SEROLOGICAL INVESTIGATION ON *ENCEPHALITOZOON CUNICULI* IN PET RABBITS IN NORTH-CENTRAL ITALY

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Abstract

This study determined the seroprevalence of *Encephalitozoon cuniculi* in pet rabbits from north-central Italy and correlated it with data (age and clinical signs) obtained from private veterinary practitioners. During the period 2007 to 2010, 826 pet rabbit sera were collected and tested using carbon immunoassay. In total, 310 out of 826 rabbits (37.53%, 95% CI: 34.23 to 40.94) displayed clinical signs consistent with infection, whereas the remaining 516 animals presented for wellness examination or to be vaccinated. The results were analyzed according to age, serological status, and the presence or absence of clinical signs. Seropositive results were observed in 59.56% (95% CI: 56.12 to 62.92) of the examined animals. Seroprevalence in the clinically ill rabbits was 70.65% (95% CI: 65.18 to 75.59), much higher than in healthy animals, which showed a prevalence of 52.91% (95% CI: 48.5 to 57.27). Clinical signs were classified into 4 groups: nonvestibular neurologic signs, vestibular signs (including head tilt), renal failure, and ocular lesions. Odds of animals presenting with clinical disease signs were higher in seropositive rabbits (odds ratio = 2.14, 95% CI: 1.59 to 2.9), and this association was particularly true for vestibular signs (odds ratio = 4.1, 95% CI: 2.48 to 7.13). The prevalence values were different with regard to age, with a peak in 3- to 4-year-old animals. Average titers in both healthy and clinically ill rabbits at various ages were different; rabbits with overt disease signs had higher titers when very young (<3 months) and from 1 to 4 years of age. In conclusion, this study provides more precise indications of the prevalence of *E. cuniculi* in pet rabbits in Italy, underlying growing interest and concern among veterinarians and pet owners regarding this organism.

Key words: rabbit; *Encephalitozoon cuniculi*; serology; CIA test; Italy

Encephalitozoon cuniculi is a Gram-positive, obligate intracellular microsporidium that can infect a wide range of mammals, including rodents (e.g., guinea pigs, mice, hamsters, rats), rabbits, horses, carnivores, and humans. The organism is considered an opportunistic pathogen of immunocompromised individuals. Infected rabbits excrete 1.5- to 2.5-μm spores intermittently in urine, with primary exposure occurring horizontally by direct contact or contamination (ingestion of contaminated food or water), or rarely by inhalation or intrauterine transmission.[1] Once ingested, the fungus reaches the internal organs via blood flow and is disseminated

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1557-5063/15/2101-$30.00

http://dx.doi.org/10.1053/j.jepm.2015.12.004

first to the kidneys, liver, lungs, and heart, with the kidneys and (subsequently) brain being the preferred sites of replication.[2] During intrauterine transmission, the spores can also be trapped in the anterior lens capsule.[3]

Rabbit encephalitozoonosis is a persistent infection that has been largely studied and controlled in experimental animal breeding facilities. According to our previous studies, infection is widespread in farmed meat rabbits, with over 50% prevalence.[4,5] Infection can cause both significant animal suffering and considerable financial loss.[6,7] In wild rabbits, *E. cuniculi* is less prevalent[8] or totally absent,[9] (Lavazza, unpublished data), possibly owing to a lower animal population density in nature.[10] In contrast, the seroprevalence rates are usually high in pet rabbit populations, with 37% to 68% of the pet population seropositive.[11-13] The increasing frequency of clinical signs in pet rabbits has generated an interest in treatment options for animals diagnosed with *E. cuniculi*.[14] No uniform treatment protocol exists for rabbits with presumptive encephalitozoonosis, as clinical manifestation varies. Although no drugs have been approved for the treatment of *E. cuniculi* infection in rabbits, benzimidazolic compounds (albendazole and fenbendazole) have been shown to prevent and treat *E. cuniculi* infections in rabbits[15] and are commonly employed.

This study focused on the determination of the seroprevalence for *E. cuniculi* in pet rabbits in north-central Italy and correlating the prevalence of this organism with anamnestic data, as age and clinical signs, obtained from private veterinary practitioners through the compilation of anamnestic sheets.

METHODS

Sampling

Between November 2007 and December 2010, samples from 826 pet rabbits were analyzed with only pretherapy antibody tests included for evaluation. All samples were accompanied by a data form reporting both the available information provided by the pet owner (pet nickname, age, sex of the rabbit, and town and province of the owner) and the anamnestic data (e.g., clinical signs, hematological values, previous serological results, and therapy) obtained by the veterinarian during evaluation.

Serological Analysis

All the sera were examined with carbon immunoassay (CIA)[16] using a commercial kit produced and distributed by Medicago AB, Uppsala, Sweden. The antigen consisted of 3×10^7 spores/mL of heat-inactivated *E. cuniculi*, which was then washed and suspended in phosphate buffer saline containing 0.1% formalin. The carbon suspension consisted of microscopic particles capable of binding nonspecifically to the immunoglobulin subtype G (IgG) of various types of mammals. The positive reference serum was hyperimmune rabbit serum to which 0.1% NaN_3 was added and used at the minimum dilution of 1:20. The sera were not inactivated and were directly assayed starting from 1:40, double the minimum significant dilution.[16] In fact, according to Suter et al.,[15] this should be considered the positive threshold titer for *E. cuniculi*. Each serum was examined at 2-fold serial dilution till the determination of the final titer. The CIA test consisted of an initial contact of an equal quantity (5 μL) of the examined sera and the antigen in a U-shaped microtiter well for 5 minutes. Then, 5 μL of this mixture was placed in contact with an equal quantity of carbon suspension on a slide and then covered. The observation was carried out 5 minutes later, at a magnification of ×600 with an ordinary light microscope. With positive sera antibodies, dark-grey spores were observed against the background of carbon particles, and if negative, translucent white on a brown background was detected. The microscopic examination was performed with an estimated count of the number of carbon-colored spores, and the titer of each serum was expressed as the highest dilution of serum at which approximately 50% of the spores were positively stained.

Statistical Analysis

Prevalence values and odds ratios (OR) were estimated with relative 95% confidence intervals (CI). Chi-squared tests were used to assess the significance of the ORs. Comparisons of prevalence levels between age classes and clinical status were performed by logistic regression. An interaction term was added to evaluate if the effect of age on seroprevalence was different in clinically ill animals compared to healthy individuals. Differences in average serological titer between age classes and clinical status were assessed using linear regression model. $P < 0.05$ (2-tailed) were