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STUDIES ON THE LIVER FLUKE FASCIOLA IN EGYPT: I- MORPHOLOGICAL AND MORPHOMETRICAL STUDIES

By

AMEEN A. ASHOUR[1], ZAKIYA ESSA[1], ABEER A. KHALIL[2] AND ELHAM A. N. EL SHERIF[3]

Departments of Zoology, Faculties of Science, Ain Shams[1] and Al Azhar[2] Universities, and Department of Parasitology, Faculty of Medicine[3], Ain Shams University, Cairo 11566, Egypt.

ABSTRACT

A comparative scanning electron microscopy and morphometrical study of the two geographically isolated species of *Fasciola* (*F. heptica* European isolate and *F. gigantica* from Egypt) were studied in order to clearify their genetic relationships and specific identification. Although the present study has revealed that most of the diagnostic morphological and morphometrical criteria in the two species are highly variable, the position of the ventral sucker relative to the whole body length produced the most significant differentiating criterion, in addition to the presence of markedly larger tegumental papillae on the ventral surface of *F. gigiantica*.

INTRODUCTION

The liver fluke *Fasciola* has long been reported to occur in Egypt. Curry et al. (1979) found this parasite in histological section from the liver of an Egyptian mummy. The first published record of *Fasciola* in Egypt goes back to 1896 when Looss reported what he considered a variety of *Fasciola hepatica (F. h. aegyptiaca)* from Egyptian cattle. Jockson (1921) demonstrated that Looss's materials are in fact *F. gigantica* which was first described from a

giraffe by Cobbold (1855). The genus *Fasciola* claimed to consist of three to six species infecting a wide range of hosts (Reddy and Subramanyam, 1973). Alicata (1938) reported that *F. gigantica* has a wide range of hosts since it was recovered from the following hosts: Buffalo, Cattle, giraffe, goat, man, sheep, zebra, horse, Guinea pigs and rabbits. Recent reports showed that *Fasciola gigantica* is wide spread in Egyptian ruminants along the Nile Valley (Gohar 1934 - 1935), Soliman and Zaki (1964) and Kendall (1974). *F. hepatica* has also been reported to be present in Egypt, but only in imported animals (Nagaty 1942; Soliman and Farid, 1960; Selim et al., 1970).

Reports on human infection with Fasciola has increased recently in Egypt (El-Gawabi et al., 1993; Ragab and Farag, 1978; Farag et al., 1979; El-Shazly et al., 1991; Farag et al., 1979; El-Shazly et al., 1993; Salem et al., 1993; Hassan et al., 1995, Osman et al., 1995 and Haridy et al., 1998). Cases of ectopic infection with *F. hepatica* was also recorded in human from Puerto Rico (Bendezu et al., 1982) and from Korea (Cho et al., 1994) on the other hand, El-Azazy and Schillhorn (1983) reported the absence of any record of *F. hepatica* from Egyptian animals. Farag et al. (1979) depending only on the egg size, collected from human stools, reported the prevalence of human infection with *Fasciola hepatica* in Egypt. Furthermore cases of human infection with both *Fasciola hepatica* and *F. gigantica* claimed to be reported from Egyptian patients (Azab, 1997 personal com.), in Southeast Asian Countries such as Japan, Taiwan and The PHilippines (Kendall, 1965), and Korea (Chu and Kim, 1967). At the extremes of this morphological range, some resemble *Fasciola hepatica,* whereas other resemble *Fasciola gigantica,* with an intermediate form also occurring. These different forms indicated the necessity of applying other techniques, in addition to the morphology, as approach to the taxonomy of *Fasicola* species. Lee and Zimmerman (1993) used a technique involving isoelectric focusing (IEF) of the whole- body protein extract of the two species *F. hepatica* and *F. gigantica* to distinguish one species from the other. Speciation within the genus *Fasciola* has been so for primarily based on the morphological characteristics of the flukes and their egg size (Alicata, 1938; Varma, 1953; Haiba and Selim, 1960 and Kendall, 1965) because these morphological characteristics are highly variable, a wide discrepancy in speciation of *Fasciola* flukes existed. In fact the criteria used for identification of the flukes are considered by several

