The Korean Journal of Parasitology
Vol. 36, No. 2, 99-107, June 1998

Viability of eggs, filariform larvae and adults of *Strongyloides venezuelensis* (Nematoda: Strongyloidea) maintained *in vitro*

Byeong-Kirl BAEK*, M. Khyrul ISLAM and Kiku MATSUDA

Biosafety Research Institute, Chonbuk National University, Chonju 561-756, Korea

Abstract: The present study was performed to check the viability of eggs, filariform larvae and adults of *Strongyloides venezuelensis* exposed to various conditions for an *in vitro* maintenance. The eggs in the feces remained viable for about 25 days at 4°C and 15 days at room temperature. However, the isolated eggs in sterile saline lost their viability within 24 hr at 4°C. The eggs in morula stage were very sensitive to air drying and rapidly lost their viability (≤ 12 hr). Filariform larvae survived for a maximum period of 45 days in fecal suspension and 28 days in 0.12% nutrient broth in polyvinyl culture bags maintained at 20°C. On the other hand, those isolated from nutrient broth cultures survived for a maximum period of 32 days in tap water and 22 days in sterile saline at 20°C. The mature adult worms obtained from experimentally infected rats survived maximally for 9 days in serum supplemented (10% rat-serum) 0.12% nutrient broth and 4 days in serum free nutrient broth at 37°C while the culture media were changed at an alternate day. The adult female worms deposited fertile eggs in serum supplemented and serum free nutrient broth cultures, however, the hatched larvae (L_1) were not able to develop to the filariform stage in the culture media and found to die within 24 hr of maintenance. The present findings on an *in vitro* maintenance of different stages of *S. venezuelensis* may provide useful information for biological and biochemical studies with *Strongyloides* species.

Key words: *Strongyloides venezuelensis*, viability, *in vitro* maintenance, free-living filariform larvae (L_3), embryonation of eggs

INTRODUCTION

Strongyloides venezuelensis (Brumpt, 1934), an intestinal nematode of rat, has recently been used as a suitable parasite model for biological and biochemical studies (Sato and Toma, 1990; Tsuji *et al.*, 1993; Tsuji and Fujisaki, 1994). Investigations involving an *in vitro* cultivation and/or maintenance of free-living and parasitic filariform larvae, juveniles and adults from experimentally infected rats generally employ various kinds of synthetic media supplemented with or without mammalian serum (Ito and Komiya, 1955; Douvres and Malakatis, 1977; Bonner, 1979; Fenglin *et al.*, 1979; McDiarmid and Matthews, 1982; Tsuji and Fujisaki, 1994; Call *et al.*, 1995). In most cases, such culture systems are complex with multi-steps and require a

• Received 23 January 1998, accepted after revision 28 April 1998.

• This study was financially supported by a Research Grant from Biosafety Research Institute, Chonbuk National University in 1997 (CNU-BSRI, No. 97-14).

* Corresponding author (e-mail: baekbk@moak.chonbuk.ac.kr)

media change at certain interval to provide an optimal environment for further development and/or maintenance. Recently, we have devised a culture method using nutrient broth for the cultivation of *S. venezuelensis* free-living filariform larvae (L_3) from eggs, which is free from mammalian serum, and simple in its composition and handling (Baek *et al.*, 1998). A large numbers of L_3 have been found to develop in the polyvinyl bags containing culture medium and survive for an extended period therein without further change of the medium.

The present study was conducted to determine the optimal conditions (*e.g.*, culture media, temperature, *etc.*) for an *in vitro* maintenance of *S. venezuelensis* in different stages such as eggs, L_3 and adults, which may facilitate the screening of wormicidal chemicals and anthelmintics, and biological studies with *Strongyloides* species. An attempt was also made to report the morphological development of eggs towards maturation and embryonation, which, at particular stage, may influence the maintenance/viable period of eggs.

MATERIALS AND METHODS

Collection of eggs and filariform larvae

In vivo maintenance of *S. venezuelensis* in Sprague-Dawley (SD) rats, collection of eggs from the infected-rat feces (IRF) and culture of L_3 were undertaken by the method of Baek *et al.* (1998).

