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Molecular detection of *Fasciola hepatica* in water sources of District Nowshehra Khyber Pakhtunkhwa Pakistan

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Abstract

Fascioliasis is spread through contamination of water sources and cause morbidity throughout the world. In the current study 300 water samples were processed by PCR for detection of *Fasciola hepatica*. The overall prevalence in different water sources was 9.66 % (29/300). Highest prevalence was recorded in drain water16 % (16/100) followed by tube well water 10% (4/40), open well water 8 % (8/100) and the lowest was recorded in tap water 1.66 %(1/60). The significant difference $P < 0.05$ was recorded during data analysis. The highest prevalence was recorded in summer. It was concluded from the study that cleaning and filtration should be adopted to avoid the health hazards against water borne zoonotic parasites.
Key words: *Fasciola hepatica*, PCR, Zoonotic parasites.

INTRODUCTION

Water is considered one of the important nutrients although it yields no energy. The structural composition of cell is based on water. Water is a prime component of diet (Baloch *et al*., 2000). The problem of water-borne parasites is widespread and turning severe. The parasites have fascinated researchers due to their ability to adjust readily to increasingly complex environments (Tauxe, 2002). Waterborne diseases occur worldwide. Contaminated water causes disease in a large number of animals. Waterborne diseases have a direct effect on the economy of the concerned population (Barwick *et al.*, 2000). The disease which occurs due to unhygienic water sources or reservoirs propagates at an alarming rate. Moreover water borne diseases produce huge economic

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hazards in most parts of the globe (Barwick *et al.,* 2000). The causative agents of water borne diseases are mainly parasites (Savioli, 2004). In the world known history about 325 waterborne parasitic outbreaks has been documented (Kramer *et al*., 2001). In order to minimize the harm caused by parasitic diseases use of healthy water is being highly emphasized (Slifko *et al*., 2000). According to WHO about 80% of diseases found in human beings originate from water. In developing countries of the world more than half of the total population is far from using pure drinking water and this tragic condition opens the way for water borne parasitic diseases (Khan *et al*., 2000).

Waterborne diseases occur throughout the world and infections due to contaminated water systems easily shift to nearby human population. In the world known history several parasitic diseases root cause is the drinking and recreational water sources (Barwick *et al*., 2000).

The common bile duct fluke or liver fluke (*F. hepatica*) is a prevalent and economically important parasite. Taxonomically *F. hepatica* belongs to the family named Fasciolidae. Mature parasites are flat and leaf-like. Parasite length range is 20-30 mm and 7-14 mm in width. *F. hepatica* has an anterior and posterior sucker for attachment to host body (Smith and Sherman, 2009).

F. hepatica is a waterborne parasite (Mas-Coma *et al*., 1999). The distribution of *F. hepatica* is cosmopolitan being reported in developed and under-developed countries. Human fascolisis is recently treated as an emerging disease (Mas-Coma *et al.,* 2005). The liver fluke causes important veterinary and public health problems worldwide (Hurtrez *et al*., 2001). This parasitic trematode, secretes specific enzymes to assist in burrowing

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through the gut wall and liver of its mammalian host before reaching in the bile ducts (Dalton and Heffernan, 1989).

Fascioliasis is an important parasitic disease in grazing animals with over 700 million production animals being at risk of infection and economic losses which exceeds US$ 2 billion per annum world-wide (Mas-Coma *et al*., 2009). Fascioliasis is more familiar in sheeps. Human beings may get the infection accidentally from contaminated water or plants in endemic areas (Mohsen and Mardani, 2008). Millions of people are infected with fascioliasis worldwide and the number of people at risk exceeds 180 million. Importance of this zoonotic food-borne disease with a great impact on human development has been emphasized by WHO and other human health authorities. Recently Fasciolosis is added to the list of important helminthiasis (WHO, 1995).

Many classes of pathogens excreted in animal and human faeces are responsible for waterborne diseases. Protozoan's cause infections like bacterial agents, which are the main cause of outbreaks of diseases worldwide. Protozoan agents are very robust in water environments and are strongly resistant to most disinfectants, including chemical procedures (like chlorination) used to disinfect drinking water (Kourenti *et al*., 2007).

