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## ORIGINAL ARTICLE

# EFFECT OF BACTERIAL INFECTION ON THE PROTOSCOLECES DEGENERATION OF HYDATID CYSTS IN SHEEP

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**Summary**

***Background*:** Hydatidosis is a deadly parasitic disease that affects both humans and animals. It has received much attention due to widespread health and economic concerns. ***Materials and Methods*:** Thirty-three hydatid cysts from the slaughterhouse and butcher shops were analyzed, 17 from the lung and 16 from the liver. The specimens were collected from hydatid fluid and grown on nutritional agar and MacConkey agar using a sterile loop. A Vitek- 2 compact instrument was used to identify bacteria. The viability of the protoscoleces was also determined in these hydatid cysts. ***Results*:** The secondary infection rate with bacteria in hepatic and pulmonary hydatid cysts was 24 (72.7%) from a total of thirty-three samples. Several types of bacteria have been isolated from hepatic and pulmonary hydatid cysts. *Aeromonas hydrophila* had the highest infection rate in hepatic and pulmonary hydatid cysts reaching 20.83% while the lowest infection rate was 4.17% for *Leuconostoc mesenteriodes*, *Lactococcus garvieae*, *Staphylococcus sciuri*, and *Staphylococcus hominis*, *Streptococcus uberis*, *Pseudomonas stutzer* and *Vibro vulnificus*. *Staphylococcus lentus* and *Lactococcus garvieae* had the highest effect on the viability of protoscoleces in liver and lung, reaching 0%, and 13% respectively. Eleven of a total of 13 types of bacteria isolated from hydatid cysts in the liver and lung: were diagnosed for the first time and had not previously been recognized by earlier investigation. The rates of bacterial infection in hepatic and pulmonary hydatid cysts were 76.47% and 68.75%, respectively. ***Conclusion*:** The results of our current study indicate that the secondary infection rate with bacteria in hepatic and pulmonary hydatid cysts reached (72.7%), and different types of bacteria in hepatic and pulmonary hydatid cysts have a clear effect on the viability of protoscoleces.

*Key words: hydatid fluid; protoscoleces; bacteria*

## Introduction

Hydatidosis, also known as hydatid cyst disease, is a deadly disease that affects both humans and animals. It has received much attention recently because of the wide-ranging health and economic concerns it creates, as it affects both humans and their pets (1).

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*Echinococcus granulosus*, a member of the Taeniide family, is responsible for the worldwide spread of unicellular hydatid cyst disease. The existence of intermediate herbivore hosts, as well as humans as occasional hosts and pet dogs as final hosts, is critical to the success of the life cycle (2).

Dogs' small intestines are habitats for adult worms. The worms' eggs are released with dog droppings that contaminate weeds, and these eggs are ingested by intermediate host, often livestock, including, goats, sheep, pigs, cattle, and camels (3).

The hexacanth embryo in the egg reaches the liver, which is the first filter for blood through the bile ducts, where the rate of infection ranges from 50-70% (2). This embryo travels from the liver to the pulmonary circulatory system, where a percentage of these embryos may be hampered (5-25%), while some embryos may change their course and spread throughout the body, such as into the like spleen, bones, muscles, brain, and eyes. (4).

Once the embryo settles inside the organ, it begins to vesicle within 4-5 days, transforming into a hollow cystic shape that tends to form the future metacestode (the hydatid cyst) (2), which consists of three layers from the outside to the inside: The damaged organ forms the outer layer (pericyst) in response to the cyst's growth and pressure, and it commonly calcifies. The flexible intermediate layer (laminated layer), allows nutrients to pass through while keeping pathogens out.

The endocyst (germinal layer) consists of a single layer of cells whose function is to form protoscoleces and hyadatid fluid (5). It contains hydatid fluid and a large number of live protoscoleces (up to one million). The definitive host acquires the infection by swallowing the dormant protoscoleces inside the hydatid cyst on which it feeds along with the infected organs (4).

