Pakistan J. Zool., vol. 43(6), pp. 1069-1077, 2011.

Phenotypic Analysis of Adult *Fasciola* spp. From Potohar Region of Northern Punjab, Pakistan*

Saira Mufti,[1] ** M. Maqbool Ahmad,[2] Yusuf Zafar[3] and Mazhar Qayyum[1]

[1]*Department of Zoology/Biology, Pir Mehr Ali Shah-Arid Agriculture University, Murree Road, Rawalpindi, Pakistan*

[2]*Department of Biological Sciences, University of Wah, Wah Cantt., Pakistan*

[3]*Agriculture and Biotechnology, Biosciences Division, Pakistan Atomic Energy Commission, Islamabad, Pakistan.*

Abstract.- Fasciolosis is caused by a trematode parasite widely distributed world over. Phenotypic analysis has been used to provide taxonomic position of *Fasciola* spp. This study highlights the taxonomical status of *Fasciola* species infecting cattle and buffalo of Potohar region. Adult gravid flukes were isolated from infected livers of the animals and were stained with Gower's carmine. The phenotypic parameters taken into consideration were BL, BW, VS-Post, VS-Vit indices and BL/BW ratios. The data was subjected to two way ANOVA and cluster analysis by using SPSS 16.1 version. Cluster analysis using hierarchical approach and similarity coefficient indicated that there were no phenotypic differences in *Fasciola* isolates of buffalo and cattle of Potohar and they were parasitized with the same liver fluke species. Results also revealed that there was a close similarity of Iranian *F.gigantica* and *F. intermedia* with that of local isolate. It was found that BL, BW and VS-Post values (mm) of *Fasciola* originating from Potohar region were similar to *Fasciola intermedia* of Iran and Egypt, whereas BL/BW ratio and VS-Vit showed that *Fasciola* isolates of Potohar region resembled with *Fasciola gigantica* of Iran. The genetic characterization of *Fasciola intermedia* using appropriate genetic markers is necessary to further confirm above findings. This study would help in developing indirect ELISA by using *Fasciola intermedia* for early diagnosis of fasciolosis in large ruminants. Furthermore, it would also facilitate to study the sero-epidemiological pattern of fasciolosis in order to develop effective control measures in Potohar region.

Key words: *Fasciola hepatica*, *Fasciola gigantica*, *Fasciola intermedia*, phenotypic analysis, fasciolosis, Potohar Region, Pakistan

INTRODUCTION

Fasciolosis is a trematode borne parasitic disease infecting liver of large ruminants widely prevalent throughout the world. Different *Fasciola* spp. *i.e.*, *Fasciola gigantica*, *Fasciola hepatica* and *Fasciola intermedia* have been characterized based on morphology. Fasciolosis results in economic losses in livestock in the form of mortalities, reduced fertility, abortions, slow growth and reduction of milk and meat production, infected livers and withered carcasses (Phiri *et al.*, 2006). Phenotypic analysis has been used most frequently to study the systematics of fasciolids infecting different vertebrate host species. Morphometrical measurements of adult *Fasciola* originating from different hosts have been investigated in various parts of the globe and regarding this different morphometrical parameters have been used to identify *Fasciola* spp. in Iran and Egypt (Valero *et al.*, 2001a, b; Ashrafi *et al.*, 2006). In current study, twenty morphometrical parameters of differentiation have been taken up. Moreover emphasis was laid on the comparison of five most valuable parameters to analyze phenotypic variations in fasciolids. These phenotypic parameters were; body length (BL), body width (BW), distance between ventral sucker and posterior end (VS-P), distance between the ventral sucker and the union of the vitelline glands (VS-Vit) indices and body length over body width (BL/BW) ratios (Valero *et al.*, 2001a,b; Lotfy *et al.*, 2002; Ashrafi *et al.*, 2006). The morphometrical measurements of *Fasciola* spp reported from Iran and Egypt were taken as reference value to find out the taxonomical status of *Fasciola* spp of bovine in Potohar region of Pakistan.

Previously no such phenotypic analysis was

* This paper is a part of the PhD thesis of first author

** Corresponding author: m_msaira@yahoo.com

0030-9923/2011/0006-1069 $ 8.00/0

carried out to investigate morphological aspects of *Fasciola* spp in Potohar region of northern Punjab, Pakistan. The purpose of present study was to identify *Fasciola* spp. accountable for fasciolosis in the Potohar region, which will facilitate to carry out an epidemiological profile that might be helpful in devising effective control measures. Besides, information gathered will provide taxonomical status of fasciolids established in different geographical regions of Pakistan.

