Parasitic contamination of drinking water and its prevalence among handlers and sheep

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Summary

The current study was designed to determine the infection rate of intestinal protozoa in sheep and their handlers, and in their drinking water, in Wasit province, so as to study the relationship between contamination of drinking water with protozoal pathogen and infection in sheep and handlers in the period from the beginning of September 2015 to the end of February 2016. Two hundred eighty fecal samples were collected: (180) samples from sheep and 100 samples from human (50 handlers and 50 from non handlers), 50 drinking water samples were collected (18 samples; human tap water and RO bottled water and 32 samples river water). All samples were examined by conventional methods (direct moist smears, flotation with saturated sugar solution and stained with Modified acid fast, Giemsa and lugol's iodine) The total infection rate in sheep, handlers and drinking water samples were 83.33%, 72%, 84.37% and 33.33% respectively. The result showed that drinking water samples of sheep and handlers were contaminated with protozoal oocysts or cysts of *Cryptosporidium* spp. (65.62%) (27.77 %), *Giardia lamblia* (6.25%) (0), *Entamoeba* spp (68.75%) (0), *Eimeria* spp (25%) (5.55%) and *Buxtonella sulcata* (40.62%)(0).

Keywords: Contamination, Water, Protozoa, Sheep, Sheep handlers.

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Introduction

Parasitic diseases play a considerable role for responsibility of infectious diseases globally and they also cause important enteric sickness (1). Several intestinal protozoan parasites cause zoonotic disease associated with livestock, domestic creatures. The open farms cause transmission of infection to humans (2). Infections usually happen through consumption of cysts/oocysts damaging raw meals or normal standard water (3 and 4). Enteric protozoa are a different group of unicellular parasites inhabiting the intestinal tract in great vertebrate hosts such as human and animals. When diarrhea is relatively a regular indication for this harmful parasite but without symptoms colonization is also typical (5). Protozoan parasite becomes a growing public health of and veterinary importance (6). Worldwide, protozoal is anticipated to be influenced by changes in rainfall quantity, intensity, regularity, and duration. Drinking water can be contaminated by sewage and waste from animal activity, or rotting animal carcasses. In addition, the parasites that contaminate open resources, such as reservoirs, ponds, and waterways have been provided by domestic and wildlife inhabiting this environment (7 and 8).

Consequently, environmental conditions in developing countries predispose to the transmitting of water-borne disease (9). The clinical symptoms of intestinal protozoal infections, include giardiasis, coccidiosis and cryptosporidiosis, showing almost similar symptoms, loss of appetite, nausea, vomiting, throwing up, soft to watery diarrhea, malabsorption, dehydration and stomach pains (10). People attacked by these diseases suffer from a significant issue of poor hygienic in the areas and loss of personal hygiene such as rural areas (11). There are several researches on pollution of drinking water with harmful protozoa in the Middle East (12-14), whereas there is scarce knowledge about them in Iraq (15-18). The aim of this study was to determine the infection rate of intestinal protozoa in sheep and their shepherds and their drinking water.

Materials and Methods

280 fecal samples were collected: (180) fecal samples from sheep whose ages ranged from 7-36 months including 37 males and 137 females, and 100 stool samples from human (50 handlers and 50 from non-handlers) whose ages ranged from 10-40 years including 68

males and 32 females, from three different regions (Al-Hafriya, Al- Al-Suwaira and Al-Aziziyah) of Wasit province and during the period from the beginning of September 2015 to the end of February 2016. In addition 50 water samples were collected (18 samples of human tap water, RO bottled water, and 32 samples of sheep river water). All samples were collected in clean sterilized and secured 100 ml plastic containers. Information was recorded (place, date of sampling, age and sex of human and animals). Then samples were transported in the cooling box to the Department of Parasitological/ Faculty of Veterinary Medicine/ University of Baghdad for examination. All samples were examined by conventional methods (direct moist smears, flotation with saturated sugar solution and stained with Modified acid fast, Giemsa and lugol's iodine) for detection of oocysts and cysts of these protozoal parasites (19 and 20).

Statistical analysis: The Chi-square test was used for the comparison between the results. Differences were considered statistically significant at P<0.05 (21).

