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Assessing the Prevalence of Hydatidosis and Fascioliasis in Slaughtered Animals and the Effectiveness of Plant Extracts Against *Echinococcus granulosus* Protoscolices in Soran City, Kurdistan Area, Iraq

Article Info.

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Abstract

Both hydatidosis and fascioliasis are prevalent diseases that affect herbivores in several countries, including Iraq. The present research evaluated the prevalence of hydatidosis and fascioliasis in livestock slaughtered at the Soran municipal abattoir in Erbil, Iraq, from December 2023 to March 2024. A total of 3,044 animals were subjected to post-mortem examination, comprising 445 sheep, 961 goats, and 1,638 cattle. The overall prevalence of hydatidosis was 1.34%, whereas fascioliasis was observed in 1.37% of the total population. Sheep demonstrated the highest infection rates for both parasitic diseases (hydatidosis: 7.6%; fascioliasis: 3.37%), followed by cattle, while goats exhibited minimal susceptibility (0% and 0.31%, respectively). Anatomical distribution indicated that 86% of hydatid cysts in sheep were situated in the lungs, whereas in cattle, cysts were found in both the liver and lungs. During the testing phase, protoscolices of *E. granulosus* were subjected in vivo to aqueous extracts of Melia azedarach and Euphorbia spp. at concentrations of 50 mg/ml and 100 mg/ml. The extract of Melia azedarach exhibited scolicidal activity that was dependent on both time and concentration, achieving a mortality rate of up to 95.8% at the highest concentration. Conversely, Euphorbia spp. The extract exhibited total scolicidal efficacy (100% mortality) across all concentrations and exposure durations evaluated. The findings underscore species-specific vulnerability to parasitic infections and designate Euphorbia spp. extract as a promising natural candidate for the formulation of effective scolicidal agents in hydatid disease management.

Keyword:*Echinococcus granulosus*, *Fasciola* spp., prevalence, Melia azedarach, Euphorbia spp.

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Introduction

Globally, intestinal helminth infections affect around one billion people. Protozoan and helminthic infections present considerable public health challenges, including diarrhea, hemorrhage, abdominal pain, malabsorption, and stunted growth, especially in children. Fasciolosis and hydatidosis are two prominent parasitic zoonoses that impact both animal health and human public health (1-3). Hydatidosis, caused by larval stages (hydatid cysts) of *Echinococcus* species (family Taeniidae), affects multiple organs such as the liver and lungs. The adult worms reside in carnivores, while the larvae infect a wide range of intermediate hosts, including humans (4). *Echinococcus granulosus* is distributed worldwide, with increased prevalence in South America, Australia, Africa, and certain regions of Eurasia. *E. multilocularis* is endemic in certain regions of the Northern Hemisphere, specifically North America and central Europe (5). The WHO estimates that echinococcosis impacts more than 1 million individuals each year, particularly in Central Asia, the Mediterranean, eastern Africa, and western China. Humans contract infections by ingesting parasite eggs from contaminated food, water, or through contact with infected animals (6, 7). The liver (75%) and lungs (15%) are the primary locations for hydatid cyst formation; however, other organs such as the spleen, kidneys, muscles, and brain may also be involved (8). Fascioliasis, induced by *Fasciola hepatica* and *F. gigantica*, is a prevalent parasitic zoonosis (9). Liver flukes reside in the bile ducts of ruminants, including sheep, goats, and cattle, and may also infect humans (10). Humans get the disease by consuming tainted water or by ingesting aquatic plants that harbor encysted organisms (11). The parasite's life cycle comprises a definitive mammalian host, an intermediate snail host, and aquatic vegetation serving as a vector (9). Hepatic damage resulting from migrating juvenile flukes can result in significant health complications and substantial economic detriment (12, 13).

Fascioliasis is common in tropical areas of Asia and Africa (associated with *F. gigantica*) and in temperate zones like Europe and the Americas (linked to F. hepatica) (14). Both species coexist in Iraq and other regions, though variation among species remains challenging. Fascioliasis results in approximately $3.2 billion in annual worldwide economic losses attributable to mortality, liver condemnation, diminished meat and milk production, and pharmaceutical expenses (14-17). Diagnosis is traditionally dependent on parasitological techniques. There is increasing demand for utilizing medicinal plants for treating parasitic infections (18, 19). Medicinal plants have historically been utilized in traditional therapies and are currently being investigated as potential anthelmintic alternatives, particularly due to increasing drug resistance (20). Various plant extracts have exhibited scolicidal properties toward *E. granulosus*, yet many species remain inadequately investigated. Medicinal substances provide various bioactive ingredients that may diminish the likelihood of resistance development and possess reduced environmental impacts compared to conventional pharmaceuticals (21-23).

