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Diagnostic study of parasites of the urinary tract infection in Nineveh governorate

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Abstract
Urinary tract infection (UTI) is one of the most widespread diseases in the world, it occurs as a result of one of the pathogens such as bacteria, viruses, fungi, as well as parasites. During this study, 305 urine samples were collected for UTI patients from hospitals and private laboratories and people suffering from UTIs from Nineveh Governorate. After conducting the examination, 27 cases of parasitic infection were recorded, at a rate of 8.90%. Among these diagnosed parasites are *Trichomonas vaginalis* with a percentage of 25.92%, *Giardia lamblia* (14.81%), *Chilomastix mesnili* (22.2%), *Entamoeba histolytica*, *Enterobius vermicularis* and *Ascaris lumbricoids* with a percentage of (7.4%), while the parasites *Schistosoma mansoni*, *H. nana*, *Entamoeba coli* and *Taenia saginata* got a percentage of 3.70%. As for the results categorized by gender, it was found that the infection rate among females was the highest among males, which amounted to 11.33%, compared to 3.92% for males, and comparing the infection rate according to age groups, the largest number of injuries was recorded in the age group (26-30) years, with a rate of 22.22%, it was noted that parasitic infections were concentrated in the poorest segment, reaching a rate of 11.03%. It was also noted that there was a significant correlation between parasitic infection and the quality of drinking water, as the highest rate of infection for users of drinking water was recorded, and it was at a rate of 9.42%, by studying the relationship of those infected with animals, it was found that the highest injuries were recorded among animal breeders, which amounted to 9%.

Keywords: Nineveh governorate, Urinary Tract Infection, bacteria, viruses, fungi

Introduction
Urinary tract infection is one of the most common health problems after respiratory infection, as it occurs as a result of infection with many pathogens such as bacteria, viruses, yeasts and parasites (Piranfar *et al*., 2014; Bennett *et al*., 2016) [27, 8]. Urinary tract infection affects all age groups, with approximately 150 million infections annually (Mazzariol *et al*., 2017) [20], in addition to physiological effects, UTIs have been associated with serious long-term complications such as kidney scarring, hypertension, and renal failure, (O'Brien, 2013) [25].

Most cases of Urinary tract infection occur as a result of the transmission of pathogens from the normal flora of the rectum to the upper urinary tract through the urethra (Handly *et al*., 2002) [16], as the recurrent Urinary tract infection causes a number of physiological damages such as cystitis glandular, urinary tract sepsis, and pyelonephritis (Zhiqiang *et al*., 2004; Andreini *et al*., 2005) [36, 5]. Parasitic infections are one of the most important pathogens of UTI, and parasites are considered as one of the causes of UTI, as *Trichomonas vaginalis* is the most common pathogenic parasite in the world being responsible for inflammation of the vagina in females and the urethra and prostate in males (Mor *et al*., 2016) [22]. Three-quarters of women infected with the parasite *Schistosom haematobium* suffer from female genital schistosomiasis when eggs accumulate in the vagina, cervix, or fallopian tubes (Kjetland *et al*., 2008; Downs *et al*., 2011) [18, 13]. In addition, there are many studies that have diagnosed many parasites, including *Chylomastix mesnili*, *Balantidium coli*, *Strongyloides stercoralis* larvae, and *Enterobius vermicularis* eggs in urine (Khurana *et al*., 2018) [17]. Microfilaria has also been seen by (Ahuja *et al*., 2012) [2] and (Vankalakunti *et al*., 2008) [35]. The infection of the larva of the parasite *Enterobius vermicularis* in urine was recorded by (sumanto *et al*., 2021) [34] as the first case in Indonesia, in addition to the infection of eggs and adult worms of the parasite in urine of one of the patients suffering from recurrent urinary tract infection (Choudhury *et al*., 2017) [12]. In North America, the first case of the Ascaris worm with a length of 15 cm was recorded in the urine (Quick *et al*., 2001) [28], followed by the diagnosis

of the parasite *Ascaris lumbricoides* in the form of an adult worm, it was the first case in which the presence of the parasite was detected in the upper part of the urinary system (Baralo *et al*., 2018) [7]. An infection with a live adult worm of the Ascaris parasite in the bladder was also recorded in Ethiopia (Mesele & Mengistu., 2021) [21]. As a result, we decided in this study to focus on the most important parasites of the urinary tract infections in the city of Mosul.

