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Research Article

First report of intra-species morphological variation of *Fasciola gigantica* from different host origins (cattle and buffalo)

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Abstract

The present study reports intra-specific morphological variation of *Fasciola gigantica* of different host origins (cattle and buffalo). Adult *Fasciola* parasites (n=80) were collected from the liver and bile duct of naturally infected cattle and buffalo slaughtered at Tangra, West Bengal. Forty *Fasciola* flukes were thoroughly chosen (twenty from cattle and twenty from buffalo) for detailed studies. The detailed morphoanatomic and morphometric study of the flukes was done using the techniques comprising staining of intact worms, histology along with gross morphology. Analysis of morphometric parameters comprising body weight, body length, body width, cephalic cone length, cephalic cone width, testes length, body length by body width ratio and the distance between ventral sucker to posterior end depicted significant difference (P<0.01) of *F. gigantica* of two host origin (cattle and buffalo). The ovarian branches were smaller, thin and light dense in cattle, while they were thick and stout, highly dense in buffalo. The testicular branches were light sinuous, simple, and minutely dense in cattle, while they were thick, more sinuous and highly dense in buffalo. All these parameters revealed overt morphological differences of *F. gigantica* collected from cattle and buffalo.

Key words: *Fasciola gigantica*, Intra-specific morphological variation, Taxonomy

Highlights

- Overt gross morphological difference of the same species (*Fasciola gigantica*) of different hosts (cattle and buffalo).
- Some significant morphometric differences of *F. gigantica* of different hosts were mentioned earlier.
- Significant microscopic morpho-anatomical difference of *F. gigantica* mentioned earlier.
- Significant differences of the body weight of the aforesaid parasite were observed.

INTRODUCTION

Fasciolosis is an important parasitic disease which causes great economic loss in the livestock industry. The two most important parasites causing fasciolosis are *Fasciola gigantica* and *F. hepatica* which predominate in tropical and temperate countries, respectively. Identification of these parasites is very important for carrying out any work. Morphological identification of these two species is the gold-standard technique in the present day. Molecular identification of parasites has been a recent trend, but before molecular identification, morphological identification is a prerequisite. Therefore, more and more morphological data of different parasites are required at this time. Several literatures are available on the morphological differences between *F. gigantica* and *F. hepatica* (Lotfy *et al*., 2002; Shafiei *et al*., 2014;

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Shaldoum *et al*., 2015; Valino *et al*., 2017). Although the two species are classified by morphological characters, especially body length and width, it is difficult accurately to discriminate between the two species because of many variations in size depending on different factors like age of the flukes, species of host and the technical difficulty in fixation of the flukes (Kendall, 1965). Among morphometric parameters, body length, body width, cephalic cone length, cephalic cone width, distance between the ventral sucker and posterior end of the body (VS-P), body roundness and body length/width ratio (BL/BW), testes length, were useful measurements for differentiating between the two species (Ashrafi *et al*., 2006; Periago *et al*., 2006, 2008). The inter-specific morphological difference between *F. gigantica* and *F. hepatica* has been reported based on these aforementioned morphological parameters. But no report is available on the morphological difference of *F. gigantica* of cattle and buffalo origin. The present work communicates intra-species morphological variation of *F. gigantica* from cattle and buffalo origin.

MATERIALS AND METHODS

Collection and preservation of flukes: Adult eighty *Fasciola* worms were collected from the liver and bile duct of five naturally infected cattle (n=50) and six naturally infected buffalo (n=30) slaughtered at the local municipal abattoir Tangra, West Bengal, Kolkata. The flukes were washed thoroughly in PBS till the flukes were free from bile, blood, and host material (Lotfy *et al*., 2002) and finally preserved in 70% alcohol for permanent mounting and 10% formalin for detailed histological studies.

Gross morphological study: Gross morphological identification was made by placing large number of flukes in a Petri dish (Fig. 1). Presumptive identification of adult flukes was made by pressing each individual fluke in between two glass slides and examined under a dissecting microscope (Yakhchali *et al*., 2015).

