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Molecular detection of Fasciola hepatica and *Toxoplasma gondii* in water sources of district Nowshehra

Imran Khan[1] Shaukat Ali Khan[2] Noorul Akbar[3]

1. College of Life science 100875 Beijing Normal University P,R China.
2. School of Zoology Anhui Normal university P,R China
3. Department of Zoology Kohat University of Science and Technology kohat 26000 Khyber Pakhtunkhwa Pakistan

For correspondence Imran_talash76@yahoo.com

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). Among different water sources 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). Toxoplasmosis 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 Toxoplasma gondii. The overall prevalence in different water sources was 6.66 % (17/300). Among different water sources highest prevalence was recorded in drain water 7% (7/100) followed by tube well water 7.5% (3/40), open well water 5 % (5/100) and the lowest was recorded in tap water 3.33 %(2/60). 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, Toxoplasma gondii, PCR, and zoonotic parasites.

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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. 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 [1]. 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. The disease which occurs due to unhygienic water sources or reservoirs propagates at an alarming rate. Moreover waterborne diseases produce huge economic hazards in most parts of the globe. The causative agents of water borne diseases are mainly parasites. In the world known history about 325 waterborne parasitic outbreaks has been documented [2-5]. To minimize the harm caused by parasitic diseases use of healthy water is being highly emphasized [6]. 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 [7].

Water-borne diseases are "dirty-water" diseases; mainly attributes to water that has been Contaminated by human, animals or chemical wastes. According to an authentic survey, it has been shown that water-borne diseases are responsible for over 12 million deaths worldwide annually. This is mainly due to poor sanitation facilities; and unsafe drinking, washing, and cooking water consumption [8].

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.

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 [9].

The disease caused by *F. hepatica* is known as fascioliasis. Formerly fascioliasis was a disease of domestic animals such as sheep and cattle, but now emerging an important chronic disease in humans, with 2.5 million people at risk in the highly endemic areas of the world [10]. *F. hepatica* is a waterborne parasite [11]. The distribution of *F. hepatica* is cosmopolitan being reported in developed and under-developed countries. Human fascolisis is recently treated as an emerging disease [12]. The liver fluke causes important veterinary and public health problems worldwide [13]. This parasitic trematode, secretes specific enzymes to assist in burrowing through the gut wall and liver of its mammalian host before reaching in the bile ducts [14].

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 [15]. Fascioliasis is more familiar in sheeps. Human beings may get the infection accidentally from contaminated water or plants in endemic areas [16]. 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 [2].

Toxoplasma gondii (*T. gondii*) is a species of parasitic protozoa in the genus Toxoplasma. This protozoan was first discovered in 1908 from an African rodent *Ctenodactylus gundii*. In 1909 the disease was differentiated from Leishmania. The first recorded congenital case of toxoplasmosis happened in 1923. The disease caused by *T. gondii* (Toxoplasmosis) is generally asymptomatic (having no symptoms) in immunocompetent individuals. Toxoplasmosis is severe in AIDS patients [17].

Infection caused by *T. gondii* is more common in human beings and less common in all warm blooded creatures throughout the world. Every third person in the world has been exposed to toxoplasmosis. However the incidence of the disease varies in different countries, different geographical areas within one country and different ethnic groups living in a common area [18]. The greatest noticeable outbreak of toxoplasmosis occurred in British Columbia in 1994 and suffered around 8000 people. This outbreak was due to contaminated drinking water. Water is contaminated by faeces of infected cats, which acts as definitive hosts for *F. hepatica* [19].

According to an estimation, 30% to 65% of people are infected with toxoplasmosis [18]. Behavioral changes also occur in infected persons such as slow reaction rate and an increased risk of traffic accidents [20]. Congenital toxoplasmosis is a significant problem that is dangerous for foetus and infants if the mother suffers from primary infection during pregnancy. People may be infected with *T. gondii* mainly by consumption of raw and undercooked meat or by ingestion of oocysts present in the environment (water, soil, fruits, and vegetables) contaminated with the faeces of infected cats, the only definitive hosts of the parasite [21]. Cats play a significant role in the epidemiology of toxoplasmosis. Oocysts are resistant to unfavourable environmental conditions and chemical inactivation. They are capabale of infection in water for up to 54 months and in soil for around an year [23]. Prevalence is higher where there is a preference for less-cooked meat and proximity to cats [22]. The basic environmental matrices like water and soil are important sources of human contamination by *Toxoplasma* oocysts.

