

Prevalence of microsporidiosis in human and cattle

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Summary

In order to identify microsporidia and other fungi in stool and urine samples of human, and in fecal and milk samples of cattle, 100 stool samples with or without diarrhea and 50 urine samples, human fecal and urine samples were obtained from certain Baghdad hospitals and certain rural areas surroundings Baghdad city, in addition to 50 fecal and 56 milk samples of cattle apparently healthy were collected from Alshula Slaughter House and directly from anal of the animal field of College of Veterinary Medicine/ Baghdad University. All samples were collected during six months from 1/10/2014 to 1/4/2015. Thin films were formed and stained by Webers Modified Trichrom stain and Modified Trichrom-Ryan Blue stain. The results showed that (23%) 23 out of 100 stool samples of human were positive for *Microsporidia spp.* and (16%) 8 out of 50 urine samples of human were positive for this fungus. While the result revealed (18%) 9 out of 50 fecal samples and (7.14%) 4 out of 56 milk samples of cattle were positive for *Microsporidia spp.* The result also explained that (25.3%) 19 cases of patients suffering from diarrhea expressed *Microsporidia spp.* after the examination of 75 stool samples, while (16%) 4 persons without diarrhea showed positive *Microsporidia*, through the examination of 25 stool samples. The study explains that the *Enterocytozoon bienersi* is a common species associated with human infection and *Encephalitozoon intestinalis* is a common Microsporidia associated with cattle infection whereas *Encephalitozoon cuniculi* is rarely identified in human but recorded in cattle.

Keywords: Microsporidiosis, Fungi, Microsporidiosis in cattle, Microsporidiosis in human.

Introduction

Microsporidia are obligate unicellular spore forming organisms infect the mammalian including human and wild range of domestic and wild animals in addition to invertebrate including insects, birds and fish (1). It is opportunistic pathogen in immunocompromised patients but it is also affected immunocompetent persons (2). Certain water treatment filters can unable to prevent passage of mature Microsporidia spores due to its small size and also this spores can resistant normal concentration of chlorine that using in treatment of drinking water, therefore Microsporidia spores may be found in drinking water and in the soil which contaminated by infected animals waste. This observation may support idea that microsporidiosis is zoonotic water borne disease, food borne disease and anthroponotic transmission (3). Microsporidia, a zoonotic pathogen, can induce gastrointestinal and ocular infection in immunocompetent individuals (4) as well as infected animals such as cows, pigs and birds (5). Previously, Microsporidia species are considered protozoa but later on, molecular phylogenetic studies

found that this organism have a relationship to fungal species (1). There are 14 species of 8 genera of Microsporidia are pathogenic organism to human and animals. Most of them are, *Enterocytozoon bienersi*, followed by the *Encephalitozoon spp.*, particularly *E. intestinalis*. Twenty six varies genotypes of *E. bienersi* have been reported in human and animals, but there is no differences between fungi isolated from human and those isolated from animals by molecular assay (6). Other *Encephalitozoon* species causing human infection are *E.cuniculi* and *E.hellem* antigenic diversity has also been demonstrated among these isolates (7). In Iraq, few reports have been published regarding the prevalence of Microsporidia species associated with diarrhea in human and animals. Therefore the aim of the present study is to determine the prevalence of Microsporidia species isolated from human stool and urine with or without diarrhea, and from fecal and milk samples from cattle apparently healthy.

Materials and Methods

Human samples: Hundred stool samples and fifty urine samples were collected from

humans, both sexes, different ages, suffering from diarrhea or from persons appear healthy and with urinary tract infection. These samples were collected from Central Teaching Hospital of Pediatric, Al Karkh Hospital, Al Yarmouk Teaching Hospital and certain rural areas surroundings Baghdad city from young and adult persons, stool samples were collect in sterile container. Few drops of urine were allowed to pass then sterile container was used to collect the stream urine, however, both urine and stool samples were transported by iceboxes containing ice to Zoonotic Unit laboratory during 2 hrs.

