

Molecular, immunological, and histological study of *Cryptosporidium parvum* in local duck *Anas platyrhynchos* in some regions of Mosul city, Iraq

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Abstract

The species and strain of *Cryptosporidium parvum* genotype in domestic ducks were recorded for the first-time using sequencing technologies in this study. In addition, it looks at the spread of *Cryptosporidium parvum* and the methods used to diagnose it in different districts of Mosul. Ninety-one duck feces were gathered from six different districts of the city: Shallalat, Bashiqa, Ali Rash, Bartella, Al-Hamdaniya, Telkaif, Felfil, and Al-Qosh. The positive result rate was 27% (25 of the original 91), with monthly variations based on geographic region and age. The statistical analysis revealed considerable disparities in scientific procedures between the northern and eastern Mosul regions. The age factor revealed a substantial difference between 9 and 12 months. Histopathological changes in the trachea and intestinal tract were recorded. The type and strain of *Cryptosporidium parvum* were recorded and compared to sequences stored in the NCBI's GenBank database. The parasite's genetic invasion, was discovered to match the strains reported in China. The study concluded that for the first time in Mosul, the strain and type of *Cryptosporidium parvum* isolate were recorded in local ducks, with a higher incidence among local ducks aged 6-12 months than at other ages. Furthermore, its incidence was higher in the city's northern areas than in its eastern areas. Duck trachea and intestines are affected by this strain's histopathological alterations.

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Introduction

Raising ducks provides valuable supplies of meat and eggs for personal consumption and sale to breeders. Many parasites can infect birds, including ducks. *Cryptosporidium parvum*, for example, can infect a range of bird species, including geese, quail, and ducks (1,2). Endoparasitic infections must be investigated to keep birds, particularly ducks, given their environmental preferences and feeding habits (3). Ducks are more likely to meet the eggs, cysts, and larvae of many parasites because they frequently live near ponds and standing water and serve as intermediate hosts for many of them. Such exposure not only introduces a plethora of parasites into the ecosystem but also exacerbates pollution, damaging soil, water, and food resources (4,5).

Cryptosporidium parvum can produce diarrhea symptoms ranging from moderate to severe. Notably, in low-income countries, it poses a significant risk of under-five mortality (6,7). According to this research, there is a modest rise in *Cryptosporidium* transmission in rural regions due to human-animal interactions. Many human cases are linked to zoonotic transmission from sick animals (8,9). Public health is endangered by infected hosts' environmental contamination of water and food (10,11). Biological environmental pollution can contribute to the spread of this disease, as river water is polluted with waste from animals infected with the disease, and the cause of the disease in humans is usually a lack of attention to hygiene, washing, and sterilization (12,13). *Cryptosporidiosis* causes gastrointestinal symptoms and abdominal cramps (14,15).

The study aimed to record the genetic sequence of the *Cryptosporidium* parasite in ducks. Through this study, researchers can know the course of this disease and find ways to treat and prevent it. It also paves the way for knowing which countries share the same parasitic strains and finding ways for cooperation between countries to get rid of these endemic diseases.

Materials and methods

Ethical approve

College of Basic Education, University of Mosul permitted this scientific work and data collection with the approval issue session 9 on 26/3/2023.

Study time and locations

The study was conducted in March and September 2022 in several different areas of Mosul, including the north and east of the city.

The animals

Fecal samples from domestic ducks Mallard (*Anas platyrhynchos*) are the principal duck breeds studied in the investigation. Each fecal sample was collected directly from the cloacal orifice and held at a constant temperature of 4°C (16). After a laboratory investigation of each duck's marked fecal samples revealed a positive result for the presence of the parasite, the ducks were euthanized. After carefully removing their internal organs, the organs were cleansed, and the samples were kept in 10% neutral formalin until the histological sections and samples were split. Tissue samples were purified: rinsed in distilled water, dehydrated in increasing concentrations of ethyl alcohol, clarified in xylene, and embedded in paraffin wax at 60°C using an ATP7000 tissue processor. A rotary microtome cut 6 µm thick sections of paraffin-embedded tissues. After that, the slices were stained with hematoxylin and eosin (17).

Diagnosis of *Cryptosporidium parvum*

Antigens of *Cryptosporidium parvum* were detected using a kit provided by Bio-x Diagnostic in Belgium. Optical density measurements were taken by the kit's instructions. This entailed subtracting the values of each sample in the ELISA plate wells from the negative control.

