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EXPERIMENTAL ENCEPHALITOZOONOSIS IN THE BLUE FOX

NEONATAL EXPOSURE TO THE PARASITE

By

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MOHN, S. F. and K. NORDSTOGA: *Experimental encephalitozoonosis in the blue fox — Neonatal exposure to the parasite.* Acta vet. scand. 1982, *23*, 344—360. — Newborn and young pups up to the age of 15 days were exposed to E. cuniculi, either by keeping the pups in cages together with orally inoculated foster-mothers and their offspring, or by oral inoculation with E. cuniculi spores. A majority of pups appeared sero-positive to E. cuniculi with the india-ink immunoreaction from 35 to 87 days post exposure; spores of E. cuniculi were detected in organs of some of the animals. The non-inoculated pups kept together with the orally inoculated pups became sero-positive from 49 to 129 days after the oral inoculations. However, the exposure of newborn and young pups failed to induce clinical encephalitozoonosis, and when killed at the time of pelting the body weights and fur quality appeared to be within the normal range in all exposed foxes. No macroscopic lesions were detected in the various organs. Histologically focal interstitial nephritis occurred in the great majority of the seropositive animals. Meningo-encephalitis was seen in some of the foxes, whereas slightly thickened walls of some arteries, mainly in the myocardium, were found in a few animals. The lesions of the brain and kidneys seem to be very similar to those seen in chronic cases of rabbit encephalitozoonosis. Polyarteritis nodosa and severe encephalitis and interstitial nephritis with extensive proliferations of plasma cells, which are almost constant findings in cases of clinically diseased foxes, were not detected in any of the subclinically infected animals. Various factors that might be of significance in the pathogenesis of the disease are discussed, and it is concluded that intrauterine infection of the pups via the transplacental route appears to be an essential supposition for the establishment of clinical fox encephalitozoonosis.

blue fox; encephalitozoonosis; neonatal exposure.

Encephalitozoon cuniculi (E. cuniculi) is an intracellular microsporidian parasite infecting homeothermic animals, including man (*Wilson* 1979). The parasite frequently infects

conventional rabbit colonies, usually with a subclinical course of disease. In rabbits the infection is generally accepted to be transmitted horizontally, almost invariably via the oral route, although evidence for vertical transmission via the placenta has been reported in some cases (*Hunt et al.* 1972, *Owen & Gannon* 1980). The natural mode of transmission of the parasite in other mammalian species is largely unknown. Vertical transmission has, however, been reported in cross-fostering studies with mice (*Perrin* 1943) and in a few cases of spontaneous encephalitozoonosis in the squirrel monkey (Saimiri sciureus) (*Anver et al.* 1972, *Brown et al.* 1973).

During the last decade encephalitozoonosis has occasionally caused great losses among young blue foxes (Alopex lagopus) in Norway (*Nordstoga* 1972, *Nordstoga et al.* 1974, *Nordstoga & Westbye* 1976). Vertical transmission of the parasite has been suggested to be the likely mode of infection in the blue fox, and a recent experiment has provided evidence for transplacental transmission in this species (*Mohn et al.* 1982).

The aim of the present experiment was to study the blue fox clinically, serologically, and post mortem after neonatal exposure of young pups to the parasite.

MATERIALS AND METHODS

Organism

A strain of E. cuniculi previously isolated from a blue fox that died from spontaneous encephalitozoonosis was propagated in monolayer cell cultures, as described elsewhere (*Mohn et al.* 1981).

Inoculates

The spore concentration in the cell culture medium was estimated according to the method described by *Waller* (1975). Volumes of 1 ml containing from 2.7×10^6 to 1.5×10^7 infective spores harvested from the cell culture medium were injected intraperitoneally into Swiss albino mice strain Bom: NMRI f(SPF). Ten days post injection some mice were killed and cut into pieces and fed to healthy vixens. From some of the other mice peritoneal exudate was harvested for oral inoculation of young blue fox pups, each pup receiving a dose of 0.75 ml undiluted exudate.

Vixens

Fifteen healthy E. cuniculi sero-negative vixens about 10 months of age were kept in wire mesh cages in a separate shed. Five of the vixens were inoculated orally with E. cuniculi by ingesting infected mice, as described elsewhere (*Mohn et al.* 1982), whereas the other vixens remained un-inoculated. The vixens were fed normal food and treated as the other vixens in the farm, including 2 matings with an interval of 2 days.

Pups

For individual identification, newborn pups were labelled by cutting the claws of various limbs. From the age of about 1 month the claw labelling was supplemented by tattooing the individual identification number inside an ear of each pup.

Cross-fostering trial

Group 1 comprised 32 pups borne and fostered by inoculated dams in 4 litters containing 4, 6, 6 and 16 pups, respectively. On the first day of life these pups were put together with newborn pups in 4 litters borne by non-inoculated mothers containing 5, 3, 4 and 4 pups, respectively.