MATERIALS AND METHODS

Samples collection

Around 300 water samples were collected from four selected places of District Nowshehra named, Akora Khattak, Ezakhel, Pubbi and Nowshehra city. Water sources comprised of Tube wells, open wells, Bore wells and Tap water. The volume of each collected sample was 1liter.

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The samples were filtered through Whatman filter paper (No, 42) by Vaccum Filtration Plant (Assembly); and then centrifuged for 15 minutes at 600 rpm. The lower residues were taken in new tubes and again centrifuged for 8 minutes at 14000 rpm. The bottom 200µl of each sample was taken in eppendorf tubes. After this DNA was extracted from the samples.

DNA Extraction

The DNA was extracted by DNA zol (Trizol) extraction Kit.

DNA Amplification (PCR)

The DNA was amplified through Polymerase Chain Reaction (PCR) using primers specific for *F. hepatica*. The primer used for detection of *F. hepatica* DNA was Fas F, 3'AGTGATTACCCGCTGAACT5' Fas R, 5'CTGAGAAAGTGCACTGACAA3'. The product size was 618 bp. PCR reaction was carried out in a thermal cycler (Tehne USA) with *Taq* DNA polymerase (Fermentas, USA). The reaction mixture consisted of 10x PCR buffer 2.3 µl, $MgCl_2$ (25mM) 2.5 µl, dNTPs (10mM) 1.0 µl, P-1 (Forward) 1.0 µl, P-2 (Reverse) 1.0 µl, dH_2O 6.8µl, *Taq* DNA polymerase 0.4µl and Extracted DNA 5.0 µl. The amplification was performed with 5µL of extracted DNA by using 10 Pico moles of forward and reverse primers. The cycle condition for PCR is given below.

PCR program for *Fasciola hepatica.*

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Table 1: PCR Cycle setup for *Fasciola hepatica*

Stage	Cycle	Step	Temperature	Time
1	1	1	94℃	3:00 min
2	35	1	94℃	30 sec
		2	60℃	30 sec
		3	72℃	60 sec
3	1	1	72℃	5:00 min
		2	4℃	2:00 min

Gel Electrophresis

The specific amplified product was compared with 50bp DNA ladder marker as size marker (Fermentas USA). The parasitic DNA was recognized by Gel Electrophoresis. The gel was prepared in 2% agarose.

RESULTS

In the current study a total of 300 water samples were collected from four selected places of District Nowshehra. The number of samples collected from different sources were as, Bore well=40, Open well=100, Tap=60, and Drain=100. The samples were examined by means of PCR for detection of *Fasciola hepatica* DNA. Out of the total 300 samples 29/300(9.66%) were found positive. Among these samples the prevalence of *Fasciola hepatica was* 10% in Tube well water, Open well water 8%, 1.66% in tap water and 16% drain water.

Prevalence of *Fasciola hepatica* in different areas of District Nowshehra

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After DNA amplification through PCR result showed variation in different areas of Nowshehra. In Pubbi all the samples collected from Bore well were negative and Open well showed 3(12%) of the total 25 samples. In Pubbi 1(6.66%) sample were positive among 15 samples collected from Tube wells while Drain water showed 4(16%) positive samples of the total 25 collected samples. In Akora Khattak out of 10 tube well samples 2(20%) were positive. Out of 25 open well 1(4%) was positive for *Fasciola hepatica* and in drain water 4(16%) were found positive while all tap samples were negative for *Fasciola hepatica*.

In Nowshehra city 1 out of 10 (10%) samples were positive for *Fasciola hepatica* collected from tube wells. In open well 2 (8%) were positive in 25 samples of open wells, while in drain water 2(8%) were positive out of 25 samples. In tap water all the 15 samples were negative. Similarly in Aza Khel 1 out of 10 (10%) samples were positive for *Fasciola hepatica* collected from tube wells. In open well 2 (8%) were positive in 25 samples, while in drain water 6(24%) were positive out of 25 samples. In tap water all the 15 samples were negative.