Preparation and examination of stained whole-mounts: Flukes collected from cattle and buffalo were fixed with 70% ethanol for at least 48 h. For staining, the parasite is removed from 70% alcohol and washed thoroughly in distilled water for about half an hour and then transferred to a dark red rose-coloured dilution of acetic borax carmine solution (2% sodium tetraborate with 1.5% carmine in 35% ethanol) and left to stain for 24 hours. The flukes were then washed thoroughly in distilled water, followed by dehydration for three to four hours through ascending grades of alcohol (30%, 50%, 70%, 90% and absolute alcohol) for 15-30 minutes depending on the size and thickness of the specimen. After sufficient dehydration with 70% alcohol, they were treated with 70% acidified ethanol solution (1 mL of concentrated HCl in 100 mL of 70% ethanol) to destain. After the specimens were removed from acidified ethanol and finally, they were then rinsed in non-acidified 70%, 90% ethanol and transferred through two to three changes of absolute ethanol. After dehydration, the specimen was cleared in xylene, and then a permanent slide was prepared following the standard procedures (Brytania, 1977), and finally, these were identified according to the keys description (Soulsby, 1982).

Preparation of flukes for histology: Five flukes collected from each cattle and buffalo were fixed for histological examination within one hour after removal from the hosts. The flukes were placed in a 10 cm-square plastic Petri dish and held flat beneath a light glass plate throughout fixation for 48 h with 10% (v/v) neutral-buffered formalin. After fixation, the tissue samples were placed under running tap water overnight in order to remove excess formalin from the tissue. After fixation, each fluke was sliced into equal right and left halves along the median plane. The two halves were dehydrated in ethanol, cleared in benzene, and

the tissue samples were passed through three liquid paraffin baths (56°C) each in one hour for impregnation and finally embedded in a melted paraffin wax block using metal moulds following conventional procedures, with the cut surfaces presented at the block face. Paraffin-embedded samples were cut with the use of a rotary microtome, and approximately 5 μm of thick slices section were cut from each block face and finally stained with haematoxylin and eosin (H and E) (Luna, 1968) to compare the difference between the shapes of organs like testes, tegumental spine, tegumental syncytium and gut region.

Morphoanatomical observation: The flukes were grossly observed by the naked eye. The variations of different morpho-anatomical structures of the flukes collected from cattle and buffalo were studied by general and histological staining.

Morphometric data recording: Each preserved worm from cattle and buffalo was thoroughly washed with normal saline for morphometric analysis. After washing, the flukes were placed in between two glass slides and gentle pressure was applied to make the flukes flatten. After sufficient flattening, the flukes are subjected to the following morphometric measurements: fluke length, fluke breath, cephalic cone length, cephalic cone breath, testes length, body weight and ventral sucker to posterior end etc. (Periago *et al*., 2006; Narva *et al*., 2011; Akhlaghi *et al*., 2017; Aryaeipour *et al*., 2017).

Statistical analysis: To compare the mean of different variables between *F. gigantica* of cattle and buffalo student's t-test was done to get whether any significant differences are there or not between the means of morphometric values of *F. gigantica* isolated from cattle and buffalo.

RESULTS

While different morphologic parameters were analysed, some differences of gross morphological features of *F. Gigantic* (collected from cattle and buffalo) were recorded. The colour of all flukes collected from cattle was grey and white combination (Fig. 1e), while the colour of the same parasites collected from buffalo was grey and greyish combination. The fresh flukes collected from buffalo were overtly thicker and larger than those collected from cattle. By finger touching, the tegument of *F. gigantica* collected from buffalo felt tough, whereas *F. gigantica* collected from cattle felt soft. Since the tegument of the flukes collected from buffalo was stout and thick, the number of marginal folds present in the whole body was very less. Table 1 represents the details of colour and general characteristics of adult *Fasciola* flukes collected from cattle and

Table 1. Morphological characteristics of adult *Fasciola* flukes collected from cattle and buffalo

Characteristics	*Fasciola gigantica* in cattle	*Fasciola gigantica* in buffalo
Colour	Reddish brown with blood-tinged soon after collection from the liver, which soon turns whitish after being washed in PBS. Later dark brown in 70% alcohol.	Golden brown with blood-tinged after immediate collection from the liver. Later turns yellowish-grey in PBS. Finally, grey and greyish in 70% alcohol.
Shape	Leaf shaped	Leaf shaped
Size	Smaller	Larger
Length	Smaller	Larger
Breadth	Smaller	Larger
Tegument	Thinner	Thicker
Ventral sucker	Elevated	Not elevated

