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THE METHOD OF RADIAL HEMOLYSIS FOR THE DETECTION OF ANTIBODIES TO ENCEPHALITOZOON CUNICULI ANTIGENS

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The method of radial hemolysis for the early detection of antibodies to Encephalitozoon cuniculi antigens was tested in rabbits. Spores obtained from tissue culture were used as the antigen. The method for determining antibodies by indirect fluorescence was used to check the specificity of radial hemolysis. Twelve rabbits with dubious and 30 with positive reactions were found by the method of radial hemolysis. The titres 1:64 and 1:128 were found in 18 (12 dubious and 6 positive by radial hemolysis) and 24 positive rabbits respectively, by the method of indirect immunofluorescence. The results of our tests indicate that radial hemolysis is suitable for use in the screening of encephalitozoonosis in rabbits.

Key words: rabbit, Encephalitozoon cuniculi, radial hemolysis test, indirect immunofluorescent antibody test

INTRODUCTION

Encephalitozoon cuniculi is a microsporidum pathogen in rabbits and other mammals, where it may induce chronic, usually latent disease (Stewart et al., 1988). The disease has great importance as a factor which complicates the interpretation of results of experimental work in laboratory animals (Canning and Lom, 1986). The selection of a suitable diagnostic method is of great importance in the diagnosis of encephalitozoonosis. The fact that antibodies to 9-10 homologous polypeptide E.cuniculi antigens were found after experimental infection of rabbits with E. cuniculi (Wicher et al.,1991) suggests that a humoral immune response may be a specific response to the infecting organism. After the detection of specific antibodies in the serum of animals examined, the culling of infected animals is possible. Moreover, it is possible to find animals suitable for breeding purposes by the method of serological diagnosis.

The aim of our work was to show the possibilities of using simple radial hemolysis in the serological diagnosis of microsporidial infections in mammals.

MATERIAL AND METHODS

Sera. Serum samples (97) came from domestic rabbits *(Oryctolagus cuniculus f. domestica)* reared on small breeding farms and in the experimental

animal breeding laboratory. The rabbit serum was obtained after taking blood from the marginal ear vein.

The isolation of E. cuniculi antigens. The spores of E. cuniculi, kept in green monkey cell cultures ("VERO E6") in RPMI medium with addition of 5% bovine serum (Vavra et al., 1972), were used as the antigen (titre min 64). The infected cellular culture was kindly provided by Ditrich (Parasitological Institute, Czech Academy of Science, Ceske Budejovice, Czech Republic).

Guinea pig lyophilized complement and ovine rinsed stabilized erythrocytes. Guinea pig lyophilized complement and ovine rinsed stabilized erythrocytes were obtained from Bioveta (Ivanovice na Hane. Czech Republic). Pig anti-rabbit antibodies conjugated with fluorescein isothiocyanate(SWAR/FITC) were obtained from the Institute of Sera and Vaccines (Prague, Czech Republic).

Determination of hemolytic antibodies. The method of radial hemolysis of antigen-bound ovine erythrocytes in agar was used to determine hemolytic antibodies (Mancini, 1965; Hiramoto, 1971). Briefly, a 5% suspension of ovine erythrocytes was prepared from a stock of ovine erythrocytes.The antigen was bound to erythrocytes by the addition of 0,5 ml of antigen (spore content 8×10^6 $ml^{-1)}$ to 3 ml of a 5% suspensio of erythrocytes. The mixture was incubated for 60 minutes at laboratory temperature, and then during the night at 4^0C. This mixture was placed on a Petri dish of diameter 6 cm. According to the template prepared, using a needle of 2mm diameter the gel was sucked out of the plate by a pump through a capture flask. Sera tested with the titre of 1:64 (inactivated) and controls (positive and negative) were added to the wells. Samples were incubated in a moist chamber for 44 h at 4^0C. After incubation, commercial guinea pig complement, diluted 1:10 was added to the wells. Results were read against a light background by evaluating the hemolytic zone diameters round the wells. An adapted micrometric gauge was used for the precise measurement of each zone in milimetres. Zone diameters up to 6.75 mm were evaluated as a negative result, and those above 6.75 mm as a positive one (Bodnar et al., 1992).

