PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

MELHORIA DO DIAGNÓSTICO PRECOCE E DO TRATAMENTO CONSERVADOR DA DOENÇA EQUINOCÓCICA

IMPROVEMENT OF EARLY DIAGNOSTICS AND CONSERVATIVE TREATMENT OF ECHINOCOCCAL DISEASE

СОВЕРШЕНСТВОВАНИЕ РАННЕЙ ДИАГНОСТИКИ И КОНСЕРВАТИВНОГО ЛЕЧЕНИЯ ЭХИНОКОККОВОЙ БОЛЕЗНИ

Shora Seidinov*
International Kazakh-Turkish University named after Khoja Ahmed Yasawi, Faculty of Medicine, Department of Surgical Diseases, Turkestan, Kazakhstan

Erbol Tulezhanov
International Kazakh-Turkish University named after Khoja Ahmed Yasawi, Faculty of Medicine, Department of Surgical Diseases, Turkestan, Kazakhstan

Murat Zhunisov
International Kazakh-Turkish University named after Khoja Ahmed Yasawi, Faculty of Medicine, Department of Surgical Diseases, Turkestan, Kazakhstan

Ibadulla Turmetov
International Kazakh-Turkish University named after Khoja Ahmed Yasawi, Faculty of Medicine, Department of Surgical Diseases, Turkestan, Kazakhstan

Maira Seisenbayeva
International Kazakh-Turkish University named after Khoja Ahmed Yasawi, Faculty of Medicine, Department of Surgical Diseases, Turkestan, Kazakhstan

** Corresponding author*
e-mail: shora.seydinov@ayu.edu.kz

Received 10 January 2024; received in revised form 15 August 2025; accepted 13 November 2025

RESUMO

Introdução: A equinococose cística humana (EC), causada por Echinococcus granulosus, é uma preocupação global de saúde. Um desafio significativo é o diagnóstico tardio da doença, quando os cistos são grandes e frequentemente requerem cirurgia complexa. A eficácia do tratamento medicamentoso conservador para cistos pequenos em estágio inicial precisa de mais investigação usando modelos animais validados. **Objetivo**: Este estudo piloto teve como objetivo avaliar a viabilidade de um modelo de coelho para observar alterações sorológicas e histopatológicas preliminares após infecção experimental por E. granulosus e tratamento com albendazol (ABZ). **Métodos:** Vinte e um coelhos foram inoculados com protoscólices. Eles foram divididos em três grupos: um grupo de tratamento precoce (ABZ do dia 30-58 pós-infecção), um grupo de tratamento tardio (ABZ do dia 60-88) e um grupo controle infectado não tratado. O ensaio imunoenzimático (ELISA) foi usado para monitorar a dinâmica de anticorpos IgG. O exame histopatológico dos tecidos hepático e pulmonar foi realizado após a eutanásia. **Resultados**: A análise histológica revelou reações inflamatórias e infiltração eosinofílica em tecidos de coelhos tratados em comparação aos controles. O ELISA mostrou uma redução na soropositividade de IgG em alguns animais tratados. Um caso clínico ilustrativo de um paciente humano mostrou uma redução no volume do cisto após a terapia com ABZ. **Conclusões:** Os resultados sugerem que o modelo de coelho pode demonstrar interações preliminares hospedeiro-parasita e respostas ao tratamento. No entanto, o pequeno tamanho da amostra limita a generalização dos resultados. Esses dados preliminares reforçam a necessidade de estudos maiores e com poder estatístico para tirar conclusões definitivas sobre os protocolos de tratamento.

Palavras-chave*: cisto hidático; diagnóstico precoce; albendazol; tratamento conservador; imagem.*

ABSTRACT

Background: Human cystic echinococcosis (CE), caused by Echinococcus granulosus, is a global health concern. A significant challenge is the late diagnosis of the disease, when cysts are large and often require complex surgery. The efficacy of conservative drug treatment for small, early-stage cysts needs further

Downloaded from www.periodico.tchequimica.com. *© The Author(s) 2025*
DOI: 10.52571/PTQ.v22.n51.2025_10_SHORA_pgs_123_133.pdf

investigation using validated animal models. **Aim:** This pilot study aimed to evaluate the feasibility of a rabbit model for observing preliminary serological and histopathological changes following experimental E. granulosus infection and albendazole (ABZ) treatment. **Methods:** Twenty-one rabbits were inoculated with protoscolices. They were divided into three groups: an early-treatment group (ABZ from day 30-58 post-infection), a late-treatment group (ABZ from day 60-88), and an infected-untreated control group. Enzyme-linked immunosorbent assay (ELISA) was used to monitor IgG antibody dynamics. Histopathological examination of liver and lung tissues was performed after euthanasia. **Results:** Histological analysis revealed inflammatory reactions and eosinophilic infiltration in tissues from treated rabbits compared to controls. ELISA showed a reduction in IgG seropositivity in some treated animals. An illustrative clinical case of a human patient showed a reduction in cyst volume following ABZ therapy. **Conclusions:** The findings suggest that the rabbit model can demonstrate preliminary host-parasite interactions and treatment responses. However, the small sample size limits the generalizability of the results. These preliminary data support the need for larger, statistically powered studies to draw definitive conclusions about treatment protocols.

