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Original article

Fasciola gigantica: Worm Recovery Rate and Adult Maturity in Experimental Host, Dwarf Hamster Infection

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Abstract: The present study was conducted to observe the worm recovery rate and adult maturity of *Fasciola gigantica*, which is plant-borne trematode of ruminants and humans. Adult worms that infect the liver of a host are mainly a causative agent of hepatic damage, which can have a serious impact on the health of the host. Sixteen dwarf hamsters *(Phodopus campbelli)* were used in this study as the experimental host. Thirty experimental encysted metacercariae were force fed to each mouse and were then sacrificed every 3 days post-infection (PI). Adult worms were observed in the intestine and liver, and then worm recovery and adult maturity were investigated. The results showed that metacercariae were excysted to young adult worms and were recovered in the intestine on days 3 and 6 PI, and until day 9 PI when they were found in the liver of the hosts. Incidence of parasitic infestation was found to be 100% and the average worm recovery rate was 36.00%. The worm recovery rate continuously decreased until day 45 PI. The highest and lowest worm recovery rates were 53.33% on days 3 and 9 PI, and 13.33% on day 45 PI, respectively. In an investigation of the development of the *F. gigantica* it was found that in accordance with the size of the body, the size of the oral and ventral suckers were also proportionally increased. The specific sizes of body were 0.24 x 0.42 mm. (day 3 PI) and 3.80 x 13.90 mm. (day 45 PI). Additionally, genital pores were discovered on day 9 PI, and caeca were found on day 18 PI, while, testes and ovary were discovered on day 27 PI and developed maturely on day 39 PI. Immature eggs were discovered on day 42 PI, which indicated that this was the point that the parasites began to mature. However, the dwarf hamsters died on day 45 PI. Therefore, it can be confirmed that *F. gigantica* metacercariae that were derived from experimental encystment could infect and develop within dwarf hamsters. This result can be applied for the treatment, monitoring, management and control program of this parasite and then be applied in other experimental hosts. **Chiang Mai Veterinary Journal 2014; 12(1): 31-39**

Keywords: *Fasciola gigantica*, Worm recovery, Adult maturity, Experimental host, Dwarf hamster

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Introduction

Fasciola gigantica is the main cause of fascioliasis in domestic ruminants of Thailand. This fluke is a causative agent of hepatic damage in ruminants and have an economic impact on the growth, development and productivity of domestic ruminants (Dargie, 1987). Several reports have been completed on the fascioliasis that is mainly found in ruminants. However, many countries have also conducted studies on humans, as humans can be an accidental host of these parasites. The species *F. gigantica* is mainly |distributed in tropical regions of Africa and Asia (Mas-Coma & Bargues, 1997). Countries such as India have reported fasciolosis in domestic ruminants, and this has been determined to have been directly caused by *F. gigantica* (Garg et al., 2009). Maqbool et al. (2002) reported that the epidemiology of *F. gigantica* at slaughter-houses, livestock farms, veterinary hospitals and in household buffalos in Punjab Province, were 25.59%, 26.16%, 13.7% and 10.5%, respectively. In Nigeria, the prevalence of *F. gigantica* infection in slaughterous cattle was 47.55% (Opera, 2005). Ulayi et al. (2007) reported that the prevalence (2.1%) of infection with *F. gigantica* was higher in the bulls (1.3%) than in the cows from slaughtered animals at Zaria abattoir. In recent years, Tsegaye et al. (2011) reported that 42.25% of 400 cattle were found to be positive for fascioliasis and that the prevalence of bovine fascioliasis was higher in male cattle than in females. In Thailand, the prevalence of *F. gigantica* in cattle and water buffalo ranged from between 4-24%, with the highest incidences in the north and north-east regions, and the lowest was found in the southern region (Sukhapesna et al., 1994; Sobhon et al., 1998).