The method ofindirect immunofluorescence of antibodies to E. cuniculi antigens. The method of indirect immunofluorescence of antibodies to E. cuniculi antigens was used according to Chalupsky et al. (1973). The spores of E. cuniculi obtained from a supernatant of monkey kidney cell culture suspension ('VERO" E6) were used as the antigen. Commercial pig anti-rabbit antibodies were conjugated with fluorescein isothiocyanate(SwaR/FITC). Following the instructions recommended by the producer, the conjugate was diluted with distilled water.

RESULTS

The detection of hemolytic antibodies by the method of radial hemolysis. Hemolytical antibodies to the E. cuniculi antigens bound in agar to ovine erythrocytes were detected by the formation of hemolytical zones round the wells with the serum tested. The animals evaluated as positive were those whose sera with the IFA titre 1:64 and higher formed hemolytic zones with a diameter above 6.75 mm. Results of the serological determination of hemolytic antibodies are illustrated in Table 1.

Table 1. Results of the serological determination of antibodies by the method of radial hemolysis

Diameter of hemolytical zone (mm)	Result evaluation	Number of animals	%
< 6.75	–	55	56.70
6.76 – 7.99	+	12	12.37
> 8.00	+	30	30.92

-negative result; + dubious result;++ positive result

The detection of antibodies to antigens of E. cuniculi by the method of indirect immunofluorescence. The method of indirect immunofluorescence of antibodies to antigens of E. cuniculi was used for comparison of the seropositivity of individual samples with the results achieved by radial hemolysis in the same animals. The animals evaluated as positive were those whose sera with the titre 1:64 reacted visually to the spores with peripheral fluorescence. The results of the serological determination of antibodies by the method of indirect immunofluorescence are given in Table 2.

Table 2. Results of the serological determination of antibodies by the method of indirect immunofluorescence

Titre of antibodies	Result evaluation	Number of animals	%
< 1 : 32	–	55	56.70
1 : 64	+	18	18.56
1 : 128	+	24	24.74

-negative result; + dubious result; ++ positive result

Comparison of the results of both methods. Sera from ninety-seven animals were examined by method of radial hemolysis and indirect immunofluorescence, individually. Twelve rabbits with dubious and 30 with positive reactions were found by the method of radial hemolysis. The titres 1:64 and 1:128 were foundin 18 (12 dubious and 6 positive by radial hemolysis) and 24 positive rabbits respectively, by the method of indirect immunofluorescence.

DISCUSSION

Several methods have been developed for diagnosing encephalitozoonosis in rabbits, such as the method of indirect fluorescence of antibodies (Chalupsky et al., 1973; Jackson, 1972), complement fixation method (Wosu et al., 1977), immunoperoxidase test (Gannon, 1978), the method of indirect microagglutination (Shadduck and Geroulo, 1979) and the method of enzymatic immunoassay (Cox et al., 1981, Hollister and Canning, 1987).

In our study, both the method of indirect immunofluorescence and that of radial hemolysis detected antibodies against E. cuniculi in 42 rabbits. The few differences in the number of animals involved with dubious or positive cases can be explained by the differences in evaluation and the principle of dual testing.

The method of radial hemolysis determines hemolytic antibodies to the antigens bound to ovine erythrocytes. Lysis of erythrocytes was observed in a

case of seropositivity. The method of radial hemolysis, is simpler and more econimical in comparison with other procedures for serological diagnostics of encephalitozoonosis. The method of indirect fluorescence was used for evaluating and comparing the precision of the method of radial hemolysis.

The results of preliminary serological examinations of rabbits in Slovakia indicate that the method of radial hemolysis is suitable for use in the screening of encephalitozoonosis in rabbits.

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METOD RADIJALNE HEMOLIZE ZA OTKRIVANJE ANTITELA PROTIV ANTIGENA ENCEPHALITOZOON CUNICULI

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SADRŽAJ

Metod radijalne hemolize za rano otkrivanje antitela protiv antigena Encephalitozoon cuniculi testiran je na kunićima. Utvrđeno je da se primenjena metoda može sa velikim uspehom koristiti za otkrivanje i izolaciju obolelih kunića.