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A CROSS SECTIONAL STUDY ON *FASCIOLA GIGANTICA* AND *GIGANTOCOTYLE EXPLANATUM* BURDENS IN NATURALLY INFECTED BUFFALOES IN BANGLADESH

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ABSTRACT

A cross sectional study on *Fasciola gigantica* and *Gigantocotyle explanatum* burdens in buffaloes in relation to their age, sex and health condition was carried out in the Laboratory of Parasitology, Bangladesh Agricultural University, Mymensingh, Bangladesh during the period from June to November 1997. A total of 460 faecal samples were examined to determine the egg per gram of faeces (epg) of *F. gigantica*. Higher epg was counted in female buffaloes (159.67 ± 6.80) than in males (153.24 ± 8.29). The epg counts were also found significantly ($p < 0.01$) higher in poor conditioned buffaloes (164.41 ± 6.67) than in buffaloes with normal health (128.57 ± 7.08). In relation to age, the young adult buffaloes were found to bear more parasitic burdens. The highest mean epg was recorded in the buffaloes of 2-3 years old (184.21 ± 19.14), followed by 3-5 years old (169.23 ± 30.77). The older buffaloes had relatively lower parasitic burden and the lowest mean epg was recorded in the growing buffaloes of up to 1 year old (125 ± 25.0). Examination of 106 livers from slaughtered buffaloes, revealed that the load of *Fasciola gigantica* and *Gigantocotyle explanatum* in the livers ranged from 13-424 (69.20 ± 6.46) and 41-427 (161.45 ± 10.79) respectively. In males the burden of *F. gigantica* (13-210) was lower than that of the females (15-424). The mean number of *F. gigantica* in the buffaloes of 5-10 years old (100 ± 15.32) was significantly higher ($p < 0.01$) than those of the buffaloes of above 10 years old (52.92 ± 4.29). In case of single infection with *F. gigantica*, the mean number of parasites per liver was significantly ($p < 0.05$) higher (88.70 ± 11.48) than that of mixed infection cases (49.74 ± 4.20). Significantly ($p < 0.01$) higher number of *G. explanatum* was recovered from the buffaloes of 5-10 years old (207.53 ± 26.72) than from the buffaloes of above 10 years old (141.58 ± 9.23) and from single infected cases than from buffaloes with mixed infection. There were almost no differences in the number of both flukes in relation to sex of buffaloes. The length and breadth of immature and mature parasites were 5-15 mm × 2-3 mm and 16-72 mm × 4-14 mm respectively. No significant variation was found in the average size of *F. gigantica* in relation to the age and sex of buffaloes. But in case of single infection, the body size of mature *F. gigantica* (38.63 mm ± 0.38 mm × 9.96 mm ± 0.12 mm) was significantly ($p < 0.05$) higher than that of the mixed infection (33.68 mm ± 0.52 mm × 8.76 mm ± 0.07 mm) cases. This study fairly suggests that the young adult buffaloes harbour more *F. gigantica* parasites than their younger and older counterparts. The concomitant infection of buffaloes with *F. gigantica* and *G. explanatum* may hamper the parasites each other in establishing their infection and attaining growth in some way as measured by their burdens and morphological dimensions.

Key words: *Fasciola gigantica*, *Gigantocotyle explanatum*, burdens, buffaloes

INTRODUCTION

The association between *Fasciola* spp. and their hosts may be traced back to the antiquity. Kendall (1954) first reported fascioliasis in cattle in East Pakistan (now Bangladesh) and stated that the economic losses due to condemnation of *Fasciola gigantica* infected liver of ruminants are enormous. In addition, *Gigantocotyle explanatum*, is an amphistome parasite of bile duct and gall bladder, immature stage of which causes serious enteritis and health hazards especially in young buffaloes (Pande *et al.*, 1968; Soulsby, 1982). Fairly a number of reports suggest the prevalence and pathology of fascioliasis in cattle, goats and buffaloes in Bangladesh (Rahman *et al.*, 1972; Nooruddin *et al.*, 1987; Islam *et al.*, 1992). Qadir (1981) and Chowdhury *et al.* (1994) reported the load of *F. gigantica* in goat and cattle livers respectively. The grazing habit of buffaloes in the low lying areas expose them to more risk of getting snail borne trematode infections especially *F. gigantica* and *G. explanatum*. This research work was undertaken to determine the egg per gram of faeces (epg) of *F. gigantica* in naturally infected live buffaloes and burdens of *F. gigantica* and *G. explanatum* in slaughtered buffaloes. The morphological dimension of *F. gigantica* in slaughtered buffaloes in relation to age, sex, health condition of the host and concomitant infection with *G. explanatum* were also determined.