Results and Discussion

Several studies on the prevalence of intestinal protozoa have been done all over the

world and have shown variations in the rate of infections depending on geographical regions and localities, sanitary environment and hygienic habits of people living there (22). The results of this study showed that the overall infection rate of intestinal protozoa in sheep collected from the three regions of Waist province was 83.33% comparable with 72% in significant human, without differences between the studied regions (Table, 1). These results were in agreement with (23), who recorded an infection rate with intestinal protozoa reaching up 85% in lambs in Baghdad Province with some protozoal infection (Cryptosporidium spp, Giardia spp, Eimeria spp) in sheep. The study recoded infection rate with intestinal protozoa (84.37%) in (27) samples from the total (32) samples of sheep drinking water (river water) and recorded a higher infection rate in Al-Suwaira (90.90%), and lower infection rate in Al-Hafriya (80%), while the study found (33.33%) in (6) samples from overall (18) handlers drinking water (Table, 1). This result agreed with (22 and 24) who detected some intestinal protozoa (G. lamblia, and C. parvum) in water sources.

Table, 1: Relation between contaminated water and infection of sheep and handlers with intestinal Protozoa according to areas.

	Types of samples		No. of Samples examined		No. of positive		Percentage %	
Area of study	Feces	Drinking water	Feces	Drinking water	Feces	Drinking water	Feces	Drinking water
Al-Hafriya	Sheep	Sheep	60	10	49	8	81.66 b	80 b
	Handlers	Handlers	18	6	10	2	55.55	33,33
	Non handlers		18		0		0	
Al-Suwaira	Sheep	Sheep	60	11	55	10	91.66 a	90.90 a
	Handlers	Handlers	16	6	14	3	87.5	50
	Non handlers		16		2		12.5	
Al-Aziziyah	Sheep	Sheep	60	11	46	9	76.66 b	81.81 b
	Handlers	Handlers	16	6	12	1	75	16.66
	Non handlers		16		2		12.5	
Total	Sheep	Sheep	180	32	150	27	83.33	84.37
	Handlers	Handlers	50	18	36	6	72	33.33
	Non handlers		50		4		8	

Different superscript refers to significant differnces at P<0.05

The infection rate of protozoal parasites in sheep and human fecal samples (50.55, 52%), (6.11, 24%), (39.44, 12%), (23.33%, 0) and (12.77%, 0) is as follows: *Cryptosporidium* spp, *Giardia* spp, *Entamoeba* spp, *Eimeria* spp and *Buxtonella sulcata*, respectively.

Moreover, results of this study approved that *Eimeria* spp and *Buxtonella sulcata* were not observed in human stool. Nevertheless, only *Entamoeba* spp was recognized in the non-handlers stool (Table, 2). Regarding the distribution of these protozoa in sheep and

human, the results of this study approved the zoonotic impact and nature of some parasites.

However, some of the protozoa didn't show this zoonotic impact (25 and 26).

Table, 2: Relation between contamination of water and infection of sheep and handlers with Intestinal Protozoa

according	to	the	genus	of	parasites
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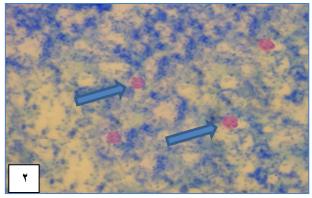
	Types of sa	amples	No. of Samples examined		No. of positive		Percentage %	
Genus of Parasites	Feces	Drinking water	Feces	Drinking water	Feces	Drinking water	Feces	Drinking water
orius of furusives								
Countagnavidium	Sheep	Sheep	180	32	91	21	50.55 a	65.62 a
Cryptosporidium	Handlers	Handlers	50	18	26	5	52	27.77
Spp	Non handlers		50		0		0	
	Sheep	Sheep	180	32	11	2	6.11 b	6.25 b
Giardia Spp	Handlers	Handlers	50	18	12	0	24	0
	Non handlers		50		0		0	
Entamoeba Spp	Sheep	Sheep	180	32	71	22	39.44 a	68.75 a
Енштоева эрр	Handlers	Handlers	50	18	6	0	12	0
	Non handlers		50		0		0	
	Sheep	Sheep	180	32	42	8	23.33 b	25 b
Eimeria Spp	Handlers	Handlers	50	18	0	1	0	5.55
Еннени эрр	Non handlers	Handicis	50	10	0	•	0	
	Sheep	Sheep	180	32	23	13	12.77	40.62
Buxtonella sulcata	Handlers	Handlers	50	18	0	0	0	0
	Non Handlers		50		0		0	

