J. Parasitol., 92(1), 2006, pp. 16–20

A QUANTITATIVE APPROACH TO THE EXPERIMENTAL TRANSMISSION SUCCESS OF *ECHINOSTOMA FRIEDI* (TREMATODA: ECHINOSTOMATIDAE) IN RATS

Rafael Toledo, Inés Carpena, Ana Espert, Javier Sotillo, Carla Muñoz-Antoli, and J. Guillermo Esteban

Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjassot–Valencia, Spain. *e-mail: rafael.toledo@uv.es*

ABSTRACT: Using a range of parameters, the ability of rats (*Rattus norvegicus*) to successfully transmit *Echinostoma friedi* to the next host was examined under experimental conditions. The concept of Experimental Transmission Success (T_M), defined as the number of hosts that become successfully infected after exposure to a number of infective stages produced by a previous host per unit of inoculation at which this latter host was exposed, was introduced. Using data for the egg output and miracidium hatching and infectivity, the T_M permits us to estimate the ability of a particular defintive host species to successfully transmit a parasite species. This concept may be also useful to compare the transmission fitness of a parasite in different definitive host species. Moreover, variations of the Experimental Transmission Success over the course of the infection were calculated by the use of the Weekly Experimental Transmission Success (T_{MW}). Overall, considering the complete duration of the experiment, the T_M of *E. friedi* using rats as definitive hosts was 0.68 infected snails/metacercaria. However, positive values of the T_{MW} were only obtained from 2 to 4 wk post-infection, with a maximum during the third wk post-infection. When comparing the T_M values of *E. friedi* in rats with those calculated in hamsters on the basis of previously published data, *E. friedi* appears to be more appropriate to move through this portion of its life cycle when using hamsters (*Mesocricetus auratus*) as the final host than rats.

Much of the interest in describing and modeling the population dynamics of helminths has concerned parasite fecundity in the definitive host. Helminthologists have long recognized that not all species capable of hosting a parasite species play equivalent roles in the maintenance of the suprapopulations of parasites (Holmes, 1979). Therefore, a quantitative assessment of the ability of a particular definitive host species to successfully transmit a helminth is essential for understanding the population dynamics of a given helminth species. However, the estimation of the transmission success is difficult because both intrinsic (evolutionary) and extrinsic (ecological) factors are involved and, further, the relative influence of these factors may be different in each host–parasite combination (Olson and Nickol, 1996).

Several methods have been developed to assess the ability of a host to transmit a helminth species to the next host and, consequently, its contribution for the maintenance of the suprapopulation of the helminth. Whitfield et al. (1986) introduced the concept of reproductive success. To assess the parasitic utilization of the host, success (R) was defined as the number of viable eggs produced per unit of inoculation to which the host was exposed. However, this parameter gives only a partial view of the ability of the host to transmit a helminth to the next host and thereby to maintain its suprapopulation. Transmission success of a parasite is also affected by other factors, i.e., the infectivity of the larval stages produced. No single estimate of either infection rate, adult life span, or the output rate of viable eggs can alone measure the significance of apparent host suitability to transmit a parasite (Holmes et al., 1977; Whitfield et al., 1986). Other approaches have attempted to evaluate the relative importance of host species by assessing the relative flow to and from the host (Esch et al., 1976; Holmes et al., 1977). However, these estimations are complicated and the information is difficult to interpret (Olson and Nickol, 1996).

Echinostoma friedi Toledo *et al.*, 2000 (Trematoda: Echinostomatidae) was recently described in the Albufera Natural Park of Valencia (Spain) (Toledo et al., 2000). In its natural habitat, *E friedi* uses *Lymnaea peregra* as the first intermediate host (Toledo et al., 1998; referred to as *Echinostoma* sp. in the study) and only rats (*Rattus norvegicus*) are known to act as the definitive host (Toledo et al., 2000). In the present study, we evaluate the ability of rats to successfully transmit *E. friedi* to *L. peregra* under experimental conditions. For this purpose, we assessed worm survival, egg output, egg viability, production of viable eggs, and infectivity of the miracidia produced. Based on these data, the concept of Experimental Transmission Success (T_M) was introduced to quantify rates of transmission of *E. friedi* in this portion of the life cycle using rats as the definitive host. Moreover, on the basis of previously published data, the results were compared with those calculated for hamsters to evaluate the utility of the Experimental Transmission Success for comparative studies on the ability of various hosts to successfully transmit a parasite.

