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ORIGINAL ARTICLE

Investigates Bacterial Species Isolated from Hydatid Cyst Fluid in Sheep Livers in Basrah, Iraq

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ABSTRACT

Background: *Echinococcus granulosus is a zoonotic parasite responsible for hydatid cyst infection, a significant public health concern prevalent in the Middle East and other regions. This infection affects both humans and various animal species such as cattle, goats, and sheep, with dogs serving as the definitive host of Echinococcus.* ***Objective:*** *This study aims to isolate and identify bacteria that invade the hepatic and bile ducts and the circulation, potentially originating from hydatid cysts.* ***Methodology:*** *A total of 78 hydatid cyst samples were collected from the Basrah province slaughterhouse in southern Iraq. Hydatid fluid was cultured on different media, including blood agar, MacConkey agar, and nutrient agar, to isolate bacterial species.* ***Results:*** *Isolates of bacteria were found that Proteus spp. Emerged as the most prevalent isolate, accounting for 47% of the total, followed closely by Shigella spp., which was detected in 37% of the samples. In contrast, Escherichia coli and Klebsiella spp. were less commonly identified, representing 13% and 3%, respectively. Furthermore, the chi-square statistical test yielded a highly significant result ($\chi^2 = 40.66$, $p<0.001$).* ***Conclusion:*** *The findings suggest that E. granulosus oncospheres may become infected with intestinal bacteria before migrating to internal organs. These findings emphasize the potential role of secondary bacterial infections in hydatid cyst pathology and raise public health and food safety concerns.*

INTRODUCTION

Echinococcus granulosus is a zoonotic parasite that causes hydatid cyst infection, a significant illness in the Middle East and other regions[1]. It belongs to the phylum Platyhelminthes and the family Taeniidae. Hydatid cyst infection occurs when an intermediate host ingests parasite eggs. This infection can damage the liver, lungs, and other organs of the intermediate hosts[2]. This public health issue affects humans and several animal species, especially in rural areas where cattle, goats, and sheep serve as the primary intermediate hosts for the larval stage of the infection, while dogs act as the definitive host[3].

Hydatid cysts develop into a single large cyst that consists of a laminated, thick outer layer and a germinal, thin inner nucleated layer, which are crucial for nutrient absorption and physiological homeostasis. They are typically filled with clear, bacteriologically sterile fluid known as hydatid fluid. Although bacterial infection of hydatid fluid can occasionally occur, it is not common[3].

Bacterial infection can arise from the introduction of pyogenic cocci or other well-organized species into the liver through various routes, playing a significant role in pathogenesis[4]. It enters the hydatid fluid via the excretion of infected carnivores. When intermediate hosts, such as sheep, consume eggs, the larvae migrate through the intestine and develop into hydatid cysts primarily in the liver or other organs[5].

During the parasite's life cycle, eggs and contact with the external environment may result in bacterial contamination of the hydatid fluid[5]. Additionally, bacterial infection of hydatid cysts can occur through bile when ruptures cause leakage of cyst contents into the bronchioles or bile ducts[6].

During parasitic infections, co-infections are common between parasites and microbiota. Pathogen-induced immunosuppression commonly occurs in parasitic diseases, cancer, and viral infections. Intestinal parasites such as helminths and protozoa can disrupt host-microbiota balance, contributing to parasite pathogenicity[7]. Modulating intestinal microbiota has even shown prophylactic protection against *Entamoeba histolytica* without antiparasitic drugs[8]. Intestinal helminths may influence the microbiota directly by secreting anti-microbial peptides or indirectly by altering intestinal physiology, permeability, and secretion[9].

Pathogenicity is often associated with protease enzymes that degrade epithelial barriers and trigger immune responses, potentially facilitating secondary infections by other pathogens[10]. Conversely, antagonism among parasites, bacteria, and viruses can protect the host by activating immune responses[11].

In mice infected with *Trichinella spiralis* followed by *Plasmodium* spp., the former activated the mononuclear phagocytic system, reducing *Plasmodium* parasite density in the blood[12]. *E. coli* infections, particularly when concurrent with intestinal parasites, can cause gastric mucosal damage, upper gastrointestinal disorders, and cardia carcinomas

When a Bacterial infection occurs, the cyst cavity will include cellular infiltration or bacterial cells, and the germinal layer and cyst wall may be damaged [14]. If a cyst ruptures, the sudden discharge of its contents may cause allergic responses ranging from moderate to severe anaphylaxis, bacterial infection, and protoscoleces spread, potentially leading to numerous secondary hydatidosis [15]. Few investigations have been conducted on the bacterial infections of hydatid cysts in animals and humans, and the kind of bacteria.

