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Detection of *Giardia lamblia* by microscopy in different water sources of district D.I Khan, KP, Pakistan

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Abstract
A total of 200 water samples were collected from different areas of District Dera Ismail Khan for the detection of *Giardia lamblia* including pond water, tap water and drain water each having 40 samples. In the present study a total of 200 water samples were examined by means of giemsa, which showed 21% (42/200) was of *Giardia lamblia,* of these samples the prevalence of *Giardia lamblia* was determined 15% in tap water, 24% pond water, and 30% in drain water.

Keywords: *Giardia lamblia*, pond water, tap water, drain water, microscopy

Introduction
Drinking water has been documented to be a possible vehicle for the spread of infections [1]. A great portion of the inhabitants in emergent countries suffers from health problems linked with either deficiency of drinking water or due to the occurrence of microbiological pollution in water [2]. Diseases caused due to contaminated water remains one of them major health problems in the world. Diarrheal diseases, due to poor water and sanitation, resulted 1.8 million deaths in 2002 and caused 62 million disability accustomed life year sperannum [3]. Estimates show that more than 3 million Pakistanis are affected by waterborne diseases each year, of which 1.2 million die [4]. In Pakistan, 30 percent of all diseases and 40 percent of all mortalities are because of poor water quality [5]. This estimation places diarrheal diseases as the sixth highest cause of deaths and third in the list of diseased cases globally and it is also estimated that 3.7% of the total worldwide disease burden are due to contaminated water, sanitation and hygiene [6]. In developed countries, the commonly occurred human parasitic protozoa spread by water are species of *Giardia* [7]. *Giardia lamblia* also called as *G. duodenalis* or *G. intestinalis*. It is a single celled flagellated, binucleated enteric protozoan parasite of humans that has been isolated worldwide and is now recognized as one of the 10 major parasites of humans [8]. *Giardia lamblia* affect both people and a broad range of household and undomesticated animals. The diseased hosts, that can be animals or humans, dispose of a large number of parasites in their feces, so rising infectivity in surroundings [9]. Water resources are polluted when faeces having the (oo) cysts are dumped into water. (Oo) cysts can enter surface waters from waste of waste water treatment plants, metropolitan overflow, or rural surplus there by spreading infectious diseases [10]. *Giardia lamblia* is the most usually reported intestinal parasite globally. It can cause acute or persistent diarrhea. *Giardia intestinalis (*synonyms: *Giardia duodenalis, Giardia lamblia)* is a protozoan Parasite that is recognized as one of the most common causes of waterborne disease in the United States. Waterborne outbreaks of giardiasis have been reported in developed countries including the U.S. and also in Europe, while the major risk factors in low developed countries were sanitation and personal hygiene [11]. The aim of the research work was to find out the detection of *giardia lamblia* by microscopy in different water sources of district D.I. Khan, KP, Pakistan.

Materials and Methods
Area of the Study
The study was conducted for the prevalence of the Zoonotic parasites in the different water sources, i.e. tap water, pond water, and drain water, for contamination and spreading of the diseases to human being and animals in district D.I. Khan.

Collection of Water Samples

A total of 200 water samples were collected from district D.I. Khan during Feb-June 2016. A sample of one liter each was collected from tap water, pond water, and drain water in clean and sterilized bottles. The samples were labeled with the date of collection, nature of water and the site of collection. Then all the samples were transported to the laboratory of the Department of Zoology immediately. The samples were collected as per the following plan shown in table-1;

Table 1: Research plan of the sample collection

Different Areas	Tap water	Pond water	Drain water	Total
Madina colony	20	10	10	40
Cantt area	20	10	10	40
Zaffarabad	20	10	10	40
Islamia colony	20	10	10	40
D.I Khan city	20	10	10	40
Total	100	50	50	200

Parasite sampling

To study the presence of parasites, water samples were collected from tap water, pond and drain. The sampling was carried out from Feb to the end of June 2016.The water samples were filtered using a whattman filter paper. A concentrated specimen of water samples, recovered from the filter was kept for the study of parasites and preserved in 10% formalin. The concentrated specimen was obtained by washing and scraping the filter with distilled water in a squirt bottle and a scalpel. The filter was not disassembled. However, some filter material was scraped into the concentrated sample.

Processing of samples

The concentrated specimens were stored at room temperature until these were processed for microscopic analysis. The specimens were further concentrated by centrifugation 800 x g for 5minutes and the pellet was re-suspended into 5ml of Phosphate Buffer Saline solution. With the help of micropipette, a drop of 5ul was taken and put on the slide and make smears, fixed and dried. Then a proper staining were given with Giemsa. Morphological characteristics, such as the dimensions of the oocysts, eggs and cysts, were used for detection/diagnosis of the parasites.