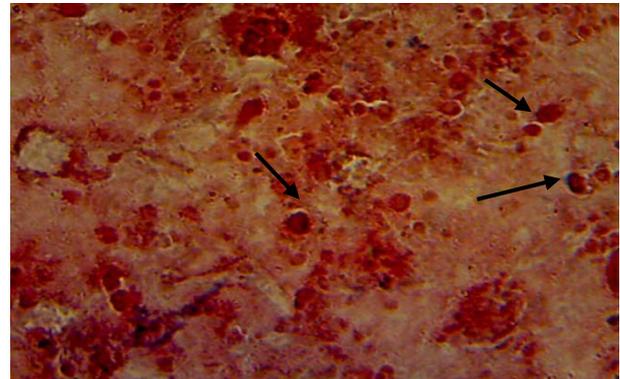
Cattle samples: Fifty fecal samples were collected directly from small and large intestinal tracts of female animals that were slaughtered in Al Shula Abattoir and directly from anal of the animals in animal field of College of Veterinary Medicine during the same period of collection human samples. 56 Milk samples were collected in sterile container directly from teat of animals apparently healthy mammary glands and both samples were transported with icebox containing ice to Zoonotic laboratory during 2 hrs.

Sample preservation: The samples were preserved and homogenized with (5%) formalin, (10ml from the solution to 10gm of feces) these samples were kept in the refrigerator 4°C till examination (8). These samples were put in sterile tubes and they were centrifuged at 9000 rpm for 30 minutes, the supernatant was neglected and the pellet was mixed with one ml of buffer normal saline. Then thin film was made on slide, dried by temperature room, and fixed by methanol and the film was stained by two types of stains: Webers Modified Trichrom stain and Modified Trichrom-Ryan stain and the diameter of the spores were measured by micrometers with an ocular micrometer under 1000 magnification (9) and photographe the spores with optical photomicroscope with a Pixera digital camera.

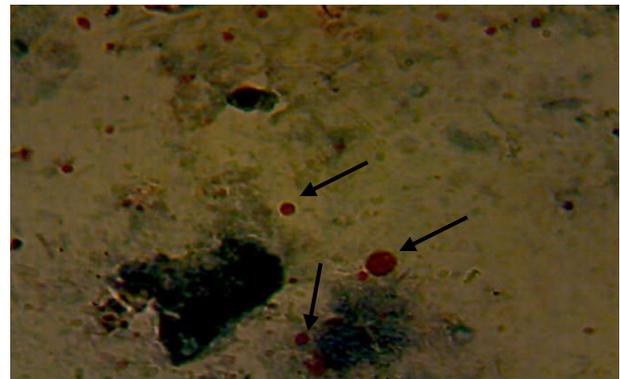
Results and Discussion

Microscopic examination showed oval structures stained purple with Webers Modified Trichrom stain and purple blue by Ryan blue stain with average measurement between 2 to 3.1 micrometer in length and 1.3

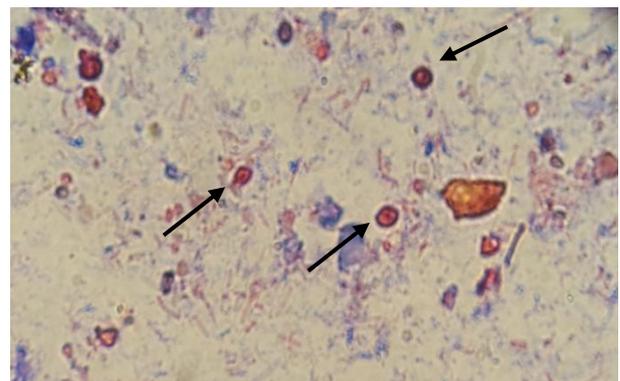
to 1,7 micrometer width (Fig. 1-5). Other bacteria, some yeast cells, and some debris will stain pink to red; the shapes and size of the various components may be helpful in differentiating the spores from other structures.



