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Strongyloides venezuelensis Brumpt, 1934 (Nematoda: Strongyloididae) Collected from *Rattus norvegicus* in Naha, Okinawa, Japan

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Abstract

A strain of *Strongyloides venezuelensis* Brumpt, 1934 was isolated from *Rattus norvegicus* trapped at Naha Port region, Okinawa, Japan. Morphological and biological characteristics of the isolate were studied in comparison with those of *S. venezuelensis* from other localities and those of *S. ratti*. The parasitic female has a longer tail and the filariform larva has a longer esophagus than those of the strains established in the United States and Israel. Free-living adults seldom developed. The majority of parasitic females located in the villi of anterior one-sixth of the small intestine of infected rat. This is the first record of *S. venezuelensis* from the Far East.

Key words: *Strongyloides venezuelensis, Rattus norvegicus,* Okinawa, morphology, new geographical record

Introduction

Strongyloides venezuelensis Brumpt, 1934 (Nematoda: Strongyloididae) has been reported from rats in Venezuela (Brumpt, 1934), Israel (Wertheim and Lengy, 1964), the United States (Little, 1966) and Brazil (Araujo, 1967). However, there has been no report of this species from the Far East, although Kamiya and Machida (1977) recorded *Strongyloides* sp. with spiraled ovaries from *Rattus rattus* on Ishigaki Island, Okinawa Prefecture, Japan. Recently the authors found concurrent infections with *S. venezuelensis* and *S. ratti* Sandground, 1925 in a brown rat on Okinawa Island, Japan. This paper reports the morphology and some biological characteristics of *S. venezuelensis* isolated in Okinawa in comparison with those of *S. venezuelensis* from other localities and *S. ratti*.

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Materials and Methods

Establishment of strains

The brown rat, *Rattus norvegicus,* was trapped at Naha Port region, Okinawa, Japan, on June 1986. The feces of the rat were incubated for 4 days by filter paper culture technique to obtain filariform larvae. A male Wister rat was infected by subcutaneous injection of the filariform larvae. Ten days later, the rat was autopsied and the living parasitic females were recovered from the small intestine by Baermann's method. The females were then separated into each species based on the difference in ovarian morphology under a dissecting microscope, and 20 parasitic females of each species were implanted surgically into the upper jejunum of male Wister rats. Then, the feces of these rats were cultured, and filariform larvae harvested were inoculated subcutaneously to male Wister rats. Afterwards, both strains were maintained by serial passage in the male Wister rats.

A strain of *S. ratti* provided by the Department of Parasitic Diseases, Kumamoto University School of Medicine was also examined for

comparison. This strain had been originally supplied by the Department of Medical Zoology, Tokyo Medical and Dental University (Tada *et al.*, 1979).

Morphological observation

The parasitic females were recovered from the small intestine of the infected Wister rats at 15th day of infection. The filariform larvae and free-living adults were harvested from filter paper cultures, after 4 days of incubation at 27°C. The modified filter paper culture (Little, 1966) and the unglazed pottery disc culture were also employed. The worms were observed both in living and fixed conditions. Fixatives used were 5% formalin for parasitic females and dilute Lugol's iodine solution for filariform larvae and free-living adults.

Distribution in rat intestine

The longitudinal distribution of parasitic females in the small intestine was studied in rats infected subcutaneously with 100, 250 and 500 larvae, respectively. The rats were killed on 8th, 10th, 13rd or 17th day of infection. The small intestine of each infected rat was dissected out, divided into 6 sections of equal length, cut open, rinsed lightly and incubated in physiological saline for 5 hrs at 37°C. Worms were collected from the incubation saline and counted under a dissecting microscope. The number of parasites in each section was expressed as percentage of the total worm burden.

The radial distribution of parasitic females in the rat intestine was also studied in the histological sections.

Results

Morphological description of S. venezuelensis *isolated in Okinawa*

a. Parasitic female (Fig. 1A): Small and filiform worm. Cuticle with extremely fine transverse striations. Mouth approximately octagonal and circumoral elevation composed of 8 small lobes; 4 papillae and 2 amphids present. Esophagus long and filiform. Nerve ring at anterior 1/5 of esophagus. Excretory pore just behind nerve ring. Reproductive system didelphic; both ovaries spiraled around intestine and terminating in prevulval level. Vagina short; vulva posterior to midbody, dividing body approximately 2:1; anterior and posterior vulval lips weakly developed. Uteri with up to 13 eggs forming one strand. Tail conical with dull tip. Eggs in fresh feces of host elliptical, thin-shelled and containing morula- to tadpole-stage embryo, 49–59 (53.1±2.2) × 26–31 (28.6±1.2) μm (Fig. 1C). Measurements are shown in Table 1 in comparison with previously reported data on *S. venezuelensis* and those of *S. ratti*.

