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Development and transmission of *Oswaldocruzia pipiens* Walton, 1929 (Nematoda: Trichostrongylidae) in amphibians

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BAKER, M. R. 1978. Development and transmission of *Oswaldocruzia pipiens* Walton, 1929 (Nematoda: Trichostrongylidae) in amphibians. Can. J. Zool. **56**: 1026–1031.

Development of *Oswaldocruzia pipiens* was similar to that of other trichostrongyles which have been studied. First-stage larvae have a valved, rhabditiform oesophagus. Infective larvae are ensheathed and have a strongyliform oesophagus. Development to the infective stage occurred in faeces and transmission was by skin penetration. In frogs, early development occurred on the mucosa of the stomach; worms then migrated to the anterior portion of the intestine. The prepatent period was 14–18 days at 14–18°C. Patent infections developed in experimentally infected tadpoles of *Rana sylvatica*. However, there was no evidence of natural infections in tadpoles. There were no significant fluctuations of prevalence and intensity between April and October, 1976 and 1977, in transformed *R. sylvatica* from a single marsh near Guelph, Ontario. Transmission apparently took place during spring and throughout the summer. Young frogs acquired infections rapidly.

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Le développement d'*Oswaldocruzia pipiens* ressemble à celui d'autres trichostrongyles déjà étudiés. Les larves de premier stade ont un œsophage rhabditiforme à valve. Les larves infectieuses sont entourées d'une gaine et ont un œsophage strongyliforme. Le développement de la larve jusqu'au stade infectieux se fait dans les fèces et le parasite se transmet par pénétration à travers la peau. Chez les grenouilles, les premières phases du développement se font sur la muqueuse de l'estomac; les vers migrent ensuite vers la portion antérieure de l'intestin. La période pré-symptomatique dure de 14 à 18 jours à 14–18°C. Des têtards de *R. sylvatica* infectés artificiellement ont manifesté des symptomes; cependant, en nature les têtards ne semblent pas sujets à l'infection. D'avril à octobre, en 1976 et en 1977, la fréquence et l'intensité de l'infection n'ont pas subi de fluctuations significatives chez des grenouilles *R. sylvatica* provenant toutes d'un même étang, près de Guelph, en Ontario. La transmission du parasite semble se faire au printemps et durant tout l'été. Les jeunes grenouilles sont vite contaminées.

[Traduit par le journal]

Introduction

Oswaldocruzia spp. in amphibians and reptiles are cosmopolitan in distribution but little is known about the biology of any species. This prompted the present investigation of the biology of *O. pipiens* Walton, 1929, which occurs in various anuran amphibians in southern Ontario (Baker 1977).

Materials and Methods

Specimens of *Rana sylvatica* and *Bufo americanus* were captured near Guelph, Ontario. All *R. sylvatica* used in the analysis of seasonal fluctuations of infection were obtained as monthly samples from a single marshy area in the Arboretum of the University of Guelph. Samples were collected between April and October, 1976 and 1977. Prevalence refers to the percentage of frogs infected and intensity refers to the mean number of worms per infected frog. Parasites were fixed in hot glycerine–alcohol and cleared in glycerine. Free-living stages of *O. pipiens* were obtained from cultures of faeces from naturally infected hosts.

Eggs of *R. sylvatica* and *B. americanus* were collected in spring and reared free of parasites. Tadpoles were fed lettuce, and young transformed frogs and toads were fed small insects.

Tadpoles were experimentally infected by placing 100 infective larvae in small beakers containing about 100 ml water and keeping tadpoles in these containers for 4 h. Tadpoles were kept at room temperature (approximately 22°C) until necropsy. To infect frogs and toads, infective larvae were placed on moist filter paper lining Syracuse dishes. Experimental hosts were restrained on contaminated filter paper for 4 h and then held in terraria at 14–18°C until necropsy.

