

Estimated egg production of *Fasciola gigantica* (Japanese strain) in goats experimentally infected with 50 metacercariae

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Summary

Egg production capacity in *Fasciola gigantica* (Japanese strain) was estimated from egg count data obtained by the experimental infection of two goats. The goats were inoculated with a single dose of 50 metacercariae. The first goat was necropsied 132 days after infection (DAI) and the second goat 732 DAI. After patency, daily faecal production was collected and weighed and number of eggs per gram was counted. At necropsy, 23 flukes were recovered from the liver of the first goat, and five from the second goat. The mean number of eggs produced per day per worm (\pm 95 % confidence limits) was 9 477.9 (\pm 764.92) for the first goat at 106 to 132 DAI, and 8,064.1 (\pm 416.49) at 195 to 561 DAI for the second goat. The number of eggs produced per day per worm in *F. gigantica* (Japanese strain) has thus reached values ranged from approximately 8 000 to 10 000 eggs.

Keywords: *Fasciola gigantica* (Japanese strain); goats; experimental infection; egg production capacity; fecundity

Introduction

Estimation of the number of flukes present in livestock based on faecal egg counts is important for the diagnosis of chronic fasciolosis. Knowledge of the egg production capacity of *Fasciola* may also facilitate the evaluation of anthelmintic treatment efficacy and provide guidelines for epidemiological studies. A variety of factors is known to influence the egg production capacity in *Fasciola*, such as age, number or isolate of the parasite (Boray, 1967; Walker *et al.*, 2006) and/or hosts species (Alicata & Swanson, 1941).

The taxonomy of *Fasciola* species circulated in Japan has not yet been thoroughly classified and is still under considerable debate. The morphological characteristics of the Japanese forms of *Fasciola* are usually corresponding to *F. gigantica* and/or *F. hepatica*, and are often similar to both

species (Itagaki & Tsutsumi, 1998). In a recent comprehensive genetic study on *Fasciola* from 16 Japanese prefectures, Itagaki *et al.* (2005) recorded 2 major forms that are identical or closely related to *F. hepatica* and *F. gigantica* and 3 minor forms, one of which was attributed to hybrid form likely originated from paternal *F. hepatica* and maternal *F. gigantica*. *Fasciola* isolate used in the present study is referred to as “*F. gigantica* (Japanese strain)” as a *Fasciola* sp. in Japan, following the method of Yoshihara *et al.* (1998) and Taira *et al.* (2000). The egg production capacity, such as the number of eggs produced per day per worm (EPDPW) of *F. gigantica* (Japanese strain), estimated using quantitative egg count data in experimental infection studies, has not been so far reported.

The aim of the present study was therefore to estimate the EPDPW in *F. gigantica* (Japanese strain) using data gathered in previous studies (Taira *et al.*, 2000). In this report, EPDPW data were extensively analysed to estimate the egg production capacity of this fluke.

Materials and methods

Infection with Fasciola metacercariae

Two goats were used in the experimental study. Information on the experimental design and the infection of the first goat (No. 802) and the second goat (No. 602) was previously described (Taira *et al.*, 2000). Briefly, goat No. 802 (18.5 kg body weight) and goat No. 602 (12.2 kg) were inoculated with a single dose of 50 metacercariae of *F. gigantica* (Japanese strain) (Taira *et al.*, 1997). The goats were raised indoors in metabolism cages with gridded floors, and fed only with cubed hay and concentrated grain to preclude natural infection. Goats were maintained with due regard for their welfare.

