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THE SUSCEPTIBILITY OF DIFFERENT GASTROPOD SPECIES TO THE INFECTION WITH VARESTRONGYLUS SAGITTATUS (MUELLER, 1890) DOUGHERTY, 1945

P. ŘEZÁČ

Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice, Czechoslovakia

Abstract. The susceptibility of 11 species of terrestrial gastropods to the infection with *Varestrongylus sagittatus* has been studied under laboratory conditions. The gastropods *Zenobiella umbrosa*, *Monachoides incarnata*, *Deroceras reticulatum*, *Cepaea nemoralis*, *Cepaea hortensis*, *Arion rufus*, and *Trichia hispida* are new potential intermediate hosts of *V. sagittatus*. The highest susceptibility and the shortest period of development of the *V. sagittatus* larvae occurred in *Succinea putris*. The development of *V. sagittatus* larvae inside *S. putris* did not proceed at the temperature of 5 °C, but at 10 °C, 15 °C, 20 °C, and 25 °C, the period of development became shorter with increasing temperature of environment. The length of *V. sagittatus* larvae reached the following values: 0.302—0.327 mm in first-stage larvae, 0.385—0.550 mm in second-stage larvae, and 0.484—0.590 mm in third-stage larvae. At the temperature of 25 °C, the larvae of *V. sagittatus* did not reach the third stage if the course of the experiment only in *Helicigona arbustorum*.

The nematode *Varestrongylus sagittatus* (Mueller, 1890) Dougherty, 1945 causes focal affections in lungs, emaciation, and anemia in susceptible members of the family Cervidae (*Cervus elaphus* L. and *Dama dama* L.) (Boev 1975). The area of its distribution includes a major part of the Palaearctic region from Middle Asia up to Europe. The occurrence of *V. sagittatus* in Czechoslovakia has been reported by Kotrlý (1958).

Similarly as other members of the family Protostrongylidae, *V. sagittatus* utilizes an intermediate host, terrestrial gastropod, in the organism of which it develops up to the infective stage. Available data on the intermediate host spectrum of *V. sagittatus* are very rare and concern mainly its life cycle in some regions of Middle Asia (Panin 1967, Lyubimov 1976).

The intermediate hosts of *V. sagittatus* in Czechoslovakia have not been studied by any author. We have therefore studied the susceptibility to experimental infection with this nematode using some common representatives of gastropod fauna from forest biotopes of South Bohemia inhabited by red deer. Besides, the course of *V. sagittatus* larva development inside the infected gastropod was observed at a constant temperature of environment.

MATERIALS AND METHODS

The first-stage larvae (L 1's) of *V. sagittatus* used in our experiment were recovered from faeces of naturally infected red deer (*Cervus elaphus hippelaphus* L.) living in South Bohemia. The larvae were isolated using a modified method after Baermann (1917), washed in water several times and concentrated by centrifugation at 800—1,000 rpm for 10 min. Before use the larvae were stored at the temperature of 5 °C not longer than for 3 weeks.

On the basis of our previous observations performed in two localities of South Bohemia, the following species of terrestrial gastropods were chosen as hosts of *V. sagittatus: Succinea putris*, *Zenobiella umbrosa*, *Helix pomatia*, *Helicigona arbustorum*, *Monachoides incarnata*, *Deroceras reticulatum*, *Cepaea nemoralis*, *C. hortensis*, *Arion rufus*, *Trichia hispida*, and *Bradybena fruticum*. The specimens used in the experiment originated from different localities in which both domestic and wild ruminants did not occur. The gastropod species were determined after Ložek (1956) and Godan (1983).

A moistened filter paper was placed on the bottom of Petri dishes (8—11 cm in diameter) and 4—6

