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Prevalence of Fascioliasis in Buffaloes under Different Agro-climatic Areas of Sindh Province of Pakistan

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ABSTRACT

Buffalo is considered as "black gold" in livestock herders in Pakistan, contributing 65% of the milk production and 26% of the total meat production of the country. Buffaloes suffer from a number of diseases, but a parasitic disease namely fascioliasis is more common and causing huge economic losses. A survey was carried out to determine the prevalence of fascioliasis in buffaloes of different agro-climatic conditions of Sindh province of Pakistan. A total of 1800 fecal samples were randomly collected from buffaloes of different sex and age groups. The overall prevalence of fascioliasis was 42.06%; and all positive samples were infected with *Fasciola gigantica*. District-wise prevalence of fascioliasis in buffaloes was recorded as 41.83, 30.83 and 53.50%, in Larkana, Hyderabad and Badin districts, respectively selected from three zones of Sindh province namely Northern, Mid and Southern Sindh, respectively. Month-wise prevalence reflected that the higher rate in colder months viz. December (58.67%) and January (61.33%), while the lowest in the warm months: May (31.33%), June (24.67%) and July (26.00%). Sex-wise data revealed more than double incidence in female (45.08%) in comparison of male (20.89%). The highest level of infection was found in older group i.e., above 6 years (62.62%) followed by in age groups of 4-6 years (57.28%), 2-4 years (42.56%) and up to 2 year (17.87%). Significant correlation was recorded in between humidity and prevalence of fascioliasis in buffaloes; however no any significant relationship could be recorded between rainfall and infection.

Key Words: Prevalence; Fascioliasis; Liver fluke: *Fasciola gigantica;* Bovine fascioliasis

INTRODUCTION

Buffalo (*Bubalus bubalis*) is the premier dairy and meat producing animal in Pakistan and contributes 64.70% (21.5 m ton) of the total milk (33.23 m ton) and 25.50% (0.55 m ton) of the total meat production (2.16 m ton) of the country (FAO, 2008; Hussain *et al*., 2010; Afzal, 2010). Buffalo is considered to be a useful animal as compared to other domesticated animal species, and suffers a large number of parasitic, viral, bacterial and fungal diseases.

Parasitic diseases are the major obstacle in the growth and development of animal health (Mahfooz *et al*., 2008; Raza *et al*., 2010). Among the parasitic diseases, helminthiasis has long been recognized and still are a problem leading to losses in ruminant production in almost all regions of the world (Alawa *et al.*, 2010).

Fascioliasis is recognized as one of the most important helminths diseases of the domesticated ruminants and is caused by genus Fasciola (Lessa *et al*., 2000). It is an emerging parasitic infection, impacts significantly on both veterinary and human health worldwide (Lazara *et al*., 2010). It causes huge economic losses in terms of reduction in milk and meat and high mortality in all ages of animals (Saleha, 1991).

Two highly infective species are identified as *Fasciola hepatica* and *F. gigantica. F. hepatica* survives in a variety of climatic conditions while *F. gigantica* is generally dominant in tropical area of many countries of the world (Urquhart *et al.*, 1988). These flukes mainly attack the liver, where they reside and graze on mucosa of the bile duct and hepatic parenchyma resulting in the massive tissue damage (Shaikh *et al*., 2004, 2005). Evidence also suggests mortalities in bovine due to fascioliasis (Irfan, 1984; Losos, 1986).

Buffalo, the prime dairy and meat producing animal has failed to fulfill meat and milk requirements of the people of Pakistan due to multi-facet problems. Among these, fascioliasis is one of the major problems for buffalo ill-health and low productivity. Prevalence of fascioliasis in buffaloes and its economic losses in Sindh province have

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not been properly documented. Therefore, no proper method of handling of animals and effective control measures are recommended. The present study was planned to investigate the prevalence of the fascioliasis in buffaloes of Sindh province of Pakistan.