Necropsy

Goats Nos. 802 and 602 were necropsied at 132 DAI and

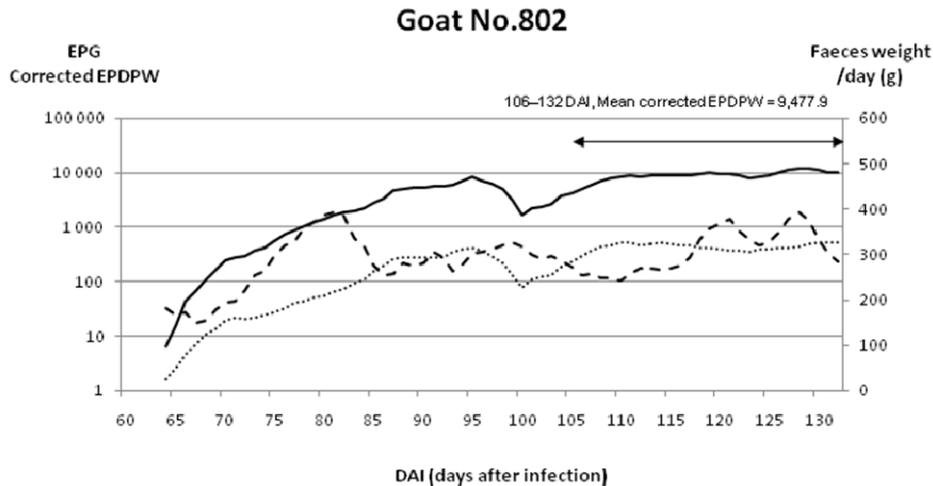


Fig. 1. Variation in daily faeces weight (broken line), eggs per gram (EPG) (dotted line) and corrected EPDPW (solid line) in a goat (No.802) experimentally infected with a single dose of 50 metacercariae of *Fasciola gigantica* (Japanese strain). Lines represent the moving average (n = 5). The goat was necropsied 132 days after infection (DAI) and 23 worms were detected.

Mean corrected EPDPW = faeces weight in a day (g) x EPG / number of worms detected at necropsy x 100/63.6 (see text).
 Mean corrected EPDPW was calculated for the period indicated by the both end arrow.

732 DAI, respectively. On the day of necropsy, the body weights of goats were measured and the macroscopical lesions of the goat livers were recorded. The number of flukes in the liver was counted at necropsy and the wet weight of the flukes from each animal was recorded.

Faecal egg counts

After patency, all faeces excreted in a day were collected and weighed. One gram of faeces of all samples was preserved in a 5 % formalin solution (Bonita & Taira, 1996). The number of eggs per gram (EPG) of faeces was counted using the beads technique which has a sensitivity of 63.6 % with respect to the egg recovery (Taira *et al.*, 1983).

The number of eggs produced per day per worm (EPDPW)
 Eggs produced per day (EPD) were calculated by multiplying EPG by the weight of all the faeces collected in one

day. The EPDPW was calculated by dividing EPD by the number of flukes detected at necropsy. The mean EPDPW values were calculated for the period 106 to 132 DAI for goat No. 802, and for the two periods 195 to 561 DAI and 707 to 732 DAI for goat No. 602; these periods were selected because the EPDPW values for these periods were relatively stable. Since the EPG count used in this study had a sensitivity of 63.6 %, the corrected EPDPW was calculated using EPG values obtained using the beads technique multiplied by 100/63.6 (Taira *et al.*, 1983).

Results

Clinical signs

Apparent clinical signs associated with *Fasciola* infestation were not observed in the two goats during the experiment.

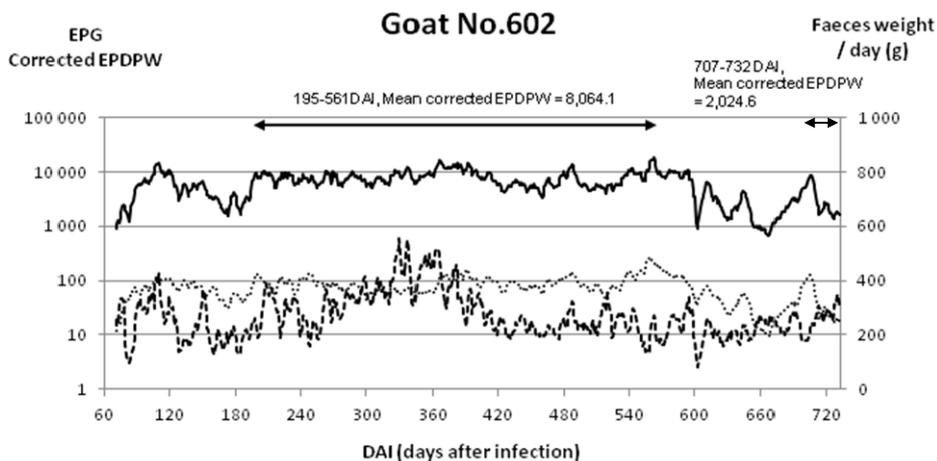


Fig. 2. Fluctuation in daily faeces weight (broken line), eggs per gram (EPG) (dotted line) and corrected EPDPW (solid line) in a goat (goat No.602) experimentally infected with a single dose of 50 metacercariae of *Fasciola gigantica* (Japanese strain). Lines represent the moving average (n = 5). The goat was necropsied 732 days after infection (DAI), and five worms were detected.

Corrected EPDPW = faeces weight in a day (g) x EPG / number of worms detected at necropsy x 100/63.6 (see text). Mean corrected EPDPW was calculated for the two periods indicated by the both end arrows.

