British Journal of Global Ecology and Sustainable Development
Volume-14, March., 2023
ISSN (E): 2754-9291

ISOLATION AND IDENTIFICATION OF HYDATID CYST FLUID-ASSOCIATED BACTERIA ISOLATED FROM CATTLE AND SHEEP LUNGS IN SOUTHERN PARTS OF LRAQ

Ghufran S. Jabber. AL-Diwan[1],
Adnan. B.G. AL-Hawash[1],
Sarmad .A.M.AL-Asadi[2]
[1]Department of Biology , Collegy of Education in Qurina,
University of Basrah, Bsarah, Iraq.
[2]Department of Biology, College of Education for Pure Sciences,
University of Basrah, Basrah. Iraq.
ghafransami1996@gmali.com[1]
adnan.ghalib@uobasrah.edu.iq[1]
sarmad.mozan@uobasrah.edu.iq[2]

Abstract

Echinococcus granulosus is a dominant disease, infection occurring during the larval stage and affecting hosts, including cattle as the intermediate host and dogs in particular as the definitive host, who become infected with hydatid cysts. Therefore, the study included the diagnosis of bacteria associated with hydatid fluids isolated from intermediate hosts, including the lungs of cows and sheep. Between February and April 2022, 75 lung samples were taken from sheep and cows infected with hydatid cysts. A set of phenotypic, diagnostic and microscopic tests was conducted, and to confirm that, the Vitek test was performed. The infection rates of bacteria isolated from sheep lungs were higher than those isolated from cow lungs, as it reached 98% in sheep compared to 88% in cows. The results of biochemical tests for *Escherichia. coli*, *Shigella spp*. *Proteus spp*. *Providencia spp*, In addition, 7 isolates were diagnosed using Vitek technology, 3 isolates belonged to cattle lungs, two isolates were identified as *Serratia ficaria* and one isolate was identified as *Aeromonas hydro/cavia*. The remaining five isolates belong to sheep lungs. Two of them belong to *Alcaligenes spp*, and three isolates belong to *Proteus hauseri*, *Planococcus massiliensis*, and *Moellerella wiscousensis*, where the results were not identical to the results of the biochemical diagnosis. Therefore, we conclude from the current study that there are types of bacteria associated the hydatid cyst that differ according to the affected organ.

Keywords: Cystic echinococcosis, bacteria associated with hydatid cyst.

Introduction

Echinococcus granulosus, which causes cystic *Echinococcosis*, is a parasite that primarily affects cattle but can infect any mammal. It is also human-transmittable. It is brought on by the tapeworm genus *Echinococcos* larval stage (Ali *et al*, 2012: Malekifard and Keramati, 2018). It is a problem for both the economy and public health. Echinococcus cysts can affect both people and various animals, particularly in rural

Journal Zone Publishing, Ilford, United Kingdom

areas where animals like cows, sheep, and other livestock are prevalent. In order for the infection to take place in the larval stage of *Echinococcus granulosus*, which serves as its intermediate host, it is crucial. But the final host of *Echinococcus granulosus*, the organism that causes hydatid cysts, which includes dogs, is a carnivore (Abdulhameed *et al.*, 2019). And herbivorous animals that represent the intermediate host, and infection occurs due to eating food, including meat, vegetables, or water contaminated with echinoderm eggs (Hijjawi et al., 2018). Sometimes hydatid cysts in the liver and even the lungs can become infected with the bacteria. Bacterial proliferation in cyst fluid causes interaction between parasites and bacteria that cause some human and animal diseases (Aitkenel *et al.*, 1978). There are two different types of relationships between parasites and bacteria, according to (Boes and Heiwigh, 2000). The first is indirect, which increases the pathogenic effects of bacteria and makes the host more susceptible to bacterial disease, particularly when bacteria and parasites coexist in the same organ, system, or tissue. The second type of relationship is direct. After the stages of parasite invasion in the environment, it happens when the bacterium is transmitted to the host by the parasite (Ziino *et al*, 2009). Significant financial losses result from the effects of cystic echinococcosis on bacteria, including a decline in this host's production (Jahed *et al.*, 2013). As a result, the study's objectives included performing the standard tests and isolating and diagnosing the bacteria linked to the fluid from hydatid cysts.