Any change in the liver's function as a result of infection with pathogenic agents such as bacteria and parasites is reflected in the animal's health, and health manifestations impacts animal health, which affects the amount of wool, meat, and milk produced, resulting in a significant economic loss for animal breeders (6).

Hydatid cyst disease initially affects the liver, and this infection may be followed by a secondary bacterial infection caused by bacteria that are carried with a hexacanth embryo that enters the biliary system through the papilla and portal vein.

The size of the cyst grows over time, and the cyst loses its ability to control the permeability of the membrane, allowing bacteria such as (*Staphylococcus. aureus*, *Pseudomonas areoginosa*, *Staphylococcus. albus* and *Staphylococcus. citreus*) to enter the hydatid fluid, which is a suitable medium for their growth because it contains lipids, cholesterol, glucose, urea, proteins, and various enzymes, as well as the presence of several minerals, including copper, iron, chlorine, phosphorous and liquid acidity up to 7, and these substances vary in their quality depending on the location of the cyst and the source of the parasite which plays a key role in the life cycle of the adult worm. (7).

The aim of the study was to detect bacterial infection concurrent with the viability of protoscoleces present in hydatid fluid.

**Material and Methods**

Sixteen hepatic and seventeen pulmonary hydatid cysts from naturally infected sheep were collected from slaughterhouses and butcher shops in Mosul city, Iraq. Under aseptic conditions, hydatid cyst fluids were extracted from these cysts.

The fluids from the hydatid cysts were centrifuged at 3000 rpm for 15 minutes, and the sediment was then grown on both nutritional agar and MacConkey agar at 37°C for 24-48 hours (8). Bacteria from positive cultures were identified after incubation using typical microbiological techniques such as Gram staining and biochemical responses (9).

The Vitek -2 compact (bioMérieux Inc. USA) instrument was used to identify bacteria (GP ID REF21342 for Gr+ and GN ID REF21341) for Gr- cards. All test procedures were carried out in accordance with the manufacturer's guidelines.

The protoscoleces were obtained from hydatid fluid according to Smith's method (10).

Eosin staining at a concentration of 0.1% was used to detect the viability of protoscoleces. Live protoscoleces appeared bright green with flame cell activity, while dead protoscoleces appeared red (11).

**Results**

There were no differences in the number of isolates infected with gram-positive and gram-negative bacteria of hepatic hydatid cysts out of a total of sixteen isolates, (Table 1), while the secondary infections with gram-negative bacteria were greater than the gram- positive bacterial isolates in the pulmonary hydatid cysts (Table 2).

**Table 1.** The types of bacteria that were isolated from hepatic hydatid cyst fluid.

| Types of bacteria | No. of isolate | Gram stain | Bacterial infection rate |
|---|---|---|---|
| *Aeromonas hydrophila* | 3 | - | 27.08% |
| *Escherichia coli* | 3 | - | 27.08% |
| *Kocuria rosea* | 1 | + | 15.38% |
| *Ameromonas sobria* | 1 | + | 7.69% |
| *Leucomostoc mesenteroides ssp cremoris* | 1 | + | 7.69% |
| *Staphylococcus hominis ssp hominis* | 1 | + | 7.69% |
| *Staphylococcus lentus* | 1 | + | 7.69% |
| *Streptococcus uberis* | 1 | + | 7.69% |
| No growth | 4 | | 0% |
| Total | 16 | | |

**Table 2.** The types of bacteria that were isolated from pulmonary hydatid cyst fluid.

| Types of bacteria | No. of isolate | Gram stain | Bacterial infection rate |
|---|---|---|---|
| *Aeromonas hydrophila* | 2 | - | 18.18% |
| *Escherichia coli* | 2 | - | 18.18% |
| *Aeromonas sobria* | 1 | - | 9.09% |
| *Citrobacter freundii* | 1 | - | 9.09% |
| *Vibro vuluificus* | 1 | - | 9.09% |
| *Leuconostoc mesenteriodes ssp* | 2 | + | 9.09% |
| *Pseudomonas stutzeri* | 1 | + | 9.09% |
| *Staphlococcus sciuri* | 1 | + | 9.09% |
| *Lactococcus garvieae* | 1 | + | 9.09% |
| No growth | 5 | | 0% |
| Total | 17 | | |

