Acta vet. scand. 1982, *23*, 99—106.

From the National Veterinary Institute, Oslo, Norway.

ENCEPHALITOZOONOSIS IN THE BLUE FOX

COMPARISON BETWEEN THE INDIA-INK IMMUNOREACTION AND THE INDIRECT FLUORESCENT ANTIBODY TEST IN DETECTING ENCEPHALITOZOON CUNICULI ANTIBODIES

By

Svein Fredrik Mohn

MOHN, S. F.: *Encephalitozoonosis in the blue fox — Comparison between the india-ink immunoreaction and the indirect fluorescent antibody test in detecting Encephalitozoon cuniculi antibodies.* Acta vet. scand. 1982, *23*, 99—106. — Sera from 32 foxes sampled at intervals varying from 20 to 70 days after oral inoculation with E. cuniculi spores were tested by the india-ink immunoreaction (IIR) and the indirect fluorescent antibody test (IFAT). Using the IFAT, antibodies were detected at low levels in sera sampled on days 20 and 29 post inoculation, whereas the IIR failed to reveal antibodies in the same sera. In sera sampled from day 35 until day 70 post inoculation, antibodies were detected by both tests, the IIR-titres reaching the magnitude of the IFAT-titres after about 50 days post inoculation.

In 14 sera sampled from foxes of at least 46 days of age and with signs of encephalitozoonosis, the tests gave almost identical results.

Comparing IIR- and IFAT-determined antibody titres using E. cuniculi antigens of blue fox and rabbit origin in the test, the antigens seemed to be closely related, supporting the suggestion that the isolates are strains of the same microsporidian species.

blue fox; rabbit; encephalitozoonosis; immunoreaction; fluorescent antibody.

Encephalitozoon cuniculi (E. cuniculi) is an intracellular microsporidian parasite affecting a variety of mammalian species including man (*Wilson* 1979). During recent years encephalitozoonosis has occasionally caused heavy losses among pups and young blue foxes (Alopex lagopus) in Norway (*Nordstoga et al.* 1974, *Nordstoga & Westbye* 1976). The indirect fluorescent antibody test (IFAT) established for the detection of humoral antibodies to E. cuniculi is a specific and sensitive, although time consuming method for diagnosing the infection in live foxes (*Mohn & Ødegaard* 1977). The india-ink immunoreaction (IIR) is a rapid and simple method for the detection of circulating E. cuniculi antibodies in rabbits. It shows complete correlation of results with the IFAT (*Waller* 1977). The IIR is not applic-

able for rat and mouse serum, but has been reported to be useful on serum from the blue fox (*Kellett & Bywater* 1980). From experience with the method in our laboratory, it seems to have some advantages to the IFAT for the diagnosis of blue fox encephalitozoonosis.

In the present study, the prevalence of antibodies to E. cuniculi in a series of sera sampled from some spontaneously — and some experimentally E. cuniculi infected foxes were determined by the IIR and the IFAT. The sera were tested against 2 different antigens prepared on strains of rabbit and blue fox origin. The results were compared in order to determine the sensitivity and reliability of the IIR applied to blue fox serum.

MATERIAL AND METHODS

Tests

Sera were examined for E. cuniculi antibodies by the IFAT (*Mohn & Ødegaard* 1977) and by the modified IIR (*Kellett & Bywater* 1978). The IFAT-titres were expressed as the reciprocal values of the highest serum dilutions showing distinct fluorescence on the periphery of most of the spores within a microscopic field and IIR-titres as the reciprocal values of the highest serum dilutions showing more than 5 % spores stained by carbon particles of at least 200 spores examined.