Materials and Methods

Sample collection

Sampling collection In February 2022, 75 samples of cows and sheep lung were collected; the samples of cows came from the Nasiriyah massacre, while the samples of sheep came from the Basra Governorate. The samples were transferred to the laboratory within hours of collection under refrigeration, where the surface of the organ was sterilized by alcohol Ethyli at a concentration of 70% before the auditory fluid was extracted using a medical syringe while taking into account the auditory system.

Cultuer and Diagnosis

Nutirnt agar , salmonella shigella agar and blood agar were used to insulate the optional bacteria, while 50 µl of the liquid from hydatid cysts was extracted and cultured directly on various cultural media. 24 hours at a temperature of 37 ° should be spent cuddling to identify bacteria (Quinn et al.,2004). Distinguished colonies have been obtained similar colonies. Developing colonies were chosen and culture on the media to get pure culture and this was described (Markey et al., 2013). The bacteria were also preserved by cultivating it, the agar and the glycerin, 20%, and it was embraced in 37 ° for a period of 24 hours, storing it with freezing and re -cultivating it monthly to maintain the activity and vitality of bacteria. Then the installation was made according to the recipe (Quinn et al., 2004), To determine the isolated bacteria, colonies and cell properties, microscopy is determined, the insulation properties are determined using chemical tests, including oxidaes, cataleas test, the IMViC test group, and negative

bacteria (Khelifal at El., 2008). Samples have been sent for the purpose of diagnosing them using the vitek technique for the purpose of confirmation of appearance diagnosis.

Discussion and Results

During the period from February 2022 to March 2022, 75 hydatid cysts were collected. After cultivating them on different culture media, we notice that some media contain bacterial colonies, and others do not notice the appearance of culture growth on those media, through which the percentages of numbers infected with bacteria associated with hydatid cysts were known. These cysts were isolated from the lungs of sheep and cows, Table (1).

Table (1) Percentage of hydatid cysts isolated isolated from cow and sheep lungs

Host	Organsim	Number	number of infected bacteria	percentage
Sheep	lung	50	49	98
Cow	lung	25	22	88

Hydatid cysts in the organs were identified by the presence of swelling on the surface of the affected organ ، With *Echinococcus granulosus*, a white or yellowish-white layer appears on the surface of the organ, resembling a bubble in the lung. It is very clear, and this is consistent with what was mentioned (Hammad, 2017). *Echinococcus granulosus* that causes cystic echinococcosis through the larval stage. It is the cause of persistent infection of medical and veterinary importance (Zeghir-Bouteldjal *et al* 2009: Ahmed *et al*.,2021) . And that opportunistic bacteria are microorganisms isolated from a host that suffers from stress as a result of parasitic infection (Al-Shemmari, 2017). Several previous studies have found a high incidence of bacterial infection, with a rate of 98% in the hydatid cysts isolated from sheep and 88% from the hydatid cysts isolated from cattle, and this is similar to the current study (Hadadi *et al*., 2020: Ziino *et al*., 2009: Khleifat *et al*., 2010) . This study used bacteria that were isolated from sheep and other cattle lungs, including cows. Each sample had growth colonies on the culture medium after it had been cultured. We observe that the majority of isolates displayed hemolysis on solid blood agar medium through phenotypic diagnosis, and the others that were not hemolytic manifested as white colonies. All colonies grow spherically and in the same color on MacConkey agar medium, but because lactose couldn't be fermented, colored colonies resulted. It was comparable to other studies (Darweesh, 2021). Then the results of the biochemical tests showed that the most common isolated bacteria, as shown in Table (2), are *E.coli, Shigella spp, Proteus spp, Providencia,* which indicates that Gram-negative bacteria are the most common in hydatid cysts, and negative bacteria were identified from the cyst fluid lungs, where its percentage in the lungs of sheep accounted for 31%, while in the lungs of cows it was the lowest percentage and reached 12%. The results were similar to what was mentioned (Ziino ., 2009: Abdullah *et al*, 2021: Fallah *et al*, 2014). When these eggs arrive, hatch, and penetrate the infected hexamerocyte embryo from the atmosphere into the mucous membrane, eventually leading to the

formation of hydatid cysts in the lungs. According to some opinions, the infection may have entered through the bile duct or enterocirculatory system (Wani *et al.*, 2010).

