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Seroprevalence of *Encephalitozoon cuniculi* in companion rabbits in Mexico City

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Abstract

Encephalitozoon cuniculi is an obligate intracellular microorganism primarily found in rabbits, although it has also been reported in various species such as horses, rodents, dogs, monkeys, foxes, cats, goats, and pigs. Immunodeficient humans are also vulnerable to this infection. This study aimed to determine the seroprevalence of *E. cuniculi* in 89 companion rabbits in Mexico City using an enzyme-linked immunosorbent assay (ELISA). The results indicated a seroprevalence of 70 %. Clinically healthy rabbits constituted 92 % of the sample, of which 68 % were positive for antibodies against *E. cuniculi*. The findings align with global reports of high seroprevalence. Despite most infections being subclinical, a substantial portion of asymptomatic rabbits were seropositive, highlighting the need for early disease recognition to prevent its spread. This study is the first to describe the seroprevalence of *E. cuniculi* in Mexico and the common associated signs.

Keywords: Pet rabbit health; Cataract; Paralysis; Synechia; Neurological signs; Preventive screening.

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Study contribution

Encephalitozoonosis is a worldwide disease with a high prevalence in pet rabbits in most of the countries where it has been reported. It is important to stress that many patients with no clinical signs of the disease are positive for antibodies, which underlines the need to recognize the syndrome in its early stages to prevent its spread. This study determined the presence of antibodies for *Encephalitozoon cuniculi* in companion rabbits. The results are consistent with reports of a high frequency of encephalitozoonosis worldwide. This agent is important in public health because it is considered a zoonosis, highlighting the risk of contagion for humans living with rabbits exposed to the agent.

Introduction

Encephalitozoon cuniculi is an obligate intracellular microorganism primarily found in rabbits;[1] however, its presence has also been reported in many other species, such as horses, rodents, dogs, monkeys, foxes, cats, goats, and pigs.[2] This pathogen is horizontally transmitted through contaminated food with urine, where it is absorbed in the small intestine, and through the inhalation of spores, which can infect the respiratory tract. Additionally, vertical transmission can occur transplacentally from mother to fetus.[1–3] The primary target sites of *E. cuniculi* spores are the central nervous system, especially the brain, and to a lesser extent, the kidneys, lungs, eyes, and myocardium. Transplacental infections frequently result in ophthalmological symptoms in young rabbits.[1–3]

Humans with immunodeficiencies are vulnerable to encephalitozoonosis.[2,4,5] In Mexico, there are no reports of *E. cuniculi* in humans, unlike in other countries;[4, 5] however, cases of *E. intestinalis* have been documented in human immunodeficiency virus patients.[6] Similarly, little is known about this disease in immunocompetent individuals, particularly those living with rabbits. Therefore, the effects of exposure to this pathogen in house rabbits will have to be investigated, since encephalitozoonosis represents a risk for other rabbits and immunocompromised humans. Moreover, the quality of life and life expectancy of affected animals are considerably compromised.

The objective of this study was to determine the seroprevalence of *Encephalitozoon cuniculi* in companion rabbits in Mexico City using an enzyme-linked immunosorbent assay (ELISA), in order to assess exposure to the pathogen, describe the clinical manifestations of encephalitozoonosis, and analyze their association with the sex and age of the rabbits. Based on the literature,[7–10] we hypothesize that the seroprevalence of *E. cuniculi* in companion rabbits in Mexico will exceed 50 %.

Materials and methods

Ethical statement

This research was approved by Comité Interno para el Cuidado y Uso de los Animales (CICUA) of the Universidad Nacional Autónoma de México (UNAM), Facultad de Medicina Veterinaria y Zootecnia (registry number 0691), and was carried out with the prior consent of the rabbits' caregivers.

