Studies on trichomoniasis in Libya and comparison between InPouch™ TV culture, wet mount examination and Giemsa staining for diagnosis of the disease

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Abstract: Trichomoniasis caused by *Trichomonas vaginalis* is the most prevalent non- viral sexually transmitted disease. In females, it causes vaginal discharge, severe pruritus, dyspareunia and dysuria. It can lead to severe reproductive health sequelae in both sexes and it has been implicated in increasing sexual transmission of human immunodeficiency virus up to two folds .Laboratory diagnosis of the disease is necessary and could be made by several methods which vary in their sensitivity and specificity. InPouch™ TV culture is highly sensitive in diagnosing the disease. Unfortunately, researches made in Libya to study the prevalence of trichomoniasis are very few; also diagnosis is based only on clinical manifestations which could be confused with those of other sexually transmitted diseases. Hence, the present work was aimed to determine the prevalence of *Trichomonas vaginalis* infection in women suffering from vaginal discharge in Zawia district, Libya. Also, to compare between InPouch™ TV culture, wet mount examination and Giemsa staining for the diagnosis of trichomoniasis. In addition, to study some sociodemographic characteristics and some hygiene practices of patients infected with *Trichomonas vaginalis* as a base to conduct control programs of the disease. Ninety eight patients suffering from vaginal discharge (Group 1) and 30 patients not suffering from vaginal discharge (Group 2 or control group) were included in this study. All participants were asked about age, level of residence and many hygiene practices. Three vaginal swabs were taken from each participant and examined by InPouch™ TV culture, wet mount examination and Giemsa staining. It was found that infection rate was relatively high in the examined patients. InPouch™ TV culture was the most sensitive method and detect 36.7% positive patients among group 1. Also, its specificity was 100%. It was superior to wet mount examination and Giemsa staining regarding ease of handling & interpretation, ease of transportation and total time of reading results. Infection rate was highest among patients in the age group 40-45 years. Also, it was relatively high in patients living in poor level, patients using tub for washing, patients who did not use to bath frequently or use soap on bathing, patients who used seat latrines and patients who were sharing the sleeping place with other members of the family. In addition, it was significantly high in patients who were sharing towels with someone else. It is concluded that infection rate of *Trichomonas vaginalis* in Libya is relatively high, so there must be a policy decision to increase awareness of this disease. InPouch™ TV culture is a very useful tool in diagnosis. It is recommended to use this culture as a routine method for diagnosis in the laboratory.
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Keywords: *Trichomonas vaginalis*, Libya, InPouch™ TV culture, wet mount examination, Giemsa staining.

1. Introduction

In Libya, assessment of the prevalence of nearly all parasitic diseases is lacking. Trichomoniasis is one of the important parasitic diseases to be discussed as it is the most prevalent non- viral sexually transmitted disease and affects an estimated 170 million people worldwide (Secor, 2012). Trichomoniasis is caused by *Trichomonas vaginalis* (TV) which is a flagellated protozoan parasite (Fernando *et al.,* 2011) that inhabits vagina & urethra in females and prostate, seminal vesicle & also urethra in males (Al Heali and Rahemo, 2006). Morphologically, TV has only the trophozoite stage. The shape of the trophozoite in culture is typically pyriform although amoeboid shapes are evident in parasites adhering to vaginal tissue *in vivo*. It is about 9x7 µm (Schwebke and Burgess, 2004). The organism possesses four anterior flagella and there is a fifth flagellum which is incorporated within its undulating membrane. The axostyle is usually extremely obvious and the undulating membrane extends about 2/3 of the distance to the posterior end of the body with no free flagellum. Nuclear chromatin is uniformly distributed (Ryu and Min, 2006). Life cycle of TV is simple in that the trophozoite is transmitted through coitus. Then, the trophozoite multiplies by binary fission and gives rise to a population in the lumen and on the mucosal surfaces of the urogenital tracts of human (Schwebke and Burgess, 2004). In women, trichomoniasis has a wide range of presentations, from an asymptomatic to an acute inflammatory disease (Fernando *et al.,*

2012). Acute infection is characterized by vaginal discharge which is copious, frothy, yellowish green and foul smelling (Patil *et al.,* 2012 and Secor, 2012). Vaginal discharge is associated with severe pruritus, dysuria and dyspareunia. Colpitis macularis (strawberry cervix) can sometimes be seen with the aid of a colposcope as "Speckling" of hemorrhagic spots on the mucosa (Garber, 2005 and Conrad *et al.,* 2012). Males though often asymptomatic can present with urethritis, urethral discharge and dysuria (Johnston and David, 2008). Trichomoniasis has been associated with severe reproductive health sequelae in both sexes, including pelvic inflammatory disease (Cherpes *et al.,* 2006) and adverse pregnancy outcomes in women (Caliendo *et al.,* 2005). In men, prostatitis, infertility and increased incidence of aggressive prostate cancers may occur (Stark *et al.,* 2009). Perhaps most importantly trichomoniasis has also been implicated in increasing sexual transmission of human immunodeficiency virus (HIV) up to two folds (McClelland *et al.,* 2007 and Van Der Pol *et al.,* 2008). The increased transmission may be because of the disruption of urogenital epithelial cells caused by TV that facilitates the passage of HIV and also due to the activation of local immune cells leading to increased replication (Guenthner *et al.,* 2005). Because of the high prevalence of trichomoniasis, this translates into a significant number of global HIV infections (Conrad *et al.,* 2012).

Diagnosis of trichomoniasis cannot be made only on the basis of clinical presentations for several reasons, for example, the clinical symptoms may be synonymous with those of other sexually transmitted diseases (STDs). Also, the classical "strawberry cervix" is seen in approximately 2% of patients and frothy discharge is seen in only 12% of women with TV infection (Patil *et al.,* 2012). It has been demonstrated that if these classical features are used alone in the diagnosis of trichomoniasis, 88% of cases will not be diagnosed and 29% of uninfected patients will be falsely diagnosed as having infection. This suggests that clinical manifestations are not reliable diagnostic parameters and hence laboratory diagnosis is necessary for early and accurate diagnosis (Patil *et al.,* 2012). Unfortunately, diagnosis of trichomoniasis in Libya is based on clinical features only.