Sera

A total of 51 sera from 34 foxes were tested. One series comprised 32 samples collected at intervals from 20 to 70 days after oral administration of E. cuniculi spores to 15 pups varying in age from 2 to 14 days. Another series of sera was taken from 19 foxes between 46 days to 2 years of age. Thirteen of these sera were collected from foxes with experimentally induced encephalitozoonosis (*Mohn*, unpublished), 1 sample originated from a spontaneous case, whereas 5 sera were collected from apparently healthy foxes without detectable clinical signs of encephalitozoonosis. In the IFAT the sera were screened undiluted and diluted 1:10 in phosphate buffered saline (PBS) pH 7.2. Positive sera were tested by serial twofold dilutions, initial dilution being 1:25. In the IIR the samples were screened on a 1:10 dilution of the serum (final dilution 1:30) prepared in 0.15 mol/l saline solution containing 0.1 % sodium azide. Positive sera were tested by serial twofold dilutions, 1:25 being the initial dilution.

Antigens

The 32 serum samples from pups orally inoculated with E. cunciuli were tested against an antigen made from a strain of E. cuniculi isolated from a spontaneous case of blue fox encephalitozoonosis. The parasite was propagated in monolayer cell cultures (*Mohn et al.* 1981), and the antigen prepared and standardized according to the procedures described by *Waller* (1977). The other sera were tested against the same antigen and against an antigen prepared from a strain of E. cuniculi isolated from rabbit and supplied commercially by Testman, Uppsala, Sweden.

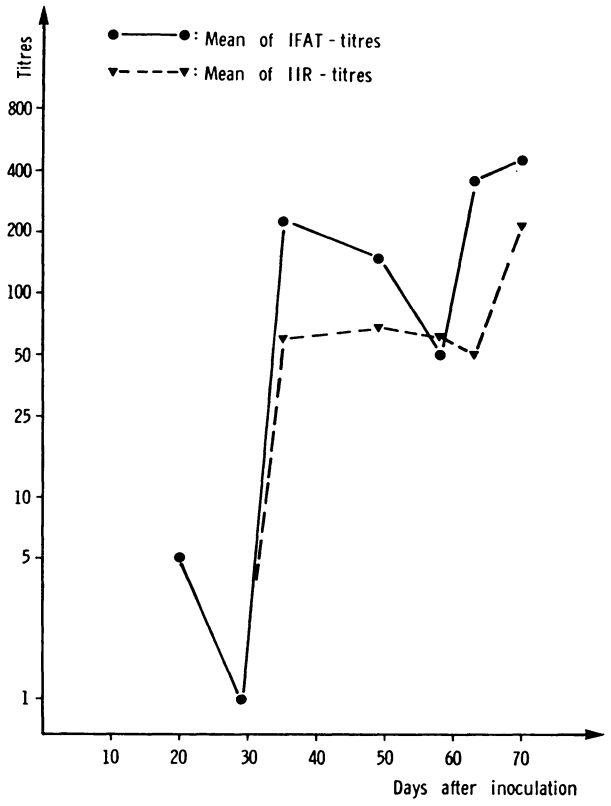

Figure 1. E. cuniculi antibody titres detected by the indirect fluorescent antibody test (IFAT) and by the india-ink immunoreaction (IIR) in 32 blue fox sera sampled at various intervals after oral inoculation of 15 pups with E. cuniculi.

RESULTS

The mean antibody titres determined by the IFAT and the IIR on 32 sera from 15 pups orally inoculated with E. cuniculi are given in Fig. 1 and ranged from 1 to 1600 and from < 10 to 400, respectively. Low level titres were detected by IFAT in 2 sera sampled on day 20 and in 4 sera sampled on day 29 post inoculation, whereas antibodies could not be detected by IIR in the same samples. In the samples collected on days 35, 49, 58, 63 and 70 post inoculation, E. cuniculi antibodies were detected by both tests.

In the sera from clinically healthy foxes examined by the IFAT and the IIR for antibodies against the 2 different antigens, no E. cuniculi antibodies were detected. In all the 14 sera sampled from foxes not less than 46 days of age and suffering from encephalitozoonosis, E. cuniculi antibodies were detected by both