Eleven types of bacteria were isolated from hydatid cysts in the liver and lung such as *Kocuria rosea*, *Aeromonas mesenteroides ssp*, *Staphylococcus lentus*, *Staphlococcus sciuri*, *Streptococcus uberis Citrobacter freundi*, *Vibrio vuluificus*, *Pesudomonas stulzerii*, *Leuconostoc mesenteriodes* spp creminis, and *Lactococcus garvieae*, were diagnosed for the first time had not previously been recognized by earlier investigations, (Tables 1 and 2).

The results showed a decrease in the viability of the protoscoleces of hepatic hydatid cysts from 87.6% in the infected cysts with *Aeromonas hydrophila* to 0% in the infected cysts with *Staphylococcus lentus*, where all the protoscoleces appeared in red when stained with eosin (Figure 1).

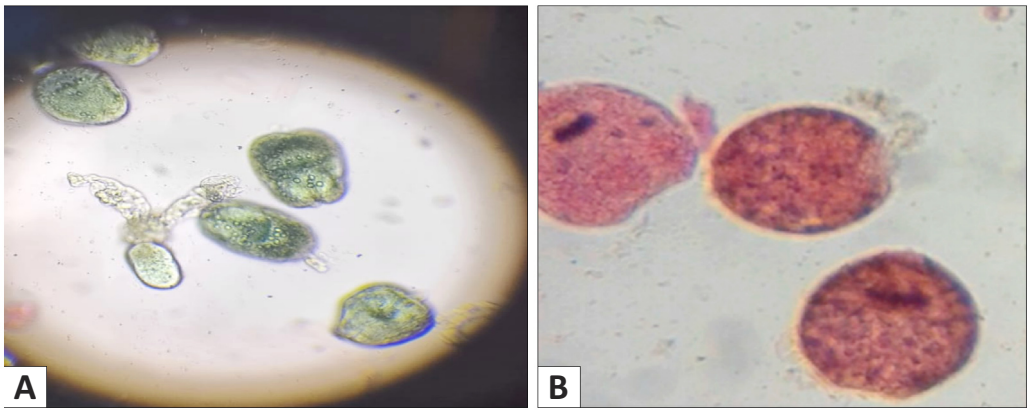


**Figure 1.**The live protoscoleces appeared bright green (A), and the dead protoscoleces appeared red (B) by staining with 0.1% eosin.

The viability of protoscoleces was measured according to their size and colors. The dead protoscoleces were red and small in size, whereas the live protoscoleces were brilliant green with flame cell movement (Table 3).

**Table 3.** Viability of *E. granulosus* protoscoleces in hepatic hydatid cysts infected with different types of bacteria.

| Types of bacteria | Viability of protoscoleces |
|---|---|
| Uninfected cyst | 87.6% |
| *Escherichia coli* | 82.2% |
| *Streptococcus uberis* | 66.2% |
| *Staphylococcus hominis ssp hominisl* | 34.6% |
| *Kocria rosea* | 33.15% |
| *Leuconostoe mesenteroides ssp cremoris* | 32.6% |
| *Aeromonas sobria* | 26% |
| *Aeromonas hydrophila* | 12.5% |
| *Staphylococcus lentus* | 0% |

A discrepancy in the viability of the protoscoleces of the hydatid cysts of the lungs was accompanied by a secondary bacterial infection, where the viability of the protoscoleces decreased from 88.5% to 13% in the cysts infected with the bacteria *Lactococcus garvieae*, while a slight decrease in the viability of the hydatid cysts was also observed during infection with a mixture of bacteria compared with infection with a single bacteria in the hydatid cysts of the lungs (Table 4).