Melia azedarach (Meliaceae), known as Mahaneem, is found in the forests of the North-West Himalayas and in tropical and subtropical regions, including India and Pakistan (24). Indigenous and traditional therapeutic systems, such as Ayurveda and Unani, utilize various plant parts—leaves, roots, and stems—to treat a diverse range of diseases (25). In vivo studies have been conducted to examine the anthelmintic efficacy of *M. azedarach*, utilizing aqueous, methanolic, and ethanolic extracts of the fruits in chickens, seeds in sheep, and fruits, seeds, and leaves in vitro against *Haemonchus contortus* (26). In similar lines, the family Euphorbiaceae, which includes the species of *Euphorbia*, is one of the largest angiosperm genera, with approximately 2,000 different species. A wide variety of bioactivities, such as antibacterial, antioxidant, anti-inflammatory, and sedative properties, are possessed by *Euphorbia* species, which are well-known for their varied morphologies and a distinctive inflorescence known as cyathium (27, 28).

The present research aimed to determine the endemic prevalence of echinococcosis and fascioliasis in Soran city by examining slaughtered livestock (sheep, cattle, and goats) and to evaluate the in vitro scolicidal efficacy of aqueous extracts from *Melia azedarach* and Euphorbia spp. against the protoscoleces of *Echinococcus granulosus*, in light of the growing interest in safe, plant-derived alternatives to traditional anthelmintics.

Material and Method

A study was performed in the city of Soran from December 2023 to March 2024. The slaughterhouse in Soran city was visited every week to carry out this investigation. The slaughtered animals underwent a comprehensive examination to verify the diseases and classify them. During this period, a total of 3,044 animals were examined, including 445 sheep, 961 goats, and 1,638 cattle**.**

Protoscolices Separation

Before being dissected, 70% ethanol was used to sterilize the hydatid cysts' exterior surfaces. Before being used in the plant extract experiment, protoscolices were extracted, purified, and stored in regular saline.Protoscolice activity was measured with 0.1% aqueous eosin staining(29).

Plant Extract Preparation

The leaves of numerous *Euphorbia* species and the fruits of *Melia azedarach* were collected in the autumn of 2024. The fruits and leaves that were collected were given a quick wash with distilled water. The fruits and leaves were then allowed to dry at room temperature before being crushed in a mortar to a powder. Following that, 16 grams of each plant were dissolved in 320 milliliters of distilled water in a different procedure to achieve the appropriate dilutions. Then, for twenty-four hours, these solutions vibrated using a magnetic stirrer. A vacuum pump was then used to filter them, and they were centrifuged for fifteen minutes at 1500 revolutions per minute. The final

method of concentration involved evaporating the extracts overnight at 30 degrees Celsius in an oven (30).

The Effect of Extract on the Viability of the Protoscolices *in Vitro*

Four plates were prepared, each containing five milliliters of hydatid fluid. Subsequently, 0.1 and 0.05 milligrams per milliliter of each extract were incorporated into the plates. After fifteen minutes, thirty minutes, forty-five minutes, and one hour, the viability of protoscolices was evaluated. In order to detect whether or not protoscolices were viable, aqueous 0.1 percent eosin was utilized. Protoscolices that were alive had a green tint, whilst those that were dead appeared red (29).

Statistical Analysis

Data were analyzed using GraphPad Prism version 10.4.2. Descriptive statistics were used to summarize infection prevalence and experimental results. Differences in parasite mortality across treatments and concentrations were assessed. The Chi-square test was applied to evaluate differences in prevalence rates among categorical groups. A p-value of less than 0.05 was considered statistically significant.

Results

Evaluation part

A total of 3,044 slaughter animals were examined. The share of animal groups and the incidence percentage of the analysed parasitic diseases in the research population were presented graphically (Figure 1).The percentages of animals infected with hydatidosis and fascioliasis were similar, accounting for approximately 1.3% of the study population (Table 1, Figure 2).

