# Prevalence of Fasciolosis in Buffaloes of Bahawalpur, Punjab,

# Pakistan

Abul Hasanat<sup>\*</sup>, Nuzhat Sial, Muhammad Shafiq Chaudhary, Muazzam Ali, Sumaira Kausar Department of Life Sciences, The Islamia University of Bahawalpur, Pakistan. \*Correspondence: <u>ahzoologist95@gmail.com</u>

#### Abstract

Fasciolosis is a trematode borne parasitic disease that infects liver of large ruminants widely prevalent throughout the world. During the present study fecal samples from buffaloes were collected on random basis from the all tehsils of Bahawalpur district form February 2012 to October 2012. Of total 1800 fecal samples, 284 (15.8%) were found to be positive. Highest prevalence was recorded in Yazman (21.7%) followed by Bahawalpur (16.7%), Khairpur (15.6%), Hasilpur (14.4%) and the lowest was recorded in Ahmedpur (10.6%). Statically chi-square ( $\chi^2$ ) showed non significant (p>0.05) difference between all areas. Monthly overall highest prevalence was recorded in September (31%), while the lowest was found in the month of May (3.5%). Statistically a significant (p<0.05) difference was recorded in all months. Overall highest seasonal wise prevalence was found in autumn (28.3%) followed by winter (21%), summer (12%) and lowest in spring (8.3%). In age wise prevalence the adult buffaloes were highly (19.9%) infected than young ones (5.3%). Statistically a significant difference (p<0.05) was found between all seasons and age groups. Gender wise the prevalence was slightly higher but statistically non significant (p>0.05) in females (15.9%) than males (15.1%). Bahawalpur (Pakistan) has a significant prevalence (%) of fasciolosis that may cause economic loss.

Keys word: Fasciolosis, baffaloes, Bahawalpur, prevalence.

#### 1. Introduction

Livestock and their products are the major source of animal's proteins. But parasitism is the main barrier which limits livestock productions. Parasitic diseases not only cause mortality of animals, and also have direct effects in term of reduced production of milk, meat, wool, hide production; infertility and loss of stamina of working animals and especially zoonotic impact on human health are considerably greater (Baker & Muller, 1988). Helminthiasis, pose a serious health threat and a limitation to the productivity of ruminants due to the associated morbidity, mortality, cost of treatment and control measures (Nwosu *et al.*, 2007). The gastro- intestinal tract (GIT) of animals harbor a large variety of parasites majorly helminthes, that causes clinical and sub clinical parasitism. These parasites severely affect the health status of animals and thus cause great economic losses to the livestock industry. In Pakistan parasitic infestation is quite prevalent and cost about 26.5 million rupees per annum to the livestock industry (Anwar *et al.*, 1995; Irfan, 1984). The prevalence of gastrointestinal helminthiasis is associated to the agro-climatic conditions like quality and quantity of pasture, humidity, temperature, rainfall and grazing behavior of the host (Pal and Qayyum, 1993; Sardar *et al.*, 2006). Fasciolosis is a trematode borne parasitic disease that infects liver of large ruminants widely prevalent throughout the world. It is caused by *Fasciola* spp. i.e., *Fasciola gigantica*, and *Fasciola hepatica* (Phiri *et al.*, 2006). In Pakistan fasciolosis is one of the major factor that limits livestock development (Kendall, 1954).

Bahawalpur is the region with semi arid condition. But still it have different type canals and which fever the prevalence of fresh water snails and hence snail borne diseases. So it is necessary to check the epidemiology of fasciolosis in economically important animals like buffaloes.

#### 2. Materials and Methods

During the present study fecal samples were collected on random basis from the five tehsils (Fig. 1) of Bahawalpur district i.e. Bahawalpur, Ahmadpur, Yazman, Khairpur, and Hasilpur from February 2013 to October 2013.

#### 2.1 Collection of fecal samples

From each tehsil a total 50 fecal samples per animal including buffaloes, and cows were sampled on random basis. For diagnostic test 1g solid excreta with less water/2g excreta with more water/4g diarrheatic excreta was collected. 1 table spoon refers to 1 g of excreta. During sampling only fresh stool were collected or these were directly collected from the rectum of the animals. The samples were collected in plastic vials with a lid, containing 5-10% formalin as preservative and to prevent hatching of the eggs. The vials were properly labeled by species, place, age and date of collection and were brought in Regional Diagnostic lab of Livestock diseases Bahawalpur for diagnosis.