Group 2 comprised 8 pups borne by 2 inoculated mothers. On the first day of life 1, 3 and 4 of these pups were transferred to 3 newborn litters of non-inoculated vixens containing 4, 7 and 9 pups, respectively.

Group 3 comprised 34 pups borne and fostered by non-inoculated dams in 5 litters containing 4, 4, 7, 9 and 10 pups, respectively. On the first day of life these pups were put together with newborn pups of 5 litters borne by inoculated mothers containing 1, 11, 3, 4 and 2 pups, respectively.

Group 4 comprised 16 pups of 4 non-inoculated vixens. On the first day of life 3, 4, 4 and 5 of these pups were transferred to 4 newborn litters of inoculated dams containing 6, 6, 16 and 4 pups, respectively.

At the age of about 8 weeks the pups were separated from their mothers and foster-mothers and divided into groups of 2—4 individuals in separate cages. Pups found dead or killed during the course of the experiment were examined for pathological

lesions. The remaining pups were euthanized at the normal pelting time during November, approximately 6 months of age, and a post-mortem examination was carried out on the skinned bodies.

Oral inoculation trial

Group 5 comprised 16 pups in 5 litters borne by non-inoculated dams. The pups were inoculated orally with E. cuniculi spores harvested from the cell culture medium or with mouse peritoneal exudate. Eight pups, 4 from each of 2 litters, were inoculated at the age of 1 day; 2 pups were inoculated when 5 days old, 2 pups when 12 days old, and 4 pups when 15 days old.

Group 6 comprised the remaining 30 non-inoculated pups of the 5 litters mentioned above: 8, 10, 2, 7 and 3 pups, respectively. The pups were kept together with the inoculated pups of Group 5, each litter in the same cage up to the age of about 8 weeks, when they were separated from their dams and divided into groups of 2—4 individuals in separate cages. At the age of 99 days 1 pup of Group 5 was killed for post-mortem examination. The remaining pups were euthanized and processed as described above.

Controls

Group 7 comprised 12 pups borne by 3 normal non-inoculated dams which had remained clinically healthy and sero-negative throughout the experimental period. The pups were selected at random and killed for necropsy at the normal time of pelting.

Clinical examinations

The pups in the experiment were observed closely, and signs of disease were recorded accordingly.

Serological examinations

Venous blood was sampled from the pups at intervals of 2—4 weeks; the first samples were taken from the pups in the cross-fostering experiment at the age of about 30 days, and on days 29 and 35 post inoculation from the pups in the inoculation experiment. Final sampling was carried out in conjunction with the euthanasia of the foxes. The sera were tested for E. cuniculi

antibodies by the modified india-ink immunoreaction (*Kellett & Bywater* 1978). The titres were expressed as the reciprocal value of the highest serum dilution showing more than 5 % spores stained by carbon particles of at least 200 spores examined.

Post-mortem examinations

Necropsies were performed according to routine schedules, recording of body weights included. Pieces of cerebrum, myocardium and kidneys were fixed in a 10 % buffered solution of formalin. The material was embedded in paraffin and sectioned at about 5 µm. Sections were stained with haematoxylin and eosin (HE), elastin van Gieson, and with a modified Gram method (*Petri* 1969). Smears from the cut surface of kidneys and from the mucosa of urinary bladders were air-dried, heat-fixed, and stained with the modified Gram method. Photographs were recorded on Agfa Pan 25 film.

Re-isolation of the parasite

Homogenized brain, heart and kidney tissues from some dead newborn pups were suspended in sterile 0.15 mol/l saline solution, and volumes of 0.5 ml of the suspension were injected intraperitoneally into groups of 4 Swiss albino mice of the outbred stock Bom:NMRI f(SPF).

Some other dead newborn pups were skinned and the stomach and the intestines removed. The rest of the bodies was cut into pieces and digested by Pepsin 1:10,000 (Difco), pH 1.3 at 37°C overnight. After neutralization the suspension was centrifuged and the sediment re-suspended in sterile 0.15 mol/l saline solution. Volumes of 0.5 ml were then injected intraperitoneally into mice, as described above. Ten days after injection the mice were killed and the peritoneal exudate examined for E. cuniculi spores according to the procedure described elsewhere (*Mohn et al.* 1982).

RESULTS

Pups

The results obtained in Groups 1—7 are summarized in Table 1.

Table 1. Clinical, serological and pathological examinations of foxes in the various groups of the cross-fostering and the oral inoculation trials.