PCR

Table 1 shows how the target area of the 18S rDNA gene was selectively amplified using a universal primer to detect *C. parvum* (Table 1) utilizing PCR amplification and electrophoresis (18). The amplification mixture was made according to the manufacturer's instructions for Promega Go Taq® Green Master Mix, USA. Each amplification reaction was carried out in a total volume of 20 l under the conditions outlined in table 2. The reaction mixture comprised 12.5 l of Promega master mix and 5 l of Pioneer master mix. In

addition, there is 2 l of sample DNA (equal to 40 ng), 1.5 l of forward and reverse primers and 10 l of nuclease-free distilled water in the mixture. A TRIO thermal cycler from Biometra, Germany, was used for gDNA amplification. Following amplification, PCR products, and a 100 bp DNA molecular ladder (from Promega) were migrated and observed on 1% (wt/vol) agarose gel electrophoresis using 1X TAE buffer and ran at 6 V/cm Constant for one hour.

Table 1: Primers were used in the study

Primer	Sequence
F	5'-AGGAATGAACGGAACGTACA-3'
R	5' -CCATTTCTTCTTCTATTGTTTCAC-3'

Table 2: PCR program

	°C	Time (m)	Cycles
Initial denaturation	94	3.5	
Denaturation	94	1	
Annealing	60	1	40
Extension	72	1	
Final extension	72	1	
Holding	4		

Statistical analysis

The Chi-square and Fisher's tests were applied, with a probability level of less than 0.05. The chi-square test found statistically significant differences in infection rates across geographic locations (X, df=Y, P<0.05). Fisher's exact test was also used, with comparable findings.

Results

PCR results

According to the ELISA results, 25 of the samples tested positive for the presence of the parasite, corresponding to a prevalence rate of 27% (Figure 1 and Table 3). Even though no extensive statistical analysis was provided, substantial variances exist between regions, including a notable disparity between the city's eastern and northern regions. Shallalat, Bashiqa, Ali Rash, Bartella, and Hamdaniya are among the former, while Tel Keif, Fafel, and Alaqoush are among the latter.

When the data are analyzed, it is evident that there is a significant difference in the prevalence of infection based on age group. As demonstrated in Table 4, the 9-12 months age group had the most significant infection rate of 50.5%, while the 3-6 months age group had the lowest infection rate of 14%. At a p-value of 0.05, statistical analysis found no significant difference between 3-6 months, 6-9 months, and 9-12 months. However, a significant difference was found between the 6-9 months and 9-12 months groups (Table 4).

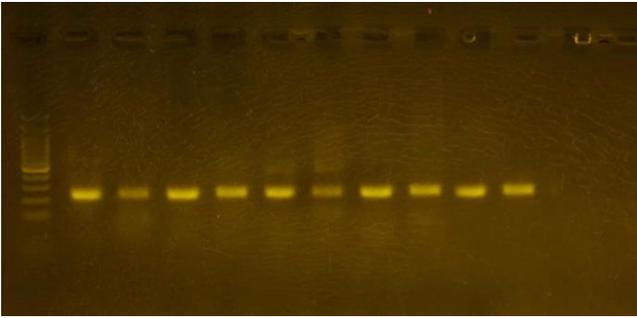


Figure 1: PCR reaction results for *Cryptosporidium parvum* samples based on an 18S rDNA gene.

Table 3: The differences in infections according to Geographic sites in Mosul city

Geographic Site	Positive sample for <i>Cryptosporidium</i>	Negative sample for <i>Cryptosporidium</i>
Al-Shallalat	4/10 (40%)	6/10 (60%)
Bashiqa	1/10 (10%)	9/10 (90%)
Ali Rash	3/10 (30%)	7/10 (70%)
Bartella	4/10 (40%)	6/10 (60%)
Al-Hamdaniyah	1/10 (10%)	9/10 (90%)
Telkaif	2/10 (20%)	8/10 (80%)
Felfel	7/10 (70%)	3/10 (30%)
Alqosh	3/15 (15%)	17/20 (85%)

Table 4: The differences in *Cryptosporidium* infection according to duck ages

Age (month)	+ve sample	-ve sample
3-6	(14%) 5/35	(86%) 30/35
6-9	(23%) 7/30	(77%) 23/30
9-12	(50.5%) 13/25	(49.5%) 12/25

Histological study

The histological examination revealed the presence of the histological changes depicted in figures 2-9, which are characterized by the accumulation of epithelial cells in the intestinal glands and the infiltration of inflammatory cells in the submucosal layer, as well as the noticeable loss of epithelial cells, infiltration of inflammatory cells in the mucous layer, and the appearance of parasite reproduction and attachment stages, with the help of intestinal villi. Histological examinations of positive specimens revealed mucosal necrosis, epithelial cell loss, infiltration of inflammatory cells in the submucosal region, intrachondral chondrocyte necrosis, and *Cryptosporidium* spp developmental stages in the trachea.