**Table 4.** Viability of E. *granulosus* protoscoleces in pulmonary hydatid cysts infected with different types of bacteria.

| Types of bacteria | Viability of protoscoleces |
|---|---|
| Uninfected cyst | 88.5% |
| *Staphylococcus sciuri* | 85.9% |
| Mixed infection: *Vibro vulnificus, Leuconostoc mesenteries ssp cremoris, Pseudomonas stutzeri* | 78.6% |
| Mixed infection: *Aeromonas hydrophila, Aeromonas caviae, Aeromonas sobia* | 76.5% |
| *Aeromonas sobria* | 42.8% |
| *Esherichia coli* | 21.5% |
| *Lactococcus garvieae* | 13% |

There was a difference in the rate of bacterial infection of both hepatic and pulmonary hydatid cysts, where the highest infection rate was 5 (20.83%) for the bacteria *Aeromonas hydrophila* and *E.coli*. The lowest infection rate was 1 (4.17%) for each of *Lactococcus garvieae*, *Staphylococcus sciuri*, *Staphylococcus hominis* spp, *Staphylococcus lentus*, *Streptococcus uberis*, *Pseudomonas stutzeri*, and *Vibro vulnificus* (Table 5).

**Table 5.** The rate of bacterial infection isolated from hepatic and pulmonary hydatid cyst fluid.

| Types of bacteria | No. of isolate | Rate of infection |
|---|---|---|
| *Aeromonas hydrophila* | 5 | 20.83% |
| *Escherichia coli* | 5 | 20.83% |
| *Aeromonas sobria* | 2 | 8.33% |
| *Kocuria rosea* | 2 | 8.33% |
| *Citrobacter freundii* | 2 | 8.33% |
| *Leuconostoc mesenteriodes ssp cremoris* | 1 | 4.17% |
| *Lactococcus garvieae* | 1 | 4.17% |
| *Staphylococcus sciuri* | 1 | 4.17% |
| *Staphylococcus hominis ssp hominis* | 1 | 4.17% |
| *Staphylococcus lentus* | 1 | 4.17% |
| *Streptococcus uberis* | 1 | 4.17% |
| *Pseudomonas stutzeri* | 1 | 4.17% |
| *Vibro vulnificus* | 1 | 4.17% |

Figure (2) shows that the rates of bacterial infection in hepatic and pulmonary hydatid cysts were 76.47% and 68.75%, respectively.

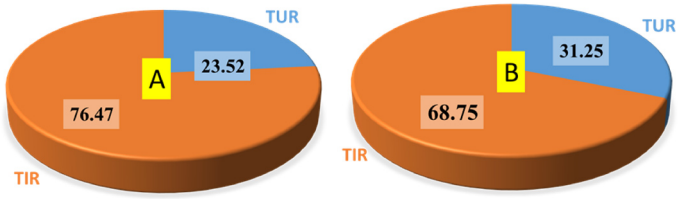


**Figure 2.** Total infection rate of bacteria (TIR) and uninfected rate (TUR) in the fluid of hepatic hydatid cysts (A) and pulmonary hydatid cysts (B**).**

The rate of infection with gram-positive and gram-negative bacteria was equal in hepatic cysts, which amounted to 37.5% compared with cysts free of bacterial infection, which amounted to 25%.

However, there was a difference in the rate of gram-positive and gram-negative bacteria in the pulmonary hydatid cysts, which amounted to 29.4% and 41.2%, respectively, compared with cysts free of bacterial infection, which amounted to 29.4% (Figure 3).