Group*	Total number of pups	Number of pups						
		examined	surviving the neonatal stage	exhibiting neurological signs	developing humoral E. cuniculi antibodies	from whom spores of E. cuniculi were detected	with widespread polyarteritis nodosa, meningo-encephalitis and diffuse nephritis with extensive plasma cell proliferations	with focal interstitial nephritis/meningo-encephalitis/thickened myocardial arteries
1	32	26	11	8	9	22	8	0
2	8	2	2	1	1	1	1	0
3	34	15	13	0	8	0	0	8
4	16	8	8	0	8	1	0	8
5	16	15	15	0	15	1	0	15
6	30	24	24	0	21	0	0	22
7	12	12	12	0	0	0	0	0

* Group 1: Pups borne and fostered by inoculated vixens and kept together with pups borne by non-inoculated vixens.
Group 2: Pups borne by inoculated mothers and transferred to non-inoculated foster-mothers and their offspring.
Group 3: Pups borne and fostered by non-inoculated vixens and kept in contact with pups borne by inoculated vixens.
Group 4: Pups borne by non-inoculated vixens and transferred to inoculated vixens and their offspring.
Group 5: Orally inoculated pups.
Group 6: Pups kept together with orally inoculated foxes.
Group 7: Pups remaining clinically healthy and sero-negative during the trial.

Cross-fostering trial

Groups 1 and 2. In the organs of 15 pups which were found dead within a period of a few hours after delivery, no macroscopic lesions could be detected. Undigested organ suspension from 13 of these pups were injected intraperitoneally into mice; spores of E. cuniculi were detected from 12 of the pups. Spores were also found by mouse inoculation after injection with pepsin-digested organ suspension from 2 other newborn pups. In modified Gram-stained sections from the newborn pups, spores of E. cuniculi, unassociated with inflammatory reactions, were detected in the brains of 2 pups. At the age of 1—2 months, 8 pups of Group 1 and 1 pup of Group 2 showed reduced growth, ataxia, and posterior weakness; some of the pups exhibited severe neurological disturbances terminating in blindness, circ-

ling behaviour with tilted heads, and convulsions. Four of the pups died or were killed in a moribund condition at the age of 42 to 56 days; E. cuniculi antibody titres of 10 and 200 were detected in 2 of these pups. In the surviving pups antibody titres of 200 were detected at the age of from 51 to 57 days; the titres ranged between 200 and 1600 when the pups were pelted at the age of from 130 to 152 days. At necropsy the 5 diseased foxes were found to be in almost normal condition although small of size, their body weights varying between 3.0 and 4.7 kg, mean 3.7 kg. The coats consisted mainly of wool with few deck hairs. The 4 foxes exhibiting no specific signs of disease had body weights varying between 5.5 and 7.1 kg, mean 6.2 kg; their furs appeared to be of almost normal quality. Hydrocephalus and enlarged pale kidneys were seen in the carcasses of the 5 clinically sick foxes. Prominent nodular lesions of the superficial coronary arteries were found in 4 of them. Hydrocephalus was observed in 1 of the apparently healthy foxes. Histological examination of the 2 categories of animals revealed meningo-encephalitis and interstitial nephritis with infiltrations of mononuclear cells; a preponderance of these cells were indistinguishable from plasma cells. Arterial alterations of the polyarteritis nodosa type were seen in the myocardium and the brain. Spores of E. cuniculi were detected in modified Gram-stained smears and sections from the kidneys of the diseased foxes. Parasitic structures were not recognized in the organs of the clinically healthy foxes. The surviving pup of Group 2 remained sero-negative throughout the trial; at necropsy the organs revealed no macroscopic or histological lesion.

Groups 3 and 4. A dull and weak pup of Group 4 died when 93 days old with an E. cuniculi antibody titre of 50. Specific macroscopic and histological lesions were not observed, and parasitic structures were not detected by mouse inoculation. In the surviving pups E. cuniculi antibody titres varying between 10 and 200 were detected at the age from 79 to 92 days. The foxes were pelted when from 147 to 153 days old, their sera revealing titres ranging from 100 to 400. At necropsy the body weights varied from 5.3 to 6.9 kg, mean 6.0 kg. No gross lesions were observed. Histological lesions were not observed in the sero-negative foxes, whereas tiny focal areas of infiltrated mononuclear inflammatory cells were found in the kidneys of all sero-positive foxes; a considerable number of these cells appeared