Consumption of meat and liver infected with hydatid cysts harbouring various bacteria may serve as a vector for zoonotic foodborne infections, leading to food poisoning in humans[5].

Because of the limited studies about bacterial isolation from sheep hydatid cyst infection in Basrah province, this study aims to isolate and identify bacteria invading the hepatic and bile ducts and bloodstream originating from hydatid cysts, which can cause harm, especially when cyst rupture allows the entry of living organisms. Causing secondary bacterial infections raises concerns regarding public health and food safety. During meat inspection and cyst removal.

METHODOLOGY

Sample collection

Veterinarians from the slaughterhouse in Basrah province, southern Iraq, conducted post-mortem examinations of slaughtered sheep through visual inspection.

Seventy-eight sheep liver samples infected with hydatid cyst were collected from infected sheep and transported in a cooling box to the Parasitology Laboratory of the Biology Department at the College of Science, Basrah University, southern Iraq. The liver surface was sterile using 70% ethyl alcohol and then rinsed with distilled water. Then, the hydatid fluid was pulled using a medical syringe, replacing the syringe after each sample. The collected hydatid fluid was then examined using the wet mount technique under a light microscope to assess the presence of viable protoscolices, stained with 0.1% eosin as a vital dye[15].

Culturing and Identification

The fluid collected from hydatid cysts was cultured on various media, including blood agar, MacConkey agar, and nutrient agar[16]. The inoculated media were incubated at 37°C for 24 hours. Following incubation, bacterial colonies were examined according to the methods outlined by Liu et al.[10] and Markey et al.[17]. Bacterial isolates were maintained by subculturing on different media and incubating at 37°C for 24 hours. Samples were then transported to the bacteriology laboratory for bacterial strain identification. After culturing, all samples that showed colony growth were purified to obtain pure isolates by further incubation at 37°C for 24 hours.

Bacteriological examination

Bacteria were cultured following the method described by Forbes et al.[18]. The isolated fluid was spread onto different agar plates using an L-shaped glass spreader and incubated at 37°C for 24 hours. After Gram staining, microscopic examination was performed to differentiate between Gram-positive and Gram-negative bacteria. Subsequently, biochemical tests were conducted on all bacterial isolates for further identification.

Staining and Biochemical tests

This study included several staining and biochemical assays, such as catalase and oxidase, performed according to the methods described by Quinn et al.[16] and Markey et al.[17].

Statistical analysis

All statistical analyses were performed using IBM SPSS version 28. The Chi-square (χ^2) test was applied to evaluate the significance of differences in the distribution of bacterial species among the hydatid cyst samples. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

All the collected (78)sheep liver samples were infected with hydatid cysts. They appeared irregular and were filled with multiple cysts, with the tissue exhibiting a loss of normal texture. The cysts were unilocular and had a clear white or yellowish-white outer surface, resembling bubbles, as shown in (Figure 1). They were filled with hydatid fluid containing both live and dead protoscoleces. After staining with 0.1% eosin vital dye, live protoscoleces appeared green, while dead ones appeared pink (Figure 2).

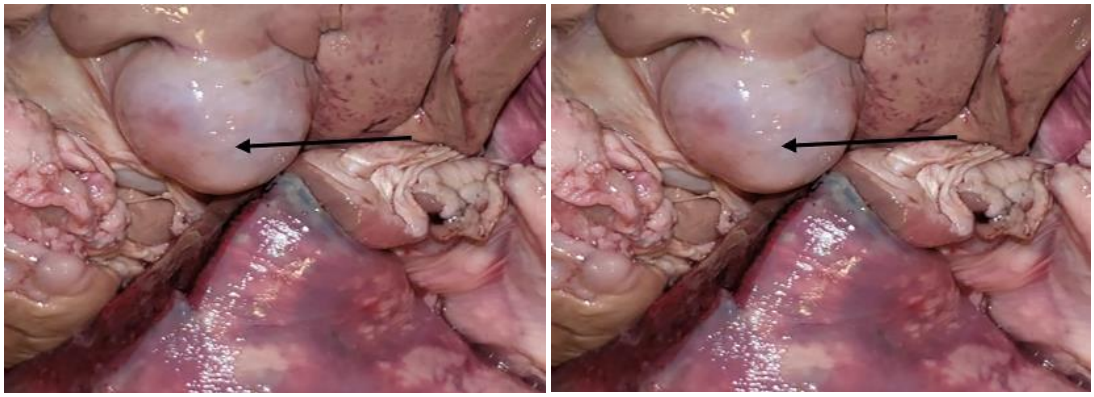

Fig. 1: Hydatid cysts in the liver of an infected sheep, showing an intact, large-sized hydatid cyst (indicated by arrow)

