, ventral sucker 1.16 mm; proportion 1 : 1.09 (*i.e.*, also within the limits).

During my investigation, "typical" specimens were cultivated of *P. pellucidus*, *P. longus morbificans*, *P. cuneatus*, *P. ovatus*, *P. rudolphii*, *P. anatinus*, and *P. fülleborni*. All were grown from cysts found in a single intermediate host (*Cordulia aenea*) from a single site (Lonnekermeer Lake). "Typical" specimens of the different "species" were cultivated by a careful selection of the final hosts and by estimating the age of various type specimens described in the literature[32]. The yield test proved that all the cysts belonged to a single species; the very high yield excludes the possibility of selection by the organ or the host.

The result of the transplantation test[32] is another proof of the marked influence of the organ on the habitus of the worm. The results of these tests, coupled with a critical consideration of the differences between, and the characteristics of, the various species, leave no shred of the species classification of the genus as it was accepted up to the present. It has become clear that *in the whole of Asia and Europe there is question of only a single species* which comprises all the species recorded for these continents. For reasons of priority I would suggest naming the species *Prosthogonimus ovatus* Rudolphi, provided it is agreed to maintain this name in a much wider sense.

No doubt *P. dogieli* from British East Africa also belongs to this species, as it has been recorded in a European migratory bird, *Hirundo rustica*. The type specimen of *P. dogieli* was an old (older than five weeks) specimen, so that the chance that the swallow had been infested in its European breeding place is great; in addition, the swallow was a young bird still possessing a fabrician sac. It could not have been in Africa for any length of time, for it was shot by an expedition that ended about august 15th.[19] *Hirundo rustica* does not breed in East Africa, but hundreds of thousands of them winter in the region of the large lakes[33].

The only species which for valid reasons may be maintained side by side with *P. ovatus* Rudolphi is *P. macrorchis* Macy 1934[1]. In his description of the latter species, MACY reported that the proportion between the sizes of the oral and the ventral suckers is constant, 1 : 1.8–1.7. This proportion was found in specimens from the fabrician sac of a crow, a sparrow, a duck and a hen, as well as in specimens from the oviduct of the duck and the hen. According to MACY, the proportion between the suckers is the most constant characteristic of the species.

Nonetheless, a shift in the proportion between the suckers must take place during growth of the trematode, as the proportion between the sucker of the *P. macrorchis*

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metacercaria is 1 : 1.25. Apparently this shift in proportion is independent of the organ in which the worm is growing and, also, the shift is less marked than in specimens of *P. ovatus* growing in the fabrician sac. (No shift in the proportion between the suckers occurs in *P. ovatus* specimens in an oviduct.)

This difference in development is a distinct differentiation between *P. macrorchis* and *P. ovatus*. Apart from this, there are differences between the cercariae, the metacercariae and the sporocysts.

P. folliculus Reid and Freeman [10] probably also belongs to *P. macrorchis*; the differences are slight and the two species occur in the same region.

P. vitellatus Nicoll 1915[24,25], from Australia, is a dubious species, the findplaces of which lie far outside those of the other species. Detailed study of the cycle and development of this species may establish whether it is indeed a species apart.

Nomenclature proposed in this paper

(1) *P. ovatus* Rudolphi 1803 (emend. Boddeke) to be identical with: *P. karausiaki* Layman 1926[6]; *P. anatinus* Markov 1902[7]; *P. furcifer* Railliet 1924[8]; *P. brauni* Skrjabin 1919[9]; *P. fülleborni* Skrjabin and Massino 1925[11]; *P. horiüchii* Mor. and Ts. 1925[12]; *P. querquedulae* Yamaguti 1933[15]; *P. putschkowskii* Skrjabin 1913[13]; *P. cuneatus* (Rud. 1809) Braun 1901[20]; *P. orientalis* Yamaguti 1933[15]; *P. skrjabini* Zakharov 1920[18]; *P. rudolphii* Skrjabin 1919[9]; *P. ovatus* (Rud. 1803) Braun 1901[20]; *P. dogieli* Skrjabin 1916[19]; *P. japonicus* Braun 1901[20]; *P. leei* Hsü 1935[21]; *P. longus morbificans* Seifried 1923[23]; *P. pellucidus* v. Linstow 1873[22]; *P. indicus* Srivastava 1938[26]; *P. macroacetabulus* Chauhan 1940[28]; *P. penni* Ku 1941[27]; *P. sinensis* Ku 1941[27]; *P. dollfusi* Jaiswal 1957[34]; *P. ketupi* Jaiswal 1957[34]; *P. mesolithicus* Jaiswal 1957[34]; *P. hyderabadensis* Jaiswal 1957[34]; *P. singhii* Jaiswal 1957[34]; *P. pseudopellucidus* Tubangui and Masilungan 1941[29].

