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Microscopic detection of *Fasciola hepatica* in drinking water sources of district Dera Ismail Khan, KP, Pakistan

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Abstract
A study was conducted to determine the presence of zoonotic parasite (*Fasciola hepatica*) in drinking water sources of five different areas madina colony, islamiacolony, deyal road, zafrabad colony and dikhan city in district D.I. Khan, Khyber Pakhtunkhwa and their zoonotic potential. A total of 200 water samples were collected from the three different sources such as tap water, pond water, drain water. The water samples were filtered through Whatt-man filter paper (USA) in water filtration assembly and the residual solid particles containing parasites were collected from the filter paper. The filtered residue was centrifuged at 4000 rpm for 5 minutes, the supernatant was discarded and the pallet was preserved in an eppendorf tubes and stored at 4 °C for further process. In the present study, overall prevalence was 5% (10/200). The presence of *Fasiola hepatica*n tap water was 1% **(**1/100**)**, in pond water was 6% (3/50) and in drain water was 12% (6/50).

Keywords: parasite, water, faciola, zoonosis, protozoan, DI khan

1. Introduction
Water is a transparent fluid which forms the world's streams, lakes, oceans and rain, and is the major constituent of the fluids of organisms. As a chemical compound, a water molecule contains one oxygen and two hydrogen atoms that are connected by covalent bonds. Water is a liquid at standard ambient temperature and pressure, but it often co-exists on Earth with its solid state, ice; and gaseous state, steam (water vapor). It also exists as snow, fog, dew and cloud. Water covers 71% of the Earth's surface [1]. It is vital for all known forms of life. On Earth, 96.5% of the planet's crust water is found in seas and oceans, 1.7% in groundwater, 1.7% in glaciers and the ice caps of Antarctica and Greenland, a small fraction in other large water bodies, and 0.001% in the air as vapor, clouds(formed of ice and liquid water suspended in air), and preparation [2, 3]. Only 2.5% of this water is freshwater, and 98.8% of that water is in ice (excepting ice in clouds) and groundwater. Less than 0.3% of all freshwater are in rivers, lakes, and the atmosphere, and an even smaller amount of the Earth's freshwater (0.003%) is contained within biological bodies and manufactured products. A greater quantity of water is found in the earth's interior [4].

Fasciola hepatica, also known as the common liver fluke or sheep liver fluke, is a parasitic trematode (fluke or flatworm, a type of helminth) of the class trematoda, phylum platyhelminthis that infects the livers of various mammals, including humans. The disease caused by the fluke is called fasciolasis or fasciolosis, which is a type of helminthiasis and has been classified as a neglected tropical diseases [5]. *F. hepatica* is distributed worldwide, has been known as an important parasite of sheep and cattle for hundreds of years and causes great economic losses in sheep and cattle. Because of its size and economic importance, it has been the subject of many scientific investigations and may be the best-known of any trematode species. It is one of the largest flukes, measuring up to 3.5 cm by 1.5 cm. The parasite lives in the liver and bile duct. Its hosts include herbivorous mammals and it is found in 46 species of domestic and wild animals as well as in man. The intermediate host is the *Lymnaea* genus of snail which lives in marshy areas and standing water. *F. gigantica* may also cause similar human disease, and several other species cause disease in animals. *Fasciolahalli* and *Fasciolacalifornica* infest sheep and cattle in the USA and may be synonymous with

Fasciolajacksoni which infests elephants in Africa and India, *Fasciolanyanzae* whose host is the hippopotamus, and *Fasciola magna* which infests mostly in deer, but also in cattle and sheep but very rare. Classic examples of parasitism include interactions between vertebrate hosts and tapeworms, flukes, the Plasmodium species, and fleas. Parasitism differs from the parasitoid relationship in that parasitoids generally kill their hosts [6, 7]. The aim of the research work was to find out the microscopic detection of *fasciola hepatica* in drinking water of D.I. Khan KP, Pakistan

2. Materials and Methods

2.1 Sample collection

A total of 200 water samples were collected from different areas of District DIK for the detection of *Fasciola hepatica* including tap water, pond water and drain water according to the research plan of sample collection. One liter of each water sample collected in sterilized bottle, labeled (date of collection, name of area and type of water) and was transported to the Laboratory of Zoology Government Degree College No 1 Dera Ismail Khan for further processing.