Most studies of *F. gigantica* have focused on the prevalence, morphology and molecular biology of the species, whereas, the life cycle, especially in the larval stages, has rarely been studied. Bitakaramire (1968) found that after forty-five days of infection of *F. gigantica* miracedium in *Lymnaea natalensis*, the snails started shedding cercariae and each snail produced 652.6 metacercariae. Rakotondravao et al. (1992) determined that in the radial generation of *F. gigantica* in *L. truncatula*, the first mature rediae did not appear until day 35 of the experiment. Dar et al. (2003) reported on the experimental infections of *F. gigantica* miracedia that were isolated from snails *L. truncatula* in Madagascar, China and Egypt in snails, and the rates of prevalence were 20.8%, 60.0% and 80.0%, respectively. In later years, Dar et al. (2004) studied the larval productivity of *F. gigantica* in two lymnaeid snails, *L. natalensis* and *Galba truncatula*, and the total number of free rediae was found to be significantly higher at day 49 post-exposure in *L. natalensis* than in *G. truncatula.*

The aims of this study elucidated on the worm recovery rate and adult maturity of experimental *F. gigantica* infection, and for which the recovery information can be applied in the monitoring, management and control program of this parasite and could be applied to other experimental hosts.

Materials and Methods

Experimental metacercaria preparation

Eggs of *F. gigantica* were recovered from the bile of the gallbladder of water buffalos *(Bubalus bubalis)*. The eggs were washed several times with dechlorinated tap water, and collected under a stereomicroscope. One thousand eggs of *F. gigantica* were placed in multiple-well plates containing dechlorinated tap water, and were then incubated at room temperature under periods of natural light and darkness to allow for the development of the miracedia. After the miracedia hatched from the eggs, free-living miracedia were placed into clay pots containing 100 snails, *Lymnaea auricularia rubiginosa*, and 2 L of dechlorinated tap water. The exposed-snails in the clay pot were cultured and given continuous aeration, and fresh lettuce leaves were supplied for the feeding of the snails. The exposed-snails were crushed and dissected for mature cercariae collection at day 43 post-infection (PI). Mature cercariae were placed into rice plant pots for cercariae encystment to develop to metacercariae. Experimental metacercariae were collected from the rice plants using a stereomicroscope.

Experimental hosts infection

Sixteen dwarf hamsters were used as the experimental hosts. Each mouse was force fed with 30 metacercariae. The infected-experimental hosts were sacrificed every 3 days post-infection (PI). The intestines and livers of the hosts were separately removed. The adult worms from the intestine and liver were dissected, collected and counted under a stereomicroscope for worm recovery rate and maturity of adult trematodes determination. For, the maturity of the adult trematode investigation, adult flukes were compressed and fixed in 5% formalin, stained with borax carmine, dehydrated in an alcohol series, cleared in xylol, and mounted in permount. The size of the body, oral sucker, ventral sucker, and development of genital pores was recorded, and the sizes of the caeca, testes and ovaries, were also determined.

All experimental hosts were managed according to the guidelines approved by the Animal Ethics Committee of Chiang Mai University (No. RE 002/13).

Results

Worm recovery rate in experimental hosts

The metacercariae were experimentally confirmed by the successful development of adult worms in the experimental hosts; dwarf hamsters. The metacercariae were excysted to young adult worms in the intestines of the host and recovered in the intestines on days 3 and 6 PI, but on day 9 PI they were recovered in the livers of the hosts.

The incidence of parasitic infection was 100% (16/16). Average worm recovery rate was 36.00% (162/450). The worm recovery rates gradually decreased until the end of the experimental infection. However, the dwarf hamsters died on day 45 PI as a result of having their livers destroyed by the migration and penetration of the worms (Fig. 1). The highest

worm recovery rate recorded in the dwarf hamsters was 53.33% in the intestines and livers on days 3 and 9 PI, respectively (Fig. 2, Table 1).

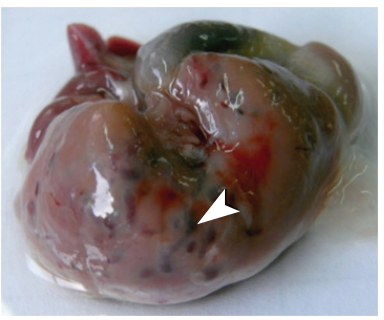

Figure 1. Infected liver of a dwarf hamster. The liver was severely destroyed due to migration and penetration of worms (arrow head)

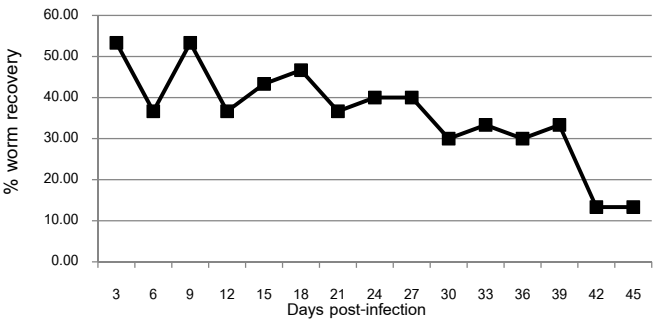

Figure 2. The worm recovery rates of *F. gigantica* adult worms in dwarf hamsters sacrificed every 3 day PI.