Experimentally infected *Rana sylvatica* frogs were examined 1, 3, 5, 7, 9, 10, 12, 14, 18, and 25 days postinfection, and experimentally infected *Bufo americanus* toads were examined 14, 18, 22, 30, and 38 days postinfection.

Results

Free-living Development

Eggs were laid by female *O. pipiens* at the 8- to 16-cell stage of development (Fig. 1). Eggs col-

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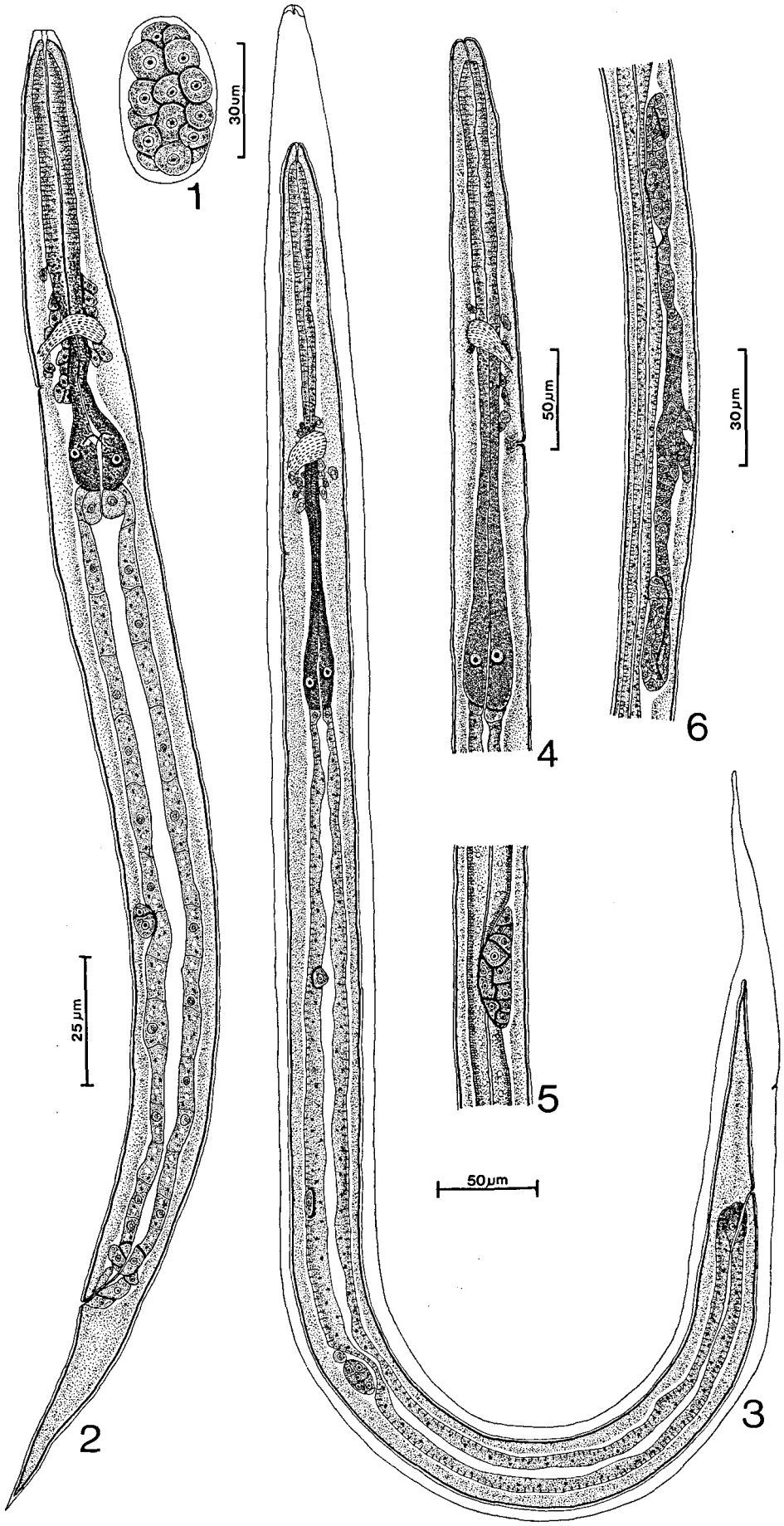
FIGS. 1–6. *Oswaldocruzia pipiens* Walton, 1929. Fig. 1. Egg at 16-cell stage of development. Fig. 2. Lateral view of first-stage larva. Fig. 3. Lateral view of ensheathed third-stage infective larva. Fig. 4. Anterior end of parasitic third-stage larva, lateral view. Fig. 5. Genital primordium of parasitic third-stage larva, lateral view. Fig. 6. Genital primordium of fourth-stage female larva, lateral view.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lected from the faeces of an infected *R. sylvatica* hatched within 24 h, releasing first stage larvae.