The present study is being conducted in order to determine the prevalence of selected Zoonotic parasites present in water sources of District Nowshehra Khyber Pakhtunkhwa Pakistan. The main objectives of this study were to detect *Fasciola hepatica* and *Toxoplasma gondii* in different water sources of District Nowshehra with molecular techniques and know the correlation of *Fasciola hepatica* and *Toxoplasma gondii* in different water sources of the above mentioned District.

MATERIALS AND METHODS

Samples collection

Around 300 water samples were collected from four selected places of District Nowshehra named, AkoraKhattak, Ezakhel, Pubbi and Nowshehra city. Water sources comprised of wells, rivers, tube wells, ponds, and ditches. The volume of each collected sample was 1liter. The samples were brought to the Zoology laboratory, Department of Zoology, Kohat University of Science and Technology (KUST) for further processing and storage.

Processes and Storage

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 it for 8 minutes at 14000 rpm. The bottom 200 µl 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 Lysis

The main steps of the method are below

Took 125µl from the sample and add 250µl DNA zol.

Mixture was mixed properly through vortex and incubated at room temperature for 5 minutes.

DNA Precipitation

Then 150µl of iso-propanol was added to the mixture and centrifuged at 7000 rpm for 10 minutes. After Centrifugation the supernatant was removed and 125µl DNA zol was added to the DNA pellet and centrifuged at 7000 rpm for 5 minutes.

DNA Wash

150µl of 70% ethanol was added to the pellet after discarding the supernatant and centrifuged at 7000 rpm for 5 minutes. Discarded the supernatant. The DNA wash step was repeated and the tubes were stored vertically to dry for 10 minutes.

DNA Preservation

Then 40µl of distilled water was added to the pallet and incubated at 55°C for 10 minutes in Hotplates, and were kept at -40 ^{0}C till use.

Amplification

The DNA was amplified through Polymerase Chain Reaction (PCR) using primers specific for *F. hepatica* and *T. gondii*. 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 first round of amplification was performed with 5 µl of DNA by using one sense primer (Primer-1) and the other with anti-sense primer (Primer-2). Reactions were carried out in a thermal cycler (Techne USA) with *Taq* DNA polymerase. The reaction mixture for a single reaction consisted of:

Components of PCR mixture for a single reaction.

1. 10x PCR buffer----------2.3 µl
2. $MgCl_2$ (25mM) ----------2.5 µl
3. dNTPs (10mM) ----------1.0 µl
4. P-1 (Forward) ----------1.0 µl
5. P-2 (Reverse) ----------1.0 µl
6. dH_2O----------6.8µl
7. *Taq* DNA polymerase----------0.4µl
8. Extracted DNA ----------5.0 µl

The reaction mixture was same for both parasites except for primers. The primer added for *F. hepatica* was Fas-F, 5'AGTGATTACCCGCTGAACT3', Fas-R, 3'CTGAGAAAGTGCACTGACAA5'. The product size was 618 bp. The primer used for detection of *T. gondii* was targeting B1 gene and the sequence was Toxo-1, 5'GGAACTGCATCGTTCATGAG 3'and Toxo-2, 3'TCTTAAAGCGTTCGTGGTC5'.

DNA Amplification (PCR)

PCR reaction was carried out in a thermal cycler (Tehne USA) with *Taq* DNA polymerase (Fermentas USA). 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.*

Table 3.1: PCR Cycle setup for *Fasciola hepatica*

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

Table 3.2: PCR program setup for *Toxoplasma gondii.*

Stage	Cycle	Step	Temperature	Time
1	1	1	95 °C	7:00 min
2	35	1	92 °C	30 sec
		2	55 °C	50 sec
		3	72 °C	30 sec
		1	72 °C	5:00 min
3	1	2	4 °C	2:00 min

Gel electrophoresis

The 10µL of PCR product mixture was mixed with 2µL loading dye and loaded in agarose gel which was prepared by dissolving 2 gram of agarose in 100 ml of 0.5X TBE buffer in reaction bottle and boiled it for 2.5 minutes. Then the bottle was cooled down at room temperature up to 45°C and 0.5µl Ethidium bromide (1µg/L) was added. Combs were fixed and flask contents were poured into gel caster. After 15 minutes gel was formed and Combs were removed. Gel caster was placed in gel tank containing 1000mL 0.5x TBE buffer. Then 10µl of each sample was loaded in the wells and 10µl of DNA Ladder (50bp). The gel was run for 25 minutes at voltage of 120 volts and 500 ampere current. Gel was then examined under UV transilluminator and Gel documentation system was utilized for Picture record.