#### 2.2 Parasitological diagnostic tests

Each sample was examined by following different types of parasitological diagnostic techniques.

A simple microscopic examination was done by taking the sample and observed under low power microscope (10 X 0.25) for *fasciola* eggs detection. For this purpose a small amount of feces was mixed with normal saline solution (0.9%) in a petri dish and a drop of it was taken on 3 X 1 glass slide in form of a thin smear as large as 1 and 1/2 X 1 and was fully covered with a coverslip and examined under microscope (Thienpont *et al.*, 1979). The positive slide (s) were further proceed by Formol-ether Sedimentation (concentration) test.

In this test 1g of feces was emulsified in 7 ml of in 10% of formol-saline solution. The emulsion was the left for 10 minutes for fixation. It is then filtered from a fine strainer and filtrate was taken in centrifuge tube. Three ml ether was then added to it and the mixture was shaken vigorously and was centrifuged at 2000 rpm for 2 minutes. The debris was loosened by a stick and the supernatant was discarded leaving few drops of sediment. Two drops of sediments was taken on glass slide, covered with coverslip and studied under microscope for the presence of *Fasciola* eggs (Foreyt, 1997; Vohra and Agarwal, 2006).

Eggs were identified based on morphology described by Soulsby (1982) and Yamaguti (1975).

The results were analysed by using Chisquare  $(\chi^2)$  test on computer statistical software SPSS version

#### 3. Results

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An overall prevalence (%) of fasciolosis was found to be 15.8% in District Bahawalpur from February 2013 to October 2013 (Tab.3.1).

The overall highest month wise prevalence was found in the September  $(31\pm0.03\%)$ , followed by October  $(25.5\pm0.031\%)$ , February  $(21\pm0.029\%)$ , August  $(20.5\pm0.029\%)$ , July  $(14.5\pm0.025\%)$ , March  $(10\pm0.021\%)$ , June  $(9.5\pm0.021\%)$ , April  $(6.5\pm0.017\%)$  and the lowest prevalence was found in May  $(3.5\pm0.013\%)$  (Tab.3.1). Statistically at 95% confidential interval the prevalence was found to be highly significant (p<0.05) in all months. Only April showed less significant difference.

Area wise overall prevalence in five areas of district Bahawalpur showed that infection was highest at Yazman (21.7 $\pm$ 0.022%) followed by Bahawalpur (16.7 $\pm$ 0.020%), Khairpur (15.6 $\pm$ 0.019%), Hasilpur (14.4 $\pm$ 0.019%) and was lowest at Ahmedpur (10.6 $\pm$ 0.16%) (Tab.3.1). Statistical analysis showed non-significant (p>0.05) difference in all areas.

Data revealed that the highest prevalence in all the areas of Bahawalpur was reported during autumn  $(28.3\pm0.023\%)$  followed by winter  $(21\pm0.029\%)$ , summer  $(12\pm0.021\%)$  and lowest in spring  $(8.3\pm0.026\%)$  (Tab.3.1). Statistically prevalence was significantly higher in autumn (P<0.05) and summer and was less significant in winter as compared to spring.

Overall sex wise prevalence showed that female  $(15.9\pm0.024\%)$  were more susceptible than males  $(15.1\pm0.017\%)$  from fasciolosis (Tab.3.1). However, statistical difference was found to be non significant (P>0.05) between the prevalence of male and female.

It was observed that overall prevalence in adult buffaloes was significantly (P<0.05) higher (19.9 $\pm$ 0.022%) than young ones (5.3 $\pm$ 0.10%) (Tab.3.1).

To find out the infection rate associated with grazing habitat of buffaloes it was observed that the highest prevalence was in Riverine areas (16.7±0.020%) followed by Plain areas (12.9±0.16%) and lowest in Dry areas (6±0.014%) (Tab.3.1). While statistical analysis showed that the prevalence was highly significant (p<0.05) in all grazing habitat groups of all areas.

