Frequency of bacterial infection in hydatid cysts

Ibrahim Faris Ali[1], Alaa Taha Younis[2], Aseel Abdul Moneim Al-Layla [3]

[1]Department of Biology, College of Education *for* Pure Science, University of Mosul, Mosul 41002, Iraq. ibrahimfali@uomosul.edu.iq

[2]Department of Biology, College of Education for Pure Science, University of Mosul, Mosul 41002, Iraq. alaatyounis@uomosul.edu.iq

[3]Department of Biophysics, College of Science, University of Mosul, Mosul 41002, Iraq. aseelallayla@uomosul.edu.iq

Abstract

Hydatidosis is a zoonotic disease caused by metacestode stage of tapeworm helminth called *E. granulosus*. Liver is the most frequent organ can be infected with this parasite, by which multiple hydatid cysts are grown and developed in the infected organ. Many of diseases, however, are reported to be accompanied with coinfections due to immunosuppression that occurred by the first infection. Thus, the current study is aimed to investigate the prevalence of bacterial infection in hydatid cyst fluid of *E. granulosus*-infected sheep. The data of current study indicated that *E. coli* and *K. pneumoniae* are the most frequent bacteria that cause coinfection in hepatic and pulmonary hydatid cysts of *E. granulosus*, respectively. The highest death percentage of protoscoleces in hepatic and pulmonary hydatid

cysts is related to infection with *E. coli.* In conclusion, *E. coli* and *K. pneumoniae* are the mean causative agents of bacterial infection of hydatid cysts in liver and lung, respectively. *E. coli* is the most lethal bacteria against protoscoleces in both hepatic and pulmonary hydatid cysts. All types of bacteria that isolated from hydatid cyst fluids in the current study are intestinal bacteria, this suggesting that *E. granulosus* oncospheres when hatch in the small intestines may be contaminated with intestinal bacteria before migration to the internal organs or may due to immunosuppression that occurred by *E. granulosus* leading to bacterial coinfection of hydatid cyst fluid. Additionally, liver abscesses that producing by bacterial infection of surrounded necrotic tissues may represent as a source of bacterial coinfection of hydatid cysts.

Keywords: *E. granulosus*, Hydatid cysts, Bacteria, Infection, Protoscoleces

Background

E. granulosus is known as a causative agent of Hydatidosis (hydatid cyst disease), it belongs to Platyhelminthes phylum and Taeniidae family. Hydatid cysts are most frequently isolated from the liver of infected intermediate hosts. However, as a part of successful development of pathogens in their host, pathogen-induced immunosuppression is occurred especially during parasitic diseases (1), cancer (2) and viral infection (3; 4), or as a result to modify in the balance of host-microbiota interaction by intestinal parasites such as helminthes and protozoa is a part of parasite pathogenicity (5; 6). Recently, alteration of intestinal microbiota components provided prophylactic protection against *Entamoeba histolytica* without using antiparasite agents (7). Additionally, intestinal helminthes can directly effect on the intestinal microbiota by secretion anti-microbiota peptides or

indirectly by alter the intestinal physiology, permeability and secretion (8). Protease enzymes are the main factors that related to pathogenicity, where these enzymes can cause breakdown of epithelial barriers, inflammatory and other immune responses which may lead to coinfection by other pathogens (9). On the other hand, antagonism effect of relationship between parasite with parasite, bacteria or virus may protect human hosts by inducing host immune responses (10). Thus, coinfection with other pathogen seems to be a quite common. For instance, in experimental infection of mice with *Trichinella spiralis* and followed by *Plasmodium berghei* infection, activation of mononuclear phagocytic system by *Trichinella spiralis* reduced *Plasmodium* parasite density in the host blood (11). Although the coinfection of bacteria in parasitic diseases has been investigated previously, but less understood is known about hydatid cyst and bacteria coinfection. It has been determined that *Helicobacter pylori* bacteria can induce denaturation in the stomach mucosa (12), but also it can protect human host from the upper gastro-oesophageal diseases (13) and carcinoma of cardia (14). Regarding to this, infection with some bacteria in particular *E. coli, Salmonella typhi and Chlamydia trachomatis* as well as parasitic protozoa including *Cryptosporidium parvum, Trichomonas vaginalis, Trypanosoma*