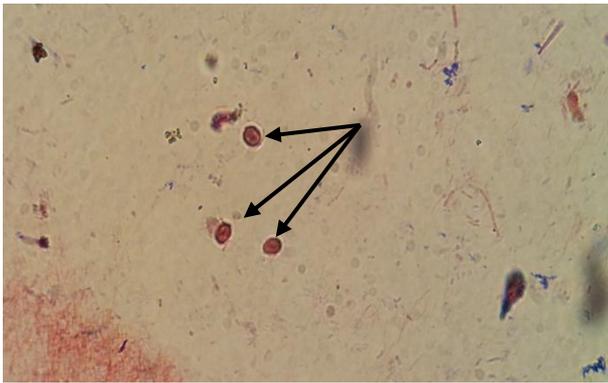
Figure, 1: Shows the *E.intestinalis* in fecal sample of cattle which appear oval pinkish in colour with clear belt like stripe stained by Modified Trichrom-Ryan stain ($\times 100$).



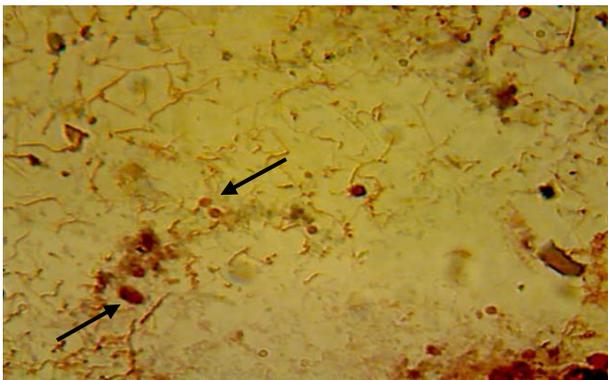
Figure, 2: Shows the *E.intestinalis* in fecal sample of cattle which appear oval pinkish in color with clear polar filament stained by Webers Modified Trichrom stain ($\times 100$).



Figure, 3: Shows the *E. Bieneusi* in stool sample of human which appear oval pinkish in color with clear polar filament stained by Webers Modified Trichrom stain ($\times 100$).



Figure, 4: Shows the *E. Bieneusi* in milk sample of cattle with polar tube stained by Modified Trichrom-Ryan Blue stain (x100).



Figure, 5: Shows the *E. cuniculin* in stool sample of human with polar tube stained by Modified Trichrom-Ryan stain (x100).

The present study revealed that 23(23%) of examined stool and 8(16%) of urine samples of human were positive for the presence of Microsporidia. It recorded that 9(18%) of fecal samples and 4(7.14%) of milk samples of cattle were positive for identified Microsporidia (Table, 1).

Table, 1: Prevalence of Microsporidia isolates from human and cattle according to the type of the samples (stool, urine) and (feces, milk).

| Species | Samples | Number of examined samples | Number of positive samples | Percentage of infection (%) | Chi-square value |
|----------------------------|---------|----------------------------|----------------------------|-----------------------------|------------------|
| Human | Stool | 100 | 23 | 23 | 3.928 * |
| | Urine | 50 | 8 | 16 | |
| Cattle | Feces | 50 | 9 | 18 | 5.267 * |
| | Milk | 56 | 4 | 7.14 | |
| Chi-square value- χ^2 | --- | --- | --- | 8.025 ** | --- |

* (P<0.05), ** (P<0.01).

The criteria used to identify Microsporidia, in the slide smear staining by Webers Modified Trichrom stain and purple blue by Ryan blue stain, included the presence of

single pink blue oval structure with blue wall and encircling by a belt like stripe. This feature was similar to those described by (10). The identification of Microsporidia from stool, urine in human and milk, feces in cattle may indicate that this pathogen can cause a wide range of infection in both human and animals. This evidence was in consistency with observation of (11) who demonstrated that this pathogen can cause broad spectrum of disease. Identification of Microsporidia in milk samples may raise public health problem and zoonotic importance of this pathogen. This idea agreed with investigation of (12) who suggested that the infection of human and animals with Microsporidia and identified of this pathogen in water source raising zoonotic importance of the Microsporidia as a waterborne transmission pathogen.