The specific DNA amplified product of each sample was determined by identifying the 618-bp band for *Fasciola hepatica* and 193-bp band for *Toxoplasma gondii* and comparing with 50-bp DNA Ladder (Fermantas Germany), used as size marker.

Statistical analysis

The data was analyzed by using the univariate ANOVA and $P<0.05$ values were considered significant.

RESULTS

In the current study a total of 300 water samples were examined by means of PCR, which indicated 9.66% prevalence of *Fasciola hepatica*. 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. Similarly *Toxoplasma gondii* was detected in 5.66% as 7.5 % in Tube well water, 5%Open well water, 3.33 % in tap water and 7 % drain water.

Table 4.1: Water samples collected from different areas of District Nowshehra

Area	Bore well	Open well	Tap	Drain	Total
Pubbi	10	25	15	25	75
Akora khattak	10	25	15	25	75
Nowshehra city	10	25	15	25	75
Aza Khel	10	25	15	25	75
Total	40	100	60	100	300

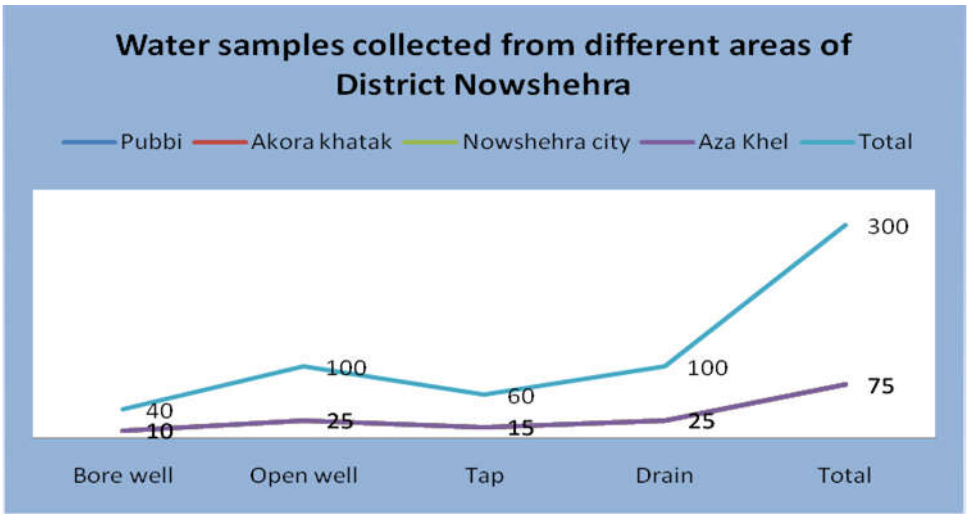

Figure 4.1 Water samples collected from different areas of District Nowshehra

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

After DNA amplification through PCR result showed variation in different areas of Nowshehra. Tube well water showed 10 % positive result for *Fasciola hepatica*. While from Pubbi out of 25, 3(12 %) samples were positive for *Fasciola hepatica* in open well, 4 (16%) were positive in drain water in the of 25 collected samples while in tap 1 (6.66%) sample was positive out of 15 and in tube well all 10 samples were found negative.

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 4.2: 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)
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)