authors unreliable. The final size of most adult digenetic trematodes is influenced by several factors, such as the age of the flukes, physiology and species of hosts and intensity of infection. Reddy and Subramanyam (1973) considered the profound variation in the elongated shape of *F. hepatica* from cows in contrast to the oval of that from sheep to be due to adaptive factors acting in the final host. Kendall (1965) reported that *F. gigantica* could be distinguished by possessing the two sides of the body parallel, the shoulders less well developed and the cephalic cone relatively shorter and caeca more branched than in *F. hepatica*. It must be mentioned that Caseby et al. (1995) used a technique involving atomic absorption spectrophotometry, to compare the ionic composition of the Bovine and Ovine biles and the flukes recovered from them and they concluded that, liver flukes from different hosts are not identical and this factor may need to be taken into account in assessment of the results of physiological and morphological studies on *F. hepatica*. So, it is clear that, the taxonomic status of Fasciola has never been discussed in depth, and the taxonomic identity of the Egyptian species of *Fasciola* is still controversial despite numerous studies. It is attepted in this work to through more light on this important issue, by defining the structural details of the flukes from different hosts. Since it is not also exactly known whther the Egyptian populations of *Fasciola* belong to a single species or several species, the present work aimed to study the morphological and anatomical variations among flukes from different Egyptian hosts to provide a detailed study of the higherto undescribed structures of this trematode, and to compare the characteristics of the Egyptian populations with those of the European origin.

The pressent is a part of a whole comparative study including morphological, anatomical and chemical characteriation of the Egyptian liver flukes.

MATERIALS AND METHODS

Adult specimens of *Fasciola* were collected from both Egyptian and European animals slaughtered at Cairo abattoirs and Giza Circus. The examined hosts included the cows *(Bos taurus)*, the buffaloes *(Bubalus bubalis)*, the sheep *(Ovis aries)* and the donkeys *(Equus asinus)*. Adult flukes

wee found in the bile ducts. After the removal of the flukes from the bile duct they were put in normal saline solution (0.75% NaCl).

Whole-Mount Preparations: The flukes were fixed in a hot mixture of 70% alcohol, and 10% formaldehyde for 24 hours, while they are pressed and stranded between 2 glass slides then stained in Gower's carmine for overnight, dehydrated in graded series of ethanol for 15 minutes, for each concentration, cleared in clove oil for 24 hours and mounted in D P X or Canada balsam. Drawings were made to scale using a camera lucida and a slide micrometer. Measurements of regular-shaped organisms were made using an ocular micrometer calibrated against a stage micrometer. All measurements are in millimeters. For scanning electron microscopy, adult flukes, were washed several times in saline (0.75%) before being fixed in 10% formaldehyde solution for 24 hours, dehydrated in graded series of ethanol and transferred into ethanol-amyl acetate (3:1), (1:1), (1:2) then 100% amyl acetate, specimens were dried in a carbon dioxide critical point dryer, coated with gold/ palladium and observed through a Jeol scanning electron microscope.

RESULTS AND DISCUSSION

The fresh live flukes of Egyptian *Fasciola* exhibit a grayish buff to brown fleshy colour, while the European flukes appear rather pale grayish and more transparent. Light and scanning electron microscope investigations of the flukes showed that both adult Egyptian and European *Fasciola* have flat and leaf-like bodies (Fig. 1,a,b,c,d and Fig. 2). The body possesses two lateral straight edges, a distinct cephalic cone gives a characteristic shouldered appearance, this cone, while it is more distinct and relatively shorter in European flukes, it is less prominent in Egyptian ones as their body gradually tapers toward the anterior extremity (figs. 3, 4). In both species of the fluke the oral sucker is directed anteroventrally, which is small and runded with a thick muscular wall (Figs. 3,4,5,6). The body is covered with well developed, posteriorly directed tegumental spines. Contact receptors were visible surrounding the oral sucker (Figs. 5,6). Several small papillae were also seen on the ventral tegumental surface between the spines. These papillae are characteristically more frequent and distinct in the Egyptian flukes comapred with those of the European ones (Figs. 3,4). They probably serve a

a
b
c
d
e
Egyptian
Buffalo
Egyptain
Cow
Egyptian
Sheep
Egyptian
Donkey
European
Cow
1
2