High percentage of Microsporidia spp. identified in stool samples of human (23%) may be indicated these fungi may cause gastrointestinal infection in human and this percentage was similar to those reported in certain countries among immunocompetent individuals. It was reported that the present result approximately near to result that reported in Spain (17%), and (13 and 14) but this percentage was higher than those reported in other countries such as Uganda sistenn (8%) (15), in Germany 0.7 (16), (17) and Nigeria 9.3 (18). The differences in the prevalence of Microsporidia between the current study and other studies may be supported observation of other authors who mentioned that Microsporidiosis is important zoonotic disease of humans worldwide but the prevalence of this disease was varies according to geographical region, population of human study, and the diagnostic methods (1;10). Also the study showed that 19(25.3%) out of 75 stool samples from patient suffering from diarrhea positively infected by Microsporidia but 4(16%) out of 25 stool samples of apparently health persons were positive for this fungus (Table, 2).

The identification of Microsporidia in stool samples of patients suffering from diarrhea but do not expressed any other illness may be indicated that this pathogen was considered as common cause of diarrhea in immune-competent persons, and this study may be

the first report in Iraq revealing that *Microsporidia* spp associated with diarrhea in immunocompetent individuals, this observation was agree with result of (19) in Korea who reported *Encephalitozoon intestinalis* infection in 7(5%) out of 139 tool samples of patients suffering from diarrhea, also (20) reported gastrointestinal and ocular infection with *Microsporidia* in immunocompetent persons, however, firstly *Microsporidia* was recognized as opportunistic pathogen of immunocompromised patients, but later on, it was recorded increasing of prevalence of *Microsporidial* infection in immunocompetent persons (2).

Table, 2: Percentage of Microsporidia identified in stool samples of individual with diarrhea or apparently healthy.

| Persons | Number of examined samples | Positive cases | Percentage (%) |
|----------------------------|----------------------------|----------------|----------------|
| Person apparently healthy | 25 | 4 | 16.0 |
| Person with diarrhea | 75 | 19 | 25.3 |
| Chi-square value- χ^2 | --- | --- | 4.571 * |

*(P<0.05).

The present study showed that 16% of apparently healthy individuals were showed positive for *Microsporidia*. This result may indicate that this fungus can asymptomatic infected immunocompetent persons ,this observation was in agreement with several researches which explained that *Microsporidia* infections were limited to HIV infection patients but few reports were published about this disease in immunocompetent individuals whom mostly showed asymptomatic infections (13 and 21). Also (22) reported that 94% of *Microsporidia* infection in nondiarrheal samples.

The study recorded that 6(20%) out of 30 stool samples of adult men were positive for *Microsporidia* while this fungi was identified in 12(26.6%) out of 45 adult women stool samples in addition 5(20%) out of 25 stool samples of children were positive for *Microsporidia* (Table, 3).

It was recorded that 6(20%) out of 30 stool samples of adult men were positive for *Microsporidia* while this fungus was identified in 12(26.6%) out of 45 of adult women stool

samples in addition 5(20%) out of 25 stool samples of children were positive for *Microsporidia*. The differences in the prevalence of identified *Microsporidia* among adult males and females and children may be due to immune status particularly in pregnant females and also this result may indicated that *Microsporidia* can infected all ages particularly the young individual this result was agreement with (19) who reported that most cases of *Microsporidial* infection occur in patients under 20 year age old .

Table, 3: Percentage of microsporidia identified in adult men and women and children stool samples.

| Infected persons | Number of examined samples | Number of positive samples | Percentage of positive samples (%) |
|----------------------------|----------------------------|----------------------------|------------------------------------|
| Adult men | 30 | 6 | 20.0 |
| Adult women | 45 | 12 | 26.6 |
| Children | 25 | 5 | 20.0 |
| Chi-square value- χ^2 | --- | --- | 2.894 NS |

NS: Non-significant.