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Subjects and sampling

Eighty-nine companion rabbits from Mexico City were selected for the study, and the number of patients was determined according to the processing capacity of the ELISA kit. Blood samples were collected at the Hospital Veterinario de Especialidades en Fauna Silvestre y Etología Clínica-UNAM between January 8th and 31st, 2024. Twenty-seven rabbits lived alone, and 62 lived with other rabbits across 15 groups, each composed of 2 to 12 individuals. Sex, age, body condition, neurological signs, and ophthalmological signs were recorded to determine correlations between variables. Rabbits were categorized into three age groups: juveniles (< 8 months), adults (9 to 60 months), and elderly individuals (> 61 months).[11] Likewise, body condition was assessed using a 5-point scale, where "1" indicated emaciation and "5" indicated obesity.[12]

All rabbits underwent a general physical examination, and venipuncture of the saphenous vein was performed to collect blood samples. These were transported to the Serology Laboratory at the Facultad de Medicina Veterinaria y Zootecnia-UNAM, where they were allowed to clot and then centrifuged at 3 000 × g for 10 minutes to obtain serum. Samples were stored at -20 °C until analysis. Sera were tested for the detection of antibodies against *Encephalitozoon cuniculi* using a commercial indirect ELISA kit (*Encephalitozoon cuniculi* (EC) ELISA, Medicago, Sweden). Diluted samples (1:100) were added to antigen-coated microplate wells, followed by incubation, washing, and the addition of an HRP-conjugated secondary antibody. After a final wash, a TMB substrate was applied, and absorbance was measured at 450 nm. Samples were considered seropositive if the optical density exceeded the threshold established by the kit controls, indicating the presence of *E. cuniculi*-specific antibodies.

Statistical analysis

Due to diagnostic capacity constraints, a sample size between 30 and 89 rabbits was proposed. Seroprevalence was defined as the proportion of individuals in the population who tested positive for *Encephalitozoon cuniculi* antibodies at the time of sampling.

A hypothesis test was performed to determine whether the proportion was greater than 50 %. The statistical test used to evaluate this hypothesis was a Z-test for a single proportion. To identify risk factors, a chi-square test of independence was performed between seroprevalence and the variables grouped by living arrangement, sex, and age. A significance level of 5 % ($\alpha = 0.05$) was used.[13] The database was processed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), and statistical analyses were carried out using IBM SPSS Statistics (version 25; IBM Corporation, 2018, Armonk, NY, USA; https://www.ibm.com/mx-es/spss).

Results

The seroprevalence of *E. cuniculi* in the studied population was 0.7 ± 0.462 (proportion ± standard deviation), indicating that approximately 70 % of the rabbits had been exposed to this pathogen. The proportion of animals positive for *E. cuniculi* was significantly greater than 50 % ($P = 0.0001$) [Table 1].

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Table 1. Seroprevalence and confidence interval

N	Prevalence	Standard deviation	95 % confidence interval	
89	0.70* ($\mu_0 = 0.5$)	0.462	0.60	0.79

* Indicates significant difference (P = 0.0001)

The rabbits were male, 56 % (n = 50/89), and 44 % (n = 39/89) were female. The most representatived age group in this study was adult rabbits (79 %). Similarly, 67 % of the rabbits had a body condition score of 3 (Table 2). The seroprevalence of *E. cuniculi* showed no statistically significant association with sex, age, or body condition (P > 0.05).

Table 2. Distribution of rabbits according to their age and body condition

Age group	% (n)	Body condition rating	% (n)
Juveniles (< 8 months)	12 % (n = 11/89)	1 emaciated	-
Adults (9 months to 60 months)	79 % (n = 70/89)	2 slim	17 % (n = 15/89)
Elderly (> 61 months)	9 % (n = 8/89)	3 suitable	67 % (n = 69/89)
		4 overweight	9 % (n = 8/89)
		5 obesity	7 % (n = 6/89)

Ninety-two percent of the rabbits were classified as clinically healthy (n = 82/89), of which 68 % tested positive. In contrast, 8 % presented neurological (n = 5/89) and ophthalmological (n = 2/89) signs. The clinical findings included synechiae, unilateral cataract, facial paralysis, seizures, paraparesis of the pelvic limbs, and head tilt (Figure 1). Six out of these seven rabbits with clinical manifestations were seropositive.