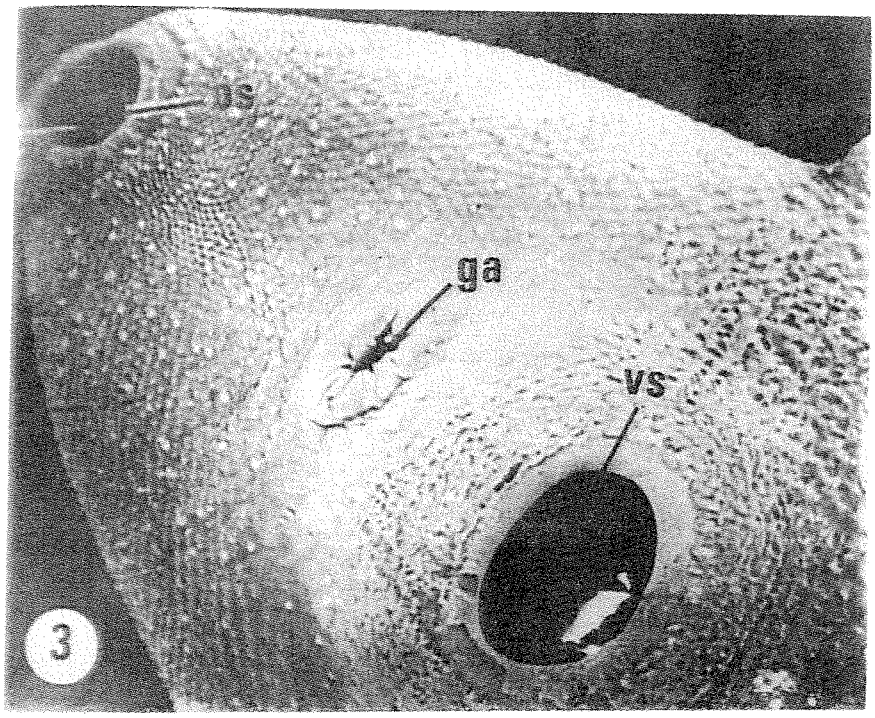

Fig. 3: S.E. micrograph of anterior region of adult Egyptian *Fasciola*, showing genital atria (ga), oral sucker (os), ventral sucker (vs). (X 160)

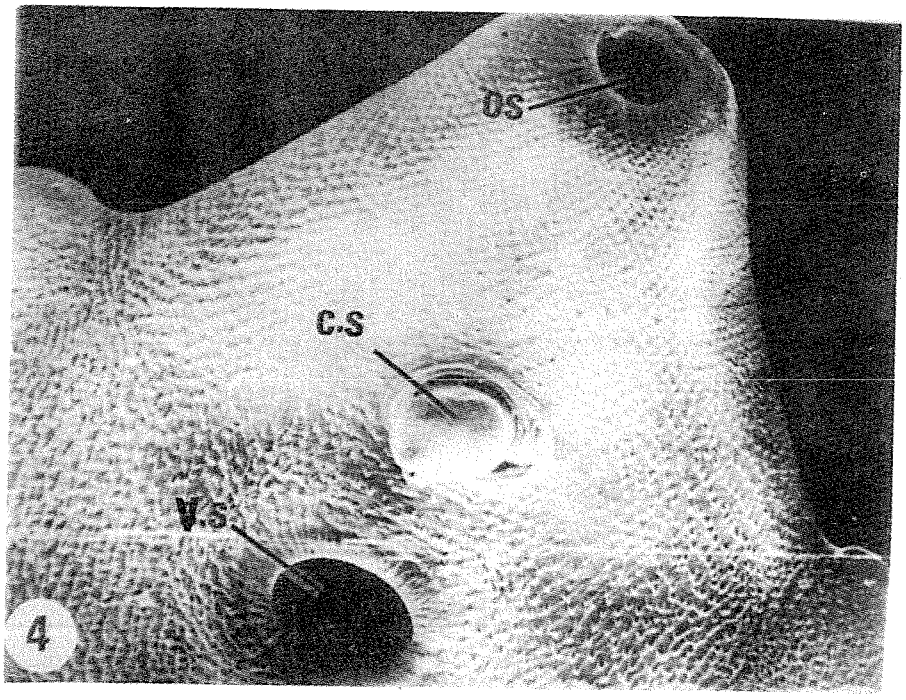

Fig. 4: S.E. micrograph of anterior region of adult Egyptian *Fasciola*, showing cirrus sac (c.s), oral sucker (os), ventral sucker (vs). (X 160)