Materials and Methods

1. Sample collection

During this study, 305 urine samples were collected from patients suffering of UTI during the period from October of 2021 to April of 2022. The samples were collected from hospitals and private laboratories from people with UTI and from different areas in the city of Mosul on its right and left sides (Al-Rafidain, Al-Zahraa, Rajm Hadid, Al-Intisar, Nablus, Al-Abar, Sumer, Al-Mithaq) as well as some villages in Nineveh Governorate (Al-Aziziyah, Al-Obour, Kokjali, Rabiea) the samples were kept in sterilized plastic containers, the name and date were recorded for each sample until transported in refrigerated conditions. They were examined in the Biology Science Laboratory of the College of Education for Girls at University of Mosul (Gupta, 2001; Adeyeba & Ojeaga, 2002) [14, 1] and the data of each sample was recorded in a questionnaire that was distributed to them.

2. Preparing the samples for examination

The physical characteristics of each of the samples to be examined were noted and recorded before being placed in the centrifuge and then 10 ml of urine sample was transferred to centrifuges after marking the tubes, then centrifuged at a speed of 2500 revolutions for 10 minutes and then, the floating part was removed and the precipitate was taken for examination. A drop was transferred from the precipitate to the slide and examined after covering it with the slide cover with 5 replicates for each urine sample. (Cheesbrough, 2007) [11].

3. Sample staining

a. Lugol's iodine dye

A drop of Lugol's iodine dye was placed on the slide and mixed with a drop of the urine sample, then examined under the microscope (Satry & Bhat, 2014) [30].

b. Giemsa stain

Clean glass slides were prepared and a drop of urine precipitate was, then methyl alcohol was placed and left for a minute to dry in room air. After the slides had dried, Giemsa stain and phosphate solution were applied in a ratio of 1:1, then left to dry for 5 minutes, then the slide was washed with water and left to dry (Paliwal *et al*., 2017) [26].

4. Microscopic examination

After staining, the glass slides containing the precipitate were placed under a microscope and examined under magnification of 400x, then an oil lens with a magnification 1000x was used for the diagnosis of parasites (Blak *et al*., 1999) [10].

Results

By following up on the results of the microscopic examination of 305 urine samples for patients coming to laboratories and suffering from urinary tract infection, they were collected from women, men and children of different ages during the study period (from October 2021 to April 2022), and by following up on the results of the microscopic examination of the samples, with 5 replicates for each sample, it was noted that there were various parasitic infections in 27 of the total samples examined. The current study recorded an infection rate of 8.90%, as shown in Table (1).

Table 1: Shows the percentage of total infections for the presence of the parasite in urine samples

No. of total samples	No. of positive samples	No. of negative samples
305	27 (%8.90)	287 (91.14%)

1. The results of diagnosing the types of parasites isolated from urine samples

During this study, 10 species of intestinal parasites were diagnosed, mostly in the examined urine samples, as it was noted that the highest infection rate was recorded with Trichomonas vaginalis, with an infection rate of 25.90% (Picture No. 1), followed by Chilomastix mesnili, which recorded an infection rate of 22.20% of the total percentages (Picture
No. 2). Among the distinctive results recorded during this study, it was noted that there were 4 infections of the *Giardia spp.* parasite, where the rate was 14.81%, which was seen in the cyst and trophozoite stage during the microscopic examination of urine samples (Fig., 3), in addition to diagnosing the cyst stage of *Entamoeba histolytica* parasite, which recorded an infection rate of 3.70% (Fig., 4), also, a cyst of *Entamoeba coli* was diagnosed in one of the cases, with a percentage of (3.70%). (Fig., 5).

The results of the study also showed the recording of parasitic worm infections, as the eggs of *Ascaris spp.* that were observed in two samples were diagnosed and the infection rate was 3.70% (Picture No. 6), and two infections of pinworm eggs were recorded at a rate of 7.40% (Fig., 7) in addition to the *Schistosoma mansoni* egg that was observed in one of the infections with a percentage of (3.70%) (Fig., 8), in addition to the diagnosis of the egg of the parasite *Taenia saginata*, which was diagnosed in the urine of a patient with a percentage of 3.70% (Fig., 9) a single egg of *H.nana* parasite in one infection (Fig., 10) and it was estimated by (3.70%) of the total infection rates, as shown in Table (2).