In month wise data, in all areas prevalence was highest in September i.e., 40%, 32.5%, 30%, 27.5% and 25% in Yazman, Bahawalpur, Khairpur, Hasilpur and Ahmedpur respectively while lowest prevelance was noted in May in at Yazman (7.5%), Bahawalpur(5%), Khairpur(2.5%), Hasilpur (2.5%) and Ahmedpur (0%) (Tab.3.2). Statistically at 99% confidential interval prevalence was highly significant (P<0.01) in the months of September, February and October in all areas while Yazman, Khairpur and Hasilpur also showed highly significant difference (P<0.01) in August. In all other months it was non significant (P>0.01).

Area and season wise data showed that highest prevalence (P<0.01) in all areas was noted in autumn followed by winter, summer and lowest in spring season (Tab.3.2). Except autumn in all other seasons prevalence was less significant.

Gender wise prevalence, in all areas it was observed a higher prevalence in females of Yazman, Bahawalpur, Khairpur, Hasilpur and Ahmedpur i.e 24.5%, 17.9%, 16.4%, 14.9% and 9.9% repectively than males i.e 12.8% (Tab.3.2) in all areas, while statistical difference was highly significant (P<0.01).in both males and females.

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In all area, it was observed that prevalence was higher in adults (Yazman 27.2%, Bahawalpur 20.6%, Khairpur 19.8%, Hasilpur 18.6% and Ahmedpur1 3.6%) than young ones (7.8%, 6.8%, 4.9%, 3.9% and 2.9% respectively) (Tab.3.2). While statistically both showed non significant (P>0.01) difference in all areas except Bahawalpur with highly significant (P<0.01) difference.

Area and grazing habitat wise data showed that prevalence was highly significant (P<0.01) in grazing habitat of all areas except the plain habitats of Khairpur, Hasilpur and Ahmedpur that showed non significant (P>0.01) difference in prevalence (Tab.3.2).

#### 4. Discussion

In the presence study overall prevalence of fasciolosis in buffloes was found to be 15.8 % of which 11, 3.61 and 1.2% prevalence was found for F. *gigentica*, F. *fasciola* and mixed infection respectively. Khan *et al.* (2009) also recorded a highest prevalence of fasciolosis in buffloes and cattles with highest prevalence rate of F. *gigentica* and lower prevalence of F. *hepatica* from Layyah, Punjab, which are in favor of results. Maqbool *et al.* (2002) in their studies reported everall 25.59, 26.16, 13.7 and 10.5 per cent, prevalence respectively in slaughtered buffaloes, buffaloes at livestock farms, veterinary hospitals and in household buffaloes in the Punjab. These results are in favour of our results as in the present study the prevalence was recorded from different environmental conditions. Our results also agree with the work of Qureshi *et al.* (2012) reported over all 14.69% prevalence in buffaloes of the Punjab by fecal analysis. Khan *et al.* (1991) have reported overall prevalence of fasciolosis in Rawalpindi division (Potohar region) to be 37.53 percent and 31.74 percent in buffalo and cattle, respectively. The difference in prevalence from our results may due the dry condition of Bahawlpur area as compare to Rawalpindi. Bhutto *et al.*, (2012) reported a total 42.06% of overall prevelane in buffalos in Sindh. They reported all positive cases for F. *gigentica* in buffaloes. The highest prevalence was also found in the reverine based areas i.e 16% as compare to other semi dry and dry areas.

In season wise prevalence Maqbool *et al.* (2002) and Khan *et al.* (2009) reported overall highest (24.0%) seasonal prevalence in all types of buffaloes was recorded during autumn, followed by spring (20.0%), winter (13.0%), while the lowest (9.0%) was recorded during summer. In our studies highest prevalence was in autumn i.e 28.2% and while the lowest was recorded in spring i.e 8.2%. The difference in our lowest prevalence may due to the difference in environmental conditions as in Bahawlapur the summer season duration in much more as compare other areas. Our results get favour form the findings of Qureshi *et al.* (2012). They also recorded highest prevalence in autumn while lowest in spring. Khan *et al.* (2009) recorded a highest prevalence in winter (39.08%) and lowest infection in summer (12.92%) form Layyah. There is difference in season wise prevalence as compare to our work. This difference may due to difference in environmental conditions. Masud and Majid, (1984) reported highest prevalence during winter season in buffaloes of Multan. These also favor our results as we found highest prevalence in cold and wet season.