Table 1. Infection, necropsy and egg output from two goats experimentally infected with *Fasciola gigantica* (Japanese strain)

Item		Goat No. 802	Goat No. 602	
Infection	Number of metacercariae administered (single dose)	50	50	
	Body weight (date)	18.5 kg (1 996.9.19)	12.2 kg (1 995.2.15)	
Necropsy	DAI ¹⁾	132	732	
	Body weight	10.5 kg	21.0 kg	
	Weight gain	-8.0 kg	+8.8 kg	
	Macroscopical lesion of livers	Fig. 3 left	Fig. 3 right	
	Number of flukes ²⁾ <a>, (recovery rate)	23 (46 %)	5 (10 %)	
	Total weight of flukes 	6.45 g	1.68 g	
	Mean weight of flukes <b/a>	0.28 g	0.34 g	
Egg output, etc.	Period to calculate EPDPW ³⁾	106 – 132 DAI (Fig. 1)	195 – 561 DAI (Fig. 2)	707 – 732 DAI (Fig. 2)
	Mean faeces weight produced/day (g) ($\pm 95\text{CL}$) <c>	307.9 (± 27.78)	297.6 (± 12.42)	288.5 (± 29.76)
	Mean EPG ($\pm 95\text{CL}$) <d>	462.4 (± 36.96)	90.8 (± 4.31)	24.1 (± 5.39)
	Mean EPD ($\pm 95\text{CL}$) <c x d>	138 642.4 (± 11189.21)	25 643.7 ($\pm 1,325.85$)	6 438.2 ($\pm 1,058.07$)
	Mean EPDPW ($\pm 95\text{CL}$) <c x d/a>	6 027.9 (± 486.49)	5 128.7 (± 265.17)	1 287.7 (± 211.61)
	Mean corrected EPDPW ($\pm 95\text{CL}$) <EPDPWx100/63.6> ⁴⁾	9 477.9 (± 764.92)	8 064.1 (± 416.94)	2 024.6 (± 332.73)

1) Days after infection

2) Flukes detected in the livers of the goats are shown in Fig. 4.

3) Eggs produced per day per worm = faeces weight in a day (g) x EPG / number of flukes detected at necropsy

4) Detectability of *Fasciola* eggs using the beads technique is 63.6% (Taira *et. al.*, (1983)

Faecal egg output

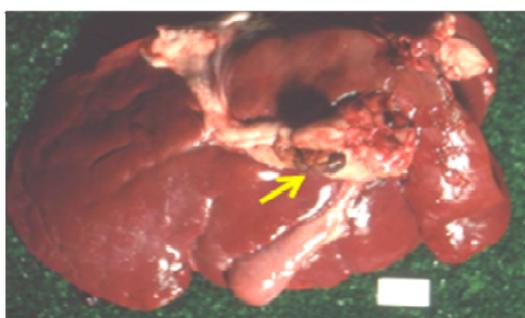
Patency started in goat No. 802 at 64 DAI and in goat No. 602 at < 70 DAI. The pattern of faecal egg output is presented in Figs. 1 and 2.

Findings at necropsy

The body weights of the goats at necropsy were 10.5 kg for goat No. 802 and 21.0 kg for goat No. 602. The weight gain of the goats after infection, calculated by subtracting

the weight of the goats at necropsy from their weight at inoculation, was -8.0 kg for goat No. 802 and +8.8 kg for goat No. 602 (Table 1). Goat No. 802 presented with an enlarged common bile duct, while goat No. 602 had a large cyst containing flukes on the diaphragmatic surface of the liver (Fig. 3). Twenty-three flukes (total wet weight of 6.45 g) were recovered from the liver of goat No. 802, and five flukes (total wet weight of 1.68 g) were recovered from the liver of goat No. 602 (Fig. 4).

Goat No. 802



Goat No. 602

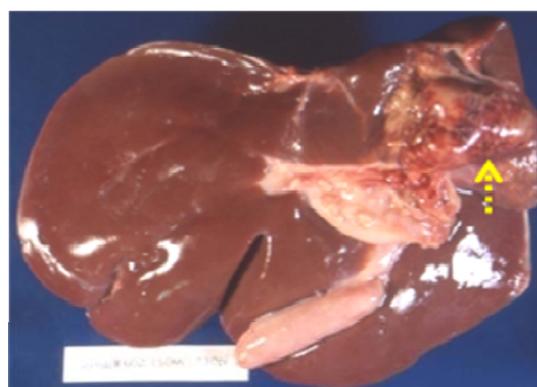


Fig. 3. Livers of goats experimentally infected with a single dose of 50 metacercariae of *Fasciola gigantica* (Japanese strain). (Left) Goat No.802 was necropsied at 132 days after infection (DAI). The bile duct was enlarged and a fluke was observed at the resected duct (solid arrow). (Right) Goat No.602 was necropsied at 732 DAI. The bile duct was enlarged and a large cyst containing worms was observed (dotted arrow).