Laboratory diagnosis of trichomoniasis is usually made by microscopic examination of a saline wet smear preparation of vaginal secretions. However, positive wet smear is diagnostic because of its high specificity, whereas a negative test cannot exclude trichomoniasis because of its low sensitivity (Fernando *et al.,* 2011). Also, Giemsa stained smears were used for diagnosis of trichomoniasis (Radonjic *et al.,* 2006). Currently, culture is clearly the most sensitive diagnostic method and various culture media have been described for cultivation of TV. Though polymerase chain reaction (PCR) technique has been found more sensitive than culture, different studies have shown its specificity to be slightly lower than culture (Lawing *et al.,* 2000). Association of trichomoniasis in women with adverse outcomes of pregnancy and increased risk of HIV suggests a need for increased control efforts which in turn depends on accurate diagnosis and specific treatment of the disease (Sood *et al.,* 2007).

As there are no available data about the prevalence of TV infection in women in Libya, the aim of the present study is to determine the prevalence of this infection in women suffering from vaginal discharge in Zawia district in Libya. Also to compare between wet mount examination, Giemsa staining and InPouch™ TV culture system (Biomed Diagnostics, San Jose, California) for the diagnosis of trichomoniasis. This culture is used for the first time in Libya. In addition, to study some sociodemographic characteristics and some hygiene practices of patients infected with TV as a base to conduct control programs of the disease.

2. Materials and Methods

A) Study design and samples:

A total of 98 women who were attending El Galaa Clinic in Zawia, Libya and were complaining of vaginal discharge were included in this study (Group 1). Each patient was interviewed face-to-face and asked about some sociodemographic characteristics such as age which ranged from 20-45 years and area of residence. They were classified according to age in three groups: group A which included patients from 20-30 years, group B which included patients from 30-40 years and group C which included patients from 40-45 years.

All patients were asked about hygiene practices such as facilities for washing, frequency of bathing, use of soap when washing, sharing towels, type of latrine used and sharing the place of sleeping. A full clinical data were obtained from each participant including history of pruritus, dysuria and dyspareunia. Also, the gynecologist was asked about presence of strawberry cervix in each patient. Then during speculum examination, three vaginal swabs (cotton tips) from the posterior fornix of the vagina were obtained from each patient (one of them was used for wet mount examination, the second was used for Giemsa staining and the third was used for inoculation of Inpouch™ TV culture system). Thirty women (20-45 years) who were attending the same place and not suffering from vaginal discharge were also included in this study to serve as controls (Group 2). They were examined with the same procedures.

B) Laboratory procedures:

1) Wet mount examination:

The first vaginal swab was placed in 10 ml screw capped plastic tube containing 0.5 ml of 0.9% saline. Swab was vigorously rotated in the saline and pressed against the side of the tube to express as much fluid as possible. One drop of expressed fluid was placed on a glass slide with a cover slip. The slide was initially scanned at a magnification of x100 looking for motile trichomonads and then at a magnification of x400 to confirm the diagnosis within one hour of collection of the sample. The positive result is defined as the presence of one or more trichomonads with the characteristic morphology and jerky motility (McCann, 1974; Kaydos *et al.,* 2002 and Sood *et al.,* 2007).

2) Giemsa staining:

The second vaginal swab which was taken for the purpose of Giemsa staining was kept in sterile tube containing one ml of physiological saline with 3 drops of 5% glucose at room temperature to ensure efficiency in transport and preparation of specimens (Manson *et al.,* 1976; Brown and Neva, 1983 and Bibbo, 1996). Then, it was brought to Parasitology Laboratory, Faculty of Medicine, 7th April University at Zawia district within 3 hours of being taken. Prior to staining with Giemsa, the tube with the swab was vigorously shaken to displace all the parasites in the saline solution. Smear of vaginal exudate was made on a clean glass slide. As soon as the smear was dry, it was fixed by immersion for one minute in absolute ethanol and allowed to dry. The following day, it was stained with Giemsa, diluted one part to 19 parts 1/15 M phosphate buffer, pH 7.2 for ten minutes. After the stain had been washed off and the slide had dried, it was scanned at a magnification of x100 and then at a magnification of x400. At least 30 fields were examined before a negative finding was recorded (Mason *et al.,* 1976).

3) InPouch™ TV culture:

InPouch™ TV culture system (BioMed Diagnostics, San Jose, California) is both a transport and culture system. It is made of a double pouched container made of soft plastic. The two pouches or chambers are separated by a channel that allows the medium to pass between them (Fig.1). The lower chamber contains 4 ml of a selective medium that is inhibitory for both yeast and bacteria. A wire tape attached to the upper chamber is used to open the pouch (Borchardt and Smith, 1991). The third vaginal swab was immediately introduced into the culture according to the manufacturer's instructions. The bag was inoculated by swirling the swab sample in the upper chamber and immediately forcing the inoculum into the lower chamber. The culture was incubated at 35°C and examined for motile TV on day 2 or 3 and again on day 5 after inoculation. A plastic viewer (Fig.2) is placed over the bottom of the pouch before microscopic evaluation. This allows the pouch to be placed on the microscope stage and immobilizes the medium for easier evaluation. The culture was examined at a magnification of x100 and then at a magnification of x400. Sometimes, the culture was examined at a magnification of x1000 to see more details of the parasites. A positive result was defined as the presence of motile trichomonads at any time; a negative result was defined as the absence of motile trichomonads at all readings (Borchardt and Smith, 1991 and Huppert *et al.,* 2007).

The above mentioned three diagnostic methods were compared regarding diagnostic yield, ease of handling & interpretation, total time of the procedure, ability to process large numbers of specimens at the same time, availability of reagents and total cost of the procedure. On calculating the sensitivity and specificity of each diagnostic method, culture is considered the "gold standard" (Patel *et al.,* 2000).

Statistical analysis

Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS version 16. Two types of statistics were done:

1) Descriptive: e.g. no, (%), mean and standard deviation SD.

2) Analytical:-

A) Student's t-test: It is a single test used to collectively indicate the presence of any significant difference between two groups for a normally distributed quantitative variable.

B) Chi-Square (χ^2): It is used to compare between two groups or more regarding one qualitative variable in 2x2 contingency table or r c complex table.

