

Water contamination with Protozoa in Kirkuk Province.

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Abstract

A total of 150 water samples examination in Kirkuk province were carried out using direct deposit examine microscopically, study parameters involves four type of water collections : direct (governmental pipes), indirect (tank vehicle) ,well water & river water (Khasah Sou), which divided in to four positions. The main aims of this study are to detect role of parasitic stages in contamination of water in Kirkuk Province & to assess the relationship between water contamination with protozoa & some water quality tests like (PH, Turbidity & chlorine percentages). The result of the study shows all rate of contamination 14%, which divided into 12 % & 2 % in urban area & rural area respectively , $P<0.05$. River water shows high rate of contamination 12 % , followed by 1.33 % & 0.66% from direct (Governmental pipes) & well water respectively $P<0.05$. The rate of water turbidity was 10.66 % with high occurrence in river water 5.33 % & the lowest rate 0.66% was in wells. $P<0.05$. Abnormal chlorine percentage was seen in 6-water sample with the rate 4 % $P>0.05$. Abnormal PH of water samples were ranged from 5.23 to 10.3 which had no role in frequency of parasitic forms in water, $P<0.05$. The more common parasitic forms that found are *Entamoeba histolytica* that was highly dominated in river water 12 % followed by *Giardia lamblia* 3.22%. Other microorganisms that are isolate include *Candida* species 12.6 % and *Paramecium* 12%. Total rate of water contamination with microorganisms is 42 %.

تلوث الماء بالأبتدائيات الطفيلية في محافظة كركوك

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المستخلص

تم إجراء فحص 150 نموذج ماء في محافظة كركوك باستخدام طريقة الفحص المجهرى لراسب نموذج الماء. جمعت النماذج من أربع مصادر : ماء الحنفية (تجهيز حكومي)، ماء السيارات (التنكرات/ طريقة غير مباشرة)، ماء الآبار و ماء النهر) نهر خاصة صو الذي يمر من وسط مدينة كركوك) التي قسمت إلى أربع مناطق لجمع المياه . الأهداف الرئيسية للدراسة هو تحديد دور الأشكال الطفيلية في تلوث الماء في محافظة كركوك وقياس العلاقة بين تلوث الماء بالأبتدائيات مع بعض الفحوصات النوعية للماء (ألباءهء، العكورة و ونسبة الكلور في الماء). أظهرت نتائج الدراسة نسبة التلوث الكلي 14 % وقسمت إلى 12 % في مياه المناطق الحضرية و 2 % في المناطق الريفية $P<0.05$, أظهر ماء النهر أعلى نسبة تلوث 12 % تلتها 1.33% & 0.66 % في النماذج من ماء الحنفية و ماء الآبار على التوالي $P<0.05$. ومن الطفيليات الأكثر شيوعا فقد ظهرت المتحولة النسيجية *Entamoeba histolytica* بنسبة 12 % وبلغت أعلى نسبتها في ماء النهر 10.6 % تلتها الجيارديا لامبليا *Giardia lamblia* وبنسبة 3.22%. أما بالنسبة للأحياء المجهرية الأخرى التي عزلت فقد ظهرت أشكال المبيضيات *Candida species* بنسبة 12.6% والبراميسيوم *Paramecium* بنسبة 12 % . وبلغت إجمالي تلوث المياه بالأحياء المجهرية نسبة 42 % . نسبة عكورة المياه بلغت 10.66% وبلغت ذروتها في ماء النهر إذ كانت النسبة 5.33% ، و اقل نسبة كانت في الماء المأخوذ من الآبار 0.66% . $P<0.05$. نسبة الكلور غير الطبيعية ظهرت في ست نماذج وبنسبة 4% وكان الفرق غير معنوي بين نسبة الكلور والتلوث بالأبتدائيات. أما بالنسبة إلى الباء هاء غير الطبيعي فقد توزعت في ست نماذج و بنسبة 6% وأن العلاقة بين توزيع الطفيليات والباء هاء لم تكن معنويا $P<0.05$.