First-stage Larvae (Fig. 2) (10 specimens)

Total length 297–317(306) μm. Maximum width 17–19(17) μm. Buccal cavity approximately 2 μm in length, lined by thin cuticle. Oesophagus 84–93(88) μm in length, rhabditiform, with valve in bulb. Corpus 46–56(51) μm, isthmus 18–24(21) μm, and bulb 14–20(16) μm in length. Nerve ring 59–71(65) μm from anterior extremity. Excretory pore at posterior margin of nerve ring. Genital primordium 6–8(7) μm in length, 166–184(175) μm from anterior extremity. Tail conical, 41–49(46) μm in length.

Infective Third-stage Larvae (Fig. 3) (10 specimens)

Total length 562–631(588) μm. Maximum width 18–22(20) μm. Larva enclosed by cuticular sheath 644–716(669) μm in length. Buccal cavity narrow, lined by thin cuticle, 5–8 (6) μm in length. Oesophagus 132–173 (153) μm in length, slender, without valve, anterior third slightly expanded. Nerve ring 72–101 (85) μm, and excretory pore 91–111 (100) μm from anterior extremity. Genital primordium 13–20 (15) μm in length, 288–388 (342) μm from anterior extremity. Tail conical, 53–66 (60) μm in length.

Development of infective larvae required 3–4 days at room temperature. In culture dishes infective larvae migrated out of faecal masses into the surrounding water.

Development in Rana sylvatica

Third-stage larvae were found in experimentally infected frogs 1 and 3 days postinfection. These worms were attached by the anterior extremity to the mucosa of the cardiac region of the stomach. A single larva collected three days postinfection had the following dimensions; total length 653 μm; buccal cavity 4 μm; nerve ring 82 μm and excretory pore 108 μm from anterior extremity; genital primordium 48 μm in length, 426 μm from anterior extremity; tail 64 μm in length. Changes during development included thickening of the oesophagus, reduction in the length of the buccal cavity, and development of the genital primordium (Figs. 4, 5).

At other intervals postinfection the following worms were recovered: (1) 5 and 7 days, 18 fourth-stage larvae; (2) 9 and 10 days, 6 fourth-stage, 8 moulting fourth-stage larvae; (3) 12 days, 1 moulting fourth-stage larva, 5 adult males, 3 subgravid adult females; (4) 14 days, 1 adult male, 2 subgravid adult females; (5) 18 days, 1 moulting fourth-stage larva, 2 adult males, 6 subgravid adult females, 1 female with eggs; (6) 25 days, 2 adult males, 1 subgravid adult female, 1 female with eggs.

Fourth-stage Larvae (Figs. 6, 7) (10 ♂, 8 ♀ worms collected 5 days postinfection)

General—Filiform worms with club-shaped oesophagus. Oral cavity lined by short, thickened cuticular ring. Buccal cavity reduced. Lateral alae thin, extending from anterior portion of oesophageal region to tail. Cuticle of body thin, smooth, not expanded into cephalic inflation. Anterior deirids not observed.