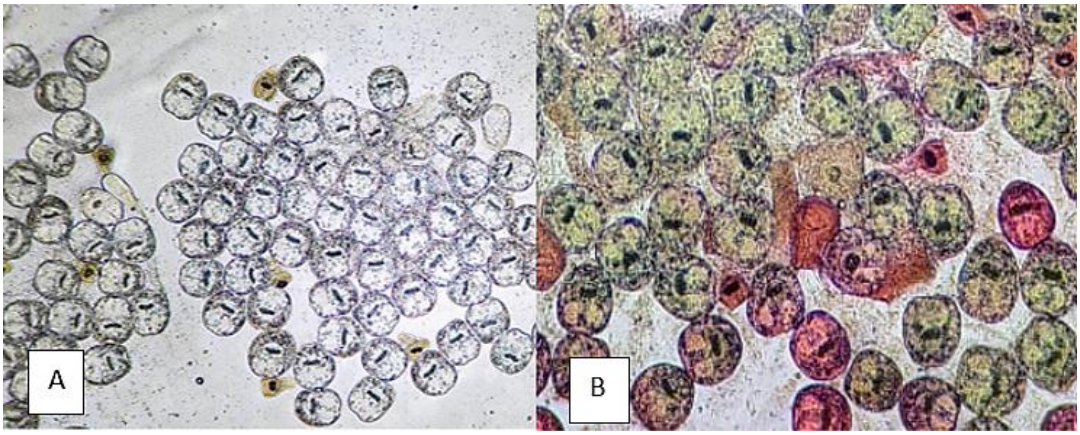

Fig. 2: Hydatid fluid containing protoscolices: (A) Unstained protoscolices, (B) Dead protoscolices appearing pink after staining with 0.1% aqueous eosin, while live, non-stained protoscolices appear green (40× magnification)

After incubating the isolated hydatid cyst fluids for 24 hours, most samples showed visible bacterial colony growth on the culture media. *Shigella* and *Proteus* produced growth on blood agar, while other isolates did not. *Klebsiella* formed round, white colonies on MacConkey agar, whereas *E. coli* grew on nutrient agar. All colonies displayed pigmentation (Figure 3), and were further examined microscopically (Figure 4).