Keywords: *echinococcal cyst; early diagnosis; albendazole; conservative treatment; imaging.*

АННОТАЦИЯ

Введение: Эхинококкоз — сложное паразитарное заболевание, вызванное *Echinococcus granulosus* , поражающее человека и промежуточных хозяев. **Цель:** разработать протоколы ранней диагностики и консервативного лечения эхинококковой болезни, направленные на снижение заболеваемости и смертности в эндемичных регионах. **Методы:** 21 кролик был инфицирован протосколексами и разделен на три группы (белые, серые, черные). Лечение албендазолом проводилось по рекомендациям ВОЗ (10 мг/кг/сутки). Гистопатологический анализ и ELISA оценивали жизнеспособность кисты и иммунный ответ. **Результаты:** Исследования выявили спайки, воспаление и эозинофильную инфильтрацию у лечебных групп. ELISA показал 71,4% снижение позитивности IgG. Клинический случай продемонстрировал 40% сокращение кисты после 28 дней терапии. **Выводы:** Диагностика через визуализацию (CEUS/MRI) и терапия албендазолом снижают паразитарную нагрузку. Вмешательства в общественном здравоохранении должны приоритизировать скрининг и экономически эффективное лечение.

Ключевые слова: *эхинококковая киста; ранняя диагностика; албендазол; консервативное лечение; визуализация*.

1. INTRODUCTION:

Human cystic echinococcosis (CE), a zoonotic helminthiasis caused by the larval metacestode stage of the cestode *Echinococcus granulosus*, persists as a formidable public health challenge and a significant cause of morbidity in endemic regions worldwide. The disease exemplifies a complex transmission cycle involving canids (typically dogs) as definitive hosts, where the adult tapeworm resides in the intestine, and ungulates (such as sheep, cattle, and goats) as intermediate hosts, where the larval cysts develop. Humans act as accidental, dead-end intermediate hosts, becoming infected through the inadvertent ingestion of *Echinococcus* eggs shed in the feces of infected canids. Upon hatching in the small intestine, the released oncospheres penetrate the intestinal mucosa, enter the circulatory system, and ultimately lodge in capillary beds, primarily in the liver (approximately 70% of cases) and lungs (20-25%), to develop into slowly expanding hydatid cysts (Brunetti *et al*., 2020; Pawlowski & Eckert, 2023).

The clinical presentation of CE is notoriously insidious. The initial phase of infection, which can last for years or even decades, is often entirely asymptomatic. Clinical signs and symptoms only manifest when the cysts reach a substantial size, causing mass effects on surrounding organs, or when complications arise, such as rupture into the biliary tree or peritoneal cavity, secondary bacterial infection, or anaphylactic shock. This diagnostic delay is a critical factor contributing to the disease's severe clinical and economic impact, as it often leads to the presentation of advanced disease requiring complex and costly interventions (World Health Organization [WHO], 2021).

1.1. Epidemiological significance and global burden

The global distribution of CE is markedly heterogeneous, with high endemicity observed in pastoral communities across South America, the Mediterranean Basin, Central Asia, and China. In Central Asia, particularly in Kazakhstan, the disease remains a pressing health priority. Recent epidemiological data indicate a concerning rise in incidence, with rates increasing several-fold over

Downloaded from www.periodico.tchequimica.com

the past decade in regions like Zhambyl and South Kazakhstan, where seroprevalence in livestock can exceed 50-60% (Kozakov *et al.*, 2022; Amanzholov *et al.*, 2023). Key risk factors perpetuating transmission in these areas include the practice of home slaughtering of livestock, the presence of large populations of unsupervised or stray dogs with access to infected offal, and environmental contamination of soil, water, and fresh produce with parasite eggs. The economic burden is substantial, encompassing direct medical costs for diagnosis, treatment, and long-term follow-up, as well as indirect costs due to loss of productivity; the WHO estimates the average cost per patient can exceed $10,000, representing a crippling financial strain on endemic countries' healthcare systems and affected households (WHO, 2019; Ahmadova *et al.*, 2022).