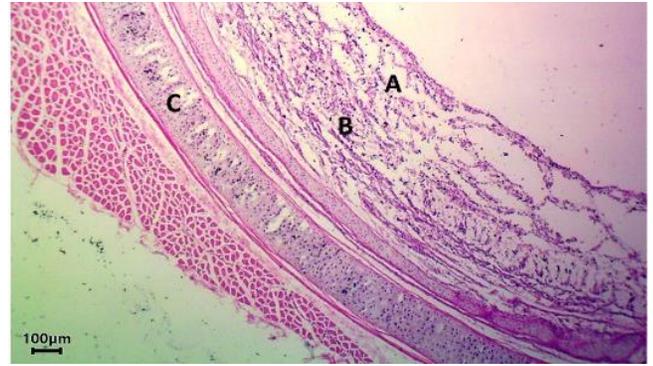


Figure 2: Photomicrograph of duck trachea infected by Cryptosporidiosis showing necrosis and loss of epithelial cells in the mucosa (A), infiltration of inflammatory cells in the submucosa (B), and necrosis of the chondrocytes in the cartilage (C). H&E stain, 40X.

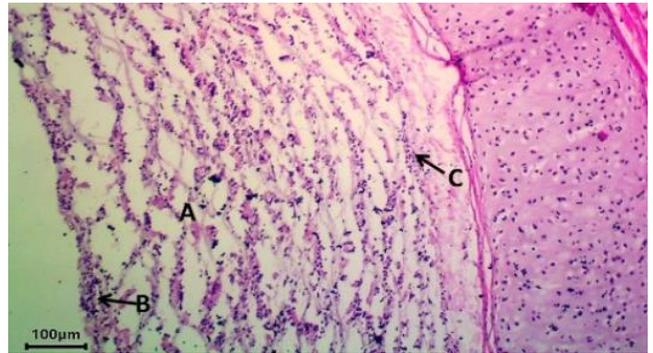


Figure 3: Photomicrograph of duck trachea infected by Cryptosporidiosis showing necrosis and loss of epithelial cells in the mucosa (A), infiltration of inflammatory cells in the mucosa (B), and submucosa (C). H&E stain, 100X.

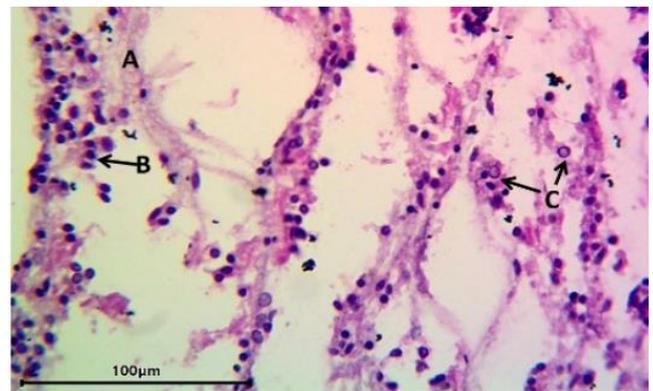


Figure 4: Photomicrograph of duck trachea infected by Cryptosporidiosis showing necrosis and loss of epithelial cells in the mucosa (A), with infiltration of inflammatory cells (B) and presence of the developmental stages of the *Cryptosporidium* spp. (C). H&E stain, 400X.