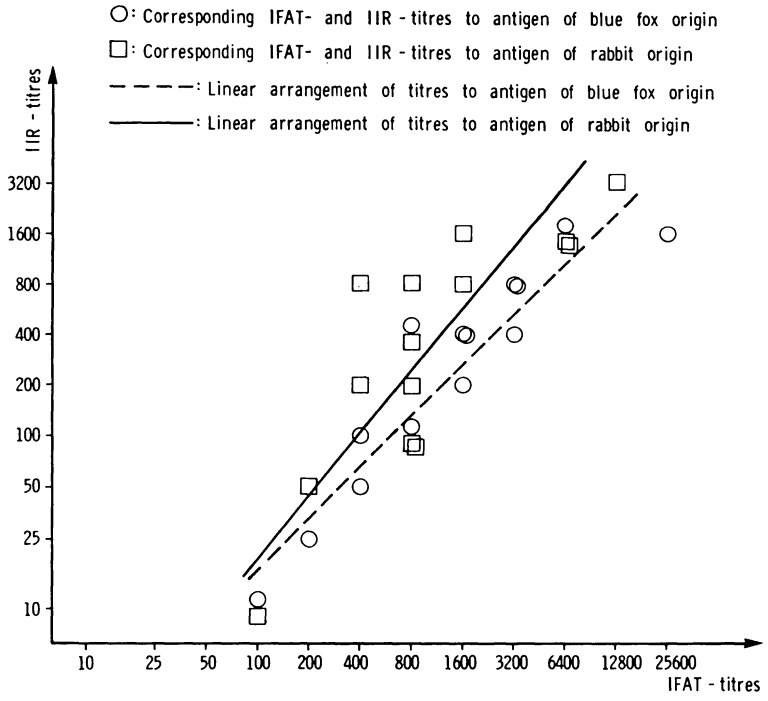

Figure 2. The indirect fluorescent antibody test (IFAT) and the india-ink immunoreaction (IIR) in detecting antibodies to E. cuniculi antigens of rabbit and blue fox origin, respectively, in sera from foxes of at least 46 days of age.

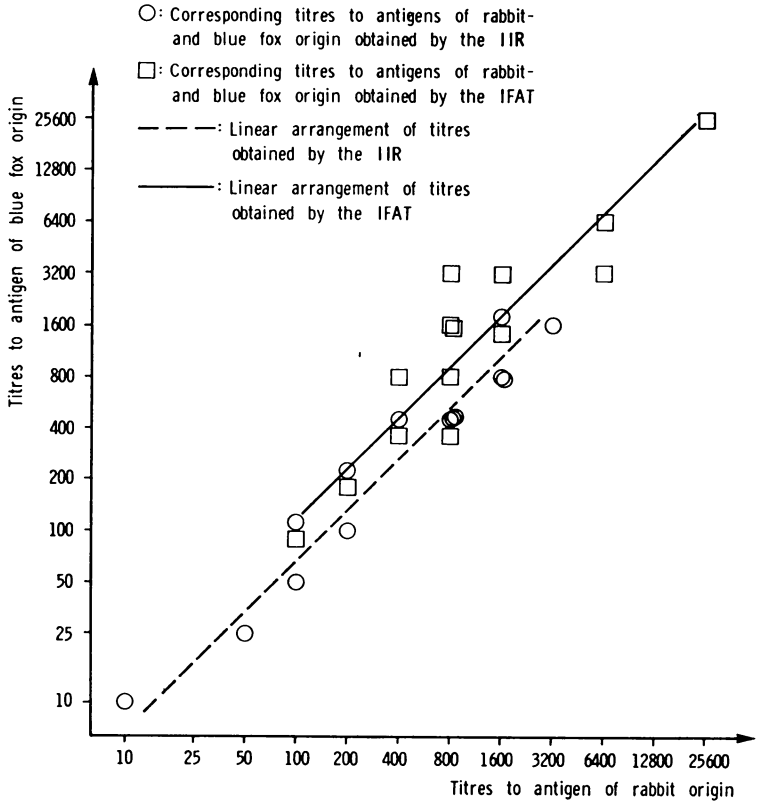

Figure 3. Comparison between E. cuniculi antigens of rabbit and blue fox origin based on antibodies detected by the indirect fluorescent antibody test (IFAT) and the india-ink immunoreaction (IIR), respectively, in sera from foxes of at least 46 days of age.

tests. The titres ranged from 10 to 3200 and from 100 to 25.600 using the IIR and the IFAT, respectively. The antibody titres recorded against the 2 different antigens are given in Figs. 2 and 3. In Fig. 2 the IFAT and the IIR are compared according to the titres detected in the various samples. The regression lines indicate approximately linear arrangement of the titres.