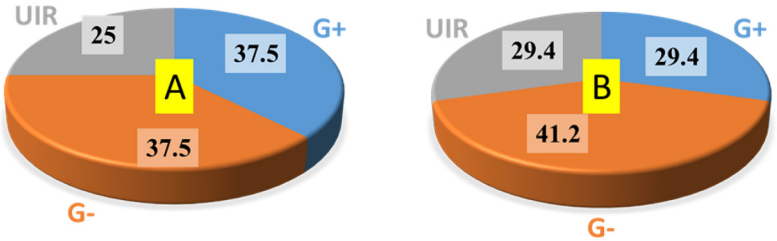


**Figure 3.** The ratio of infection with G+, G-, and uninfected rate (UIR) in the fluid of hepatic hydatid cysts (A) and pulmonary hydatid cysts (B).

**Discussion**

Hydatid cysts can infect any organ in the intermediate host body (animals and humans), but they most commonly infect the liver as the first station (12). The second station for these cysts is the lung due to hematogenous dissemination from hepatic lesions (13). It is possible for embryos to travel through the intestinal lymphatic system, the thoracic duct, the right heart, and eventually the lung (14) or by direct pulmonary exposure (inhalation of air infected with *Echinococcus* eggs (13).

The results of our current study showed a high rate of secondary infection of bacteria in hepatic and pulmonary hydatid cysts, which may be because the gastrointestinal tract is home to the most complex animal microbial ecology, colonized by approximately one trillion bacteria of various species (15) and when the gut barrier is damaged for several reasons, the cyst wall's selective permeable property may be absent, allowing some bacteria species to penetrate the cyst (16, 17).

The bacterial infections that infect the liver vary depending on the point of entry and gut microbe concentrations vary across the gastrointestinal lumen depending on pH, O2, tension, digestion flow rates, and digestive product type (18).

Because the liver is the primary filter for blood, it is the first site of infection in hydatid cysts, and the greatest hazard posed by parasite migration is the creation of favorable conditions for secondary bacterial infection (19).

On the other hand, when the hydatid cyst expands in size, the permeability of the adventitious capsule, lamination layer, and germ layer of the hydatid cyst rises, allowing bacteria to enter the hydatid fluid in greater numbers (15). The hydatid fluid structure can also help bacteria survive, the fluid has a pH of approximately 7, is richer in amino acids than serum, and is high in lipids and carbohydrates (20).

Bacterial infection of hydatid cysts, in addition to the numerous symptoms commonly associated with this serious parasite infection in humans, could be regarded as a potentially dangerous risk factor (21).

In both hepatic and pulmonary hydatid cysts, the current investigation demonstrated a high incidence of *Escherichia coli*, which is consistent with (22, 23). Because there is a correlation between damage to the intestinal lining and bacterial infections, these bacteria could enter through the damage created by the hexacanth embryo's production of digestive enzymes that facilitate penetration into the intestinal wall. This allows bacteria to pass through the intestinal wall and into the liver, resulting in secondary infection (3). This is also supported by the findings of (24).

The results of our current study indicate that the secondary infection rate with bacteria in hepatic and pulmonary hydatid cysts amounted to a total of 33 samples, 24 (72.7%), which is consistent with the findings of (25), where the rate of secondary infection with bacteria in isolated hydatid cysts from sheep reached 70%.

The *E.coli* bacteria recorded the highest infection rate in liver and lung cysts, reaching 27.08% and 18.18%, respectively, which is consistent with what was found (Najm *et al*. 2020) (26), where they recorded the highest infection rate of this bacteria in sheep liver, which amounted to 31.48%.

The infection rate in both types of Gr+ and Gr- bacteria in the liver in the current investigation, is consistent with what was discovered in a previous study (22). The presence of bacteria that function as killers on protoscoleces was also discovered through experimental research, revealing that *E. coli* is the most efficient bacterial species, which could be due to the lethal action of endotoxins, exotoxins, or both (25). Intestinal Staphylococcus bacteria were also isolated from the infected liver and lung.