Table 2: Shows the types and percentages of parasites diagnosed from urine samples tested in the laboratory

Species of the diagnosed parasites	Number of positive samples	Percentage%
Trichomonas vaginalis	7	25.92%
Giardia lamblia	4	14.81%
Chilomastix mesnili	6	22.22%
Schistosoma mansoni	1	3.70%
Entamoeba coli	1	3.70%
Entamoeba histolytica	2	7.40%
Enterobius vermicularis	2	7.40%
Ascaris lumbricoids	2	7.40%
H. nana	1	3.70%
Taenia saginata	1	3.70%

2. The results of the distribution of the percentages of positive infections according to gender

By following up on the results of the current study, it was noted that the highest rates of infection were recorded in females compared to male infections, as females recorded 23 infections with a rate of 11.33% compared to males who recorded only 4 infections with a percentage of 3.92% in the urine of the patients as shown in Table (3).

Table 3: Shows the distribution of infection rates according to gender (males and females)

Gender	No. of samples	No. of positive samples	Percentage%
Males	102	4	3.92%
Females	203	23	11.33%

3. Distribution of infection rates according to age groups

By following up on the distribution of infection rates according to the ages of the patients, it was noted that the highest rate of infection was recorded in the category (26-30) years with a rate of 22.22%, followed by the age group (5-10) years in which 5 infections were recorded at a rate of (18.51%), and the category (36-40) also recorded 5 infections, with a rate of 18.51% and most of the cases of this group were infected with the *Trichomonas vaginalis* parasite, while the group (31-35) years recorded three cases infected with parasites and they were at a rate of 11.11%, while in the age group (51-55) years, which recorded three parasitic infections were also recorded with a rate of 11.11%, while the group (11-15) years recorded two infections with a rate of 7.40%, one of which was for the *Trichomonas* parasite at the age of 14 years and the other at the age of 12 years for the parasite *Taenia saginata*, and it was noted through the study that the least infections were recorded within the age group (56-60) years, with a single infection rate of 3.70%, as shown in Table No. (4).

Table 4: Shows the distribution of infection rates according to age groups in the samples of the examined patients

Age groups	No. of samples	No. of positive samples	Percentage
(5-10)	82	5	18.51%
(11-15)	40	2	7.40%
(16-20)	23	0	0%
(21-25)	30	2	7.40%
(26-30)	29	6	22.22%
(31-35)	28	3	11.11%
(36-40)	21	5	18.51%
(41-45)	15	0	0%
(46-50)	13	0	0%
(51-55)	9	3	11.11%
(56-60)	7	1	3.70%
(61-65)	3	0	0%
(66-70)	3	0	0%
(71-75)	2	0	0%

4. Distribution of the infection rate according to the economic level

Most of the infections were concentrated in people of low economic level, as the study recorded 16 parasitic infections out of a total of 145 urine samples that were examined, which was 11.03%, while the number of infections in the middle-income group was 11 infections out of a total of 148 samples, at a rate of 7.43%, as in Table No. (5).

Table 5: Shows the distribution of infection rates according to the economic level of urine samples for the patients

Social level	No. of samples	No. of positive samples	Percentage%
High	12	0	0%
Average	148	11	7.43%
Low	145	16	11.03%

5. Distribution of infection rates according to receiving treatment

Through conducting a questionnaire during the current study, 26 infections were recorded out of a total of 295 samples examined with a rate of 9.77% in people who did not use treatment, while one infection was recorded at a rate of 10% for people who received treatment before the test, as shown in Table No. (6)

Table 6: Shows the rates of infection among the examined persons, according to whether they received treatment or not.

Distribution of infection rates according to receiving treatment	No. of samples	No. of positive samples	Percentage%
The patient used a treatment before the examination	10	1	10.00%
The patient did not use any medication prior to the examination	295	26	9.77%

6. Distribution of infection rates according to marital status

The infections were distributed between married and unmarried people, with 10 infections out of a total of 137 samples with a rate of 7.29% for the unmarried people examined, while the highest infection rate for married people was 10.11% as shown in Table No. (7)

Table 7: Shows the distribution of parasite infections rates according to social status

Social status	No. of samples	No. of positive samples	Percentage%
Unmarried	137	10	7.29%
Married	168	17	10.11%

7. Distribution of infection rates according to the type of drinking water

By following up on the type of drinking water that the examined people during this study depend on, it was noted that the infection rates were the highest among the people who depended on liquefied water, as the largest number of infections was 26 positive samples, with an infection rate of 9.42%, while the infection rates were lower in the people who depend on liquefied water. Mineral water increased by 6.66%, while no infection was recorded in people who depend on well water for drinking, as shown in Table No. (8).