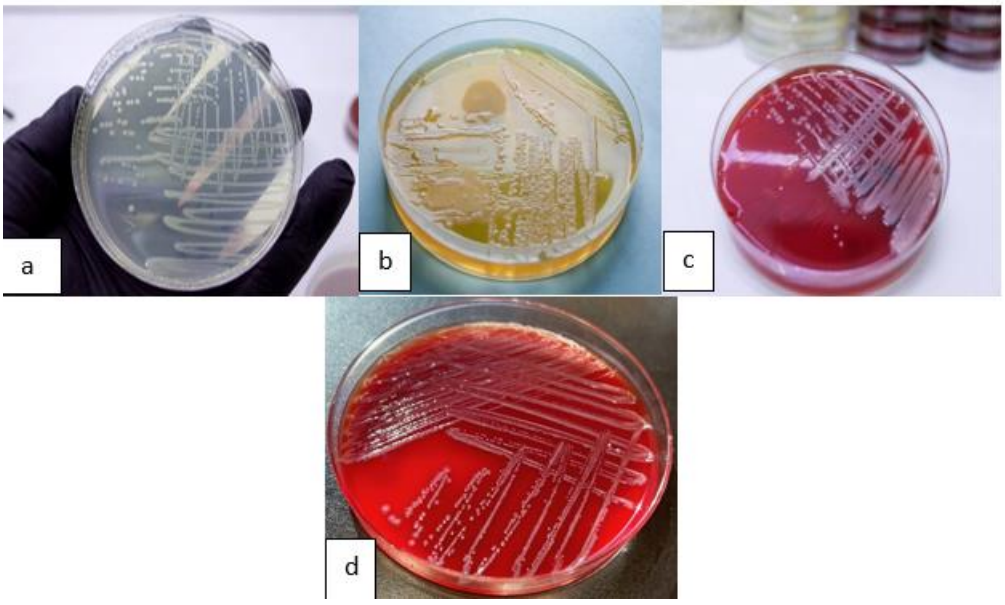

Fig. 3: Bacterial colonies cultured from hydatid cyst fluid isolated from infected sheep liver: (a) *Escherichia coli*, (b) *Klebsiella* spp., (c) *Proteus* spp., (d) *Shigella* spp.

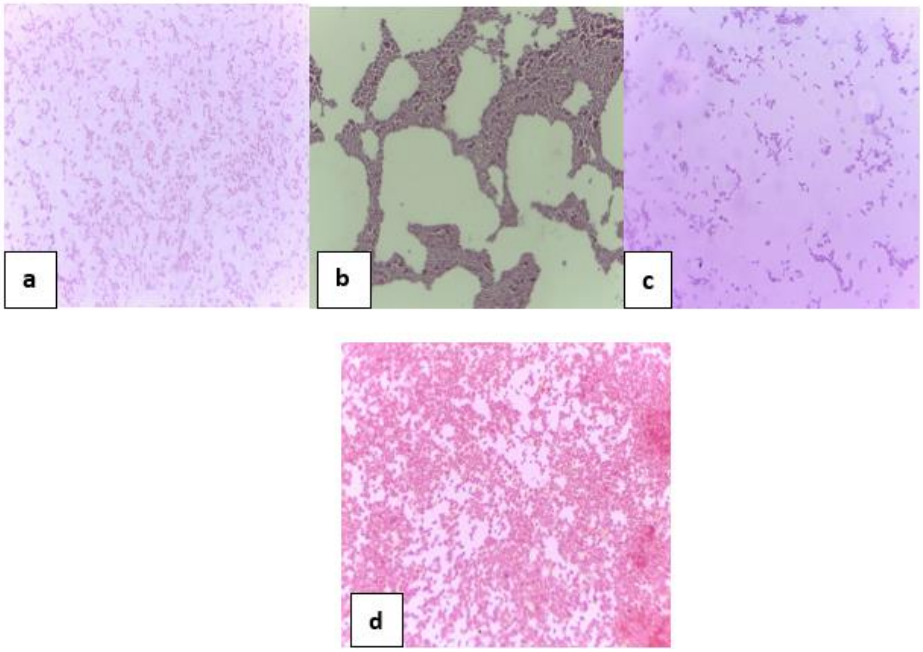

Fig. 4: Types of bacteria isolated from the hydatid cyst fluid of sheep livers infected with hydatidosis: (a) *Escherichia coli*, (b) *Klebsiella* spp., (c) *Proteus* spp., (d) *Shigella* spp.

Biochemical testing revealed that *E. coli* was positive for the Methyl Red, Indole, and Hemolysis tests, while it tested negative for the Citrate, Oxidase, Catalase, and Voges-Proskauer tests. *Klebsiella* spp. the Citrate and Voges-Proskauer tests showed positive results, whereas the Oxidase, Catalase, Methyl Red, Indole, and Hemolysis tests were negative. In the case of *Proteus* spp., all tests were negative except for the Methyl Red test, which was positive; however, the Citrate and Voges-Proskauer tests showed heterogeneous results. For *Shigella* spp., all tests were negative except for the Methyl Red test, which was positive, and the Indole test, which yielded heterogeneous results. These findings are summarized in (Table 1).

The frequency and distribution of bacterial isolates identified from hydatid cyst fluid collected from infected animal livers. Revealed that Among the 78 samples analyzed, *Proteus spp*. emerged as the most prevalent isolate, accounting for 47% of the total, followed closely *by Shigella spp.*, which was detected in 37% of the samples. In contrast, *Escherichia coli* and *Klebsiella spp*. were less commonly identified, representing 13% and 3%, respectively. Furthermore the chi-square statistical test yielded a highly significant result ($\chi^2 = 40.66$, $p<0.001$), as illustrated in (Table 2) and (Figure 5).