MATERIALS AND METHODS

This study was carried out from June to November 1997 in the Laboratory of Department of Parasitology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Examination of faecal samples

A total of 460 faecal samples were collected from 460 buffaloes of Mymensingh, Jamalpur, Sylhet and Bangladesh Agricultural University Dairy Farm. The faecal samples were collected within 8-9 am and directly from the rectum of the animals or from the freshly voided faeces when the animal was found in the act of defecation. The samples were examined by Stoll's Method as described by Thienpont *et al.* (1986) to determine eggs per gram of faeces (epg) of *F. gigantica*. Before collection of the faecal samples, the age, sex and health condition of the buffaloes were recorded properly.

Examination of liver samples

A total of 106 liver samples of the slaughtered buffaloes were collected from the slaughter houses of Sylhet, Jamalpur and Dhaka. The livers were collected as a whole organ. In the laboratory, the main bile ducts and the gall bladders were opened first and then the liver was cut into small slices of approximately 2 cm thickness. The sliced pieces were squeezed with the thumb and fingers to remove the *Fasciola gigantica* that might be present in the small ductules and parenchyma. Both the mature and immature parasites were collected. The *Gigantocotyle explanatum* those were found in the bile ducts and gall bladder were collected in the same way. The *F. gigantica* and *G. explanatum* recovered from each liver were counted and recorded. Both mature and immature *F. gigantica* were laid on the smooth glass surface and the length and breadth were measured in millimeters using the calipers (Ross *et al.*, 1966). An aliquot of 500 mature and 500 immature *F. gigantica* were measured for each parameter of study. The parasites were identified by following the standard key (Yamaguti, 1958).

Statistical analysis

The epg, number and size of the flukes in different sex, age, body condition of buffaloes and in case of single and mixed infection were analyzed by Student's '*t*' test and '*z*' tests (Gupta, 1990).

RESULTS AND DISCUSSION

Faecal egg counts of F. gigantica

The epg of *F. gigantica* among the different sex, age and health condition of buffaloes varied markedly (Table 1).

Table 1. Faecal egg counts of *Fasciola gigantica* in buffaloes

Factors	Factor level	No. of faecal samples examined	No. of infected animals	Egg per gram (epg) of faeces (Mean ± SE)	Range
Sex	Male	196	94	153.24 ± 8.29	100-500
	Female	264	139	159.67 ± 6.80	100-500
Health condition	Normal	201	56	128.57 ± 7.08^{b}	100-300
	Poor	259	177	164.41 ± 6.67^{a}	100-600
Age	0-1 year	51	04	125.00 ± 25.00^{c}	100-200
	> 1-2 year	38	13	131.71 ± 8.86^{c}	100-500
	> 2-3 year	38	19	184.21 ± 19.14^{a}	100-600
	> 3-5 year	78	41	169.23 ± 30.77^{b}	100-300
	> 5-10 year	159	93	162.37 ± 10.13^{b}	100-400
	> 10 year	96	63	155.56 ± 9.0^{c}	100-400
Overall		460	233	156.65 ± 5.52	100-600

^{a,c}Values with different superscript letters within the same group differ ($p < 0.01$), ^{b,c} Values with different superscript letters within the same group differ ($p < 0.05$).