MATERIALS AND METHODS

Parasites

Echinostoma friedi (originally obtained from the Albufera Natural Park of Valencia, Spain) was maintained in the laboratory using *Lymnaea peregra* (Müller, 1774) as the first intermediate host. This snail species also was used as the second intermediate host and golden hamsters (*Mesocricetus auratus*) and Wistar rats (*R. norvegicus*) as definitive hosts. Further techniques for maintaining *E. friedi* in the laboratory were described in a previous study (Toledo et al., 2000).

Experimental infections

Each of 60 outbred male Wistar rats (*R. norvegicus*), weighing 110–140 g, was infected by stomach tube with 100 metacercariae of *E. friedi* collected from the kidney and pericardial cavity of experimentally infected *L. peregra*. Those rats that became infected were randomly allocated to groups A (8 rats) and B (21). Group A was used to study the kinetics of egg production of *E. friedi* in rats. Group B was used to study the worm recovery and the production of viable eggs over the course of the infection. All the infected rats were maintained under conventional conditions with food and water ad libitum.

Kinetics of egg release

This experiment was designed to investigate the kinetics of egg output of *E. friedi* during the course of the infection in golden hamsters. For this purpose, the egg output was determined daily in the rats of group A during 49 consecutive days post-experimental infection. Twenty-four hr fecal production was collected individually from each animal according to the method described by Keymer and Hiorns (1986). The

Received 25 January 2005; revised 1 April 2005, 2 June 2005; accepted 3 June 2005.

fecal samples from each day and rat were individually pooled and weighed. The number of eggs of *E. friedi* per g of feces was determined according to Brindley and Dobson (1981). Briefly, fecal samples were emulsified in the ratio 1 g/30 ml of 0.1 N in NaOH. This mixture was shaken at room temperature for 2 hr and the sediment homogenized in 2 ml. The eggs held in 200 μl were counted. Five replicated samples were analyzed for each day and rat to determine the eggs per g of feces; the average was considered as an estimate of the total number of eggs released daily per rat.

Worm recovery and egg viability

This experiment was designed to investigate the worm establishment of *E. friedi* in rats and the effect of worm age on the viability of the eggs produced during the first 7 wk after infection. Egg viability was measured in terms of miracidia hatching.

At each wk post-inoculation (wpi), 3 rats in group B were necropsied and the number of worms recovered per rat was recorded. The uteri of 10 worms were teased to obtain eggs and the eggs were placed in petri dishes containing 10 ml of spring water. Egg cultures were maintained at 20±1 C in darkness. At day 10–15 of incubation, the egg cultures were exposed daily to artificial light (60W) for 2 hr. The number of swimming miracidia was counted at 15-min intervals. In total, 2,000 eggs were studied for each wk that comprised the experiment, except for the second wpi in which 1,000 eggs were studied. Miracidia hatching was expressed as the percentage of miracidia hatched each wk.

Weekly reproductive success

In the present study, we calculated the Reproductive Success (R) of *E. friedi* in experimentally infected rats according to Whitfield et al. (1986). The reproductive success of a parasite species in particular host (R) is defined as:

$$R = E_{s+1}/C_0,$$

where E_{s+1} represents the total number of viable eggs produced by the cohort of adult parasites resulting from host exposure to C_0 metacercariae.

To estimate the variations in the fecundity of *E. friedi* during the experimental infection in rats, we calculated the Weekly Reproductive Success (R_w) according to Toledo et al. (2003). The Weekly Reproductive Success was defined as the total number of viable eggs produced each wk per unit of inoculation (metacercariae) at which the host was exposed and can be calculated as:

$$R_{wi} = E_i/C_0,$$

where E_i is the total number of viable eggs produced on wk i and C_0 is the number of metacercariae at which the hosts were exposed (100 metacercariae in the present study).