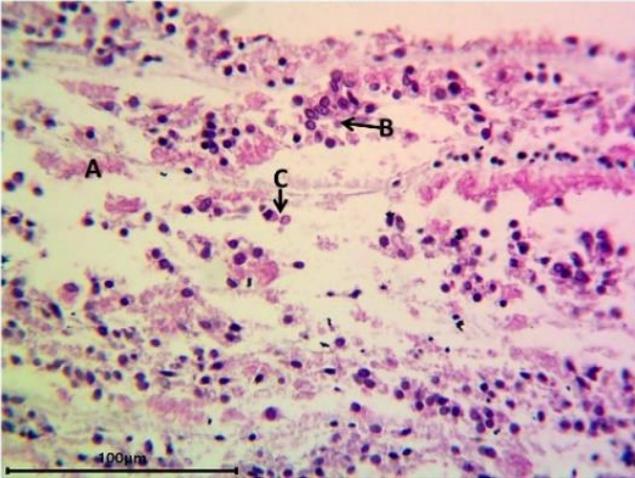


Figure 5: Photomicrograph of duck trachea infected by Cryptosporidiosis showing necrosis and loss of epithelial cells in the mucosa (A), with infiltration of inflammatory cells (B) and presence of the developmental stages of the Cryptosporidium spp. (C). H&E stain, 400X.

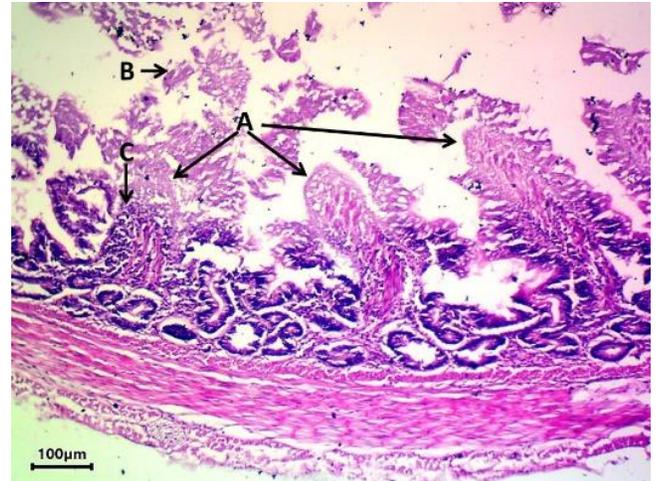


Figure 7: Photomicrograph of duck intestine infected by Cryptosporidiosis showing shortening of the villi due to necrosis and loss of epithelial cells in the mucosa (A), with sloughing in the lumen (B) and infiltration of inflammatory cells in the submucosa (C). H&E stain, 100X.

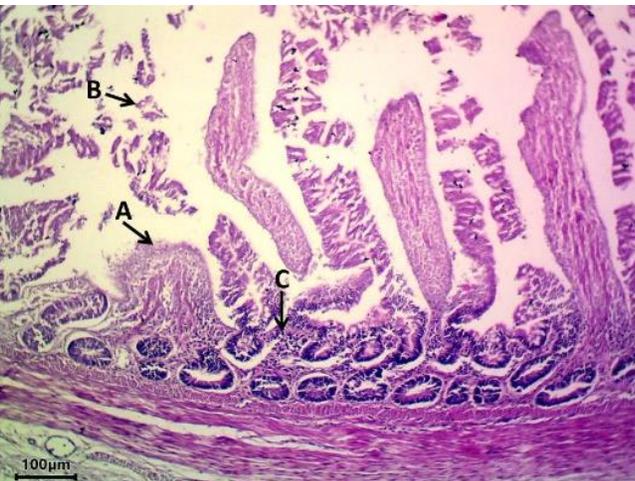


Figure 6: Photomicrograph of duck intestine infected by Cryptosporidiosis showing shortening of the villi due to necrosis and loss of epithelial cells in the mucosa (A), with sloughing in the lumen (B) and infiltration of inflammatory cells in the submucosa (C). H&E stain, 100X.

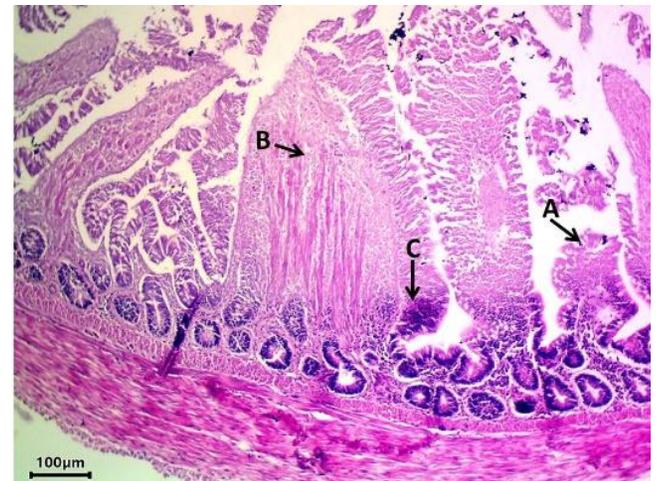


Figure 8: Photomicrograph of duck intestine infected by Cryptosporidiosis showing shortening of the villi due to necrosis and loss of epithelial cells in the mucosa (A), coalesce of the two villi (B) and infiltration of inflammatory cells in the submucosa (C). H&E stain, 100X.