Introduction

Water is an essential resource for living systems, industrial processes, agricultural production and domestic use. The principal factors that are taken into consideration when determining water quality are dissolved oxygen content (DO) turbidity, acidity, alkalinity trace elements and nutrients such as nitrogen, phosphorus, halogens (chloride and fluoride ions), alkali metals (sodium and potassium ions), calcium and magnesium ions & microorganisms.(1).Surface (rivers, Stream ,reservoirs), ground water(wells)& tanker water are readily contaminated with fecal matter from humans ,agricultural animals & birds fecal material may contain many pathogens (viruses, bacteria & parasites) (2). Processing of water for human consumption requires filtration to remove organic debris & some types of potentially pathogenic organisms & chlorination to inactivate other potentially pathogenic microorganisms (3). If there is a failure in the water treatment process or unrecognized break in the integrity of the distribution system very large number of people may be exposed to pathogenic microorganism in a short space of time, large outbreaks of infection have occurred because of such problems (4). In less undeveloped countries, lack of access potable water is one of the major causes of ill health in particular diarrheal diseases (5). Among the more important water-borne infectious agents are the following protozoan parasites such that causing gastroenteritis like *Giardia lamblia*(6) .*Entamoeba histolytica* (7& 8). *Cryptosporidium parvum* (9), *Cyclospora* (10), *Isospora* , *Microsporida* (11)& *Balantidium coli* (12),others like *Toxoplasma gondii* (13) can cause serious damage to fetus during pregnancy (congenital abnormalities), *Naegleria fowleri* (14), *Acanthamoeba gilberstoni* (15) can causes primary meningo-encephalitis & keratitis respectively .Since 1979, Kirkuk province shows more development & revolution of building which extend to

reach around Khasah Sou river without uncontrolled planning, most of factories discharge their waste directly to the river, in addition to extension of building on the sites of river after the event of 2003 (after return of departure to Kirkuk city) so, for these reasons this study was conducted to determine the Water quality to human consumption & to detect distribution of microorganism's especially protozoan parasites in water. In addition to determine the relationship between some water quality tests(PH,Turbidity&chlorine percentage) & distribution of water contaminants .

Materials & methods

From 1st October 2003 to 30th April 2004 a total of 150 water samples were collected from different area of Kirkuk province & each sample was placed in a special container (standard universal bottle 200 ml) with a preservative Solution of thiosulfate (16). These specimens have been differ in their original sources from pipes water (direct), tank water(indirect) wells & different parts from river Khasah Sou water. Each container was labeled (name of area, time of collection & source of sample). As soon as it is collecting, it was proceeded for examination & when delay is suspected, the specimen will kept in refrigerator until the time of examination .**Turbidity examination:-**Is detected by two ways, necked eye & by using the instrument (211A 2424 turbidimeter)Using nephelometer turbidity unit(NTU).**Estimation of chlorine percentages:-** was detected by measuring of chlorine percentage in water, either by using orthotoludin solution or Palint test by using DPD tablets No.4 , which can yield a pink color.**PH of water :-**Two methods were used in adjusting the PH, either by using gradient PH paper (Germany manufactured) & confirmed by checking PH by instrument (PH-meter. Germany manufactured).**Sample examination for parasites:-**Macroscopic examination:-its done by using hand lens & light source to observe parasitic forms (segment of

Cestoda, larvae of other helminthes & insect larvae (*Osterus Species*) & other inclusion bodies. While microscopic examination was done by using long period of centrifugation of the specimen, then the supernatant & deposit were examined by preparing double wet preparations from both using 0.085% of NaCl for detecting trophozoites & Lugol's iodine solution 5% for detecting cystic stages of protozoan parasites.(17) .**Statistical analysis:-**All obtained data are tabulaized by the aid of computer, statistical analysis was done using Chi-square & t- student test to determine source of variances between different type of water sampling & distribution of parasites among samples (18).

Results

Water examination of 150 samples from different areas of Kirkuk province shows the all rate 14% of water contamination with parasitic stages, which distributed in 21 water samples .The all rate was divided into 12 % (18 +ve sample) in urban area & 2 % (3 +ve samples) in rural area. $P < 0.05$, Table 1. Location & type of water collection are clearly seen in table(2) through which, the all rate 14% were divided into 12% of river water (Khasah Sou), followed by governmental pipes water with the rate 1.33% & 0.66 % in the water from wells, $P < 0.05$. Table 2 .In regard of water turbidity the result of the present study shows significant relationship in distribution of parasitic stages & turbidity especially with river water, which reveals 5.33 % of parasitic stages & 5.34 % of turbidity, followed by 0.66 % in well water with turbidity rate 0.66 %, while five samples of direct water & two samples of indirect water shows 3.33% & 1.33 % of turbidity respectively with negative percentages for parasitic stages in both, $P < 0.05$, table 3. Table (4) is showing determination of chlorine percentage & its effects on distribution of parasitic forms in water sample, the abnormal chlorine % were seen in 6 samples (three samples of direct & wells water) with the rate 1.3 % for each one. Normal chlorine