The overall epg count was 156.65 ± 5.52 with a range of 100-600 which was higher than that of the findings of Chowdhury *et al.* (1994), who recorded 100-400 epg with an average of 138.30 ± 4.27 in cattle of Bangladesh. Of course, the other scientists, Howlader *et al.* (1991) and Contreras *et al.* (1979) recorded 100-900 epg in goats and 200-

2800 epg in cattle respectively. The variation might be due to the variation in the species and breed of the host animals, load of the parasites in the liver and the techniques of faecal examination applied. The epg of *F. gigantica* rises rapidly for 7-10 weeks after the first eggs are observed and thereafter it is very variable and may be very low even in a heavily infected animals which greatly reduces the values of faecal egg counts as a diagnostic technique (Sewell, 1966). This may also be true for the variation of our findings with the earlier scientists in relation to epg count as because the sample in this study were collected from the naturally infected animals where it was not possible to determine the time of exposure of buffaloes to the infection. The time and part of faecal sample collection also greatly influences the epg counts of *Fasciola hepatica* (Hagens and Over, 1966; Dorsman, 1967). Hagens and Over (1966) noted the highest epg from noon to midnight. Dorsman (1967) found that there was a clear fluctuation in the epg count of *Fasciola hepatica* in cattle for each defecation either the last portion of each sample or the entire sample was examined. He also reported that there was fluctuation when the first or the random portion was used and suggested that faecal samples used for liver fluke egg counts should be collected in the middle of the day. The samples for his study were collected before noon (within 8–9 am) without bothering the part of the faeces of each defecation, which may be a factor of the variation of the epg count with the earlier scientists. The mean epg count of the female buffaloes (159.67 ± 6.80) was higher than that of males (153.24 ± 8.29). The causes of higher epg count in the female buffaloes than that of the males are difficult to explain but it might be due to the alteration in the body physiology during pregnancy and lactation, which may lead to lowering of body resistance to combat the establishment of infection leading to more *F. gigantica* burdens. On the other hand the mean epg was much higher in the poor conditioned buffaloes (164.41 ± 6.67) than that of the normal conditioned buffaloes (128.57 ± 7.08). Significantly ($p < 0.01$) higher epg count in poor conditioned buffaloes than in buffaloes of normal health is the reflection of higher *F. gigantica* burdens as the parasites cause severe damage of the liver, which may result in diarrhoea and inappetance, leading to loss of the body weight and emaciation. The fluctuation of epg count was observed in different age groups of buffaloes. The buffaloes of 2-5 years old were found to harbour more parasitic burden than their younger and older counterparts. The highest mean epg was recorded in the buffaloes of 2-3 years old (184.21 ± 19.14), followed by 3-5 years old buffaloes (169.23 ± 30.77). The older buffaloes had comparatively lower parasitic burdens where mean epg were counted 162.37 ± 10.13 in 5-10 years old and 155.56 ± 9.0 in buffaloes of above 10 years old (Table 1). The buffaloes of 1-2 years old had mean epg 131.71 ± 8.86. The lowest mean epg was recorded in the buffalo calves of up to 1-year-old (125 ± 25.0). The highest epg count in buffaloes of 2-3 years old group followed by 3-5 years old group buffaloes might be due to the less resistance of the animals to the new exposure of infection resulting in establishment and maturity of a large number of parasites in the hosts. The facts behind lower epg in the older animals of 5 years and above are difficult to explain. Due to the continuous exposure to infection there is fibrosis and calcification of the livers and bile ducts, which might be a factor of establishment of less number of parasites in the liver. Some immunological phenomenon or any other body mechanisms of the older buffaloes might have role in causing fewer establishments or reducing fecundity of the parasites. The lowest epg in the animals of up to 1 year of age can simply be explained by the fact that these animals get very little chance to contract massive infection through grazing. However, the epg of *G. explanatum* could not be determined, as it was not possible to identify the *G. explanatum* eggs on the basis of their morphology through faecal examination (Soulsby, 1982).