Table 1: Biochemical test types used in bacterial species identification

Test / Bacteria type	*E. coli*	*Klebsiella*	*Proteus spp*	*Shigella spp.*
Citrate test	–	+	V	–
Oxidase test	–	–	–	–
Catalase test	–	–	–	
Methyl red test	+	–	+	+
Indol test	+	–	–	V
Voges Proskauer test	–	+	V	–
Haemolysis test	+	–	–	–

+: The test is positive, -: The test is negative, V: The test is heterogeneous +/-/-The results presented in

Table 2: Frequency and Percentage Distribution of Bacterial Isolates with statistical significance (Chi-Square Test)

	Shigella spp.	*Proteus* spp.	*Escherichia coli*	*Klebsiella* spp.
N (%)	29(37%)	37(47%)	10(13%)	2(3%)
Chi-squared test (p-value)	40.66 < 0.001			

*significant difference between groups (p-value < 0.05)

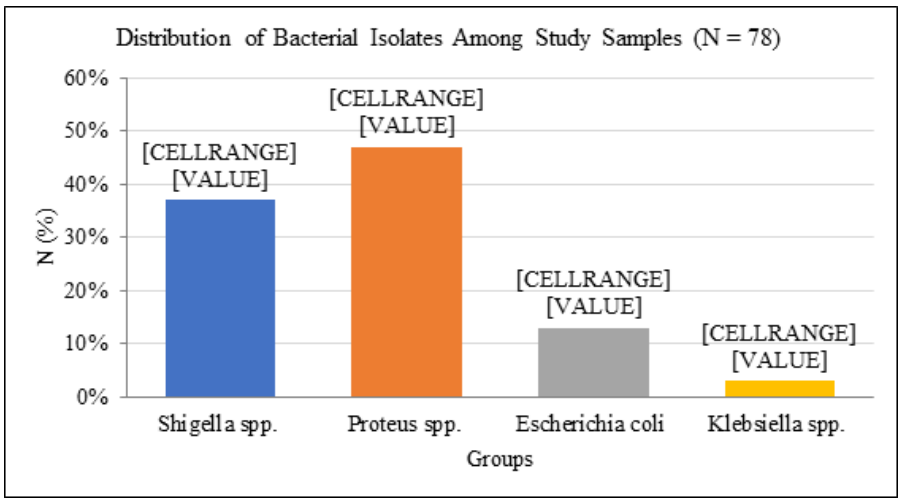

Fig. 5: Distribution of Bacterial Isolates Among Study Samples (N = 78)

DISCUSSION

The liver is an essential meat by-product that must be kept free of all pathogens, including bacteria and parasites, as these can severely damage liver tissue and render it unfit for human consumption, often resulting in partial or complete condemnation during meat inspection in abattoirs[7]. The morphological characteristics of the infected livers, which were filled with hydatid cysts, indicated that the sheep livers were extensively affected, consistent with the findings of De Biase et al.[19]. While previous research has explored bacterial coinfections in parasitic diseases, little is known about the relationship between hydatid cysts and bacteria. This study focused on bacteria isolated from hydatid cyst fluid obtained from infected liver cysts. Bacteria associated with helminthes are more frequently found, with pathogenic bacteria being the most prevalent among positive isolates[20]. The human intestine harbors a complex ecosystem of normal microbiota, including bacteria and yeast, which play crucial roles in maintaining host-pathogen interactions[20]. These microbiota contribute to host immune responses and metabolic homeostasis, protecting against invading pathogens[20].

Interactions between the intestinal microbiota and host responses are not limited to intestinal protozoans; they can also potentially influence infections in the blood and other tissues[21,22]. Problems arising from hydatid cysts in humans are generally rare unless the cysts rupture spontaneously[23]. However, recent research has indicated that complications may also occur in intact cysts.

Klebsiella isolated in the current study could be attributed to its well-known role as a common respiratory pathogen, especially in individuals with chronic illnesses. The current study also indicates the highly significant result of the chi-square statistical test (χ^2 =40.66, p<0.001), in which the variation in bacterial distribution among the different genera was statistically significant and not due to random chance. This significant difference supports the hypothesis that particular bacterial species may have a preferential association with hydatid cysts, possibly due to differences in tissue tropism, virulence mechanisms, or translocation routes within the host.

As shown in (Figure 2), the data are visually represented to highlight the disproportionate dominance of *Proteus spp*. and *Shigella spp*., which accounted for 84% of all isolates. These organisms are primarily known as enteric bacteria and may have entered the hepatic tissue either through hematogenous spread or via secondary infection of necrotic liver parenchyma. This finding aligns with the study's hypothesis that *Echinococcus granulosus* oncospheres might acquire bacterial contamination during or after their intestinal transit phase, particularly from commensal or opportunistic intestinal flora.