Infectivity of the miracidia produced

The Reproductive Success is a measure of worm fecundity. However, in terms of parasite transmission to the next host, the infectivity of the miracidia produced should also be considered. To examine the infectivity of *E. friedi* miracidia produced in rats, laboratory-reared specimens of *L. peregra* (size range: 3–5 mm) were singly exposed to 5 newly hatched miracidia (maximal age: 15 min) derived from adults of *E. friedi* collected each wk that comprised the experiment. Exposures were conducted in Petri dishes for 12 hr in 3 ml of spring water at 20±1 C. After exposure, snails were maintained in a day–night scheme of 12:12 hr at 20±1 C; snails were fed with washed lettuce ad libitum. At 5–6 wk post-exposure, individual snails were investigated daily to determine the release of cercariae. Those snails that failed to release cercariae were crushed and examined for infection. Twenty replicate exposures were conducted for miracidia derived from each adult age class.

Experimental Transmission Success(T_M)

Using the concepts developed by Whitfield et al. (1986) and Toledo et al. (2003), and the results obtained herein, we formulated a simple model to describe the ability of rats to successfully transmit *E. friedi* to the next host in the life cycle. Our aim was to provide a framework to estimate quantitatively the transmission of a parasite from host to host. In the context of transmission from a host A to a host B, the Experimental Transmission Success (T_M) may be defined as the number of hosts B that become successfully infected after exposure to a number (M) of infective stages produced by host A per unit of inoculation at which host A was exposed. In the present study, the Experimental Transmission Success could be simply defined as the number of snails that became infected per unit of inoculation at which rats were exposed (100 metacercariae). The Experimental Transmission Success can be calculated as:

$$T_M = R\, I_M/S_M\, M,$$

where R is the Reproductive Success, I_M is the number of snails exposed to ''a'' miracidia that became successfully infected, S_M is the number of snails that were individually exposed to miracidia, and M is the number of miracidia used to expose individually the snails.

Furthermore, the variations of the Experimental Transmission Success over the course of the infections can be easily estimated using this model. The Weekly Experimental Transmission Success (T_{MW}), defined as the Experimental Transmission Success for each wk of the infection, can be calculated as:

$$T_{MW} = R_{Wi}\, I_{Mi}/S_{Mi}\, M,$$

where R_W is the Weekly Reproductive Success on wk i (Toledo et al., 2003), I_{Mi} is the number of snails that were individually exposed to M miracidia derived from adults collected on wk i that became successfully infected, and S_{Mi} is the number of snails that were exposed to miracidia derived from adults collected on wk i.

Although the difficulty of evaluating the correspondence between experimental results and those obtained in the field was recognized, the Experimental Transmission Success may be useful to obtain an estimation of the ability of a host to successfully transmit a parasite and to compare this ability between different host species. If possible, the miracidia dose should be 1, though other miracidia doses can be used. In the present study, M was 5 miracidia/snail to allow further comparisons of previous studies on the transmission dynamics of *E. friedi* in hamsters in the same part of the life cycle (Toledo, et al., 2003, 2004).

RESULTS

Pre-patent period and worm recovery

Only 29 (48%) of the rats experimentally exposed to 100 metacercariae of *E. friedi* became infected. The duration of the pre-patent period studied in the rats of group A was rather variable. Egg release began 13–18 (15.9 ± 2.4) days post-infection. The number of worms recovered weekly in the rats belonging to group B ranged from 7 to 26 (15.2 ± 6.7) worms/rat (Table I).

Kinetics of egg release

Daily egg output was not uniform over time and there was an important variation in daily egg counts (Fig. 1). The maximum number of released eggs per day was 389 per rat at day 17 p.i. During days 13–15 post-infection, the number of eggs released was low, probably in relation to the relatively low number of mature adult worms and rats that had started to release eggs (only 2 rats). At 17 days post-infection, 7 of the rats were positive and the egg release reached the maximum value. Thereafter, the egg counts declined. One of the rats was negative from 28 days post-infection, whereas 3 other rats reverted to negative values at 29 days post-infection. All the samples analyzed were negative for egg examination from 33 days post-infection. No worm was recorded at necropsy of the rats at 35 days post-infection.