In month wise prevalence our study shows highest prevalence in September (31%) and lowest prevalence in May (3.5%). All areas also showed the same pattern of prevalence in month wise. Similar findings were recorded by Qureshi *et al.* (2012) and also by Maqbool *et al.* (2002) and Khan *et al.* (2009) in buffaloes.

In gender wise statistically no significant difference, but a slightly higher prevalence was recorded in females buffaloes (male 15.2%, female 16%) in our studies. In age wise prevalence animals above 2 years (20%) found to be highly susceptible then below 2 (5.3%). These results could get favor from the findings of Qureshi *et al.* (2012) and also by Maqbool *et al.* (2002). They recorded similar results in different areas of Punjab. The results also could favor from the work of Bhutto *et al.* (2012) and Khan *et al.* (2009).

In area wise highest prevalence in buffaloes was found in Yazman (21.7%) and lowest was recorded in ahmadpur (10.6%), the results could favor the findings of Qureshi (2009). They recorded a different but statiscaly non significant difference precalene in buffaloes in differenct area of Punjab. Iqbal *et al.* (2007) and Khan *et al.* (2009) also recorded the same results for fasciolosis from different areas of Pakistan.

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| Tab.3.1: ( | Over all | Prevalence | of fasciolosis | s in buffaloes | of Bahawalpur | from feb-2013 to |
|------------|----------|------------|----------------|----------------|---------------|------------------|
| oct-2013.  |          |            |                |                |               |                  |

| Factors         |  |                                     |                                     |                       |  |
|-----------------|--|-------------------------------------|-------------------------------------|-----------------------|--|
|                 |  | total no. of<br>observed<br>samples | total no. of<br>positive<br>samples | Prevalence<br>(%)±S.E |  |
|                 | February                                     | 200                                 | 42                                  | 21 <b>±</b> 0.029     |  |
|                 | March  | 200                                 | 20                                  | 10 <b>±</b> 0.021     |  |
|                 | April  | 200                                 | 13                                  | 6 <b>±</b> 0.017      |  |
|                 | Mav  | 200                                 | 7                                   | 3 5 <b>+</b> 0 013    |  |
|                 | wiay   | 200                                 | ,                                   | 3.3=0.015             |  |
| Month (s)       | [onth (s) .June                              |                                     | 19                                  | 9.5 <b>±</b> 0.021    |  |
|                 | -  |                                     |                                     |                       |  |
|                 | July   | 200                                 | 29                                  | 14.5 <b>±</b> 0.025   |  |
|                 | August                                       | 200                                 | 41                                  | 20.5 <b>±</b> 0.029   |  |
|                 | September                                    | 200                                 | 62                                  | 31 <b>±</b> 0.033     |  |
|                 | October                                      | 200                                 | 51                                  | 25.5 <b>±</b> 0.031   |  |
|                 | Winter                                       | 200                                 | 42                                  | 21 <b>±</b> 0.029     |  |
| Season          | Spring                                       | 400                                 | 33                                  | 8.25±0.026            |  |
|                 | Summer                                       | 800                                 | 96                                  | 12±0.021              |  |
|                 | Autumn                                       | 400                                 | 113                                 | 28.25±0.023           |  |
| Gender          | Male   | 430                                 | 65                                  | 15.11±0.017           |  |
|                 | Female                                       | 1370                                | 219                                 | 15.98±0.024           |  |
| Age             | 0-2 years                                    | 513                                 | 27                                  | 5.26±0.10             |  |
|                 | > 2 years                                    | 1287                                | 257                                 | 19.96±0.022           |  |
|                 |  | 360                                 | 78                                  | 21.66 <b>±</b> 0.022  |  |
|                 | Yazman<br>Bahawalpur<br>Khiarpur<br>Haslipur |                                     | 60                                  | 16.66+±0.020          |  |
|                 |  |                                     | 56                                  | 15.55±0.019           |  |
|                 |  |                                     | 52                                  | 14.44 <b>±</b> 0.019  |  |
| Area (s)        | Ahmand pur                                   | 360                                 | 38                                  | 10.55±0.16            |  |
|                 | riverine area                                | 360                                 | 60                                  | 16.66±0.020           |  |
| Grazing habitat | Plain area                                   | 450 58                              |                                     | 12.88±0.16            |  |
|                 | Dry area                                     | 450                                 | 27                                  | 6±0.014               |  |
| Total           |  | 1800                                | 284                                 | 15.8%                 |  |