C) Fisher's exact test (FE): It is used to compare between two groups or more regarding one qualitative variable when the count of any of the expected cells less than 5.

D) Z test: It is used to compare the proportions from different groups to determine if they are significantly different from one another.

E) * Sensitivity or true positive rate (TPR) =
True +ve / (True +ve + False -ve)
*** Specificity (SPC) or True Negative Rate=**
True -ve / (True -ve + False +ve)= 1- False +ve rate
*** Accuracy (ACC) =**
(True +ve) + (True –ve) / (Positive + Negative)
***Positive predictive value (PPV) =**
True +ve / (True +ve + False +ve)
*** Negative predictive value (NPV) =**
True -ve / (True -ve + False -ve)

F) P value:

- Insignificant difference if $P > 0.05$.

- Significant difference if $P < 0.05$.
- Highly significant difference if $P < 0.001$.

3. Results

Diagnosis of TV infection by InPouch™ TV culture revealed that 36 patients out of 98 patients of group1 (patients who were suffering from vaginal discharge) were found to be infected (36.7%) while no positive cases (0%) were found among group 2 (control group: 30 women who were not suffering from vaginal discharge). Difference between infection rate of the two groups was highly statistically significant ($p<0.001$) {Table 1& Graph 1}. Regarding diagnosis of TV infection by wet mount examination, 22 out of 98 patients of group 1 were found to be infected (22.4%) while infection rate of group 2 was found to be 0%. Difference between infection rate of the two studied groups was statistically significant ($p<0.01$) {Table 1& Graph 1}. Concerning diagnosis of TV infection by Giemsa staining, 20 out of 98 patients of group 1 were found to be infected (20.4%) while one case of group 2 was found to be infected (3.3%). Difference between the infection rate of the two groups was statistically significant ($p <0.05$) {Table 1& Graph 1}.

Regarding infection rate of TV among different age groups, it was found that the highest rate was recorded in group C (patients between 40-45 years) {72.72%} followed by group A (patients between 20-30 years) {33.33%} and then group B (patients between 30-40 years) {30.95%} {Table 2 & Graph 2}. There was a significant difference between group C and both of group A and B regarding infection rate while there was no significant difference between group A and B.

Concerning level of residence, infection rate of TV was found to be higher in patients living in poor level (52.8%) than in patients living in relatively good level (47.2%){Table 3 & Graph 3}. There was no significant difference between them ($p >0.05$).

As regards relation between hygiene practices of patients of group 1 and infection rate of TV, it was found that patients using tub for washing had a higher rate (55.6%) than patients using shower for washing (44.4%){Table 4 &Graph 4} with no significant difference between the two groups ($p >0.05$).

Patients who used to bath infrequently had a higher infection rate of TV (52.8%) than those who used to bath frequently (47.2%){Table 4 & Graph 4}. Difference between the two groups was not significant ($p >0.05$).

Patients who used to use soap when bathing had a lower infection rate of TV (47.2%) than those who did not use to use soap when bathing (52.8%){Table 4 & Graph 4}. Difference between the two groups was not significant ($p >0.05$).

Patients who were sharing towels with someone else had a higher infection rate of TV (80.6%) than those who were not sharing towels (19.4%){Table 4 & Graph 4). Difference between the two groups was highly statistically significant ($p <0.001$).

Patients who were using seat latrines had a higher infection rate of TV (58.3%) than those who were using pit latrines (41.7%){Table 4 & Graph 4}. Difference between the two groups was not significant ($p >0.05$).

Infection rate of TV was higher among patients who were sharing sleeping place with other members of the family (52.8%) than those who were not sharing sleeping place (47.2%){Table 4 & Graph 4}. Difference between the two groups was not significant ($p >0.05$).

The most frequent clinical manifestation reported by infected patients was pruritus (36.1%), followed by dysuria (22.2%) then dyspareunia (14.3%) and lastly strawberry cervix (2.8%){Table 5 &Graph 5}. There was a high significant difference between positive and negative patients related to group 1 regarding dysuria, pruritus and dyspareunia ($p <0.001$). On the other hand, there was no significant difference between positive and negative patients related to group 1regarding strawberry cervix ($p >0.05$) {Table 5 Graph 5}.

In InPouch™ TV culture, *Trichomonas vaginalis* appeared as oval or pear shaped flagellated bodies. It was slightly larger than a white blood cell, measuring approximately 9X7 µm. There was a large nucleus usually located at the wider anterior end of the parasite. The axostyle was seen as a thin rod that started adjacent to nucleus and finished with a sharp end penetrating from the posterior extremity. Occasionally, flagella appeared. They were in the form of four anteriorly directed flagella and a fifth flagellum that wrapped backwards along the surface of the organism (Figs. 3, 4, 5, 6, 7). Also, jerky movement of the parasite could be detected.

In wet mount examination, TV was seen with the same characters mentioned above regarding InPouch™ TV culture. Moreover, jerky movement of the parasite could be clearly detected (Fig. 8)

In Giemsa staining, TV was seen with the same characters mentioned above but flagella were not obvious. Also, parasites were stained purple (Figs. 9, 10).

Regarding sensitivity and specificity of the diagnostic methods, wet mount examination had a sensitivity of 61% and specificity of 100% when compared to InPouch™ TV culture (Table 6). Giemsa staining had a sensitivity of 50% and a specificity of 97% as compared to InPouch™ TV culture (Table 7).

Regarding ease of handling & interpretation, the easiest diagnostic method was InPouch™ TV culture as it required less hands on time, no slide preparation was needed, could be self-administered and then sent to laboratory for analysis. Moreover, it was so easily transported to laboratory. Wet mount examination was less easy than InPouch™ culture and the least easy method was Giemsa staining.

Concerning total time of the procedures, wet mount examination had the shortest time from taking the specimen till confirmation of infection and then Giemsa staining but InPouch™ TV culture took the longest period of time to know the results. However, this long period was consumed on waiting the growth of the parasite but procedure itself and reading of results did not take a long time. So, regarding reading of results, it took about 15 minutes/slide to examine the sample using wet mount or Giemsa but it took only 5 minutes to read the results of the sample examined by InPouch™ TV culture as the parasites were more abundant.

As regards ability to process large number of specimens at the same time, all the three diagnostic methods had this benefit.