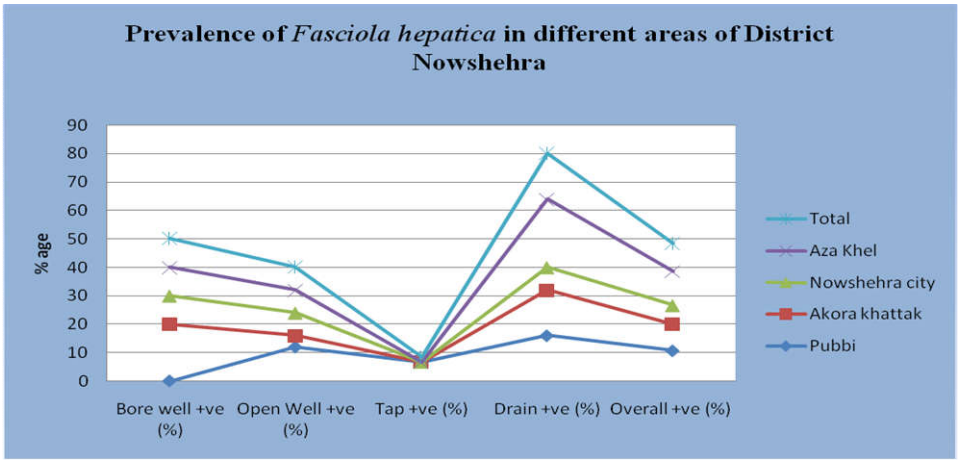

Fig 4.2; Prevalence of *Fasciola hepatica* in different areas of District Nowshehra

Prevalence of *Toxoplasma gondii* in different areas of District Nowshehra

The total 300 water samples collected from district Nowshehra showed the following results for *Toxoplasma gondii* in different areas. Drain and open well water were more contaminated than tap and tube well water. In drain water 7 samples out of 100 (7%) were positive for *Toxoplasma gondii* and in open well water 5 (5%) out of 100 samples were positive for *Toxoplasma gondii*. While tap water showed 2 positive out of 60 (3.33%) for *Toxoplasma gondii* and tube well water showed 3 (7.5) positive samples out of 75.

In Akora Khattak drain and open well water showed 4% and 8% positive results respectively for *Toxoplasma gondii* while tube well and tap water failed to show any positive results for the parasite.

In Nowshehra City 75 samples of water containing 10 from tube well, 25 from well, tap 15 and drain 25 respectively showed high prevalence rate 12% for *Toxoplasma gondii* in drain, well, 8%, tube well showed 10% and 0% in tap water for the parasite.

In Aza Khel the results were as 4%, 6.66%, 0.0% and 10% in drain, tap, open well and tube well water respectively for *Toxoplasma gondii* as shown in table 4.3.

Table 4.3: Prevalence of *Toxoplasma gondii* 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	1/10 (10)	1/25 (4)	1/15 (6.66)	2/25 (8)	5/75 (6.66)
Akora khattak	0/10 (0.0)	2/25 (8)	0/15 (0)	1/25 (4)	3/75 (4)
Nowshehra city	1/10 (10)	2/25 (8)	0/15 (0)	3/25 (12)	6/75 (8)
Aza Khel	1/10 (10)	0/25 (0)	1/15 (6.66)	1/25 (4)	3/75 (4)
Total	3(7.5)	5(5)	2(3.33)	7(7)	17(5.66)

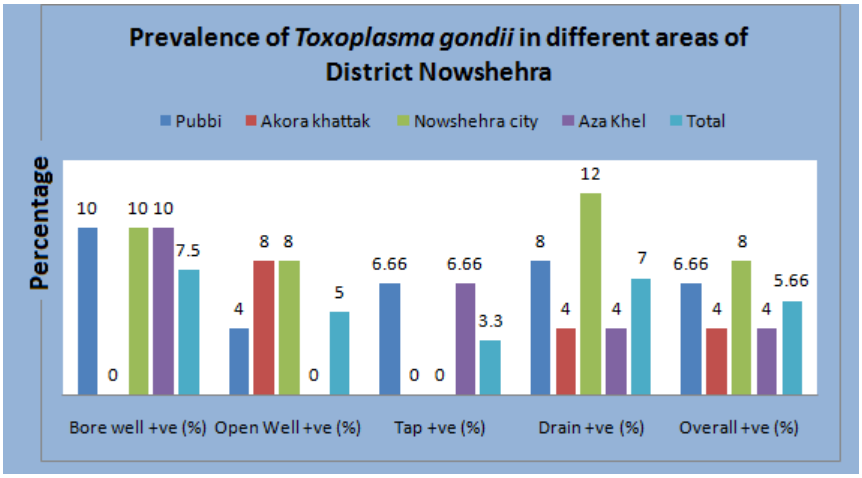

Figure 4.3 Prevalence of *Toxoplasma gondii* in different areas of District Nowshehra

Over all prevalence of *Fasciola hepatica* and *Toxoplasma gondii* in different water sources of district Nowshehra

In district Nowshehra out of 300 water samples showed high prevalence rate 16% in drain water 1.66% in tap water, 8% in open well water and 10 % in tube well water for *Fasciola hepatica*.