cruzi, *Toxoplasma gondii* can impact positively on cancer progression (15; 16). The aim of the current study is to investigate the frequency of bacteria type, bacterial infection rate in hepatic and pulmonary hydatid cyst fluid and the effect of bacterial infection on protoscoleces viability in naturally *E. granulosus*-infected sheep.

Materials and Methods

Under sterilized conditions, hydatid cyst fluids of 150 hepatic and 64 pulmonary cysts were collected from the livers and lungs isolated from slaughtered sheep in the sheep open market in the Mosul city, Iraq. The hydatid cyst fluids were underwent to centrifugation at 3000 rpm/10 min, following by cultivation of sediment in broth agar for 24 hrs at 37Cº. The bacteria samples were subcultured on MacConkey agar and eosin methylene blue (EMB) for determination of lactose-fermenting bacteria (gram negative bacteria). Additionally, IMVIC analysis including indole, methyl red, and Voges-Broskauer and Simmons citrate as well as hydrogen peroxide (H2O2), motility, urease, potassium cyanide (KCN), oxidase and H2S detection test were obtained for biochemical detection of gram negative bacteria. Gram stain technique was applied for differentiation between gram

positive and gram negative bacteria (17). For protoscoleces viability determination assay, 0.1% of eosin stain was used (18).

Results

The current data indicated that the majority of bacterial infection of hydatid cyst fluid is caused by gram negative bacteria (Table1). *E. coli* and *K. pneumoniae* are the most frequent bacteria cause infection in hydatid cyst fluid in hepatic and pulmonary hydatid cysts, respectively (Table1). The frequency of other bacteria was as follows; *E. coli, K. pneumoniae, P. mirabilis, S. aureus, P. aeruginosa* and *K. kristinae* in the hepatic hydatid cysts, whilst the bacteria frequency in the pulmonary hydatid cysts was as following; *K. pneumoniae, E. coli, S. aureus, P. aeruginosa, P. mirabilis and B. subtilis* (Table-1).

Table 1: Indicated infection rate of different types of isolated bacteria from hydatid cyst fluid of liver and lung.

	Liver			Lung		
No.	**Type of bacteria**	**Gram stain**	**Bacterial infection rate%**	**Type of bacteria**	**Gram stain**	**Bacterial infection rate%**
1.	***E. coli***	**-**	**41.9%**	***K. pneumoniae***	**-**	**35.4%**
2.	***K. pneumoniae***	**-**	**26.6%**	***E. coli***	**-**	**29.1%**
3.	***Proteus mirabilis***	**-**	**12.1%**	***S. aureus***	**+**	**14.5%**

4.	***S. aureus***	**+**	**10.4%**	***P. aeruginosa***	**-**	**10.4%**
5.	***P. aeruginosa***	**-**	**6.4%**	***P. mirabilis***	**-**	**6.2%**
6.	***Kocuria kristinae***	**+**	**2.4%**	***B. subtilis***	**-**	**4.1%**

The current data indicated 82.6% and 70.5% of total bacterial infection rate in hepatic and pulmonary hydatid cysts, respectively (Fig.1). Additionally, the gram stain technique showed that 87.1% and 12.8% of gram negative and gram positive bacterial infection, respectively, in the hepatic hydatid cysts versus 85.4% and 14.5% of gram negative and gram positive bacterial infection, respectively, in the pulmonary hydatid cysts (Fig.2).

Fig.1: Total bacterial infection rate of hepatic (A) and pulmonary hydatid cysts (B).