MATERIALS AND METHODS

A total of 1800 fecal samples were collected randomly from male and female buffaloes of different age groups (Up to 2 year, 2 to 4 years, 4 to 6 years & above 6 years) from October, 2004 to September, 2005. The samples were collected from commercial and non-commercial dairy farms and house-hold animals of three different districts of Sindh namely Larkana, Hyderabad and Badin representing three agro-ecological zones of Sindh viz. Northern, Mid and Southern Sindh, respectively.

Stratified sampling method was applied for selection of animal farms categorized as commercial, non-commercial, and households based farming (4-5 animals per household). These farms were selected from urban, suburban, and rural settlements (villages) within periphery of 10 km in surrounding areas of district headquarters. List of sampling frames were prepared along-with locations during preliminary survey. It was observed that quite a large majority of commercial farms were in urban, non-commercial in suburban, household based farming in villages. Proportion of farms by the above mentioned stratification revealed that there were about 10% commercial farms, 10% non-commercial farms and 80% house hold based farms. However, in terms of total number of animals in the above categories deviated as about 30% commercial, 30% non-commercial and 40% in rural settlement particularly due to more number of animals per farm in commercial/non-commercial farms in comparison to house hold based farms. Two stage sampling was done for the selection of animals from villages. In the first stage, 24 villages (2 villages per month) were selected and in the second stage 11 house-based farms per village were selected. From each house, two (2) animals were selected (Table I).

Fecal samples were collected per rectum from the animals. Each samples of 5-10 g of fecal material was collected in wide-mouthed screw capped glass bottle or polythane bag containing 10% formalin as preservative. The samples were properly labeled and brought to the laboratory of Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam. Direct Smear and Sedimentation techniques were followed to detect the fluke eggs in fecal samples as described by Thienpont *et al.* (1979). The eggs of fasciola were identified according to the keys described by Thienpont *et al.* (1979) and Soulsby (1983). The flukes were collected from the bile duct of the slaughtered buffaloes at the main slaughter houses of Larkana, Hyderabad and Badin in wide-mouthed bottles containing normal saline solution. The flukes were vigorously shacked in 1% salt solution and fixed in 10% formalin and identified according to the keys given by Soulsby (1982) and Urquhart *et al.* (1988).

Meteorological data were collected from the routine record of the Meteorological Stations of study area. The relationship of meteorological data with prevalence of fascioliasis was investigated. The retrospective data were analyzed using SPSS V.17. Pearson Chi-square Test was used to compare the effect of season, age and sex on the prevalence of fascioliasis in buffaloes. Correlation of temperature, relative humidity and rainfall with infection of fascioliasis was calculated on MS Excel 2010.

RESULTS

The overall prevalence of fascioliasis was 42.06%, (768 out of 1800) in study area, where buffaloes were found infected with *F. gigantica*. The prevalence of fascioliasis in buffaloes of Larkana, Hyderabad and Badin was recorded as 41.83, 30.83 and 53.50%, respectively. Statistically significant difference ($P<0.01$) was observed for the prevalence of infection among districts (Table II).

The results revealed that overall highest rate of infection in colder month as 61.33% was recorded in January, whereas the lowest rate of prevalence in warm months as 24.67% was recorded in June. A significant difference ($P<0.01$) in infection was recorded among the months. District-wise highest infection rate of 66.00 and 50.00% were recorded in January in Larkana and Hyderabad, respectively; whereas the highest infection of 72.00% was observed in month of December in Badin. The lowest rate of 20.00 and 16.00% was recorded in July in Larkana and Hyderabad, respectively. The lowest 32.00% was recorded in the month of June in Badin. The proportion of prevalence of fascioliasis in animals among months in Larkana was observed highly significant at $P<0.01$ as compare to Hyderabad and Badin (Table III).

A significantly inversely proportional relation was recorded between temperature and infection [Infestation (%) = 96.75 - 2.021(^oC); $r^2=0.932$] in Larkana district. The results indicated that when temperature increased, the infection rate decreased. A negative correlation of temperature with infection rate was recorded [Infestation (%) = 66.12 - 1.304 (^oC); $r^2=0.721$] in district Hyderabad. A negative correlation [infestation=106 -1.970 (^oC); $r^2=0.805$] of temperature with the rate of infection was observed in Badin district. On an overall basis, it was observed that infection rate decreased with the increased temperature in three districts. A significantly inversely proportional relation [y=99.71 - 1.759 (^oC); $r^2=0.797$] of temperature with the rate of infection was observed during study period (Table IV).