Table 8: Shows the distribution of infection rates according to the type of drinking water

Type of drinking water	No. of samples	No. of positive samples	Percentage%
Well water	12	0	0%
Municipal water	278	26	9.42%
Mineral water	15	1	6.66%

8. Distribution of infection rates according to animal presence

The study was concerned with following up the distribution of infections according to the presence or absence of animals in the environment of the infected people from whom urine samples were isolated, as it was noted that the highest percentage of parasite infection was 9% for people interested in animal husbandry, while the result of examining 205 urine samples collected from people who did not raise animals, 18 samples from them appeared with a positive result were infected with parasites as shown in Table No. (9)

Table 9: Shows the distribution of infection rates according to animal presence among the infected

	No. of samples	No. of positive samples	Percentage%
Contact animal	100	9	9.00%
Not contact with animal	205	18	8.78%

9. Distribution of percentages according to educational level

By studying the relationship of education level of the patients under study, it was noted that the uneducated category had 17 infected cases out of 188 samples, with a higher infection rate than the educated, estimated at 9.04%, as shown in Table No. (10).

Table 10: Shows infection rates according to educational level

Educational level	No. of samples	No. of positive samples	Percentage%
Educated	117	10	8.54%
Uneducated	188	17	9.04%

10. Studying the effect of infections on color in the examined urine samples

By linking the diagnosis of urine samples with the color of the sample, it was noted that the highest rate of infection for urine samples with
a pale-yellow color was recorded at 10%, while the lowest rate of infection for urine samples with natural color was recorded at 5.91%, as in Table No. (11).

Table 11: Shows the study of the effect of infections on color in the examined urine samples

Color	No. of samples	No. of positive samples	Percentage%
Pale-yellow	19	2	10%
Red	11	1	9.09%
Dark yellow	106	8	7.54%
Natural yellow	169	10	5.91%

11. Studying the effect of infection on the turbidity of the urine of the examined samples:

Through the physical examination of the turbidity of urine samples, it was noted that most of the positive samples had a pure appearance with a rate of 7% and were higher than the rate of infections with a turbid appearance as in Table No. (12)

Table 12: Shows the effect of infection on the turbidity of the urine of the examined samples

Turbidity	No. of samples	No. of positive samples	Percentage%
Pure	200	14	7%
Turbid	105	7	6.6%

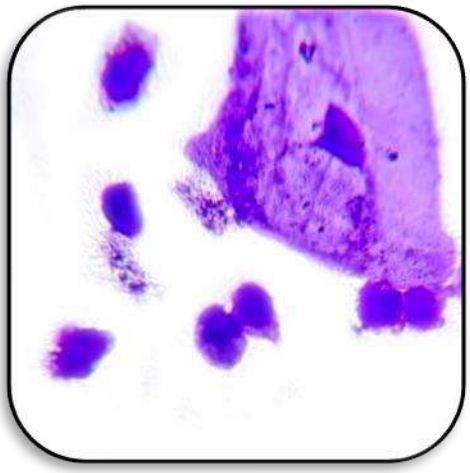

Fig 1: Shows the parasite *Trichomonas* with a magnification of 1000x, dyed with Giemsa stain

