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# An Epidemiological and Therapeutic Study of the *Echinococcus granulosus* Parasite in Sheep of Anbar Province, Iraq

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**Abstract**

The primary objective of this study was to determine the incidence of (*Echinococcus granulosus*) infections in sheep in Iraq's Anbar Province. A total of 626 sheep of various ages and both sexes were examined in slaughterhouses during 2023; the infection rate was 33.7%, with a notable difference ($p \leq 0.05$) observed between females (44.7%) and males (23.2%). The highest infection rate (42.3%) occurred in sheep aged 3–5 years, and the liver was the most commonly affected organ. The research also evaluated the in vitro impact of the Alhamid (*Rumex vesicarius*) plant's aqueous extract on protoscoleces' viability; concentrations of 5–20 mg/ml significantly reduced protoscoleces' viability, with 20 mg/ml achieving 100% mortality after 24 hours; This research highlights the need for a comprehensive control strategy, including safe alternative treatments like plant extracts, while acknowledging the necessity for further in vivo studies to confirm their safety and efficacy in live animals.

**Keywords:** *Rumex vesicarius* plant, *Echinococcus granulosus*, Sheep, protoscoleces.

## 1. Introduction

The hydatid cyst is a significant zoonotic parasite found in dogs' and other carnivores' intestines, with humans and livestock serving as intermediate hosts. It causes Cystic Echinococcosis (CE), a severe parasitic disease with health and economic consequences (Issa *et al.*, 2018; Salih *et al.*, 2019). Sheep are examples of intermediate hosts that get infected. The larvae develop in various organs notably the lungs and liver (Muqbil *et al.*, 2012; Amni *et al.*, 2021). Humans and animals become infested by ingesting eggs of parasites via tainted food, drink, or direct contact with dogs that are affected (Ahmed *et al.*, 2023).

In Iraq, CE remains endemic, exacerbated by the numerous stray dogs and the practice of feeding infected offal to dogs. Although contagion can occur through environmental contamination (e.g., pastures), direct transmission via dogs is the main route (Xu *et al*., 2025).

The parasite that causes hydatid disease is widespread in Iraq, particularly in the governorates of Erbil, Sulaymaniyah, Baghdad, Karbala, Kirkuk, Anbar, and Basra. Infection rates vary among the population, ranging from 2–4 % in some areas (Abdulla, 2024). Reports from animal slaughterhouses indicate that infection rates range from 2% to more than 15 % among sheep and cattle, depending on the governorate (Jasim *et al.*, 2024). Infection rates among stray dogs in Erbil ranged from 24% to 70% (Wahab, 2022). These data demonstrate that Iraq is a region with a high epidemic burden of this condition, which implies that awareness, monitoring and treatment of the parasite need to continue.

Medicinal plants, rich in biologically active compounds (e.g., alkaloids, tannins, and glycosides) are being studied as alternative therapies for parasite illnesses (Al-Baba *et al.*, 2015; Mukhlif *et al.*, 2023). The Alhamid (*Rumex vesicarius*) plant is renowned for its traditional therapeutic virtues and bioactive components, including anthraquinones, phenols and flavonoids (ACSAD, 2008).

However, its specific efficacy against *E. granulosus* protoscoleces has not been previously studied. This research, therefore, aimed to assess Anbar Province's sheep *E. granulosus* infection epidemiology and to explore the in vitro impact of *R. vesicarius* extracts on protoscoleces' viability.

## 2. Materials and Methods

Six hundred twenty-six sheep slaughtered in several abattoirs in Anbar province, including Ramadi, Fallujah, Heet, and Al-Qaim, of either gender and various ages, spanning 6 months to 8 years, were examined in 2023. The lungs, spleen, kidneys, heart, brain, and other parts of the body of the sheep were examined, removing the tissues surrounding the hydatid cysts with scalpels and scissors, and writing down the complete information about the animal's sex, age, type of affected organ, and months of the year.

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### *2.1. Hydatid Cysts and Protoscolece Collection*

Hydatid cysts and protoscoleces (Figure 1) were obtained from the sheep livers and lungs of the infected at slaughterhouses in Anbar Governorate. After disinfecting the cysts with 70% ethyl alcohol, they were transported to the Laboratory of Parasites to extract protoscoleces, according to Smyth's procedures (1985). A medical syringe of 10 ml was used to withdraw the fluid with protoscoleces and put it in a sterile 250 ml bottle; the germinal layer was removed after the cysts were cut open using medical scissors and tweezers (Figure 2). Then, it was put in a sterile glass dish containing a physiological saline solution. Then, it was washed several times with a washing bottle containing sterile saline phosphate buffer (PH=7.2) to extract as many protoscoleces as possible. This suspension, which we obtained after washing, was collected and added to the Hydatid fluid withdrawn previously. The protoscoleces were placed in sterile test tubes to be deposited with a Centrifuge apparatus 3 times at 2500 rpm for 10 minutes each time. Subsequently, phosphate buffer with sterile sodium was introduced to the sediment after pouring the filtrate. The number of protoscoleces was determined, and their viability was evaluated.