Table 1. The worm recovery rates of *F. gigantica* adult worms in dwarf hamsters sacrificed every 3 day PI.

Day PI	No. metacercaria	Dwarf hamster		
		No. worms	Site of infection	(%) Worm recovery
3	30	16	intestine	53.33
6	30	11	intestine	36.67
9	30	16	liver	53.33
12	30	11	liver	36.67
15	30	13	liver	43.33
18	30	14	liver	46.67
21	30	11	liver	36.67
24	30	12	liver	40.00
27	30	12	liver	40.00
30	30	9	liver	30.00
33	30	10	liver	33.33
36	30	9	liver	30.00
39	30	10	liver	33.33
42	30	4	liver	13.33
45	30	4	liver	13.33
Total	**450**	**162**		**36.00**

Maturity of adult trematodes in experimental host

The developmental patterns of *F. gigantica*, including measurements of body width, body length, and the oral and ventral suckers of the *F. gigantica* observed in the hosts, continuously increased. In an examination of the development of the *F. gigantica*, it was found that the minimum and maximum sizes of the bodies were 0.24 x 0.42 mm. (day 3 PI) and 3.80 x 13.90 mm. (day 45 PI) (Fig. 3, Table 2). Additionally, a study of the organ developments showed that the genital pores were initially recovered on day 9 PI, while the caeca was found on day 18 PI. But, the testes and ovaries were discovered on day 27 PI and developed to maturity on day 39 PI. Immature eggs were discovered on day 42 PI, which indicated that the parasites had begun to mature. However, the dwarf hamsters died on day 45 PI. Therefore, it can be confirmed that *F. gigantica* metacercariae that were derived from the experimental encystment could be infected and developed in experimental hosts, as well.