Table 2. Morphometric characteristics of adult *F. gigantica* isolated from cattle and buffalo

Fresh worms	CattleN=10	BuffaloN=10	t- Test (p-value)
Body weight	81-135	137.6-165.2	P= 0.007
Range Mean (±S.D.)	102.8 (±16.78)	147.51(±8.16)	
Fluke length	20.4-29.43	32.5-44.5	
Range Mean (±S.D.)	25.22 (±3.15)	37 (±3.74)	
Fluke breadth	7.12-10.92	14-17.5	
Range Mean (±S.D.)	8.31 (±1.18)	15.95 (±1.23)	
Cephalic cone length	1.91-3.01	4-6.5	
Range Mean (±S.D..)	2.46 (±0.41)	5.52 (±0.83)	
Cephalic cone breadth	2.1-5.0	6-7	
Range Mean (±S.D.)	3.49 (±0.76)	6.43 (±0.40)	
Testes length	8.2-11.98	13-19	
Range Mean (±S.D.)	10.26 (±1.07)	15.5 (±2.33)	
BL/BW	2.51-3.70	1.91-2.96	
Range Mean (±S.D.)	3.02 (±0.37)	2.33 (±0.33)	
Ventral sucker to posterior end	16.75-25.93	28.1-40.7	
Range Mean (±S.D.)	21.63 (±2.87)	33.35 (±3.96)	

buffalo.

The morphometric criteria taken up in this present study consisted of eight parameters like body weight, body length, body width, cephalic cone length, cephalic cone breadth, testes length and body length/body width ratio and ventral sucker to posterior end etc., which are very much suitable for getting intra-species variations of *Fasciola* sp. in between two hosts. The morphometric characteristics of *F. Gigantic* isolated from cattle and buffalo are represented in Table 2.

The morphometric data of several parameters differ significantly. Analysis of morphometric criteria with t-test showed that the difference between the body weight, body length, body width, cephalic cone length, cephalic cone width, testes length, body length by body weight ratio, and the distance between ventral sucker to posterior end in between the two hosts were highly significant statistically ($P<0.01$). While analysing the data of body length, higher value was found in case of *F. gigantica* collected from buffalo than the value recorded from the cattle. The mean value of body weight (147.51±8.16), fluke length (37±3.74), fluke breadth (15.95±1.23), cephalic cone length (5.52±0.83), cephalic cone breadth (6.43±0.40), testes length (15.5±2.33), body length/ body width ratio (2.33±0.33) and ventral sucker to posterior end (33.35±3.96) was higher in buffalo than the mean value of body weight (102.8±16.78), fluke length (25.22±3.15), fluke breadth (8.31±1.18), cephalic cone length (2.46±0.41), cephalic cone breadth (3.49±0.76), testes length (10.26±1.07), body length/ body width ratio (3.02±0.37) and ventral sucker to posterior end (21.63±2.87) in cattle.

The present study primarily focused on ovarian branches and testicular branches for detailed morpho-anatomy. While analysing the detailed ovarian branches, smaller, thin and light dense branches were observed in cattle (Fig. 4a), while they were thick and stout and highly dense in buffalo (Fig. 4b). While analysing the testicular branches of *F. gigantica*, they were light sinuous, simple, and minutely dense in cattle (Fig. 4c), whereas they were thick, extremely sinuous, highly dense and more complicated in buffalo (Fig. 4d).