Males—Total length 1.13–1.85 (1.44) mm. Width at midbody 27–37 (32) μm. Oesophagus 195–258 (220) μm in length. Nerve ring 90–123 (103) μm and excretory pore 120–152 (133) μm from anterior extremity. Spicule primordium prominent; body slightly expanded in anal region near spicule primordium. Bursa undeveloped. Tail 57–76 (68) μm in length, tapering to terminal spike 8–12 (10) μm in length. A moulting fourth-stage male worm collected 9 days postinfection had the following dimensions: total length 2.2 mm; oesophagus length 267 μm; nerve ring 116 μm and excretory pore 163 μm from anterior extremity.

Females—Total length 1.21–1.71 (1.53) mm. Width at midbody 28–36 (33) μm. Oesophagus 207–242 (221) μm in length. Nerve ring 93–108 (101) μm and excretory pore 127–141 (135) μm from anterior extremity. Vulva primordium prominent, 0.84–1.15 (1.03) mm from anterior extremity. Tail 73–88 (81) μm in length, tapering to terminal spike 8–14 (12) μm in length. A moulting fourth-stage female worm collected 9 days postinfection had the following dimensions; total length 3.00 mm; oesophagus length 289 μm; nerve ring 127 μm, excretory pore 164 μm, and vulva 1.9 mm from anterior extremity; tail 104 μm in length.

Development in Bufo americanus

Development of *O. pipiens* in *B. americanus* was similar to that observed in *R. sylvatica*. At 14 days postinfection, five worms were recovered from a single toad. All were adult worms (one male, four female) but none of the female worms had eggs. At 18 days postinfection two toads were examined. In one toad there were three adult male worms and two adult females with eggs. In the second toad there was one male worm, two female worms containing eggs, and two without eggs. At 22 days postinfection four infected toads were examined. In one of these only three male worms were found. In each of the other three toads adult male and female worms were recovered and the infections were patent. Similarly, a single toad examined 30 days postinfection and two toads examined 38 days postinfection contained patent infections.

Cross Infection

Toads (*B. americanus*) were exposed to infective

larvae of *O. pipiens* from cultures of faeces of infected *R. sylvatica*. From each of three toads examined 14 days postinfection 1–5 worms were recovered, and 13–18 worms were recovered from two toads examined 18 days postinfection. All worms were mature and each infection was patent.

Transmission to Tadpoles

A total of five fourth-stage larvae (three female, two male) and two moulting fourth-stage larvae (one male, one female) of *O. pipiens* was recovered from two *R. sylvatica* tadpoles examined 7 days postinfection. One worm was located in the gall bladder, while the others were in the anterior portion of the intestine. Five adult male worms and seven adult female worms containing eggs were recovered from two tadpoles examined 14 days postinfection. All these worms were located in the anterior portion of the intestine and each infection was apparently patent.

Twenty wild *R. sylvatica* and 20 wild *B. americanus* tadpoles were uninfected, although they were from localities where adult amphibians commonly harboured *O. pipiens*.

Observations on Natural Infections in Rana sylvatica

Seasonal variation in prevalence and intensity of *O. pipiens* in a single population of *R. sylvatica* was followed for 2 years (Figs. 8, 9). Fluctuations in prevalence in both years were similar in that a peak observed in spring (May–June) was followed by a decline in summer and an increase in fall. Intensity increased to a peak in May of both years. However, in 1976 intensity declined in late spring and stayed relatively low for the rest of the summer and fall, while in 1977 there was an initial decline in summer followed by an increase in September.

All worms collected in April of both years were mature, whereas worms collected in May and subsequent months included some fourth-stage larvae and immature adults. This indicated worms overwinter in frogs and that transmission occurs in early spring and throughout the summer and early fall.