MATERIALS AND METHODS

Study area

The study area lies in the northern part of the Punjab province of Pakistan known as the Potohar region, lying between 32º - 30" N to 34º latitude and from 71º - 45" E to 73º - 45" E longitudes. It is situated at an elevation of 472.2 to 609.6 meter above sea level lying to the south of Pakistan's majestic northern mountain range (Kala Chitta Range and the Margalla Hills); the salt range is present on the southern side whereas in the west is the River Indus and in the east is the River Jhelum (Fig. 1).

Fig. 1. Map of Punjab showing Potohar region.

Adult fluke's collection

The bovines (buffaloes and cattle) were brought from different parts of Potohar region for slaughter at local abattoirs during March 2006 to March 2007. The livers along with gall bladders including the bile duct were incised and examined for the presence of adult gravid flukes. The flukes were removed with the help of rubber coated forceps to avoid any damage. Each worm was washed separately 2-3 times in 0.9 percent saline to remove the debris. *Fasciola* specimens were then transported to the Parasitology laboratory and preserved in 70 percent ethyl alcohol for later phenotypic analysis.

Morphometry

Fluke's staining

In this study 54 adult gravid flukes from 7 cattle and 153 from 12 buffalo were collected and processed for staining. Staining procedure was carried out with Gower's carmine adopted by Bukhary (1988), with slight modifications in dehydration process by considering thickness of fluke specimen.

Morphometric measurement

All morphological measurements of adult flukes taken were based on diagram (Fig. 2) sketched by Periago *et al.* (2006). The stained adult flukes were examined under dissecting microscope and dimensions of the body were assessed using a stereomicroscope/light microscope to measure internal organs. All measurements were taken in millimeters (mm) by the help of filar micrometer (OSM-4, Olympus). Furthermore ratios of body parts were also calculated where ever required.

Biometric parameters

The parameters considered (Fig. 2) for phenotypic analysis were; Length of body (BL), maximum width of body (BW), length of cone (CL), width of cone (CW), diameter of the oral sucker; maximum (OS max), diameter of the oral sucker; minimum (OS min), diameter of the ventral sucker maximum (VS max), diameter of the ventral sucker minimum (VS min), distance between the anterior end of the body and the ventral sucker (A-VS), distance between the oral sucker and the ventral sucker (OS-VS), distance between the ventral sucker and the union of the vitelline glands (VS-Vit),

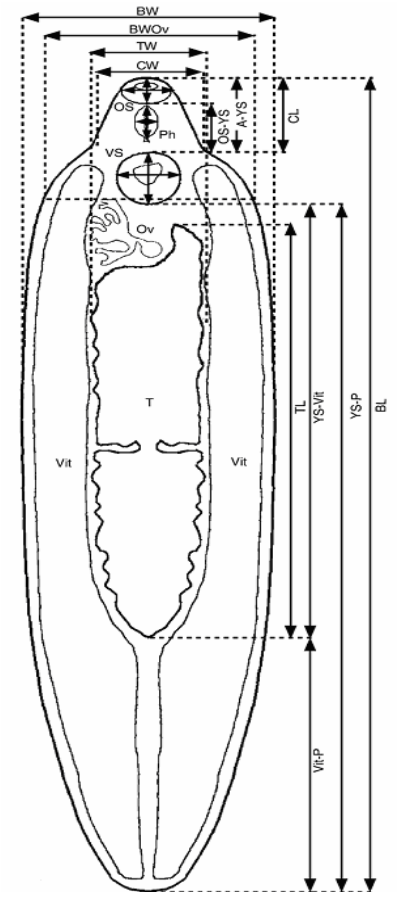


Fig. 2. Standardized measurements in gravid *Fasciola gigantica* (Periago *et al.,* 2006). Length of body (BL), maximum width of body (BW), length of cone (CL), width of cone (CW), diameter of the oral sucker; maximum (OS max), diameter of the oral sucker; minimum (OS min), diameter of the ventral sucker maximum (VS max), diameter of the ventral sucker minimum (VS min), distance between the anterior end of the body and the ventral sucker (A-VS), distance between the oral sucker and the ventral sucker (OS-VS), distance between the ventral sucker and the union of the vitelline glands (VS-Vit), distance between the union of the vitelline glands and the posterior end of the body (Vit -P), distance between the ventral sucker and the posterior end of the body (VS-P), pharynx length (Ph L), pharynx width (Ph W), Body area (BA), oral sucker area (OSA), ventral sucker area (VSA); BA was calculated as the product of BLxBW; OSA and VSA were calculated as the product of their diameters, body length over body width (BL/BW) and oral sucker area over ventral sucker area (OSA/VSA).