The histological study is mainly focused on the study of tegumental spine, shape of tegumental syncytium and the gut region (Prasetya *et al*., 2019; Fadavi *et al*., 2020). Tegumental spines were highly pointed and long in cattle (Fig. 5a), whereas they were less

Intra-specific morphological variation of *F. gigantica* from two different host

Fig. 1. Fresh adult *Fasciola* flukes collected from the infected liver and bile ducts of naturally infected cattle (A) & buffalo (B). C&D- represents adult *Fasciola* flukes of cattle and buffalo. Complete *Fasciola* flukes having grey and greyish appearance in buffalo and grey and white appearance in cattle preserved in 70% alcohol. E- showing highly elevated ventral sucker area of adult *Fasciola* flukes collected from cattle (right) & elongated in buffalo (left). F- scale bar showing adult *Fasciola* flukes collected from cattle & buffalo

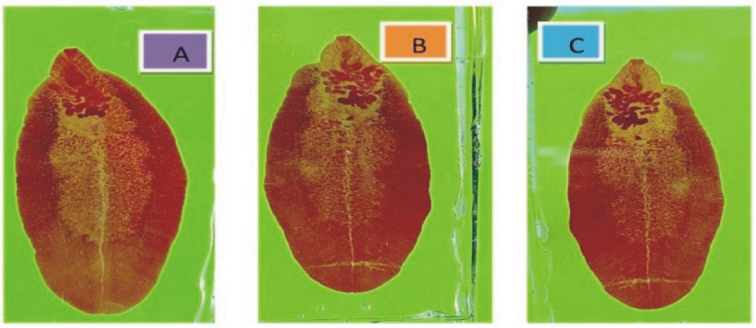

Fig. 2. Borax carmine stained showing whole mounts of adult *F. gigantica* giving light coloured appearance under low stage illumination isolated from cattle & identified by detailed morphoanatomy

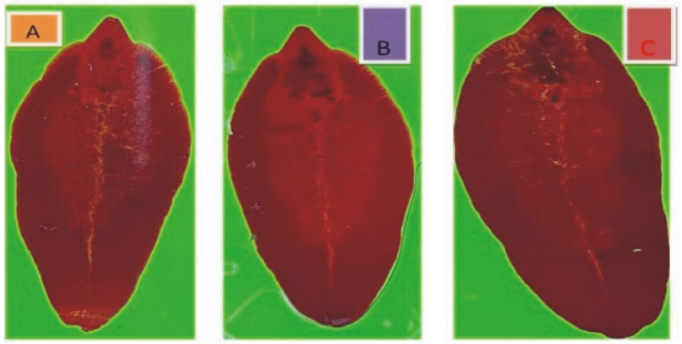

Fig. 3. Borax carmine stained showing whole mounts of adult *F. gigantica* giving dark coloured appearance under low stage illumination isolated from buffalo & identified by detailed morphoanatomy

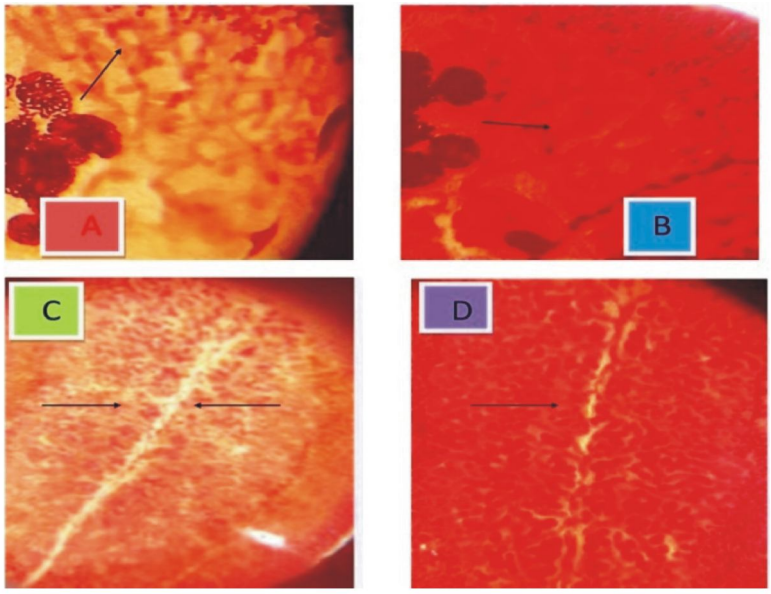

Fig. 4. Borax carmine stained showing detailed morphoanatomic differences between the internal organs of adult *F. gigantica* isolated from cattle and buffalo. a& b: ovarian branches of cattle & buffalo. c& d: testicular branches