Regarding the availability of reagents, chemicals used in wet mount examination and Giemsa staining were readily available, however, culture was less available.

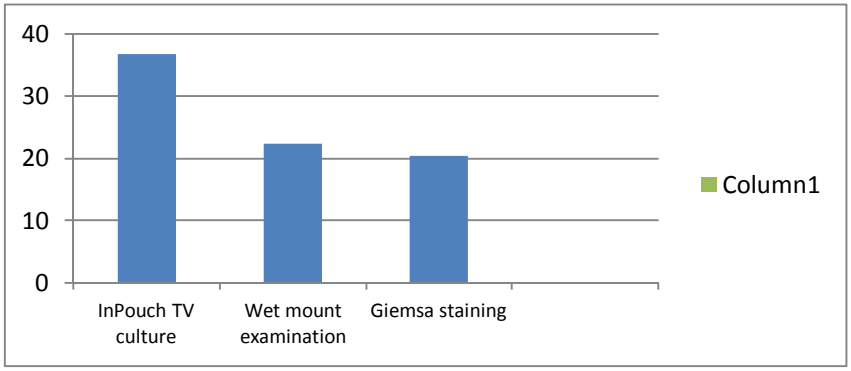
Concerning total cost of the procedure, wet mount examination and Giemsa staining were cheaper than culture. However, culture had the availability to use it for wet mount examination and culture at the same time.

There were more benefits of culture making it superior to wet mount examination and Giemsa staining, for example, no expensive equipment was required to complete it though its high sensitivity. Also, its reagents could be stored for one year at room temperature. This actually reduces storage costs. It eliminates operator exposure to pathogen and reduces volume of media which in turn reduces volume of hazardous waste.

Table 1: Infection rate of *Trichomonas vaginalis* in studied groups as detected by the three diagnostic methods.

	Groups				χ^2	*P* value
	Group 1 (n=98)		Group 2 (n=30)			
	no	%	no	%		
In Pouch TV culture						
Positive	36	36.7	0	0.0	15.33	(0.00) <0.001
Negative	62	63.3	30	100.0		
Wet mount preparation						
Positive	22	22.4	0	0.0	8.13	(0.004) <0.01
Negative	76	77.6	30	100.0		
Giemsa staining						
Positive	20	20.4	1	3.3	4.88	(0.026) <0.05
Negative	78	79.6	29	96.7		

Group 1: 98 patients who were suffering from vaginal discharge.
Group 2: 30 women who were not suffering from vaginal discharge.



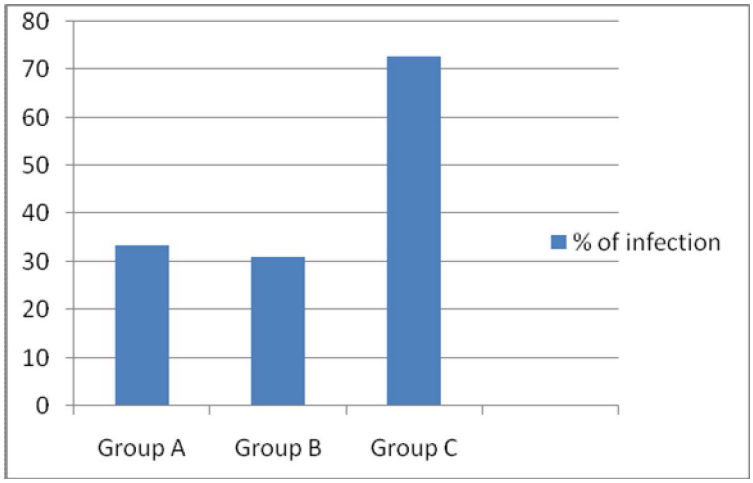
Graph 1: Infection rate of *Trichomonas vaginalis* in studied groups as detected by three diagnostic methods.

Table 2: Relation between age and infection rate of *Trichomonas vaginalis* among patients related to group 1 (98 patients).

Age group	Number of patients	Number of positive patients	% of infection
Group A	45	15	33.33%
Group B	42	13	30.95%
Group C	11	8	72.72%

By z test, it was found that:

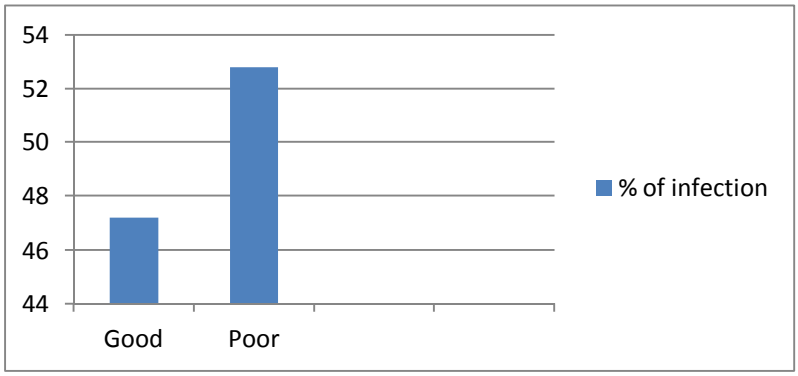
* No significant difference between infection rate of group A and B.
* Significant difference between infection rate of of group C and both of group A and B.



Graph 2: Relation between age and infection rate of *Trichomonas vaginalis* among patients related to group 1 (98 patients).

Table 3: Relation between level of residence and infection rate of *Trichomonas vaginalis* among patients of group 1(98 patients).