In 1976, 93 of 153 (61%) frogs examined were infected with *O. pipiens*, while in 1977, 81 of 131 (62%) were infected. However, of the total of 174 infected frogs only 93 (53%) contained patent infections. This varied from 57% of infected frogs in 1976 to 49% in 1977. Thus only 33% of the frogs examined in the study contained patent infections. The intensity during the study was only 4.7 (812 worms in 174 infected frogs) and a lack of male or female worms in many frogs contributed significantly to the low proportion of infections which were patent. Males constituted 42.5% and females 57.5% of the worms recovered. The number of

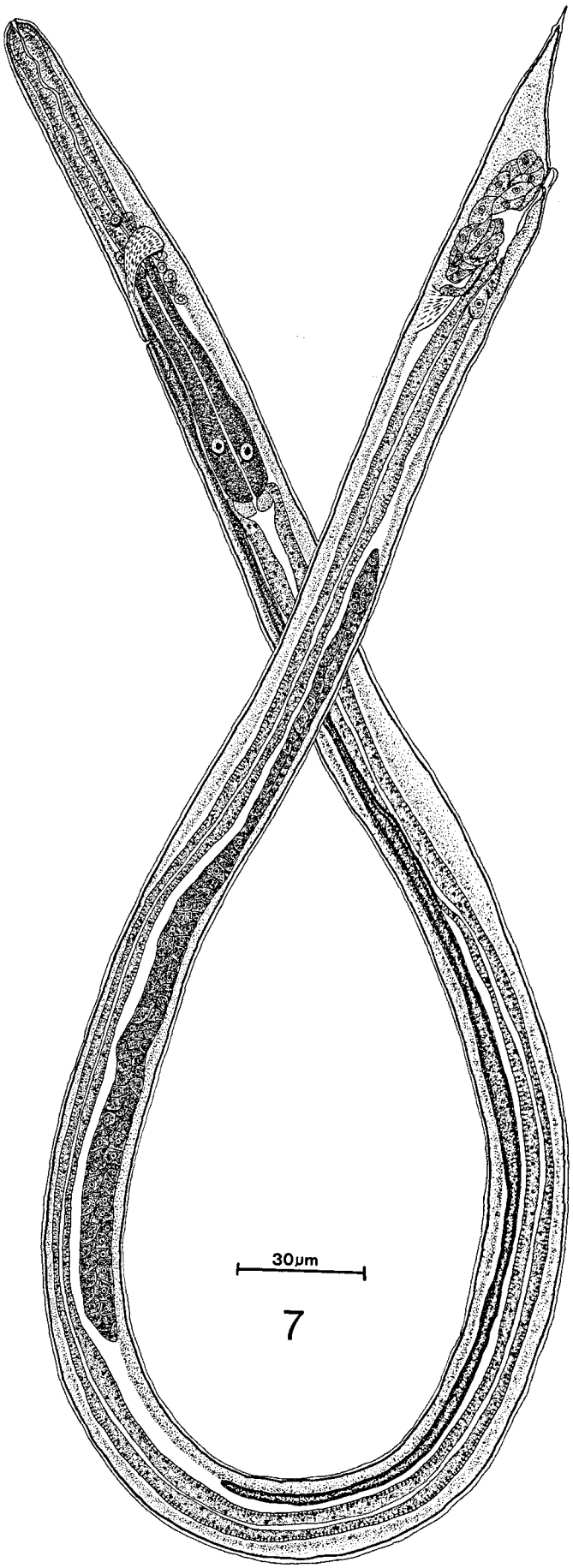

FIG. 7. *Oswaldocruzia pipiens* Walton, 1929. Lateral view of male fourth-stage larva.