(2) *P. macrorchis* Macy 1934[14] probably identical with *P. folliculus* Reid and Freeman 1936[10].

(3) *P. vitellatus* Nicoll 1914[24] (no opinion).

ECONOMIC ASPECTS OF THE *PROSTHOGONIMUS* GENUS

Since 1921 much has been published on Prosthogonimiasis, a name covering the pathological symptoms caused by an infection of the oviduct in poultry by *Prosthogonimus* spec. The majority of the publications deal with chickens and the finding of *Prosthogonimus* specimens in an egg. To our knowledge, none of the publications mentioned the species of hen in which the disease was observed.

SZIDAT[35] stated that a few worms may give rise to serious disturbances and that 40 to 50 worms mean the death of the hen, often before the worms have reached maturity.

Experiments in test hens RO 11 and 12 revealed 55 and 64 worms, respectively[32]. Apart from the fact that laying stopped abruptly, the birds showed no clinical symptoms, and they were lively and ate well; the comb was red and the feathers smooth and glossy. The oviduct was normal and undamaged. In another test with hen RO 32, 33 mature worms were found, but the hen showed no symptoms apart from the fact that it stopped laying within 48 hours.

It appeared improbable that the resistance in the laboratory hens would be stronger than in barnyard chickens because of better care, as the hens did not arrive in the laboratory until a few days before they were infested. It is more logical to assume that the race of chicken played a part in this.

In MACY's[1] diagram of the life cycle of *P. macrorchis*, a White Leghorn is depicted as the final host. The only natural victim of dragonfly disease I observed in the summer of 1957 was also a White Leghorn. Poultry farmers near the Lonnekermeer Lake who often had dragonfly disease victims among their hens all declared that the latter were always White Leghorns, as this was the only species they ever kept. In the twenties and thirties the White Leghorn was practically the only race of poultry-farm chicken in the Netherlands.

In the last few years this race has been supplanted by crossbreeds of White Leghorns and Rhode Island Reds.

As our experiments in chickens were all made with Rhode Island Reds it was decided to carry out a control test with White Leghorns in order to establish whether the latter is less tolerant to infestation with *Prosthogonimus* than are Rhode Island Reds.

On June 23rd, 1959, two White Leghorns (WO 11 and 12) were infested with 62 and 77 + 18 cysts, respectively (WO 12 was infested a second time on June 26th). Shortly before, an experiment had been carried out in Rhode Island Reds which showed no clinical symptoms although they harboured respectively 7 and 33 large *Prosthogonimus* specimens.

During the experiment with WO 11 and 12, the birds did not give the impression that they were ill, although in both the comb became blueish. Section after 24 days revealed that WO 12 had a semi-functioning oviduct; there were only three large P. specimens. WO 11 had a well-developed oviduct containing 35 large P. specimens. It was remarkable that the worms were scattered through the entire oviduct; in tests in Rhode Islands Reds and tame ducks, the worms were lodged chiefly in the upper portion of the oviduct when the specimens were mature.

In the abdominal cavity we found five soft-shelled eggs and a quantity of egg-white and thin liquid. The outside of the oviducts had a white, flaky aspect.

The finding of soft-shelled eggs in the abdominal cavity of a hen infested with *Prosthogonimus* is not uncommon; there are several preparations of this kind in the Laboratory for Veterinary Parasitology, Utrecht.

It may be concluded from the foregoing that the White Leghorn is very sensitive to P. infection, whereas the Rhode Island Red is much more tolerant to the parasite.