Load of F. gigantica and G. explanatum in the slaughtered buffaloes

The immature and mature *F. gigantica* recovered from liver samples ranged from 13 to 424 with a mean of 69.20 ± 6.46 which was higher than that of report of Qadir (1981) who isolated 1-120 parasites from goat livers. Chowdhury *et al.* (1994) recovered 4-132 *F. gigantica* from cattle liver. This variation in the findings might be due to the species variation of the hosts, size of the animals as well as the liver, grazing habit of the host animals, season of the years, degree of pasture infestation, management factors, topography of the area, availability of the snail intermediaries and age of the animals when slaughtered. In the male buffaloes, the burden of *F. gigantica* (13-210) was lower than those in the females (15-424). In all cases (single and mixed infection), the number of parasitic burden was higher in the female buffaloes than in the males which is substantiated by the previous report (Chowdhury *et al.*, 1994) and it can be interpreted as it is described in case of epg count. In the buffaloes of 5-10 years old the mean number of *F. gigantica* (100 ± 15.32) with a range of 29-424 was significantly higher ($p < 0.01$) than those of buffaloes of above 10 years old where it ranged from 13 to 163 with an average of 52.92 ± 4.29 (Table 2). In case of single infection with *F. gigantica,* the average number of parasites per liver (88.70 ± 11.48 with the range of 19-424) was significantly ($p < 0.05$) higher than that of mixed cases of infection (49.74 ± 4.20 with a range of 13-121 parasites). An average 161.45 ± 10.79 with a range of 41-427 *G. explanatum* was recovered from infected livers. Like *F. gigantica,* more parasites were isolated from the female buffaloes than in males. Significantly ($p < 0.01$) higher number of *G. explanatum* were isolated from

Table 2. Counts of *F. gigantica* and *G. explanatum* in the livers of slaughtered buffaloes

Factors	Factor level	Livers examined	Livers infected		Counts of *F. gigantica* worms (Range / Mean ± SE)			Counts of *G. explanatum* (Range/Mean±SE)
			Fg	*Ge*	Mature	Immature	Total	
Sex	Male	46	30	24	13-185 60.77 ± 7.92	0-25 8.0 ± 1.48	13-210 69.02 ± 9.1	41-383 155.33 ± 14.43
	Female	60	48	31	13-397 61.94 ± 8.13	0-93 8.08 ± 2.04	15-424 69.77 ± 8.52	45-427 166.19 ± 15.70
Age (Year)	5-10	39	32	16	23-397 90.33 ± 14.14	0-25 13.68 ± 1.68	29-424 100 ± 15.32[a]	41-427 207.53 ± 26.72[a]
	> 10	67	46	39	13-141 45.84 ± 3.32	0-93 7.08 ± 1.92	13-163 52.92 ± 4.29[b]	45-280 141.58 ± 9.23[b]
Infection type	Single	106*	39	16	19-397 78.23 ± 10.49	0-93 10.46 ± 2.57	19-424 88.70 ± 11.48[c]	87-427 225.25 ± 24.26[a]
	Mixed		39	39	13-108 44.28 ± 3.47	0-23 5.46 ± 0.94	13-121 49.74 ± 4.20[d]	41-225 13.28 ± 8.71[b]
Overall		106	78	55	19-397 61.25 ± 5.82	0-93 7.96 ± 1.39	13-424 69.20 ± 6.46	41-427 161.45 ± 10.79

*Indicates total number of livers examined for detection of both single and mixed infection, [a,b] Values with different superscript letters within the same group differ ($p < 0.01$), [c,d] Values with different superscript letters within the same group differ ($p < 0.05$), Fg = *Fasciola gigantica*, Ge = *Gigontocotyle explanatum*.

the buffaloes of 5-10 years old than from buffaloes of above 10 years old and from single infected cases than from buffaloes with mixed infection (Table 2). The inverse relationship of more parasitic burden in the lower age groups (5-10 years) than in the older animals of above 10 years may be explained due to an immunological reaction or due to the mechanical barrier by fibrosis in hepatic parenchyma and bile ducts which results from repeated exposure to parasites and may hinder the establishment of the flukes.

