Isolation of *Strongyloides venezuelensis* from *Rattus norvegicus* in Kagoshima Prefecture

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ABSTRACT. On 30 August 1984, *Strongyloides* sp. specimens were isolated from a rat (*Rattus norvegicus*) trapped at a pig farm in Kiire-cho, Ibusuki-gun, Kagoshima Prefecture. Both anterior and posterior ovaries of parasitic female were spirally coiled. The means of body length and width, esophagus length, and the distances from mouth to vulva and from anus to tail end were 2,493, 34.5, 719.8, 1,682.8 and 54.4 μ m respectively. Filariform larvae measured 544.2, 15.6 and 238.5 μ m in the means of body length and width, and esophagus length respectively. These measurements of the present *Strongyloides* species agreed with those described for *S. venezuelensis*. Infection rate by oral administration of larvae was low, while subcutaneous inoculation and percutaneous exposure to larvae produced high infection rate to rats which discharged many egg in the feces. The present parasite has been passaged through rats (SD strain) in these 8 years. This isolate is designated as the Kagoshima strain of *S. venezuelensis*.—**KEY WORDS:** distribution, *Rattus*, *Strongyloides venezuelensis*.

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Strongyloides venezuelensis (SVZ) was first reported from rats by Brumpt (1934) [4], and has been found in North America (New Orleans), South America (Venezuela) [2], and Israel [7]. In Japan, this species of parasite has not been found in other districts than Naha, Okinawa [1]. Experimental infections with SVZ, however, has not been reported.

On 30 August 1984, *Strongyloides* sp. specimens were isolated from a rat (*Rattus norvegicus*) trapped at a pig farm in Sesegushi, Kiire-cho, Ibusuki-gun, Kagoshima Prefecture. The present report deals with morphology of the worm and influence of inoculation route on infectivity of larvae to rats (SD strain).

MATERIALS AND METHODS

The parasitic females examined were recovered from the small intestine of a rat (SD strain) 14 days after inoculation with *Strongyloides* sp. which had been passaged in the laboratory through rats since 1984 [6]. Worms recovered were fixed in 5% formalin. Twelve worms were measured for body length and width, esophagus length, the distances from mouth to vulva and from the anus to

tail end. Filariform larvae examined were recovered from fecal cultures, which consisted of a small piece of cotton immersed in suspension of infected feces and incubated at 25°C for 3 to 5 days [5]. Twenty larvae killed by heating were measured for body length and width and esophagus length.

Rats were inoculated with larvae by three routes: oral administration with a stomach catheter, subcutaneous inoculation, and percutaneous exposure. In percutaneous exposure, rats were kept in a beaker on the bottom layered with wet cotton to which determined numbers of larvae had been disseminated. A total of 18 rats was used with in three experimental groups with 6 animals each, each groups being subdivided into three with 2 rats. The subgroups of rats were infected with 500, 1,000 and 2,000 larvae, respectively.

Rats were examined for larval counts in the feces 7 and 11 days after infection by the polyethylene tube culture. The procedure of culture was as follows: 1 g of fecal suspension was absorbed with a piece of dry cotton. The cotton was then wrapped in a piece of gauze and was set in a triangle polyethylene bag 16 cm in depth and 9 cm in width. The bag was filled with tap water and was

Fig. 1. Parasitic female of *Strongyloides venezuelensis* (Kagoshima strain); aov, anterior ovary; an, anus; es, esophagus; int, intestine; nrv, nerve ring; pov, posterior ovary; vu, vulva.

incubated at 25°C. At 24 and 48 hr after incubation, the sediment in the bag was transferred onto a grass slide for counting larvae.

All the rats were necropsied 11 days after infection to make count of worms in the small intestine.

RESULTS

Both the anterior and posterior ovaries of parasitic female were spirally coiled (Fig. 1). The means (ranges) of body length and width, esophagus length, distance from mouth to vulva, and from anus to tail end were 2,493.3 (2,035–2,959), 34.5 (29.0–41.0), 719.8 (667–775), 1,682.8 (1,367–1,960) and 54.4 (41–69) μm respectively (Table 1). Filariform larvae measured the means (ranges) of body length and width, and the esophagus length were 544.2 (482.3–573.1), 15.6 (14.4–17.4) and 238.5 (213.7–262.5) μm respectively (Table 2).

Four of 6 rats orally inoculated with larvae showed comparatively slight infection and two rats among them developed no infection. On the other hand, all the rats of subcutaneously inoculated and percutaneously exposed groups were severely infected and discharged many eggs

which developed into larvae at 7 and 11 days after infection. Additionally, in these rats comparatively large numbers of parasitic females were detected at necropsy (Table 3).

DISCUSSION

The measurements of adult worms and larvae of the present *Strongyloides* species agreed with those described for *S. venezuelensis* by Hasegawa [1], Little [3] and Wertheim [8], so the species was designated as the Kagoshima strain of *S. venezuelensis*.