b. Filariform larva: Body slender. Cuticle with fine transverse striations. Double lateral alae present. Mouth dorsoventrally elongated, surrounded by 4 papillae and amphids. Esophagus filiform, occupying about 45% of body length. Nerve ring at anterior 3/10 of esophagus. Excretory pore just behind nerve ring. Genital primordium minute, sometimes hardly discernible, anterior to mid-intestine. Tail conical with notched tip. Phasmids in middle of tail. Measurements are stated in Table 2 being compared with previously reported data on *S. venezuelensis* and those of *S. ratti*.

c. Free-living adult: Free-living adults were rarely observed in cultures in spite of repeated attempts using various methods. Only females were collected from the filter paper cultures.

Female (Fig. 1B): Small and long spindle-shaped. Cuticle with extremely fine transverse striations. Mouth elongated dorso-ventrally, and lateral cephalic lobes present; 4 papillae and 2 amphids present. Esophagus rhabditoid. Nerve ring at posterior part of isthmus. Excretory pore just behind nerve ring. Reproductive system didelphic; both ovaries reflected. Vagina weakly developed: vulva at midbody; anterior and posterior vulval lips slightly elevated; prevulval constriction of body absent. Tail long conical with pointed tip. Morphometric data are given in Table 3 in comparison with previously reported data on *S. venezuelensis* and those of *S. ratti*.