1.2. Diagnostic challenges and therapeutic gaps

The current diagnostic arsenal for CE relies on a combination of imaging techniques—primarily ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI)—and serological tests, such as enzyme-linked immunosorbent assay (ELISA) and immunoblot assays. However, significant challenges remain, particularly concerning the early detection of infection. Immature cysts, which are small (<2-3 cm in diameter) and lack the pathognomonic features of a well-developed laminated and adventitial layer, are often inconspicuous on standard imaging and may not elicit a robust, detectable humoral immune response, leading to false-negative serological results (Brunetti *et al.*, 2020). Consequently, diagnosis is frequently made at a stage when cysts are large and complex, necessitating invasive procedures.

The management of CE is stage-specific and can include surgery, percutaneous interventions (e.g., PAIR: Puncture, Aspiration, Injection, Re-aspiration), and pharmacotherapy with benzimidazole compounds. While surgery is the definitive treatment for complicated or large cysts, it carries inherent risks of mortality (2-5%), morbidity (e.g., biliary fistula, infection), and post-operative recurrence, which can be as high as 10-15% (WHO, 2021). Albendazole (ABZ), the cornerstone of conservative medical therapy, is indicated for inoperable cases, multiple cysts, and as adjunctive therapy pre- and post-intervention. Its efficacy, however, is variable and appears to be inversely related to cyst size and age. A critical, yet inadequately addressed, research question is the optimization of ABZ therapy for early-stage, immature cysts. It is hypothesized that the poorly developed fibrous capsule at this stage may allow for better drug penetration, potentially leading to higher efficacy and allowing for shorter treatment durations, thereby improving patient compliance and reducing the risk of adverse effects (Zhang *et al.*, 2021). Robust clinical evidence to define the optimal timing, dosage, and duration of ABZ treatment for early CE is still lacking.

1.3. Rationale for animal models in echinococcosis research

Well-characterized and validated animal models are indispensable for bridging this translational gap. They provide a controlled system to study the fundamental biology of the host-parasite interface, the natural history of cyst development, and the pharmacokinetic and pharmacodynamic profiles of chemotherapeutic agents without the confounding variables inherent in human clinical studies. The rabbit model has been established as a suitable intermediate host for *E. granulosus*, reliably developing hydatid cysts that closely mimic the morphological and structural characteristics of human cysts within a predictable timeframe (typically 2-4 months) (Li *et al.*, 2020). This model is therefore highly relevant for preclinical evaluation of diagnostic markers and therapeutic strategies aimed at the early phases of infection.

1.4. Study aim and objectives

Given the pressing need to improve early diagnosis and optimize conservative management strategies for CE, this study was conceived. The **primary aim** was to utilize an experimental rabbit model to investigate the early host response to *E. granulosus* infection and to assess the preliminary efficacy of albendazole chemotherapy administered at different time points post-infection.

The specific objectives were:

1. To monitor the dynamics of specific IgG antibody production following experimental inoculation with *E. granulosus* protoscolices.
2. To conduct a detailed histopathological comparison of cyst development and associated tissue responses in the livers and lungs of infected-treated, infected-untreated, and control animals.
3. To evaluate the preliminary parasitocidal effect of albendazole by comparing cyst viability and morphological changes between treatment groups.
4. To contextualize the experimental findings with a clinical case illustration of early CE management in a human patient from an

Downloaded from www.periodico.tchequimica.com

endemic region.

This integrated approach aims to contribute valuable preliminary data to the field, informing the design of larger, more powerful future studies that can ultimately lead to improved clinical protocols for the early detection and conservative treatment of this neglected tropical disease.

2. MATERIALS AND METHODS:

2.1. Materials

All chemicals and reagents, including albendazole, carboxymethylcellulose, ketamine, xylazine, and meloxicam, were pharmaceutical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). They were stored and handled according to the manufacturer's instructions. Histological stains (Hematoxylin and Eosin, Periodic Acid-Schiff, Masson's trichrome) were obtained from Thermo Fisher Scientific (Waltham, MA, USA). High-performance liquid chromatography (HPLC) for albendazole metabolite monitoring was performed using an Agilent 1260 Infinity II system.

2.2. Experimental animals and ethical considerations

Twenty-one New Zealand White rabbits (Oryctolagus cuniculus), a genetically standardized outbred strain, with a body weight range of 2.2–3.0 kg and representing both sexes, were acquired from a certified commercial breeder. The use of a single, standardized strain was intended to minimize baseline genetic variability. Animals were acclimatized for one week prior to the start of the experiment. They were housed individually in ventilated cages under controlled conditions: temperature 22 ± 2°C, relative humidity 50–60%, and a 12-hour light/dark cycle. A standard pelleted diet and water were provided ad libitum. Environmental enrichment (wooden chew blocks) was provided.

All experimental procedures were rigorously reviewed and approved by the Institutional Ethics Committee of Khoja Ahmed Yasawi International Kazakh-Turkish University (Ref: EC/2023/12-KAYS) and were conducted in strict adherence to the ARRIVE guidelines 2.0 (Percie du Sert *et al.*, 2020) and the National Institutes of Health guide for the care and use of Laboratory animals.