In district Nowshehra out of 300 water samples showed high prevalence rate 7% in drain water 3.33% in tap water, 5% in open well water and 7.5% in tube well water for *Toxoplasma gondii*.

Table 4.4: Overall Prevalence of *Fasciola hepatica* and *Toxoplasma gondii* in different water sources of District Nowshehra.

Source of water	Bore Well Water, n=40					Open Well Water, n=100					Tap Water, n=60					Drain Water, n=100					Grand Total, n=300
Area	**Pubbi (10)**	**Akora Khatak (10)**	**Nowshehra city (10)**	**Aza Khel (10)**	**%**	**Pubbi (25)**	**Akora Khatak (25)**	**Nowshehra city (25)**	**Aza Khel (25)**	**%**	**Pubbi (15)**	**Akora Khatak (15)**	**Nowshehra city (15)**	**Aza Khel (15)**	**%**	**Pubbi (25)**	**Akora Khatak (25)**	**Nowshehra city (25)**	**Aza Khel (25)**	**%**	**%**
F.hepatica	0	2	1	1	10%	3	1	2	2	8%	1	0	0	0	1.66%	4	4	2	6	16%	9.66%
T.gondii	1	0	1	1	7.5%	1	2	2	0	5%	1	0	0	1	3.33%	2	1	3	1	7%	5.66%

Prevalence of *Fasciola hepatica* and *Toxoplasma gondii* in different sources

In the study area different water sources were examined for *Fasciola hepatica* through PCR. The results revealed that *Fasciola hepatica* was found 10% (4/40) in tube well, 8% (8/100) in open well water, 1.66% (1/60) in tap water while drain water showed high prevalence 16% (16/100).

Similarly the different water samples were examined for *Toxoplasma gondii* which showed that the prevalence was 7.5% (3/40) in tube well water, 5 % (5/100) in open well water, 3.33% (2/60) in tap water, while drain water showed 7% (7/100) prevalence rate.

Table 4.5: Over all prevalence of *Fasciola hepatica* and *Toxoplasma gondii* in different areas of District Nowshehra.

Area	Pubbi, n= 75					Akora Khattak, n= 75					Nowshehra city, n=75					Aza Khel n= 75					Grand Total
Source of Water	Bore well (6)	Open well (43)	Tap (18)	Drain (8)	%	Bore well (10)	Open well (46)	Tap (16)	Drain (8)	%	Bore well (11)	Open well (35)	Tap (35)	Drain (9)	%	Bore well (7)	Open well (25)	Tap (30)	Drain (13)	%	n= 300 (%)
Fasciola hepatica	0	3	1	4	10.66	2	1	0	4	9.33	1	2	0	2	6.66	1	2	0	6	s12	29/300 (9.66%)
Toxoplasma gondii	1	1	1	2	6.66	0	2	0	1	4	1	2	0	3	8	1	0	1	1	4	17/300 (5.66%)

DISCUSSION

In the present study, *Fashiola hepatica* and *Toxoplasma gondii* were found in Tube well, Open well, tap and drain water in Nowshehra district of Khyber Pakhtunkhwa province of Pakistan. Of all the samples, 15.33% (46/300) contained parasites. Amongst these *Fasciola hepatica* and *Toxoplasma gondii* were 9.66% (29/300) and 6.66 % (17/300) respectively.

The results of the study confirmed the findings of clinical studies conducted that had shown the presence of these two parasites in the human population [24]. Both *Fashiola hepatica* and *Toxoplsma gondii* were considered two of the leading causes of waterborne diseases in the studies conducted by [24, 19].

This study is one of the first investigations describing the development and optimization of a sensitive molecular detection method for *T. gondii*oocyst DNA. Little information has been gathered on the presence of oocysts in environmental waters, and evaluation of *T. gondii*oocyst contamination of water has been limited by a lack of reliable detection methodologies [19].