Research plan of sample collection

Areas	Tap Water	Pond water	Drain water	Total
Madina Colony	20	10	10	40
Islamia Colony	20	10	10	40
Deyal Road	20	10	10	40
Zafrabad Colony	20	10	10	40
DIKhan City	20	10	10	40
Total	100	50	50	200

2.2 Processing of water samples

The water samples were filtered through Whatt-man filter paper (USA) in water filtration assembly and the residual solid particles containing parasites were collected from the filter paper. The filtered residue centrifuge at 4000 rpm for 5 minutes, the supernatant was discarded and the pallet was preserved. The pallet were further centrifuged at 4000 rpm for 5 minutes, Then the supernantant was discarded and the pallet were preserved in an eppendorf tubes and stored at 4°C for further process.

2.3 Giemsa stain preparation

- Dissolve 3.8g of Gimsa powder into 250ml of ethanol.
- Heat solution fromstep 1 to 60 °C.
- Slowly add in 250ml of glycerin tp the solution from step 2.
- Filter the solution from step 3.
- The solution needs to stand a period of time prior to use. Although times varies based on who you ask a minimum of two months is usually recommended.

2.4 Slide preparation

- First 2 micro liter water were taken with the help of pipette on glass slide.
- Now air dry the drop of water on the slide.
- Add a drop of methanol for fixation.
- Add a drop of gimsa stain.
- Place the slide for 5 minute to dry.
- Now check the slide under microscope.

2.5 Microscopy

The prepared slides were examined under microscope 10X magnification power to detect the zoonotic parasite on the basis of size and morphology (Lindsay *et al*., 2000) and compared with positive slides of parasites.

2.6 Statistical analysis

SPSS 16.0 software is used for data analysis. P values less than 0.05 were considered to be statistically significant.

3. Results and Discussion

Fasciola trophozoites and eggs were found in all the water sources. The disease caused by the fluke is called fasciolasis or fasciolosis, which is a type of helminthiasis and has been classified as a neglected tropical diseases. *F. hepatica* is distributed worldwide, has been known as an important parasite of sheep and cattle for hundreds of years and causes great economic losses in sheep and cattle. Because of its size and economic importance, it has been the subject of many scientific investigations and may be the best-known of any trematode species.

It is one of the largest flukes, measuring up to 3.5 cm by 1.5 cm. The 1 ml of concentrated specimen represents a 1 liter of collecting raw water. The parasite lives in the liver and bile duct. Its hosts include herbivorous mammals and it is found in 46 species of domestic and wild animals as well as in man. The intermediate host is the *Lymnaea* genus of snail which lives in marshy areas and standing water. *F. gigantica* may also cause similar human disease, and several other species cause disease in animals. *Fasciola hepatica* has an indirectlifecycle with amphibious snails as intermediate hosts, typically from the genus *Lymnaea*.

Adult flukes produce eggs in the biliary ducts of their hosts. These eggs reach the gall bladder and are passed to the host's gut when the gall bladder is emptied. They are passively transported to the anus and are expelled with the feces. A single liver fluke can produce up to 25'000 eggs a day. The numbers of *f.hepatica* eggs in all the sites showed no observable pattern over the study period (March to July). Overall prevalence of *Fasciola hepatica* was 5% (10/200), while high prevalence was recorded in Drain water 12% (6/50), followed by pond water 6% (3/50) and the lowest was recorded in tape water 1% (1/100). (Table No.1 and fig, 1). The present study on zoonotic parasites detection were based on microscopic examination. The present study reported that *F. hepatica* were widely present in dikhan. This was a novel work which was carried out for first time in DIKhan. A total of 200 samples were examined, amongst these 100 samples were of tape, 50 were drainage and 50 were pond water each. Overall prevalence of parasite *f. hepatica* is 5%. In which tape water prevalence is 1% (1/100) and pond water prevalence is 6% (3/50) while the drain water prevalence is 12% (6/50).