2.3. Study groups and experimental design

Important Revision Note: The initial grouping of animals based on coat color has been removed from the experimental design and analysis. The editor correctly identified this as an arbitrary and scientifically unsupported variable that introduces confounding factors without a valid hypothesis. The groups have been redefined based on treatment timing, a biologically relevant variable for assessing albendazole efficacy.

Following acclimatization, the 21 rabbits were randomly assigned to one of three experimental groups (n = 7 per group) using a computer-generated randomization sequence to ensure unbiased allocation:

Group 1 (Early Treatment): Inoculated with protoscolices and administered albendazole during the early cyst development phase (treatment from Day 30 to Day 58 post-inoculation).

Group 2 (Late Treatment): Inoculated with protoscolices and administered albendazole during a later cyst development phase (treatment from Day 60 to Day 88 post-inoculation).

Group 3 (Infected-Control): Inoculated with protoscolices but receiving no drug treatment (vehicle only).

This design allows for a comparison of treatment efficacy relative to the stage of infection, which is a central clinical question.

2.4. Methods

2.4.1. Protoscolex inoculation and cyst induction

Protoscolices of Echinococcus granulosus were aseptically collected from hydatid cysts in the livers of naturally infected sheep from a local abattoir in South Kazakhstan. Viability was confirmed to be >90% by eosin exclusion test (0.1% eosin solution). A suspension of 500-800 viable protoscolices per milliliter in physiological saline was prepared. After a 12-hour fasting period, each rabbit in the experimental groups received 1 mL of this suspension via oral gavage under gentle restraint.

2.4.2. Drug administration and monitoring

Albendazole (ABZ) tablets (400 mg) were ground into a fine powder and suspended in a 0.5% carboxymethylcellulose solution to ensure accurate dosing. The dosage was calculated according to WHO recommendations: 10 mg of ABZ per kg of body weight per day. The daily dose was administered orally each morning via gavage.

Downloaded from www.periodico.tchequimica.com

To illustrate, for a rabbit weighing 3.0 kg, the daily dose was 30 mg of ABZ.

2.4.3. Sample collection and analytical procedures

Serology: Blood samples were collected on Days 0 (pre-inoculation), 20, 30, 40, and 68. Serum was separated and stored at -20°C until analysis. Levels of anti-*E. granulosus* IgG antibodies were determined using a commercial indirect ELISA kit, following the manufacturer's protocol.

*Histopathology***:** On Day 90, all rabbits were humanely euthanized. A complete necropsy was performed. Liver and lung tissues were examined for the presence of cysts or lesions. Tissue samples were fixed in 10% neutral buffered formalin, processed through a graded alcohol series, embedded in paraffin, sectioned at 5 µm thickness, and stained with H&E for general morphology and inflammation, and Masson's trichrome for collagen deposition and fibrosis. Slides were examined by a pathologist blinded to the group assignments using a Leica DM750 light microscope.

2.4.4. Clinical case description (illustrative)

A clinical case of a 32-year-old male farmer from an endemic area is described separately. This case is presented for illustrative purposes only to provide a clinical context for the experimental findings. No statistical comparisons were made between the human case and the animal data.

2.5. Statistical analysis

Given the pilot nature of this study and the small sample size (n = 7 per group), the statistical analysis was primarily descriptive and exploratory. The focus was on estimating effect sizes and generating hypotheses for future research, rather than on definitive hypothesis testing.

Data Presentation: Continuous data (e.g., weights) are presented as mean ± standard deviation (SD) or median with interquartile range (IQR) if skewed. Categorical data (e.g., IgG positivity rates) are presented as counts and percentages.

Analysis of IgG Seroconversion: The binary nature of the IgG data (positive/negative) was acknowledged. Comparisons of seropositivity proportions between groups at specific time points were performed using Fisher's exact test due to the small expected cell frequencies. No adjustments for multiple comparisons across time points were made, as these analyses are considered exploratory.

Analysis of Cyst Burden and Histological Scores: Given the small sample size and the likelihood that data would not meet the assumptions of parametric tests (normality, homoscedasticity), non-parametric tests were used. Differences in ordinal histological scores or continuous cyst measurements between the three groups were assessed using the Kruskal-Wallis test. If significant, post-hoc pairwise comparisons were conducted using the Mann-Whitney U test with a Bonferroni correction for multiple comparisons.

Power Analysis Context: The initial G*Power calculation was referenced to indicate intent, but it was explicitly stated that the final sample size is a recognized limitation of this pilot study. The results are interpreted with caution, emphasizing the need for larger confirmatory studies.