Morphology and viability of eggs

Eight-week-old SD male rats previously infected with 10,000 L_3 were killed by overdose ether anesthesia on day 7 post-infection (PI). Anterior 10 cm of the small intestine was opened longitudinally and the mucous membranes were scraped with a surgical blade. The mucosal scrapings were placed in a petri dish containing sterile saline (SS) pre-heated to 37°C and shaken gently for 10 min to allow adult worms to migrate out of the mucosa. The adults were examined under a light microscope for eggs in the primary stage of development in the ovary and measured *in situ*. The eggs in different cell division stages were collected from the uterus by crushing the worms in SS. The mucosal scrapings were carefully examined for the presence of eggs deposited in a strand. The eggs were also collected from both the intestinal contents and freshly passed feces by flotation method. The latter were incubated at 25°C according to the methods described by Baek *et al.* (1998) until the embryo fully developed. The embryonated eggs and newly hatched larvae were collected. Morphological characteristics of eggs were observed in the primary stage in the ovary, immature and mature stages in the uterus, deposited eggs in the intestinal mucosa, in the intestinal lumen, and in freshly passed feces. Eggs isolated from freshly passed IRF suspended in SS (approximately 10^4 eggs/ml) in 15 ml Falcon conical tube (Becton Dickinson Company, USA) as well as freshly passed IRF (5 g feces/tube) were stored at 0°C, 4°C and room temperature. The preserved samples in each conditions were transferred to a petri dish with SS incubated at 25°C for 48 hr to observe the hatch rate. Additionally, the fecal sample of 0.5 g was placed in 10 × 12 cm polyvinyl culture bag (PB) containing 10 ml SS and incubated as above. The isolated eggs in SS as well as the IRF were exposed to air drying by transferring a small volume of the samples to a petri dish and left for 12 hr at room temperature. Upon complete drying, the samples were incubated in SS similarly as mentioned above to assess the effect of drying on egg hatch rate. The end point of the viable period of the isolated eggs/IRF was determined when the eggs did not hatch on incubation and became degenerated.

Viability of filariform larvae

The viability of the L_3 in PB was assessed by maintaining them at 4°C, 10°C, 15°C, 20°C, 25°C and 37°C. Two types of culture media were prepared: (i) 0.12% nutrient broth and (ii) fecal suspension (0.2 g freshly passed uninfected rat feces in 10 ml SS/PB). A numbers of PBs containing each culture media separately were inoculated with 15,000 eggs and incubated at 25°C for the development of L_3 as described by Baek *et al.* (1998). As soon as the L_3 developed in the PBs (usually on day 4 post-incubation), they were shifted to

different temperatures as mentioned above and maintained. The PBs were examined daily under a light microscope to check the viability of the L_3, which was recorded until the larvae lost motility. The viability of the isolated L_3 from the PBs was examined by maintaining them in tap water and in SS. The L_3 were isolated from nutrient broth cultures on day 5 post-cultivation by Baermann method, washed five-times in SS containing antibacterial (penicillin G potassium salt 500 units/ml, streptomycin sulphate 500 units/ml) and antifungal agents (amphotericin B 2 μg/ml) obtained from Sigma, by centrifugation at 1,500 rpm for 7 min. The larval suspensions, approximately 5,000 L_3 in 0.5 ml SS, were placed in 15 ml conical tube containing 5 ml tap water and SS, respectively. Both types of culture tubes were maintained at above mentioned temperatures.

Collection of non-immune rat sera

Sera were collected from 6-8 week-old parasite-free SD male rats and stored at –20°C until used. They were inactivated by heating at 56°C for 35 min and used as supplements in nutrient broth for the maintenance of adults recovered from experimentally infected rats.

Collection of adult worms

Adult worms were collected from SD rats infected with 20,000 L_3. The small intestine of rats was dissected out, opened longitudinally and incubated in SS at 37°C for 3 hr in a Baermann device using two-folds gauze. The worms capable of migrating through the gauze and having settled at the bottom of the apparatus were collected and washed five-times in SS containing antibacterial and antifungal agents by centrifugation at 1,000 rpm for 7 min. Approximately 30% of the L_3 were recovered as adults on day 7 PI. Finally, 500 adults were suspended in 0.5 ml SS and used as an inoculum.