Statistical analysis

In the present study a two way ANOVA (5% significance level) was used to find out the difference in morphological parameters. Moreover, Cluster Analysis was also done by using SPSS Version 16.1 to find out the resemblance and relationship between fasciolid species.

RESULTS

Morphometric indices taken up in this study consist of twenty different parameters based on lineal biometric characters, areas and ratios. It was observed that measurements of nine parameters (BL, VS min, VS max, PhW, OS-VS, BA, OSA, VSA, BL/BW) resemble with *F. gigantica*, six parameters (CL, CW, VS-Vit, VS-Post, Vit-Post, OSA/VSA) with *Fasciola* spp and five parameters (BW, OS min, OS max, PhL, A-VS) with *F.hepatica* (Table I). In the present study five useful and most favorably used differentiating morphometrical parameters (BL, BW, BL/BW, VS-Vit, VS-Post) for species differentiation were considered for phenotypic characterization of fasciolids (Valero *et al.*, 2001; Lotfy *et al.,* 2002; Ashrafi *et al*., 2006). These parameters were used for species differentiation (Table II) as coefficient of variation was 21.43% (<0.05). The results revealed that BL, BW values and distance between VS-Posterior of the liver flukes reported in the Potohar region resembles with *F. intermedia* of Egypt and Iran (Figs. 3A-C), whereas the BL/BW ratio and distance between VS-Vit showed the liver fluke of Potohar region resembling with *F. gigantica* of Iran (Figs. 3D,E).

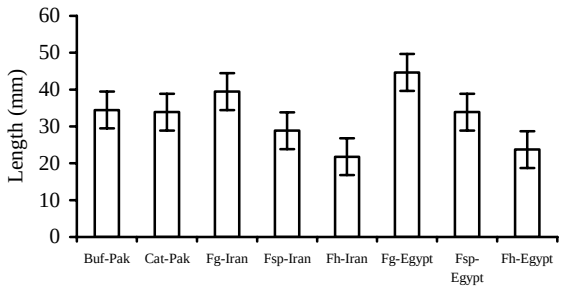
Cluster analysis using the hierarchical approach, was also used to evaluate the relationship

Table I.- Morphometric data (mm) of liver flukes hosted in cattle and buffalo from Potohar (Pakistan) compared with available data of *Fasciola* present in bovines from Iran and Egypt (Periago *et al.*, 2006).