ml of *V. sagittatus* L 1's suspension was dropped on each of them. About 5—20 gastropod specimens (according to their size) were placed on each dish. The infection dose was about 100 *V. sagittatus* L 1's per one specimen. The gastropods were allowed to move freely in order that the sole of their foot was always in contact with the bottom of the dish. The contact of gastropods with larvae at room temperature (22—24 °C) lasted 2.5 h. After this time the gastropods were transferred to glass vessels (volume 800—1,000 ml) with a soil layer on their bottom and the vessels were placed in an environment with constant temperature. The course of development of larvae in *Succinea putris* was observed at the temperatures of 5 °C, 10 °C, 15 °C, 20 °C, and 25 °C, whereas the development of larvae in other gastropod species was observed at 25 °C. The gastropods were fed with fresh vegetables and nettle (*Urtica dioica*) during the experiment. Two to five specimens of each gastropod species were killed at 7-day intervals, soft tissues of their bodies were cut into small pieces, crushed between compressor glasses and examined in a light microscope. Developmental stages of larvae were differentiated according to the description by Gerichter (1948). *V. sagittatus* L 1's whose morphology was studied originated from the faeces. Second-stage (L 2's) and third-stage (L 3's) larvae used for morphological studies were isolated from experimentally infected *S. putris*. The larvae obtained from crushed gastropod tissue by water washing (L 2's) or by means of digestion with an artificial digestive solution (6 g pepsin, 7 ml conc. HCl, 1,000 ml dist. H_2O) (L 3's) were photographed in a native preparation by Docuval (Zeiss—Jena) and their measurements were taken from working photographs. The morphology of L 1's was studied in the same manner. A total of 20 larvae of each stage were measured.

RESULTS

We have succeeded in infecting all of the 11 terrestrial gastropod species with *Varestrongylus sagittatus* larvae. The susceptibility of individual gastropod species to infection arranged according to the infection abundance (mean number of *V. sagittatus* larvae per 1 gastropod in the experiment) is shown in Table 1.

Table 1. Prevalence, intensity and abundance of *Varestrongylus sagittatus* infection in terrestrial gastropods experimentally infected with first-stage larvae

Gastropod species	No. of specimens in experiment	Percentage of infected gastropods	Intensity of infection	Abundance of infection
Succinea putris	30	100.0	1—107	35.6
Zenobiella umbrosa	20	100.0	24— 54	34.8
Monachoides incarnata	22	100.0	11— 56	31.8
Helicigona arbustorum	20	100.0	2— 56	22.9
Trichia hispida	18	100.0	1— 53	20.6
Deroceras reticulatum	20	100.0	1— 32	15.6
Helix pomatia	20	100.0	2— 47	14.2
Cepaea hortensis	15	91.7	2— 41	12.3
Cepaea nemoralis	20	94.1	1— 27	9.5
Arion rufus	20	66.7	1— 30	6.1
Bradybena fruticum	20	100.0	1— 10	4.4

The following morphological changes were observed during the development of *V. sagittatus* larvae. After the penetration into the gastropod tissue, the larvae first increased in size and gradually lost their original mobility. The contours of intestine became more marked and structures of the character of dark granules started to appear first in the region of proximal part of intestine, later in the whole intestine and partly also in the region of oesophagus (Fig. 2B). A larva with this morphological characteristics is termed by Ždárská (1960) "transitive stage of development". Then the first sheath of the larva loosed and the second stage of development started (Fig. 2C). Since this time the dark granules gradually became less numerous and the inner organs previously covered by them started to become more distinct. Loosening of the

second sheath and transition to the third stage of development occurred only after a complete disappearance of the dark granules, when individual organs of the larvae (excretory pore, nerve ring, oesophageal glands, genital primordium, anus) were already well visible. After the lost of the two sheaths the mobile larvae became infective (Fig. 2D). The percentage of individual developmental stages of *V. sagittatus* during their development inside infected gastropods is illustrated in Fig. 3. The measurements of these stages are given in Tables 3—5.

The speed of *V. sagittatus* larvae development in individual gastropod species was compared on the basis of three following criteria:

1. Number of days post infection (DPI), when all (most) larvae were at the third stage of development.

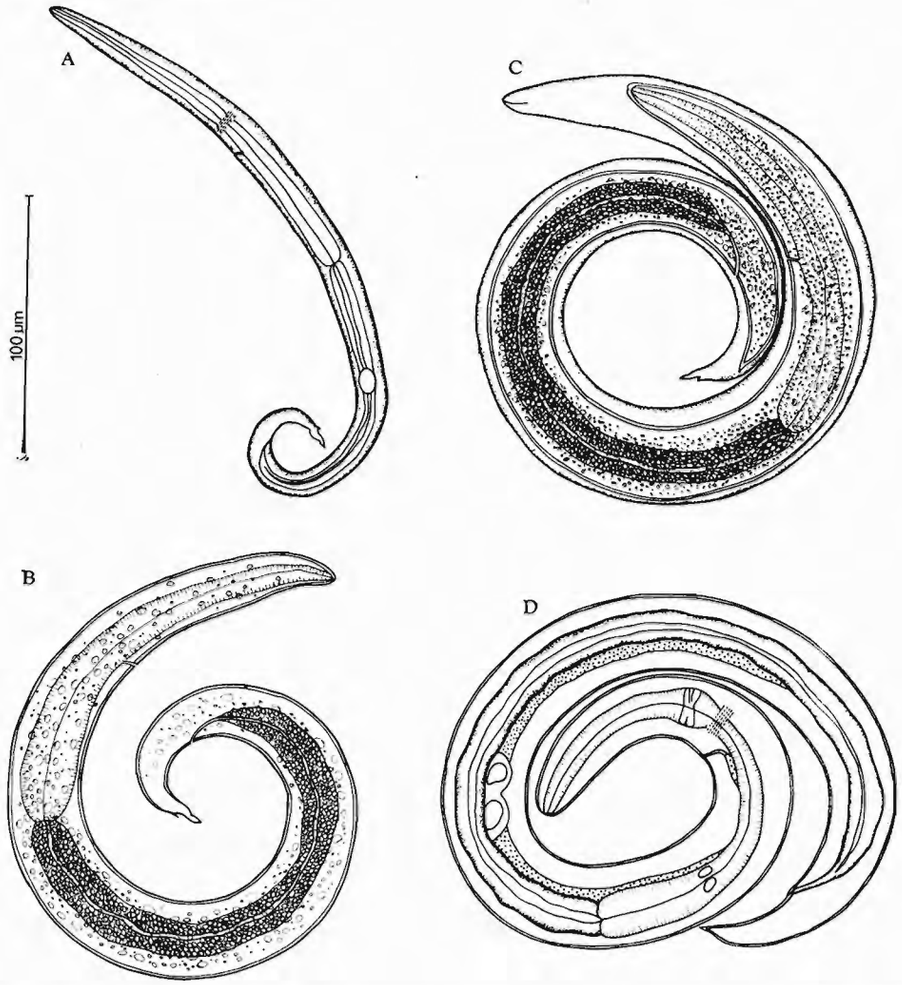

Fig. 1. Larval stages of *Varestrongylus sagittatus* (lateral view): *A* — first-stage larva isolated from faeces, **B** — first-stage larva before first moulting, **C** — second-stage larva, **D** — third-stage (infective) larva.

2. Number of DPI when first L 3's were found.
3. Number of DPI when first L 2's were found (Table 2).

According to these criteria the development of *V. sagittatus* larvae at the temperatu-