Viability of adult worms

To determine the viability of adult worms, three different types of culture media were prepared as follows: (i) autoclaved 0.12% nutrient broth, (ii) 0.12% nutrient broth plus inactivated rat sera to a final concentration of 10%, and (iii) fecal suspension (5% freshly passed uninfected rat feces in SS). Antibacterial and antifungal agents to an half-concentration were added to all culture media. An inoculum of adult worms was placed in 15 ml conical tube containing 5 ml culture media. The culture tubes were then maintained at different temperatures *viz.* 4°C, 10°C, 20°C, 25°C and 37°C without further exchange of the media. The viability of adults was monitored at 24 hr interval. In another setting, culture media were changed at an alternate day and monitored as mentioned above. The numbers of eggs deposited by parasitic females per day in culture tube were counted in order to assess the egg-laying capacity of *S. venezuelensis in vitro*.

RESULTS

Morphological characteristics of eggs

Eggs of *S. venezuelensis* in the primary stage of development in the ovary of a parasitic female were oval to rectangular in shape, covered with a thin layer of envelope, and measured 32.59 ± 4.69 × 24.21 ± 1.52 μm (Fig. 1). Those in the uterus of parasitic females in various developmental stages were elongated oval or elliptical in shape, covered with a thin layer of egg-shell containing 2-32 germinal cells, and measured 51.75 ± 4.26 × 22.45 ± 1.86 μm (Fig. 2). The female worms in the intestinal villi, layed mature eggs in a strand (Fig. 3). A single egg-strand was found to contain 3-15 eggs. The eggs obtained from the intestinal contents of rats measured 52.35 ± 2.43 × 25.39 ± 1.08 μm. Further development of eggs occurred in the intestinal tract during their passage towards the rectum at which stage the germinal cells developed to a blastular or morula stage embryo. The eggs excreted in fresh feces were in morula or tadpole stage of embryonic development and measured 53.00 ± 3.50 × 25.75 ± 3.15 μm (Fig. 4). The fully embryonated eggs containing a motile 'S-shaped' larva measured 52.63 ± 2.69 × 27.88 ± 1.72 μm (Fig. 5), and the newly hatched larvae were 176.78 ± 15.35 μm × 14.34 ± 1.87 μm in body size (Fig. 6).

Viability of eggs exposed to various conditions

Eggs in IRF survived for a maximum period of 25 days at 4°C and 15 days at room temperature, respectively (Table 1). The isolated eggs suspended in SS did not hatch at 0°C and at 4°C, and lost viability within 24 hr, while a few eggs (5%) hatched at room temperature and survived only for 24 hr. The isolated eggs as well as those in the IRF lost viability rapidly (≤ 12 hr) when exposed to air drying at room temperature.

Viability of larvae

Filariform larvae of *S. venezuelensis* survived for a maximum period of 45 days in fecal suspension and 28 days in 0.12% nutrient broth in PB at 20°C, respectively (Table 2). On the other hand, the viable period of the L_3 in both type of culture media in PB was 2 days at 4°C and at 37°C. In contrast, the isolated L_3 survived maximally for 32 days in tap water and 22 days in SS maintained at 20°C, and minimally for 1-2 days at 4°C and at 37°C (Table 2).

Morphology of larvae

The dead larvae in the PBs and those in tap water and SS were easily distin-guished from the alive by the absence of free movement/ motility. Their body were relaxed and straight with degeneration of buccal capsule, esophageal-intestinal junction, intestinal tract and anus (Fig. 7). In addition, the dead larvae did not coil their body when a drop of 10% formalin was added while the alive did (Fig. 8). The alive larvae showed an intact buccal capsule, digestive tract and anus.