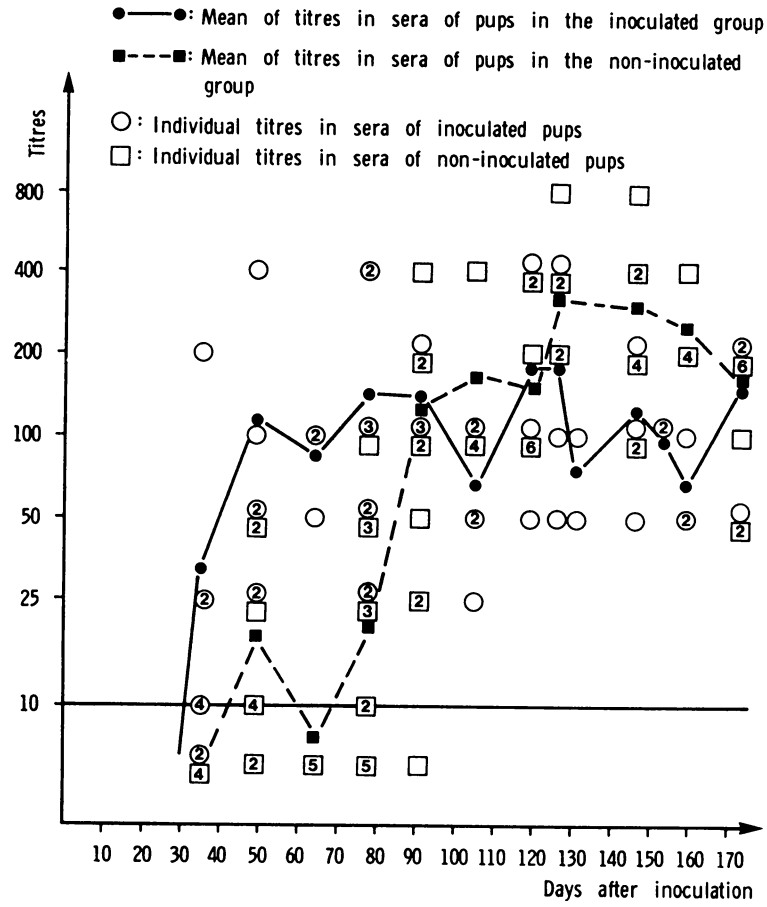

Figure 1. Antibodies to E. cuniculi in sera of 9 pups inoculated orally with E. cuniculi spores at the age of 1—5 days and in sera of 14 non-inoculated pups kept together with the inoculated pups. The figures in the symbols indicate number of samples with same antibody titre.

to be indistinguishable from plasma cells (Figs. 3 and 4). Mononuclear cuffings and incipient and scattered infiltrations of mononuclear cells were seen in the leptomeninges of 2 foxes in each group. Similar cuffings occurred in the cortical layer of the brains of 5 foxes of Group 3, whereas minor granulomatous lesions (Figs. 5 and 6) were seen in the same area of the brains from 1 fox in each group. Moderate thickened walls of some arteries of the myocardium were found in 1 fox of Group 4 (Fig. 7).

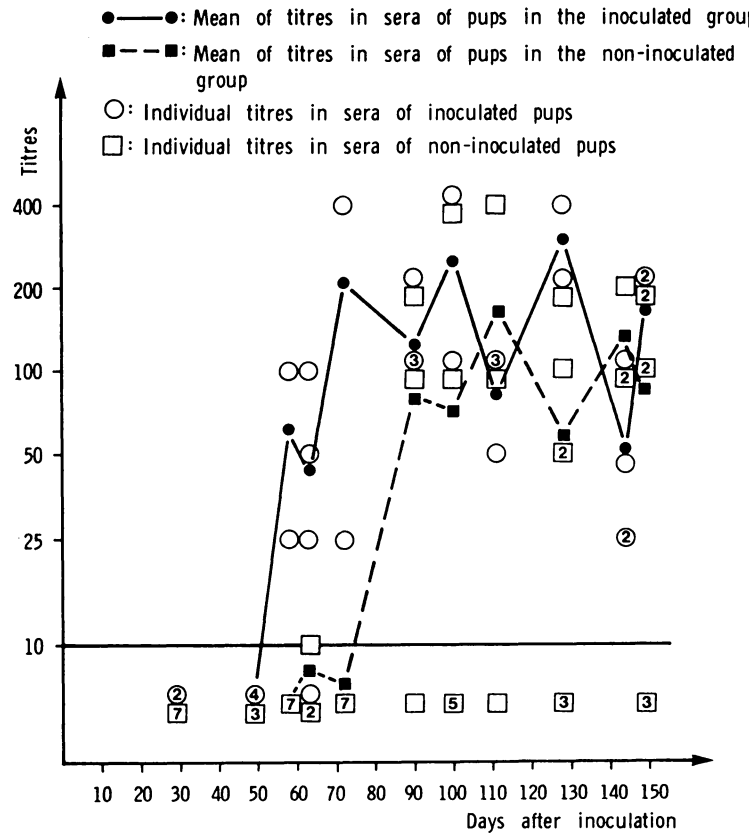

Figure 2. Antibodies to E. cuniculi in sera of 6 pups inoculated orally with E. cuniculi spores at the age of 12—15 days and in sera of 10 non-inoculated pups kept together with the inoculated pups. The figures in the symbols indicate number of samples with same antibody titre.