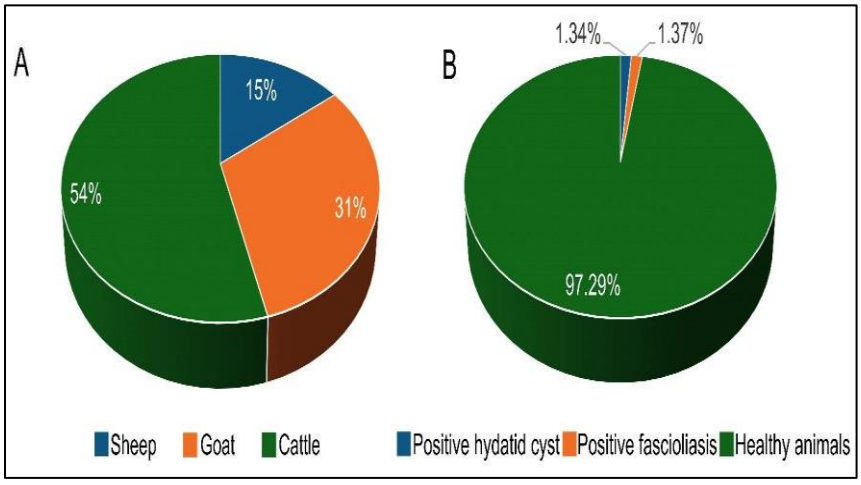

Figure. 1: Distribution of slaughtered animal species and prevalence of hydatidosis and fascioliasis among examined animals.

Pie charts illustrating (A) the proportional distribution of slaughtered animal species (sheep, goats, and cattle) and (B) the prevalence of hydatidosis and fascioliasis among the examined animals, with the remaining proportion representing healthy animals. Percentages indicate the relative contribution of each category.

Tab.1.The prevalence of *Echinococcus granulosus* Batsch hydatid cyst and fascioliasis *Fasciola* sp. in different animals in Soran city abattoir.

Animals	Total number of slaughtered animals	Positive hydatid cyst	Percentage [%]	Positive fascioliasis	Percentage [%]
Sheep	445	34	7.6	15	3.37
Goat	961	0	0	3	0.31
Cattle	1638	7	0.42	24	1.46
Total number	3044	41	1.34	42	1.37

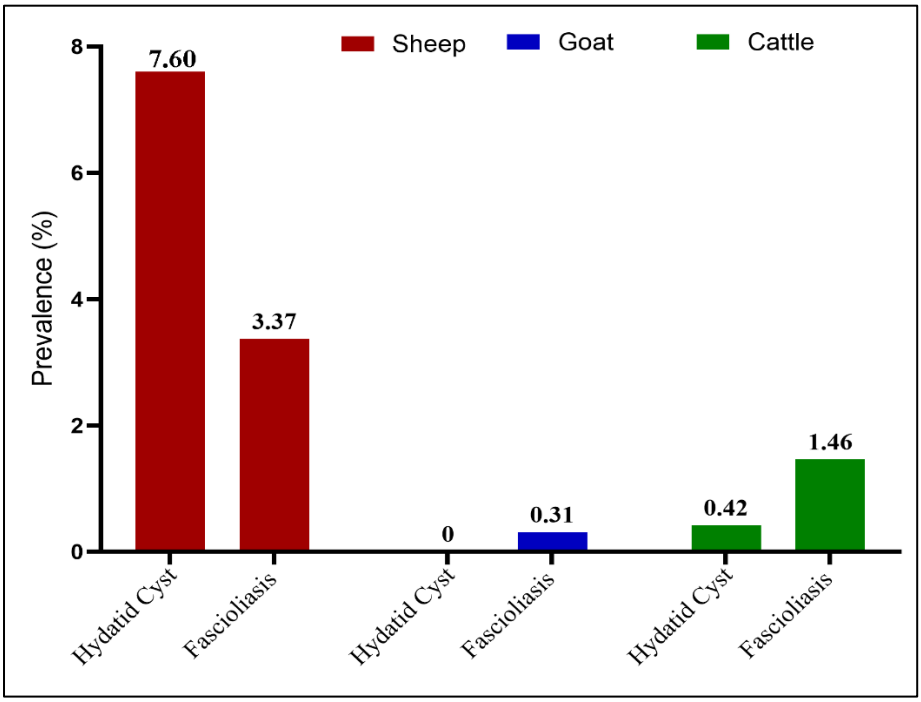

Figure.2. Prevalence of Hydatid Cyst and Fascioliasis in Livestock Slaughtered at Soran Slaughterhouse.