For this reason, *poultry-farmers in areas where dragonfly disease occurs frequently, should be dissuaded from keeping the vulnerable White Leghorn.*

Although in Rhode Island Reds the egg production is temporarily stopped, the animals remain healthy and in due course they start laying again.

In order to restrict the economic loss to a minimum, the chickens should be kept in coops with finely-meshed wire-netting.

Effect of the Prosthogonimus infestation on egg production

The effect of a P. infestation on the egg production was striking in all experiments with full-grown hens. Often one more egg was laid on the day after infestation, but that was the end of it. Occasionally some egg-white and once a soft-shelled egg was found.

The severity of the infestation does not play a part; infestation with a small number of *Prosthogonimus* (RO 21 and 31) also stopped laying on the day after the infestation. Nor had the rate of growth of the worm any appreciable effect (RO 21).

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In tame ducks, the effect on the egg production was not so instantaneous as in hens, as evidenced by the experiment with birds KO 11, 12 and 13 from September 15th to October 20th, 1958. The ducks arrived on September 9th and they were infested on Sept. 15th and 16th. The birds were put in one coop so that in most instances it was impossible to ascertain which duck had laid an egg.

Two eggs were found on September 10th, one on the 13th, one on the 15th, and one on the 16th, laid by KO 13; the infestation took place on September 15th and 16th, after which two eggs were laid on the 17th, the 19th and 20th, and one on the 22nd. No more eggs were produced from September 23rd to October 20th.

This experiment showed that the effect of P. infestation on the egg production in ducks manifests itself considerably later than in hens, although the final result—complete cessation of laying—is the same as in chicken race that are tolerant to the infestation.

Ecological considerations on Prosthogonimus infestation

Which are the causes which lead to the occurrence of dragonfly disease in poultry?

(1) A rather dry region with few pools and ponds is conducive to the appearance of dragonfly disease. Dragonflies are predatory insects and they may occur in large numbers also in a dry area where they find less places suitable for breeding than they would in a region abounding in water. Consequently, enormous numbers of dragonfly larvae may be collected in the few favourable spots in a dry area, whereas in watery areas the larvae are scattered over large surfaces of water.

(2) A dense population of dragonfly larvae demands that the water be rich in food and that it be clear. Should the water be poor in food the larvae devour each other; this results in a regular, but thin distribution over the entire surface of a pool. This state of affairs is found in moor fens. No Anisoptera larvae are found in very polluted waters.

(3) The first intermediate host, *Bithynia tentaculata*, is not particular as regards its environment; it may occur in large numbers in polluted waters. However, like all snails, they cannot survive in acid water.

(4) An important factor for a severe infestation in dragonfly larvae is a large population of breeding birds; it is immaterial whether the birds are tame or wild. In cases of a large population of breeding waterbirds at the moment when the dragonflies get on the wing in the beginning of May, there will be many young birds with a fabrician sac, as well as full-grown birds with a functioning oviduct, that will become heavily infested. This will result in an enormous production of *Prosthogonimus* ova in the spring and summer.

The infestation of the oviduct is of major importance in the development of massive infection in a particular place, as *Prosthogonimus* reaches maturity sooner and the maximal number of adult worms is much higher in an oviduct than in a fabrician sac; so is the resulting production of P. ova.

The effect of a large number of birds on the degree to which dragonfly larvae are infested is illustrated by an observation by MACY[1]. In a pond in Minnesota, the dragonfly larvae on the side where the ducks were regularly fed by the public were much more heavily infested than elsewhere in the pond.

When a pond is occupied by tame ducks which lay eggs for the greater part of the

year (such as Peking ducks and Khaki Campbells) and which, therefore, have a functioning oviduct, the P. infestation in snails and dragonfly larvae is greatly increased. However, a pond housing large numbers of tame ducks will become badly polluted, the Anisoptera larvae disappearing as a result thereof, so that the pond ceases to be a focus of Prosthogonimiasis.