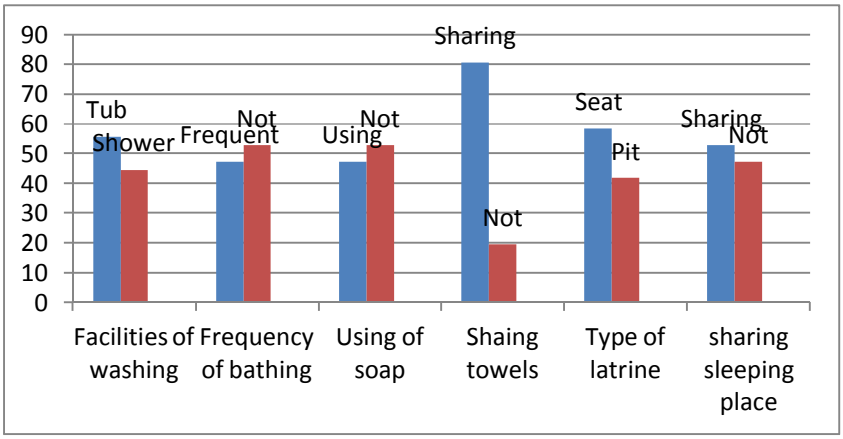
Residence	*Trichomonas vaginalis* infection					
	Positive (n=36)		Negative (n=62)			
	no	%	no	%	χ^2	*P* value
Good	17	47.2	28	45.2	0.039	(0.844) >0.05
Poor	19	52.8	34	54.8		



Graph 3: Relation between level of residence and infection rate of *Trichomonas vaginalis* among patients of group 1 (98 patients).

Table 4: Relation between hygiene practices of patients of group 1 and infection rate of *Trichomonas vaginalis*.

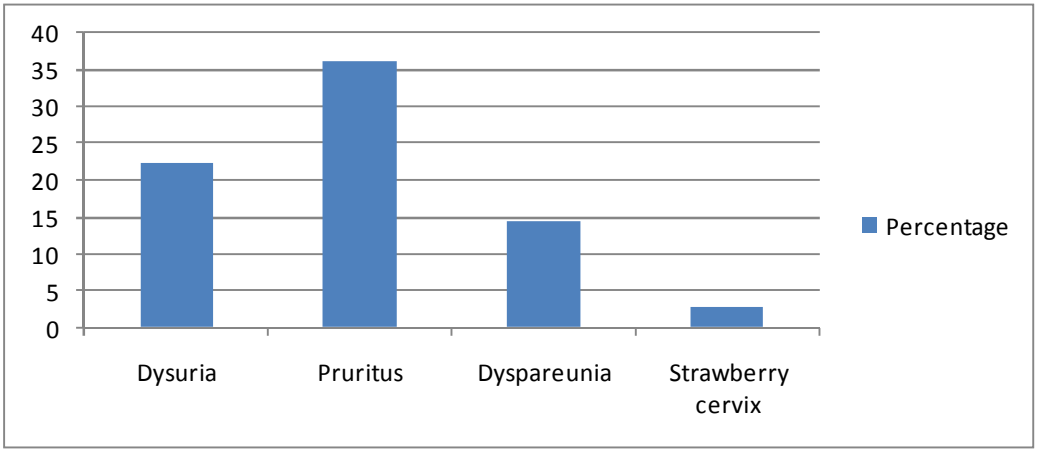
Hygiene practices	*Trichomonas vaginalis* infection				χ^2	*P* value
	Positive (n=36)		Negative (n=62)			
	no	%	no	%		
Facilities of washing						
Tub	20	55.6	34	54.8	0.005	(0.945) >0.05
Shower	16	44.4	28	45.2		
Frequency of bathing						
Frequent	17	47.2	30	48.4	0.01	(0.911) >0.05
Not frequent	19	52.8	32	51.6		
Using of Soap						
Using	17	47.2	37	59.7	1.43	(0.232) >0.05
Not using	19	52.8	25	40.3		
Sharing towels						
Sharing	29	80.6	12	19.4	25.06	(0.00) <0.001
Not sharing	7	19.4	50	80.6		
Type of latrine						
Seat	21	58.3	30	48.4	0.90	(0.342) >0.05
Pit	15	41.7	32	51.6		
Sharing sleeping place						
Sharing	19	52.8	33	53.2	0.002	(0.966) >0.05
Not sharing	17	47.2	29	46.8		



Graph 4: Relation between hygiene practices of patients of group 1 and infection rate of *Trichomonas vaginalis*.

Table 5: Clinical manifestations of *Trichomonas vaginalis* infection as detected by examination of patients of group 1

Clinical manifestations	*Trichomonas vaginalis* infection				Test	*P* value
	Positive (n=36)		Negative (n=62)			
	no	%	no	%		
Dysuria						
Positive	8	22.2	0	0.0	FE=15.0	(0.00) <0.001
Negative	28	77.8	62	100.0		
Pruritus						
Positive	31	36.1	0	0.0	χ^2=78.09	(0.00) <0.001
Negative	5	63.9	62	100.0		
Dyspareunia						
Positive	13	14.3	1	1.6	χ^2=22.14	(0.00) <0.001
Negative	23	85.7	61	98.4		
Strawberry cervix						
Positive	1	2.8	0	0.0	FE=1.74	(0.367) >0.05
Negative	35	97.2	62	100.0		



Graph 5: Clinical manifestations of *Trichomonas vaginalis* infection as detected by examination of patients of group 1

Table 6: Sensitivity and specificity of wet mount examination for diagnosis of *Trichomonas vaginalis* infection.

Wet mount examination	InPouch TV culture		Total
	Positive	Negative	
Positive	22	0	22
Negative	14	62	76
Total	36	62	98

Prevalence : 0.37 Sensitivity: 0.61 Specificity: 1.00 Accuracy : 0.86
Predictive value of +ve result: 1.00 Predictive value of -ve result: 0.82

Table 7: Sensitivity and specificity of Giemsa staining for diagnosis of *Trichomonas vaginalis.*

Giemsa staining	InPouch TV culture		Total
	Positive	Negative	
Positive	18	2	20
Negative	18	60	78
Total	36	62	98

Prevalence : 0.37 Sensitivity: 0.50 Specificity : 0.97 Accuracy: 0.80
Predictive value of +ve result: 0.90 Predictive value of -ve result : 0.77

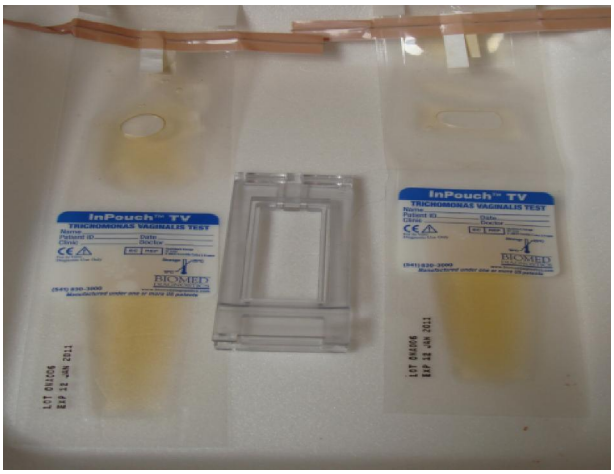


Fig. (1): Bags and viewer of InPouch™ TV culture system.