Discussion

Various studies from around the world have reported the prevalence of encephalitozoonosis. Only in South Korea[9] and Germany[14] has a prevalence below 50 % been observed. In contrast, the remaining authors report prevalences greater than 50 %,[7, 8, 10, 15, 16] which may suggest a high level of contagiousness of the disease among companion rabbit populations (Table 3). Berguer et al.[15] in Brazil and Chilón[7] in Peru reported prevalences of 87.7 % and 56.6 %, respectively. In the present study, a seroprevalence of 70 % was found, indicating that the occurrence of encephalitozoonosis in Mexico is comparable to that reported in other countries.

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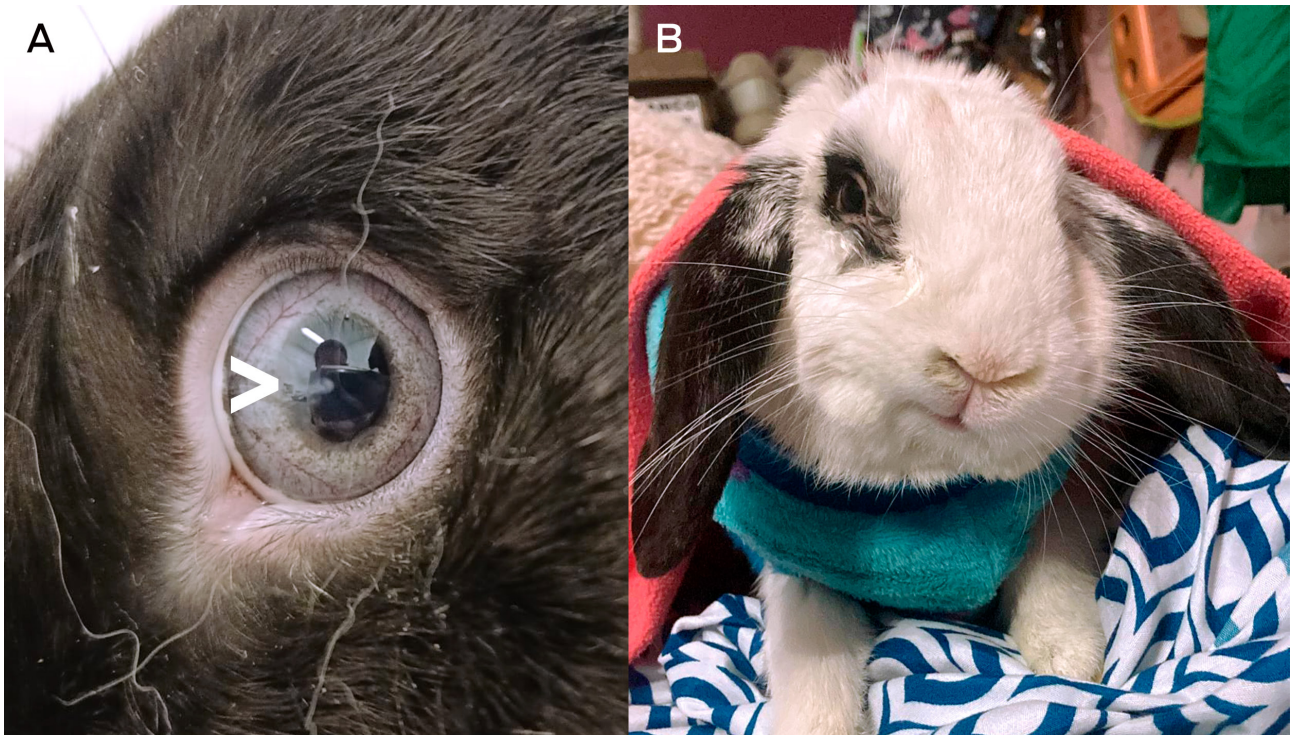

Figure 1. Clinical findings in the rabbits: (A) synechia in the left eye located toward the medial ridge (arrow), and (B) facial paralysis.