percentages in 146 water samples shows 19 positive result for parasitic forms, while each three abnormal chlorine percentages in direct & well water shows one positive sample for parasitic with the 1.3 % for each one. Relationship between chlorine percentage & distribution of parasitic stages is not significant $P > 0.05$.Normal PH of water is between 6.5---9.2, while the value below & above is consider as abnormal PH. This factor was studied ,from a total of 150 only 9 samples shows abnormal PH with the rate 6% this rate was divided in to 3.34 % in water sampled from river, followed by 1.33 % in both direct & indirect water samples, although taste of wells water in normal seems to be slightly salient ,but all well water sample not shows abnormal PH , Relationship between water PH according to different mode of sampling is not significant $P > 0.05$. Also same relationship was obtained when abnormal PH of water is take in consider with distribution of parasitic forms, only six samples shows parasitic stages(five from river & one sample from direct sampling with the rates 3.34% & 0.66 in both respectively), table 5 .Water contamination with parasites & other microorganisms is the main target of the present study, the common parasitic forms which were found includes *Entamoeba histolytica*, in 19 samples with the rate 12.6 %, this rate also distributed as high rate 10.6 % in water from river, followed by 1.3 % in direct water samples, & the lowest rate 0.66 % was seen in one well water sample, $P < 0.05$. The second parasite is *Giardia lamblia* which was found in 5 samples with the rate 3.2 %, also high rate 2.6 % of giardia was obtained from river water followed by 0.66% in well water, while no *Giardia* stages be obtain in direct & indirect samples.. Other microorganisms that are found includes *Candida* species & *paramecium*, which were found in 19 samples (16 from river, & 1 sample from well) with following rates 12.0 % & 0.66 % respectively. Total rate of water contamination in Kirkuk province with Microorganisms is 42 %, with high frequency in Khasah Sou 38.6 % , $P < 0.05$. Table 6. Khasah Sou as river passes through kirkuk

city was divided into four locations: Raheem awa to Qala'a, Albaladya, Alkurniesh & Alwakfa to doomez. High rate 36.84 % of parasite isolation was obtained in both Raheem awa & Alkurniesh followed by 15.78 % in Alwakfa ,while the lowest rate 5.26 % was in Albaladiyah region ; $P < 0.05$. Table 7. The parasites that isolated were *Entamoeba histolytica* in 16 samples & *Giardia lamblia* in 3 samples. Other two positive samples shows double protozoan parasites.

Discussion

The subject of protozoa or parasite which can cause contamination to water samples in Kirkuk city is the first attempt, pervious studies, literatures, or any formal information are absent, so the following discussion to the results of this study was suggestive. The all rate of water contamination 14 % is very high according to the small size of samples 150, this can be attributing to ignoring of water treatment & poor practice in water examination in this province. The result of the present study was agree with those recorded in Unites State by (6 & 19), & with that in southwest of England by (20), whom they record the rates from 1.5 to 20% of water contamination with protozoa specially high rates of giardiasis. Considering high rate of water contamination in urban area 12 % , this may reflect the degree of population exposure to diarrhea & gastroenteritis caused by protozoan parasites, the causes can be explained by several factors , such as continues of water interruption in this province & hence the people try to use other or alternative source of water(to bring residual water from Khasah' sou which normally contaminated by microorganisms as it was shown in table 7, or even not to take complete care in washing hands after working in gardens or in farm at that time the rate of exposure becomes high with parasitic stages. Although the rate of water contamination in rural area 2% was low within 15 villages, this not meant the exact percentage because the number of sampling is very low, which due absence of facilities of movements among villages ,increasing size of

sampling may prove that. Concerning two type of water & parasite stages distribution, tightly fitted tank water(indirect type) is good potable or direct water can be consumed by human being due its clearance from parasitic forms , this finding is not agree with that recorded by (21),who found 44.69 % & 37.87 % of *Giardia lamblia* stages in governmental water & tank water (indirect) respectively . While the water from river (Khasah Sou) is not potable, due to high rate of contamination, which contains protozoan contaminants specially *Entamoeba histolytica* 10.6 % followed by *Giardia lamblia* with the rate 2.6 % , the causes of this high rates may be due to several factors low level of water flow in the river because its restricted by illegal building on the sites of river , most of men are without work, so they takes parts on the side of river to seal fishes, vegetables, they discharge their wastage directly to river, in addition to direct discharge of animal offal or animal fecal contact with water. Further more that no control or special program by ecology directorate could be applied to control or reduce the water exposure for contamination. Regarding the rate of well water contamination with parasitic stages, although the rate 0.66 % is low, but it has important, this may be due that, most of wells are not properly covered which can give rise in more susceptibility for contamination. Although the relationship between water contamination with parasitic form & chlorine percentage is not significant , but the rate 2.6 % (1.3 % in both direct & well water) is important & reflect bad or incomplete treating of water with chlorine or due to low level of sanitation & practices in labors in the origin source of water supply. Water appearance as clear as requirement for drink & consume, so the turbidity is not desirable, the high occurrence of parasitic rates 3.34 % & 0.66 % in river & well water respectively is not related to parasites, this may be due to wrong usage of river via the waste materials or disposable & even the offal of animals were discharged, the result of the present study is not agree with that recorded by (21), who record 8.33 % of parasitic contamination of

well water. Growth & development most of the protozoan parasites are achieved at isotonic PH 7.0--- 7.6 with some exception to *Entamoeba histolytica* which survive in slightly acidic media (22), but the causes of abnormal PH also related to human being interference artificially .Crowded area, poor hygienic condition, low socio-economic levels of peoples close to river in addition to

animal Park in Alwakfa region close to river & slaughter house, which discharge directly the waste products to the river, had role in explaining high rate of water contamination with protozoan parasites in this area .