Oral inoculation trial

Groups 5 and 6. At the age of about 12 weeks 4 pups of Group 5 inoculated on their first day of life appeared dull with rough hair coats and reduced appetite, being slightly smaller in size than the other pups in the same litter. One of these pups was killed and necropsied when 99 days old, its serum revealing an E. cuniculi antibody titre of 50. At necropsy slightly swollen kidneys was the only gross lesion found in its organs. Histological examination of the kidneys revealed interstitial infiltrations of inflammatory cells, a majority of which appeared to be plasma cells. In the cerebral cortical layer focal accumu-

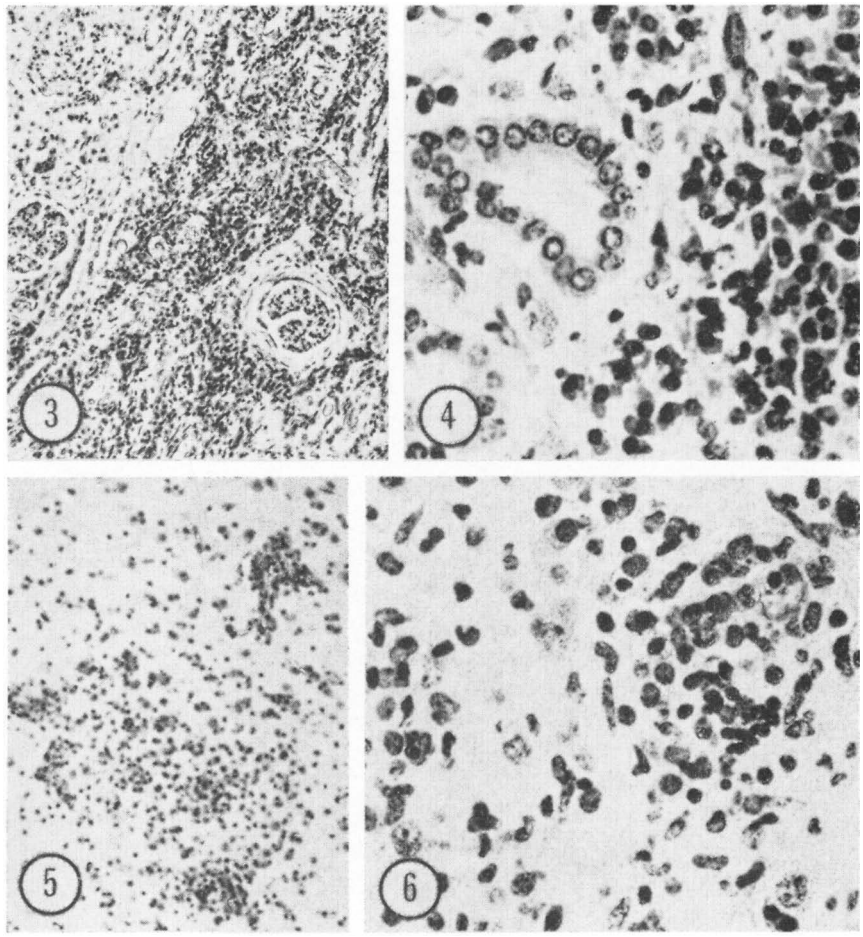

Figure 3. Focal interstitial nephritis in a fox with subclinical encephalitozoonosis killed 153 days after oral inoculation with E. cuniculi spores (Group 5). HE, × 106.

Figure 4. Detail of Fig. 3 showing infiltration of mononuclear inflammatory cells, some of which appear to be plasma cells. HE, × 425.

Figure 5. Granuloma in the cerebral cortex of a fox with subclinical encephalitozoonosis killed 149 days after oral inoculation with E. cuniculi spores (Group 5). HE, × 106.

Figure 6. Detail of Fig. 5 showing accumulation of mononuclear inflammatory cells in the cerebral cortical layer. HE, × 425.

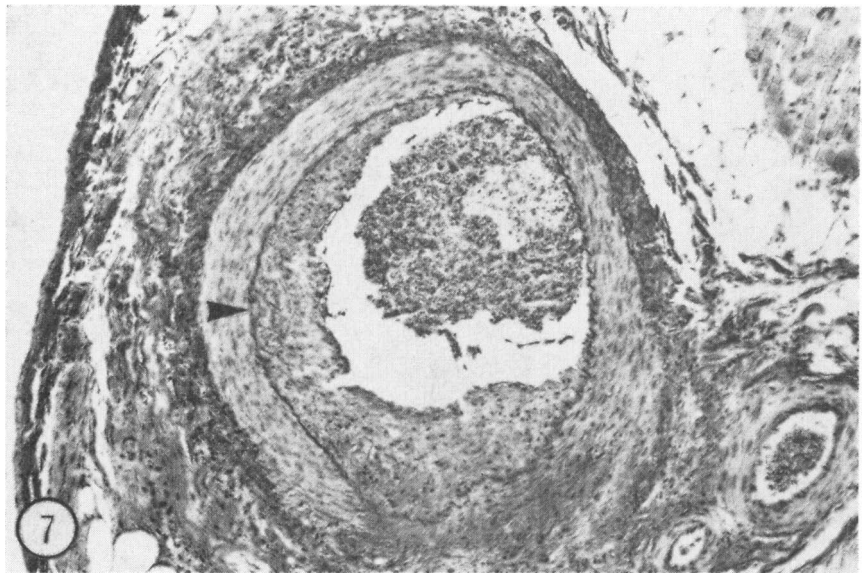

Figure 7. A superficial artery in the myocardium of a fox with subclinical encephalitozoonosis killed 173 days after oral inoculation with E. cuniculi spores (Group 5). The arterial wall is thickened mainly due to fibrocellular intimal changes; accumulation of red blood cells in arterial lumen. Arrow points to the internal elastic membrane. Elastin van Gieson, × 106.