This graph displays the prevalence (%) of *Echinococcus granulosus* (hydatid cyst) and *Fasciola* spp. (fascioliasis) infections among sheep, goats, and cattle slaughtered at the Soran city abattoir. The data indicate that sheep have the highest infection rates for both parasites, with 7.6% for hydatid cyst and 3.37% for fascioliasis. Goats show the lowest prevalence, while cattle have intermediate levels. These findings suggest species-specific differences in susceptibility to these parasitic diseases in the study area.

In both parasitic diseases, the percentage of infected sheep was the highest. However, sheep did not constitute the largest group in the research population. Goats were the most resistant to infections with the examined parasites – no hydatoid cysts were found in them, and only 0.31% were infected with fascioliasis (Tab. 1). Cows were an intermediate group in terms of infection resistance with the analysed parasites. Regarding the infected organs with hydatidosis the results showed that lungs had the most infection in sheep (86%), whereas the infection was distributed between liver and lungs in cattle (Figure 2, Figure 3).

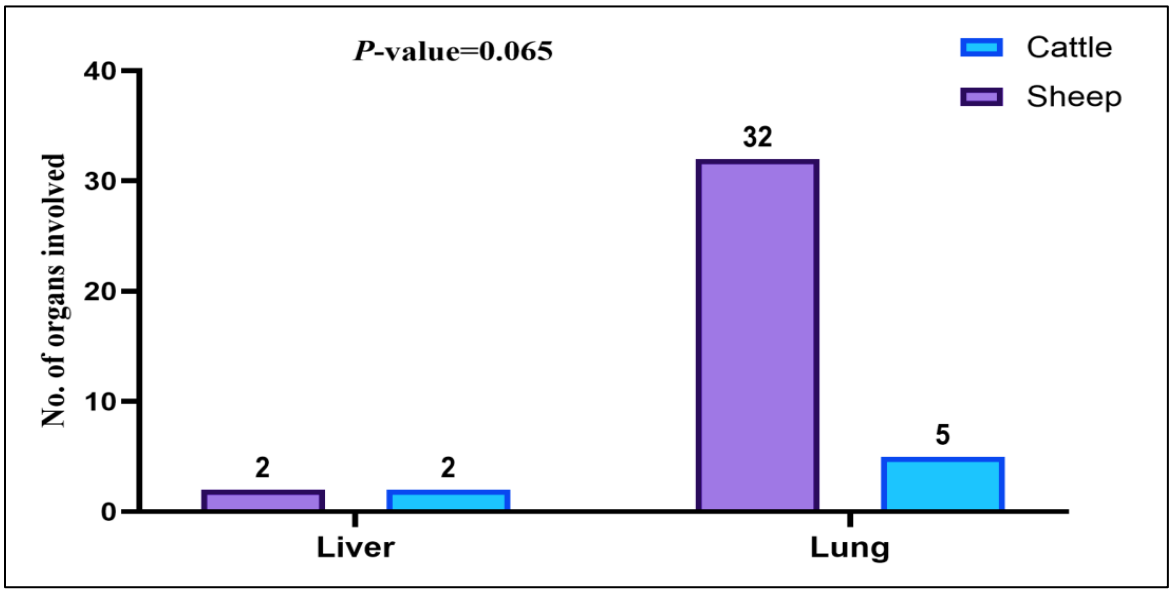

Figure 3. Distribution of hydatid cysts of *Echinococcus granulosus* in different organs of infected animals: cysts were detected in the lungs in 37 cases and in the liver in 4 cases.

The bar graph displays the number of organs affected by infection in various animal species. Despite sheep exhibiting a greater number of affected organs relative to other animals, this disparity was not statistically significant (p = 0.065). This indicates that the differences in organ involvement among species may be attributable to randomness or necessitate further examination with an expanded sample size.