Similar studies conducted in Sri Lanka also showed the levels and concentrations of *Fashiola hepatica* and *Toxoplsma gondii* species although these were higher than the result of the present studies from other countries [25, 26]. This could be due to the different environmental and geographical distribution of the country and locality. In the present study, *Fashiola hepatica* and *T. gondii* oocysts were found in all the water sources and were most numerous in drain water. According to the recent report that water borne transmission of *T. gondii* is uncommon but a large human outbreak linked to contamination of a municipal water reservoir by wild felids and the widespread infection by marine mammals [27]. In the current study, *Fasciola*eggs and *T. gondii* cysts were recovered from all the water sources. The recent longitudinal studies reported the finding of these parasites in the water sources throughout the year [28-

31]. *T. gondii* is responsible for approximately 100,000 deaths worldwide each year, making it second only to malaria as a cause of mortality due to parasite [29]. In other studies, *T. gondii* and *Fasciola hepatica* was recovered from the sewage waters and stool [8]. Possible sources of water contamination including both human and animal sources are known to be important in the introduction of parasites to water systems [25].

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 [32]. In Baluchistan province, Naseer Ahmed [33] 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 [34]. Similar, findings were previously reported by Kendall [25]. 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 [35].

Molecular techniques such as PCR show promise for the rapid detection of oocysts from the environment. However, prior to environmental isolation and detection of *T. gondii* oocysts, 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. The described *T. gondii* oocyst DNA recovery, amplification, and detection system will facilitate the development and optimization of a method for the isolation of low numbers of *T. gondii* oocysts from large-volume water samples.

CONCLUSIONS AND RECOMMENDATIONS

The burden of parasitic diseases is high, and water-borne diseases can be prevented at community level, instead of managing them at a tertiary hospital. Therefore, health education and provision of safe drinking water and adequate sanitation should be the priority. Outbreak or rapid response teams should be established at all levels of the health care system.

REFERENCES

1. Tauxe, R.V. (2002). Emerging food borne pathogens. *International Journal of Food Microbiology.* **78**: 31-41.
2. World Health Organization (1995). Control of foodborne trematode infections. WHO Tech.Report Series, **849**: 1-157.
3. Aramini, J.J., Stephen, C., Dubey, J.P., Engelstoft, C., Schwantje, H. and Ribble, C.S. (1999). Potential contamination of drinking water with *Toxoplasma gondii* Oocysts. *Epidemiology Infection*. **122**:305–315.
4. Andrews, S.J. (1999). The Life Cycle of *Fasciola hepatica*, in: Dalton J.P. (Ed.), CAB International, Oxon, pp. 1–29.
5. Bahia-Oliveira, L.M., Jones, J.L., Azevedo-Silva, J., Alves, C.C., Orefice, F and Addiss, D.G (2003). Highly endemic waterborne toxoplasmosis in North Rio de Janeiro State, Brazil. Emerg Infect Dis **9**: 55-62.
6. Beazley, D.M and Egerman, R.S. (1998). Toxoplasmosis. Semin Perinatology. **22**:332–338.
7. Benenson, M.W., Takafuji, E.T., Lemon, S.M., Greenup, R.L and Sulzer, A.J (1982). Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. N Engl J Med **307**: 666-669.
8. Hernandez, C.F and L, Avendendo, (2001). Simple modification of the Baermann method for diagnosis of Strongyloidiasis. Mem Inst Oswaldo Cruz, Rio de Janeiro, **96**: 805-807.
9. Smith, M. C. and Sherman, D. M. (2009). *Goat Medicine* (2nd ed.). Ames, Iowa: Wiley-Blackwell.
10. Mas-Coma, S., M. D. Bargues and M. A. Valero. (2005). Fascioliasis and other plant- borne trematode zoonoses. Int. J. Parasitol., **35**: 1255–1278.
11. Mas-Coma, S., Valero, M.A. and Bargues, M.D. (2009). Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, genetics, molecular epidemiology and control. Adv Parasitol 69:41
12. Mas-Coma, M.A., Valero, M.D. and Bargues. (2009). Fasciola lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. 69: 41–146.
13. Hurtrez-Boussès, S., Meunier, C., Durand, P. and Renaud, F. (2001). Dynamics of host-parasite interactions: the example of population biology of the liver fluke (Fasciola hepatica). Microbes and Infection. 3: 841–849.
14. Dalton, J.P. and Heffernan, M. (1989). Molecular Biochemistory. Parasitology.**35**: 161-166.
15. Mas-Coma, M. S., Esteban, J. G. and Bargas, M. D. (1999). Epidemiology of human fasciolosis: a review and proposed new classification. Bull World Health Organization. **77**: 340–346.
16. Mohsen, M. and Mardani, M. (2008). *Fasciola hepatica*: A cause of obstructive Jaundice in an elderly man from Iran. *Saudi Journal of Gastroenterology.* **14**: 208-210.
17. Israelski, D.M. and Remington, J.S. (1993). Toxoplasmosis in the non-AIDS immuno-compromised host. *Current Clinical Infectious Diseases*. **13**: 322–356.
18. Tenter, A.M., Heckeroth, A.R. and Weiss, L.M. (2001). *Toxoplasma gondii* from animals to humans. International Journal of Parasitology 31(**2**), 217-220.