lations of mononuclear cells were frequently observed together with numerous spores of E. cuniculi. The leptomeninges were thickened and infiltrated by mononuclear cells; cuffings occurred frequently, while arterial lesions were not detected in the myocardium. The other 3 pups gradually became healthy, and at the time of pelting they had a normal appearance.

The results of the serological examinations are shown in Figs. 1 and 2.

The foxes were killed on day 144 to 174 post inoculation; their body weights ranged from 4.6 to 7.0 kg, mean 5.9 kg for Group 5, and 4.9 to 7.5 kg, mean 6.3 kg for Group 6. At necropsy no macroscopic lesions were detected. Focal interstitial infiltrations of mononuclear cells were found in the kidneys of 12 foxes of Group 5 and 22 foxes of Group 6; a considerable number of these cells were indistinguishable from plasma cells (Figs. 3 and 4). Focal fibrosis was seen in the kidneys of 1 fox from Group 5, whereas a few periarterial infiltrations with eosinophilic cells were observed in the kidneys of 1 fox from Group 6. Histological lesions in the cerebral cortical tissue and in the leptomeninges, similar to those described in the foxes of Groups 3 and 4 (Figs. 5 and 6), occurred in 13 foxes of Group 5 and in 19 foxes of Group 6. Scattered proliferations of glial cells were seen in the cortex of 1 animal of Group 5. Thickened arteries were observed in the myocardium of 4 foxes of Group 5 and 3 foxes of Group 6 (Fig. 7).

Controls

Group 7. At necropsy the body weights of the foxes ranged from 6.5 to 7.0 kg, mean 6.7 kg. Further results are given in Table 1.

Vixens

Clinical signs of disease were not observed in the mothers and the foster-mothers of pups within the 2 trials. In the group of inoculated vixens antibody titres to E. cuniculi, ranging from 10 to 50, appeared during a period of from 43 days before to 16 days after whelping. All inoculated vixens remained sero-positive throughout the experiment, showing maximum titres from 50 to 400. Three non-inoculated vixens, 2 in the cross-fostering trial and 1 in the oral inoculation trial, remained sero-negative during

the experimental period, whereas antibody titres of 10 to 25 appeared from 80 to 159 days after whelping in the other 7 non-inoculated vixens, maximum titres reaching the magnitude of 10 to 100 during the final weeks of the experiment. Necropsy of 6 of the vixens killed at the end of the experiment revealed no pathological lesions in any organ.

DISCUSSION

Neonatal exposure of newborn and young pups, either by oral inoculation with infective E. cuniculi spores, or by keeping the pups in direct contact with E. cuniculi infected animals, does not seem to induce clinical encephalitozoonosis. Antibodies to E. cuniculi appeared, however, in a majority of the exposed pups during the experimental period. In Groups 4 and 5 all pups surviving the neonatal stage became sero-positive, whereas 8 out of 13 surviving pups in Group 3 and 21 out of 24 surviving pups in Group 6 developed antibodies (Table 1). The high infection rate of the pups in the various groups may reflect exposure to heavy infectious doses. Our findings do not, however, seem to indicate significant differences between the infectious risks presented to the pups by oral inoculation or through contact with the various groups of infected foxes.

In some of the non-inoculated dams, sero-negative reactions were found throughout the trial, whereas antibodies appeared at low levels in the other vixens of this group late in the course of the experiment; antibodies at higher levels were found around the time of whelping in the group of vixens orally inoculated before mating. This seems to exclude congenital infection of pups borne by the non-inoculated vixens. It is, therefore, likely that the development of E. cuniculi antibodies in these pups reflects neonatally acquired infection of E. cuniculi. The appearance of antibodies in pups exposed to direct contact with infected animals (Groups 3, 4 and 6) at an age of not less than 50 days also supports neonatal infection.

The antibodies in the group of pups borne by non-inoculated vixens and kept together with orally inoculated pups (Group 6) (Figs. 1 and 2), and in pups kept in contact with inoculated vixens and their offspring (Groups 3 and 4), appeared from 14 to 42 days later in the course of the experiment than antibodies in the inoculated pups (Group 5) and in the pups borne by

inoculated vixens (Groups 1 and 2). This observation indicates that the infected pups, and possibly the inoculated vixens, were the source of infection for the pups kept together with these animals. Furthermore, it seems obvious that the infected foxes contaminated the environment rather soon after exposure, or after birth, presumably through urine, vaginal excretions, or probably through milk and saliva. In this way infection of the pups and vixens kept together with infected animals may have occurred through close contact in the same cage.