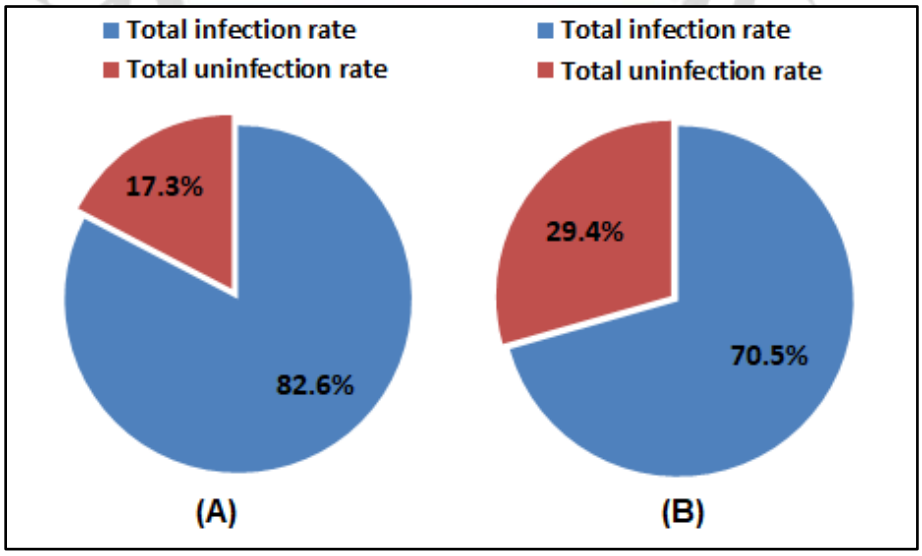
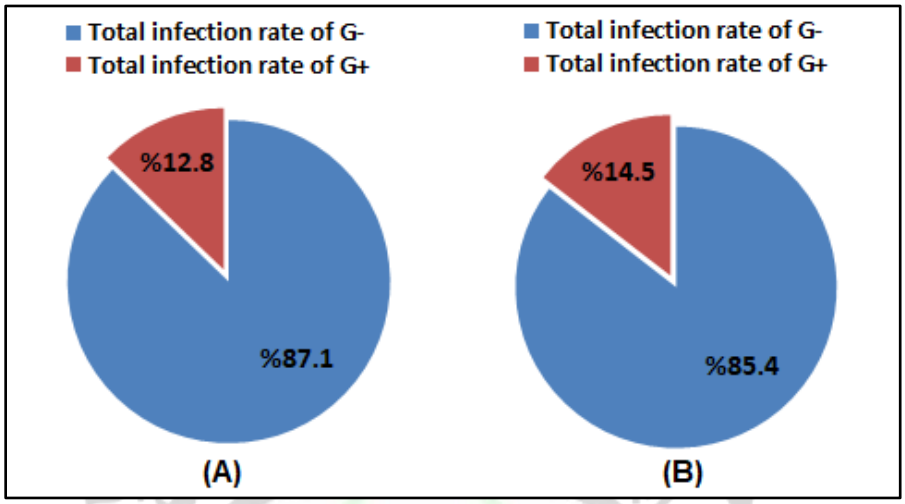


Fig.2: Total infection rate of gram negative (G-) and gram positive (G+) bacteria in the hepatic (A) and pulmonary cysts (B).



The viability test of protoscoleces indicated that the most deadly isolated bacteria is *E. coli* where it reported 67.4-100% (mean: 73.04%) and 26.8-100% (mean: 68.56%) of dead protoscoleces in the hepatic and pulmonary cysts, respectively (Table2). Cytocidal effect were determined as following; *E. coli, K. pneumoniae, P. mirabilis, S. Aureus, P. aeruginosa* and *K. kristinae* in the hepatic hydatid cysts, while the death% of protoscoleces in the pulmonary hydatid cysts was as follows; *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, P. mirabilis and B. subtilis* (Table-2).