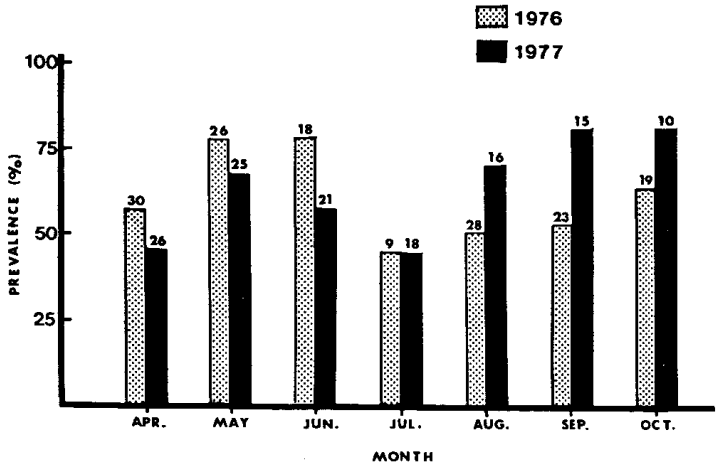

FIG. 8. Monthly fluctuations in prevalence of *O. pipiens* in *Rana sylvatica* from Guelph, Ontario, examined in spring, summer, and fall of 1976 and 1977. Numbers above each prevalence bar indicate sample size of frogs.

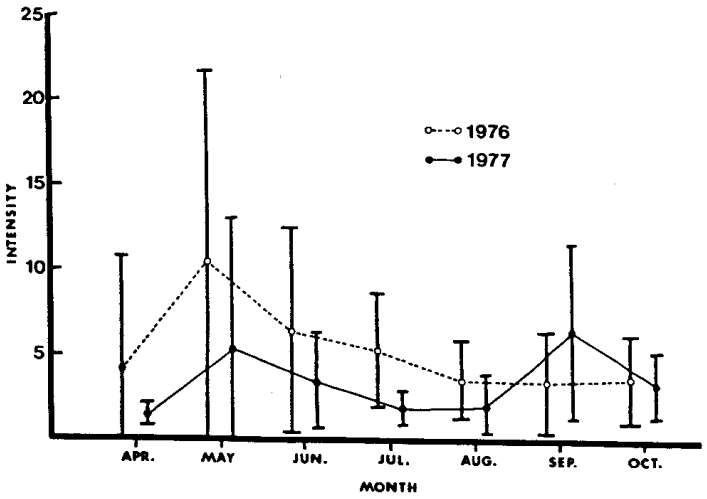

FIG. 9. Monthly fluctuations in intensity ± one standard deviation of *O. pipiens* in *Rana sylvatica* from Guelph, Ontario, examined in spring, summer, and fall of 1976 and 1977.

worms recovered from individual frogs varied from 1 to 45, but most infected frogs contained 1–5 worms (Fig. 10). Prevalence and intensity varied little with size of frog in frogs longer than 35 mm in snout–vent length (Table 1).

In 1976, newly transformed *R. sylvatica* were first observed on June 22. None of 10 young frogs

TABLE 1. Variations in prevalence and intensity of *Oswaldocruzia pipiens* in *Rana sylvatica* of different size classes from Guelph, Ontario

Size class of frogs*	Number examined	Prevalence	Intensity ± 1 SD
<31	60	50%	2.4±1.8
31–35	65	63%	3.6±4.9
36–40	43	79%	6.1±4.6
41–45	66	55%	5.5±8.7
46–50	41	59%	5.8±5.6
>50	9	67%	5.8±7.1

*Snout–vent length measured in millimetres.