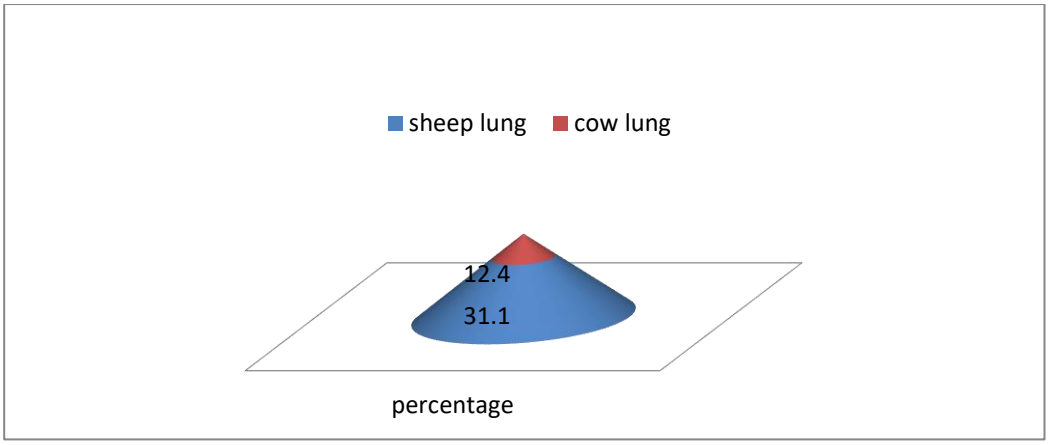

Figure (1) shows the percentage of Gram-negative bacteria, which is the most common, as its percentage was higher in the lungs of sheep than in the lungs of cows, where it constituted the lowest percentage.

Table (2) shows the types of biochemical tests that were tested to identify bacterial species

Type of bacteria ,test	***E.Coli***	***Shigela. Spp***	***Proteus. spp***	***Providncia. spp***
Indol test	+	**V**	-	+
Methyl red test	+	+	+	+
Voges Prosker test	-	-	**V**	-
Citrate test	-	-	**V**	+
Oxidase test	-	-	-	-
Catalase test	+	-	-	-

+: the test result is positive -: the test result is negative **v**: the test result is +/-

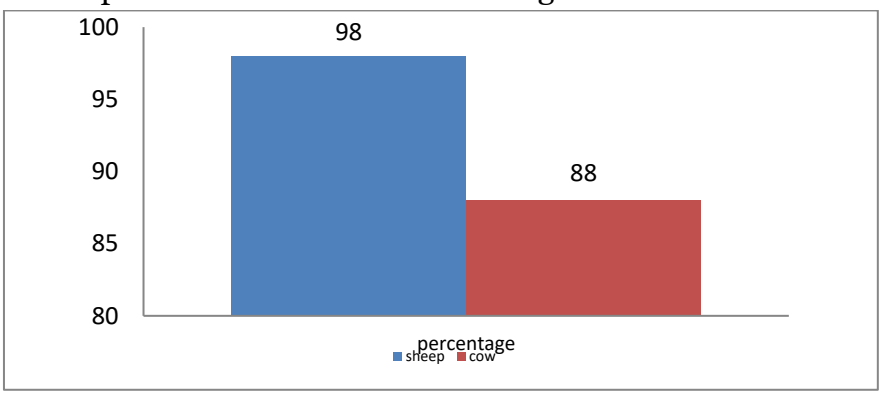

Figure (2) shows the percentage of bacteria associated with the hydatid cyst isolated from the lungs of sheep and cows, where the percentage of bacteria associated with the

hydatid cyst in sheep was higher than that of cows, while the percentage of cows was the lowest.