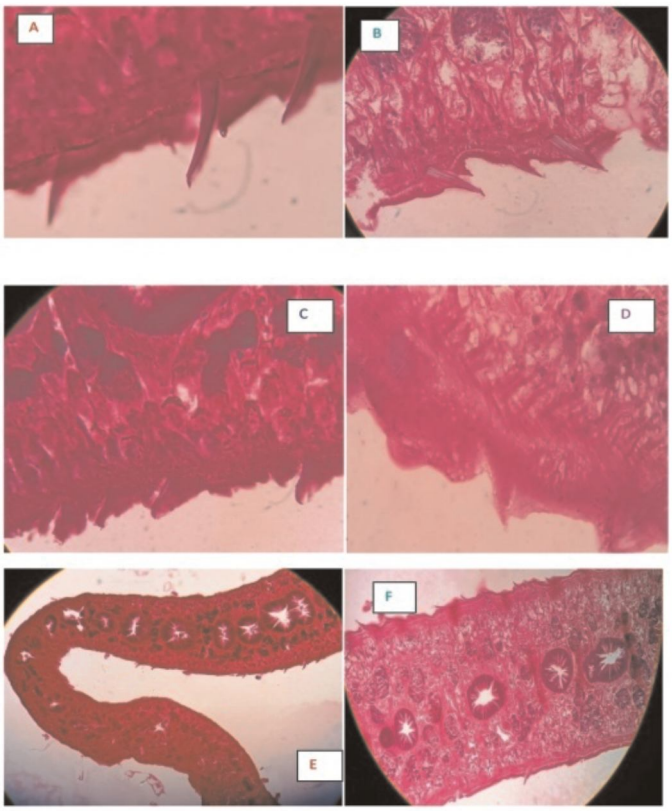

Fig. 5. A)- Tegumental spine in cattle, B)- buffalo, C)- Tegumental syncytium in *F. gigantica* of cattle, D)- *F. gigantica* of buffalo, E)- simple & uniform gut in *F. gigantica* of cattle, F)- highly complicated gut in *F. gigantica* of buffalo

pointed, thick and stout in buffalo (Fig. 5b). Tegumental syncytiums were stout, thicker, and more striated at their roots in flukes isolated from buffalo (Fig. 5c), whereas they were thin, long, pointed and less striated at their roots in flukes isolated from cattle (Fig. 5d). The gut area was highly striated and ellipsoidal shaped in flukes collected from buffalo (Fig. 5e), whereas they were elongated and uniform in flukes collected from cattle (Fig. 5f).

DISCUSSION

While scrutinizing available literatures on morphological features of *Fasciola* sp., it surfaced out that most of the studies were related to morphological differences between *F. gigantica* and *F. hepatica,* which indicates inter-specific morphological differences (Shahbazi *et al*., 2011). Our finding is very interesting as the study reveals intra-specific morphological variation of *F. gigantica* of cattle and buffalo origin. Our sincere search failed to find a single literature on intra-specific morphological variation of *F. gigantica* induced by different host's internal environments. The reason for this intra-specific morphologic variation might be the host's internal environment, host's immunity, long time host-parasite co-evolution, ecological factors etc. In this regard, the present finding bears worth to communicate large audience for further research.

Different parameters, i.e., body weight, body length, body width, cephalic cone length, cephalic cone width, testes length, body length by body weight ratio and the distance between ventral sucker to posterior end etc., were used for the morphometric study of *Fasciola* species by different workers (Periago *et al*., 2006; Shafiei *et al*., 2014). In the present study, we also used those parameters for the morphological study of *F. gigantica* of cattle and buffalo origin. The results of these parameters overtly indicate the morphological difference of *F. gigantica* of different host origins.

In conclusion, by comparing the morphologic, morpho-anatomic and histologic criteria of the flukes isolated from cattle and buffalo, it is concluded that there is a significant intra-specific variation of *F. gigantica* of two host origins (cattle and buffalo).

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: SD, SCM, SB, RJ, SP, SB, SNJ: Planning and designing of the experiment; SCM, SD: Final shape of the manuscript; SD, SD, Subham D: Sample collection; SD, AD: Staining of flukes; RM, NC: Histology.

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