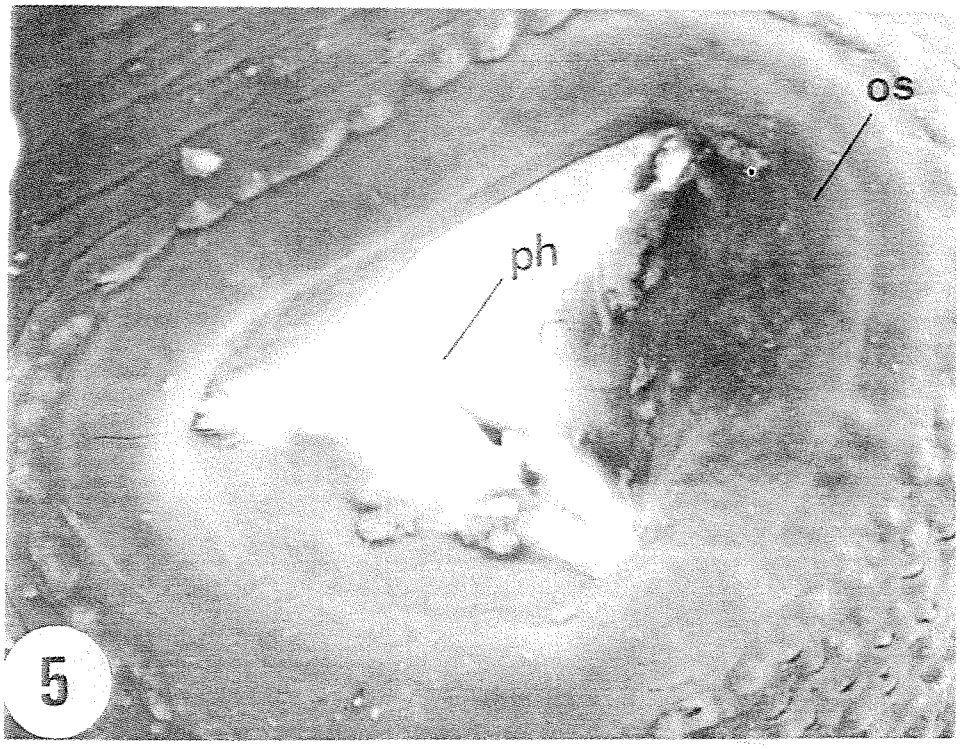

Fig. 5: S.E. micrograph of oral sucker of adult Egyptian *Fasciola* showing: oral sucker (os), pharynx (ph) protruding inside oral sucker. (X 280)

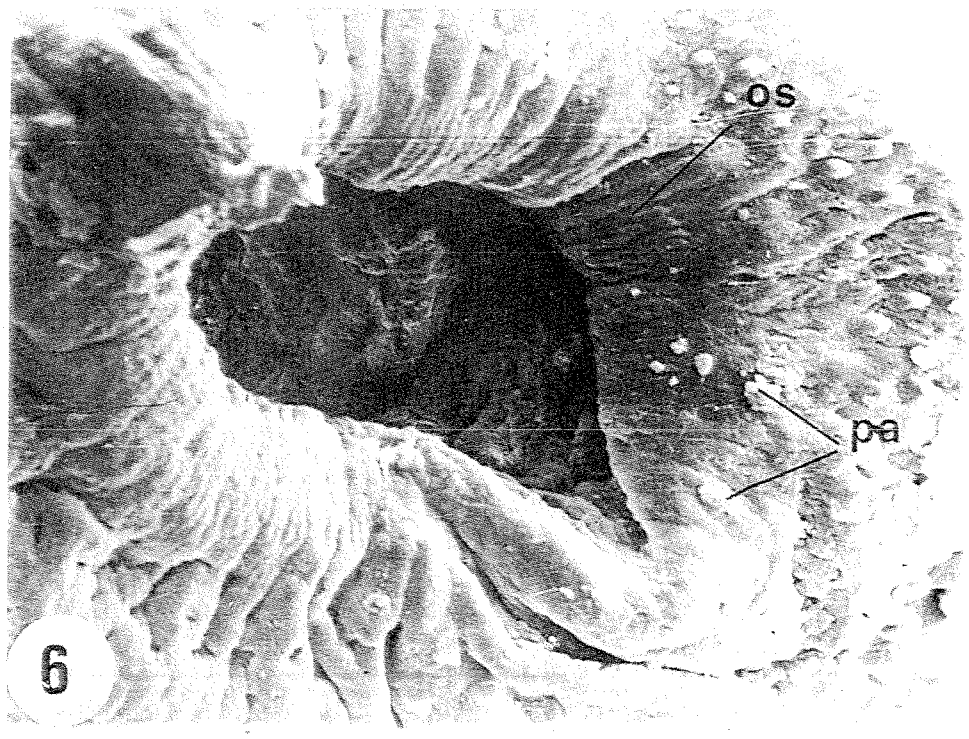

Fig. 6: S.E. micrograph of region of the oral sucker of adult European *Fasciola* showing, oral sucker (os), sensory papillae (pa) on dorsal lip of oral sucker. (X 200)