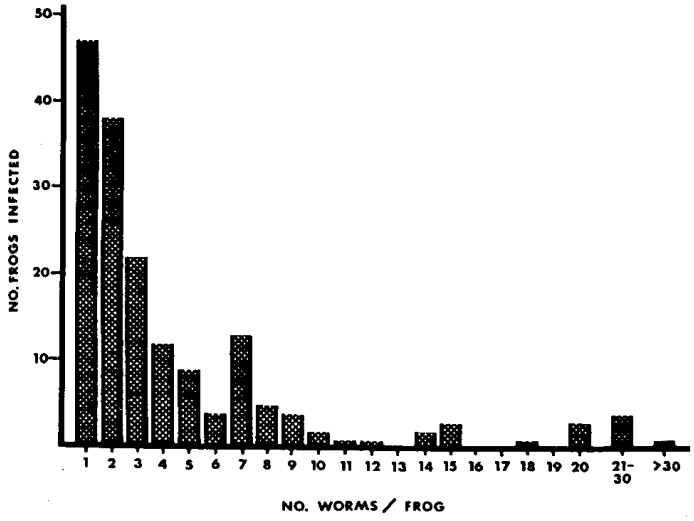

FIG. 10. Variation in numbers of *O. pipiens* recovered from *Rana sylvatica* from Guelph, Ontario, examined in 1976 and 1977.

examined at this time was infected with *O. pipiens*. However, between August 8–13, 1976, 6 of 11 young of the year frogs examined were infected and on August 23, 6 of 9 young frogs were found infected.

Discussion

The rhabditiform oesophagus with a valved bulb in the first-stage larvae and strongyliform, unvalved oesophagus in infective larvae and parasitic stages of *O. pipiens* is similar to that observed in other trichostrongyles. Also, early parasitic development of *O. pipiens* on the mucosa of the stomach of *R. sylvatica* prior to migration to the lumen of the intestine is similar to the histiotrophic phase observed in other trichostrongyles which have been studied.

The development of patent infections of *O. pipiens* in the intestine of experimentally infected *R. sylvatica* tadpoles is interesting considering differences in digestive physiology and morphology and feeding habits of tadpole and adult frogs. Tadpoles were not infected in enzootic areas probably because of ecological factors. Nematodes are poor swimmers and larvae would be dispersed in open water. Also it is unlikely they could penetrate the skin of tadpoles in water.

The successful cross transmission of *O. pipiens* from *R. sylvatica* to *B. americanus* demonstrates that a single species of trichostrongyle infected these hosts in the Guelph area. *Oswaldocruzia pipiens* occurs commonly in *R. sylvatica*, *R. pipiens*, *Pseudacris triseriata*, and *Bufo americanus* in the Guelph area (Baker 1977). Since these amphibians overlap to some degree in the use of habitat, transfer of parasites between them is likely to occur.

In England, Lees (1962) reported prevalence and

intensity of *Oswaldocruzia filiformis* (=*O. goezei*) in *Rana temporaria* fluctuated seasonally similar to *O. pipiens* in *R. sylvatica*. Apparently the late summer and fall are important periods for transmission of both parasites in a temperate climate. In the marsh where seasonal fluctuations of infections in *R. sylvatica* were studied frogs were usually observed in summer at the edge of open water with nearby tree or bush cover. In both years of the study the marsh shrunk in size in July and August. This corresponded to the period when the population of frogs was at a peak because of transformation of tadpoles. Thus the density of frogs was probably highest at the most favourable period for free-living development and transmission of *O. pipiens*. Thus many young of the year frogs become infected before winter.

Rana sylvatica may attain a snout–vent length of approximately 35 mm in the same summer they transform from tadpoles. Although age of frogs from about 2 months posttransformation could not be determined with certainty, size is probably related to age as has been observed in other ranid species. Therefore, there was little evidence of increase in prevalence and intensity of infection with age of frogs after the 1st year of life (Table 1). Adult worms are large in size relative to the intestine of *R. sylvatica* and crowding probably keeps worm burdens relatively low. Most frogs parasitized by more than about 15 worms contained many small, immature specimens.

It is not known if infective larvae of *O. pipiens* can survive winter in the Guelph area and remain infective to frogs. Overwintering of infective larvae of several trichostrongyle parasites of cattle on pasture has been shown to contribute significantly to the spring rise in intensity of infections in southwestern Ontario (Slocombe 1974) and New Brunswick (Smith 1974). However, this is unlikely to be true of *O. pipiens* in the Guelph area because much of the favoured summer habitat for frogs is usually flooded in spring and larvae located there would not come into contact with frogs. Thus, the species is probably dependent upon survival in overwintering frogs.

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