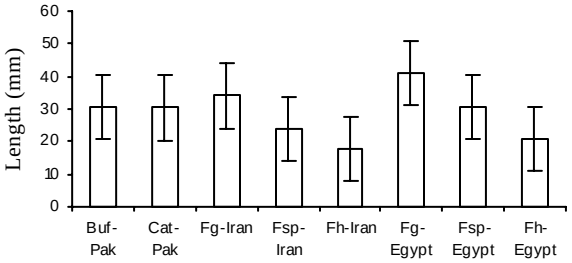
Parameters	*Fasciola s*pp	*Fasciol*a spp	*F.gigantica*	*Fasciola* spp	*F.hepatica*	*F.gigantica*	*Fasciola* spp	*F.hepatica*
Host/Country	BUF-PAK	CAT-PAK	BOV-IRAN	BOV-IRAN	BOV-IRAN	BOV-EGYPT	BOV-EGYPT	BOV-EGYPT
No.	153	54	154	66	121	12	126	82
Body length								
Range	21.2-52.1	20.1-41.3	29.97-62.39	21.46-36.41	11.47–30.02	35.25-48.71	23.46-45.40	15.48–28.71
Mean	34.46±0.51	33.89±0.76	39.45± 0.57	28.84±0.38	21.76 ± 0.40	44.65± 1.15	33.88± 0.34	23.73 ± 0.33
Body width								
Range	4.5-9.1	4.1-8.99	3.49-9.08	3.40-10.55	4.59–11.38	6.27-10,14	5.90-14.04	8.21–14.27
Mean	5.84±0.09	6.01±0.17	5.41± 0.07	6.01±0.15	7.22 ± 0.12	7.77± 0.29	9.70± 0.13	10.54 ± 0.15
Cone length								
Range	2.10-4.27	2.0-3.07	2.63-4.15	2.19-3.69	1.63–3.48	2.61 -3.68	1.68-3.56	1.36–2.98
Mean	2.71±0.05	2.72±0.09	3.21±0.02	2.88±0.04	2.53 ± 0.03	3.16± 0.11	2.62± 0.03	2.23 ± 0.04
Cone width								
Range	2.58-4.29	2.13-3.09	2.73-4.05	2.58-4.13	2.14–4.13	3.25-4.34	2.62-4.59	2.05–3.99
Mean	2.76±0.03	2.78±0.06	3.30± 0.02	3.21±0.03	3.29 ± 0.04	3.81 ±0.10	3.77± 0.03	3.18 ± 0.04
Diameter of oral sucker (Minimum)								
Range	0.36-1.22	0.31-1.12	0.52-1.01	0.49-0.85	0.36–0.88	0.72-0.88	0.47-0.95	0.45–0.89
Mean	0.58±0.00	0.69±0.01	0.75± 0.01	0.69±0.01	0.66 ± 0.01	0.79 ±0.01	0.73± 0.01	0.70 ± 0.01
Diameter of oral sucker (Maximum)								
Range	0.64-1.25	0.61-1.22	0.70-1.03	0.75-1.03	0.65–0.95	0.84-1.05	0.75-1.12	0.69–1.01
Mean	0.82±0.01	0.84±0.02	0.90± 0.01	0.84±0.01	0.83 ± 0.01	0.95± 0.02	0.91± 0.01	0.86 ± 0.01
Diameter of ventral sucker (Minimum)								
Range	1.14-2.04	1.06-2.01	1.14-1.81	0.93-1.57	0.80–1.24	1.26-1.52	0.90-1.47	0.82–1.37
Mean	1.45±0.02	1.52±0.03	1.48± 0.01	1.28±0.02	1.05 ± 0.01	1.43± 0.02	1.12± 0.01	1.04 ± 0.01
Diameter of ventral sucker (Maximum)								
Range	1.15-2.31	1.12-2.10	1.26-1.86	0.95-1.68	0.90–1.26	1.35-1.67	0.97-1.56	0.97–1.49
Mean	1.53±0.02	1.62±0.03	1.55±0.01	1.34±0.03	1.11 ± 0.01	1.53± 0.03	1.22± 0.01	1.14 ± 0.01
Pharynx length								
Range	0.34-1.44	0.31-1.08	0.65-0.98	0.59-0.93	0.46–0.95	0.75-0.94	0.62-1.12	0.58–1.02
Mean	0.66±0.01	0.72±0.03	0.82±0.01	0.77±0.01	0.76 ± 0.01	0.84± 0.02	0.84± 0.01	0.79 ± 0.01
Pharynx width								
Range	0.36-1.14	0.31-1.11	0.36-0.82	0.26-0.62	0.28–0.57	0.45-0.59	0.32-0.60	0.32–0.55
Mean	0.55±0.01	0.58±0.01	0.47±0.004	0.44±0.01	0.40 ± 0.01	0.50± 0.01	0.45± 0.01	0.42 ± 0.01
Distance bw interior end of body and VS								
Range	1.22-3.53	1.12-3.51	1.88-3.38	1.99-3.33	1.70–3.02	2.44-3.39	2.25 4.04	2.01–3.52
Mean	2.35±0.11	2.42±0.19	2.60±0.02	2.49±0.03	2.50 ± 0.03	2.98± 0.08	3.14± 0.03	2.78 ± 0.03