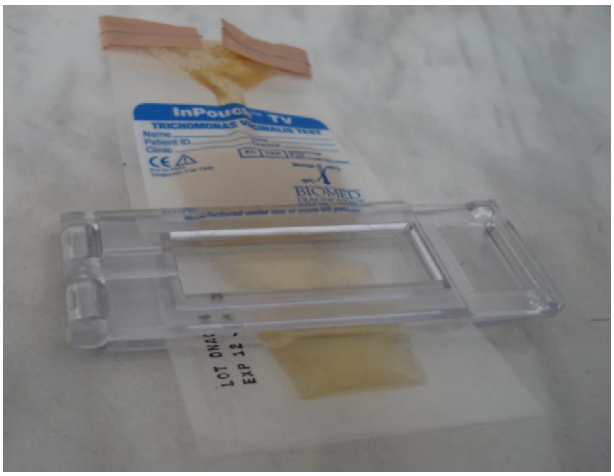


Fig. (2): Plastic viewer of InPouch™ TV culture system.

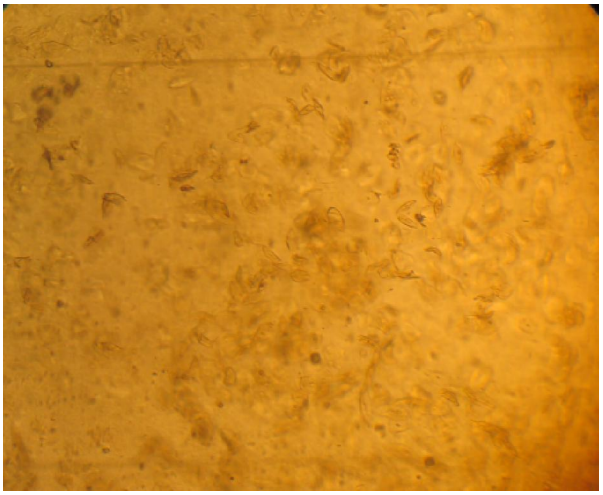


Fig. (3): *Trichomonas vaginalis* trophozoites seen by InPouch™ TV culture (x100).

Fig. (4): *Trichomonas vaginalis* trophozoites seen by InPouch™ TV culture (x100).

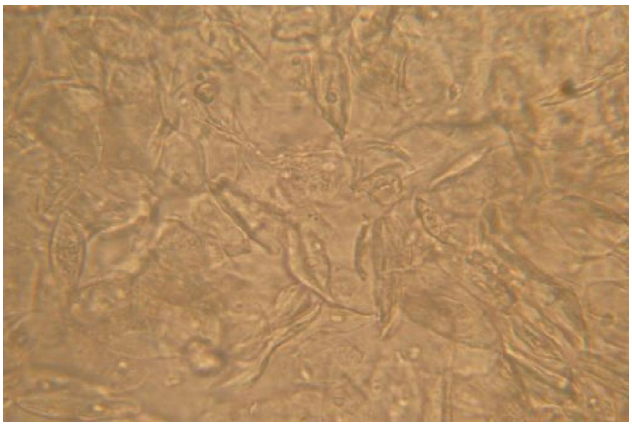


Fig. (5): *Trichomonas vaginalis* trophozoites seen by InPouch™ TV culture (x400).

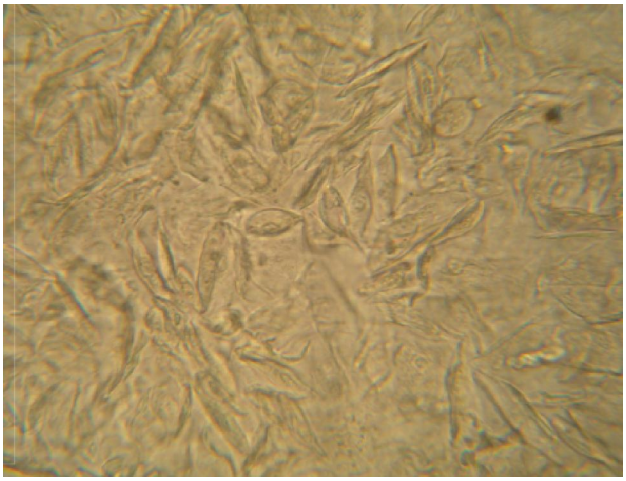


Fig. (6): *Trichomonas vaginalis* trophozoites seen by InPouch™ TV culture (x400).

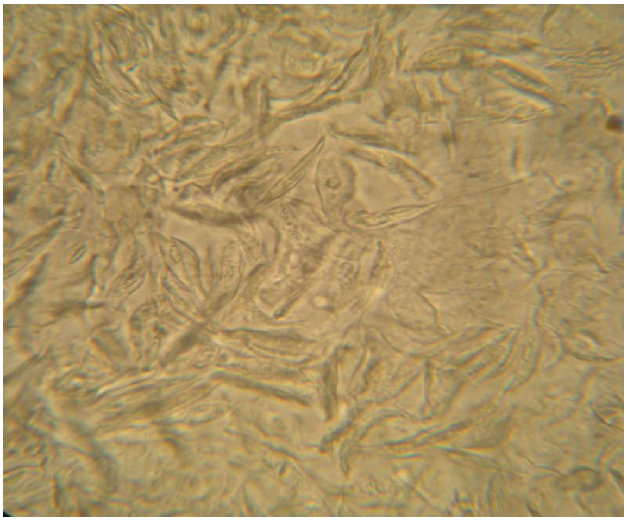


Fig. (7): *Trichomonas vaginalis* trophozoites seen by InPouch™ TV culture (x400).