Table 2: Range and mean of death percentage of *E. granulosus* protoscoleces isolated from hydatid cysts infected with different types of bacteria from liver and lung.

	Liver			Lung		
No.	**Type of bacteria**	**Range of death % of protos.**	**Mean of death% of protos.**	**Type of bacteria**	**Range of death % of protos.**	**Mean of death % of protos.**
1.	**Uninfected cyst**	**(4.1-10.4)**	**6.71%**	**Uninfected cyst**	**(8.8-18.4)**	**11.85%**
1.	***E. coli***	**(67.4-100)**	**73.04%**	***E. coli***	**(26.8-100)**	**68.56%**
2.	***K. pneumoniae***	**(20.8-93.7)**	**61.02%**	***K. pneumoniae***	**(27.9-47.9)**	**37.62%**
3.	***P. mirabilis***	**(29.7-88.6)**	**54.24%**	***P. aeruginosa***	**(22.1-44.3)**	**26.36%**
4.	***S. aureus***	**(36.3-63.4)**	**48.57%**	***S. aureus***	**(24.1-42.4)**	**30.12%**
5.	***P. aeruginosa***	**(21.3-50.4)**	**32.6%**	***P. mirabilis***	**(20.0-34.1)**	**27.80%**
6.	***K. kristinae***	**(20.6-32.4)**	**26.13%**	***B. subtilis***	**(14.5-28.2)**	**21.35%**

In terms of multiple bacterial infections, four cases of double bacterial infections were represented by *E. coli* and *P. aeruginosa* in a single hydatid cyst with two cases of *E. coli* and *P. mirabilis* coinfection reported in a single hepatic hydatid cysts. Four cases of double bacterial infections represented by *E. coli* and *B. subtilis* with one case of triple infection represented by *P. mirabilis*, *B. subtilis* and *S. aureus* were reported in pulmonary hydatid cysts.

Discussion

Human Intestines represent a complex ecosystem, where content different normal microbiota such as bacteria and yeast which play a critical role in the maintenance of host-pathogen interactions, where they involve in the host immune responses and metabolic homeostasis by which providing protection against invading pathogens (19). Interaction between intestinal microbiota and host defenses is not directed against intestinal protozoan only, but it is also can influence on blood and tissue pathogens (20; 21). Although, complications in hydatid cysts in humans is uncommon if hydatid cysts did not spontaneously rupture (22), but the current study indicated that complications can be frequently occurred in intact hydatid cysts as well. High frequency of *K. pneumoniae* comparing to other types of isolated bacteria that reported in the current study maybe due to *K. pneumoniae* is a common respiratory pathogen particularly in the chronically diseases. Previously, high frequency of bacterial infection (approximately 50%) in hydatid cyst isolated from cattle, coat and sheep was reported (23). Moreover, the frequency of bacteria had been shown as follows; *S. aureus* (18%), *P. aeruginosa* (12%), *E. coli* (6%) and *Streptococcus pneumoniae* (4%) in the human hepatic hydatid cysts (24). Bacterial contamination of parasites during their life cycle was determined previously, where *Staphylococcus* spp., *Micrococcus tetragenes, Sarcina lutea,*

Bacterium coli, *Proteus vulgaris* and *Streptococcus brevis* were isolated from the Cestoda of *Echinococcus polymorphus* and *Echinococcus multilocularis*, this lead to suggest that the isolated bacteria from and within parasitic infection may be intestinal microbiota (25). Coinfection of *Schistosoma* and *Salmonella* seems to be associated with *salmonella* resistance against most antibiotics due to sequestration of bacteria in the groove of *Schistosoma* which may reduce the affectivity of antibiotics and anthelmintic monotherapy leading to increase the burden of bacteria (26).