In the group orally inoculated when 1—5 days old, antibodies were detected from the 35th day after inoculation, whereas antibodies appeared about 55 days after inoculation in the group of pups inoculated when 12—15 days old (Figs. 1 and 2). In the group of non-inoculated pups kept together with pups orally inoculated when 1—5 days old, antibodies were detected 14 days later in the course of the trial, compared to antibodies in the inoculated group. In the group kept in contact with pups inoculated when 12—15 days old, the antibodies appeared 42 days later than the antibodies in the inoculated group. Three individuals in the contact group of pups inoculated when 12—15 days old remained non-infected, since no antibodies were detected in these animals throughout the experiment (Fig. 2), whereas all animals in the group of pups inoculated when 1—15 days old became sero-positive during the course of the experiment (Fig. 1). These observations may indicate a probable higher degree of resistance to the parasite in pups infected when 2 weeks old than in pups infected when less than 1 week old.

Pathological lesions were not detected in the sero-negative control foxes and in 2 sero-negative foxes exposed neonatally, whereas lesions occurred in all but 1 of the neonatally exposed foxes developing antibodies to E. cuniculi during the trial. In organs of 3 of the sero-positive foxes spores of E. cuniculi were detected. These findings indicate that the lesions are attributed to the parasite infection. A majority of the sero-positive foxes showed lesions of focal interstitial nephritis (Figs. 3 and 4). Granulomatous encephalitis with meningitis and perivascular cuffings occurred concomitantly with the nephritis in some of the foxes (Figs. 5 and 6). The histological findings appeared to be very similar in the various groups of neonatally exposed foxes, except for thickened walls of myocardial arteries (Fig. 7) which were mainly seen in orally infected animals. The pathological

alterations of the brains and the kidneys of the subclinically infected foxes seem to be of the same type as those described in these organs of rabbits suffering from chronic encephalitozoonosis (*Flatt & Jackson* 1970, *Cox & Gallichio* 1978). These organs are likely to be the predilection sites also for the subclinical infection of the blue fox. Focal interstitial nephritis of unknown etiology, almost indistinguishable from the renal lesions described above, are in our experience commonly seen in blue foxes that have died from various causes; these lesions could probably be due to subclinical encephalitozoonosis. Severe encephalitis and nephritis with extensive proliferations of plasma cells and polyarteritis nodosa as seen in "spontaneous" and in clinical cases of induced encephalitozoonosis of pups borne by experimentally inoculated vixens, were not found in the neonatally exposed foxes. The introduction of E. cuniculi neonatally to pups seems to result in a subclinical infection, which for a shorter period of time, may give a rough coat and stunted growth in some of the affected animals. These foxes, however, gradually seem to overcome the infection, and at the normal time of pelting the infection appears to have had no apparent influence on body weights and fur quality.

Intrauterine infection appears to be an essential supposition for the establishment of experimentally induced fox encephalitozoonosis. Further knowledge about the pathogenesis of the disease is needed before the failure to induce the disease by neonatal infection can be fully understood. However, the possibility of a high degree of natural resistance to E. cuniculi in the blue fox might be of some importance for establishing the disease. This suggestion might also explain the ability of the blue fox to overcome the infection (*Mohn & Nordstoga* 1975), which is also indicated by a gradual decrease of antibodies to undetectable levels during some months after infection (*Mohn*, unpublished). This observation seems to contrast with the general concept of chronic rabbit encephalitozoonosis (*Wilson* 1979), which may indicate that the parasite is more adapted to the rabbit than to the blue fox.

A probable higher susceptibility of E. cuniculi in prenatally infected than in neonatally infected foxes could be a factor of significance in the pathogenesis of the disease, although it presumably does not give the full explanation of the failure to induce clinical encephalitozoonosis in neonatally exposed pups.

Plasma cell proliferations, hypergammaglobulinaemia, and polyarteritis nodosa, which are almost constant findings in fox encephalitozoonosis (*Mohn & Nordstoga* 1975, *Nordstoga & Westbye* 1976), have been suggested to represent a hypersensitive condition. Although the mechanism behind the development of this presumed hypersensitivity is unknown, it is reasonable to suggest that it is in some way immunologically induced during the fetal stage of the infection. The infection established through transplacental transmission of the parasite does not seem to have caused detectable morphological lesions in the organs of pups delivered perinatally by caesarian section, or in the organs of normally born pups that died a few hours old. It looks, however, obvious that the pups do not become immunologically tolerant to the parasite, since humoral antibodies to the agent are almost constant findings in pups suffering from clinical encephalitozoonosis (*Mohn,* unpublished). Although neonatal infection with E. cuniculi does not seem to result in a manifest disease and heavy losses among affected pups, the subclinical infection of pups and adult foxes may be of some importance for the spreading of the agent among the population. Thus the parasite may remain within an affected farm from one breeding season to another or even spread to other farms through purchase of subclinically infected foxes. Therefore, selection of breeders for the next breeding season based on sero-negative individuals must be recommended as an important measure for the control of the disease, together with destruction of all sero-positive reagents which may be a potential reservoir for the parasite.