Continued

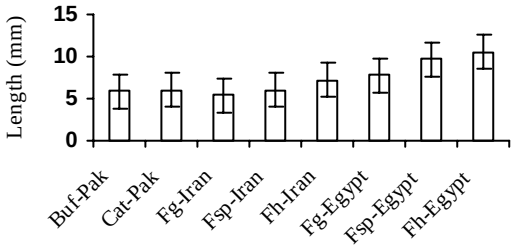
Parameters	***Fasciola s*pp**	***Fasciol*a spp**	***F.gigantica***	***Fasciola* spp**	***F.hepatica***	***F.gigantica***	***Fasciola* spp**	***F.hepatica***
Distance bw sucker OS-VS								
Range	0.94-3.36	0.91-3.3	1.16-2.50	1.32-2.55	1.16–2.35	1.63-2.52	1.38-3.16	1.44–2.62
Mean	1.65±0.04	1.75±0.08	1.85±0.02	1.80±0.03	1.84 ± 0.03	2.18± 0.08	2.40± 0.02	2.07 ± 0.03
Distance bw sucker VS and union								
Range	10.5-29.5	10.1-28.0	12.13-32.51	11.61-21.08	6.09–18.03	21.15-30.76	14.39-28.11	8.07–19.00
Mean	19.79±0.35	19.3±0.56	20.05±0.35	14.80±0.22	11.91 ± 0.24	26.04± 0.82	21.10± 0.23	14.24 ± 0.25
Distance bw VS and posterior end of body								
Range	19.10-44.81	18.51-41.60	26.39-54.49	18.81-31.30	8.54–24.54	31.01-45.39	20.60-41.11	12.40–25.08
Mean	30.71±0.48	30.37±0.75	34.08±0.54	23.87±0.31	17.73 ± 0.34	41.02 ±1.21	30.59 ±0.33	20.79 ± 0.31
Distance bw vit. and posterior end of body								
Range	5.00-16.90	4.90-16.70	8.18-25.46	5.42-12.51	2.45–12.02	9.86-19.72	5.26-15.01	3.30–10.40
Mean	10.93±0.22	11.1±0.33	13.96±0.25	9.08±0.26	5.81 ± 0.15	14.98± 0.81	9.49± 0.37	6.55 ± 0.16
Body area								
Range	126.10-369.00	108.9-327.08	138.49-387.46	113.07-306.74	49.93–281.31	226.16-475.95	137.00-467.20	92.73–303.96
Mean	204.87±5.29	207.29±9.09	217.34±5.03	162.66±4.29	152.31 ± 4.87	359.20 ±19.05	319.46 ±5.12	180.92 ± 4.70
Oral sucker area								
Range	0.49-1.66	0.45-1.16	0.43-0.99	0.39-0.80	0.26–0.81	0.27-0.53	0.28-0.76	0.27–0.69
Mean	0.49±0.01	0.52±0.03	0.67±0.01	0.58±0.01	0.55 ± 0.01	0.35± 0.02	0.50± 0.01	0.49 ± 0.01
Ventral sucker area								
Range	2.05-4.82	1.71-4.60	1.44-3.35	0.89-2.60	0.77–1.53	1.34-2.02	0.70-1.81	0.69–1.61
Mean	2.3±0.06	2.51±0.12	2.31±0.03	1.76±0.06	1.17 ± 0.01	1.74± 0.06	1.09± 0.01	0.94 ± 0.02
BL to BW ratio								
Range	3.88-8.40	3.37-8.01	3.81-8.41	2.60-6.33	1.57–2.79	3.43-5.50	1.86-3.37	1.65–2.76
Mean	6.01±0.09	5.78±0.15	5.74±0.07	3.94±0.12	2.24 ± 0.03	4.37± 0.17	2.61 ±0.03	2.27 ± 0.03
OSA to VSA ratio								
Range	0.13-0.33	0.12-0.32	0.20-0.46	0.17-0.59	0.25–0.65	0.27-0.53	0.28-0.76	0.25–0.72
Mean	0.22±0.00	0.21±0.00	0.29±0.004	0.36±0.01	0.47 ± 0.01	0.35± 0.02	0.50± 0.01	0.53 ± 0.01



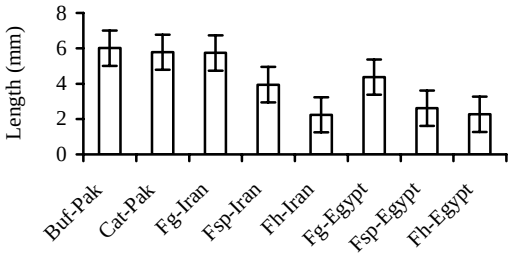
A



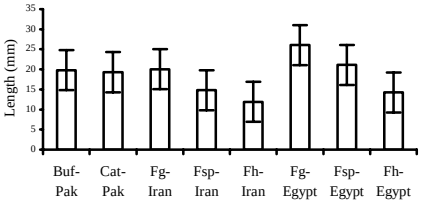
B



C



D



E

Fg. 3. Various measurement of Potohar *Fasciola intermedia* compared with *Fasciola gigantica* (Fg), *Fasciola hepatica* (Fh), *Fasciola* spp. / *intermedia* of Iran and Egypt. A, body length (BL); B, ventral Sucker to Posterior end (VS-Post); C, body width (BW); D, Ratio of Body length/Body width (BL/BW); E, length range from Ventral Sucker to union of Vitelline glands (VS-Vit);

Table II.- The ANOVA table showed that the p-values of phenotypic parameters and *Fasciola* groups (P<0.05).