In Fig. 3 the antibody titres against the 2 antigens are compared according to the titres obtained by the IFAT and the IIR, respectively. The regression lines indicate the approximately linear arrangement of the results.

DISCUSSION

The IFAT with rabbit-anti-fox-gammaglobulin conjugate makes it possible to detect specific humoral E. cuniculi antibodies within the gammaglobulin fraction, mainly the IgG and the IgM classes (*Waller et al.* 1978). The mechanism of the IIR adapted to rabbit serum is based on the assumption that the carbon particles in the ink attach to the heavy chains of the IgG molecules which specifically react with homologous antigenic sites on the surface of the E. cuniculi spores. The IIR therefore detects IgG only (*Waller et al., Bywater & Kellett* 1979). The mechanism of the IIR adapted to fox serum has not been investigated. However, it is likely to be similar to that suggested for rabbit serum. As the antibody response in the fox early after infection probably is more confined to the IgM than to the IgG, as shown for the rabbit (*Waller et al.*), the IFAT may detect E. cuniculi immunoglobulins at an earlier stage than the IIR in the course of the infection. The results recorded in Fig. 1 show specific antibodies detected by IFAT on day 20 post inoculation, whereas the IIR failed to detect antibodies in sera earlier than 35 days post inoculation. These findings correspond with the results reported on sera from orally inoculated rabbits (*Waller et al.*). At the early stage of the infection the titres recorded by IFAT are on a higher level than those recorded by IIR, while later in the course of the infection the 2 tests seem to show titres almost of the same magnitude (Fig. 1), as the antibodies gradually increase in the IgG class and decrease in the IgM class.

The minimum serum dilution in the IIR was 1:10 (final dilution 1:30). Dilutions beneath this level induced spontaneous agglutination of the carbon particles in the ink, as described for rabbit serum (*Waller* 1979), making the test difficult to read. Theoretically lower levels of IgG can be detected by the IFAT than by the IIR as the IFAT is applicable on undiluted serum. For the purpose of detecting seropositive individuals in a fox population the difference between the tests in ability to detect antibodies seems to be of less practical importance if the control programme is based on repeated testing with monthly intervals.

E. cuniculi antibodies were not detected in sera from 5 healthy foxes, the results showing complete correlation between the 2 tests. The positive sera originated from foxes which most likely had been infected in utero (*Mohn et al.* 1982). At sampling the individuals were at least 46 days of age. At this stage of the infection the antibodies are expected to be found mainly

in the IgG fraction. As shown in Fig. 2, corresponding IFAT- and IIR titres run almost linearly, especially in the tests with antigen of fox origin. This finding indicates good correlation between the tests. When the 2 tests are to be compared in respect of sensitivity, a correction factor of 3 for IIR-titres should be taken into account. When antigen of fox origin is used, the IIR appears slightly less sensitive than the IFAT, whereas the tests seem to be almost equal with respect to sensitivity when run with rabbit antigen.

The antigens of fox and rabbit origin seem to be closely related as the corresponding titres in the various sera detected by the 2 tests appear along lines which almost coincide with the ideal 45° line for complete correlation (Fig. 3). The fox strain has been shown to be morphologically identical with microsporidian parasites previously isolated from a variety of other mammalian species (*Mohn et al.* 1981). The close antigenic relationship between the parasites of fox and rabbit origin also supports the suggestion that these isolates are strains of the same parasite species.

In conclusion the IIR seems to be a reliable and specific test for the detection of humoral E. cuniculi antibodies in the blue fox. The IIR is rapid and technically simple, requiring uncomplicated reagents and a minimum of laboratory equipment. This test shows almost complete correlation with the IFAT in the detection of antibodies in sera sampled from individuals of at least 46 days of age, whereas the IFAT has advantage in detecting antibodies at an early stage of the infection.