Viability of adult worms

The highest survival period of the mature adults was 9 days in serum supplemented nutrient broth and 4 days in serum free nutrient broth at 37°C with exchange of the culture media at an alternate day (Table 3). However, they did not survive at 4°C and 10°C irrespective of the culture media (Table 3). The lowest viable period (< 24 hr) was observed in fecal suspension irrespective of temperatures

Table 1. Viability of *S. venezuelensis* eggs exposed to different temperatures and air drying at room temperature

Temperature (°C)	Viability of eggs	
	Isolated eggs in sterile saline	Infected feces
0	≤ 24 hr	24 hr
4	≤ 24 hr	25 days
Room temp. (25°C)	24 hr	15 days
Air drying at room temp.	≤ 12 hr	≤ 12 hr

Table 2. Viability of filariform larvae of *S. venezuelensis* maintained in various culture media at different temperatures

Temperature (°C)	Viability (days) of larvae in			
	NB[a)]	FS[b)]	TW[c)]	SS[d)]
4	2	2	1	1
10	7	15	4	3
15	25	30	18	12
20	28	45	32	22
25	7	10	12	8
37	2	2	2	2

[a)]nutrient broth; [b)]fecal suspension; [c)]tap water; [d)]sterile saline.

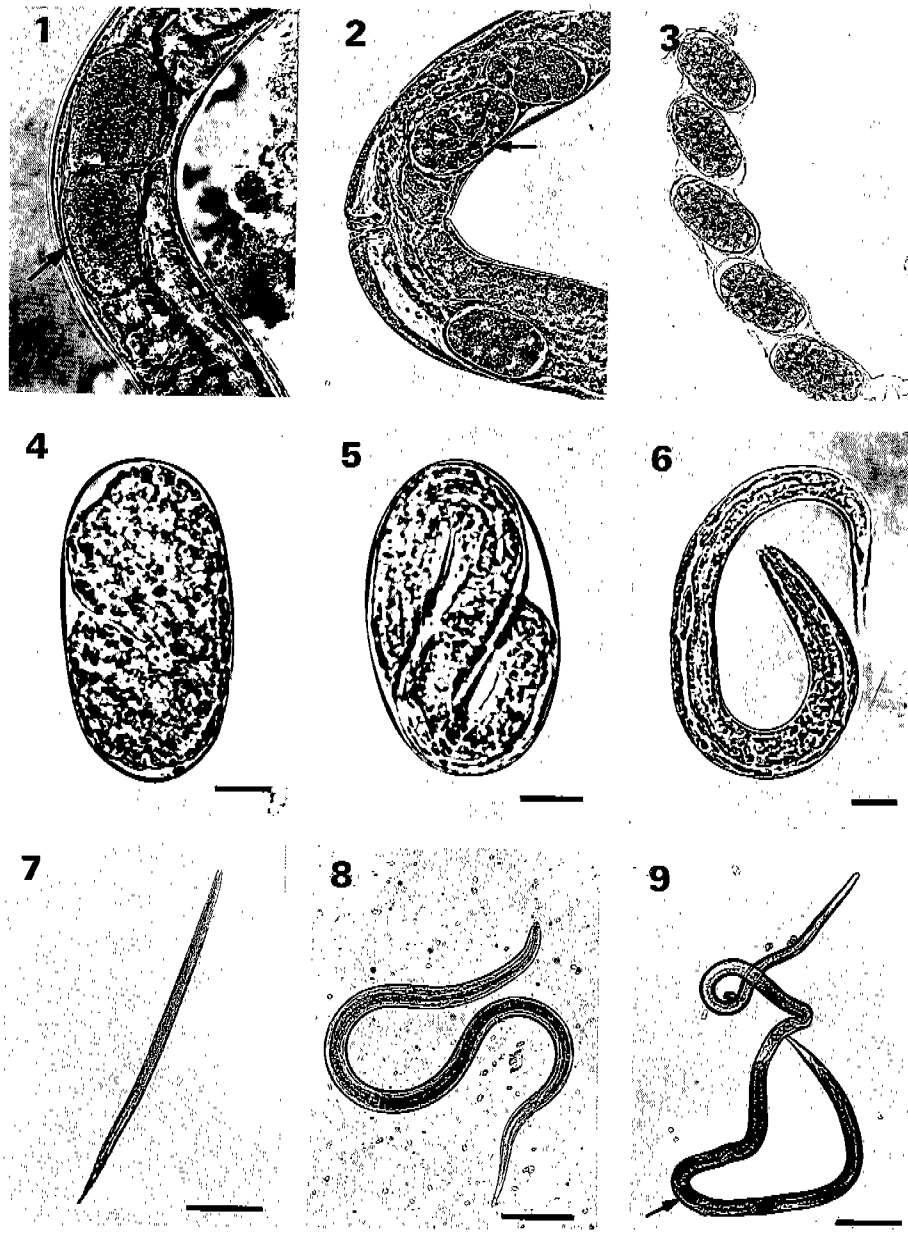

Fig. 1. Eggs of *S. venezuelensis* (arrow) in the primary stage of development in the ovary of a parasitic female (× 600). **Fig. 2.** Eggs (arrow) in different developmental stages in the uterus of a parasitic female (× 300). **Fig. 3.** An egg-strand isolated from mucosal scrapings (× 300). **Fig. 4.** An egg collected from freshly passed feces. Bar = 10 μm. **Fig. 5.** An embryonated egg. Bar = 10 μm. **Fig. 6.** A newly hatched larva of *S. venezuelensis*. Bar = 10 μm. **Fig. 7.** A free-living filariform larva recovered from the polyvinyl culture bag after death. Bar = 100 μm. **Fig. 8.** A free-living filariform larva recovered from the polyvinyl culture bag before death (fixed in 10% formalin). Bar = 50 μm. **Fig. 9.** A mature parasitic female maintained in serum supplemented nutrient broth at 37°C. Note the eggs in the uterus (arrow). Bar = 200 μm.

used for maintenance. The female worms deposited fertile eggs (3.34 ± 0.72 eggs per worm by 48 hr) in serum supplemented and serum free nutrient broth cultures, those

Table 3. Viability of *S. venezuelensis* adult worms in nutrient broth and fecal suspension maintained at different temperatures

Temperature (°C)	Viability of adult worms (days)					