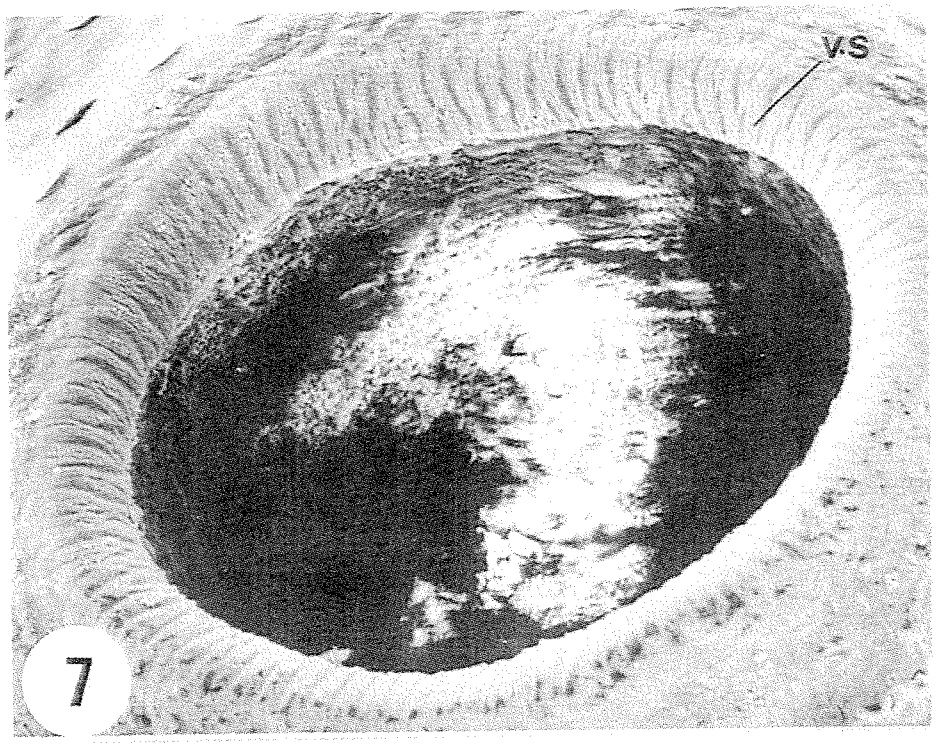

Fig. 7: S.E. micrograph of ventral sucker (vs) of adult Egyptian *Fasciola*. (X 560)

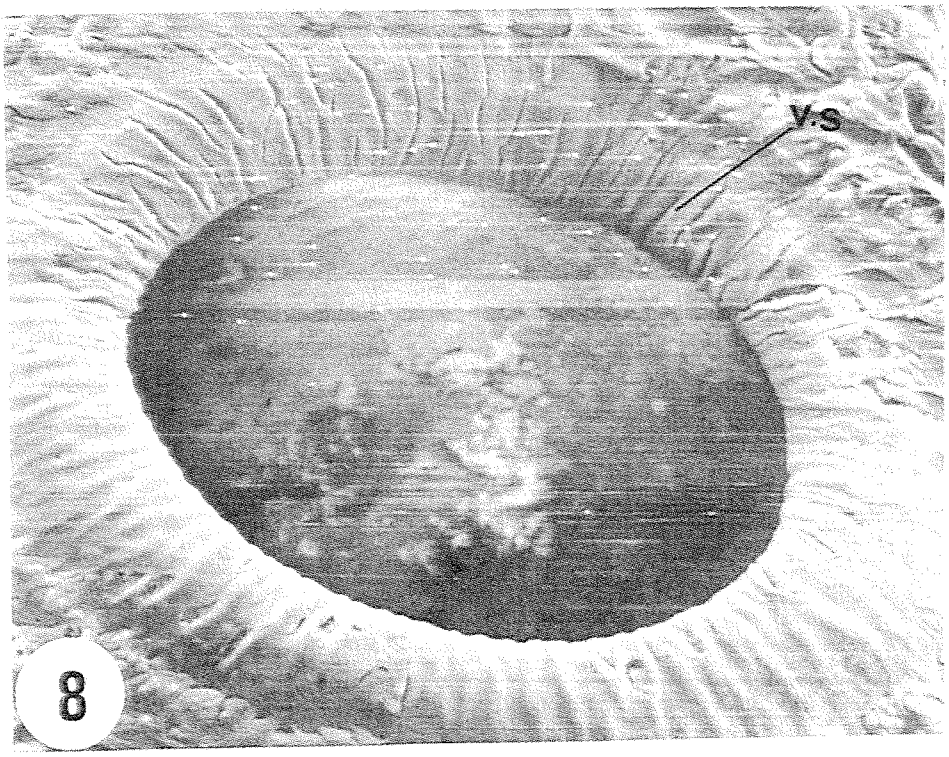

Fig. 8: S.E. micrograph of ventral sucker (vs) of adult European *Fasciola*. (X 560)

sensory function. The possible functions of the tegumental papillae was discussed by Ashour (1995). The acetabulum in both adult Egyptian and European flukes is quite rounded in shape and is provided with thick muscular wall (Figs. 7,8). It lies at about the level of the shoulders (Figs, 3,4), and is more anterior in position in Egyptian flukes (Table 3).