Table 3. Prevalence of *Encephalitozoon cuniculi* around the world

Author	Year	Country	Prevalence
Keeble et al.[10]	2006	United Kingdom	52 % (ELISA) Companion rabbits
Dipineto et al.[16]	2007	Italy	67.2 % (ELISA) Companion rabbits
Chilón[7]	2014	Peru	56.6 % (ELISA) Companion rabbits
Hein et al.[14]	2014	Germany	43 % (indirect immune fluorescence antibody test)
Shin et al.[9]	2014	South Korea	22.6 % (ELISA) Companion rabbits
Berguer et al.[15]	2015	Brazil	81.7 % (ELISA) Companion rabbits
Maestrini et al.[8]	2016	Italy	70.5 % (ELISA) All farm, laboratory, zoo, and companion rabbits 44 % only the companion rabbits

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On the other hand, encephalitozoonosis frequently occurs subclinically,[9, 10, 14, 15] with infected individuals acting as silent disseminators[8, 9] In this study, 92 % of the rabbits were clinically healthy, yet 68 % of these individuals tested positive. Keblee et al.[10] conducted a study with 97 clinically healthy companion rabbits, reporting that 52 % of the specimens were seropositive for *E. cuniculi*. Similarly, other studies that evaluated clinically healthy populations found antibody presence ranging from 18 % (n = 39/218)(14) to 86% (n = 160/186)[15] of the study subjects.

The patients showing clinical manifestations consistent with encephalitozoonosis in this study represented only 8 % (n = 7/89) of the rabbits, presenting visible neurological and ophthalmological signs. The most common clinical signs reported in the literature are head tilt[1] and phacoclastic uveitis.[17] However, hemiparesis, paresis, pelvic limb paralysis, cataracts, and synechiae, among other signs, have also been documented[1, 9, 15, 17] Likewise, Berger et al.[15] reported that among their population of seropositive rabbits, 3.75 % exhibited neurological signs (head tilt and pelvic limb paralysis), and 3.71 % showed ophthalmological signs, such as uveitis, cataracts, and glaucoma—findings consistent with the present study.

Finally, this research has important limitations that must be considered when interpreting the results. First, the number of clinically affected rabbits was insufficient to determine whether age, sex, or body condition were significant risk factors. Future research should include a larger sample of symptomatic rabbits to obtain more representative results and conclusions. Furthermore, the ELISA employed in this study detects antibodies indicating prior exposure to *E. cuniculi*, but does not confirm active infection. Therefore, it is essential to conduct longitudinal monitoring and complement serological testing with more sensitive methods, such as PCR, to confirm active disease and monitor its progression. These methodological improvements will provide a more accurate and comprehensive understanding of the impact of *E. cuniculi* on rabbit health.

Conclusions

The seroprevalence of *Encephalitozoon cuniculi*, determined by ELISA, in 89 companion rabbits from Mexico City was 70 %. *Encephalitozoonosis* is a globally recognized disease, with a high prevalence reported in most countries. A significant proportion of asymptomatic rabbits were seropositive, underscoring the importance of early disease recognition to prevent its dissemination. This is the first study to report the seroprevalence of *E. cuniculi* in Mexico and to describe the most frequent clinical signs associated with the infection.

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Data availability

All relevant data are within the manuscript and its supporting information files.

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Conflicts of interest

The authors have no conflict of interest to declare regarding this publication.

Author contributions

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Data curation: E Cortés-Pérez, MG Sánchez-González, I Maldonado-Reséndiz.

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Funding acquisition: MG Anaya-Santillán, I Maldonado-Reséndiz.

Investigation: E Cortés-Pérez, I Maldonado-Reséndiz.

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Writing-review and editing: E Cortés-Pérez, A Rodríguez-Hernández, MG Anaya-Santillán, MG Sánchez-González, I Maldonado-Reséndiz.

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