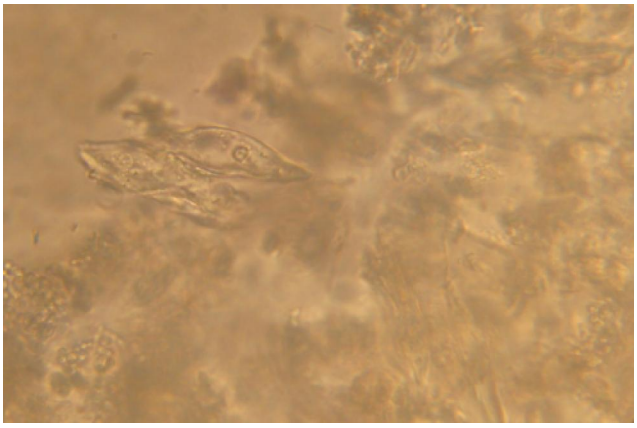


Fig. (8): *Trichomonas vaginalis* trophozoites seen by wet mount preparation (x400).

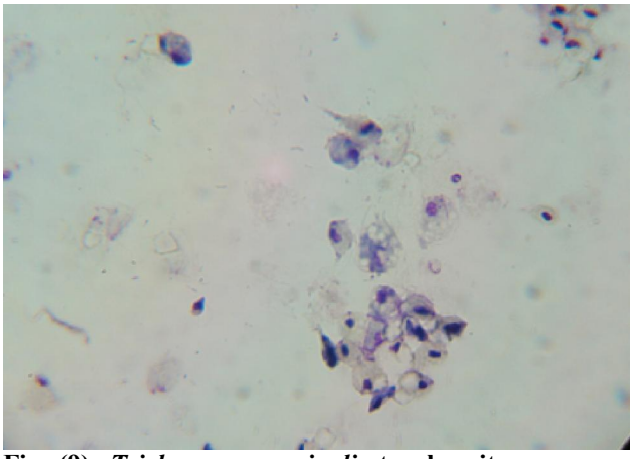


Fig. (9): *Trichomonas vaginalis* trophozoites seen by Giemsa stain (x100).

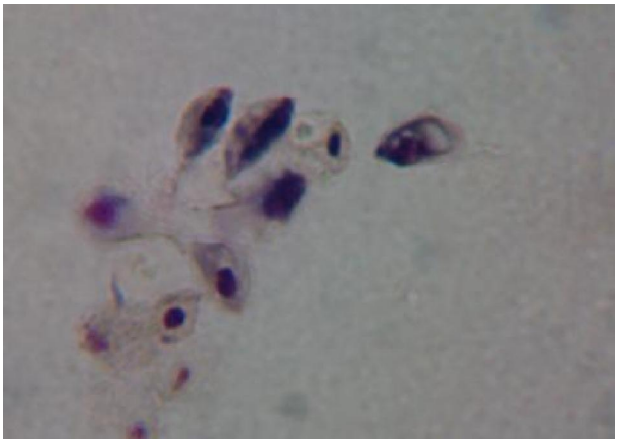


Fig. (10): *Trichomonas vaginalis* trophozoites seen by Giemsa stain (x400).

4.Discussion

Trichomonas vaginalis is the parasite of the human urogenital tract that causes trichomoniasis. It is the most prevalent non -viral sexually transmitted disease (Frasson *et al.,* 2012). The incidence of trichomoniasis has declined sharply in developed countries in the recent years probably due to early diagnosis, use of better diagnostic techniques, proper management and emphasis on behavioral changes. In contrast, in developing countries and amongst disadvantaged groups in developed countries, the infection appears to be widespread (Fernando *et al.,* 2012). Till today, in developing countries as Libya, little emphasis has been laid on the importance of decreasing the rates of TV infection even though it has been associated with human immunodeficiency virus acquisition. Because vaginal discharge which is the major symptom of TV infection is a non- specific symptom, laboratory diagnosis is must for detecting the infection (Patil *et al.,* 2012). Although effective therapeutic agents for treatment of trichomoniasis are widely available, diagnostic difficulties have become the limiting factor in reducing the disease burden (Fernando *et al.,* 2012). Various laboratory methods have been employed for the detection of TV in vaginal discharge which vary in their sensitivity and specificity (Patil *et al.,* 2012). Hence, the present work was aimed to study the infection rate of TV in Libya as researches made previously to study this item are very few. Also, to study some sociodemographic characteristics and some hygiene practices of the infected patients. Moreover, it was aimed to compare between wet mount examination, Giemsa staining and InPouch™ TV culture method to diagnose infection with TV.

Regarding infection rate in patients with vaginal discharge in the examined area, it was found to be significantly high (36.7%) as detected by InPouch™ TV culture. This finding coincides, to some extent, with those found in some African countries. Mhlongo *et al.* (2010) recorded that prevalence of TV infection was 19% in Cape Town and 34% in Johannesburg, South Africa in patients with vaginal discharge. Also, Anorlu *et al.* (2001) reported that the prevalence of TV among patients with vaginal discharge was 74.5% in Lagos, Nigeria. On the contrary, Kassem and Majoud (2006) reported that the prevalence of TV infection in Benghazi city, Libya was 1.2%. However, this result is not unexpected as there was a great difference between patients of the two studies. Patients of the present study were highly selected from patients already suffering from vaginal discharge while in Benghazi; all patients who were attending the gynecologic department were included in the study without special selections. Paul *et al.* (2012) found a low prevalence also in South India and they explained that the prevalence varies from region to region according to several factors, for example, they stated that the low prevalence of their study could be attributed to the selection of patients for the study as it was set up in tertiary care center which was relatively expensive, only patients who can afford the management might come to that hospital.

As regards infection rate of TV among different age groups, patients in the age group 40-45 years had significantly higher rate than other patients. This result is in agreement with the finding of Bowden *et al.* (1999). On the contrary, Patil *et al.* (2012) reported that the majority of infected patients belonged to the age group of 26-35 years. This discrepancy is probably attributed to difference in other sociodemographic characteristics of the patients related to the two studies or due to difference in hygiene practices of patients.

Concerning level of residence, infection rate of TV was found to be higher in patients living in poor level (52.8%) than in patients living in relatively good level (47.2%) although the difference between them was not significant. This result supports that of Crucitti *et al.* (2011) who stated that infection rate of TV was higher in patients living in poor or very poor areas (28.9%) than in patients living in better areas (23.5%).