The results of the biochemical test showed that *Providencia* bacteria had positive results for the test of indole and the test of methyl red, as well as the consumption of citrate, while the rest of the tests were negative. While *Protues spp*., the results were negative for all tests except for the test of methyl red, the result was positive, and the test of vogs-Proscore, the result was different. Also, *shigella* bacteria were also positive for the red methyl test, and the results were negative for the rest of the tests, and finally *E.coli* bacteria, where the results were positive for the test of indole, catalase, and methyl red, while it was negative for the other tests. Most of the isolates were negative for the oxidase test. The results showed that the isolates possess the enzyme catalase, which converts H_2O_2 into water and oxygen gas. Positive for each of the indole test as a result of the appearance of a red ring on the surface of the medium. This test is important to differentiate between Escherichia coli and Providncia. spp and other members of the Enterobacter family This is consistent with what was presented by (Tille, 2017). And to confirm that it was identifaction using the Vitakll, 8 isolates were diagnosed using Vitek, where three isolates belonged to cow lungs, *Aeromonaas hydro/cavia*, *Serratia ficaria* isolate, and the other isolate was undiagnosed, and five isolates belonged to sheep lungs. The result was two isolates belonging to the genus *Alcaligenes spp*., and three different isolates belonging to *Proteus hauseri, Planococcus massiliensis, Moellerella wiscousensis*, where the results were contrary to the phenotypic diagnosis.

Conclusion

Echinococcus granulosus is a zoonotic disease that affects herbivorous animals through the larval stage. Infection occurs by ingesting water and food contaminated with *Echinococcus granulosus* eggs. Therefore, methods must be followed to prevent infection with this disease. The bacteria accompanying hydatid cysts are gram-negative bacteria, and the types of bacteria differ according to the organism and organ formed. The percentage of bacteria associated with hydatid cysts in the lungs of sheep was 98%, while the percentage was lowest in cows, reaching 88%.

Reference

1. Abdulhameed,M. F., Robertson, I.D., Al-Azizz, S. A., & Habib,I. (2019). Neglected Zoonoses and the Missing Opportunities for One Health Education: The Case of Cystic Echinococcosis among Surgical Operated Patients in Basrah, Southern Iraq.Diseases, 7(1),4.
2. Abdullah, M., Ali, I., Haleem, K. S., Rehman, A. U., Qayyum, S., Niaz, Z., ... & Tauseef, I. (2021). Molecular and biochemical chara cterization of echinococcus spp. in hydatid cyst fluid collected from human and livestock in northern Khyber pakhtunkhwa and gligit baltistan. JAPS: Journal of Animal & Plant Sciences, 31(5).
3. Ahmed, A.B.Ras,R.,Mahmond,A.F.,El-Ghazaly E.,Widmer, Dah shan,H,&Elsohaby,I. (2021). Prevalence and bacterial isolation from hydatid cysts

in dromedary camels slaughteredat sharkia abattoirs,Egypt. J Parasit Dis 45(1):236-243.