Several studies have supported the possibility of bacterial co-infection within hydatid cysts. For example, a study by Kalkan et al. [24] reported the isolation of bacteria, including *E. coli* and *Proteus spp*., from infected hydatid cysts, suggesting that these infections might exacerbate cyst degeneration and host inflammatory responses. Moreover, it emphasizes the public health risks of improper cyst handling in abattoirs, where cross-contamination can occur and threaten meat safety[25].

Limited studies have documented bacterial isolation from hydatid cyst infection of sheep in Basrah province. A study of Al-Diwan et al.[26] concluded the identification of bacteria isolated from buffalo in southern Iraq. Another study, done by Yahya and Mohi-Aldeen[27], included the Isolation and documentation of hydatid cyst bacteria collected from diseased goat and sheep lungs and livers in Mosul abattoir/ Nineveh/ Iraq,

while a study by Al-Ouqaili et al.[28] focused only on human infections in Al-Ramadi province.

E. coli in hepatic cysts suggests that hydatid cyst fluid may become contaminated with intestinal bacteria during the hatching of the oncosphere (the hexacanth embryo of *E. granulosus*) within the intestines of the intermediate host. This finding aligns with the observations reported by Al-Ouqaili et al.[28] in which they isolated bacteria from hydatid cysts in Al-Ramadi province.

The ability of specific isolates, such as *Shigella* and *Proteus*, to produce hemolysis on blood agar medium corresponds with their colony appearance in culture, supporting that the liver is their preferred site during coinfection. This is consistent with Al-Ouqaili et al.[28] findings, which noted that blood provides abundant nutrients that facilitate bacterial growth. Other isolates, including *Klebsiella*, which formed round white colonies on MacConkey agar, and *E. coli*, which grew on nutrient agar, did not exhibit lactose fermentation, consistent with the study of Khodair et al.[29].

Biochemical testing has also identified different bacterial species, including *Proteus spp.*, *E. coli*, *Shigella spp.*, and *Klebsiella spp.*, suggesting a strong association between bacteria and parasitic infection. This is notable even when bile is sterilized, potentially due to bacterial invasion via bile flow or circulatory system infections. These findings agree with earlier studies by Ziino[5] and Fallah[6], which linked such infections to biliary tract obstruction and stasis.

Most isolates tested positive for catalase, an enzyme that converts hydrogen peroxide (H_2O_2) into water and oxygen gas. The indole test showed positive results, indicated by a red ring at the medium's surface. The methyl red test, differentiating *Escherichia coli* from other Enterobacteriaceae, produced a positive red coloration[30].

Positive citrate test results indicate bacterial citrate utilization as the sole carbon source due to citrate permease. This metabolic activity causes the medium to change color to bluish-green, reflecting the production of citric acid and a stable pH, consistent with the findings reported by Procop et al.[31]. Additionally, *Proteus spp* has a higher prevalence. (54%) compared to *Klebsiella* (3%) isolated from hydatid cyst fluid may relate to various virulence factors, including urease activity, alkaline adaptation, motility, and swarming behavior. *Proteus spp.* is commonly found in soil, water, and fecal environments, facilitating its transmission[16].

CONCLUSION

The investigation identified several bacterial species in hydatid fluid, although the exact origin of these microorganisms remains unknown. The data underscore the importance of routine microbiological surveillance of hydatid cysts in slaughterhouses, especially in endemic areas such as southern Iraq. Therefore, Particular attention must be paid to sterile procedures to prevent bacterial dissemination and contamination of carcasses intended for human consumption, as this poses a significant public health risk. Improving sanitary conditions in slaughterhouses, properly disposing of rejected tissues, and treating parasitic infections in animals before slaughter are crucial. Additionally, controlling parasite transmission through eradicating stray dogs can substantially reduce the prevalence of hydatid disease in animals. Immunosuppression induced by *Echinococcus granulosus* in the host may further contribute to coinfections.

Ethical approvals

The current study did not need ethical approval or permission under European Directive 2010/63/EU because all organ collection procedures were post-mortem and supervised by the University of Basrah's Ethics Committee.

Conflict of Interest

We state no conflict of interest.

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