Calculation of mean corrected EPDPW

The mean weight of faeces produced per day ($\pm 95\%$ confidence limits) was 307.9 g (± 27.78 g), mean EPG was 462.4 (± 36.96), mean EPD was 138 642.4 ($\pm 11 189.21$) and mean EPDPW was 6 027.9 (± 486.49) at 106 – 132 DAI for goat No. 802. These values for goat No. 602 at 195 – 561 DAI were 297.6 (± 12.42), 90.8 (± 4.31), 25 643.7 ($\pm 1 325.85$) and 5 128.7 (± 265.17), and at 707 – 732 DAI were 288.5 g (± 29.76 g), 24.1 (± 5.39), 6 438.2 ($\pm 1 058.07$) and 1 287.7 (± 211.61), respectively. The mean corrected EPDPW was 9 477.9 (± 764.92) for goat No. 802, and 8 064.1 (± 416.49) at 195 – 561 DAI and 2 024.6 (± 332.73) at 707 – 732 DAI for goat No. 602 (Table 1). The weight of faeces produced per day (g), EPG and corrected EPDPW for goat No. 802 are plotted with a moving average ($n = 5$) in Fig. 1 and those of goat No. 602 are plotted in Fig. 2, using a broken line, dotted line and solid line, respectively. EPG and corrected EPDPW were converted to natural logarithm values ($\log(x + 1)$) in Figs. 1 and 2.

(1969) reported a gradual decrease in the egg production of flukes in response to an increase in the number of flukes in the liver of sheep, possibly due to the overcrowding effect. These authors also characterized liver infection by 1 to 100 flukes as being a light infection that causes chronic sub-clinical fasciolosis. The two goats herein used, each inoculated with 50 metacercariae, harboured 23 and 5 flukes in the liver and did not show any clinical signs. Since the light infection in this study may not have resulted in the overcrowding effect, the fecundity of flukes in the goats in the present study may not have been affected by the number of flukes in each host.

Rather, it appears that differences in either species or isolates of *Fasciola* are responsible for the differences in fecundity. Walker *et al.* (2006) reported differences in the EPDPW of different fluke isolates recovered from experimentally infected rats *in vitro*. These authors documented that the Fairhurst isolate, which was triclabendazole-susceptible, had EPDPW of 10 200, while the Oberon isolate, which is triclabendazole-resistant, was 3 623. The mean

Goat No. 802



Goat No. 602



Fig. 4. Flukes detected at necropsy in goats experimentally infected with a single dose of 50 metacercariae of *Fasciola gigantica* (Japanese strain). (Left) Goat No.802 was necropsied at 132 days after infection (DAI); 23 worms were found in the liver and the mean weight of the fluke was 0.28 g. (Right) Goat No.602 was necropsied at 732 DAI; five worms were found in the liver and the mean weight of fluke was 0.34g.

Discussion

The egg count data obtained in the present study was used to determine the fecundity of *F. gigantica* (Japanese strain). The mean corrected EPDPW calculated for goat No. 602 at 195 – 561 DAI was not markedly different from that obtained from goat No. 802 in the period 106 – 132 DAI. However, the corrected EPDPW value for goat No. 602 at 707 – 732 DAI constituted approximately 25 % of that observed in the same goat at 195 – 561 DAI (Table 1). This reduction in EPDPW observed in goat No. 602 was likely due to senescence of the flukes in this animal. The comparatively large fluctuation was observed in the EPDPW in goat No. 602 at around 600 DAI, indicating that the activity of flukes appears to decline approximately 1.5 years after infection in goats.

The number of flukes in the liver also appears to affect the egg output capacity of *Fasciola*. Happich and Boray (1969) reported a gradual decrease in the egg production of flukes in response to an increase in the number of flukes in the liver of sheep, possibly due to the overcrowding effect. These authors also characterized liver infection by 1 to 100 flukes as being a light infection that causes chronic sub-clinical fasciolosis. The two goats herein used, each inoculated with 50 metacercariae, harboured 23 and 5 flukes in the liver and did not show any clinical signs. Since the light infection in this study may not have resulted in the overcrowding effect, the fecundity of flukes in the goats in the present study may not have been affected by the number of flukes in each host.

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Using data of *Fasciola* egg output obtained from goats that had been experimentally infected with a single inoculum of 50 metacercariae, the EPDPW of *F. gigantica* (Japanese strain) was estimated to range between approximately 8 000

– 10 000 eggs. Although several factors may influence the fecundity of *Fasciola* in the field, the knowledge of the EPDPW estimated in the present study may assist faecal egg counts in the diagnosis of chronic fasciolosis caused by *F. gigantica* (Japanese strain).

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