19. Isaac-Renton, J., W. R. Bowie, A. King, G. S. Irwin, C. S. Ong, C. P. Fung, M. O. Shokeir, and J. P. Dubey. (1998). Detection of *Toxoplasma gondii* Toxoplasma gondii oocysts in drinking water. Appl. Environ. Microbiol. 64**:**2278-2280.
20. Flegr, J., Klose, J., Novotna, M., Berenreitterova, M. and Havlicek, J. (2009). Increased incidence of traffic accidents in Toxoplasma-infected military drivers and protective effect RhD molecule revealed by a large-scale prospective cohort study. *BMC Infectious Diseases.* **9**: 72.
21. Fayer, R., Dubey, J.P. and Lindsay, D.S. (2004) Zoonotic protozoa from land to sea. Trends Parasitol **20**:531–536.
22. Meerburg, B.G. and Kijlstra, A. (2009). Climate changing pathogens: *Toxoplasma gondii* in North-Western Europe. *Parasitology Research.* **1**: 17.
23. Dumetre, A. and Darde, M.L. (2003). How to detect *Toxoplasma gondii* oocysts in environmental samples. *FEMS Microbiology Review*. **27**:651–661.
24. Guerrant, R.l (1970). Cryptosporidiosis: an emerging, highly infectious threat. Emerging Infectious Diseases, **3**: 51-57.
25. World Health Organization,(2004). Guidelines for Drinking-water quality. 3rd edition, Geneva. pp: 121-144.
26. Solo, G.H.M, A.A LeRoy, L.J Fitzgerald, J.M Dubon, S.M Neumeister and M.K Baum, (1998).Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in water supplies of San Pedro Sula, Honduras. Revista Panamericana de Salud Publica, 4: 398-400.
27. Dubey, J.P., D.E. Hill and C. Sreekumar. (2005). Toxoplasmosis, In Kahn, C.M. Eds. The Merck Veterinary Manual Ninth Edition, Publication Services, Merck and CO., White House Station,New Jersey. **1**: 549.
28. Wallis, P.M, S.L Erlandsen, R.J.L lsaac, M.E Olson, W.J Robertson and H Vankeulen, (1996).Prevalence of Giardia Cysts and Cryptosporidium oocysts and characterization of Giardia spp. Isolated from drinking water in Canada. Applied and Environmental Microbiolgy, 62: 2789-2797.
29. Walsh, J.A, (1986). Problems in recognition and diagnosis of amebiasis: estimation of the Global magnitude of morbidity and mortality. Reviews of Infectious Diseases, **8**:228–238.
30. Black, R.E, Dykes, A.C, Sinclair, S.P and Wells, J.G, (1977). Giardiasis in day-care centers: evidence of person-to-person transmission. Pediatrics, 60: 486–491.
31. Chapman, P.A, (1988). Cryptosporidiosis: recent trends in epidemiology, diagnosis, and treatment. Serodiagnosis and Immunotherapy in Infectious Disease, 2: 311–317.
32. Hashmi, H.A and M. A. Muneer. (1981). Subclinical mastitis in buffaloes at Lahore. Pak.Vet. J. **1**(4): 164.
33. Naseer, Ahmed. (1984). Fasciolosis in Baluchistan. Pak. Vet. J., **4**(1): 44.
34. Buriro, S. N., M. S. Phulan and B. M. Junejo. (1984). Epizootiology of fascioliasis in Livestock in Sind. Pak. Vet. J., 4(1): 29-30.
35. Hayat, C. S., Z. Iqbal, B. Hayat and M. Nisar Khan. (1986). Studies on the seasonal Prevalence of fasciolosis and lungworm disease in sheep at Faisalabad. Pak.Vet. J., 6 (3): 131.

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