The analysis of the number of eggs released weekly per rat showed that the maximal egg output occurred at the third wpi (Table I). From this week onwards, the values decreased, becoming negative at 6 wpi.

TABLE I. Values of the worm recovery, egg output, percentage of egg viability, Weekly Reproductive Success, miracidia infectivity and Weekly Experimental Transmission Success estimated for *Echinostoma friedi* at increasing age in infections with 100 metacercariae in rats.

Week post-infection	1	2	3	4	5
Worms recovered/rat (mean ± SD)	14 ± 9	18 ± 8	20 ± 10	14 ± 5	0
Eggs released/rat (mean ± SD)	0	17 ± 10	1101 ± 456	548 ± 123	28 ± 15
Percentage of viable eggs	0	11	47	69	0
Weekly Reproductive Success (viable eggs/metacercaria)	0	0.02	5.12	3.76	0
Percentage of miracidia infection	0	35	70	40	0
Weekly Experimental Transmission Success (infected snails/metacercaria)	0	0.001	0.72	0.31	0

Egg viability

The egg viability was measured in terms of miracidia hatching. Overall, viable eggs constituted about 42% of the total egg production. However, the percentage of viable eggs varied with worm age (Table I). Percentage of viable eggs progressively increased from the second to reach a maximum at 4 wpi. The maximum percentage of egg viability was 69% at 4 wpi.

Reproductive success

Overall, considering the complete duration of the experiment, the value of the Reproductive Success of *E. friedi* in rats was 7.02 viable eggs/metacercaria. However, the Weekly Reproductive Success was not uniform throughout the experiment. The values of the Weekly Reproductive Success, expressed as the number of viable eggs produced per metacercaria each wk of the infection, is shown in Table I. Positive values of the Weekly Reproductive Success were obtained from 2 to 4 wpi though the value was almost insignificant at 2 wpi (0.019 viable eggs/metacercaria). The maximum value was observed at 4 wpi (5.12 viable eggs/metacercaria).

Miracidia infectivity

Considering the complete experiment, the percentage of infected snails was 48.3%. However, variations were observed in relation to the adult worm age that produced the miracidia. The infectivity observed from miracidia derived from adult worms collected each week of the experiment is shown in Table I. The percentage of infection was not uniform over the course of the experiment and ranged from 35 to 70%. The maximum was observed at 3 wpi, whereas the minimum was observed at 2 wpi.

Experimental Transmission Success

The Experimental Transmission Success of *E. friedi* in rats, using miracidia doses of 5 per snail, was 0.68 infected snails/metacercaria. The Weekly Experimental Transmission Success observed during the course of the infection is shown in Table I. Positive values were obtained from the second wpi to 4 wpi, though the value at 2 wpi was almost insignificant (0.001 infected snails/metacercaria). The maximum value of the Weekly Experimental Transmission Success was observed at 3 wpi (0.72 infected snails/metacercaria) probably due to the higher egg output and the greater percentage of the miracidia infectivity during this week.

DISCUSSION

Infection of rats with *E. friedi* is characterized by low infection, worm recovery and survival, and egg output. Overall, considering the complete duration of the *E. friedi* infection in rats, the value of the Experimental Transmission Success was low, suggesting that more than 1 metacercaria is required to infect a single snail under the conditions employed. As previously shown in *E. friedi* infections in hamsters, the infectivity of the miracidia produced depends on the adult worm age (Toledo et