The detection of E. cuniculi spores in the peritoneal exudate of mice after injection of pepsin-digested tissues shows that the parasite resists the treatment by the enzyme at a very low pH over a period of at least 12 h. The high resistance, which is likely to be due to the thick capsule of the spores, indicates the oral route to be a natural mode of infection for this agent, since it seems to be so protected as to pass the stomach without being damaged, and may then finally enter the gut epithelium. This is in agreement with the general acceptance of the oral route as a major infection mechanism of encephalitozoonosis in laboratory animals (*Wilson* 1979).

The methods for detecting spores by mouse inoculation using digested and undigested organ suspension have not been compared; the enzyme digestion is, however, probably a better

method for emancipating spores from infected cells than mechanical grinding of the tissues, thus concentrating free spores in the residual sediment after the digestion of the material. For the detection of E. cuniculi spores at low tissue concentrations the pepsin digestion could, therefore, be the method of choice. The detection of E. cuniculi spores by histology and mouse inoculation from organs of pups borne by inoculated vixens and dead a few hours after delivery parallels the results of a previous experiment that provided evidence for transplacental transmission of the parasite (*Mohn et al.* 1982).

Some pups of the clinically affected litters (Groups 1 and 2) appeared clinically normal without developing detectable antibodied to E. cuniculi during the present experiment (Table 1). This shows that some pups may escape the intrauterine infection, and this observation also coincides with previous results (*Mohn et al.* 1982). However, pups born free from the infection may develop subclinical infection with sero-positive findings after neonatal contact with individuals infected in utero. This explains the common findings of E. cuniculi antibodies around the time of pelting in apparently healthy members of litters in which some of the pups have suffered from encephalitozoonosis either in experimentally induced cases or in natural field cases (*Mohn,* unpublished).

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SAMMENDRAG

Eksperimentell encephalitozoonose hos blårev — Neonatal eksponering overfor parasitten.

Nyfødte og unge valper inntil en alder av 15 dager ble eksponert for Encephalitozoon cuniculi enten ved at de ble holdt i bur sammen med oralt smittede fostermødre og deres avkom, eller ved at de ble inokulert oralt med E. cuniculi sporer. Hos et flertall av disse valpene ble det ved hjelp av india-ink immunoreaksjonen påvist E. cuniculi antistoffer fra 35 til 87 dager etter eksponeringen. Sporer ble dessuten påvist histologisk i organer fra noen av disse dyrene. Upodete kullsøsken holdt i bur sammen med oralt podete valper ble funnet seropositive fra 49 til 129 dager etter podningen.

Det lyktes ikke å fremkalle klinisk encephalitozoonose hos noen av revene utsatt for neonatal smittepåkjenning. Enkelte av de eksponerte valpene viste moderat nedsatt tilvekst i 2—3 måneders alderen, men alle revene hadde ved pelsing kroppsvekter og skinnkvalitet innenfor normalt variasjonsområde.

Alle serologisk positive rever eksponert neonatalt viste histologiske forandringer i form av fokale interstitielle infiltrater i nyrene. Hos en del av revene sås meningo-encephalitt i tillegg til nyreforan-

dringene. Moderat fortykkete arterievegger, vesentlig i myokardiet, ble påvist hos noen få rever. Nyre- og hjerneforandringene var av samme type som de lesjoner man ser i tilsvarende organer hos kanin ved kronisk encephalitozoonose. Uttalte betennelsesforandringer i nyrene med massive plasmacelleproliferasjoner — som nærmest er konstante funn ved klinisk encephalitozoonose hos blårev — ble ikke påvist hos de neonatalt smittede revene, heller ikke karforandringer av typen polyarteritis nodosa eller uttalte betennelsesforandringer i hjernen som også er vanlig ved klinisk encephalitozoonose hos blårev.

Intrauterin infeksjon av valpene etter transplacental overføring av parasitten synes å være en vesentlig forutsetning for at klinisk encephalitozoonose skal kunne etableres og utvikles hos blårev. Sykdommens patogenese er i detaljer ukjent, men man antar at infeksjonen i fosterstadiet resulterer i en hypersensitiv tilstand manifestert ved polyarteritis nodosa, uttalte plasmacelle-proliferasjoner og hypergammaglobulinemi. Disse forandringene synes å være av vesentlig betydning for sykdommens spesielle manifestasjon og forløp.

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