Giemsa stain procedure

1. Fresh smear was made on the slide and allow the smear to air dry at room temperature.
2. Fix with absolute methanol for three minutes.
3. Dip the slide in the stain for 30 minutes.
4. Remove the slide and wash with tap water, then air dry.
5. Slides were examined under the 10x, 40x and 100x magnification of the microscope

Prevalence rate

The prevalence rate was determined by the formula

$$\text{Prevalence rate}=\left(\frac{\text{N0.of parasites detected in water sample}}{\text{Total no. of water samples examined}}\right)\times 100$$

Statistical analysis

Statistical package SPSS4 was utilized for data analysis.

Fig 1: *Giardia lamblia* microscopic view

Results

A total of 200 water sample were collected from different areas of District Dera Ismail Khan for the detection of *Giardia lamblia* including pond water, tap water and drain water each having 40 samples. In the present study a total of 200 water samples were examined by means of giemsa, which showed 21% (42/200) was of *Giardia lamblia*. In these samples the prevalence of *Giardia lamblia* was determined 15% in tap water, 24% pond water, and 30% in drain water.

Table 2: Water samples collected from different areas of District D.I. Khan

Different Areas	Tap water	Pond water	Drain water	Total
Madina colony	20	10	10	40
Cantt area	20	10	10	40
Zaffarabad	20	10	10	40
Islamia colony	20	10	10	40
D.I. Khan city	20	10	10	40
Total	100	50	50	200

Prevalence of *Giardia lamblia* in different areas of District D. I. Khan

The Prevalence of *Giardia lamblia* was found in all the water sources collected from D.I. Khan City during study period (2016). Overall presence of *Giardia lamblia* was 15% (6/40), while high prevalence was observed in drain water 30% (3/10) and the lowest observed in pond water 10% (1/10).The tap water showed 10% (2/20) results. Madina colony showed high prevalence of *Giardia lamblia*25% (5/20) in tap water, followed by 30% (3/10) in pond water, 10% (1/10) in each drain water. Cantt Area 15% (3/20) results for the *Giardia lamblia* in each water sample collected from tap water. While high prevalence was recorded 50% (5/10) in drain water and pond water showed lowest positive results for *Giardia lamblia* 20% (2/10). Zafarabad showed 60% (6/10) results in drain water for the *Giardia lamblia*, 50% (5/10) in samples from pond water and 20% (4/20) in Tap water. Islamia colony showed 15% (1.5/10) result in drain water 6.7% (1.35/20) in tap water and 10% (1/10) in pond water.

Prevalence of *Giardia lamblia* in different water samples of District D. I. Khan

Giardia lamblia was also detected in different water sources collected from different areas of district D. I. Khan. Tap water showed 10% (2/20) results collected from D. I. Khan City. Madina colony, Cantt area, Zafarabad and Islamia colony showed positive results for *Giardia lamblia* 25% (5/20), 15% (3/20), 20% (4/20) and 6.7% (1.35/20) respectively. Similarly, pond water showed 10% (1/10) results collected from D.I. Khan City. While Madina Colony, Cant Area, Zafarabad and

Islamia Colony also showed positive results for *Giardia lamblia*, 30% (3/10), 20% (2/10),50%(5/10) and 10%(1/10) respectively in pond water. The drain water showed 30% (3/10) result for *Giardia lamblia* in D.I. Khan City in out of 10 samples. The drain water of Madina colony showed 10% (1/10), Cantt area 50% (5/10) Zafarabad showed 50% (5/10) and Islamia colony showed 10% (1/10) positive results for *Giardia lamblia.*

Overall prevalence of *Giardia lamblia* different water sources of District D. I. Khan

Giardia lamblia was also observed in different water sources in the studied areas. Out of 200 water samples collected tap water, pond water, and drain water each which showed positive results for *Giardia lamblia* containing 15%, 24% and 30% having 15, 12,and 15 positive samples respectively.

Table 3: Prevalence of *Giardia lamblia* in different areas of District D.I. Khan

Area (n)	Tap water Positive/total (%)	Pond water Positive/total (%)	Drain Water Positive/total (%)	Over All Samples Positive/total (%)
D.I. Khan City	2/20 (10%)	1/10 (10%)	3/10 (30%)	6/40 (15%)
Madina colony	5/20 (25%)	3/10 (30%)	1/10 (10%)	9/40 (22.5%)
Cantt area	3/20 (15%)	2/10 (20%)	5/10 (50%)	10/40 (25%)
Zafarabad	4/20 (20%)	5/10 (50%)	5/10 (50%)	14/40 (35%)
Islamia colony	*1/20 6.7%*	*1/10 10%*	*1/10 10%*	*3/40 8.4%*
Total	15.35/100 (15.35%)	12/50 (24%)	15/50 (30%)	42/200 (21%)

Table 4: Prevalence of *Giardia lamblia* in different areas of District D.I. Khan