Source	Degree of freedom	Sum of Squares	Mean square	F-value	P-value
Rep	4	5062.23	1265.558	83.09	0.0000
Type	7	567.38	81.054	5.32	0.0006
Error	28	426.48	15.231		
Total	39	6056.09			

Coefficient of variation = 21.43%
Grand mean, 18.213; Grand sum, 728.510; Total count, 40.

and resemblance between the Pakistani *Fasciola* with three fasciolids reported in Iran and Egypt. The proximity table of five morphometrical characters (Table III) depicted the distance from one worm species group to another group whereas; the proximity matrix (dissimilarity matrix) showed similarities by furnishing the distances between the cases. The Squared Euclidean distance (SED) demonstrated the similarity index among all the fasciolids (Table III). Simultaneously, SED showed that there was no phenotypic differences among the *Fasciola* prevalent in buffalo and cattle (SED is 0.007) of Potohar region. It was inferred that there is a close similarity of *F. gigantica* and *Fasciola* intermedia of Iran with that of Potohar region (SED is 0.192, 0.615), respectively. In agglomerative clustering the similarity coefficients indicated that Potohar buffalo and cattle are exhibiting lowest

proximity distance which showed that both hosts were parasitized with same liver fluke species (Table IV). The dendrogram represents the similarities among the different fasciolids (Fig. 4).

Table III.- Proximity matrix of BL, BW, BL/BW, VS-Vit, VS-Post morphometrical parameters between *Fasciola* groups (1, Buf-Pak; 2, Cat-Pak; 3, Bov-Iran-Fg; 4, Bov-Iran Fsp; 5, Bov-Iran-Fh; 6, Bov-Egypt-Fg; 7, Bov-Egypt-Fsp; 8, Bov-Egypt-Fh) showing Squared Euclidean Distance (SED)

Proximity Matrix
Squared Euclidean Distance

Case	1:Buf-Pak *Fasciola*	2:Cat-Pak *Fasciola*	3:Bov-Iran Fg	4:Bov-Iran Fsp	5:Bov-Iran Fh	6:Bov-Egypt Fg	7:Bov-Egypt Fsp	8:Bov-Egypt Fh
1:Buf-Pak *Fasciola*	.000	.007	.192	.615	2.319	1.111	1.414	2.611
2:Cat-Pak *Fasciola*	.007	.000	.200	.516	2.125	1.090	1.272	2.392
3:Bov-Iran Fg	.192	.200	.000	1.075	3.212	.674	1.718	3.478
4:Bov-Iran Fsp	.615	.516	1.075	.000	.595	2.193	.974	1.122
5:Bov-Iran Fh	2.319	2.125	3.212	.595	.000	4.331	1.415	.477
6:Bov-Egypt Fg	1.111	1.090	.674	2.193	4.331	.000	1.258	3.733
7:Bov-Egypt Fsp	1.414	1.272	1.718	.974	1.415	1.258	.000	.749
8:Bov-Egypt Fh	2.611	2.392	3.478	1.122	.477	3.733	.749	.000

This is a dissimilarity matrix

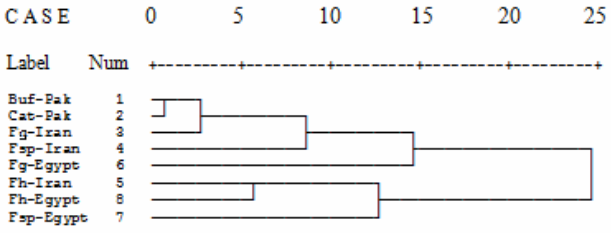


Fig. 4. Dendrogram using average linkage (Between Groups) based on 5 morphometrical parameters among 8 groups (Rescaled Distance Cluster Combine).

DISCUSSION

The morphological data based on *F. gigantica*, *F. hepatica* and *F. intermedia* from Iran and Egypt (Periago *et al.*, 2008), was used as a reference standard for comparison of *Fasciola* spp, prevalent in Potohar region. Ashrafi *et al.* (2006) initially outlined BL/ BW, VS-Vit followed by VS-P as an important index of differentiation between

Table IV.- Agglomeration Schedule of BL, BW, BL/BW, VS-Vit, VS-Post morphometrical parameters *Fasciola* groups (1, Buf-Pak; 2, Cat-Pak; 3, Bov-Iran-Fg; 4, Bov-Iran Fsp; 5, Bov-Iran-Fh; 6, Bov-Egypt-Fg; 7, Bov-Egypt-Fsp; 8, Bov-Egypt-Fh)