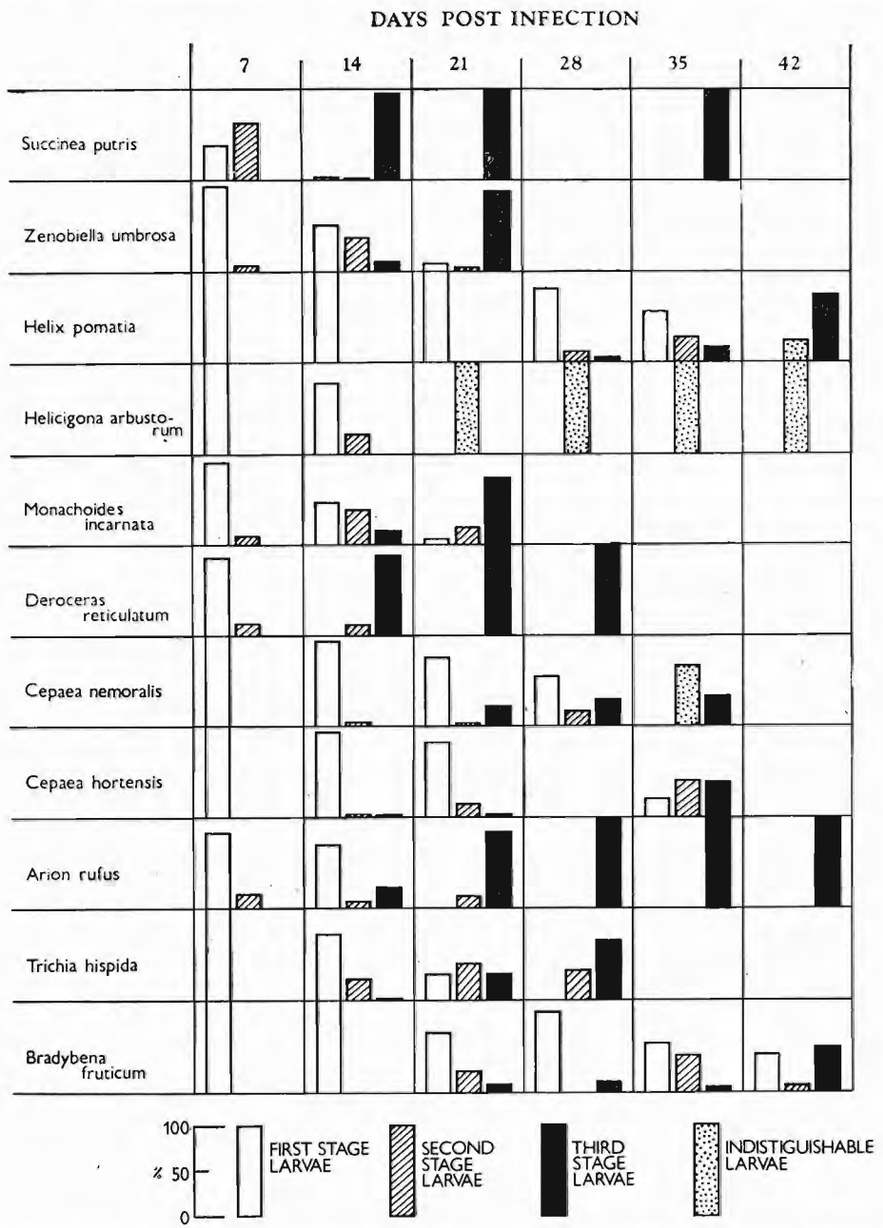

Fig. 2. Percentage of *Varestrongylus sagittatus* larvae developmental stages in individual gastropod species during the experiment.

Table 2. Number of days post infection (DPI) when the first second-stage and third-stage *V. sagittatus* larvae were found and when all (most) larvae reached the third stage of development in individual gastropod species at the temperature of 25 °C

Gastropod species	First second-stage larvae (DPI)	First third-stage larvae (DPI)	All (most) larvae at the third stage (DPI)
Succinea putris	7	14	21 (100 %)
Zenobiella umbrosa	7	14	21 (88 %)
Helix pomatia	28	28	42 (74 %)
Helicigona arbustorum	14	Since DPI 21 the developmental stage could not be distinguished	
Monachoides incarnata	7	14	21 (88 %)
Deroceras reticulatum	7	14	21 (100 %)
Cepaea nemoralis	14	21	—
Cepaea hortensis	14	14	—
Arion rufus	7	14	21 (86 %)
Trichia hispida	14	14	28 (67 %)
Bradybena fruticum	21	21	—

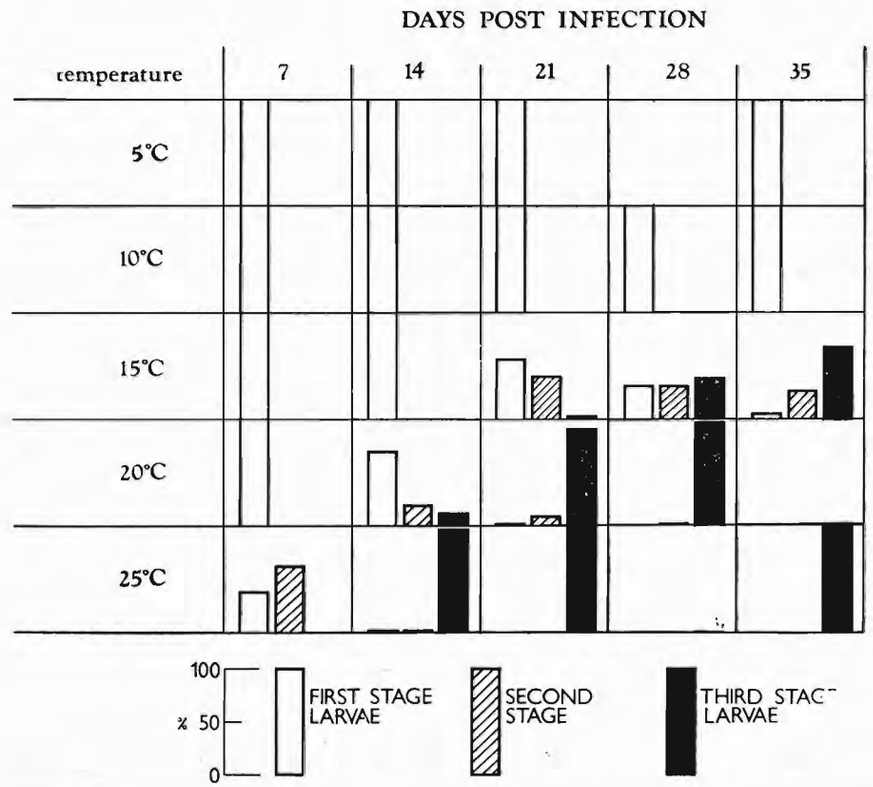

Fig. 3. Percentage of *Varestrongylus sagittatus* developmental stages in *Succinea putris* at different temperatures.