All these factors determine the measure of infestation with *Prosthogonimus* in snails, dragonfly larvae and for the most part in wild birds. However, this need not have a clearly discernible effect on the health of birds which are of importance for the economics of the area. This happens only if in the vicinity of such a focus there are many large-scale poultry-farms with a stock of White Leghorns, or another chicken race that is not tolerant to the parasite. When there are few chickens in an area there may be incidental cases of Prosthogonimiasis, but they will remain unnoticed as farmers who do not specialize in poultry-farming pay little attention to these birds.

The value of a hen is less than the cost of consulting a veterinarian. The normal course of things is that the chicken is slaughtered at an early stage of the infestation and the (gratis) advice of a chick-sexer is asked.

When more tolerant races of chickens are kept instead of White Leghorns, for instance Rhode Island Reds, the externally visible result of the infestation remains restricted to a cessation of laying and an occasional soft-shelled egg. As the laying of soft-shelled eggs may have a great many causes, it is improbable that the *Prosthogonimus* infestation is detected.

Apart from *Prosthogonimus*, there is still another trematode that may cause soft-shelled eggs and make the hens ill. This is *Plagiorchis arcuatus* STROM 1924[36] the life-cycle of which was studied by PASKALSKAJA[37]. She demonstrated that Agrionides belonging to the genera *Coenagrion* and *Lestes* may act as second intermediate host of *Plagiorchis arcuatus*. This trematode has not yet been observed in the Netherlands. Nonetheless, the possibility that in incidental cases it may cause symptoms resembling those of prosthogonimiasis cannot be excluded.

Poultry-farmers in the vicinity of Lonnekermeer Lake were conversant with the fact that the eating of dragonflies is harmful to the chickens. However, one of them stated that "the small blue ones are even more dangerous than the big ones", although he could not give exact data to support his opinion. As "small blue ones" can apply only to Agrionides, this might be an indication of the presence of *Plagiorchis arcuatus* in the Netherlands, as *Prosthogonimus* cysts cannot possibly be harboured by Agrionides, because the larva has a different system of respiration.

The life cycle of Prosthogonimus ovatus (Fig. 8).

The mature trematode lives in the oviduct and the fabrician sac of birds.

The ova of *P. ovatus* are excreted in embryonated condition with the faeces of the host. When the ova enter the water their development is possible only if they are ingested by the snail *Bithynia tentaculata*.

This snail acts as first intermediate host in which the larva emerges from the cyst. In the central intestinal gland a sporocyst develops from the larva.

Cercariae are formed inside the sporocyst; they emerge from it into the water. These cercariae are sucked into the intestine of Anisoptera larvae—that may belong to various species—by the latter's respiratory movements (second intermediate host).

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Inside the dragonfly larva, the cercariae develop into metacercariae which after vigorous growth form a radially striated cystic wall.

When the larvae or imagines of dragonflies containing *Prosthogonimus* cysts are ingested by a bird with a functioning oviduct or a fabrician sac, the metacercariae emerge from the cyst, due to the effect of trypsin in the avian intestine. The liberated metacercariae probably creep into the oviduct or fabrician sac via the cloaca. In these organs the metacercariae grow into mature trematodes.

SUMMARY

Taxonomy. In a critical consideration of the characteristics on which the taxonomy of *Prosthogonimus* is based and which have been tested experimentally for their taxonomical value, the author makes it clear that all these characteristics are useless in differiating the species, the only exception being the proportion between the diameters of the ventral and oral suckers. Provided the conditions are comparable—the same host, worms of the same age, developed in the same season—this proportion may be of value in species determination.

All species that have been described in Eurasia are classified by the author into a single species: *Prosthogonimus ovatus* (Rudolphi).

Economical aspects. The tolerance to *Prosthogonimus* infestation is much weaker in White Leghorn than in Rhode Island Red hens. For this reason it is recommended that the latter species be bred in areas where *P. ovatus* occurs frequently.

The factors leading to the outbreak of Prosthogonimiasis are discussed in detail.

A summary is given of the life cycle of *P. ovatus*.