### *2.2. Protoscolece viability estimation and number quantification*

An aqueous eosin dye drop (0.1%) was applied to a similar volume of protoscoleces suspension. The solution was shaken, and a droplet was taken and inspected with a microscope. The proportion of living protoscoleces that were stained green was calculated, while the dead ones were colored red according to Smyth and Barrett's method (1980). A 10 µl fine pipette was used to compute the number of protoscoleces using the fixed volume transfer method, and a microscope was utilized to calculate the overall quantity of heads for each volume. The average number of five replications was relied on and was calculated for every milliliter as indicated below: An average protoscoleces amount per fixed volume used 10 mL = 20 protoscoleces, so the number of protoscoleces per milliliter = 100 × 20 = 2000 protoscoleces, according to Wangoo *et al.*'s method (1989).

**Figure 1.** Removed Hydatid cysts

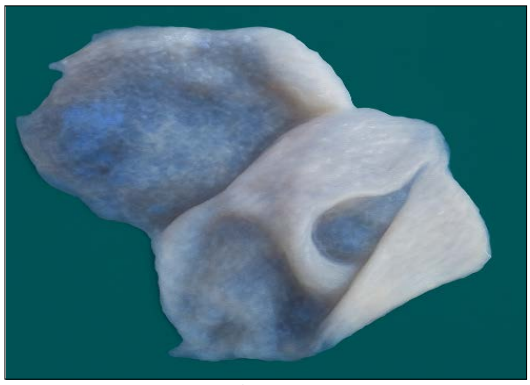

**Figure 2.** Germinal layer of hydatid cyst

### *2.3. Preparing the plant's aqueous extract*

The plant *R. vicarious* (Figure 3) was collected from the Al-Sufiyya district in Ramadi, and its identification was confirmed in the Herbarium's Anbar University. The plant components were washed and dried; an extract was made using the Veisi *et al.* (2018) method, which involved Crush 40 g of the plant and blending it with 160 ml of purified water in a 4:1 proportion with a blender device. The crushing was done in an ice bath to prevent the high temperature from damaging the plant's active ingredients. After 60 minutes of mixing with an electric magnetic stirrer, the mixture was allowed to soak for 24 hours at 40 °C before filtering through filter papers; the resulting powder was stored in glass vials at -10°C after the plant extract was freeze-dried under controlled pressure. To produce the primary extract, 2 grams of the crude Extract are combined with 20 ml of sterile water to obtain a standard 100 mg/ml solution. Solutions of 5, 10,15, and 20 mg/ml were then prepared.

### *2.4. Treatment of protoscoleces with aquatic extract concentrations*

Once the number of protoscoleces and their viability were estimated, test tubes containing 1 ml of the protoscoleces suspension each were treated with plant aqueous extract at 5, 10, 15, and 20 mg/ml concentrations. The average vitality of the protoscoleces was then determined following treatment for 24, 48, 72, and 96-hour periods.

**Figure 3.** Alhamid plant *Rumex vesicarius*

*2.5. Chemical identification of active plant compounds (Brusotti et al., 2014)*

The identification of alkaloids: Fifty milliliters of purified water with HCl (4%) were used to boil 10 grams of plant extract. After cooling, it was filtered. Then, 0.5 ml of leachate was examined with half a milliliter of Reagent of Meyer in a watch glass. The appearance of a white deposition verified that alkaloids were present.

The identification of glycosides: After adding a few Fehling's reagents to 10 milliliters of distilled water and one gram of a dry plant extract, the mixture became crimson, demonstrating the existence of glycosides.

The identification of Saponins: Five milliliters of the extract were mixed with three milliliters of a one per cent mercuric chloride solution; the formation of white sediment suggested Saponins.

Tannins detection: Five millilitres of the plant extract were mixed with several drops of lead acid (1%); tannins are available when a white, gelatinous deposit forms.

The identification of Flavonoids: When ten milliliters of (50%) ethanol solution were combined with 10 milliliters of 50% potassium hydroxide and blended equally, flavonoids were indicated by the appearance of a yellow tint.