Stage	Cluster Combined		Coefficient
	Cluster 1	Cluster 2	
1	1	2	.007
2	1	3	.196
3	5	8	.477
4	1	4	.735
5	5	7	1.082
6	1	6	1.267
7	1	5	2.170

the fasciolids species. The present study shows that *Fasciola* in cattle and buffalo are morphologically identical as indicated by Ashrafi *et al.* (2006) and Periago *et al.* (2008). Previous studies consider buffalo and cattle as bovines, on the other hand some earlier studies suggest conflicting data on the size of the liver fluke hosted in different animals, with occasional details signifying, flukes from cattle are larger than from sheep, but without attaining any valid conclusion (Panaccio and Trudgett, 1999). Moreover, some studies concluded that the flukes in sheep grow faster, more uniformly and reach a larger size as compared to cattle (Valero *et al.*, 2001a,b). It has been reported that morphometric patterns are dependent upon the host species (Sahba *et al.*, 1972; Terasaki *et al.*, 2000; Valero *et al.*, 2001a; Lotfy *et al.*, 2002; Mas-Coma *et al.*, 2005; Ashrafi *et al.*, 2006). Later on Ghavami (2009) showed that flukes isolated from sheep are larger than those of cattle, may be because of low resistance in sheep than in cattle exhibiting moderate resistance against parasite. Besides, differences in morphometry of organs of fasciolids can be influenced by intensity of infection, host species, age and immune reactions due to a possible previous exposure to the infection.

The main difference between *F. gigantica* and *F. hepatica* is the larger size of the former, as well an elongated body and a narrow posterior end.

Whereas, *F. hepatica* has a different shape. The simplest and common way to differentiate between the two species is the length and body shape (Kendall, 1965). Earlier studies outlined by Cobbold (1855) and Varma (1953) show the presence of numerous secondary intestinal ramifications in *F.gigantica*. Though it is emphasized that external morphology, especially the size and shape of the body are helpful in identification, nevertheless, many authors agree that it is very difficult to achieve a precise classification criterion as many variations exist in their morphological characteristics (Kimura *et al*., 1984). The patterns of the reproductive organs and intestines serve as a differentiating factor however, natural ramifications makes this characteristic unreliable for identification (Watanabe, 1962; Bergeon and Laurent, 1970). Many morphometric studies have been carried out but none have emphasized on the comparison of both species (Srimuzipo *et al*., 2000). Nevertheless, differentiating between fasciolids in areas where both species overlap is quite intricate, although different computer programs have been used in previous studies for analyzing morphometrical parameters.

The present study concludes that morphometrical indicators used for differentiating between *Fasciola* species indicate that liver fluke specimens from Potohar are not pure *F. gigantica or F. hepatica*, but an intermediate form of *Fasciola spp* which apparently looks more like *F. gigantica.* Furthermore the Potohar species resembles more to the Iranian *Fasciola* species as compared to the Egyptian isolates. This might be due to introduction of metacercariae through bird migration and cross border movement of livestock.

The three species of *Fasciola (F. hepatica*, *F. gigantica*, *F. intermedia*) are phenotypically very similar to each other (Srimuzipo *et al*., 2000; Terasaki *et al*., 2000) and due to the presence of intra-species morphometric differences and significant overlaps of these indices between the two species, it is emphasized that morphometric measurements alone are insufficient for differential diagnosis of the fasciolid species. Therefore, it is suggested to genetically characterize the reported *Fasciola intermedia* using appropriate genetic markers. This study would help in developing indirect ELISA by using *Fasciola intermedia* for early diagnosis of fasciolosis in large ruminants. Furthermore, it would also facilitate to study the sero-epidemiological pattern of fasciolosis in order to develop effective control measures in Potohar region.

REFERENCES

ASHRAFI, K., VALERO, M., PANOVA, A., M., PERIAGO, M.V., MASSOUD, J. AND MAS-COMA, S., 2006. Phenotypic analysis of adults of *Fasciola hepatica*, *Fasciola gigantica* and intermediate forms from the endemic region of Gilan, Iran. *Parasitol. Int.,* **55:** 249–260.

BERGEON, P AND LAURENT, M., 1970. Difference entre la morphologie testiculaire de *Fasciola hepatica* et *Fasciola gigantica*. *Rev. Elev. Med. Vet. Pays Trop*., **23:** 223-227

BUKHARY, Z. S. H., 1988. *Immunotaxino studies in F. hepatica.* M. Phil thesis, QAU, Islamabad.

COBBOLD, T.S., 1855. Description of a new trematode worm (*Fasciola gigantica*). *Edinbourg N. Phil. J., N. S.*, **2:** 262–266.

GHAVAMI, M.B., RAHIM, P., HANILOO, A. AND MOSAVINASAB, S. N., 2009. Genotypic and phenotypic analysis of *Fasciola* isolates. *Iranian J. Parasitol.*, **4**: 61-70.

ITAGAKI, T., KIKAWA, M., SAKAGUCHI, K., SHIMO, J., TERASAKI, K., SHIBAHARA, T. AND FUKUDA, K., 2005. Genetic characterization of parthenogenic *Fasciola* sp. in Japan on the basis of the sequences of ribosomal and mitochondrial DNA. *Parasitology,* **131:** 679–685.