Table 2: Prevalence of *Fasciola hepatica* in different areas of District Nowshehra

Area	**Bore well +ve/Total (%)**	**Open Well +ve/Total (%)**	**Tap +ve/Total (%)**	**Drain +ve/Total (%)**	**Overall +ve/Total (%)**
Pubbi	0/10 (0.0)	3/25 (12)	1/15 (6.66)	4/25 (16)	8/75 (10.66)
Akora khattak	2/10 (20)	1/25 (4)	0/15 (0)	4/25 (16)	7/75 (9.33)

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Nowshehra city	1/10 (10)	2/25 (8)	0/15 (0)	2/25 (8)	5/75 (6.66)
Aza Khel	1/10 (10)	2/25 (8)	0/15 (0)	6/25 (24)	9/75 (12)
Total	4/40 (10)	8/100 (8)	1/60 (1.66)	16/100 (16)	29/300 (9.66)

Discussion

In the present study, *Faschiola hepatica* was found in Tube well, Open well, tap and drain water in Nowshehra district of Khyber Pakhtunkhwa province of Pakistan. Of all the samples, 9.66% (29/300) contained parasites. *Fasciola hepatica* DNA was found in 9.66% (29/300).

The results of the study confirmed the findings of clinical studies conducted that had shown the presence of this parasite in the human population (Guerrant, 1970). *Fashiola hepatica* is considered one of the leading cause of waterborne diseases in the studies conducted by (Guerrant, 1970; Furness *et al.*, 2000).

Similar studies conducted in Sri Lanka also showed the levels and concentrations of *Fashiola hepatica* species although these were higher than the result of the present studies from other countries (WHO, 2004; Solo *et al.*, 1998: Quintero and Ledesma 2000). This could be due to the different environmental and geographical distribution of the country and locality. In the present study, *Fashiola hepatica* was found in all the water sources and were most numerous in drain water. In the current study, *Fasciola* eggs

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were recovered from all the water sources. The recent longitudinal studies reported the finding of these parasites in the water sources throughout the year (Wallis *et al*., 1996: Black *et al*., 1977; Walsh, 1986; Chapman, 1988). In other studies, *Fasciola hepatica* was recovered from the sewage waters and stool (Hernandez-Chavarria and Avendano, 2001). Possible sources of water contamination including both human and animal sources are known to be important in the introduction of parasites to water systems (WHO, 2004). In Jhelum valley (AJK), sheep and goats were found to be infected with a variety of parasites from July to August. Among these, fasciolosis (73.2 percent) was most prevalent (Hashmi and Muneer, 1981). In Baluchistan province, Naseer Ahmed (1984) concluded five million sheep and goats were suffering from fasciolosis. Similarly, domesticated animals in Sindh province revealed heavy infection of *F. hepatica*. Moreover, *F. gigantica* was reported at high altitudes in N.W.F.P province; whereas *F. hepatica* occurred in deltoic regions of Punjab and Sindh provinces, Pakistan (Bureiro *et al*., 1984). Similar, findings were previously reported by Kendall (1954). In Faisalabad district (Central Punjab), overall prevalence of fasciolosis was found to be 17.55 percent, of which *F. hepatica* was 5.7 percent. However mixed infection was revealed in 2.02 percent animals (Hayat *et al.*, 1986).

Molecular techniques such as PCR show promise for the rapid detection of oocysts from the environment. However, prior to environmental isolation and detection of *F. hepatica* eggs a robust and sensitive detection method must first be developed.

This method can provide positive confirmed results in less than 1 day and detect fewer than 50 oocysts.

Conclusions and Recommendations

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Water sources acts as vehicles for spread of parasites. This study concludes that zoonotic parasites can easily reach water sources and causes different ailments like fascioliasis. To minimize the health hazards due to zoonotic parasites pure and filtered water should be used. Moreover water sources may have proper sanitary measures to avoid spread of parasites.

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