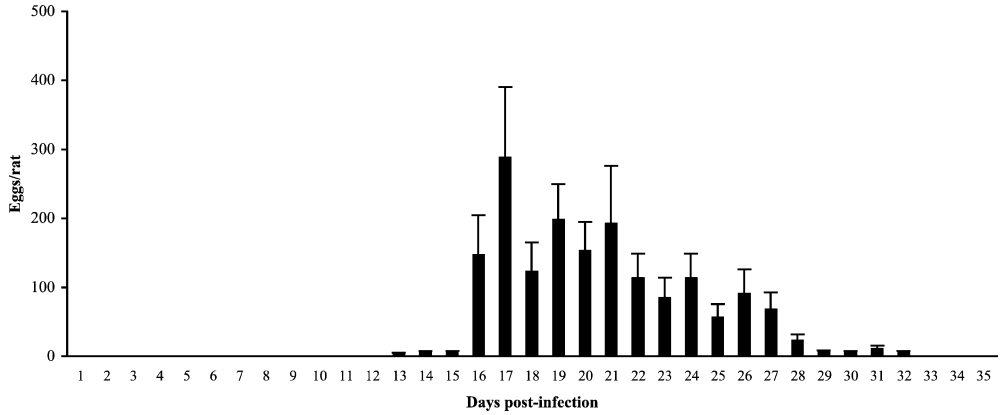

FIGURE 1. Average daily egg output of *Echinostoma friedi* at increasing age in infections with 100 metacercariae in rats. Vertical bars represent the standard deviation.

TABLE II. Values of the Weekly Experimental Transmission Success (T_{MW}) estimated for *Echinostoma friedi* at increasing age in infections with 100 metacercariae in rats and hamsters. Values for hamsters predicted from previously published data.

	Weekly Experimental Transmission Success (T_{MW}) (infected snails/metacercariae)											
Week post-infection	1	2	3	4	5	6	7	8	9	10	11	12
Rat	0	0.001	0.72	0.31	0	0	0	0	0	0	0	0
Hamster	0	0	0	25.20	7.51	15.08	5.66	26.93	2.10	0	0	0

al., 2003). This fact, together with time-related changes in the egg output and egg viability, results in variations in the Experimental Transmission Success values over the course of the infection. Positive values of the Weekly Experimental Transmission Success were observed from 2 to 4 wpi, though the values at 2 wpi were almost insignificant. The maximum value of the Weekly Experimental Transmission Success was observed at 3 wpi. This is of interest since the maximum percentage viable egg production was not concomitant with the maximum values of miracidia infectivity and Weekly Experimental Transmission Success, suggesting the existence of specific mechanisms determining the production of infective miracidia (Toledo et al., 2004). Adult worms that are capable of releasing eggs require further maturation to yield infective miracidia.

The application of the concept of the Experimental Transmission Success may be useful to compare the suitability of different host species and their respective ability to successfully transmit a particular helminth. In the present study, we have calculated the Reproductive Success and the Experimental Transmission Success of *E. friedi* in hamsters on the basis of the data previously published (Toledo et al., 2003, 2004). The Experimental Transmission Success of *E. friedi* in hamsters, calculated over the first 7 wpi, was 62.11 infected snails/metacercaria. The values of the Weekly Experimental Transmission Success of *E. friedi* in hamsters and rats are compared in the Table II. Positive values of Experimental Transmission Success in the hamster were only observed from 4 wpi, suggesting that maturation of *E. friedi* in hamsters is slower than in rats. This could be related to functional changes induced by the host species because the worm recovery rates were similar in both host species. However, hamsters are able to transmit *E. friedi* for a longer period of time and with a considerably higher Experimental Transmission Success values than rats. The advantages of hamsters over rats as definitive hosts are the result of a greater life span, egg output, and viable egg production, which result in a Experimental Transmission Success rate of 91:1 with respect to rats. In the context of our experimental conditions, the host specific values of Experimental Transmission Success provide a measure of the host ability to successfully transmit the parasite. This points to the conclusion that *E. friedi* is better adapted to successfully pass through this portion of its life cycle when using hamsters as final host than rats.

Rattus norvegicus is the only known natural host for *E. friedi* (Toledo et al., 2000). However, the low values of the Experimental Transmission Success obtained in the present study suggest that other definitive hosts species are probably involved in the maintenance of the suprapopulation of this parasite in the wild. Holmes (1979) defined 3 categories of hosts in relation to their ability to maintain the suprapopulation of a parasite species: (1) required hosts, or those species which are capable of maintaining the parasite suprapopulation in the absence of other host species; (2) suitable hosts, or those hosts in which the parasite develops and matures but at an insufficient level to maintain the parasite suprapopulations without the participation of other host species; and (3) unsuitable hosts, or those in which the parasite can occur but does not mature. Although the difficulty of evaluating the correspondence between experimental results and those obtained in the field is recognized, rats are predicted to be a suitable host for *E. friedi*, whereas other host species with higher Experimental Transmission Success should play the role of required hosts. Based on the experimental results obtained herein, *E. friedi* is able to recruit, mature, and produce eggs in rats but at an insufficient level to maintain the suprapopulation of the parasite in natural conditions.