The high frequent of gram negative bacteria in both hepatic and pulmonary cysts in particular *E. coli* may indicate that contamination of hydatid cyst fluid with intestinal bacteria may due to contaminate oncosphere (hexacanth embryo of *E. granulosus*) when hatch in the intestines of intermediate host. In the current study, the highest rate of dead protoscoleces in hydatid cysts was showed with *E. coli* coinfection in both liver and lung. This current data is in agreement with that reported by Fallah *et al.* (27) in experimental case. In vitro cultivation of protoscoleces of *E. granulosus* parasite with isolated bacteria showed cytocidal effect of bacteria on protoscoleces, where *E. coli* was the most deadly bacteria and

this was following by *K. pneumoniae* and *Enterobacter aerogenes*. The cytocidal effect of bacteria on protoscoleces viability may be due to secretion endotoxin, exotoxin or both of them. Additionally, some enzymes and other biochemical mediators may involve in the virulence of bacteria (27).

Conclusion

E. coli and *K. pneumoniae* are the most frequent agents that cause bacterial infection of hydatid cyst fluid in liver and lung of *E. granulosus*-infected sheep, respectively. The majority of bacterial infections were caused by gram negative bacteria, suggesting that protoscoleces may be contaminated with intestinal microbiota before migration into the internal organs or immunosuppression that occurred in the host by *E. granulosus* may lead to coinfection. The highest death percentage of protoscoleces was connected to infection with *E. coli* in both hepatic and pulmonary hydatid cysts. Finally, exposure protoscoleces of *E. granulosus* to the isolated bacteria from hydatid cysts or their components may interpret the mechanism of cytocidal effects of isolated bacteria on protoscoleces viability.

Acknowledgment

The authors are very thankful to the university of Mosul and college of Education for pure Science for providing free access to the facilities which allowed to finish this research properly and limited time.

References

1. Mabbott, N.A. 2018. The influence of parasite infections on host immunity to coinfection with other pathogens. Frontiers in immunology, 9, p.2579.

2. Kawakami, Y., Qian, L., Kawamura, N., Miyazaki, J., Tsubota, K., Kinoshita, T., Nakamura, K., Ohmura, G., Satomi, R., Sugiyama, J. and Nishio, H., 2015. Cancer Induced Immunosuppression and Its Modulation by Signal Inhibitors. In *Resistance of Cancer Cells to CTL-Mediated Immunotherapy* (pp. 287-301). Springer, Cham.

3. Kumar, N., Sharma, S., Barua, S., Tripathi, B.N. and Rouse, B.T., 2018. Virological and immunological outcomes of coinfections. Clinical microbiology reviews, 31(4), pp.e00111-17.

4. Palefsky, J.M. and Holly, E.A., 2003. Chapter 6: Immunosuppression and co-infection with HIV. JNCI Monographs, 2003(31), pp.41-46.

5. Aivelo, T. and Norberg, A., 2018. Parasite–microbiota interactions potentially affect intestinal communities in wild mammals. *Journal of Animal Ecology*, *87*(2), pp.438-447.

6. Berrilli, F., Di Cave, D., Cavallero, S. and D'Amelio, S., 2012. Interactions between parasites and microbial communities in the human gut. Frontiers in cellular and infection microbiology, 2, p.141.

7. Sarjapuram, N., Mekala, N., Singh, M. and Tatu, U., 2017. The potential of Lactobacillus casei and Entercoccus faecium combination as a preventive probiotic against Entamoeba. *Probiotics and antimicrobial proteins*, *9*(2), pp.142-149.

8. Zaiss, M.M. and Harris, N.L., 2015. The interaction of commensal intestinal bacteria with helminth parasites. Parasite Immunology, 38(1), pp.5-11.

9. Liu, J., Ma'ayeh, S., Peirasmaki, D., Lundström-Stadelmann, B., Hellman, L. and Svärd, S.G., 2018. Secreted Giardia intestinalis cysteine proteases disrupt intestinal epithelial cell junctional complexes and degrade chemokines. *Virulence*, *9*(1), pp.879-894.