Source of Water	*Giardia lamblia* % in different Area					Grand Total, n=200
	D.I. Khan city, n=40	Madina colony, n=40	Cantt Area, n=40	Zafarabad, n=40	Islamia colony, n=40	
Tap Water, n=40	10%	25%	15%	20%	6.7%	15.35%
Pond Water, n=40	10%	30%	20%	50%	10%	24%
drain Water, n=40	30%	10%	50%	50%	10%	30%
(%)	15%	22.5%	25%	35%	8.4%	21%

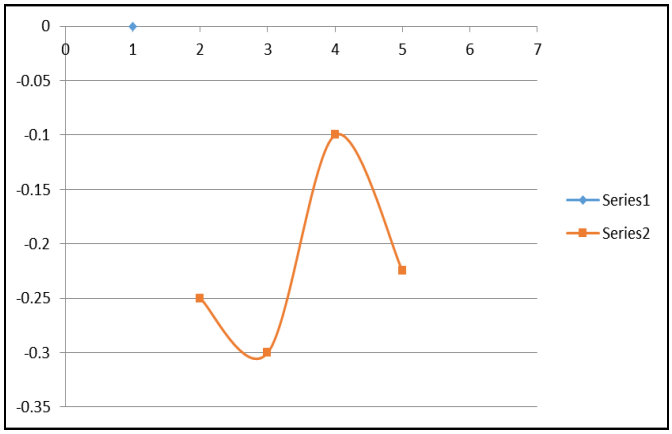

Fig 1: Prevalence of *Giardia lamblia* in different water sources of Madina colony

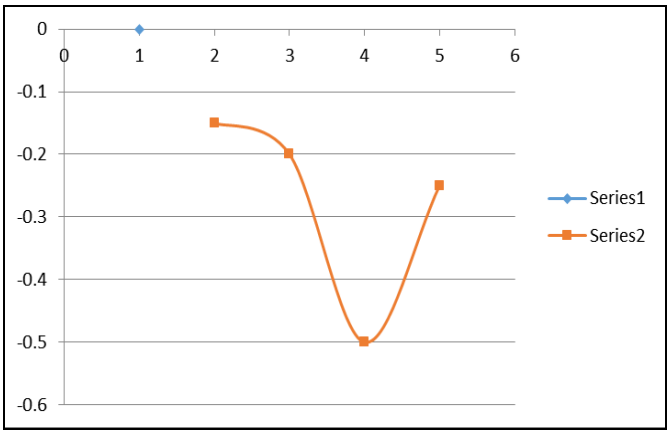

Fig 3: Prevalence of *Giardia lamblia* in different water sources of Cantt area.

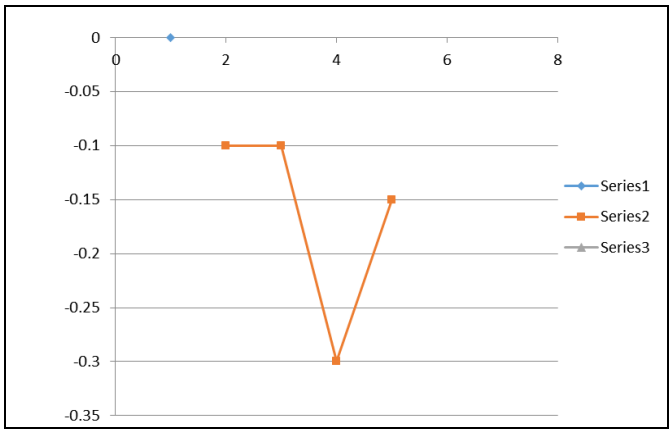

Fig 2: Prevalence of *Giardia lamblia* in different water sources of D.I Khan City

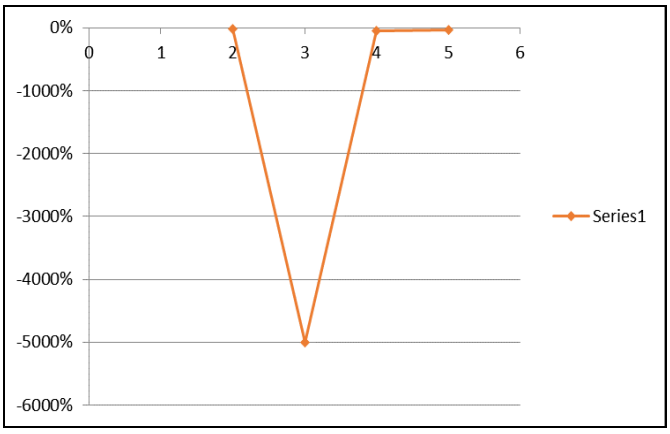

Fig 4: Prevalence of *Giardia lamblia* in different water sources of Zafarabad colony