Table (1): percentages of water contaminated with parasitic stages in Kirkuk Province.

Districts	Total number examined	Number of +ve Positive sample	Percentage % Of positive
Urban area	122	18	12 *
Rural are	28	3	2
total	150	21	14

P<0.05 = significant.

Table (2): Distributions of parasitic contamination of water according to type of water samples .

Type of sample collection	Total number examined	Number of +ve Positive sample	Percentage % Of positive
Direct	73	2	1.33
Indirect	50	0	0
Well	8	1	0.66
River Khasah Sou	19	18	12 *
total	150	21	14

P<0.05 = Significant

Direct means water from governmental pipes .

Indirect means water from tanker vehicles

Table (3) Relationship between parasitic contamination of water &the Turbidity percentage

Type of sample collection	Total No. examined	No. of Abnormal turbidity	% of Abnormal turbidity	No. of Sample Positive e For parasites	% of Sample Positive e For parasites
Direct	73	5	3.33	0	0
Indirect	50	2	1.33	0	0
Well	8	1	0.66	1	0.66
River Khasah Sou	19	8	5.33	8	5.34 *
total	150	16	10.66	9 **	6.0

P<0.05 =significant

** The other 12 positive samples are clear (not turbid).

The abnormal turbidity in our result is from (26.0-- 280)NTU.

NTU means nephelometric turbidity unit

Table (4) Relationship between parasitic contamination of water & the chlorine percentages .

Type of sample collection	Total No. Examined	No. of Sample with abnormal Chlorine percentages	% of Sample with abnormal Chlorine percentages	No. of Sample Positive For parasites	% of Sample Positive for parasites
Direct	73	3	1.3	1	0.66
Indirect	50	0	0	0	0
Well	8	3	1.3	1	0.66
River Khasah Sou	19	0	0	0	0
total	150	6	2.6	2 **	1.32

*P>0.05 = not significant.

*The other positive water samples with parasites are with Normal chlorine percentages.

** Normal chlorine percentage in water sample is ranged from 0.1--2.5 ppm(part per million).

Table (5): Relationship between parasitic contaminations of water & PH.

Type of Sample collection	Total No. examined	No. of Sample with Abnormal PH	% of Sample with Abnormal PH	No. of Sample Positive for parasites	% of Sample Positive For parasites
Direct	73	2	1.33	1	0.66
Indirect	50	2	1.33	0	0
Well	8	0	0	0	0
River Khasah Sou	19	5	3.34	5	3.34 *
total	150	9	6.0	6 **	4.0

*P>0.05 = not significant .

**Other 15 positive samples with parasitic forms are with normal PH.Normal PH is between 6.5-9.2.

Abnormal PH in present study is ranged from 5.6-- 10.3.

Table (6): Percentages of protozoan parasites & other microorganisms in different type of water samples.

Type of sample collection	Total No. EXAMINED.	protozoa				Other microorganisms				Total contamination With protozoa & microorganisms	
		Giardia lamblia		Entamoeba histolytica		Candia species		Paramecium		No. +ve	% +ve
		No.	+ve	No.	+ve	No.	+ve	No.	+ve		
Direct	73	0	0	2	1.32	0	0	0	0	2	1.32
Indirect	50	0	0	0	0	0	0	0	0	0	0.0
Well	8	1	0.66	1	0.66	1	0.66	0	0	3	1.98
River Khasah Sou	19	4	2.6	16	10.6 *	18	12	18	12	58	38.6 *
total	150	5	3.22	19	12.6	19	12.6	18	12	63	42.0

Table (7): Positive percentages of parasites distribution in river (Khasah Sou) Correlation with some water quality test (Turbidity, Chlorine & PH).

Districts *	Total No. examined	Abnormal Turbidity	Abnormal chlorine	Abnormal PH	No. of isolated parasites	% Of * isolated parasites
Raheem Awa	7	3	0	3	7	36.84
Albaladiah	2	0	0	0	1	5.26
Alkurniesh	7	2	0	1	7	36.84
alwakfa	3	3	0	1	3	15.78
Total	19	8	0	5	18	94.73

*P<0.05 = Significant.

The parasites that isolated were Entamoeba histolytica in 16 samples & Giardia lamblia in 3 samples. Other two positive samples shows double Protozoan parasites.

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