ACUTE PHASE PROTEIN LEVELS IN RABBITS WITH SUSPECTED *ENCEPHALITOZOON CUNICULI* INFECTION

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Abstract

The objective of this study was to evaluate the application of acute phase protein assays for C-reactive protein (CRP), haptoglobin (HP), and serum amyloid A (SAA) in the diagnosis of *Encephalitozoon cuniculi* (ECUN) infection in pet rabbits. Serum samples from 48 pet rabbits were submitted from veterinary clinics within the United States. Participating veterinarians completed a questionnaire that was used to classify rabbits as either non-ECUN suspect ($n = 19$) or suspected of having ECUN infection ($n = 29$). A previously described enzyme-linked immunosorbent assay diagnostic test was used to detect immunoglobulin G (IgG) titers against ECUN. Samples were additionally tested for levels of CRP, HP, and SAA. A nearly 10-fold mean increase in CRP levels was observed in the ECUN-suspect group. This increase was significant ($P < 0.05$). There was no significant difference in HP or SAA levels between the clinical groups. These data support the use of CRP as an adjunct test in the diagnosis of ECUN infection in pet rabbits.

Key words: acute phase protein; C-reactive protein; *Encephalitozoon cuniculi*; haptoglobin; protein electrophoresis; serum amyloid A

Infection of the obligate intracellular microsporidian *Encephalitozoon cuniculi* (ECUN) in rabbits can result in subclinical and clinical diseases.[1-5] Notably, there is considerable evidence indicating that ECUN and other microsporidia are actually early divergent members of the fungi taxonomy.[6,7] There are 3 main manifestations of clinical disease caused by ECUN: neurological, ocular, and renal. The common neurological manifestation often presents with a head tilt and ataxia, which necessitates the inclusion of otitis media/interna, toxoplasmosis, viral infection, lymphoma, inherited abnormalities, toxicities, and central nervous system lesions as differential diagnoses.[8] Ocular presentation of uveitis may result from ECUN or other infections.[9] Renal disease associated with clinical signs of polyuria and polydypsia may also be secondary disease conditions that result from ECUN, urinary stone formation, or bacterial infection.[10] Some inflammatory lesions are identified in clinically normal rabbits that are subsequently diagnosed with subclinical disease conditions related to ECUN infection.[11] Postmortem diagnosis of ECUN is often difficult in infected rabbits because spores are infrequently detected by routine histological examination techniques, although detection of ECUN in tissues can be aided by immunohistochemical testing.[12]

Diagnosis of ECUN infection is difficult because of the presence of high anti-ECUN immunoglobulin G (IgG) titers in clinically normal rabbits; seroconversion may represent acute infection, subclinical infection, or exposure.[13,14] In a previous study, although samples from ECUN-suspect rabbits demonstrated 1.7-fold higher IgG titers than clinically normal or non-ECUN-suspect rabbits, the latter groups had mean titers more than 1:700.[15] It has also been determined that IgG titers are especially long lived in rabbits.[16] Thus, although positive titers are

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understandably difficult to interpret, a negative titer may be helpful in ruling out ECUN as an apparent cause of the patient presenting with the clinical disease condition. Recently, IgM titers have been described as a correlative immune response to ECUN in infected rabbits, and IgM-positive serostatus has also been previously described in experimentally infected rabbits.[17-19] A positive IgM serostatus for ECUN in rabbits is an encouraging diagnostic possibility, given the expected early expression of IgM that then fades as IgG class switching occurs and the immune response matures.

The use of protein electrophoresis (EPH) as an adjunct test in the diagnosis of ECUN infection in rabbits has been previously described.[15] In that study, clinically abnormal rabbits had higher concentrations of gamma globulins than rabbits with no overt disease conditions. Although EPH provides valuable information on the concentration of albumin and the globulin fractions, it does so as a broad measure of the acute phase response.[20,21] Analysis of specific acute phase proteins (APP) has been the focus of many studies in veterinary medicine.[22-26] Many specific APP assays are available that can provide increased sensitivity for the detection of underlying inflammatory processes.[27] In the current study, C- reactive protein (CRP), haptoglobin (HP), and serum amyloid A (SAA) levels were quantitated in samples from non-ECUN-suspect and ECUN-suspect rabbits. The purpose of this investigation was to assess whether APP quantitation may aid in the diagnosis of ECUN infection in rabbits.

MATERIALS AND METHODS

Animals and Sample Collection

Blood samples from pet rabbits were submitted from veterinarians within the United States to the reference laboratory of the Division of Comparative Pathology at the University of Miami, Miller School of Medicine (Miami, FL USA). Veterinarians completed a questionnaire that provided general patient information, clinical signs, clinical diagnosis, and response to treatment, which could be inclusive of benzimidazoles, antibiotics, and anti-inflammatory therapeutic agents. Additional information was provided after diagnostic test results were obtained, including any definitive test results (e.g., necropsy) and response to traditional ECUN-based treatment. Samples were categorized as either non-ECUN suspect ($n = 19$) or ECUN suspect ($n = 29$). The non-ECUN-suspect group included animals that presented with similar clinical disease signs as ECUN-infected rabbits but were diagnosed with otitis media/interna or bacterial infections. ECUN-suspect animals included those with clinical signs consistent with ECUN (e.g., head tilt, ataxia, paresis, urinary incontinence, and ocular changes) and were ultimately diagnosed with the disease. Specifically, there were 13 (44.8%) animals with neurological signs, 6 (20.7%) animals with ocular signs, and 10 (34.5%) animals with renal signs. All animals in the study presented with a recent onset of clinical disease signs that have been associated with ECUN. In the ECUN-suspect group, a positive response to ECUN treatment regimens (e.g., benzimidazoles) was also observed and the participating clinician confirmed that the working diagnosis was ECUN infection. Two (6.9%) animals were strongly suspected to have ECUN infection because of the presence of severe inflammatory lesions found on histological examination of tissues collected during the necropsy procedure; spores were observed by histological examination in the eye of one additional animal. Non-ECUN-suspect animals responded to treatment regimens recommended for their specific diagnosed disease conditions (e.g., otitis media/interna and bacterial infections). All enzyme-linked immunosorbent assay (ELISA) assays were performed within 24 hours after refrigeration of collected samples. The samples were then stored in freezer-appropriate vials at −20°C until CRP, HP, and SAA testing was performed.

ELISA

The ELISA was performed as previously described.[15] Briefly, commercially available ELISA plates (Charles River Laboratories, Wilmington, MA USA) were used, which contain paired wells with ECUN antigen and negative-control antigen. Samples were serially diluted starting at 1:32. Horseradish peroxidase–conjugated anti–rabbit IgG (Sigma Chemical Corporation, St. Louis, MO USA) was used as the antibody conjugate and ABTS (Kirkegaard & Perry Laboratories, Gaithersburg, MD USA) as the substrate. Absorbance was determined at 405 nm using a Molecular Devices plate reader (Sunnyvale, CA USA). Titers were determined based on the index of reactivity by dividing the absorbance of the ECUN antigen well by the absorbance of the negative-control antigen well; an index of >1.5 was considered a positive reaction.

CRP

CRP levels were measured using an automated analyzer (RX Daytona Analyzer, Randox,