RESUMEN

III. Taxonomía y aspectos económicos.

Taxonomía. Después de haber realizado un estudio crítico de las características que forman la base de la taxonomía de *Prosthogonimus*, el autor pone en claro que todas estas características no pueden ser usadas para la diferenciación de las especies. La única excepción válida sería la proporción entre los diámetros de la ventosa adhesiva oral y la ventral. Concluye que dicha proporción tiene valor para la determinación de las especies, siempre que las condiciones – el mismo huésped, vermes de la misma edad, desarrollados en la misma estación – sean comparables. El autor clasifica todas las especies descritas en Eurasia, como una sóla especie *Prosthogonimus ovatus Rudolphi.*

Aspectos económicos. La resistencia contra una infección con *Prosthogonimus* es mucho menor en gallinas "White Leghorn" que en gallinas "Rhode Island". Por esta razón el autor recomienda criar ésta raza en áreas donde el *Prosthogonimus ovatus* ocurre con frecuencia.

Se discuten detalladamente los factores que favorecen el brote de Prosthogonimiasis. Además se ofrece un sumario del ciclo vital de *P. ovatus*.

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KORTE SAMENVATTING

(1) Het is uit infectieproeven gebleken, dat alle soorten van het geslacht *Prosthogonimus* die in Europa en Azië beschreven zijn, samengebracht moeten worden tot een enkele soort voortaan te noemen: *Prosthogonimus ovatus* (Rudolphi) Boddeke.

Deze soort komt voor in de eileider of in de bursa fabricii van diverse vogelsoorten. Voor deze proeven zijn in totaal 38 vogels gebruikt behorende tot de volgende soorten: *Sturnus vulgaris, Corvus monedula, Corvus corone, Anas platyrhynchus* en *Gallus domesticus.*

De vogels werden geïnfecteerd met cysten van *Prosthogonimus* uit larven van *Cordulia aenea.*

(2) Uit proeven waarbij exemplaren uit de bursa fabricii in de eileider werden gebracht is gebleken, dat in de bursa fabricii de mondzuignap klein blijft. In de eileider wordt een uitgroeien van de mondzuignap gestimuleerd.

De parasieten bewegen zich in de eileider kruipend voort, beurtelings gebruikmakend van de beide zuignappen, in de bursa fabricii heeft de mondzuignap uitsluitend een zuigende functie.

(3) Een mannelijk stadium bij jonge exemplaren komt bij een snelle groei sterker tot uiting dan bij een langzame.

(4) De groeisnelheid van *Prosthogonimus ovatus* is afhankelijk van minstens 5 factoren:

(a) het jaargetijde. Dit is van grote betekenis. In de winter groeien de wormen uiterst traag. (b) Het aantal exemplaren dat in het orgaan aanwezig is. Hoe kleiner het aantal des te sneller is de groei. (c) Het orgaan. In de eileider groeien de wormen sneller dan in de bursa fabricii. (d) De aanwezigheid van oudere exemplaren remt de groei van de jonge exemplaren bij een nieuwe infectie. (e) De soort gastheer is van weinig belang. In de eileider van de kip gaat de ontwikkeling van de parasiet iets sneller dan in de eileider van de eend.

(5) De eerste tussengastheer van *Prosthogonimus ovatus* is de prosobranchiaat *Bithynia tentaculata.* De slak infecteert zich door het eten van de (geëmbryoneerde) eieren.

(6) Uit slakken die deze eieren hadden opgegeten, kon de *Prosthogonimus* cercarie gekweekt worden. Dece cercarie is ook op de vindplaats van de besmette libellenlarven gevangen. De *Prosthogonimus* cercarie komt overeen met *Cercaria helvetica* XII Dubois, 1929.

Met deze cercariën is met succes een infectieproef op libellenlarven gedaan. Na 4 weken bevatte een der libellenlarven 2 typische, practisch volgroeide metacercariën.

(8) Naast de reeds bekende tweede tussengastheren *Cordulia aenea* en *Libellula quadrimaculata* werden in het Lonnekermeer een aantal nieuwe gevonden: *Leucorrhinia pectoralis, Orthetrum cancellatum* en *Aeshna cyanea.*

(9) De afmetingen van de cysten zijn bij verschillende tweede tussengastheren niet constant. De afmetingen van de cysten uit *Aeshna cyanea* en de taxonomische positie van deze libel vormen een fraaie verbinding tussen *P. putschkowskii* en *P. ovatus.*

(10) De infectie van de libellenlarven vindt toevallig plaats. De beweging van de cercariën is niet gericht. De cercariën worden passief opgezogen door de ademhalingsbewegingen uitgaande van het rectum van de libellenlarve.