4. Aiteken,M.M.,Jones,P.W,Hall,G.A.,Hughes,D.L.,&Collis,K.A.(1978).Effects of experimental salmonella Dublin in infction in cattle given fasciola hepatica thirteen weeks previously. J Comp Pathol 88(1),75-84.
5. Ali,S.A,Dawood,K.A.,& Al-Oumashi,G.B.(2012). Hydatidosis of cattle with Secondary bacterial invaders.Kufa J Vet Med Sci 3(2).
6. Aiteken,M.M.,Jones,P.W,Hall,G.A.,Hughes,D.L.,&Collis,K.A.(1978).Effects of experimental salmonella Dublin in infction in cattle given fasciola hepatica thirteen weeks previously. J Comp Pathol 88(1),75-84.
7. Al-Shemmari, N.A. (2017).Isolation and Diadnosis of Bacteris Associated with some disease infection in some fish in Basra Governorate, Iraq. Masters Disseration, College of Agriculture, University of Basra,Iraq.
8. Boes,J., &H lwigh, A.B.(2000). Animal models of intestinal nematode infectios of human, Parasitologh, 121(S1),S97-S111.
9. Darweesh AO, Shareef AA, Alriyahee F.A.A.(2021). Isolation and Identification of Cellulytic Ruminan\t Pseudomonas Aeruginosa by classical and Molecular Methods in Basrah Province. Ann Rom Soc Cell Biol.4672-80
10. Fallah ,M, Kavand A, Yousefi Mashouf R(2014). Infected hydatid Cysts bacteria in Slaughtered linestock and their effects on protoscoleces degeneration. JAUNDISHAPUR J Microbiol 7:e10135.
11. Jahed Khaniki G.R.,Kia E.B, Raei M.(2013).Liver condemnation and economiclosses due to parasitic in Slaughtered animal in Iraq. J.Parasit Dis 37:240-244.
12. Hadadi, Z.Nematzadeh ,G. A., & Ghahari ,S.(2020). Astudy on the antioxidant and antimicrobial activites in the chloroformic and methanolic extracts of 6 important medicinal plants collected from north of Iran. BMC Chemistry, 14(1),1-11.
13. Hammad, S.J. (2017). Detection of Echinococcus granulosus strains using morphological parameters and molecular methods. Ph.D.Thesis, College of Science, Univeersity of Tikrit. Iraq.pp:48-150.
14. Hijjawi, N.S.,Al-Radaideh, A.M.,Rababah,E.M.,Al-Qaoud, K.M.,& Bani-Hani, K.E.(2018). Cystic echinococcosis in Jordan : a review of causative Species, Previos studies, serological and radiological diadnosis. Acta Tropica 179:10-16.
15. Malekifard, F.,& Keramati ,F. (2018). Susceptibility of protoscles of Hydatid Cyst to Various Concentaration of oak Gall Extract at Different Exposure Time In Vitro.Zahedan. J. Res .Med. Sci 20(5).
16. Markey,B.; Leonard, F.; A rchambanlt , M,; Cullinane, A. and Maguire, D.(2013). Clinical veterinary microbiology E- Book-Elsevier Health sciences.
17. Noaema, A. H., AlKafaji, M. H., & Alhasany, A. R. (2020). Effect of Nano Fertilization on growth and yield of three varietals of wheat bread (Triticum aestivum L.). Int. J. Agricult. Stat. Sci, 16, 1269-1274.

Journal Zone Publishing, Ilford, United Kingdom

18. Khleifat, K.M., Halasah,R.A.,Tarawne, K.A.,Halasah,Z.,Shawabkeh,R.,& Wendyan, M. A.(2010).Biodegradation of line-alkylbenzene Sulfonate by Burkholderia sp:Effect of some growth Conditions.Int J Agr Biol 12:17-25.
19. Quinn, P.J.; Markey,B. K. Carter,M.E.; Donnelly,W.J. and Leonard, F. C. (2004). Veterinary microbiology and microbial Diseases . 1 st Ed.; Blackwell Science Ltd.; PP:327.
20. Tille ,P.M.(2017). Baily And Scotts Diagnostic Microbiology. 14 th ed. Elsevier,Inc. China. 1115pp.
21. Wani, I.,Bhat, Y., Khan, N.,Mir, F.Nanda,S.,& Shah, O.J. (2010). Concomitant rupture of hydatid cyst of liver in hepatic duct and gallbladder :Case report. Gastroenterol Res 3(4):17S.
22. Zeghir-Bouteldja, R., Amri, M.,Aitaissa,S.,Bouaziz,S., Meziouy,D., & Touil-Boukofa,C.(2009). In vitro study of nitric oxide metabolites effects on human hydatid of Echinococcus granulosus J Parasitol Res 2009.
23. Ziino G. Giuffrida, Bilei .S, Panebianco ,A. (2009). Bacterial isolates from25 hydatid cysts in sheep, cattle, and goats, Vet Rec 165:234-6.

Journal Zone Publishing, Ilford, United Kingdom