Regarding hygiene practices of patients related to group 1, it was found that infection rate of TV was higher among certain groups of patients such as patients using tub for washing, patients who did not use to bath frequently, patients who did not use to use soap when bathing, patients who used seat latrines and patients who were sharing sleeping place with other members of the family. However, it was found that although the infection rate among those patients was higher than other patients but there was no significant statistical difference between them. On the other hand, the most striking observation was that patients who were sharing towels with someone else had a very high infection rate (80.6%) when compared to those who were not sharing towels (19.4%) with a high statistical difference between them ($p<0.001$). The above mentioned findings concerning hygiene practices of infected patients were in accordance with those of Crucitti *et al.* (2011) who mentioned the same observations.

In this work, the most frequent clinical manifestation reported by infected patients was pruritus (36.1%), followed by dysuria (22.2%), then dyspareunia (14.3%) and lastly strawberry cervix (2.8%). These findings coincide to a great extent with the findings of several previous authors who stated the same observations such as Petrin *et al.* (1998), Nam *et al.* (2011), Frasson *et al.* (2012) and Patil *et al.* (2012).

Analysis of results given by wet mount examination, Giemsa staining and InPouch™ TV culture showed that wet mount examination had a sensitivity of 61% and specificity of 100% when compared to InPouch™ TV culture. These results are not unexpected and were reported by many pervious authors. Barenfanger *et al.* (2002) stated that wet mount method is insensitive (36-70%) compared to culture. Schwebke and Burgess (2004) mentioned that sensitivity of wet preparation was about 56%. CDC (2006) reported that wet mount examination had a sensitivity of 60-70% as compared to culture. Sood *et al.* (2007) reported that the sensitivity of wet preparation was 55% and the specificity of was 100%. Patil *et al.* (2012) also mentioned that sensitivity was 60% and specificity was 100%.

In this work, Giemsa staining had a sensitivity of 50% and specificity of 97% when compared to culture. This result is in agreement with that of Radonjic *et al.* (2006) who have demonstrated a low sensitivity of 52.4% for diagnosis of TV with Giemsa stain.

From the above mentioned data, it is observed that wet mount examination and InPouch™ culture had 100% specificity; this could be attributed to the fact that the presence of trichomonads was determined by its characteristic size, shape and mobility, so there are no possibilities of misdiagnosis (Sood *et al.*, 2007). However, Giemsa staining diagnosed two positive cases which were found to be negative by both wet mount examination and InPouch™ culture. These could be considered false positives as diagnosis by the latter two methods depends not only on size and shape but also on mobility of the parasite.

In the present work, it was found that InPouch™ TV culture was superior to wet mount examination and Giemsa staining regarding ease of handling & interpretation, ease of transportation and total time of reading results. Also, other benefits were observed as that no expensive equipment was required and reagents could be stored for one year at room temperature. On the other hand, it took a relatively long period from taking the specimen till confirmation of the infection although examination itself didn't take more than five minutes. Also, culture itself is not readily available in Libya and had to be imported. Moreover, its cost was relatively higher than that of wet mount examination and Giemsa staining. However, its cost is comparable to the ordinary culture tube.

These findings coincide with the findings of several authors. Borchardt and Smith (1991) stated that InPouch™ TV culture for diagnosis of TV offers many unique advantages when compared to other temporary in vitro laboratory diagnostic tests. Its 6 months stability at room temperature offers significant savings in media. The pouch has the versatility of being used both for specimen transport and culture. Specimens may be mailed and maintain trichomonads viability for approximately one week. The medium is selective with effective antibacterial and antifungal activity. Moreover, because the pouch is so simple to view microscopically, positive tests can be observed with counts less than 10 trichomonads/ml. Draper *et al.* (1993) and Sood *et al.* (2007) added that there is an additional advantage of InPouch which is that microscopy could be performed on the pouch itself. This decreases contamination and eliminates slide preparation which in turn speeds up the examination time. However, they mentioned that there are some

limitations of using the culture like that 2-7 days are needed for identification of positive culture. Moreover, the medium is relatively expensive.

Regarding wet mount examination, results and observations of the present work are in accordance with those of Heine and McGregor (1993) who mentioned that wet mount examination depends on an experienced microscopist and requires the presence of viable trichomonads. Also, Kingston *et al.* (2003) and Schwebke & Burgess (2004) added that specimens must be examined within 20 minutes of collection and stated that sensitivity of the wet mount microscopy declines substantially with even short time delays between collection and examination. Moreover, Patil *et al.* (2012) explained that although wet mount examination of stool is inexpensive, rapid, requiring only a microscope and a trained personnel, the sensitivity of this method is low compared to culture. It is highly dependent on the expertise of the microscopist, prompt transport and laboratory processing of the sample before organisms lose their motility or become nonviable.

As regards Giemsa staining, the results and observations of the present work are contradictory with those obtained by Fernando *et al.* (2011) who stated that Giemsa staining was the most feasible and cost effective test in their study and added that it was more sensitive when compared to wet mount examination. This discrepancy is probably attributable to the fact that they compared Giemsa staining with wet mount examination and a culture other than InPouch™ TV culture. In the present study, the swabs after being taken were kept in sterile tubes containing physiological saline with drops of 5% glucose. This mixture acts as an extracellular energy source to preserve the viability of the organisms in vitro (Jayaram, 2002). Thereby, smears could be prepared up to 24 hours later. The fact that smears could be examined after a lag period is a definite advantage over the immediate examination of a wet smear preparation. In addition, this modification of the used technique allows general practitioners and even patients themselves to take blind vaginal swabs (without speculum use) and preserve the sample for later inspection (Fernando *et al.,* 2011).

In conclusion, this study showed that the infection rate of *Trichomonas vaginalis* in Libya is relatively high, so there must be a policy decision to increase awareness of STDs in the community via mass media and public institutions. Also, it is recommended that laboratory diagnosis of trichomoniasis is a must as diagnosis of the diseases depending on clinical manifestations only is not reliable. In addition, it is concluded that this study confirms the usefulness of InPouch™ TV culture in the diagnosis of trichomoniasis as it was superior to both wet mount examination and Giemsa staining. So, we recommend that InPouch ™ TV culture may be used as a routine method for diagnosis in the laboratory. Lastly, we recommend that health education must give attention to advice females to not share towels with others either indoor or outdoor.

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