Microsporidial infection associated with protein energy malnutrition (23), however, shedding *Microsporidia* in stools is intermittent therefore the stools samples examination do not provide a good data about the intensity of *Microsporidia* infection (24), the present result is inconsistent with the result of (25 and 26) who reported no significant differences in prevalence of *Microsporidia* infection between males and females. The current study revealed that the prevalence infection in adult female patients was higher than those in children. This result may be due to active behavior and eating habit of adult person as compared with children or the shedding of *Microsporidia* was low in children, this result is agree with (26) who reported the prevalence of *Microsporidia* infection was high in adult more than fifteen year as compared to those in less fifteen years also (27) reported highest prevalence rates of shedding of *Microsporidia* in patient with age fifty and above. (28) Also reported a high prevalence (57.2%) of *microsporidiosis* among adults more than 31 years.

The study revealed that *Microsporidia* spp identified in 7(23.4%) out 30 stool samples of patients in the countryside as compared with

16 (22.8) out of 70 stool samples from patients in the city (Table, 4).

Table, 4: Percentage of Microsporidia identification in stool samples of patients in the countryside and in the city.

| Area | Number of examined samples | Number of positive samples | Percentage of positive samples (%) |
|----------------------------|----------------------------|----------------------------|------------------------------------|
| Country side | 30 | 7 | 23.4 |
| City | 70 | 16 | 22.8 |
| Chi-square value- χ^2 | --- | --- | 0.0946 NS |

NS: Non-significant

The study showed that Microsporidia identified in stool samples of human included *E.bieneusi* (69.2%), *E. intestinalis* (21.2%) and *E. cuniculi* (8.6%) and these form 62%, 25.5% and 12.5% respectively in urine samples of human. While the fecal samples of cattle express the Microsporidia including *E. intestinalis* (66.61%) and *E.cuniculi* (33.4%). While the milk samples showed (25%) of *E. bieneusi*, (75%) *E.intestinalis*, (Table,5).

Table, 5: Show the percentage of identified Microsporidia spp. from human and cattle samples according to type of the sample (stool, urine) and (feces, milk).

| Species | Samples | <i>E. bieneusi</i> Positive (%) | <i>E. intestinalis</i> Positive (%) | <i>E. cuniculi</i> Positive (%) | Chi-square value - χ^2 | No. of Microsporidia isolates |
|----------------------------|---------|---------------------------------|-------------------------------------|---------------------------------|-----------------------------|-------------------------------|
| Human | Stool | 69.2 | 21.2 | 8.6 | 11.955** | 23 |
| | Urine | 62.0 | 25.5 | 12.5 | 9.742** | 8 |
| Cattle | Feces | 0.0 | 66.61 | 33.4 | 9.351** | 9 |
| | Milk | 25.0 | 75.0 | 0.0 | 12.863** | 4 |
| Chi-square value- χ^2 | --- | 12.597** | 13.041** | 10.569** | --- | --- |

** (P<0.01).

The present study showed high percentage of *E.bieneusi* identified in stool samples of the patients followed by *E. intestinalis*, this observation may indicated that human mostly infected with this pathogens, this idea was in consistent with observation of (29) who found that *E.bieneusi* followed by *E.intestinalis* causes the most human microsporidiosis also (30) showed that *E.bieneusi* infected upper gastrointestinal tract and cause chronic diarrhea and weight losses.

Also the current result was agreement with (31) who identified of this pathogen in non HIV infected patients suffering from chronic diarrhea. Identified of *E.bieneusi* with high prevalence in human samples may be indicated this fungi was considered a major species of Microsporidia associated with gastrointestinal and urinary tract infection of immunocompetent human and this pathogen does not associated only with immunocompromised patients, it was firstly, reported that *Enterocytozoon bieneusi* as opportunistic pathogen of acquired immunodeficiency syndrome (32). Intestinal infections with this pathogen were recorded in organ transplant recipients, travelers, children and elderly (33).