Different superscript refers to significant differnces at P<0.05

The study recorded infection rate with Cryptosporidium spp (Fig. 1 and 2) in drinking water of sheep and handlers (65.62, 27.77%) respectively, while recorded infection rate with Giardia spp (Fig, 3 and 4) and Entamoeba spp (Fig. 5 and 6) in drinking water of sheep only (6.25) and 68.75%). Also the result showed an infection rate with Eimeria spp (Fig, 7 and 8) in sheep and handlers drinking water reaching (25, 5.55%) respectively, while the result recorded an infection rate with Buxtonella sulcata (Fig. 9 and 10) in sheep fecal samples and sheep drinking water only (12.77, 40.62%) respectively, (Table, 2). These results agreed with (27) in Basrah who recorded contamination of drinking water samples (36.4%), and also agreed with (28) in Baghdad province who recorded rate of contamination of piped water samples (25.3%). (29) recorded (19.34%) in river water and (30) recorded rate (25%) in tap water in Baghdad city contamination with intestinal protozoa, (31) in India recorded contamination of plain water with intestinal protozoa (41.66 %), due to low socio-economic status and contamination of water sources of sheep drinking water with Waste of humans and animals which is considered an important source of prevalence of several intestinal parasites. As well the emergence of infection among people who use piped drinking water may be attributable to retro

gradation in the water networks in the cities (31).





Figure, 1 and 2: *Cryptosporidium* spp. oocyst in fecal sample of sheep, direct wet film (1), stained with Modified acid fast (2), (100x).





Figure, 3 and 4: Giardia spp cyst (3), trophozoite in sheep fecal sample stained with Lugols Iodine and Giemsa stain (4), (100x).





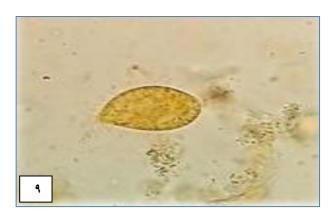
Figure, 5 and 6: *Entamobea* spp cyst in handlers stool sample (5), Trophozite in fecal sample of sheep stained with Lugols Iodine stain (6), (100x).

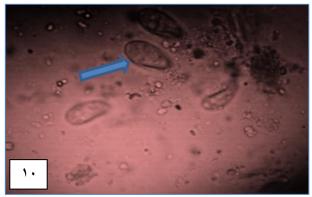




Figure, 7 and 8: *Eimeria* spp oocyst (7) unsporulated, sporulated oocyst stained with lugols Iodine (8), (100x) in sheep fecal samples.

The results of this study recorded the highest infection rate (100%) in sheep and sheep drinking water contamination in September, November and December, while the lowest infection rate was during February (36.66%)with significant differences (P>0.05). The results also showed that the sheep handlers revealed highest infection rate with intestinal protozoa in September and December (85.71%), while the lowest infection rate was during November and February (55.55%) with significant differences (P>0.05) (Table, 3). This result agreed with (32) in Diyala province who recorded highest infection rate in November and lowest in February, and also agreed with (23) who recorded the highest infection rate in December (100%) and January (95.65%), while the lowest infection rate in June (66.67%) in Baghdad province, and (33) who recorded highest infection in Autumn season. Also the results are compatible with (33) who recoded same results in same months, and explained these variations in infection rate as being due to the existence of suitable environmental factors such as temperature and moisture which facilitated spread and completing the life cycle of these parasites.





Figure, 9 and 10: *B.Sulcata* trophozoite in sheep drinking water (9), Trophozoite in sheep fecal samples stained with lugols Iodine (10), (100x).

Table, 3: Relation between contaminated water and infection of sheep and handlers with Intestinal Protozoa according to months.

Months of	Types of samples		No. of Samples examined		No. of positive		Percentage %	
study	Feces	Drinking water	Feces	Drinking water	Feces	Drinking water	Feces	Drinking water
September	Sheep	Sheep	30	6	30	6	100 a	100 a
	Handlers	Handlers	18	3	6	2	85.71a	66.66
	Non handlers		18		0		0	
October	Sheep	Sheep	30	5	25	4	83.33 a	80 b
	Handlers	Handlers	9	3	7	2	77.77 b	66.66
	Non handlers		9		0			
November	Sheep	Sheep	30	5	30	5	100 a	100 a
	Handlers	Handlers	16	3	5	1	55.55b	16.66
	Non handlers		16		0		0	
December	Sheep	Sheep	30	5	30	5	100 a	100 a
	Handlers	Handlers	16	3	6	1	85.71 b	16.66
	Non handlers		16		0		12.5 c	
January	Sheep	Sheep	30	6	24	4	83.33 b	66.66 b
		Handlers	9	3	7	0	77.77 b	0
	Handlers		9		2		22.22 c	
	Non handlers							
February	Sheep	Sheep	30	5	11	3	36.66b	60b
	Handlers	Handlers	9	3	5	0	55.55b	0
	Non handlers		9		2		22.22c	
Total	Sheep	Sheep	180	32	150	27	83.33	40.62
	Handlers	Handlers	50	18	36	6	72	33.33
	Non handlers		50		4		8	

Different superscript refers to significant differnces at P<0.05

The highest infection rate with intestinal protozoa was in age group (7-12 month) (91.42%), while the lowest infection rate (76.19%) in age group (19-24 month) with significant differences (P>0.05). While the highest infection rate of human with intestinal protozoa were recorded in the age groups (21-30 years) and (10-20 years) (83.33, 80%) respectively, and the lowest infection rate

(25%) was recorded in age group (31-40 years) (Table, 4). These results are compatible with (26 and 34) in Baghdad and Diyala governorates who found that younger animals showed higher susceptibility to infection than adults and this is mostly related to lower immune status, crowding stress and managements in the farm, where much of sheep get in contact in or access into the

infected fields without any preparation plan against the infection.

