



SEROPREVALENCE OF *ENCEPHALITOZOON CUNICULI* INFECTION IN PET RABBITS IN BRAZIL

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Abstract

Encephalitozoon cuniculi is an obligate intracellular parasite responsible for encephalitozoonosis, primarily an opportunistic infection of rabbits but also other animals, including humans, which affects the nervous system, kidney, liver, and eyes. This article reports the results of a research investigation that examined the seroepidemiological data from rabbits diagnosed with encephalitozoonosis in Brazil. A total of 186 pet rabbits (*Oryctolagus cuniculus*) either with subclinical infection or presenting with ophthalmic or neurological signs suggestive of encephalitozoonosis were included in the study. Using enzyme-linked immunosorbent assay, antibodies to *E. cuniculi* were detected in 81.7% of the animals. Serological status did not correlate with place of birth, age, gender, breed, or fur color of the animals. Of the 152 seropositive rabbits, 89% (136 rabbits) were subclinical, supporting their role as *E. cuniculi* reservoirs. Of the 16 seropositive animals with clinical signs, 62.5% (5 rabbits) showed ophthalmic signs, 60% (6 rabbits) presented with neurological signs, and 62.5% (5 rabbits) had a combination of ophthalmic and neurologic signs. These results indicate a high prevalence of *E. cuniculi* infection in pet rabbits being cared for in Brazil, thus highlighting the importance of improving diagnostic and control measures. Further research studies are required to determine if the parasite strain is a significant factor for zoonotic transmission. Copyright 2015 Published by Elsevier Inc.

Key words: *Encephalitozoon cuniculi*; rabbit; zoonosis; serology; clinical signs; ELISA

Encephalitozoon *cuniculi* is a single-celled eukaryote belonging to the *Microsporidia* phylum within the *Fungi* kingdom.¹ A wide range of mammals, including humans, can be infected with the *E. cuniculi* organism through the ingestion or inhalation of spores that can be shed in the feces, urine, or respiratory secretions of infected animals.^{1,2} Humans have been infected with the same *E. cuniculi* organism that has been isolated from dogs, cats, rabbits, and birds, which suggests zoonotic transmission,³⁻⁶ which likely occurs through exposure to contaminated water, food, or air.^{1,7}

The clinical course of encephalitozoonosis depends on the host's immunological competence. Immune-competent hosts are usually subclinical carriers, show mild signs of disease, or have reproductive problems, whereas immune-compromised individuals often present with a disseminated infection.⁷⁻⁹ The disease is of clinical

significance in pet and laboratory rodents and rabbits. Rabbits can be chronically subclinical or show signs of renal insufficiency, including polyuria, polydipsia, pollakisuria, and azotemia, and nervous system lesions, including head tilt, vestibular syndrome, and hind limb paralysis. The eye, which is immune privileged, can develop

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uveitis, secondary glaucoma, and cataracts.¹⁰⁻¹² Immune-competent hosts are able to often control but not completely eliminate the infectious organisms. Treatment may contribute little to total resolution of disease, rather helping to reduce inflammation, secondary bacterial infections, and prevent spore formation.¹³ Conversely, immune-compromised hosts are considerably more susceptible to fatal infection.

Despite the fact that *E. cuniculi* has been reported to infect animals and humans worldwide, only 2 prevalence studies have been conducted in Brazil, and they involved animals other than rabbits.^{9,14} The present work was conducted to provide more information regarding encephalitozoonosis in rabbits, specifically in Brazil, by determining the seroprevalence of antibodies to *E. cuniculi* in rabbits from the southeastern and southern country.

MATERIALS AND METHODS

Animals, Sample Sources, and Collection

In the present study, 186 pet rabbits (*Oryctolagus cuniculus*) were evaluated from 3 Brazilian states including São Paulo state (Presidente Prudente city; 22°07'33"S, 51°23'20"W), Paraná state (Curitiba city; 25°25'47"S, 49°16'19"W), and Santa Catarina state (Federal Institute of Education and Pomerode's Zoo, Pomerode city; 26°44'27"S, 49°10'37"W). These cities are located at the center of the most developed, densely populated, and industrialized region of Brazil (southeast and southern) (Fig. 1). The linear distance from Presidente Prudente to Curitiba is 600 km and from Curitiba to Pomerode is 200 km (Fig. 1).

A clinical history including husbandry, age, gender, diet, and health status followed by an external physical examination including neurological and ophthalmic examinations was obtained for each animal. During the physical examination, overt neurological signs (e.g., urinary incontinence, head tilt, ataxia, paralysis, circling, rolling, spontaneous nystagmus, tremors, and opisthotonus) and signs of renal insufficiency (e.g., polyuria, polydipsia, pollakisuria, and azotemia) and/or ophthalmic abnormalities (e.g., cataracts, uveitis, and glaucoma) were evaluated. Animals that exhibited one or more of the clinical signs often associated with rabbit encephalitozoonosis were considered clinically infected.

Although hematology and biochemistry analyses were performed for a thorough assessment of general health, another objective of the study was to correlate possible changes in the



FIGURE. Map of Brazil demonstrating the different states and regions. Dots indicate cities in the 3 states in which a population of rabbits (*Oryctolagus cuniculus*) was investigated: red dot—Presidente Prudente (São Paulo); black dot—Curitiba (Paraná); and yellow dot—Pomerode (Santa Catarina).

blood results with seropositive *E. cuniculi* cases. Approximately 8 mL of blood was collected from the jugular vein from each animal for hematology (complete blood count and platelet count), serum biochemistry (serum alanine amino transferase, gamma glutamyl transpeptidase, alkaline phosphatase, urea, and creatinine), and disease-specific serological tests. Complete blood counts were performed on an automated hematology analyzer BC-2800 Vet (Shenzhen Mindray Bio-Medical Electronics Co, LTD, Shenzhen, China). Serum chemistries were measured using a chemistry analyzer BS-200 (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen City, China). Serum samples were frozen at -20°C before serology testing.

Enzyme-Linked Immunosorbent Assay Examination

The frozen sera collected for this investigation were shipped overnight packed with dry ice to the Comparative Pathology Laboratory at the School of Medicine, University of Miami, FL, for *E. cuniculi* testing. Commercially available enzyme-linked immunosorbent assay (ELISA) plates (ECUN ELISA plate, Charles River Laboratories, Wilmington, MA USA) were coated with rabbit *E. cuniculi* (ATCC 50503, American Type Culture Collection, Manassas, VA USA) that had been

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