In both Egyptian and European flukes the ventral sucker is some what larger than the oral sucker (Figs, 3,4). However, in Egyptian flukes it is slightly larger relative to the body length (Figs. 7, 8), (table 2). On the ventral surface and between the two suckers lies the genital atrium (Figs, 2,3 & 4) which contains the muscular cirrus of the male and the female genital opening. At the most posterior point of the body the excretory pore is located medially. The eggs of both adult Egyptian and European *Fasciola,* collected from adult flukes obtained from different hosts, have a more or less similar morphology and all of them have a nearly similar colour (light gold brown), unioperculum, oval, egg shaped, and finely or coarsely knobbed broader opposite pole.

LEGENDS FOR FIGURES

Fig. 9: S.E. micrograph of ventral surface anterior to ventral sucker of adult Egyptian *Fasciola* showing division of spine tips (di). (X 5620)

Fig. 10: S.E. micrograph of anterior ventral surface of adult European *Fasciola* showing ventral sucker (vs), invaginated genital atrium (ga) and spines (sp) anterior to ventral sucker. (X 375)

Fig. 11: S.E. micrograph of the spines on ventral surface posterior to ventral sucker of Egyptian *Fasciola* showing division of spine tips, (di); spines (sp), sensory papillae, (pa) and transverse fold of tegument due to body flexure, (tf). (X 3750)

Fig. 12: S.E. micrograph of spines on ventral surface posterior to ventral sucker of adult European *Fasciola* showing, division of spine tips, (di); spines, (sp); transverse fold, (tf) of the tegument due to body flexure. (X2810)

pa
sp
10

sp
tf
12

de
9

de
sp
pa
11

The overall body size of the adult Egyptian populations of the liver flukes, obtained from all hosts, was found to be conspicuously large compared to that of the European flukes (Table 1). In Egyptian populations the mean body size was (3.95 cm x 1.22 cm) while in European populations the mean body size was distinctly smaller (2.20 x 1.08 cm). Differences in body size were also noticed among adult Egyptian flukes collected from different hosts (Table 1). In Egyptian populations the mean body size was (4.77 x 1.13 cm) in flukes from cow, (3.39 x 1.01 cm) in flukes from sheep, (4.08 x 1.3 cm) in flukes from buffalo and (3.59 x 1.47 cm) in flukes from donkey. Oral sucker measures (1.06 x 0.72 mm) in Egyptian flukes while it measures only (0.96 x 0.58 mm) in European ones. In the Egyptian flukes, the acetabulum is distinctly larger in diameter (1.23 - 1.84 mm) than that of the European ones (1.0 - 1.1 mm) (table 2). One of the most important morphological charateristics that can differentiate between the Egyptian and the European forms of *Fasciola* is the relative location of the ventral sucker to the whole body length. While this figure is small in Egyptian populations (only 0.07 to, 0.11 mm), it is large in the European ones (0.13) i.e. the acetabulum is more anteriorly situated in the Egyptian populations of *Fasciola* (table 3). On the other hand, the measurements of the egg-size of both Egyptian and European flukes were rather overlapped and did not show a significant variation between the two populations, the egg-sizes of Egyptian flukes were found to be (0.12 - 0.14 mm) x (0.06 - 0.08 mm) and those of the European flukes (0.12mm x 0.07 mm) (Table 4).

It is quite clear that the Egyptian populations of *Fasciola* flukes from hosts are highly-polymorphic (table 1), and vary morphologically depending upon the host being parasitized. The fact that *Fasciola* flukes are highly polymorphic was confirmed by Stunkard (1957); Haiba and Selim (1960) and Sazanov (1973). Stunkard (1957) reported that specimens from different hosts manifest differences far greater than those usually employed to distinguish beteen species. Haiba and Selim (1960) made a comparative study on the morphological status of the liver flukes from Egyptian buffaloes, cows and sheep, and they recorded that the Egyptian flukes displayed a quite clear environmental size increase conspicuous enough to separate these flukes from other *Fasciola* recorded in other countries. The present description of the *Fasciola* from Egyptian cows, sheep and buffaloes significantly differs from

Table (I) : Comparative measurements of adult European and Egyptian *Fasciola* collected from different hosts) .