Het gedrag van de cercarie is zeer doelmatig voor het bereiken van de gastheer, aangezien zij onder invloed van beweging in het water van het substraat waarop zij zich bevinden, vrijkomen en gaan rollen, zwemmen of zweven.

(11) In Nederland is *Prosthogonimus ovatus* tegenwoordig zeer zeldzaam.

(12) Het Lonnekermeer bij Hengelo (Ov.) bleek een belangrijke, nieuwe haard van de parasiet.

(13) Er bleek een groot verschil te zijn in de tolerantie voor een infectie van de eileider met *Prosthogonimus ovatus* tussen de kippenrassen: Rhode Island Red en Witte Leghorn. Rhode Island Red kippen worden van een infectie niet ziek, al stopt de leg. Witte Leghorns worden ernstig ziek. Zij speciaal, vertonen de verschijnselen die bekend staan als libellenziekte (prosthogonimiasis).

(14) De invloed van een infectie met *Prosthogonimus* van de eileider op de eierproductie is bij tamme eenden na 6 dagen merkbaar, bij kippen reeds na 24–48 uur.

(15) Gunstig voor het optreden van Prosthogonimiasis onder pluimvee zijn de volgende factoren: (a) een vrij droge streek met weinig plassen en vijvers met voedselrijk niet vervuild water; (b) een grote broedvogelstand in en om de plassen; (c) talrijkheid van de beide tussengastheren; (d) het op grote schaal houden van (Witte Leghorn) kippen in de omgeving van de infectie haard.

КРАТКОЕ СОДЕРЖАНИЕ

1. Опыты искуственного заражения показали, что все виды рода Prosthogonimus, описанные в Европе и в Азии, должны быть соединены в один вид, в дальнейшем называемый Prosthogonimus ovatus (Rudolphi) Boddeke.

Этот вид находится или в яйцеводе или в фабрицевой сумке у различных видов птиц.

В этих опытах всего использовано 38 птиц следующих видов: скворец (Sturnus vulgaris L.), галка (Corvus (Coloeus) monedula L.), черная ворона (Corvus corone L.), домашняя утка-кряква (Anas platyrhynchos L.) породы Пекинг/молод./и породы Хаки Камбел/взросл./, домашняя курица (Gallus domesticus L.) породы Белая легхорн/молод. и взросл./и Род айленд ред/молод.и взросл./

Эти птицы были заражены инцистированными метацеркариями паразита Prosthogonimus, обнаруженными в личинках стрекозы Cordulia aenea.

2. Опыты трансплантации показали, что ротовая присоска паразитов, находящихся в фабрицевой сумке, остается небольших размеров. В яйцеводе, однако, рост ротовой присоски стимулируется. При ползущих движениях в яйцеводе паразиты пользуются обоими присосками попеременно. В фабрицевой сумке же ротовая присоска повидимому функционирует только как орган сосания.

3. Мужеская стадия проявляется у молодых экземпляров сильнее при быстром росте, чем при медленном.

4. Скорость роста Prosthogonimus ovatus зависит минимум от следующих факторов:

а. времени года. Этот фактор имеет большое значение. Зимой паразиты растут чрезвычайно медленно.

б. количества паразитов, находящихся в органе. Чем их меньше, тем быстрее их рост.

в. органа, где Prosthogonimus паразитирует. В яйцеводе паразиты растут быстрее чем в фабрицевой сумке.

г. присутствия более старых экземпляров. Эти тормозят рост молодых при новой инфекции.

д. вида хозяина, значение которого, правда, не велико. В яйцеводе курицы развитие паразита идет немного быстрее, чем в яйцеводе утки.

5. Первый промежуточный хозяин паразита Prosthogonimus ovatus — моллюск: Bythinia tentaculata. Этот моллюск заражается посредством питания неповрежденными яйцами паразита.