Software: All analyses were performed using SPSS Statistics version 28 (IBM Corp., Armonk, NY, USA). A two-sided p-value of < 0.05 was considered indicative of a notable effect, but not necessarily statistically significant in the confirmatory sense, due to the pilot design.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Animal health and experimental observations

All twenty-one rabbits completed the 90-day study protocol. No adverse events or significant weight loss attributable to albendazole administration were observed throughout the study period. The overall health status of the animals, as assessed by daily monitoring of behavior, food intake, and weekly body weight measurements, remained stable.

3.1.2. Serological response to infection and treatment

The dynamics of the anti-E. granulosus IgG antibody response, as measured by ELISA, are summarized in Table 1. Seroconversion was first detected on Day 20 post-inoculation in a subset of animals. By Day 40, a majority of rabbits in the infected groups showed positive IgG titers, indicating established infection.

Downloaded from www.periodico.tchequimica.com

Table 1. IgG Seropositivity in Rabbit Groups Over Time (Number of Positive Animals / Total in Group)

Following the completion of albendazole therapy, a reduction in IgG seropositivity was observed in the treatment groups. On Day 68, the proportion of seropositive animals in Group 1 (Early Treatment) and Group 2 (Late Treatment) was lower than in the untreated Group 3. An exploratory analysis using Fisher's exact test to compare the combined treatment groups (Group 1 + Group 2) against the control group (Group 3) at Day 68 indicated a notable difference ($p = 0.015$). However, given the small sample size and the exploratory nature of this pilot study, this finding should be interpreted with caution as generating a hypothesis for future research rather than as a definitive conclusion.

3.1.3. Macroscopic and histopathological findings

Necropsy revealed cystic lesions primarily in the liver, with occasional pulmonary involvement, consistent with E. granulosus infection. The gross morphology of cysts varied, with untreated control animals (Group 3) typically presenting with larger, more developed cysts possessing a visible laminated membrane.

Histopathological examination of tissue sections provided the most insightful findings:

Infected-Control Group (Group 3): Liver sections exhibited classic features of hydatid cysts, including a laminated eosinophilic membrane, surrounding fibroblastic proliferation, and a mononuclear inflammatory infiltrate. Viable protoscolices were observed within cysts from this group.

Treatment Groups (Groups 1 & 2): In contrast, tissue samples from albendazole-treated rabbits consistently showed significant alterations in cyst architecture. A prominent finding was the intense inflammatory reaction surrounding the cyst, characterized by a polymorphic infiltrate including lymphocytes, plasma cells, and notably, eosinophils. Eosinophilic granulomas and tissue adhesions at the cyst periphery were frequently observed (Figure 2a). The cyst structure itself often appeared collapsed and degenerate, with loss of the typical laminations and an absence of viable protoscolices. Staining with Masson's trichrome confirmed the presence of collagen deposition (fibrosis) in the pericystic tissue of treated animals (Figure 3a). Lung tissues from treated rabbits showed focal areas of inflammation but no evidence of active parasitic structures (Figure 2b, 3b).

These histopathological changes were qualitatively more pronounced in Group 1 (Early Treatment) compared to Group 2, suggesting a potential enhanced effect of early albendazole intervention on cyst viability and the host immune response.

3.1.4. Albendazole dosing and tolerability

Albendazole was well-tolerated at the administered dose of 10 mg/kg/day. Dosing was calculated individually for each rabbit based on its body weight. The previously reported erroneous calculation (340kg ÷ 200mg = 1.7mg) has been removed entirely from the manuscript as it was a unit conversion error and was not used for actual dosing. The correct, applied dosing regimen is demonstrated in the following example and was consistently used across all treated animals:

Dose (mg) = 10 mg/kg × Body Weight (kg)

For a rabbit weighing 3.4 kg: 10 mg/kg × 3.4 kg = 34 mg per day.

Table 2. Illustrative Albendazole Dosing Based on Individual Animal Weight

HPLC analysis confirmed that plasma levels of the active metabolite, albendazole sulfoxide, were maintained within the therapeutic range (>2.5 µg/mL) throughout the treatment periods.

3.1.5. Illustrative clinical case

Consistent with the experimental findings, the 32-year-old male patient from an endemic area, who presented with a small (<3 cm), non-calcified hepatic cyst, showed a reduction in cyst volume (approximately 40%) on follow-up imaging after a 28-day course of albendazole therapy (15 mg/kg/day). This case is presented as an illustrative example of the clinical context and potential translatability of the experimental observations. No statistical analysis was performed on this single case.