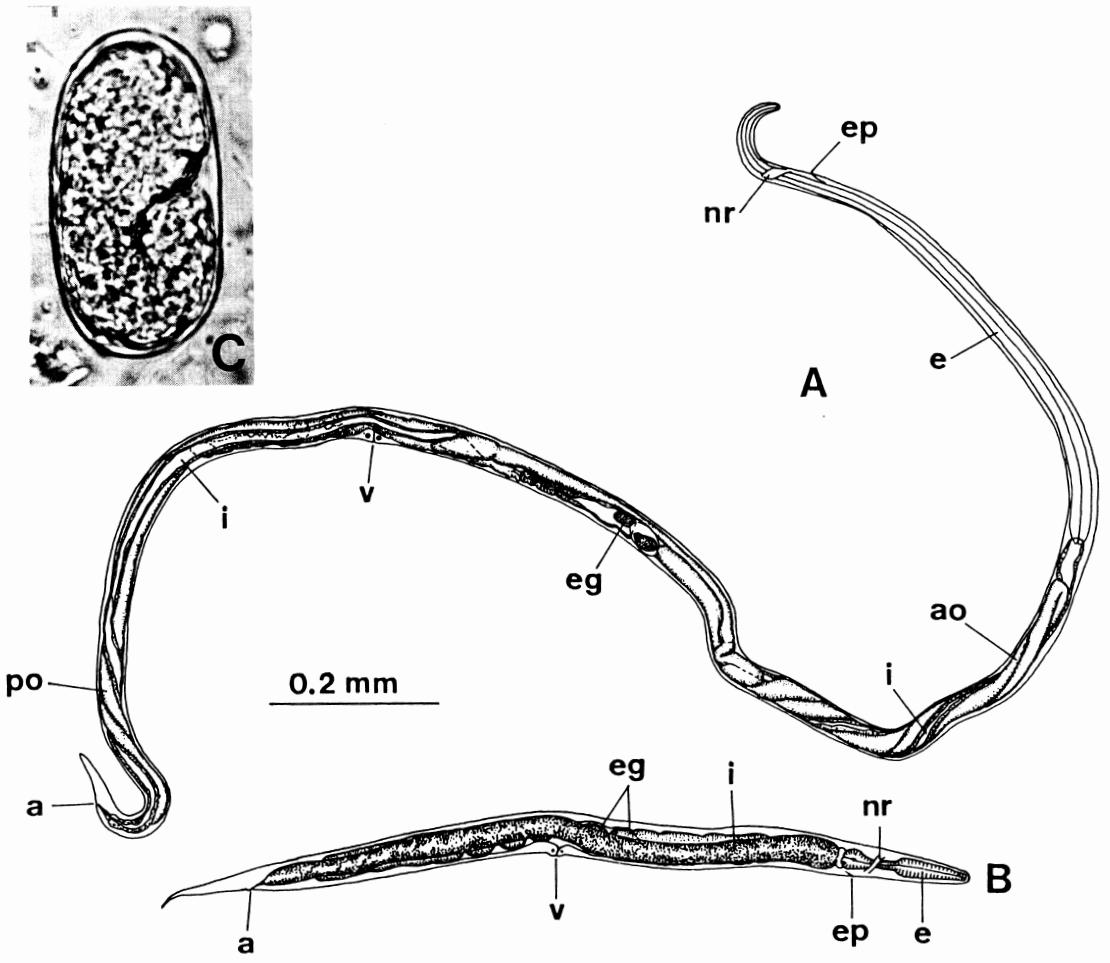

Fig. 1. *Strongyloides venezuelensis* Brimpt, 1934 isolated from *Rattus norvegicus* in Okinawa, Japan. A. Parasitic female, B. free-living female and C. Egg passed in fresh feces of infected rat (× 800). Abbreviations: a. anus, ao. anterior ovary, e. esophagus, eg, egg(s), ep. excretory pore, i. intestine, nr. nerve ring, po. posterior ovary, v. vulva.

Distribution of parasitic females in rat intestine

a. Longitudinal distribution. The total recovery rate at 10th and 17th day of infection was 20.0 to 38.0 (mean 30.4)% (n = 5) and 60.4 to 62.0 (61.2)% (n = 2), respectively. At 10th day of infection, 90.0 to 100.0 (mean 95.3)% of *S. venezuelensis* were recovered from the anterior-most (1st) section of intestine and only 0.0 to 10.0 (mean 5.0)% were detected in 2nd section. No worm was found in the posterior 4 sections. At 17th day of infection, 86.1 to 89.4 (mean 87.8)% were detected in 1st section and 10.6 to 13.9 (mean 12.3)% were in 2nd section. Again, no worm was recovered from the posterior 4 sections. On the other hand, in the rats infected with *S. ratti* and autopsied at 8th and 13th days of infection (n = 3), 29.5 to 39.0 (mean 33.2)% of worms were recovered from 2nd section and 3.9 to 4.9 (4.5)% and 0.3 to 2.0 (1.0)% were also found in 3rd and 4th sections, respectively, although 54.5 to 65.9 (61.3)% were detected in 1st section.

b. Radial distribution. Parasitic females of *S.*

Table 1 Comparison of measurements among parasitic females of *Strongyloides venezuelensis* and *S. ratti*

Species	*Strongyloides venezuelensis*			*Strongyloides ratti*		
Locality	Okinawa	USA	Israel	Okinawa	Kumamoto*	USA
Reporter	Present authors	Little (1966)	Wertheim (1970a)	Present authors	Present authors	Little (1966)
No. worms measured	20	13	20	20	20	10
Body length, mm	2.97±0.22	2.59	2.86	3.19±0.13	2.35±0.10	2.37
	(2.48-3.49)	(2.0-3.2)	(2.65-3.09)	(2.97-3.45)	(2.17-2.56)	(2.1-3.1)
Body width, μm	40. ±2.0	38	31	42.6±2.0	37.7±0.86	34
	(36-43)	(33-41)	(29-35)	(40-49)	(36-40)	(30-38)
From cephalic apex to:						
Nerve ring, μm	151.1±11.2			190.6±10.9	162.1±14.0	
	(130-169)			(169-208)	(125-183)	
Excretory pore, μm	196.3±12.9			262.8±14.5	230.6±20.9	
	(177-221)			(220-283)	(168-260)	
Vulva, mm	1.97±0.15	1.74		2.09±0.09	1.59±0.08	1.60
	(1.61-2.27)	(1.4-2.2)		(1.95-2.27)	(1.39-1.77)	(1.4-1.9)
Esophagus length, mm	0.77±0.05	0.68	0.78	0.78±0.04	0.81±0.03	0.74
	(0.65-0.85)	(0.60-0.78)	(0.75-0.84)	(0.72-0.86)	(0.77-0.86)	(0.73-0.76)
Tail length, μm	66.5±5.0	44	53	68.1±4.9	71.7±7.7	55
	(57-75)	(38-58)	(45-61)	(60-78)	(58-83)	(45-65)

Mean ±SD (Range)
* A strain provided by the Department of Parasitic Diseases, Kumamoto University School of Medicine.