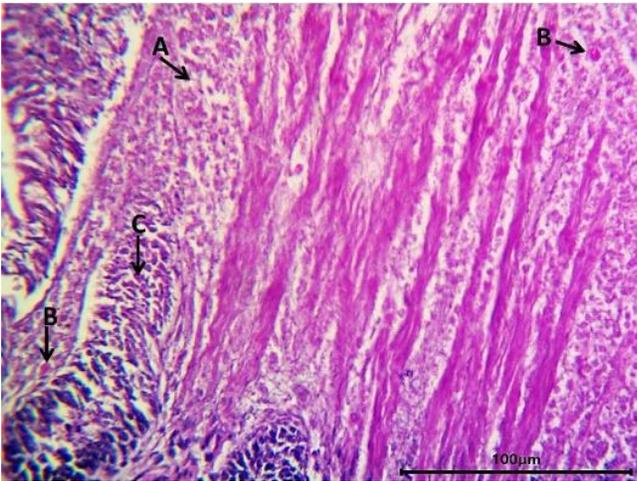


Figure 9: Photomicrograph of duck intestine infected by Cryptosporidiosis showing shortening of the villi due to necrosis of epithelial cells in the mucosa of the coalescing two villi (A) present in the developmental stages of the *Cryptosporidium* spp. (B) and necrosis of the intestinal glands' epithelial cells (C). H&E stain, 400X.

The isolates from local ducks in Mosul were identified as LC757381 by sequencing, which is highly similar to isolates KX926463 and KX926462 discovered and reported in China (Figures 10).

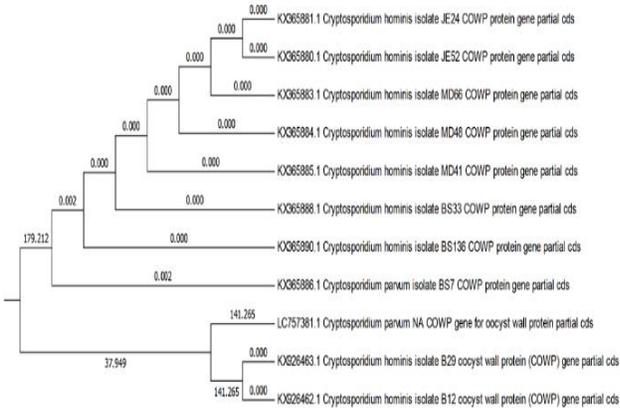


Figure 10 represents the Phylogenetic tree plotted from the 18S rDNA gene sequence of the *Cryptosporidium Parvum* isolate LC757381.

Discussion

The results demonstrated a noticeable difference in the infection rate between the different locations, with a high rate of infection appearing in the city's northern sections (Felfel), at 70%, compared to 10% in the Bashiqa region's eastern side. According to these findings, the geographical location and surrounding environment may have affected the

illness. Water supply sources may also influence illness dissemination (19,20).

According to the ELISA results, 25 of the samples tested positive for the parasite's presence, corresponding to a prevalence rate of 27% (21-23), although it is close to the prevalence reported by Jarad (24). According to Roellig *et al.* (25), there is variation in infection rates across different locations. Host vectors are responsible for the regional variation in infection rates. For example, McReynolds *et al.* (26) identified calves as essential hosts for parasite transmission. The presence of agricultural lands fed with organic fertilizers may play a role in parasite transmission to animals in that area (27-29).

The infection rate was 50.5% among ducks aged 9 to 12 months. This could be due to issues with decreased immunity due to physiological reasons during this age period or the frequent exposure of ducks at this age to parasites in contaminated food and water, in addition to ducks at this age being exposed to stressful conditions that lead to weak immunity (30,31).