Fig 2: Shows the parasite *Chilomastix* at a magnification of 1000x

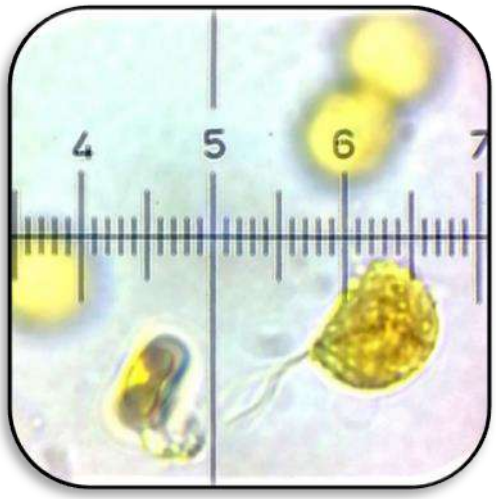

Fig 3: Shows the parasite *Giardia* with a magnification of 1000x

Fig 4: Shows the parasite *Entamoeba histolytica* with a magnification of 1000x

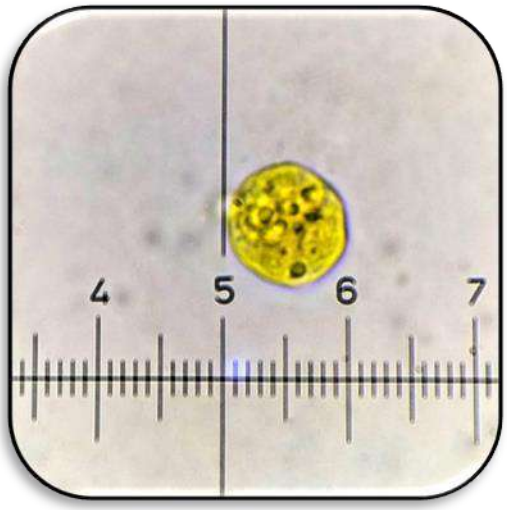

Fig 5: Shows a cyst of the parasite *Entamoeba coli* with a magnification of 1000x

Fig 6: Shows an egg of the parasite *Ascaris lumbricoids* with a magnification of 1000x

Fig 7: Shows an egg of the parasite *Enterobius vermicularis* with a magnification of 1000x

Fig 8: Shows an egg of the parasite *Schistosoma mansoni* with a magnification of 1000x

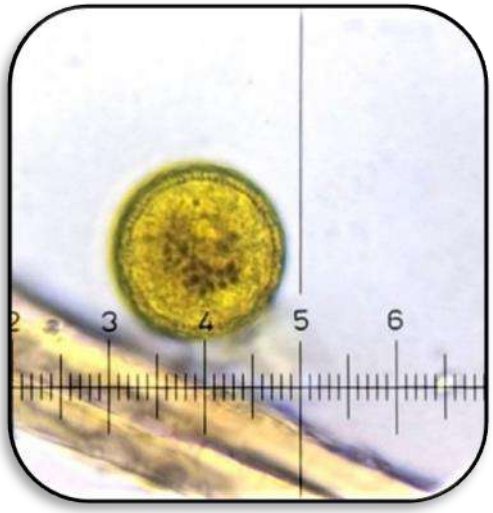

Fig 9: An egg of the parasite *Taenia saginata*, with a magnification of 1000x

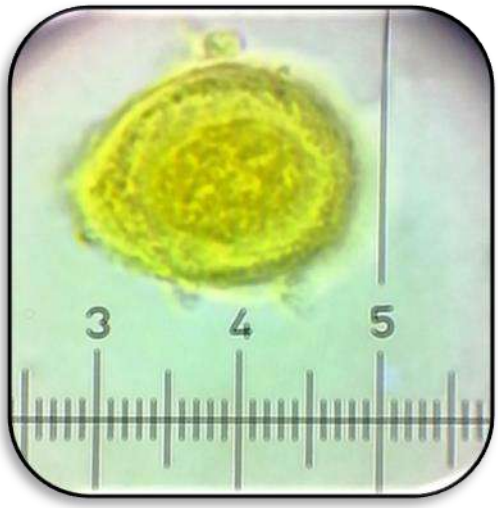

Fig 10: Shows an egg of the parasite *H.nana* with a magnification of 1000x

Discussion

The prevalent idea in the past was that most of the causes of UTI are due to the presence of pathogens such as bacteria, yeasts and viruses of the urinary system (Piranfar *et al*., 2014) [27], as nearly 150 million UTI cases are recorded annually (Mazzariol *et al*., 2017) [20], but with the successive research studies, it was found that some UTI cases occur due to the presence of parasites in the urinary tract, it has been observed that many pathological effects left by parasitic organisms in the urinary system, such as cervical cancer and pelvic inflammation and prematurity due to preterm rupture of placental membranes (pprom) pregnancy, as well as immunodeficiency and other symptoms as a result of infection with parasites such as the parasite *Trichomonas vaginalis* or *Schistosomes* species, as females suffer from female genital schistosomiasis when parasite eggs accumulate in the vagina, fallopian tubes or cervix (Kjetland *et al*., 2008; Downs *et al*., 2011) [18, 13].

Microscopic diagnosis of parasitic infections from the urine of UTI patients

The current study recorded a number of parasites that were associated with urinary tract infection in 27 (8.9%) cases of parasitic infection. These results are close to what was obtained in the Indian study, as it diagnosed 25 parasitic infections, three of which were consistent with what was recorded in our current study, which are: the eggs of pinworms, the parasite *Chilomastix* and the parasite *Trichomonas vaginalis*, which were recorded as the highest recorded parasitoid species in both the current and Indian studies (Khurana *et al*., 2018) [17]. While a Nigerian study recorded 20 parasitic infections, most of the infections were due to the urinary schistosomiasis parasite, followed by the trichomoniasis parasite (Ayoade *et al*., 2013) [6].