KENDALL, S.B., 1965. Relationships between the species of *Fasciola* and the molluscan hosts. *Adv. Parasitol*., **3:** 59–98.

KIMURA, S., SHIMIZUAND, A., KAWANO, J., 1984. Morphological observation on liver fluke detected from naturally infected Carabaos in the Philippines. Scientific *Rep. Facul. Agric. Kobe Univ*., **18:** 353-357.

LOTFY, W. M., EL-MORSHEDY, H. N., EL-HODA, M. A., EL-TAWILA, M. M., OMAR, E. A. AND FARAG. H. F., 2002. Identification of the Egyptian species of *Fasciola*. *Vet. Parasitol.,* **103:** 323–332.

MAS-COMA, S., BARGUES, M. D. AND VALERO. M. A., 2005. Fascioliasis and other plant-borne trematode zoonoses. *Int. J. Parasitol.*, **35:** 1255–1278.

PANACCIO, M. AND TRUDGETT. A., 1999. Molecular biology. In: *Fasciolosis* (ed. J.P. Dalton). CABI Publishing, Wallingford, Oxon, UK, pp. 449–464.

PERIAGO, M.V., VALERO, M. A., PANOVA, M., MAS-COMA, S., 2006. Phenotypic comparison of allopatric

populations of *Fasciola hepatica* and *Fasciola gigantica* from European and African bovines using a computer image analysis system (CIAS). *Parasitol. Res.*, **99:** 368–378.

PERIAGO, M. V., VALERO, M. A., EL-SAYED, M., ASHRAFI, K., ELWAKEEL, A., MOHAMED, M. Y., DESQUESNES, M., CURTALE, F. AND MAS-COMA, S., 2008. First phenotypic description of *Fasciola hepatica/Fasciola gigantica* intermediate forms from the human endemic area of the Nile Delta, Egypt. *Infect. Genet. Evol.*, **8:** 51–58.

PHIRI, I. K., PHIRI, M. A. AND HARRISON, J. L., 2006. Serum antibody isotype responses of *Fasciola*-infected sheep and cattle to excretory and secretory products of *Fasciola* species. *Vet. Parasitol*., **141**: 234–42.

SAHBA, G.H., ARFAA, F., FARAHMANDIAN, I. AND JALALI, H., 1972. Animal fascioliasis in Khuzestan, South western Iran. *J. Parasitol.,* **58:** 712-716.

SRIMUZIPO, P., KOMALAMISRA, C., CHOOCHOTE, W., JITPAKDI, A., VANICHTHANAKORN, P., KEHA, P., RIYONG, D., SUKONTASAN, K., KOMALAMISRA, N., SUKONTASAN, K. AND TIPPAWANGKOSOL. P., 2000. Comparative morphometry, morphology of egg and adult surface topography under light and scanning electron microscopies, and metaphase karyotype among three size-races of *Fasciola gigantica* in Thailand. *Southeast Asian J. trop. Med. Publ. Hlth.*, **31:** 366-73.

TERASAKI, K., NODA, Y., SHIBAHARA, T. AND ITAGAKI, T., 2000. Morphological comparisons and hypotheses on the origin of polyploids in parthenogenetic *Fasciola* sp. *J. Parasitol.*, **86:** 724–729.

TERASAKI, K., AKAHANE, H., HABE, S. AND MORIYAMA, N., 1982. The geographical distribution of common liver flukes (the genus *Fasciola*) with normal and abnormal spermatogenesis. *Japan J. Vet. Sci*., **44:** 223– 231.

VALERO, M. A., PANOVA, M. AND MAS-COMA, S., 2001a. Developmental differences in the uterus of *Fasciola hepatica* between livestock liver fluke populations from Bolivian highland and European lowlands. *Parasitol. Res.*, **87:** 337– 342.

VALERO, M. A., DARCE, N. A., PANOVA, M. AND MAS-COMA, S., 2001b. Relationships between host species and morphometric patterns in *Fasciola hepatica* adults and eggs from the Northern Bolivian Altiplano hyperendemic region. *Vet.Parasitol*., **102**: 85–100.

VARMA, A. K., 1953. On *Fasciola indica* (n. sp.) with some observations on *F. hepatica* and *F. gigantica. J. Helminth*., **27**: 185—198.

WATANABE, S., 1962. Fascioliasis of ruminants in Japan. *Bull. Off. Int. Epizoot*., **58**: 313–322.

(Received 8 November 2010, revised 8 January 2011)