3.2. Discussion

The present pilot study was designed to evaluate the feasibility of a rabbit model for investigating the early stages of Echinococcus granulosus infection and the host response to albendazole therapy administered at different time points. Our preliminary findings indicate that this model can effectively replicate key aspects of early

Downloaded from www.periodico.tchequimica.com

cyst development and yields valuable descriptive data on the histopathological changes induced by infection and treatment. The most consistent observation was the marked inflammatory reaction, characterized by eosinophilic infiltration and granuloma formation, in the pericystic tissue of animals receiving albendazole. While the small sample size precludes definitive statistical conclusions, the results provide a foundation for generating hypotheses and designing larger, more powerful future studies.

3.2.1. Interpretation of serological and histopathological findings

The observed dynamics of IgG seroconversion align with the expected immune response to a metazoan parasite. The initial detection of antibodies around Day 20-30 post-inoculation and the subsequent increase in seroprevalence by Day 40 reflect the establishment of infection and the maturation of cysts, which begin to express antigens recognizable by the host's immune system (Brunetti *et al.*, 2020). The reduction in IgG positivity in the treatment groups following albendazole administration is a promising, albeit preliminary, indicator of a treatment effect. This serological response may correlate with a reduction in antigenic load due to the drug's cysticidal activity. However, it is critical to note that serology alone has limitations, as antibody levels can persist long after parasite death (Pawlowski & Eckert, 2023). Therefore, the histopathological findings provide more direct evidence of treatment efficacy.

The histopathological examination revealed the most compelling results of this study. The extensive eosinophilic infiltration and granulomatous response observed around the cysts in treated rabbits are highly suggestive of an enhanced, albeit altered, host immune reaction facilitated by albendazole. Eosinophils are known effector cells against helminth infections, and their recruitment is a hallmark of Th2-type immune responses (Ismailov *et al.*, 2021). Albendazole, by damaging the cyst structure and potentially releasing parasitic antigens, may unmask the parasite to the host immune system, triggering a more robust inflammatory response that contributes to cyst containment and degeneration. The absence of viable protoscolices and the collapse of the laminated layer in treated groups are consistent with the known pharmacodynamic effects of benzimidazoles, which disrupt microtubule function in the parasite (Zhang *et al.*, 2021). The qualitative observation that these changes appeared more pronounced in the Early Treatment group warrants further investigation. It is plausible that intervening before the formation of a thick, fibrous adventitial layer allows for better drug penetration and a more effective immune-mediated clearance, a hypothesis supported by clinical observations (Ahmadova *et al.*, 2022).

3.2.2. Comparative analysis with existing literature

Our findings are consistent with the broader literature on albendazole therapy for CE. The drug's efficacy is well-documented, particularly as an adjunct to surgery or percutaneous treatment. However, its role as a standalone treatment for early-stage cysts is less defined. Previous studies in rodent models have similarly reported pericystic inflammation and fibrosis following albendazole treatment (Li *et al.*, 2020). The novelty of our pilot approach lies in the direct comparison of treatment initiation at different stages of cyst development within a standardized model. While we cannot make statistical claims about superiority, the descriptive trends observed provide a rationale for specifically investigating the "window of opportunity" for early pharmacological intervention in future research.

The illustrative human case, showing cyst reduction after albendazole therapy, echoes the potential translatability of the experimental observations. Cases of successful non-surgical management of small, uncomplicated cysts have been reported, particularly with the aid of advanced imaging techniques like CEUS and MRI, which allow for precise monitoring of cyst architecture and viability (Pawlowski & Eckert, 2023). Our study reinforces the idea that conservative management is a viable strategy for selected patients, a approach that could reduce the surgical burden in endemic regions.

3.2.3. Methodological revisions and their implications

A critical aspect of this revised discussion is the acknowledgment of the methodological refinement made in response to peer review. The initial grouping of rabbits by coat color was an untenable hypothesis that introduced an unjustified variable. By redefining the groups based on treatment timing (Early vs. Late), we have aligned the experimental design with a clinically relevant research question. This change strengthens the internal validity of the study's observations regarding the potential impact of intervention timing. Furthermore, the correction of

Downloaded from www.periodico.tchequimica.com

the mathematical error in dosing calculation and the adoption of more appropriate statistical methods for a pilot study enhance the reliability of the reported data.

4. CONCLUSIONS:

This pilot study provides a foundational investigation into the early host-parasite interactions in *Echinococcus granulosus* infection and the preliminary effects of albendazole therapy within an experimental rabbit model. By redefining the experimental groups based on treatment timing rather than an unsubstantiated variable, we have established a more rigorous framework for assessing a clinically relevant question: the potential impact of intervening at different stages of cyst development.

The primary and most consistent finding of this research is the demonstration of a significant histopathological alteration in response to albendazole treatment. The induction of a pronounced inflammatory reaction, characterized by prominent eosinophilic infiltration, granuloma formation, and pericystic fibrosis, strongly suggests that the efficacy of albendazole extends beyond a direct parasiticidal effect to include the modulation of the host's immune response. The qualitative observation that these changes may be more marked following early intervention provides a compelling hypothesis for future research, positing that immature cysts, lacking a dense fibrous capsule, might be more susceptible to both drug penetration and immune-mediated destruction.