Finding the phenols: When 1 milliliter of the extract was mixed with one milliliter of (1%) Ferric Chloride Solution, phenols were present because a green hue emerged.

*2.6. Analysis of Statistics*

The SAS statistical application, Chi-square, Analysis of Variance, and least significant difference (LSD) were used to analyze statistics (SAS, 2012).

## 3. Results and Discussion

The hydatid cyst infection rate in 626 sheep was 33.7%. The female infection rate (44.7%) was significantly higher than that of males (23.2%) (Figure 4). When compared to previous studies, this rate is lower than the 50% reported by Hashim (2021) in certain Iraqi regions. Conversely, it is notably higher than the rates documented in earlier studies from other provinces. For instance, Jawad *et al.* (2018) recorded an infection rate of 1.9% among sheep in Karbala, while Abdulhameed *et al.* (2018) reported a prevalence of 7.3% in Basra during a study involving 631 sheep. In a more recent study, Radhwan and Al-Nasiri (2021) found a 2.25% infection rate in sheep in Kirkuk.

Such disparities in infection rates across various regions of Iraq could be attributed to multiple contributing factors; these include variations in animal husbandry practices, the density and management of stray dog populations (which serve as the definitive hosts), and differences in slaughterhouse hygiene and waste disposal systems, as well as public knowledge and awareness regarding the transmission cycle of *E. granulosus*. Furthermore, many important factors contribute to the differences in results compared to previous studies. These factors include environmental conditions, the different diagnostic methods used by each study, the age and sex of the sheep being studied by the researcher, the number of animals, their proximity to the breeding pens and their contact with dogs, and the type and strain of the *E. granulosus* parasite (Aregawi *et al.*, 2024; Dixit *et al.*, 2024).

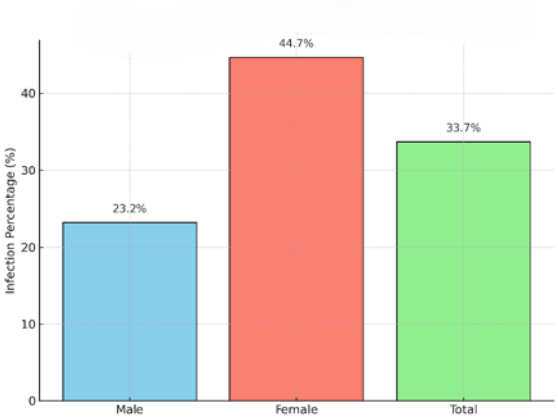


**Figure 4.** Percentage of *E. ganulosus* infections by gender in sheep.

The infection rates between females (44.7%) and males (23.2%), which showed significant differences, were consistent with the results of a Pakistani research carried out by Muqaddas *et al.* (2019). The higher occurrence of *E. granulosus* infection among female sheep compared to males is attributed to differences in proper management practices, particularly in livestock systems. In Iraq, female sheep are usually kept longer, mainly for breeding and milk production. In contrast, male sheep are often sold or slaughtered at a youthful age to meet local meat demand. This extended lifespan in females results in a greater cumulative exposure to *E. granulosus* eggs (Al-Dhurani *et al.*, 2024). According to the FAO (1996), Awassi ewes, the dominant breed in Iraq, can produce over 100 kg of milk during a 142-day lactation period, making them economically valuable and justifying their longer retention in flocks. These national-level practices strongly support the explanation for the higher infection rate in females.

Concerning the relationship between infection and animal age, Figure 5 shows that the highest infection rate (42.3%) was recorded in sheep between 3 and 5 years of age, while the lowest percentage (16.8%) was noted in sheep between six months and two years of age. These findings align with those of Alvi *et al.* (2020) in Pakistan. The high rates of infection at older ages are explained by the fact that large age groups are more vulnerable to infection from animals that pass through during many seasons and come into direct contact with dogs, as well as from their increased exposure to infection as they age and continue to eat weeds contaminated with parasite eggs (Shahatha, 2019). Moreover, younger animals have stronger immunity against parasitic infections because they acquire antibodies from colostrum while feeding from their mothers (Baghezza *et al.*, 2024).