| Factors         |               | Yazman     |            | Bahawalpur |            | Khairpur   |            | Hasilpur   |            | Ahmadpur   |            |
|-----------------|---------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
|                 |               | Inf./total | Prevl. (%) |
|                 | February      | 10/40      | 25         | 9/40       | 22.5       | 9/40       | 22.5       | 8/40       | 20         | 6/40       | 15         |
|                 | March         | 5/40       | 12.5       | 5/40       | 12.5       | 4/40       | 10         | 4/40       | 10         | 2/40       | 5          |
|                 | April         | 4/40       | 10         | 3/40       | 7.5        | 3/40       | 7.5        | 2/40       | 5          | 1/40       | 2.5        |
|                 |               |            |            |            |            |            |            |            |            | -,         |            |
|                 | May           | 3/40       | 7.5        | 2/40       | 5          | 1/40       | 2.5        | 1/40       | 2.5        | 0/40       | 0          |
|                 |               |            |            |            |            |            |            |            |            |            |            |
| Month (s)       | June          | 5/40       | 12.5       | 4/40       | 10         | 4/40       | 10         | 4/40       | 10         | 2/40       | 5          |
|                 | July          | 8/40       | 20         | 6/40       | 15         | 6/40       | 15         | 5/40       | 12.5       | 4/40       | 10         |
|                 | August        | 14/40      | 35         | 7/40       | 17.5       | 7/40       | 17.5       | 8/40       | 20         | 5/40       | 12.5       |
|                 | September     | 16/40      | 40         | 13/40      | 32.5       | 12/40      | 30         | 11/40      | 27.5       | 10/40      | 25         |
|                 | October       | 13/40      | 32.5       | 11/40      | 27.5       | 10/40      | 25         | 9/40       | 22.5       | 8/40       | 20         |
|                 | Winter        | 10/40      | 25         | 9/40       | 22.5       | 9/40       | 22.5       | 8/40       | 20         | 6/40       | 15         |
| Season          | Spring        | 9/80       | 11.25      | 8/80       | 10         | 7/80       | 8.75       | 6/80       | 7.5        | 3/80       | 3.75       |
|                 | Summer        | 30/160     | 18.75      | 19/160     | 11.87      | 18/160     | 11.25      | 18/160     | 11.25      | 11/160     | 6.87       |
|                 | Autumn        | 29/80      | 36.25      | 24/80      | 30         | 22/80      | 27.5       | 20/80      | 25         | 18/80      | 22.5       |
| Gender          | Male          | 11/86      | 12.79      | 11/86      | 12.79      | 11/86      | 12.79      | 11/86      | 12.79      | 11/86      | 12.79      |
|                 | Female        | 67/274     | 24.45      | 49/274     | 17.88      | 45/274     | 16.42      | 41/274     | 14.96      | 27/274     | 9.85       |
| Age             | 0-2 years     | 8/103      | 7.766      | 7/103      | 6.796      | 5/103      | 4.854      | 4/102      | 3.92       | 3/102      | 2.94       |
|                 | > 2 years     | 70/257     | 27.23      | 53/257     | 20.62      | 51/257     | 19.84      | 48/258     | 18.60      | 35/258     | 13.56      |
|                 | riverine area | 52/180     | 28.88      | 44/180     | 24.44      | 40/180     | 22.22      | 38/180.    | 21.11      | 25/180     | 13.88      |
| Grazing habitat | Plain area    | 20/90      | 22.22      | 12/90.     | 13.33      | 10/90      | 11.11      | 9/90.      | 10         | 7/90       | 7.77       |
|                 | Dry area      | 6/90.      | 6.66       | 4/90.      | 4.44       | 6/90       | 6.66       | 5/90.      | 5.55       | 6/90       | 6.66       |
| Total           |               | 78/360     | 21.66      | 60/360     | 16.66      | 56/360     | 15.55      | 52/360     | 14.44      | 38/360     | 10.55      |

## Tab. 3.2: Areas wise prevalence (%) of fasciolosis in buffaloes

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