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Effect of Storage on the Infectivity of *Vampirolepis nana* var. *nana* Eggs to Swiss Albino Mice

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Abstract

Vampirolepis nana is the most common cestode in humans especially children. Domestic mice and rats can serve as definitive hosts for *V. nana*. Confusion exists over the species status and host-specificity of this tapeworm. In a previous study done by the same authors it was found that the Egyptian human isolates of *V. nana* could be used to infect mice for experimental work. Eggs in human feces and in the fecal pellets excreted by mice probably lose their infectivity sooner or later. Such information is very important from the epidemiologic and epizootic points of view. The aim of this work was to study the infectivity of *V. nana* var. *nana* eggs isolated from human feces, murine fecal pellets and worm gravid proglottids after storage for different periods of time. According to the results, the transmission potential capacity of the human strain of *V. nana* by mice can not be ignored. The relative infectivity of the eggs isolated from the murine rectal fecal pellets is higher than that isolated from the worm because some immature eggs are found in the gravid segments. It was clear that storage of eggs had a deteriorating effect and that the egg infectivity was dependent upon storage time. After 2 wk of storage in dechlorinated water some eggs were still viable and infective. Such a group of eggs present a health risk for people living in the wastewater-exposed areas like Egyptian rural areas, or when wastewater is reused for agricultural purposes as in countries with water scarcity.

Keywords: Vampirolepis nana, *Cestode, Swiss albino mice*

Introduction

Vampirolepis nana (Syn. *Hymenolepis nana*) or the dwarf tapeworm was discovered by Theodor Bilharz in Cairo in 1851 (1). It is now a cosmopolitan species that is the most common cestode of humans in the world, especially among children (2). Besides humans, domestic mice and rats can serve as definitive hosts for *V. nana* (3). However, confusion exists over the species status and host-specificity of this tapeworm. It has been described as one species, *V. nana,* found in both humans and rodents. Some authors suggest that because higher rates of infection result from eggs obtained from the same host species than from the other, two subspecies exist (4). Others reported that the human strain of *V. nana* (var. *nana*) is essentially non-infective to rodents, while a subspecies *V. nana* var. *fraterna*, which is morphologically identical to the human form, was only found in rodents (5). Al-Baldawi *et al.* (1989), in Iraq, reported that six attempts were made to infect mice by feeding them by eggs of the human strain of *Hymenolepis nana*, but none was successful (6). Yet, in a previous study done by the present authors it was found that the Egyptian human isolates of *V. nana* could be used to infect mice for experimental work (7). The life cycle of *V. nana* is unique among tapeworms in that an intermediate host is optional. Thus *V. nana* is usually maintained in the laboratory by inoculation of mice either with eggs isolated from adult worms collected from mice or from murine excreted fecal pellets (8). Eggs in human feces and in the fecal pellets excreted by mice probably lose their infectivity sooner or later.

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Such information is very important from the epidemiologic and epizootic points of view. The World Health Organization (WHO) has reported that *V. nana* could persist long enough and present a health risk when wastewater is reused for agricultural purposes (9). Field studies found that *V. nana* infection is higher among children living in the wastewater-exposed areas (10).

The aim of this work was to study the infectivity of *V. nana* var. *nana* eggs isolated from human feces, murine fecal pellets and worm gravid proglottids (mature segments) after storage for different periods of time.

Materials and Methods

Obtaining human isolates of* V. nana *eggs

Vampirolepis nana eggs were isolated from human stools by simple sedimentation. Briefly, pooled feces were mixed with suitable amount of physiological saline in a large container and stirred well with a stirring rod. The solution was strained though a large mesh sieve to a second container. The sample was allowed to stand for 10 min and then the supernatant was discarded and the sediment was re-suspended in saline. The sedimentation process was repeated at least four times till the supernatant became clear, which means all the small debris were removed. After the last sedimentation and decantation, the sediment was recovered in minimal amount of dechlorinated water and the concentration of eggs was adjusted as 140 eggs/ml. *Vampirolepis nana* eggs were stored in total darkness at 4 ^{o}C for 1 h, 2 h, 24 h (1d), 72 h (3 d), 168 h (1 wk) or 336 h (2 wk).

Mice infection with human isolates of* V. nana *eggs A total of 54 parasite free Swiss albino mice aged 4-5 wk were infected with human isolates of *V. nana*. According to the storage time schedules of *V. nana* eggs mentioned above, this group was divided into 6 subgroups each of 9 mice. Each mouse received 0.5 ml of egg suspension in dechlorinated water (approximately 70 eggs) by a stomach tube. Fifteen days after infection, mice were sacrificed and *V. nana* worms were collected from the gut, rinsed in physiological saline, identified according to maturity and counted (7,11).

Mice infection with murine passaged V. nana eggs:

Infection with* V. nana *eggs obtained from murine rectal fecal pellets From the first subgroup of the above mentioned sacrificed mice (infected with eggs stored for only one hour), *V. nana* eggs were collected from the rectal fecal pellets, pooled, suspended, washed and concentrated by simple sedimentation in saline.