A positive correlation between humidity and infection in animals was recorded [Infestation (%) =-13.83 + 0.869 (relative humidity); $r^2=0.512$] in district Larkana. It was observed that the infection was positively correlated with

Table I: Two stage sampling plan for selection of animal farms and animals

	Commercial (Urban)	Non-commercial (Peri-urban)	House based farms (Villages)	Total
Farms selected/district	24 (13%)	24 (13%)	132 (76%)	180 (100%)
Farms/District/month	2	2	11	15
Animals/farm/month	7	7	2	----
Animals/district/month	14	14	22	50
Animals/district/year	168	168	264	600
Animal in 3 districts/ year	504 (28%)	504 (28%)	792 (44%)	1800 (100%)

Table II: Prevalence of fascioliasis in buffaloes of Larkana, Hyderabad and Badin districts

District	No. of Animals Examined	No. Animal Infected	Percentage of Infection
Larkana	600	251	41. 83
Hyderabad	600	185	30.83
Badin	600	321	53.06
Total	1800	757	42.06

Df = 2, Pearson Chi-Square = 63.268 and P-Value = 0.000

Table III: Month-wise prevalence of fascioliasis in buffaloes of Larkana, Hyderabad and Badin districts

Month	Percentage of Infection			
	Larkana*	Hyderabad**	Badin***	Overall****
Oct., 2004	44.00	24.00	52.00	40.00
Nov., 2004	44.00	26.00	60.00	43.33
Dec., 2004	64.00	40.00	72.00	58.67
Jan., 2005	66.00	50.00	68.00	61.33
Feb., 2005	60.00	46.00	64.00	56.67
March, 2005	46.00	40.00	52.00	46.00
April, 2005	36.00	30.00	56.00	40.67
May, 2005	28.00	24.00	42.00	31.33
June, 2005	22.00	20.00	32.00	24.67
July, 2005	20.00	16.00	42.00	26.00
Aug., 2005	32.00	22.00	50.00	34.67
Sept., 2005	40.00	32.00	52.00	41.33

Percentages of infection were calculated on the number of sample examined each month

* df = 11, Pearson Chi-square = 53.994 and P-Value = 0.000

** df = 11, Pearson Chi-square = 30.471 and P-Value = 0.001

*** df = 11, Pearson Chi-square =29.263 and P-Value = 0.002

**** df = 11, Pearson Chi-square = 99.387 and P-Value = 0.000

humidity as humidity increased, the infection rate also increased. Statistically [Infestation (%) = 5.524 + 0.407 (relative humidity); r^2=0.086] non-significant effect of relative humidity on fascioliasis was recorded in district Hyderabad (Table IV).

A non-significant negative correlation [Infestation (%) = 79.74 - 0.319 (relatively humidity); r^2=0.004] of humidity was observed on infection in buffaloes in Badin district. The humidity was recorded higher in Badin district in comparison of other districts under this study i.e., Hyderabad and Larkana round the year, however a slightly positive correlation [Infestation (%) = -6.176 + 0.837 (relative humidity); r^2=0.164] between overall humidity of the study area and prevalence of fascioliasis in buffaloes was recorded.

A highly positive correlation of rainfall on infection rate was found in animals in Larkana district [infestation (%) = 39.07 + 4.254 (rain fall); r^2=0.074]. Non-significant negative correlation [infestation (%) = 33.32 - 2.642 (relative humidity); r^2=0.111] was found between infection and rainfall in Hyderabad. No significant [y= -0.033 x +53.98, r^2=0.009] effect was noted on the infection rate in buffaloes in district Badin, whereas on an overall basis, no significantly correlation between prevalence of infection and rainfall was determined in all districts. The correlation values [Infestation = 55.01 - 0.389 (Rainfall); r^2= 0.136] indicated no effect of rainfall on the overall prevalence of fascioliasis in the study area (Table IV).