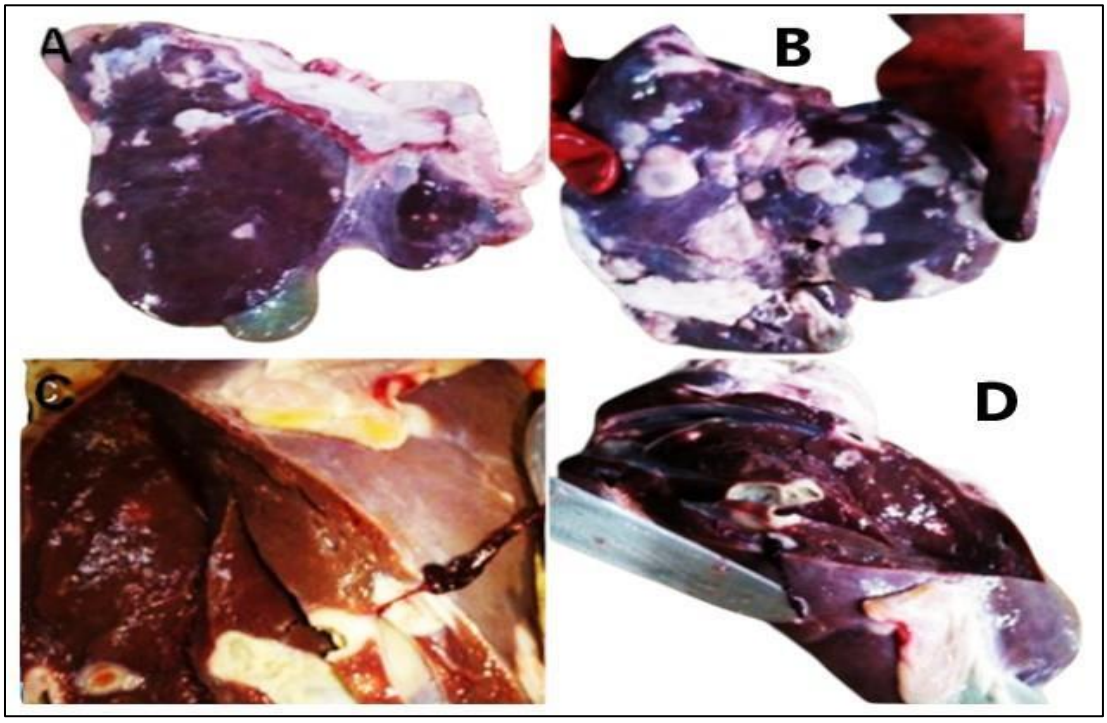

Figure.4. Liver infected with *Echinococcus granulosus* Batsch hydatid cyst A, B; liver infected with *Fasciola* sp. C, D Gross pathological lesions of hepatic hydatidosis in slaughtered animals. Panels (A–D) show livers with multiple hydatid cysts of varying sizes within the hepatic parenchyma.

Experimental part

Analyses of the effect of aqueous extracts of *Melia azedarach* and *Euphorbia* sp. on protoscolices of *Echinococcus granulosus* showed that both *Melia* and *Euphorbia* exhibit antiparasitic activity (Tab. 2).

In the case of *Melia*, higher concentrations of aqueous extracts (100 mg/ml) caused higher parasite mortality than when using 50 mg/ml extracts. To the best of our knowledge, the *Euphorbia* extracts showed notable scolicidal activity, causing 100% death of protoscolices across all tested concentrations—an outcome not before reported in the scientific literature.

Discussion

Humans and domestic animals are both susceptible to the cyclo-zoonetic illness hydatid cyst. In Iraq and other parts of the world, the disease is endemic and one of the most significant health and economic issues (31, 32). *Fasciolasis*, on the other hand, is regarded as one of the most significant parasitic diseases affecting domestic ruminants. It is available all around the world

(33).Additionally, 51 counties have seen an increase in the frequency of human fascioliasis, with current studies estimating that up to 2-4 million people are infected (34)**.**

Table 2. The effect of *Melia azedarach* L. and *Euphorbia* sp. aqueous extract against protoscolices of *Echinococcus granulosus* Batsch *in vivo* (average ±SD, n=3)

Species Concentrate/ Time [min]	***Melia azedarach* aqueous extract 50 [mg/ml]**		***Euphorbia* sp. aqueous extract 50 [mg/ml]**	
	Dead	**Alive**	**Dead**	**Alive**
15	11	2	all	0
30	5	7	all	0
45	9	6	all	0
60(LD)	6	2	all	0
Concentrate/ Time [min]	100 [mg/ml]		100 [mg/ml]	
15	12	1	all	0
30	11	1	all	0
45	23	1	all	0
60(LD)	23	1	all	0

The current study's findings indicated that the total prevalence of hydatid cyst infection was 1.34 percent. In comparison to other animals, such as cattle, which had a 0.42% infection rate, sheep had the highest incidence of hydatid cyst infection (7.6%), whereas goats had no infection, as shown in Figure 1 and Table 1. The results of the study contradicted previous research that found a 2% frequency of sheep illness in Mosul and Iran (32). Additionally, the percentage of cattle infected with hydatidosis was recorded in the current study at 0.42%, which was comparable to the findings of other Mosul researchers who found the same number at 0.55% (32). In contrast, the percentage of cattle infected with the disease in Iran was 8.6% (35).