Eggs in a concentration of 140 eggs/ml of dechlorinated water were stored in total darkness at 4^{o} C for the different time schedules mentioned above, and used to infect 54 Swiss albino mice aged 4-5 wk divided into 6 subgroups as mentioned.

Infection with* V. nana *eggs obtained from worm gravid proglottids *Vampirolepis nana* eggs were isolated from worms found in the first subgroup of the above-mentioned first group of mice (infected with human isolates of eggs stored for only one hour). Eggs were collected from gravid proglottids (mature segments) by squeezing of the last 10-20 proglottids in the posterior part of the strobila (body) and suspended in saline. They were transferred to a watch glass to remove small debris and floating eggs (immature eggs). Eggs in a concentration of 140 eggs/ml of dechlorinated water were stored for different time schedules, and used to infect 54 Swiss albino mice aged 4-5 wk divided into 6 subgroups as mentioned above.

Results

Table 1 presents the results of experimental infection of mice with human isolates of *V. nana* eggs after storage as mentioned earlier. According to the present results the infection rate in the Swiss albino mice was 100% after 1 h of storage in dechlorinated water and the worm

recovery percentage was 35%. Also it was clear that there was a gradual decrease in the infectivity of eggs to mice after storage in dechlorinated water for the subsequent 2 wk (67% infection rate and 12% worm recovery percentage). No immature worms were recovered.

Table 2 presents the results of experimental infection of mice with *V. nana* eggs isolated from murine rectal fecal pellets after mentioned storage time. Also here there was a gradual decrease in the infectivity of eggs to mice after storage in dechlorinated water for 2 wk. The infection rate decreased from 89% after 1 h storage to 67% after 2 wk, and the worm recovery percentage decreased from 30% after storage for 1 h to 5.6% after 2 wk (immature and mature worms). Only in the subgroup of mice infected with the egg stocks stored for 2 wk, four mice were found to harbor both immature and mature worms.

Table 3 presents the results of experimental infection of mice with *V. nana* eggs isolated from gravid proglottids after storage time. Also, there was a gradual decrease in the infectivity of eggs to mice after storage in dechlorinated water for 2 wk. The infection rate decreased from 67% after 1 h to 44% after 2 wk, and the worm recovery percentage decreased from 8.6 % after storage for 1 h to 2.1% after 2 wk (immature and mature worms). Nine mice were found to harbor both immature and mature worms. Those were only in the subgroups of mice infected by egg stocks stored for 3 d, 1 wk and 2 wk.

Table 4 compares the number of adult worms recovered from mice infected with *V. nana* eggs isolated from murine rectal fecal pellets and those from mice infected with eggs isolated from worm gravid proglottids. After each storage time, the number of worms recovered from mice infected with eggs isolated from murine rectal fecal pellets was significantly higher than the other group.

Table 1: Experimental infection of mice with human isolates of *V. nana* eggs after storage for different periods of time

Storage Period	Mice		Worms/mouse(*)		
	Exposed	Infected	Range	Mean (*p*-value of t-test)	Recovery %(§)
1 h	9	9 (100%)	11-32	24.7	35
2 h	9	8 (89%)	0-31	21.4 (0.42)	31
24 h (1 d)	9	8 (89%)	0-27	17.2 (0.06)	25
72 h (3 d)	9	7 (78%)	0-23	14.2 (0.01)	20
168 h (1 wk)	9	7 (78%)	0-19	8.7 (0.00)	12
336 h (2 wk)	9	6 (67%)	0-18	8.3 (0.00)	12

(*) All recovered worms were mature.
(§)Worm recovery % = worms recovered X 100/eggs administrated

Table 2: Experimental infection of mice with *V. nana* eggs isolated from murine rectal fecal pellets after storage for different periods of time

Storage Period	Mice		Worms/mouse					
			Immature			Mature		
	Exposed	Infected	Range *(n)*	Mean	Recovery %(§)	Range *(n)*	Mean (*p*-value of t-test)	Recovery %(§)
1 h	9	8 (89%)	0	0	0	0-37 *(8)*	21.3	30
2 h	9	7 (78%)	0	0	0	0-30 *(7)*	17.3 (0.44)	25
24 h (1 d)	9	6 (67%)	0	0	0	0-20 *(6)*	11.2 (0.04)	16
72 h (3 d)	9	6 (67%)	0	0	0	0-17 *(6)*	8.8 (0.00)	13
168 h (1wk)	9	5 (56%)	0	0	0	0-18 *(5)*	6.6 (0.00)	9
336 h (2wk)	9	6 (67%)	0-4 *(4)*	1.1	1.6	0-7 *(6)*	2.8 (0.00)	4