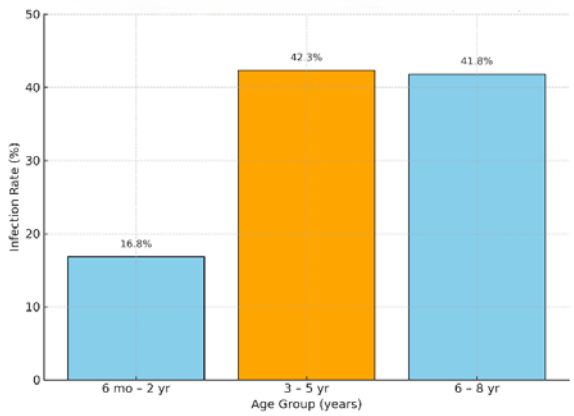


**Figure 5.** Percentage of *E. granulosus* infections by Age group in sheep.

There is a notable variance in the connection between infection and the months ($p \leq 0.05$). Infection rates were at their lowest in July at 7.8% and highest in January and February at 64% and 61.6%, respectively (Figure 6). This is consistent with the results documented in the study of Al-Alo (2019) conducted in Najaf Governorate. In addition to the fact that parasites become more active in colder climates with higher humidity, the transmission of parasite eggs through direct contact between animals and dogs in barns and breeding fields during the winter months is the cause of the increased infection rates in winter as opposed to summer (Sultan *et al.*, 2023).

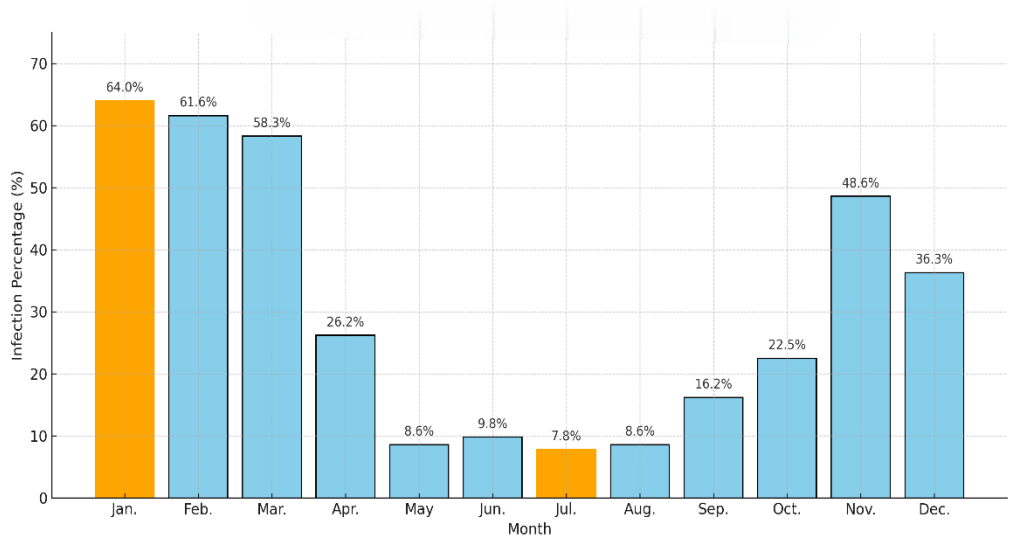


**Figure 6.** Percentage of *E. ganulosus* infections by months in sheep.

The Hydatid cysts were found in 72 affected livers (34.1%) and the lungs (31.7%) (Figure 7). The kidney had the lowest percentage at 13.2%, which aligns with research by Li *et al.* (2020) and Haleem's study (2018). This indicates that the liver is the most impacted organ; as shown in Figure 8. This is because the embryos landed in the liver and lungs after crossing through the blood.

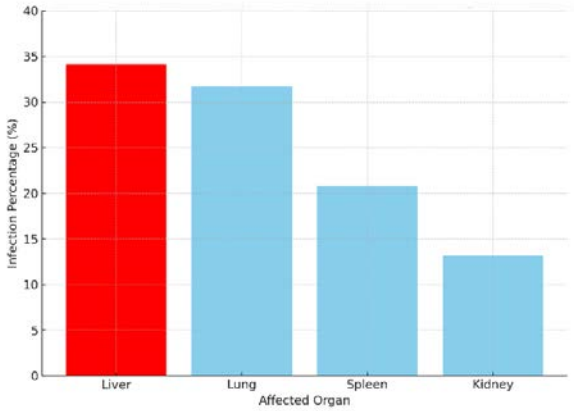


**Figure 7.** The proportions of Hydatid cyst infections affecting each body organ.


**Figure 8.** Sheep liver, lungs, and kidneys are affected by hydatid cysts.

(Table 1) shows slight differences between the parameters when using the fixed volume in calculating the protoscoleces of the parasite, where the average protoscoleces vitality at zero hours of treatment was 20. At the same time, it reached 19 at 96 hours.

The results of calculating the percentage of protoscoleces' vitality of five replicates showed that the total rate of protoscoleces per drop ranges between 97 and 108 heads, while the rate of live protoscoleces was 84-98. Therefore, the percentage of protoscoleces' vitality reached 89% (Table 2).