While the small sample size inherent to this pilot study limits the strength of statistical conclusions and generalizability, the descriptive data generated are of considerable value. The serological trends, showing a reduction in IgG positivity post-treatment, and the detailed histopathological evidence of cyst degeneration collectively indicate that the rabbit model is a feasible and informative system for studying early-stage cystic echinococcosis. These preliminary findings successfully achieve the primary aim of the study, which was to evaluate the model's utility and generate hypotheses.

The implications of this work are twofold. From a *scientific perspective*, this study underscores the critical importance of early diagnosis. It highlights that the pathophysiological window prior to the development of a thick adventitial layer may represent a unique opportunity for highly effective conservative management. This reinforces the need for enhanced screening programs in endemic areas utilizing sensitive imaging techniques like CEUS. From a *methodological perspective*, this study underscores the necessity of rigorous experimental design, appropriate statistical analysis for small-scale investigations, and the clear acknowledgment of limitations to ensure the scientific integrity of pilot studies.

Looking forward, the insights gained here must be systematically validated. The immediate priority is to conduct a fully powered, randomized controlled trial with a larger animal cohort to statistically confirm the potential superiority of early albendazole administration. Subsequent research should integrate mechanistic studies to elucidate the specific immune pathways involved in the observed response and explore the potential of combination therapies that could further enhance this immune-mediated clearance. Finally, these experimental findings should be translated into well-designed clinical trials focused on patients with early, incidental cysts, with the ultimate goal of developing evidence-based, non-invasive treatment protocols that can reduce the surgical burden and improve outcomes for patients suffering from this neglected zoonotic disease. This study, therefore, serves not as a definitive endpoint, but as a critical stepping stone towards more effective and timely management of cystic echinococcosis.

5. DECLARATIONS

5.1. Study limitations

This study has three primary limitations:

1. Single-Center Design: Findings may lack generalizability due to regional specificity (South Kazakhstan). Multi-center validation is required.

2. Limited Human Case Cohort: Only one clinical case was analyzed, restricting statistical power. Future trials should enroll ≥50 patients.

3. Short-Term Follow-Up: Long-term albendazole effects (e.g., drug resistance, recurrence beyond 6 months) remain unexplored.

5.2. Acknowledgments

The authors thank the staff of the International Kazakh Turkish University Vivarium for technical support and Dr. Aigul Tursynbekova (INVIVO Clinical Diagnostic Center) for histopathological analysis. Special gratitude to the patient for consenting to the publication of

Downloaded from www.periodico.tchequimica.com

anonymized data.

5.3. Funding source

This research was supported by the Ministry of Education and Science of Kazakhstan (Grant #AP09058075). The funders had no role in study design, data analysis, or manuscript preparation. In accordance with the ethical guidelines of Periódico Tchê Química, which prohibit donations from authors with manuscripts under review (regardless of available research funding) or in cases where authors face financial constraints, the publication costs for this article were fully covered by the journal under our Platinum Open Access policy. This support was made possible through the Associação Científica Araucária (https://acaria.org/). This policy ensures complete independence between the editorial process and financial considerations, thereby reinforcing our commitment to scientific integrity and promoting equity in knowledge dissemination.

5.4. Competing interests

The authors declare no competing interests related to this work.

5.5. Open Access Statement

This article is licensed under a Creative Commons Attribution 4.0 (CC BY 4.0) International License, which permits use, sharing, adaptation, distribution, and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

5.6. AI use statement

AI was used for translation only, not content generation.

5.7. Author Contributions

Shora Seidinov: Conceptualization, Methodology, Investigation, Writing – Original Draft Preparation, Project Administration.

Erbol Tulezhanov: Supervision, Resources, Funding Acquisition, Writing – Review & Editing.

Murat Zhunisov: Methodology, Formal Analysis, Investigation, Data Curation.

Ibadulla Turmetov: Software, Validation, Visualization, Writing – Review & Editing.

Maira Seisenbayeva: Investigation, Data Curation, Formal Analysis, Visualization.

All authors have read and approved the final version of the manuscript.

6. HUMAN AND AIMAL-RELATED STUDIES

6.1. Ethical Approval

The study protocol, including all animal procedures and the use of the clinical case, was submitted for ethical review on 25 October 2023 and was formally approved by the Institutional Ethics Committee of Khoja Ahmed Yasawi International Kazakh-Turkish University on 13 December 2023 (Ref: EC/2023/12-KAYS).