(§) Worm recovery % = worms recovered X 100/eggs administrated

Table 3: Experimental infection of mice with *V. nana* eggs isolated from gravid proglottids after storage for different periods of time

Storage Period	Mice		Worms/mouse					
			Immature			Mature		
	Exposed	Infected	Range *(n)*	Mean	Recovery %(§)	Range *(n)*	Mean (*p*-value of t-test)	Recovery %(§)
1 h	9	6 (67%)	0	0	0	0-11 *(6)*	6	8.6
2 h	9	6 (67%)	0	0	0	0-10 *(6)*	4 (0.34)	5.7
24 h (1 d)	9	6 (67%)	0	0	0	0-8 *(6)*	3.7 (0.24)	5.2
72 h (3 d)	9	5 (56%)	0-3 *(3)*	0.9	1.3	0-5 *(5)*	1.4 (0.02)	2.1
168 h (1 wk)	9	5 (56%)	0-3 *(3)*	0.7	1	0-4 *(5)*	1.2 (0.01)	1.7
336 h (2 wk)	9	4 (44%)	0-3 *(3)*	0.7	1	0-3 *(4)*	0.8 (0.00)	1.1

(§) Worm recovery % = worms recovered X 100/eggs administrated

Table 4: Adult worms recovered from mice infected with two different *V. nana* egg isolates after storage for different periods of time

Storage Period	Source of *V. nana* eggs	
	Murine rectal fecal pellets	Worm gravid proglottids
	Mean of adult worms (§) (*p*-value of t-test)	Mean of adult worms (§) (*p*-value of t-test)
1 h	21.3	6 (0.00)
2 h	17.3	4 (0.00)
24 h (1 d)	11.2	3.7 (0.03)
72 h (3 d)	8.8	1.4 (0.00)
168 h (1 wk)	6.6	1.2 (0.03)
336 hr (2 wk)	2.8	0.8 (0.04)

(§) Each group composed of nine mice.

Discussion

According to the results of the present study and a previous study done by the same authors (7), it could be concluded that the Egyptian human isolates of *V. nana* could be used to infect mice for experimental studies. However, further studies on the comparative infectivity of the Egyptian human and murine isolates of *V. nana* are needed. Most studies on *V. nana* egg infectivity testing in laboratory animals have been qualitative, and the few quantitative studies are difficult to compare. No standardization has been developed to define the factors that influence susceptibility to infection, such as the infection technique employed or the experimental host used.

According to the present results, although the infection rate of *V. nana* var. *nana* to the Swiss albino mice was 100% after 1 h of storage in dechlorinated water, only 35% of the eggs passed in human feces infected the mice and the infection proceeded to maturity. After being passaged in mice the infection rate decreased to 89% and 67%, and the worm recovery was 30% and 8.6% for the eggs isolated from the murine rectal fecal pellets and worm proglottids respectively. According to the present results, *V. nana* var. *nana* may not be fully adapted to the Swiss albino mice. However, from an epidemiological point of view the transmission potential capacity of the human strain of *V. nana* by mice cannot be ignored. Astaf'ev and Fedina (1975) studied the adaptability of different strains of *V. nana* from man, and Norway and white rats to mice. In the course of successive passages the infectivity of these strains, devel-

opmental rates of tissue larvae, localization of cysticercoids in the small intestine and mesenteric lymph glands were compared. The strains were found to possess different adaptability to white mice. The strain from white rats had the highest rate of adaptation, the strains from Norway rats and man showed lower adaptation rates, respectively (12). Subsequent studies of the inbred lines of mice A/He, AKR and CBA infected with different strains of *V. nana* have shown that the worm when changing the host for another one of the same species but with different hereditary characters happens to be insufficiently adapted to the new host. However it does not prevent the start of the infectious process. With increasing number of passages the parasite's adaptation level to the organism of the new host rises gradually. Possibilities and the adaptation level of the agent to the new host are defined as well by the adaptational mechanisms common to each specific strain of *V. nana* and the host's characters (13).

Based on the results of the present study, it could be concluded that the relative infectivity of the eggs isolated from the murine rectal fecal pellets was higher than that of eggs isolated from the worm. Comparable results were described by Maki and Yanagisawa (1987) (14). Such a result could be explained by the fact that not all the eggs in the posterior proglottids are mature as some immature eggs are found (15).

It was clear results that in all the studied groups, there was a gradual decrease in the infectivity of eggs to mice after storage in water for 2 wk. From such a finding it could be concluded that storage of eggs has a deteriorating effect and that the egg infectivity is dependent upon storage time. Such a deteriorating effect affected the viability and the rhythm of future development of the eggs in the vertebrate host, as the period of 2 wk after infection was not enough for some worms to reach maturity. Also, based on this finding it could be concluded that after 2 wk of storage some *V. nana* eggs were still viable and infective. Such a group of eggs present a health risk for people living in the wastewater-exposed areas like Egyptian rural areas, or when wastewater is reused for agricultural purposes as in countries with water scarcity.

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