**Table 1.** Calculation of live protoscoleces with a constant volume method of 10 µl.

| Duration of examination (hour) | The number of protoscoleces calculated for five replications | | | | | The average number of protoscoleces ±Standard deviation |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| 0 | 22 | 20 | 19 | 21 | 18 | 20±0.60 |
| 24 | 21 | 20 | 21 | 22 | 21 | 21±0.62 |
| 48 | 20 | 21 | 19 | 20 | 20 | 20±1.72 |
| 72 | 21 | 18 | 19 | 19 | 18 | 19±0.80 |
| 96 | 20 | 22 | 18 | 17 | 18 | 19±1.00 |

**Table 2.** Frequency for calculating the percentages of protoscoleces in a single drop of 50 µl.

| Repetition | 1 | 2 | 3 | 4 | 5 | Total |
|---|---|---|---|---|---|---|
| Average of the total number of protoscoleces | 108 | 104 | 97 | 102 | 108 | 519 |
| The average number of living protoscoleces | 98 | 93 | 84 | 92 | 95 | 462 |
| % of protoscoleces vitality | 90.7 | 89.4 | 86.5 | 90.1 | 87.9 | 89.0 |

Table 3 demonstrates the effect of the concentrations (5, 10, 15, and 20 mg/ml) of the *R. vesicarius* aqueous extract on the survival of *E. granulosus* in vitro. After 96 hours of treatment, protoscoleces' viability decreased significantly: from 93% to 21% at 5 mg/ml and from 91% to 0% at 10 mg/ml. The results also showed that a 15 mg/ml concentration of the plant's watery extract decreased protoscoleces' viability from 90% to 0% after 96 hours of treatment. After only 72 hours, a higher concentration reduced protoscoleces' viability from 85% to 0%, indicating a more substantial effect of higher concentrations. These findings align with previous studies (Vakili *et al.*, 2019; Al-Taei *et al.*, 2019), and this inhibitory effect is attributed to Various active components in the extract, such as sterols, alkaloids, terpenes, tannins, flavonoids, and glycosides that cues obstructing the respiration process through its effect on the mitochondria, these compounds also cause the breakdown of the cell membrane and the fats and proteins, resulting in the parasite's death (Shahatha, 2024). This is because they interfere with the chain of metabolic reactions of the necessary proteins that help the parasite's vitality to continue (Tandon and Sirohi, 2010; Shahatha *et al.*, 2022). Eosin dye penetration into primary cells is used to assess their viability, as this process is closely linked to membrane permeability. Any physiological disruption in the membrane can increase its permeability, allowing dye entry; however, living primary cells will maintain their original color. A physiological saline solution supported the primary cells by providing the essential nutrients (Nasr *et al.*, 2014; Al-Sabawi *et al.*, 2023).

Among the many phytochemicals that are present in the extract, flavonoids and tannins are regarded as the key contributors to its powerful scolicidal action. These chemicals have been shown to disrupt mitochondrial function, increase membrane permeability and denature structural proteins, together resulting in protoscolex mortality (Almutairi *et al.*, 2022). This shows a strong mechanistic relationship between the extract's phytochemical composition and its observed efficacy.

**Table 3.** Average viability of protoscoleces following exposure to concentrations of aqueous extract over several periods.

| concentration Mg/ml | Average viability of protoscoleces during periods (hours) | | | |
|---|---|---|---|---|
| | 24 | 48 | 72 | 96 |
| Control | 94 | 88 | 70 | 61 |
| 5 | 93 | 82 | 62 | 21 |
| 10 | 91 | 73 | 42 | 0 |
| 15 | 90 | 62 | 12 | 0 |
| 20 | 85 | 38 | 0 | 0 |

Our research demonstrated that the plant extract showed promising activity against protoscoleces in vitro. However, its effectiveness and safety in live animals have yet to be assessed; Key factors like optimal dosage, pharmacokinetics, and potential side effects are still uncertain. Thus, more in vivo studies are needed to determine suitable dosing strategies, evaluate safety, and verify therapeutic effectiveness in sheep.

## 4. Conclusion

This study highlights a high occurrence of hydatid cyst infection among sheep in Anbar Province, with significant differences related to sex, age, and seasons. The aqueous extract of *R. vesicarius* demonstrated a strong in vitro inhibitory effect on protoscoleces' viability. However, further research, including in vivo testing in sheep, is essential to determine practical applications and ensure safe use in parasite control strategies.

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## Conflict of Interests

The authors declare no conflict of interest.

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