10. Shen, S.S., Qu, X.Y., Zhang, W.Z., Li, J. and Lv, Z.Y., 2019. Infection against infection: parasite antagonism against parasites, viruses and bacteria. Infectious diseases of poverty, 8(1), p.49.

11. Ngwenya, B.Z., 1982. Enhanced resistance to Plasmodium berghei in mice previously infected with Trichinella spiralis. *Parasite immunology*, *4*(3), pp.197-207.

12. Wang, F., Meng, W., Wang, B. and Qiao, L., 2014. Helicobacter pylori-induced gastric inflammation and gastric cancer. Cancer letters, 345(2), pp.196-202.

13. Labenz, J. and Malfertheiner, P., 1997. Helicobacter pylori in gastro-oesophageal reflux disease: causal agent, independent or protective factor?. *Gut*, *41*(3), pp.277-280.

14. Molloy, R.M. and Sonnenberg, A., 1996, April. History of peptic ulcer in cardiac and non-cardiac cancer of the stomach. In *Gastroenterology* (Vol. 110, No. 4, pp. A200-A200). 1600 John F Kennedy Boulevard, STE 1800, Philadelphia, PA 19103-2899 USA: WB Saunders Co-Elsevier INC.

15. Fulbright, L.E., Ellermann, M. and Arthur, J.C., 2017. The microbiome and the hallmarks of cancer. PLoS Pathogens, 13(9), p.e1006480.

16. De Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F., Forman, D. and Plummer, M., 2012. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. The lancet oncology, 13(6), pp.607-615.

17. Pacarynuk, L.A. and Danyk, H.C., 2005. BIOLOGY 3200 Principles of Microbiology.

18. Smyth, J.D. and Barrett, N.J., 1980. Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *74*(5), pp.649-652.

19. Mumcuoglu, I., 2019. Interactions between Parasites and Human Microbiota. Eur. J. Ther. 25(1), pp.6-11.

20. Bar, A.K., Phukan, N., Pinheiro, J. and Simoes-Barbosa, A., 2015. The interplay of host microbiota and parasitic protozoans at mucosal interfaces: implications for the outcomes of infections and diseases. PLoS neglected tropical diseases, 9(12), p.e0004176.

21. Keselman, A., Li, E., Maloney, J. and Singer, S.M., 2016. The microbiota contributes to CD8+ T cell activation and nutrient malabsorption following intestinal infection with Giardia duodenalis. Infection and immunity, 84(10), pp.2853-2860.

22. Garcia, M.B., Lledías, J.P., Pérez, I.G., Tirado, V.V., Pardo, L.F., Bellvís, L.M., Varela, G. and Sánchez, M.C., 2010. Primary super-infection of hydatid cyst-clinical setting and microbiology in 37 cases. The American journal of tropical medicine and hygiene, 82(3), pp.376-378.

23. Ziino, G., Giuffrida, A., Panebianco, A. and Bilei, S., 2009. Bacteria isolated from 25 hydatid cysts in sheep, cattle and goats. *Veterinary Record*, *165*(8), pp.234-236.

24. Al-Ouqaili, M., Al-Khamesi, M.B. and Al–Hadithi, I.A.W., 2010. Effect of some species of bacteria on viability of human hydatid cysts. Baghdad Science Journal, 7(3), pp.1153-1158.

25. Dollfuss, R. (1946) Enciclopedie Biologique, XXVII, Parasites (animaux et vegetaux) des Helminthes. Paul Lechevalier Editeur. pp 128-140.

26. Barnhill, A.E., Novozhilova, E., Day, T.A. and Carlson, S.A., 2011. Schistosoma-associated Salmonella resist antibiotics via specific fimbrial attachments to the flatworm. *Parasites & vectors*, *4*(1), p.123.

27. Fallah, M., Kavand, A. and Mashouf, R.Y., 2014. Infected hydatid cysts bacteria in slaughtered livestock and their effects on protoscoleces degeneration. *Jundishapur journal of microbiology*, *7*(6).