The sex-wise highest (57.71%) prevalence of fascioliasis was determined in Badin followed by 45.21 and 33.27% in female buffaloes in Larkana and Hyderabad districts, respectively. Whereas 30.85, 19.23 and 5.66% of fascioliasis cases were observed in male in Badin, Larkana and Hyderabad districts, respectively. The percentage of infection for sex of the buffaloes enumerated highly significant (P< 0.01). The overall prevalence of fascioliasis in three districts by sex was recorded as 45.08 and 20.89% in female and male, respectively. On an overall basis, fascioliasis between male and female was estimated as highly significant (P<0.01) (Table V).

The age-wise distribution of the fascioliasis in buffaloes revealed that the highest prevalence (62.62%) was recorded for the animals above 6 years of age group, the lowest (17.87%) prevalence was found in animals of age ranged from 1 month to 2 years, whereas 42.56 and 57.28% were recorded in animals of 2-4 and 4-6 years old, respectively. The rate of prevalence of fascioliasis for age groups was recorded significant at the P<0.01 level of significance between Larkana, Hyderabad, Badin and on an overall basis in all districts (Table VI).

DISCUSSION

Infestation of Fascioliasis causes heavy losses in terms of milk and meat production and due to their sporadic and infectious spread irrespective of geographical boundaries have imposed a serious concern on the socio-economic status of livestock farming. The occurrence of fascioliasis in an area is influenced by a multifactorial system which comprises hosts, parasite and environmental effects. In the natural foci of fascioliasis, the *Fasciola* and their intermediate and final hosts form an association posing a potential epidemiological threat and it is important that the existence and localization of such an association should be recognized beforehand so that the situation can be brought under control (Maqbool *et al*., 2002).

Table IV: Correlation of temperature, relative humidity and rainfall with the prevalence of fasciolisis in buffaloes of Larkana, Hyderabad and Badin districts

S. No.	Correlations of infection between	Larkana		Hyderabad		Badin		Overall	
		Regression equation	R^2	Regression equation	R^2	Regression equation	R^2	Regression equation	R^2
1	Temp.	y= -2.107x+98.214	0.9293	y=-1.4621x+69.275	0.7727	y=-1.9706x+106.08	0.8057	y=-1.7596x+99.711	0.7979
2.	Humidity	y=0.9242x-18.205	0.5307	y=0.5235x-2.9857	0.1216	y=-0.3191x+79.743	0.0045	y=-0.3191x+79.743	0.0045
3.	Rainfall	y=4.3298x+38.507	0.08	y=-2.6188+32.208	0.0958	y=-0.0337x+53.988	0.0098	y=-0.3897x+54.995	0.1363

Table V: Sex-wise infection of fascioliasis in buffaloes of Larkana, Hyderabad and Badin districts

SEX	Percentage of Infection			
	Larkana*	Hyderabad**	Badin***	Overall****
Male	19.23	5.66	30.85	20.89
Female	45.21	33.27	57.71	45.08

Percentages of infection were calculated on the number of samples examined for each gender

* df =1, Pearson Chi-square = 18.823 and P-Value = 0.000

** df =1, Pearson Chi-square = 17.274 and P-Value = 0.000

*** df =1, Pearson Chi-square =22.984 and P-Value = 0.000

**** df =1, Pearson Chi-square = 47.276 and P-Value = 0.000

Table VI: Age-wise infection of fascioliasis in buffaloes of Larkana, Hyderabad and Badin districts

Age Groups	Percentage of Infection			
	Larkana*	Hyderabad**	Badin***	Overall****
Up to 2 year	17.51	9.13	27.73	17.87
2-4 years	48.33	24.73	50.41	42.56
4-6 years	48.54	45.05	77.98	57.28
Above 6 years	56.00	58.06	76.35	62.62

Percentages of infection were calculated on the number of samples examined for each age group