	Media not changed			Media changed at an alternate day		
	NB[a]	NBS[b]	FS[c]	NB	NBS	FS
4	< 1	< 1	< 1	nd[d]	nd	nd
10	< 1	< 1	< 1	nd	nd	nd
20	3	3	< 1	4	5	nd
25	3	4	< 1	4	5	nd
37	3	5	< 1	4	9	nd

[a]nutrient broth; [b]serum supplemented nutrient broth; [c]fecal suspension; [d]not done

maintained at 20°C, 25°C and 37°C for the first two days only (Fig. 9). However, the newly hatched larvae were not able to develop to the L_3 stage in the culture media and were found to die within 24 hr of maintenance.

DISCUSSION

There have been numerous reports on the development of an *in vitro* culture techniques for parasitic helminths to obtain excretory/secretory products of parasites (Healer *et al.*, 1991), to screen the effects of anthelmintics (Rew *et al.*, 1986), to evaluate nutritional requirements of larvae (Urban *et al.*, 1984), and to determine biochemical changes associated with parasite development (Gamble and Mansfield, 1996). An *in vivo* maintenance of adult stage parasites for experimental investigation requires a serial passage in susceptible laboratory animals, which is not only an expensive work but also a time consuming one. Therefore, the present study was designed to develop a simple *in vitro* culture system free from mammalian serum for maintenance of L_3 and adult worms as well as to develop the technique(s) for extending the viability/longevity of the parasite and eggs on preservation, which ultimately reduce the frequency of serial passage and/or can meet up the necessity of *in vivo* maintenance of the adult parasites.

In many parasitic nematodes including *S. venezuelensis*, eggs are layed to the external environment through feces, and under optimal conditions (*e.g.*, temperature, humidity, *etc.*) they develop to the L_3 stage which can invade the definitive hosts. The eggs of *S. venezuelensis* are produced through folliculogenesis in the ovary of a parasitic female, enter into the uterus where they undergo repeated cell division producing mature eggs, and expelled onto the villi epithelium of the small intestine. During passage in the intestinal tract, eggs developed to blastular or morula stage embryo covered with a thin shell, and excreted in the host feces, which corresponds with the findings of previous authors (Little, 1966; Hasegawa *et al.*, 1988; Taira *et al.*, 1994).