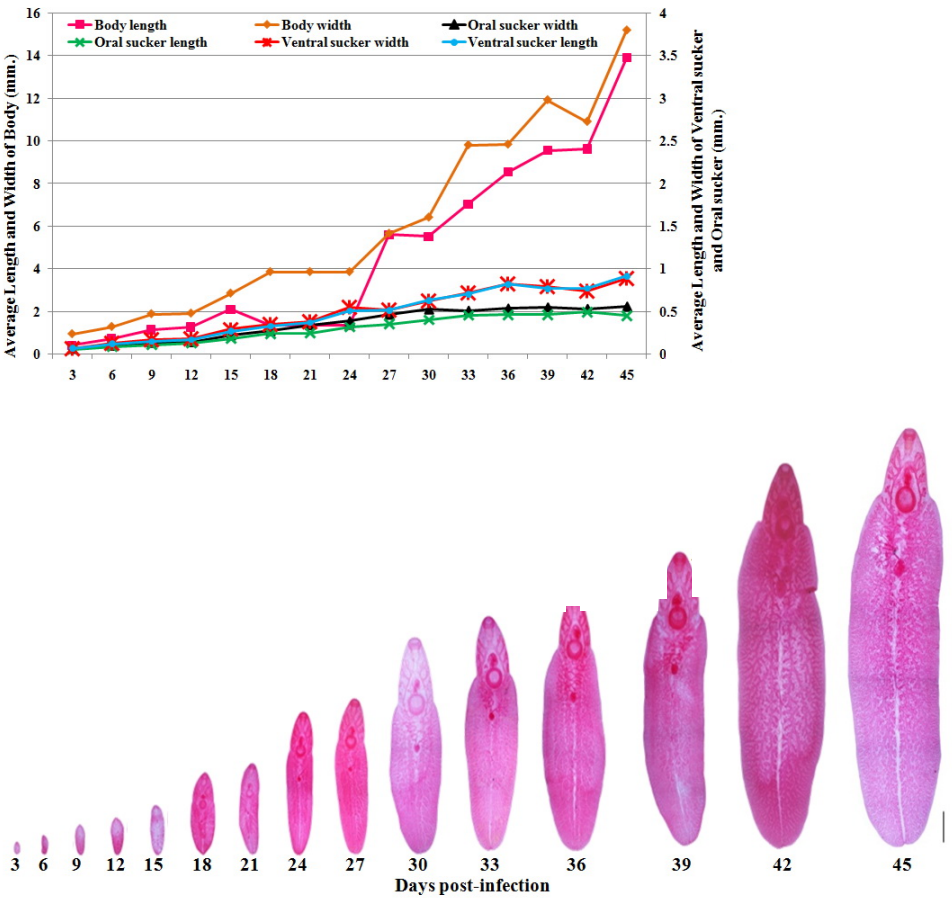

Figure 3. Morphological changes and average measurements of *F. gigantica* recovered from dwarf hamsters (scale bar: 1 mm.)

Table 2. Average measurements (mm.) of young to mature adult of *F. gigantica* in dwarf hamsters

Day PI	No. worms	Body		Oral sucker		Ventral sucker	
		width	length	width	length	width	length
3	4	0.24	0.42	0.07	0.06	0.06	0.07
6	3	0.32	0.72	0.10	0.09	0.13	0.12
9	11	0.47	1.14	0.13	0.11	0.17	0.15
12	3	0.48	1.27	0.14	0.13	0.19	0.17
15	5	0.72	2.11	0.22	0.18	0.29	0.27
18	6	0.97	1.35	0.27	0.24	0.35	0.33
21	6	0.97	1.35	0.34	0.25	0.38	0.37
24	6	0.97	1.35	0.39	0.32	0.55	0.51
27	5	1.42	5.60	0.46	0.35	0.52	0.52
30	7	1.61	5.52	0.53	0.41	0.62	0.63
33	9	2.45	7.04	0.51	0.46	0.72	0.71
36	7	2.46	8.54	0.54	0.46	0.83	0.82
39	9	2.98	9.56	0.55	0.46	0.79	0.77
42	3	2.73	9.62	0.53	0.50	0.74	0.77
45	2	3.80	13.90	0.56	0.45	0.89	0.92

Discussion

This study is the first report on the worm recovery and maturity of *F. gigantica* in experimental hosts: dwarf hamsters. The developmental model can be applied to other experimental hosts, and for which the acquired information can be used in the management, control and treatment of this parasite in ruminants and humans. The rate of incidence of this parasite in dwarf hamsters was found to be 100%. The results confirmed that experimental metacercariae were successfully developed in dwarf hamsters. The average worm recovery rate was found to be 36.00%, and the trend of discovery continuously declined. As a result, it was determined that hamsters are also suitable for use as models for parasitic infections. But the disadvantages are their inherent weakness and lowered resistance to parasitic infections. Therefore, some studies used bison as the experimental definitive host for *F. hepatica* (Foreyt & Drew, 2010). Moreover, the previous study used mice, black rat and hamster as the experimental hosts (Davies & Smyth, 1978; Valero et al., 1998; Keiser et al., 2006). The differences between worm recovery rates of *F. gigantica* in these experimental hosts have not been described in the previous study, and then in the further study should be more investigate. It could be seen that on day 45 PI, the dwarf hamsters died because of the heavy liver damage caused by the adult worms. Previous reports have involved the culturing of *F. hepatica* derived from mice and showed that the flukes revealed no further development in the genital rudiments (Davies

& Smyth, 1978).

Developmental patterns of this parasite in dwarf hamsters found that sizes of the body, oral and ventral suckers all continuously increased. On day 42 PI, the immature eggs were observed, which indicated that the *F. gigantica* had matured. An examination of the parasitic infection in goats found the parasite's eggs in the feces on days 64-70 PI (Taira & Saitoh, 2010). In this study, the parasite's eggs were not found due to the fact that the hosts had died before the adult worms could expel the eggs. The above results were different because the results were related to the liver fluke species, of which *F. hepatica* showed a period of maturity requiring 37 days (Dawes, 1962) and 40 days (Davies & Smyth, 1978).

Conclusion

The present study reported on the worm recovery rate and the maturity of *F. gigantica* in experimental hosts; dwarf hamsters, and indicated that *F. gigantica* can be successfully developed to maturity in other hosts besides ruminants and humans. This result can be applied in the treatment, monitoring, management and control programs of this parasite and can be applied to studies involving other experimental hosts.

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