Size of *F. gigantica*

For the immature parasites, the average length and breadth were 5-15 mm (8.98 ± 0.10 mm) and 2-3 mm (2.16 ± 0.01 mm) respectively whereas in case of mature parasites they were 16-72 mm (36.28 ± 0.12 mm) and 4 – 14 mm (9.13 ± 0.02 mm) respectively. No significant variation could be measured in the average body size of the mature parasites recovered from male (35.32 ± 0.51 mm × 8.32 ± 0.08 mm) and female buffaloes (37.34 ± 0.32 mm × 9.38 ± 0.09 mm). Similarly, in case of buffaloes of 5-10 years old the length and breadth (38.86 ± 0.34 mm × 9.58 ± 0.09 mm) of mature parasites were almost equal to the parasites recovered from the buffaloes of above 10 years of age group (35.68± 0.57 mm × 8.13 ± 0.09 mm). Again, in case of single infection, the body size of *F. gigantica* (38.63 ± 0.38 mm × 9.96 ± 0.12 mm) was significantly ($p < 0.05$) higher than that of the mixed cases of infection (33.68 ± 0.52 mm × 8.76 ± 0.07 mm). No significant differences were measured in size of the immature *F. gigantica* in any group of buffaloes (Table 3). The size of the mature parasites (16-72 × 4-14 mm) as measured in this study is in close agreement with Chowdhury *et al.* (1994) who recorded *F. gigantica* of 12 - 72 mm × 2 - 12 mm in cattle. Nooruddin *et al.* (1987) measured *F. gigantica* of 15-48 mm × 3-13 mm in Black Bengal goats in Bangladesh. According to Soulsby (1982), mature *F. gigantica* measures 25-72 × 2-12 mm and according to Boray (1985), the measurement is 24-76 × 5-13 mm which is more in length than the present finding and it can be concluded that the parasites prevailing in this country is relatively a shorter strain and this variation might be due to the strain variation, species of the hosts and the climatic factors. A little average shorter size of *F. gigantica* in the animals of above 10 years of age might be due to the calcification and mechanical barrier by the fibrous tissue formed in the process of healing of the damaged hepatic tissues during the repeated migration of the immature flukes. The exact cause of the significantly smaller size of the parasites in the mixed infection is difficult to explain but might be due to the competition among the parasites for accommodation, and food or effects of some biochemical reactions in the liver. The *G. explanatum* counted in this

Table 3. Size of *Fasciola gigantica* recovered from livers of slaughtered buffaloes

Factors	Factor level	Mature worms (n = 500)		Immature worms (n = 500)	
		Length (mm) (Range/Mean±SE)	Breadth (mm) (Range/Mean±SE)	Length (mm) (Range/Mean±SE)	Breadth (mm) (Range/Mean±SE)
Sex	Male	16-72 35.32 ± 0.51	4-14 8.32 ± 0.08	6-15 8.58 ± 0.23	2-3 2.16 ± 0.06
	Female	16-72 37.34 ± 0.32	4-14 9.38 ± 0.09	5-15 8.88 ± 0.29	2-3 2.12 ± 0.06
Age (Year)	5-10	16-72 38.86 ± 0.34	4-14 9.58 ± 0.09	5-15 9.15 ± 0.19	2-3 2.23 ± 0.04
	> 10	14-71 35.68 ± 0.57	4-14 8.13 ± 0.09	5-15 8.36 ± 0.23	2-3 2.08 ± 0.04
Infection type	Single	16-72 38.63 ± 0.38[a]	4-14 9.96 ± 0.12	5-15 8.78 ± 0.25	2-3 2.31 ± 0.06
	Mixed	16-72 33.68 ± 0.52[b]	4-14 8.76 ± 0.07	5-15 8.28 ± 0.25	2-3 2.10 ± 0.06
Overall		16-72 36.28 ± 0.12	4-14 9.13 ± 0.02	5-15 8.98 ± 0.10	2-3 2.16 ± 0.01

[a,b] Values with different superscript letters within the same group differ ($p < 0.05$).

study is in close conformation with Hafeez and Rao (1989). The parasites also varied in number with the sex and age of he animals and in single and mixed infected cases as it was recorded for *F. gigantica*. The reasons behind may be as it has been explained in case of *F. gigantica*.

It may be concluded that the load of *F. gigantica* and *G. explanatum* varies in relation to the age and sex of the buffaloes. The type of infection either single (with *F. gigantica* or *G. explanatum*) or mixed infection may influence the establishment of the number of either of the flukes. The age and sex of the buffaloes and concomitant infection with *G. explanatum* influence the morphological dimension of the mature *F. gigantica*.

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