The prevalence of *f. hepatica* in water samples of Madina colony is 2.5% (1/40), while the high prevalence was recorded in drain water is 10% (1/10), but the prevalence of f. hepatica in pond water and tape water was recorded as 0% (0/10) and 0% (0/20). The prevalence of *f. hepatica* in water samples of Islamia colony is 10% (04/40), while high prevalence was recorded in drain water is 30% (03/10), followed by pond water 10% (1/10) and the lowest prevalence was recorded in tape water 0% (0/20).

The prevalence of *f. hepatica* in water samples of Deyal road is 2.5% (1/40), while the higher prevalence was recorded in pond water is 10% (1.10), but the prevalence of *f. hepatica* in tape water and drain water is lowest is about 0% (0/20) and 0% (0/10). The prevalence of *f. hepatica* in water samples of Zafrabad colony is 5% (2/40), while the higher prevalence was recorded in pond water and drain water is 10% (1/10) and

10%(1/10), but the lowest prevalence was recorded in tape water is 0% (0/20).

The prevalence of *F. hepatica* in water samples of Dikhan city is 5% (2/40), while the high prevalence was recorded in tape water and drain water is 5%(1/20) and 10%(1/10), but the lowest prevalence was recorded in pond water is 0% (0/10).

In contrast result of studies conducted in Dikhan have marked differences, as out of 200 samples only 10 were positive for *Fasciola Hepatica*. While *f. hepatica* was examined in 1 % (1/100) and 12 % (12/50) of tape and drainage water samples as well as the *f. hepatica* was examined in 6% (03/50) of pond water respectively.

Table 1: Prevalence of *f. hepatica* in different water sources of District DIKhan (Madinacolony, Islamia colony, Zafrabad colony, Deyal road, Dikhan city) in KPK (March-July, 2016).

S. No	Location	*F. Hepatica* in water samples, %			
		Tape water	Pond water	Drain water	
1	Madina colony	0 20	0 10	1 10	01 40 2.5%
2	Islamia colony	0 20	1 10	03 10	04 40 10%
3	Deyal road	0 20	1 10	0 10	01 40 2.5%
4	Zafarabad colony	0 20	1 10	1 10	02 40 5%
5	DIKhan city	1 20	0 10	1 10	02 40 5%
Grand result + Total%		01 100 1%	03 50 6%	06 50 12%	10 200 5%

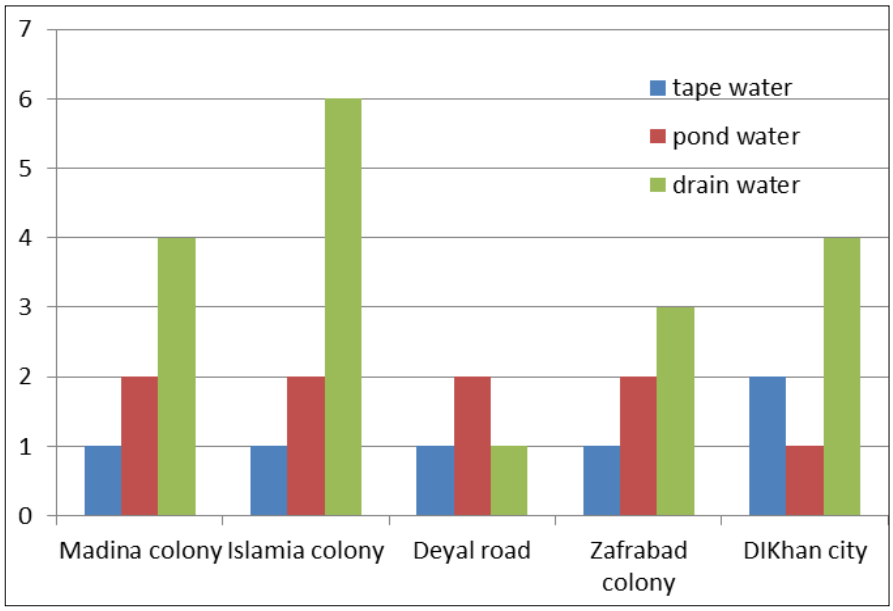

Fig 1: Prevalence of *F. hepatica* in drinking water of district D.I. Khan

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