Table, 4: Infection rate of intestinal protozoa

according to age groups.

Age	Types of	No. of	No. of	Percentage
groups	samples	samples Samples p		%
		Examined		
Sheep	7-12	70	64	91.42 a
(months)	13-18	15	12	80b
	19-24	42	32	76.19 b
	25-30	27	22	81.48 a
	31-36	26	20	76.92 b
Handlers	10-20	30	24	80b
(years)	21-30	12	10	83.33 a
	31-40	8	2	25 c
Non	10-20	18	0	0
handlers	21-30	20	2	10 b
(Years)	31-40	12	2	16.66 a

Different superscript refers to significant differnces at P<0.05.

This study recorded highest infection rate of intestinal protozoa in male sheep and male handlers as (90.69, 75%) respectively, while the lowest infection rate in females (81.02, 66.66%) respectively, while the highest infection rate of intestinal protozoa in non handlers (14.28%) in females (Table, 5). This result was in accordance with (34) who recorded highest infection rate in males in Diyala Province, while it did not agree with (35) who recorded highest infection rate in females in Diwaniyah province.

Table, 5: Infection rate of intestinal protozoa

according to sex.

Sex	Types of samples	No. of Samples	No. of positive	Percentage %			
DOM.	examined						
	Sheep	43	39	90.69 a			
Male	Handlers	32	24	75 a			
	Non handlers	36	2	5.55 c			
	Sheep	137	111	81.02 b			
Female	Handlers	18	12	66.66 b			
	Non handlers	14	2	14.28 c			
	Sheep	180	150	83.33			
Total	Handlers	50	36	72			
	Non handlers	50	4	8			

Different superscript refers to significant differnces at P<0.05.

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التلوث الطفيلي لمياه الشرب وانتشارها بين العاملين والأغنام

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صُممت الدراسة الحالية لمعرفة نسب الإصابة بالأوالي المعوية التي تصيب الأغنام والإنسان ومياه الشرب لكليهما في محافظة واسط مع دراسة العلاقة الموجودة بين تلوث مياه الشرب بالأوالي الطغيلية الممرضة وإصابة كل من الأغنام والعاملين على رعايتها ضمن المدة من بداية شهر أيلول 2015 إلى نهاية شهر شباط 2016. جُمعت (280) عينة براز (50 عينة براز أغنام و 100 عينة براز (50 عينة من الأشخاص الذين كانوا بتماس مع الأغنام (المربين) و 50 عينة من الأشخاص الذين ليسوا بتماس مع الأغنام (المربين و 23 عينة ماء شرب للأغنام) وقد خضعت جميع الغينات للفحص المجهري بالاختبارات التقليدية. أظهرت نتائج الدراسة الحالية أن نسبة الإصابة الكلية بالأوالي المعوية في الأغنام والمربين ونسبة تلوث مياه الشرب للأغنام والمربين كانت (83.33 و 72%)، (41.25 و 6.66 %) على التوالي. وأظهرت الدراسة نسب تلوث مياه شرب الأغنام والمربين بطفيلي و 83.33 و 77%)، (55.62 و 77.77%)على التوالي، والإصابة بطفيلي و 65.63 و 65.63%) على التوالي، والمربين بطفيلي و 65.6% و 65.5%) على التوالي، الماطفيلي و 84 و 55.5%) على التوالي، اماطفيلي المعوية في مياه الشرب الأغنام والمربين بطفيلي و 65.5%) على التوالي، اماطفيلي المعوية في مياه الشرب المربين. الأغنام والمربين بطفيلي 65.5%) على التوالي، اماطفيلي المعوية في مياه الشرب المربين. المؤلكية الموالية المفتاحية و 65.5%) على التوالي، المأخنام، مربي الأغنام والمربين الأغنام والمربين بطفيلي المعادية في مياه الشرب المربين. المفتاحية و 100% أولى، أغنام، مربي الأغنام والمربين المؤلكية المؤلكية المؤلكية المؤلكية و 100% و 100% المؤلكية و 100% و 100%