* df =3, Pearson Chi-square = 63.506 and P-Value = 0.000

** df =3, Pearson Chi-square = 119.267 and P-Value = 0.000

*** df =3, Pearson Chi-square = 116.539 and P-Value = 0.000

**** df =3, Pearson Chi-square = 271.230 and P-Value = 0.000

The findings recorded in this survey are in accordance with the reports of other countries as 48.70% (Jithendran & Bhatt, 1999), 50.00% (Molina *et al.,* 2005), 44.70% (Liu *et al.,* 2009) and 50.00% (Siddiki *et al.,* 2010). Whereas similar findings were recorded by Tongson (1978) and Mahato and Harrison (2005), observed the prevalence ranged from 34.00 -100.00% and 30.00 -70.00% in buffaloes in endemic areas of Philippines and Nepal, respectively. Buffaloes provide the major draught power in rice farming activities in the Philippine (Molina *et al.,* 2005). In low land tropical irrigated rice field, the main infection is caused by *F. gigantica* (Spithill *et al.,* 1999) and prevalence of fascioliasis in buffaloes in rice cultivated areas of Indonesia was observed usually higher (Copeman, 1999). The results of the present study are in agreement with the findings that the higher infection rate was recorded in rice cultivating areas of the province of the Sindh. Whereas, the lower prevalence of fascioliasis in bovine in Brazil (Luz, *et al.,* 1992), China (Wang BingYun, *et al.,* 2001), Egypt (El-Shazly *et al.,* 2002), Iraq (Al-Khafaji *et al.,* 2003), Brazil (Marques & Screferneker, 2003), Kenya (Mungube *et al.,* 2006), Cambodia (Sothoeun *et al.,* 2006) and in Toba Tek Singh district of Pakistan (Khan *et al.,* 2010) was recorded as 19.80, 29.19, 9.73, 7.00, 20.00, 26.00, 24.00 and 25.61%, respectively. In Punjab, Pakistan, Suhail *et al.* (2003) recorded the prevalence of bovine fascioliasis as 6.80%. This variation in infection may be incremented to management practices, meteorological differences and presence of intermediate hosts in that area. Maha (2008) reported 59.50% prevalence of fascioliasis in North Sinnaria Dakahlia governarte, higher as compared to the present study. This difference may be attributed to humidity, temperature, rainfall, presence of intermediate hosts and management.

It was observed that the higher prevalence was recorded in winter than in summer, these results are in agreement with the findings of Singh (2001). It was further observed that the infection persisted round the year in study area; this may be due to the suitability of the climate and presence of canal and ponds for intermediate host and their egg masses to persist throughout the year. El-Bahy (1998) reported that, the presence of infection throughout the year may be due to resistance of metacercariae for dissociation, especially with the presence of the shallow water, enough vegetation and humidity, continued exposure of the animals to encysted metacercariae from the banks of canal or ponds, especially during the dry season and no restriction on animal importation, grazing habits and movement between the infected and treated localities.

A negative correlation between temperature and infection rate and a positive correlation between relative humidity and infection rate was recorded. The relative humidity of the Larkana and Hyderabad increased in the cold months that resulted in an increase in infection rate. However, in Badin, there was no effect of the relative humidity on the prevalence of fascioliasis because relative humidity of the Badin was high in all months of the year.

The prevalence of fascioliasis in buffaloes was higher in females than males; similar trend was observed district-wise in both sexes. This may be due to age factor because mostly male animals are sold for slaughter at the age of 2-3 years and infection rate was higher in animals falling in the age group of 4-6 years. However, the results of the present study are in conformity with the findings of Molina *et al.* (2005) who observed higher prevalence (50.00%) in females as compared to (37.50%) male buffaloes.

The age of the animals is considered as a major factor in the prevalence of fascioliasis. During present study, the overall higher infection was recorded in animals of above 6

years of age group. These findings are very similar to the finding of Soesetya (1975) and Molina *et al.* (2005), they also observed that animals above 6 years of age were more infected with the fascioliasis. The higher infection rate in older animals could be due to long time exposure to disease entity and their grazing habit close to submerge areas (Hossain *et al.*, 2011).

In conclusion, *F. gigantica* infection in buffaloes is endemic and wide spread in the all three districts particularly in rice cultivated areas of Larkana and Badin. Control measures should be taken for destruction of intermediate host (Snail population). Periodic anthelmintic treatment should be given for getting maximum productivity from the buffaloes.

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