The present study shows a way in which the ability of a host to successfully transmit a helminth and its contribution to the maintenance of a parasite population can be estimated under experimental conditions. Moreover, this ability can be compared between various taxonomically distinct hosts. Although the transmission of helminths in the wild is subjected to a great variety of factors, the Experimental Transmission Success provides a quantitative approach to assess the relative importance of host species to maintain the parasite suprapopulations.

ACKNOWLEDGMENTS

Work supported by project GV04B-107 de la Conselleria de Cultura, Educació i Esport de la Generalitat Valenciana (Valencia, Spain) and the Project UV-AE-20050201 de la Universitat de València (Spain). This work has been carried out while the third author (A.E.) was recipient of a pre-doctoral fellowship from the Ministerio de Educación, Cultura y Deportes, Madrid (Spain). This research complied with the current laws for animal health research in Spain.

LITERATURE CITED

BRINDLEY, P. J., AND C. DOBSON. 1981. Genetic control of liability to infection with *Nematospiroides dubius* in mice: selection of refractory and liable populations of mice. Parasitology **83:** 51–65.

ESCH, G. W., G. C. CAMPBELL, R. E. CONNERS, AND J. R. COGGINS. 1976. Recruitment of helminth parasites by bluegills (*Lepomys macrochirus*) using a modified live-box technique. Transactions of the American Microscopical Society **105:** 486–490.

HOLMES, J. C. 1979. Population regulation and parasite flow through communities. *In* Host-parasite interfaces, B. B. Nickol (ed.). Academic Press, New York, New York, p. 27–46.

———, R. P. HOBBS, AND T. S. LEONG. 1977. Population in perspective: Community organization and regulation of parasite populations. In Regulation of parasite populations, G. W. Esch (ed.). Academic Press, New York, New York, p. 209–246.

KEYMER, A., AND R. W. HIORNS. 1986. Faecal egg counts and nematode fecundity: *Heligmosomoides polygyrus* and laboratory mice. Parasitology **93:** 189–203.

MAHLER, H., N. Ø. CHRISTENSEN, AND O. HINDSBO. 1995. Studies on the reproductive capacity of *Echinostoma caproni* (Trematoda) in hamsters and jirds. International Journal for Parasitology **25:** 705–710.

OLSON, P. D., AND B. B. NICKOL. 1996. Comparison of *Leptorhynchoides thecatus* (Acantocephala) recruitment into green sunfish and largemouth bass populations. Journal of Parasitology **82:** 702–706.

TOLEDO, R., A. ESPERT, I. CARPENA, C. MUÑOZ-ANTOLI, AND J. G. ESTEBAN. 2003. An experimental study of the reproductive success of *Echinostoma friedi* (Trematoda: Echinostomatidae) in the golden hamster. Parasitology **126:** 433–441.

———, ———, ———, M. TRELIS, C. MUÑOZ-ANTOLI, AND J. G. ESTEBAN. 2004. *Echinostoma friedi*: the effect of age of adult worms on the infectivity of miracidia. Journal of Helminthology **78:** 91–93.

———, C. MUÑOZ-ANTOLI, AND J. G. ESTEBAN. 2000. The life cycle of *Echinostoma friedi* n. sp. (Trematoda: Echinostomatidae) in Spain and a discussion on the relationships within the ''*revolutum*'' group based on cercarial chaetotaxy. Systematic Parasitology **45:** 199–217.

———, ———, M. PÉREZ, AND J. G. ESTEBAN. 1998. Larval trematode infections in freshwater gastropods from the Albufera Natural Park in Spain. Journal of Helminthology **72:** 79–82.

WHITFIELD, P. J., R. M. ANDERSON, AND D. A. P. BUNDY. 1986. Host-specific components of the reproductive success of *Transversotrema patialense* (Digenea: Transversotrematidae). Parasitology **92:** 683–698.