Despite the fact that, most of the intestinal parasites recorded in our study are common and their presence was recorded in stool samples examined in many studies conducted in Iraq, such as *Entamoeba spp*., *Enterobius vermicularis*, *Ascaris*, *lumbricoides*, *Tania saginata*, *Hymenolepis nana*, *Giardia intestinalis* (Al-Bayati, 2007) [3] This indicates that there are factors that help in the spread and presence of these parasites, such as social and economic conditions, as well as the environment and poor personal hygiene. The transmission of worms such as pinworms can also occur through contaminated soil or through fecal-oral contamination, as for the parasite *Entamoeba spp*., it can be

transmitted through contaminated food and water (Manganelli *et al*., 2012; Someshwaran *et al*., 2015; Gyang *et al*., 2017) [19, 33, 15] the presence of these intestinal parasites in an unnatural place may be due to contamination during the defecation process, as well as their coexistence in the urinary tract in an unknown way so far.

The record of the parasite *Schistosoma mansoni* in our results is for the first time in Al-Mosul city, as the case was diagnosed in the urine of a patient who was suffering from severe inflammation of the urinary tract in addition to her diabetes, and this result is consistent with what was recorded by (Ratard *et al*., 1991) [29], which attributed the reason to the density of the eggs of the parasite resulting from the acute infection, which caused the leakage of these eggs into the urinary tract. It is worth noting that the cause of acute infection with the parasite may be due to the fact that the patient works as an animal breeder and that the environment in which she lives contains ponds that would be a cause of infection.

And our recording of pinworm eggs is consistent with what was recorded by (Saeed & Al-Saeed, 2015) [4] in Iraq, in which the parasite in its adult worm form was seen in association with urinary tract infection. The study attributed the relationship between parasitic infection and urinary tract infection to the hyperactivity and movement of the female worm searching for a way to pass through it such as the urethra to reach the urinary bladder, and through this process, the worms make the way for bacteria that are naturally present in the colon and rectum to the new location (bladder) and then the bacteria begin to multiply and cause cystitis and urethritis (Serpytis & Senin, 2012) [31]. Also, the parasitic infection with Ascaris worm was consistent with what was recorded in North America and Ethiopia, where the parasite was observed in the form of an adult worm (Quick *et al*., 2001; Baralo *et al*., 2018) [28, 7]. The reason for the presence of the parasite in the urinary tract was attributed to several reasons, including that there is a fistula that is between the urinary canal and the alimentary canal or the migration of the worm through the urethra to reach the urinary system (Baralo *et al*., 2018) [7], but the most acceptable reason is that the worms were forced to migrate and exit the anus due to anthelminthic treatments and then move towards the perineum to reach the urethra and the bladder (Singh *et al*., 2010) [31].

From comparing the results of rates between males and females, our study recorded parasitic infections in females with a rate of 11.33%, which was higher than what was recorded in males by 3.92%. These results are consistent with what was recorded in Nigeria, this may be due to the prevalence of urinary tract infection in women more than in males, which facilitates parasite infection and the proximity of the anus to the urinary tract and the narrowing of the urethra, in addition to the short specific structure of the urinary tract (Nitzan *et al*., 2015) [24].

While parasitic infections recorded the highest percentage in the age group (26-30) years, in which the majority of infections were caused by *Trichomonas vaginalis*, which is an important cause of vaginitis and the fact that this group is sexually active, this explains the association of urinary tract infections with age and gender, in addition to the infection history (Neild, 2003) [23]. The areas that suffer from a lack of health care and poor infrastructure, causing recurrent infection with parasites as well as the availability of intermediate hosts for them (Bennett *et al*., 2019) [9], this explains the emergence of the highest rate of infection among the poorest people, as it obtained a rate of 11.03%, in addition to the use of municipal water for drinking, which also recorded the highest percentage of parasitic infection, reaching 9.42%, which may be due to the inability to use this water for drinking because it may contain pathogens that may kill individuals unnoticed, in addition to not filtering and purifying them by severe control methods.

Conclusion

Through these studies, it was concluded that the importance of the role of parasites associated with urinary tract infection and using molecular methods to obtain a more accurate view for these parasites and their impact on the tissues of the urinary system, including the kidneys.

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