Table 2 Comparison of measurements among filariform larvae of *Strongyloides venezuelensis* and *S. ratti*

Species	*Strongyloides venezuelensis*			*Strongyloides ratti*	
Locality	Okinawa	USA	Israel	Okinawa	Kumamoto*
Reporter	Present authors	Little (1966)	Wertheim (1970a)	Present authors	Present authors
No. worms measured	20	41	50	20	20
Body length, mm	0.59±0.02	0.57	0.57	0.60±0.02	0.61±0.03
	(0.54-0.61)	(0.47-0.64)	(0.51-0.64)	(0.56-0.64)	(0.54-0.64)
Body width, μm	17.9±0.6	17.7	17	18.3±0.5	18.0±0.6
	(16-19)	(16-19)	(15-18)	(18-19)	(16-19)
From cephalic apex to:					
Nerve ring, μm	85.7±4.0			92.2±4.2	87.5±3.6
	(75-91)			(88-104)	(83-93)
Excretory pore, μm	105.2±3.7			113.0±8.9	105.6±6.3
	(95-109)			(104-120)	(98-130)
Esophagus length, μm	270.3±7.7	254	251	256.8±8.6	252.0±11.0
	(255-283)	(220-290)	(218-272)	(237-280)	(228-295)
Tail length, μm	63.7±2.4	60	60	63.8±2.6	65.2±4.2
	(60-68)	(45-70)	(58-66)	(60-68)	(55-70)

Mean ±SD; (Range)
* A strain provided by the Department of Parasitic Diseases, Kumamoto University School of Medicine.

Table 3 Comparison of measurements among free-living females of *Strongyloides venezuelensis* and *S. ratti*

Species	*Strongyloides venezuelensis*		*Strongyloides ratti*	
Locality	Okinawa	USA	Okinawa	Kumamoto*
Reporter	Present authors	Little (1966)	Present authors	Present authors
No. worms measured	20	12	20	10
Body length, mm	1.03±0.05	1.0	1.12±0.07	1.00±0.06
	(0.94-1.11)	(0.81-1.2)	(0.95-1.20)	(0.92-1.07)
Body width, μm	51.9±2.3	53	66.4±6.3	49.8±3.3
	(48-55)	(40-70)	(53-78)	(45-55)
From cephalic apex to:				
Nerve ring, μm	114.8±5.11		105.2±5.8	102.0±4.9
	(104-125)		(94-117)	(95-113)
Excretory pore, μm	146.4±7.5		163.4±13.9	138.9±7.7
	(130-157)		(122-187)	(123-163)
Vulva, mm	0.53±0.03	0.51	0.56±0.04	0.49±0.03
	(0.49-0.58)	(0.41-0.57)	(0.48-0.62)	(0.42-0.55)
Esophagus length, μm	148.3±8.6	140	144.4±7.5	138.9±7.7
	(135-164)	(125-155)	(127-156)	(125-151)
Tail length, μm	111.6±5.3	115	128.1±13.1	128.1±10.3
	(101-120)	(80-130)	(101-148)	(100-143)

Mean ±SD; (Range)
* A strain provided by the Department of Parasitic Diseases, Kumamoto University School of Medicine.

venezuelensis were located in the villi, and not entered deep into the Lieberkühn crypts, while *S. ratti* was usually located in the crypts.

Discussion

Strongyloides venezuelensis is easily distinguishable from *S. ratti* in that the parasitic female has spiraled ovaries, and the eggs are in early developmental stages when passed in the host feces (Little, 1966). The distances from the cephalic apex to the nerve ring and to the excretory pore in *S. venezuelensis* of Okinawa are larger than those in *S. ratti* of Okinawa. The parasitic female of *S. venezuelensis* collected in Okinawa has longer tail than that of *S. venezuelensis* reported from the United States and Israel (Little, 1966; Wertheim, 1970a) (Table 1). The filariform larva of *S. venezuelensis* of Okinawa has longer esophagus than that of the United States and Israel (Little, 1966; Wertheim, 1970a) (Table 2). These morphometric differences are supposed to be geographical and/or strain variations. The longitudinal and radial distributions of *S. venezuelensis* in the rat intestine were identical with those reported by Wertheim (1970b).

As all of recorded localities of *S. venezuelensis* are situated between 35° North and 35° South Latitude (Brumpt, 1934; Wertheim and Lengy, 1964; Little, 1966; Araujo, 1967), it is suggested that this nematode requires a warm climate for the development in the external environment. The presence of *S. venezuelensis* in the Naha Port region is considered to be natural since Okinawa has a subtropical climate, and many commercial and military ships, by which rats spread their distribution occasionally, frequently arrive from various parts of the world.

It is of interest that free-living adults, especially male, were seldom developed in the

cultures of *S. venezuelensis* of Okinawa. Further study is required to elucidate the factors which prevented the development of free-living adults.

It is suggested that *S. venezuelensis* may be distributed in Southeast Asia and the mainland of Japan, especially in harbor regions. Because of morphological similarity, *S. venezuelensis* might have been misidentified and/or confused with *S. ratti*. Careful examination of rat *Strongyloides* species in these areas may reveal wider distribution of *S. venezuelensis*.

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