This indicates that the transmission of these bacteria with hexacanth embryo or extension of the cyst from the gut is consistent with what was found by (27).

*Salmonella spp*. were the most common bacteria detected in hydatid fluid collected from the lungs. *Staphylococcus spp*., *Pseudomonas spp*. with *Enterococci Staphylococci spp*. were isolated from hepatic hydatid fluid (28).

Eleven of a total of 13 types of bacteria isolated from hydatid cysts in the liver and lung were diagnosed for the first time and had not previously been recognized by earlier investigation such as, *Kocuria rosea*, *Aeromonas mesenteroides ssp*, *Staphylococcus lentus*, *Staphylococcus sciuri*, *Streptococcus uberis Citrobacter freundi*, *Vibro vuluificus*, *Pseudomonas stulzerii*, *Leuconostoc mesenteriodes* spp creminis, and *Lactococcus garvieae*, as shown in the results, which may be due to the environmental conditions and the parasite stain.

This study also demonstrated the presence of *E.coli*, in hepatic and pulmonary hydatid cysts, which is consistent with findings from several earlier investigations (22, 25, 26, 29-32).

We also noticed, through our results, a variation in the decrease in the viability of protoscoleces of hepatic hydatid cysts infected with secondary bacterial infection according to the different types of bacteria, where they reached 0% with *Staphylococcus lentus*, so all protoscoleces appeared red, while the percentage of viability of protoscoleces was 28% in hydatid cysts infected with the bacterium *Staphylococcus aureus* The presence of a large number of bacteria in the hydatid fluid may cause the hydatid cyst to transform from a living cyst to a sterile cyst (17).

While the viability of the protoscoleces in the pulmonary hydatid cysts infected with bacteria reached 13%, there was also a small decrease in protoscoleces viability in cysts infected with a variety of bacteria. This could be due to the interaction of multiple toxins from distinct types of bacteria, which hinder each other's potency and thus have a small influence on protoscolece viability compared to single types of bacteria. These bacteria may have an influence on protoscoleces by lowering their vitality or pathogenicity in a variety of ways, or perhaps the bacteria employed as probiotics have the capacity to stimulate the immune system, which results in the production of antibodies or cells that aid in the reduction of parasite infection (33).

There are few studies on the subject of hydatid cyst secondary infection of microorganisms (34).

Despite the effect of bacteria on the dissolution of the protoscoleces and their spread inside the hydatid cyst, there are not enough studies on their effect outside the hydatid cyst, and the negative effect of these bacteria on the protoscoleces is due to their ability to penetrate the outer membrane and secret toxins.

The polysaccharide extracted from K*lebsiella pneumoniae* was found to have an effect as an immunological modulator against hydatid cysts in an experimental investigation on white mice, with an effective effect in reducing the quantity and diameter of hydatid cysts when compared to the control group (35).

Despite the lack of studies in the field of secondary bacterial infections in hydatid cysts, it is obvious that bacteria have an effect on the breakdown of protoscoleces and their proliferation inside the hydatid cyst, as the current study's findings revealed. It is likely that by binding to receptors on the cell membrane, holes are created in the membrane, and the selective influx and influx of ions across the plasma membrane are disrupted (36). It may be possible to utilize bacterial toxins as protoscoleces killers before performing specific surgery if it is definitely established through extensive experimental research that bacterial toxins have an effect on the breakdown of the protoscoleces. Perhaps it will also serve as a springboard for a series of studies involving the extraction of toxins from diverse bacteria and determining their efficacy in destroying protoscoleces *ex vivo*. Perhaps as a replacement for chemical medications, which have adverse effects such as killing protoscoleces during surgical operations.

## Conclusion

It appears from current results that secondary bacterial infection of different types of bacteria in hepatic and pulmonary hydatid cysts has a clear effect on the viability of protoscoleces.

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**Conflict of interest**

Authors declare no competing interest related to this study.

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**Adherence to Ethical Standards**

Not applicable.

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