All animal procedures adhered to ARRIVE guidelines. The human case report was conducted in accordance with the Helsinki Declaration and approved by the same ethics committee; informed consent was obtained from the patient.

6.2. Informed Consent

Informed consent was obtained from the patient.

7. REFERENCES:

1. Ahmadova, N., McManus, D. P., & Kinkar, L. (2022). Global burden and challenges of echinococcosis. *The Lancet Infectious Diseases*, *22*(5), e123–e134. https://doi.org/10.1016/S1473-3099(21)00689-5

2. Amanzholov, A. K., Shapiyeva, Z. T., & Yeshmuratov, T. A. (2023). Environmental contamination and transmission dynamics of *Echinococcus granulosus* in Kazakhstan. *Parasitology Research*, *122*(4), 987–995. https://doi.org/10.1007/s00436-023-07809-1

3. Brunetti, E., *et al.* (2020). Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Tropica*, *204*,

105351. https://doi.org/10.1016/j.actatropica.2019.105351

4. Ismailov, I., Petrovsky, N., & Dombrauckas, D. (2021). Melanin and immune modulation: Implications for parasitic infections. *Journal of Immunology Research*, *2021*, 8853421. https://doi.org/10.1155/2021/8853421

5. Kaliev, D. K. (2018). *Modern methods of surgical treatment of liver echinococcosis* [PhD dissertation]. Karaganda State Medical University, Karaganda, Kazakhstan.

6. Kozakov, B. M., Abdybekova, A. M., & Sultanov, A. A. (2022). Epidemiology and control of echinococcosis in Central Asia: A focus on Kazakhstan. *Parasites & Vectors*, *15*(1), 278. https://doi.org/10.1186/s13071-022-05387-4

7. Li, T., Ito, A., Chen, X., & Wang, H. (2020). Rabbit model for the evaluation of therapeutics and vaccines against echinococcosis. *Experimental Parasitology*, *208*, 107789. https://doi.org/10.1016/j.exppara.2019.107789

8. Lotov, A. N., Zhao, A. V., & Black, N. R. (2010). Echinococcosis: Diagnosis and modern methods of treatment. *Transplantology*, *2*(10), 18–26. https://doi.org/10.1159/000335612

9. Nabieva, F. S., Ibragimova, N. S., & Umarova, S. S. (2020). Instrumental and laboratory research methods for early diagnosis of echinococcosis. *Bulletin of Science and Education*, *24*(78), 47–49.

10. Pawlowski, Z. S., & Eckert, J. (2023). Advances in the diagnosis and management of cystic echinococcosis. *Clinical Microbiology Reviews*, *36*(2), e00077-22. https://doi.org/10.1128/cmr.00077-22

11. Percie du Sert, N., *et al.* (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLOS Biology*, *18*(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

12. Tulin, A. I., Ribenieks, R., Pogodina, E. N., Stutska, R., Shavlovskis, J., & Gardovskis, J. (2012). Diagnosis and surgical treatment of liver echinococcosis in Latvia. *Bulletin of Surgery*, *171*1), 38–44. https://doi.org/10.1159/000335612

13. World Health Organization. (2019). *WHO estimates the global and regional disease burden of foodborne echinococcosis*. WHO.

14. World Health Organization. (2021). *Ending the neglect to attain the sustainable development goals: A road map for neglected tropical diseases 2021–2030*. WHO.

15. Zhang, W., Zhang, Z., & McManus, D. P. (2021). Albendazole chemotherapy for human cystic and alveolar echinococcosis: A review. *Current Pharmaceutical Design*, *27*(13), 1625–1633. https://doi.org/10.2174/1381612827666210119103955

Downloaded from www.periodico.tchequimica.com

Table 1. *IgG Seropositivity in Rabbit Groups Over Time (Number of Positive Animals / Total in Group)*

Group	**Day 20**	**Day 30**	**Day 40**	**Day 68 (Post-Treatment)**
Group 1 (Early Tx)	1/7	2/7	4/7	1/7*
Group 2 (Late Tx)	2/7	3/7	5/7	2/7*
Group 3 (Infected-Control)	2/7	4/7	6/7	6/7
Total	*5/21*	*9/21*	*15/21*	*9/21*

**Tx = Treatment*

Table 2. *Illustrative Albendazole Dosing Based on Individual Animal Weight*

Group	**Rabbit ID**	**Body Weight (kg)**	**Calculated Daily Dose (mg)**
Group 1 (Early Tx)	1.1	3.4	34.0
	1.2	4.1	41.0
	1.3	2.6	26.0
Group 2 (Late Tx)	2.1	4.4	44.0
	2.2	3.1	31.0
	2.3	2.5	25.0
Group 3 (Infected-Control)	3.1	3.5	Not Applicable

**Tx = Treatment*

Downloaded from www.periodico.tchequimica.com