The current study showed that Microsporidia identified in stool samples of non-diarrhea individuals this result may indicated that *E.bieneusi* can cause asymptomatic infection, this idea was in consistent with (22) who recorded that *E.bieneusi* can induced asymptomatic infection in both immunocompromised and immunocompetent persons, however, Microsporidia firstly recognized as a cause of pebrine disease of silkworms in 1857 (34), later one, this pathogen identified a major cause of human and animals infection particularly in human with immunodeficiency virus HIV, in 1985, (22) recorded that *E.bieneusi* can induced asymptomatic infection in both immunocompromised and immunocompetent persons in developing countries (22) reported *E bieneusi* in 2.5 % of urine, 11.5% fecal of HIV seronegative patients suffering from diarrhea .

E. bieneusi is the most commonly identified species in human, therefore ,identified of this fungus in fecal and milk samples of cattle in the present study may indicated that cattle may be considered importance reservoirs for this pathogen and they act as zoonotic potential in public health of this fungus. This idea was in agreement with result of (6) who recorded that over 90 genotypes identified based on the ITS nucleotide sequence of *E.bieneusi* spores recovered from fecal samples of human and animals, also (22) found that this pathogen can infected animals particularly mammals and

cause public health problem as zoonotic water transmission pathogen.

The present study showed that low percentage of *E.cuniculi* identified in both stool and urine samples of human as compared with other identified species but identified in high percentage in fecal samples of cattle ,this idea may indicated this fungus considered less importance in human infection but it may important in animals, this idea is agree with (35) who recorded that *Encephalitozoon cuniculi* is an important Microsporidial infection in domestic animals including cattle, sheep, horse in addition to dogs, cats, and rabbits but this pathogen rarely induced symptomatic infection in humans.

The present study showed that *E.intestinalis* form a high percentage of Microsporidia which were identified in both fecal and milk samples of cattle, thus may be that this fungi is a common spices infected cattle and it may be associated with bovine mastitis and it may be transmitted to human through contact with or consumption of milk simples.

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انتشار الأبواغ الدقيقة في الإنسان والأبقار

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الخلاصة

من أجل تحديد الأبواغ الدقيقة في عينات البراز والبول في الإنسان، وعينات البراز والحليب في الأبقار. أخذت 100 عينة من براز أشخاص يعانون من الإسهال وأشخاص سليمين وخمسين عينة من البول تم الحصول على عينات الإنسان من عدد من مستشفيات بغداد و من المناطق الريفية المحيطة بمدينة بغداد، فضلاً عن 50 عينة براز و 56 عينة حليب للأبقار جمعت من مجزرة الشعلة ومن حقل كلية الطب البيطري/ جامعة بغداد وقد جمعت العينات خلال 6 اشهر من 2014/10/1 الى 2015/4/1. حضرت شرائح زجاجية وصبغت بصبغات خاصة (صبغة ترائي كروم المحورة- ريان الزرقاء وصبغة وبيرز ترائي كروم المحورة). أظهرت النتائج 23 % (23 من 100) عينة من براز الإنسان كانت موجبة للأبواغ الدقيقة و 16 % (8 من أصل 50) عينة بول إنسان أظهرت نتائج موجبة لهذه الفطريات، في حين كانت النتيجة 18 % (9 من أصل 50) عينة براز ابقار و 7.1 % (4 من أصل 56) عينة حليب من الماشية إيجابية للأبواغ الدقيقة. اوضحت النتائج أن هناك 25.3 % (19 من أصل 75) حالة موجبة لعينات مأخوذة لأشخاص يعانون من الإسهال بفحص عينات البراز. في حين كانت هناك 16 % (4 من أصل 25) عينة موجبة للأبواغ الدقيقة عند فحص عينات البراز لأشخاص لا يعانون من الإسهال. وأظهرت النتائج أن *E.bieneusi* هو أكثر الأنواع الشائعة من الأبواغ الدقيقة في إصابة الإنسان و *E.intestinalis* الأكثر شيوعاً من بقية أنواع الأبواغ الدقيقة في إصابة الأبقار بينما في حين تشخيص *E.cuniculi* نادراً في عينات الإنسان لكنه سجل في الأبقار.

الكلمات المفتاحية: داء الأبواغ الدقيقة، فطريات، داء الأبواغ الدقيقة في الأبقار، داء الأبواغ الدقيقة في الإنسان.