The goats in this investigation showed no evidence of hydatid cysts; however, research conducted in Karbala indicated a 2.4% infection rate in goats. This may suggest that sheep were more vulnerable to hydatidosis than goats (36). Additionally, 3% of cattle in Zambia had a hydatidosis infection overall (37). Additionally, the findings indicated that the liver and lungs were the most severely impacted organs, which is explained by the fact that *Echinococcus* oncoshpheres migrates via these capillary beds first via the portal hepatic pathway. Our findings may be explained by the lung's larger capillary bed than any other organ (38). Regarding the fascioliasis infection, the present study reported (1.37%) of slaughtered animals were infected, and the highest infection rates were in sheep, followed by cattle and goats as (3.37, 1.46 and 0.31%) respectively. In India Swarnnakar and Sanger (2014) reported that (12.68%) of buffaloes were infected with fascioliasis, followed by cow (9.7%) and goat (7.5%)(39).

It is important to note that fascioliasis is a serious issue that affects many different types of productivity, including meat, milk, and others (40). This study also demonstrated that goats have a lower vulnerability to liver flukes. Cattle, sheep, and goats are also susceptible to the illness in Iran (34). Because of the negative effects and problems of chemotherapy and surgery, there has been a recent tendency toward the use of plants in therapy (back to nature). An important medicinal plant is *Euphorbia* spp., and one of the most beneficial traditional medicinal herbs is *M. azedarach*. The current study's findings demonstrated that the aqueous extract of *Euphorbia* spp. was highly effective against *E. granulosus* protoscolices. Additionally, other investigations have verified the impact of various plants, such as *Nigella sativa*, *Prosopis farcta*, and *Pistacia khinjuh*, against the *E. granulosus* protoscolices (41). Additionally, the aqueous extract of *M. azedarach* leaves showed an anti-parasitic action against protoscolices of *E. granulosus*. Furthermore, the impact is found to grow in proportion to the concentration of the extract. *M. azedarach* leaves are utilized in the treatment of leprosy.

Conclusion

This study confirms the occurrence of echinococcosis and fascioliasis in slaughtered animals in Soran city, with sheep as the most impacted species. Hydatid cysts predominantly occur in the lungs, particularly in sheep. In vitro assays demonstrated significant scolicidal activity of Euphorbia spp. extract and dose-dependent effects of Melia azedarach. The results indicate that both plants possess potential as alternative antiparasitic agents, warranting further investigation for therapeutic applications.

Acknowledgement

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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تقييم انتشار داء الكيسات المائية وداء المتورقات في الحيوانات المذبوحة وفعالية المستخلصات النباتية ضد رؤيسات المشوكة الحبيبية في مدينة سوران، إقليم كردستان، العراق

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الخلاصة

يعد كل من داء الكيسات المائية وداء المتورقات من الأمراض الشائعة وواسعة الانتشار التي تصيب الحيوانات العشبية في العديد من البلدان، بما في ذلك العراق. أُجريت هذه الدراسة خلال الفترة من ديسمبر إلى مارس 2024 بهدف تحديد مدى انتشار هذين المرضين في الحيوانات المذبوحة في مدينة سوران أربيل. من بين 3,444حيواناً تم فحصها، تبين إصابة (41) حيواناً بداء الكيسات المائية و (42 حيواناً) بداء المتورقات. وأظهرت النتائج أن نسبة الإصابة بداء الكيسات المائية كانت أعلى بشكل ملحوظ في الأغنام مقارنة بالأنواع الأخرى، بينما كانت نسبة الإصابة بداء المتورقات أعلى بكثير في الماشية (الأبقار). علاوة على ذلك، وُجدت أكياس مائية في الكبد (4 حالات) والرئتين (37 حالة).وفيما يتعلق بالمستخلصات المائية، أظهرت النتائج أن مستخلص نبات الفربيون كان أكثر فعالية من مستخلص نبات الميليا في مكافحة رؤيسات طفيل المشوكة الحبيبية أكدت هذه الدراسة وجود داء الكيسات المائية وداء المتورقات في الحيوانات المذبوحة في مدينة سوران، كما أثبتت النتائج أن مستخلص الفربيون له تأثير فعال ضد الرؤيسات، حتى عند استخدامه بجرعات مميتة.

الكلمات المفتاحية :المشوكة الحبيبية، أنواع المتورقة، الانتشار، الميليا، أنواع الفربيون.