6. Из моллюсков поедающих эти яйца выращивались церкарии вида Prosthogonimus ovatus. Те же самые церкарии были найдены и в местах где находились зараженные личинки стрекоз. Церкарии вида Prosthogonimus ovatus соответствуют описанию Cercaria helvetica XII Dubois, 1929.

Этими же церкариями проводились с успехом опыты искуственного заражения личинок стрекоз. Через четыре недели в одной из личинок найдены были две почти вполне развившиеся метацеркарии.

7. Рядом с известными вторичными промежуточными хозяевами: Cordulia aenea, Libellula quadrimaculata, найдены были новые: Leucorrhinia pectoralis, Orthetrum cancellatum и Aeshna cyanea.

8. Размеры инцистированных метацеркарий у различных вторичных промежуточых хозяев не постоянны. Размеры инцистированных метацеркарий стрекозы Aeshna cyanea и таксономическое положение у этой стрекозы образуют явную связь между Prosthogonimus putschkowskii и P. ovatus.

9. Заражение личинки стрекозы происходит случайным образом. Движения церкарий не направленьы. Церкарии пассивно засасываются движением дыхания исходящего из прямой кишки личинки стрекозы. Поведение церкарий очень целесообразно для достижения хозяина. Под влиянием движения воды они освобождаются от субстрата на котором находились и начинают плавать и двигаться.

10. В настоящее время в Голландии Prosthogonimus ovatus находят редко.

11. Новый и значительный очаг паразита был обнаружен в Оверейсель/Лоннекер мер около г. Хенгело/.

12. Найдена большая разница в резистенции яйцевода, домашних кур породы Род айленд ред и белой легхорн, против заражения паразитом Prosthogonimus ovatus. Куры породы Род айленд ред не заболевают после инфекции хотя носка яиц прекращается. Белый легхорн серьезно заболевает. У этой породы особенно проявляются симптомы известные под названием простогонимоза.

13. Влияние заражения яйцевода паразитом Prosthogonimus на носку яиц у домашних уток заметно через 6 дней, а у кур уже через 24–48 часов.

14. Следующие факторы влияют положительно на заражение простогонимозом домашних птиц.

а. сухая местность с небольшим количеством прудов и озер. Пруды с евтрофной, не загрязненной водой.

б. большое количество гнездящихся в прудах птиц.

в. большое количество обоих промежуточных хозяев.

г. интенсивное птицеводство в окрестностях таких очагов заразы.

STELLINGEN

1

In Johannes 21: 1–12 is sprake van de vis Tilapia galilaea.

Nieuwe Testament

2

De overbevolking van de wereld is biologisch gezien geen toekomstprobleem, doch heeft reeds een geschiedenis van 8000 jaar.

3

De opmerking van Punt over de dwarse streping van insectenspieren is onjuist. Punt, *Inaugurele rede*, p. 15, 2e al.

4

Het verdient aanbeveling, om in het leerplan voor H.B.S.b en Gymnasium b, het frans te vervangen door het russisch.

5

De bij bepaalde takken van sport opvallende verschillen in prestatie en speelwijze tussen bepaalde naties, zijn voor een belangrijk deel te wijten aan de gerichte selectie op karaktereigenschappen die bij vele volkeren eeuwenlang heeft plaatsgevonden.

6

Een Leviathan loopt op vier poten. Slijper, *Walvissen*, p. 66

7

Indien de eieren van een Trematode minder dan 30 micron lang en ten hoogste 20 micron breed zijn, heeft deze Trematode een kieuwslak (Prosobranchiaat) als eerste tussengastheer.

8

De door Havinga en Tiews gebruikte methode van geslachtsbepaling bij de Garnaal (Crangon crangon) is onjuist.

Havinga, *Krebse und Weichtiere*, 1929

Tiews, *Ber. Dtsch. Wiss. Komm. Meeresforsch.*, 1954, 13, 3, 235–269

9

De wettelijk vastgestelde minimummaten van zoetwatervissen zijn volgens een verouderd principe opgesteld en dienen grondig te worden herzien. Het is aan te bevelen, de minimummaat van Snoek op 55 cm (nu 40 cm), die van Brasem op 35 cm (nu 22 cm) te stellen.

R. Boddeke