Isolated eggs of *S. venezuelensis* showed a lower viability when suspended in SS and, on the contrary, they were highly viable in the IRF stored at 4°C and at room temperature. This result strongly suggests that the viability of nematode eggs depends on the physiological status of the eggs and/or the environmental factors such as temperature, type of media, *etc.* This study also demonstrated that a lower temperature (4°C) served as an optimum condition for maintenance of eggs in the IRF but not for the isolated eggs. The latter could not survive even in a lower concentration of formalin (0.05%) and lost viability rapidly (< 24 hr) when stored at 4°C (data not shown). However, a longer viability (over one year) of infective eggs of *Ascaris suum* has been observed in 0.1% formalin at 4°C (Douvres and Urban, 1983). An egg of *A. suum* containing an infective-stage larva (L_2) is covered with a thick egg-shell, which may help the egg resist the effect of formalin in lower concentration, and larval metabolic activity at 4°C is diminished in the presence of formalin and thus gain

longer viability. By contrast, *S. venezuelensis* egg containing a morula stage embryo (*i.e.*, early stage embryo) is covered with a very thin egg-shell, which is not appropriate for egg protection from formalin, and therefore the egg die rapidly. These findings suggest that morphological characteristics of nematode eggs such as thickness of egg-shell, stage of embryo within an egg may play an important role in viability of eggs exposed to a physical or chemical environment. Eggs of *S. venezuelensis* were found very sensitive to air drying and degenerated rapidly. Based on the present findings, therefore, it is assumed that the viability of *S. venezuelensis* eggs at each stage of development is variable when exposed to different conditions (*e.g.*, temperatures, moistures, drying, chemicals) on preservation.

The viability of L_3 in both types of PBs was found highest at 20°C and lowest at 4°C and at 37°C. Interestingly, it was greatly increased when a suspension of freshly passed uninfected rat feces in SS was used in the PBs instead of 0.12% nutrient broth medium and maintained at the same incubation temperature. The longer viability of the L_3 in fecal suspension is thought to be due to the presence of enormous populations of microbial species in rat feces which might serve as nutrients for the L_3. It is known that the free-living stage of many species of parasitic nematodes hatchs and develops in host feces during which stage they are microbivorous (Weinstein, 1996). In our previous paper, we have reported that incubation temperature as well as concen-tration of nutrient broth significantly influence the growth and development of the *S. venezuelensis* filariform larvae (Baek *et al.*, 1998). The isolated L_3 survived signifi-cantly for a longer period in tap water (32 days) than in SS (22 days) maintained at 20°C. Increased viability of the isolated L_3 in tap water is attributed to the presence of numerous microbial organisms and/or organic substances in tap water. A shorter viability (two days) of L_3 in both PBs and in tap water/SS at 37°C contradicts with the findings of Tsuji and Fujisaki (1994) who maintained the L_3 for a week in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) at 25°C and at 37°C. The reason for such difference in viability of L_3 is supposed to be due to the absence of adequate nutritional components in culture media used in this study. The L_3 were viable for two days at 4°C, however, those of other gastrointestinal nematodes such as *Ostertagia ostertagi*, *Trichostrongylus axei* and *Oesophagostomum radiatum* have been shown to survive for over 45 days in tap water at 5°C (Douvres, 1979 & 1983; Douvres and Malakatis, 1977). An increased viability of microfilariae of *Onchocerca gutturosa* has also been observed at lower temperatures (4°C and 11°C) than 37°C in Tyrode's solution supplemented with 20% human serum (McDiarmid and Matthews, 1982). Taken together, it is suggested that the viability of the L_3 maintained *in vitro* is highly variable depending on parasite species, micro-environment where the larvae naturally grow and develop (*e.g.*, microfilariae in insect vector and in definitive host), nutritional components of the culture media and temperatures.