According to Song *et al.* (8), samples of older birds indicate a steady reduction in the growth of both the peripheral and central immune systems, which ends when sexual maturity is reached. While the data showed that the age group 3 to 6 months had the lowest infection rate at 14%, this could be attributed to physiological immunity being better at this age than at older ages (32,33).

Cryptosporidium Parvum produced pathological and histological alterations in duck intestines and tracheas. This is due to the parasite's capacity to infect certain sporozoites, which aid in the attachment of cryptosporidium to the surface following transmission by contaminated water and food or through animals carrying the disease. When the parasite hits the target tissue, it attaches itself to it before penetrating it. It begins to multiply within the tissues, and epithelial cells release cytokines, causing phagocytic cells to become activated (34).

This means that the target tissues and neighboring tissues may be damaged. Then, depending on the bird's condition, inflammatory effects appear on the tissues, and a different immune response begins to appear, resulting in damage and destruction of the intestinal barrier, as well as affecting the natural mucosal barriers in the intestine, which increases exposure to harmful polluted environmental factors (35,36). The isolates from ducks were identified as LC757381, which is highly similar to isolates KX926463 and KX926462 found in China.

This similarity may be due to the presence of genomic similarity between the parasite strains found in Mosul and China, and this may be due to the occurrence of hereditary mutations in the genes or a natural or abnormal genetic mutation in the *Cryptosporidium parvum* gene (37,38). Furthermore, as it occurs in response to environmental variables and host stresses, the transfer of genetic variants across strains and natural evolutionary pressure may affect

the similarity in isolates (39,40). In addition, cross-species Transmission between humans and animals can result in breed similarities. An in-depth genetic investigation and comparison of different locations are required to discover the primary basis for this resemblance.

Conclusion

Cryptosporidium is a parasite illness that affects indigenous ducks in Mosul. For the first time in Mosul, the study recorded the phylogenetic tree of *Cryptosporidium parvum* in local ducks, revealing a higher incidence among local ducks at 6-12 months than at other ages. Furthermore, its incidence was higher in the city's northern sections than in its eastern areas. This strain was found to be responsible for histological abnormalities in duck trachea and intestines.

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Conflict of interest

None.

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دراسة جزيئية ومناعية ونسجية لطفيلى المكورات الخبئية في البط المحلي في بعض مناطق مدينة الموصل، العراق

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الخلاصة

سجل في هذه الدراسة لأول مرة نوع وسلالة النمط الوراثي لطفيلى الأبوغ الخبيثة في البط المحلي باستخدام تقنية التسلسل الجيني، كما بحثت هذه الدراسة في مدى انتشار وطريقة تشخيص طفيلي الأبوغ الخبيثة في البط في مناطق مختلفة من مدينة الموصل. جمعت ٩١ عينة براز من البط من ثماني مناطق مختلفة داخل المدينة، وهي الشلالات، بعشيقية، على راش، برطلة، الحمداية، تلكيف، فليل، والقوش. وأظهرت النتائج معدل إيجابي بنسبة ٢٧% (٢٥ من أصل ٩١)، مع وجود اختلافات في الإصابة على أساس المنطقة الجغرافية والعمر. وأبرز التحليل الإحصائي وجود اختلافات كبيرة في معدلات الإصابة بين مناطق شمال وشرق الموصل. وأظهر عامل العمر تسجيل فرق معنوي بعمر ٩-١٢ شهرا. كشفت الفحوصات النسيجية للعينات الإيجابية عن تغييرات مرضية نسيجية في القصبه الهوائية، وفي الغدد المعوية. تم تسجيل نوع وسلالة الأبوغ الخبيثة ومقارنته مع التسلسلات المسجلة في قواعد البيانات في المركز الوطني للمعلومات الجينية، وقد تبين وجود تطابق في التسلسل الجيني للطفيلي مع العتر الموجودة في الصين. خلصت الدراسة الى تسجيل سلالة ونوع عزلة الأبوغ الخبيثة في البط المحلي لأول مرة في مدينة الموصل والذي اظهر انتشارا بين البط المحلي في الأعمار ٦-١٢ شهر بنسبة أكبر من بقية الأعمار كما أن نسبة انتشاره كانت أكبر في مناطق الشمالية من المدينة أكثر من المناطق الشرقية وأظهرت هذه السلالة أحداث تغييرات مرضية نسيجية في القصبه الهوائية والأمعاء في البط المحلي.