Table 3. Measurements of first-stage *Varestrongylus sagittatus* larvae (in mm); comparison with literature data

	Our observations		Panin 1967	Kutzer and Prosl 1975		Demiaszkiewicz 1986	
	range	mean	range	range	mean	range	mean
Body length	0.302—0.327	0.313	0.260—0.305	0.272—0.344	0.309	0.280—0.339	0.310
Body width	0.012—0.015	0.013	0.014—0.017			0.012—0.016	0.014
Oesophagus length	0.145—0.159	0.152	0.115—0.151	0.124—0.168	0.150	0.132—0.172	0.149
Distance of nerve ring from anterior body end				0.077—0.106	0.090	0.069—0.097	0.085
Distance of excretory pore from anterior body end	0.088—0.108	0.093	0.081—0.084				
Distance of genital primordium from anterior body end			0.179—0.201	0.160—0.225	0.199		
Distance of anus from posterior body end	0.031—0.040	0.034	0.025—0.031	0.024—0.043	0.037	0.028—0.041	0.033

re of 25 °C was most rapid in *Succinea putris* and *Deroceras reticulatum* and then followed *Zenobiella umbrosa, Arion rufus, Monachoides incarnata, Trichia hispida, Cepaea hortensis, Cepaea nemoralis, Bradybena fruticum,* and *Helix pomatia.* The speed of development in *Helicigena arbustorum* could not be detected, because since DPI 21, there appeared marked morphological changes in all larvae characterized particularly by deformations of body and yellow-brown colour of intestine contents, which made the identification of the developmental stage impossible. Similar changes, but in a smaller extent, were observed also in *Cepaea nemoralis* and *Helix pomatia.*

The speed of development of *V. sagittatus* larvae in *Succinea putris* in relation to the environmental temperature is shown in Fig. 3. At the temperature of 5 °C, no marks of development were observed during 35 days. At the temperature of 10 °C, increased

Table 4. Measurements of second-stage *Varestrongylus sagittatus* larvae (in mm); comparison with literature data

	Our observations		Panin 1967
	range	mean	range
Body length	0.385—0.550	0.492	0.426—0.484
Body width	0.023—0.035	0.030	0.032—0.038
Oesophagus length	0.146—0.192	0.176	0.154—0.168
Distance of excretory pore from anterior body end	0.090—0.098	0.093	0.098
Distance of anus from posterior body end	0.032—0.060	0.044	0.038—0.041
Length of separated outer sheath	0.415—0.579	0.544	0.472—0.502

Table 5. Measurements of third-stage *Varestrongylus sagittatus* larvae (in mm); comparison with literature data

	Our observations		Panin 1967	Demiaszkiewicz 1986	
	range	mean	range	range	mean
Body length	0.484—0.590	0.536	0.576—0.640	0.531—0.649	0.607
Body width	0.024—0.031	0.028	0.038—0.041	0.027—0.033	0.029
Oesophagus length	0.167—0.206	0.192	0.192—0.204	0.163—0.207	0.187
Distance of nerve ring from anterior body end	0.053—0.090	0.079		0.085—0.111	0.095
Distance of excretory pore from anterior body end	0.061—0.102	0.092	0.102—0.109	0.088—0.119	0.104
Distance of genital primordium from anterior body end	0.228—0.276	0.252			
Distance of anus from posterior body end	0.031—0.050	0.042	0.044—0.048	0.039—0.053	0.048

size of L 1's, more marked intestine contours, and first dark granules in the region of proximal part of intestine were observed at the end of the studied period (28 DPI and 35 DPI). At the temperature of 15 °C, 68.4% of larvae reached the third stage by 35 DPI. At the temperature of 20 °C, all larvae were at the third stage on 35 DPI, and at the temperature of 25 °C, all larvae reached the third stage of development already by 21 DPI.