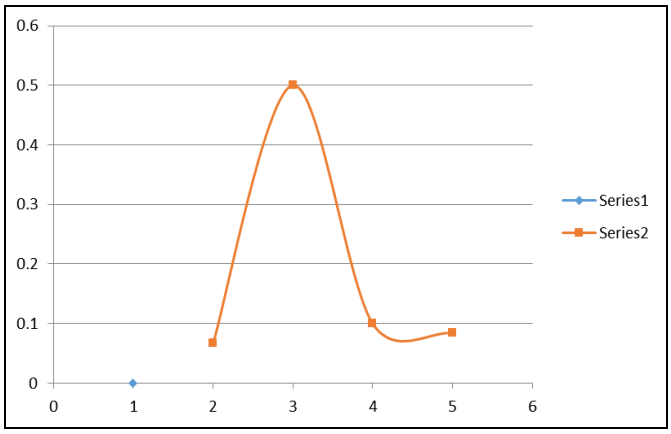

Fig 5: Prevalence of Giardia *lamblia* in different water sources of Islamia colony.

Discussion

Waterborne diseases occur all over the world, and outbreaks caused by the contamination of community water systems have the probable to cause diseases in large numbers of consumers. These outbreaks have been associated with drinking and recreational water worldwide, but waterborne parasites are global protozoan' parasites that infect humans, domestic animals and wildlife worldwide [12] (Barwick *et al.*, 2000). Microscopic studies were commonly used for identification of zoonotic parasites, but our present work is based on the molecular detection of *Giardia lamblia* by means of giemsa staining.

Giardia lamblia were found in tap water, pond water, well water and drain the water in distract Dera Ismail Khan. The total of 200 samples 40 each from tap water, pond water and drain water were examined using Giemsa stain. Overall prevalence of *Giardia lamblia* was 21% (42/200), among these 16.7% (10/40) in tap water, 18.3% (11/40) pond water, 25% (15/40) in drain water. The highest prevalence was recorded in drain water in which 25% for *Giardia lamblia* and was considered significant during statistical analysis similarly study was conducted in Srilanka that showed the levels and concentrations of *Giardia lamblia* were higher than the result of the present studies from other countries [13] (Quintero *et al.*, 2000) detected which was a higher rate of prevalence. The prevalence of *G. lamblia* depends on location of sample collection, study area, season, climatic condition, volume of sample; size of population etc.

The drain water showed high level of contamination with *Giardia lamblia* than tap water, well water and pond water. The 40 and 15 samples were found positive for *Giardia lamblia*. The presence of these parasites in the water samples was supported by Wallis *et al.* [14] (1996) who claimed that the parasitic protozoa increased in late winter and the beginning of spring in spite of its presence all over the year. The different water samples were examined through Giemsa stain to detect *Giardia lamblia*. Tap water showed 10% (2/20) results collected from D.I. Khan City. Madina colony, Cantt area, Zafarabad and Islamia colony showed positive results for *Giardia lamblia 25% (5/20),15% (3/20),20% (4/20) and 6.7% (1.35/20)* respectively. Similarly, pond water showed 10% (1/10) results collected from D.I. Khan City, Madina colony, Cantt area, Zafarabad and Islamia colony also showed positive results for *Giardia lamblia*, 30% (3/10), 20% (2/10), 50% (5/10) and 10% (1/10) respectively in pond water. The drain water showed 30% (3/10) result for *Giardia lamblia* in D.I. Khan City in out of 10 samples. The drain water of Madina colony showed 10% (1/10), Cantt area 50% (5/10) Zafarabad showed 50% (5/10) and Islamia colony showed 10%(1/10) positive results for *Giardia lamblia*. Similar results were obtained by Ayaz *et al.* [15] (2011) in which the three different sources of water were contaminated with eggs, cysts or oocytes of the *Giardia* parasites. The results specify overall prevalence of *Giardia* spp; 21% (42/200)**.** Probable sources of water contamination in this study area include both human and animals, since animal sources are known to be important in the introduction of these protozoa to a water system.

Conclusion and Recommendation

As the portable water in District Dera Ismail Khan is highly contaminated and is hazardous for the health of the common man, the present research center's round one such predominant source of contamination which causes debilitating diseases to man and animals. The highest level of contamination was reported in drain water of Zafarabad for *Giardia lamblia*. The outcome of this study will help the population of D.I. Khan specially Zafarabad colony which is in great need of access to safe and clean water and can pave the way for further research work. This will also cause the awareness among the public, mostly the poor, who share their drinking water sources with their cattle. Keeping in view the present study results, it is recommended for a further study to differentiate between water contamination by human and animal feces. It is highly recommended to develop methods to improve pit-latrines for reducing the protozoans load in the local water supplies and reduce animal feces from entering the water system. The filter and boiled water may use for drinking purposes and advance methods may be recommended for diagnosis of waterborne parasites.

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