REFERENCES

Bywater, J. E. C. & B. S. Kellett: Humoral immune response to natural infection with Encephalitozoon cuniculi in rabbits. Lab. Anim. 1979, *13*, 293—297.

Kellett, B. S. & J. E. C. Bywater: A modified india-ink immunoreaction for the detection of encephalitozoonosis. Lab. Anim. 1978, *12*, 59—60.

Kellett, B. S. & J. E. C. Bywater: The indirect india-ink immunoreaction for detection of antibodies to Encephalitozoon cuniculi in rat and mouse serum. Lab. Anim. 1980, *14*, 83—86.

Mohn, S. F. & Ø. A. Ødegaard: The indirect fluorescent antibody test (IFAT) for the detection of Nosema cuniculi antibodies in the blue fox (Alopex lagopus). Acta vet. scand. 1977, *18*, 290—292.

Mohn, S. F., T. Landsverk & K. Nordstoga: Encephalitozoonosis in the blue fox — Morphological identification of the parasite. Acta path. microbiol. scand. Sect. B 1981, *89*, 117—122.

Mohn, S. F., K. Nordstoga & O. M. Møller: Experimental encephalitozoonosis in the blue fox — Transplacental transmission of the parasite. In press 1982.

Nordstoga, K., S. F. Mohn & G. Loftsgård: Nosematose hos blårev. (Nosematosis in the blue fox). Proc. 12th Nordic Vet. Congr., Reykjavik 1974, pp. 183—186.

Nordstoga, K. & Kr. Westbye: Polyarteritis nodosa associated with nosematosis in blue foxes. Acta path. microbiol. scand. Sect. A 1976, *84*, 291—296.

Waller, T.: The india-ink immunoreaction: a method for the rapid diagnosis of encephalitozoonosis. Lab. Anim. 1977, *11*, 93—97.

Waller, T.: Serology and sensitivity of Encephalitozoon cuniculi in relation to diagnosis and control of encephalitozoonosis in domestic rabbits. Thesis, Swedish University of Agriculture Sciences, Uppsala 1979.

Waller, T., B. Morein & E. Fabiansson: Humoral immune response to infection with Encephalitozoon cuniculi in rabbits. Lab. Anim. 1978, *12*, 145—148.

Wilson, J. M.: The biology of Encephalitozoon cuniculi. Med. biol. 1979, *57*, 84—101.

SAMMENDRAG

Encephalitozoonose hos blårev — Sammenligning mellom india-ink immunoreaksjonen og den indirekte fluoreserende antistofftest for påvisning av Encephalitozoon cuniculi antistoffer.

Sera fra 32 blårever tatt ut i tidsrommet 20 til 70 dager etter oral podning med Encephalitozoon cuniculi sporer ble undersøkt ved hjelp av india-ink immunoreaksjon (IIR) og indirekte fluoreserende antistofftest (IFAT). I sera tatt ut på 20. og 29. dagen etter podning ble det påvist lave E. cuniculi-antistofftitre ved hjelp av IFAT, mens det i de samme sera ikke kunne påvises tilsvarende antistoffer med IIR. I sera tatt ut i perioden 35 til 70 dager etter podning ble det påvist E. cuniculi-antistoffer ved hjelp av begge testene. Antistofftitrene var i begynnelsen av perioden lavere ved bruk av IIR enn når IFAT ble benyttet, men fra omkring 50 dager etter podning påviste begge testene titre av omtrent samme størrelsesorden.

I 14 sera tatt fra rever minimum 46 dager gamle og med tydelige tegn på encephalitozoonose var det god overensstemmelse mellom resultatene av de 2 testene.

Sammenligning av antistofftitre mot E. cuniculi-antigener fremstilt av parasitter isolert fra henholdsvis rev og kanin, tydet på at disse antigener ved bruk av både IFAT og IIR, er nær beslektet. Denne observasjon støtter antagelsen om at de isolerte parasitter er stammer av den samme mikrosporidieart.

(Received January 11, 1982).

Reprints may be requested from: S. F. Mohn, the National Veterinary Institute, P.O. Box 8156, Dep., Oslo 1, Norway.