DISCUSSION

A part of *V. sagittatus* life cycle taking place inside the organism of intermediate host at the temperature of 25 °C was terminated by the development of invasive larvae in *Succinea putris, Zenobiella umbrosa, Monachoides incarnata, Deroceras reticulatum, Trichia hispida, Cepaea nemoralis, Cepaea hortensis, Arion rufus, Bradybena fruticum,* and *Helix pomatia.* The development of *V. sagittatus* in *B. fruticum* has previously been demonstrated in red deer pasturelands in southern regions of the Altai Mountains (Panin 1967), in *B. fruticum* and *S. putris* in conditions of red deer farms of Middle Asia (Lyubimov 1976), and in *H. pomatia* after experimental infection (Demiaszkiewicz 1986). *Z. umbrosa, M. incarnata, D. reticulatum, T. hispida, C. nemoralis, C. hortensis,* and *A. rufus* are new intermediate hosts for *V. sagittatus.* Considering the susceptibility to infection and the time necessary for the development of larvae, *S. putris* was found to be the most suitable of the studied intermediate hosts for *V. sagittatus,* while *Z. umbrosa, M. incarnata, D. reticulatum,* and *T. hispida* are regarded as very suitable intermediate hosts. *H. arbustorum,* however, cannot be considered a potential intermediate host of *V. sagittatus* under the given conditions, since no L 3's were found in it during the experiment.

The measurements of *V. sagittatus* L 1's found by us are almost identical with those recorded by Kutzer and Prosl (1975) and Demaszkiewicz (1986), but are greater than those published by Panin (1967) (Table 3). The measurements of L 2's do not markedly differ from those described by Panin (1967) (Table 4). The measurements of L 3's do not reach the values reported in the literature (Panin 1967, Demiaszkiewicz 1986) (Table 5). These disproportions may be caused by different geographic and climatic conditions during the development of *V. sagittatus* larvae.

According to our records, at the temperature of 5 °C *V. sagittatus* larvae did not develop for 35 days in *S. putris,* whereas at the temperatures of 10—25 °C, the time of development decreased with the increasing temperature of environment. This is in agreement with the data published by other authors who studied the effect of the temperature on the time of development of protostrongylid larvae (Gerichter 1948, Rose 1957, Halvorsen and Skorping 1982, Samson and Holmes 1984).

Our studies have provided some knowledge of the course of *V. sagittatus* larvae development in their intermediate hosts — terrestrial gastropods in Czechoslovakia. In further studies of this subject it will be necessary to include another species of terrestrial gastropods, as well as some of the fresh-water ones, to study the development of larvae at a wider temperature spectrum including variable temperatures of environment, and to compare the results with cases of natural infection with *V. sagittatus* in localities inhabited by red deer and fallow deer.

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ВОСПРИИМЧИВОСТЬ РАЗНЫХ ВИДОВ НАЗЕМНЫХ МОЛЛЮСКОВ К ЗАРАЖЕНИЮ НЕМАТОДОЙ *VARESTRONGYLUS SAGITTATUS* (MUELLER, 1890) DOUGHERTY

П. Ржезач

Резюме. В лабораторных условиях изучали восприимчивость 11 видов наземных моллюсков к заражению личинками *Varestrongylus sagittatus.* Моллюски ***Zenobiella umbrosa,*** *Monachoides incarnata, Deroceras reticulatum, Cepaea nemoralis, Cepaea hortensis, Arion rufus* и *Trichia hispida* являются новыми потенциальными промежуточными хозяевами *V. sagittatus.* Самая высокая восприимчивость к заражению и самое короткое время развития личиночных стадий *V. sagittatus* были обнаружены у *Succinea putris.* Развитие личинок *V. sagittatus* и *S. putris* остановилось при температуре 5 °C, тогда как при температурах 10 °C, 15 °C, 20 °C время развития сокращалось согласно повышению температуры среды. Длина личиночных стадий *V. sagittatus* была следующая: 0,302—0,327 мм у личинок 1-ой стадии, 0,385—0,550 мм у личинок 2-ой стадии и 0,484—0,590 мм у личинок 3-й стадии. При температуре среды 25 °C в течение эксперимента личинки *V. sagittatus* не развивались до 3-й стадии только в *Helicigona arbustorum.*

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P. Ř., Parazitologický ústav ČSAV,
Branišovská 31,
370 05 České Budějovice, ČSSR