Animal	Body length	Body width
European cow n = 50	2.20 (1.4-3.2) # 0.52 cm SE = 0.07	1.08 (0.7-1.3) # 0.21 cm & SE = 0.03
Egyptian cow n = 35	4.77 (3.8-6.5) # 0.72 cm SE = 0.12	1.13 (1.0-1.4) # 0.11 cm & SE = 0.01
Egyptian sheep n = 80	3.39 (2.0-5.1) # 0.87 cm SE = 0.09	1.01 (2.7-1.3) # 0.22 cm & SE = 0.02
Egyptian buffalo n =6	4.08 (3.0-4.7) # 0.86 cm SE = 0.35	1.3 (1.3-1.5) # 0.08 cm & SE = 0.03
Donkey n = 10	3.59 (2.9-4.6) # 0.31 cm & SE = 0.11	1.47 (1.0-1.6) # 0.23 cm & SE = 0.07

n = the number of flukes

Table (II) : Measurements of the acetabulum of adult European and Egyptian *Fasciola* collected from different hosts.

Animal	The acetabulum length	The acetabulum width
European cow n = 20	1.0 (0.72-1.48) # 0.20 mm SE = 0.04	1.1 (0.72-1.35) # 0.20 mm & SE = 0.04
Egyptian cow n = 20	1.81 (1.57-2.11) # 0.14 mm SE = 0.03	1.84 (1.48-2.02) # 0.17 mm & SE = 0.03
Egyptian sheep n = 20	1.79 (1.57-2.25) # 0.18 mm SE = 0.04	1.78 (1.57-2.02) # 0.16 mm & SE = 0.03
Egyptian buffalo n =6	1.68 (1.4-1.89) # 0.18 mm SE = 0.07	1.67 (1.4-1.89) # 0.21 mm & SE = 0.08
Donkey n = 10	1.31 (1.12-1.62) # 0.14 mm & SE = 0.04	1.23 (1.12-1.80) # 0.44 mm & SE = 0.1

Table (III) : Measurements of Anterior end of body to middle of (v.s) mm, and anterior end of body to middle of v.s/length of body

Animal	Anterior end of body to middle of (v.s) mm	Anterior end of body to middle of (v.s) mm/length of body (mm)
European cow n = 20	2.26 (2.25-4.2) # 0.41 mm SE = 0.09	0.13 (0.1-0.2) # 0.28 mm & SE = 0.006
Egyptian cow n =20	3.66 (3.15-4.27) # 0.32 mm SE = 0.07	0.07 (0.06-0.1) # 0.01 mm & SE = 0.002
Egyptian sheep n = 20	3.75 (3.15-4.5) # 044 mm SE = 0.09	0.08 (0.07-0.1) # 0.01 & SE = 0.004
Egyptian buffalo n =6	4.56 (4.05-4.95) # 0.30 mm SE = 0.12	0.11 (0.09-0.15) # 0.02 mm & SE = 0.008
Donkey n = 10	4.2 (3.37-6.7) # 0.89 mm SE = 0.28	0.11 (0.08-0.16) # 0.02 mm & SE = 0.007

Table (IV) : Measurements of the eggs of adult Egyptian and European *Fasciola*

Animal	Length of eggs	Width of eggs
European cow n = 100	0.12 (0.10-0.16) # 0.01 mm SE = 0.001	0.07(0.06-0.08)#0.009mm& SE = 0.001
Egyptian cow n =50	0.14 (0.12-0.18) # 0.01 mm SE = 0.001	0.08(0.07-0.09)#0.008mm& SE = 0.001
Egyptian sheep n = 100	0.14 (0.12-0.16) # 0.01 mm SE = 0.001	0.08(0.08-0.09)#0.007mm SE = 0.0007
Egyptian buffalo n =50	0.12 (0.12-0.14) # 0.007 mm SE = 0.001	0.06(0.06-0.08)#0.006mm SE = 0.0009
Donkey n = 50	0.12 (0.11-0.16) # 0.01 mm SE = 0.002	0.066(0.04-0.08)#0.01mm& SE = 0.001

the descriptions given by Haiba and Selim (1960). Where the present specimens showed a larger body size (Table 5).

In fact, the present measurements of the liver fluke, to a large extent, agree with those given by Railliet (1895); Looss (1896); Kendall (1965); Alicata and Swanson (1937) and Li, et al. (1988) concerning the body length while it is slightly larger than those given by Railliet (1895) and Looss (1896) concerning the body width (Table 5).