An *in vitro* maintenance of L_3 either in PBs or in tap water/SS could give us the opportunity of at least reducing the frequency of serial passage (biological passage) for *in vivo* maintenance of adults which requires infinite labor and great expense. Moreover, *in vitro* maintenance of L_3 as bio-bar/bioassay system could facilitate the screening tests for new chemical products, anthelmintics (Jenkins, 1982) and pesticides (Patel and Wright, 1996).

The adult worms did not survive at lower temperatures (4°C and 10°C) irrespective of the culture media used in this study. This might be due to the impairment of metabolic activity of the adults in culture media maintained at lower temperatures. This investigation revealed that supplements of rat serum to nutrient broth and application of fresh culture media at an alternate day in association with the increasing culture temperature (37°C) could extend the viable period at least to double (9 days) than in serum free nutrient broth (4 days). Some undefined media supplemented with dog serum have been used to maintain *in vivo* derived *Ancylostoma caninum* and *A. duodenale* juveniles and adults for over 1 month (Fenglin *et al.*, 1979). The results of the present study suggest that mature adult *S.*

venezuelensis recovered from experimentally infected rats is able to produce fertile eggs *in vitro* in serum supplemented and serum free nutrient broth cultures maintained at 20°C, 25°C and 37°C, but the larvae do not develop further in those culture media. Although Tsuji and Fujisaki (1994) reported for the first time to culture parasitic stage of *S. venezuelensis* from L_3 in a chemically defined media, however, there has been no report so far on the cultivation of adult *S. venezuelensis in vitro*.

The present study on an *in vitro* maintenance of *S. venezuelensis* in different stages reveals that eggs can be maintained as long as 25 days at 4°C, L_3 in culture bags for 45 days at 20°C and adult worms in serum supplemented nutrient broth for 9 days at 37°C. Our results provide the useful information on the maintenance of parasite as bio-bar system, for screening wormicidal chemicals and anthelmintics, and for biological studies with *Strongyloides* species.

REFERENCES

Baek BK, Islam MK, Kim JH (1998) Development of an *in vitro* culture method for harvesting the free-living infective larvae of *Strongyloides venezuelensis*. *Korean J Parasitol* **36:** 15-22.

Bonner TP (1979) Initiation of development *in vitro* of third-stage *Nippostrongylus brasiliensis*. *J Parasitol* **65:** 74-78.

Call JL, Pilcher JB, Freeman Jr GL, Tsang VCW (1995) Serum-free culturing of adult *Schistosoma mansoni* in dialysis bags for the production of excretory/secretory antigens. *J Parasitol* **81:** 742-746.

Douvres FW (1979) *In vitro* development of *Trichostrongylus axei*, from infective larvae to young adults. *J Parasitol* **65:** 79-84.

Douvres FW (1983) The *in vitro* cultivation of *Oesophagostomum radiatum*, the nodular worm of cattle. III. Effects of bovine heme on development to adults. *J Parasitol* **69:** 570-576.

Douvres FW, Malakatis GM (1977) *In vitro* cultivation of *Ostertagia ostertagi*, the medium stomach worm of cattle. I. Development from infective larvae to egg-laying adults. *J Parasitol* **63:** 520-527.

Douvres FW, Urban Jr JF (1983) Factors contributing to the *in vitro* development of *Ascaris suum* from second-stage larvae to mature adults. *J Parasitol* **69:** 549-558.

Fenglin W, Rongjun H, Xianxiang H, Xihui Z (1979) *In vitro* cultivation of *Ancylostoma caninum* and *A. duodenale*. *Chin Med J* **92:** 839-846.

Gamble HR, Mansfield LS (1996) Characterization of excretory-secretory products from larval stages of *Haemonchus contortus* cultured *in vitro*. *Vet Parasitol* **62:** 291-305.

Hasegawa H, Orido Y, Sato Y, Otsuru M (1988) *Strongyloides venezuelensis* Brumpt, 1934 (Nematoda: Strongyloididae) collected from *Rattus norvegicus* in Naha, Okinawa, Japan. *Jpn J Parasitol* **37:** 429-434.