The present *S. venezuelensis* shed no free larvae but only embryonated or blastular eggs in the fresh feces. Free-living stages of worm in the indirect life cycle were seldom detected in fecal cultures. The prepatent period ranged from 6 to 7 days. These findings also supported the premise that this isolate is *S. venezuelensis*. This species of worm was easily passaged in the laboratory through rats because large numbers of eggs and larvae were obtained from rats exposed to 10,000–30,000 larvae. The Kagoshima strain of *S. venezuelensis* may be a useful parasite model for fundamental studies on strongyloidiasis.

Table 1. Measurements of *Strongyloides venezuelensis* parasitic females (Kagoshima strain) compared with those by previous authors

Parameter		Present authors n=12	Hasegawa <i>et</i> <i>al.</i> (1988) n=20	Little (1966) n=13	Wertheim (1970) n=15
Body length in μm	Mean	2,493.3	2,970	2,590	2,856
	Min.	2,035	2,480	2,000	2,652
	Max.	2,959	3,490	3,200	3,087
	SD ^{a)}	287.34	220		
	C. limits ^{b)}	182.57			
Body width in μm	Mean	34.5	40	38	31
	Min.	29.0	36	33	29
	Max.	41.0	43	41	35
	SD	3.44	2		
	C. limits	2.19			
Esophagus length in μm	Mean	719.8	770	680	779
	Min.	667	650	600	748
	Max.	775	580	780	843
	SD	39.52	50		
	C. limits	25.11			
Ratio (%) ^{c)}		28.9	25.9	26.3	27.3
Mouth to vulva in μm	Mean	1,682.8	1,970	1,740	
	Min.	1,367	1,610	1,400	
	Max.	1,960	2,270	2,200	
	SD	189.87	150		
	C. limits	120.64			
Anus to tail end in μm	Mean	54.4	66.5	44	53
	Min.	41	57	38	45
	Max.	69	75	58	61
	SD	7.69	5		
	C. limits	4.89			

a) Standard deviation.

b) 95% confidence limits ($t_{(11,0.05)} \times \text{Standard error}$).

c) Mean of esophagus length / Mean of body length.

Table 2. Measurements of *Strongyloides venezuelensis* filariform larvae (Kagoshima strain) compared with those by previous authors

Parameter		Present authors n=20	Hasegawa <i>et al.</i> (1988) n=20	Little (1966) n=41	Wertheim (1970) n=50
Body length in μm	Mean	544.2	590	565	568
	Min.	482.3	540	470	505
	Max.	573.1	610	640	639
	SD ^{a)}	21.10	20		
	C. limits ^{b)}	9.90			
Body width in μm	Mean	15.6	17.9	17.7	17
	Min.	14.4	16	16	15
	Max.	17.4	19	19	18
	SD	0.80	0.6		
	C. limits	0.40			
Esophagus length in μm	Mean	238.5	270.3	254	251
	Min.	213.7	255	220	218
	Max.	262.5	283	290	272
	SD	12.90	7.7		
	C. limits	6.60			
Ratio (%) ^{c)}		43.8	45.8	45.0	44.2

a) Standard deviation.

b) 95% confidence limits ($t_{(19,0.05)} \times \text{Standard error}$).

c) Mean of esophagus length / Mean of body length.

Table 3. Effect of the route of administration on establishment of *Strongyloides venezuelensis* infection in rats

Route of infection	Number of larvae given	Rat No.	Days after infection				Number ^{c)} of worms recovered
			7 days		11 days		
			LPG-1 ^{a)}	LPG-2 ^{b)}	LPG-1	LPG-2	
A Oral	500	1	0	0	60	0	1
		2	90	16	127	10	0
	1000	3	116	1	118	2	2
		4	0	0	0	0	0
	2000	5	62	3	335	0	1
		6	1,360	415	1,175	46	8
	Mean		271.3	72.5	302.5	9.7	2.0
B Subcutaneous	500	7	3,760	274	4,475	146	28
		8	5,910	160	3,050	340	20
	1000	9	3,310	240	3,825	90	23
		10	4,160	530	7,875	177	43
	2000	11	4,890	580	4,750	240	36
		12	11,540	370	7,976	303	154
	Mean		5,595.0	359.0	5,325.2	216.0	50.7
t-value to A		4.40**		3.46**		2.32**	
C Percutaneous	500	13	670	48	1,900	30	7
		14	100	25	475	6	5
	1000	15	3,970	380	2,250	56	19
		16	4,150	640	2,050	25	28
	2000	17	2,220	800	3,200	70	23
		18	13,900	342	4,850	100	146
	Mean		4,168.3	372.5	2,454.2	47.8	38.0
t-value to A		1.97 ^{NS}		3.44**		1.64 ^{NS}	
t-value to B		0.60 ^{NS}		2.75*		0.42 ^{NS}	

a) Polyethylene tube culture at 25°C for 24 hr.

b) Polyethylene tube culture at 25°C between 24 and 48 hr.

c) Parasitic females in the small intestine (11 days after infection).

NS=Not significant, *= $P<0.05$, **= $P<0.01$.

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