In the present specimen, the body length is slightly larger than those given by Varma (1953) for *Fasciola indica* (Table 5). Table 6 gives a comparison between egg measurements of the adult flukes from hosts from different geographical locations. Linnaeus (1758); Cobbold (1855) thomas (1883); Railliet (1895) and Farag et al. (1979), gave more or less variable egg measurements, which are nearly similar to those reported in the present study, while those of Alicata (1938) and Varma (1953) appear to be slightly wider (Table 6).

The egg measurements of Fasciola flukes from different geographical locations, as given by Cobbold (1895), Looss (1896), Alicata, (1938) and Li, et al. (1988) respectively from Africa, Egypt, Hawaii and China were more or less similar (Table 6). The present egg measurements were confined to all the previous measurements from different hosts and different countries. However, the present egg size is larger than those given by Abdel Ghani (1955) and Haiba and Selim (1960).

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Table (V) : Comparative measurements of adult liver flukes collected from different hosts as given by various authors.

	Authors	Species	Adult body length of the liver fluke	Adult body width of the liver fluke
1	Railliet (1895)	*F. hepatica var. angusta* from Senegal	2.6-3.8 cm	0.6-0.8 cm
2	Looss (1896)	*F. hepatica var. aegyptiaca* fromEgypt	2.5-3.1 cm	0.6-0.75 cm
3	Alicata & Swanson (1937)	*F. giganica* from Hawaii	4.0-5.0 cm	----
4	Varma (1953)	*F. indica* from Indian buffalo	2.0 cm	1.1 cm
5	Haiba & Selim (1960)	Based on materials collected from Egyptian hosts: a) Egyptian buffalo b) Egypt. cow c) Egypt sheep	 3.18 cm* 3.03 cm* 3.60 cm*	 0.75 cm* 0.69 cm* 0.98 cm*
6	Kendall (1965)	*F. hepatica* *F. gigantica*	1.8-5.1 cm* 2.5-7.5 cm*	0.4-1.3 cm* 0.3-1.2 cm*
7	Pike & Condy (1966)	*F. tragelaphi sp. nov.* from cho be swamps in Rhodesia	11.4* (10.2-12.0 cm)	0.66* (0.6-0.7 cm)
8	Li et al. (1988)	*F. hepatica* from China	2.88 cm	1.06 cm
9	Farag & El-Sayad (1995)	*F. gigantica* based of experimental infection rabbits	1.56 (1.3-1.8 cm) # 0.13 cm	----

* The mean

Table (VI) : Comparative measurements of the liver fluke eggs as given by various authors.

	Authors	Species	The eggs length	The eggs width
1	Linnaeus (1758)	*F. hepatica* from Europe	0.133 (0.125-0.155 mm)# 0.009	0.079 (0.070-0.088 mm)# 0.004
2	Cobbold (1855)	*F. gigantiaca* from Africa	0.166* (0.150-0.190#0.011mm	---
3	Thomas (1883)	*F. hepatica*	0.105 - 0.145 mm	0.066 - 0.09 mm
4	Railliet (1895)	*F. hepatica*	0.147* (0.143-0.151mm)	0.082* (0.082-0.088mm)
5	Looss (1896)	*F. h. var aegyptaca* from Egypt	0.150-0.190mm	0.075-0.09mm
6	Alicata (1938)	*F. hepatica*	0.013-0.14mm	0.075-0.09mm
		F. gigantica from Hawaii	0.156-0.197mm	0.090-0.104mm
7	Varma (1953)	*F. indica* from India	0.155* (0.140-0.165mm # 0.007	0.103* (0.095-0.112mm # 0.004
8	Abdel Ghani. (1955)	*F. gigantica* from Egyptian buffalo	0.143 - 0.187mm	0.065 - 0.130mm
9	Haiba & Selim (1960)	Based on materials collected from Egyptian hosts:		
		a) *F.* from Egyptian buffalo	0.154* mm	0.083* mm
		b) *F.* from Egypt. cow	0.150* mm	0.073* mm
		c) *F.* from Egypt sheep	0.141* mm	0.082* mm
10	Farag (1979)	*F.* from Egyptian patient	0.128-0,167mm	0.060-0,084mm
		F. from Egyptian cow	0.128- 0.175mm	0.060- 0.100mm
11	Li et al. (1988)	*F.* hepatica from China	0.151* mm	0.079* mm

* The mean

gigantica, the common liver fluke of cattle in Hawaii, and the intermediate host, *Fossaria ollula*, Bull. (**80**), (Hawaii Exp. stn). 1-22.

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