Healer J, Ashall F, Maizels RM (1991) Characterization of proteolytic enzymes from larval and adult *Nippostrongylus brasiliensis*. *Parasitology* **103:** 305-314.

Ito J, Komiya Y (1955) Studies on the survival of *Schistosoma japonicum in vitro*. 2. Survival in a chemically defined medium. *Jpn J Parasitol* **4:** 258-261.

Jenkins DC (1982) *In vitro* screening tests for anthelmintics. *In* Animal Models in Parasitology, Owen D (ed.), p173-186, Macmillan, London, UK.

Little MD (1966) Comparative morphology of six species of *Strongyloides* (Nematoda) and redefinition of the genus. *J Parasitol* **52:** 69-84.

McDiarmid SS, Matthews BE (1982) Survival and behavior of *Onchocerca gutturosa* microfilariae *in vitro*. *J Parasitol* **68:** 470-477.

Patel MN, Wright DJ (1996) The influence of neuroactive pesticides on behaviour of entomopathogenic nematodes. *J Helminthol* **70:** 53-61.

Rew RS, Urban Jr JF, Douvres FW (1986) An *in vitro* screen for anthelmintics using larvae of *Ascaris suum*. *Am J Vet Res* **47:** 869-873.

Sato Y, Toma H (1990) *Strongyloides venezuelensis* infections in mice. *Int J Parasit* **20:** 57-62.

Taira N, Hirooka M, Saeki H (1994) Isolation of *Strongyloides venezuelensis* from *Rattus norvegicus* in Kagoshima Prefecture. *Jap J Vet Med Sci* **56:** 255-258.

Tsuji N, Fujisaki K (1994) Development *in vitro* of free-living infective larvae to the parasitic stage of *Strongyloides venezuelensis* by temperature shift. *Parasitology* **109:** 643-648.

Tsuji N, Nakamura Y, Taira N (1993) Long-lasting

parasitism of *Strongyloides venezuelensis* in Mongolian gerbils (*Meriones unguiculatus*). *J Parasitol* **79:** 305-307.

Urban Jr JF, Douvres FW, Xu S (1984) Culture requirements of *Ascaris suum* larvae using a stationary multi-well system: increased survival, development and growth with cholesterol. *Vet Parasitol* **14:** 33-42.

Weinstein PP (1996) Vitamin B_{12} changes in *Nippostrongylus brasiliensis* in its free-living and parasitic habitats with biochemical implications. *J Parasitol* **82:** 1-6.

=초록=

베네수엘라분선충 (*Strongyloides venezuelensis*)의 충란, 감염자충 및 성충의 실험관 내 배양

백병걸, M. Khyrul Islam, Kiku Mastuda

전북대학교 부속 생체안전성연구소

베네수엘라분선충의 충란, 자충 및 성충을 장기간 보존할 수 있는 방안을 마련하기 위하여 여러 가지 배양환경에 대한 연구를 수행하였던 바, 다음과 같은 결과를 얻었다. 분변 내의 충란은 4°C에서 25일간, 실온에서는 15일간 생존하였으며, 분변에서 분리한 충란은 4°C의 생리적 식염수에서 24시간 생존하였으나, 상실배기의 충란은 건조한 공기에 민감하여 12시간 내에 활성을 잃었다. 감염형 자충을 polyvinyl bag에 넣어 20°C에 보관하였을 경우, 분변 물질이 첨가된 0.12% 영양배지에서는 45일, 영양배지는 28일간 생존하였다. 한편, 영양배지에서 배양된 감염 자충은 상수에서는 32일간, 멸균 식염수에서는 22일간 생존하였다. 인공감염시켜 얻은 성충은 37°C에서 9일간, 영양배지 (10% 쥐 혈청을 첨가)와 혈청을 첨가하지 않은 영양배지를 매일 교환하여 주어도 4일동안 밖에 생존하지 못하였다. 성충 암컷은 실험관 내에서 산란하고, 자충으로 부화되었지만 혈청을 첨가한 배지에서도 감염자충으로 발육하지 못하였다.

〔기생충학잡지 36(2): 99-107, 1998년 6월〕