Prevention and treatment of *Encephalitozoon cuniculi* infection in rabbits with fenbendazole

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The efficacy of fenbendazole for preventing an experimental infection of *Encephalitozoon cuniculi* and for eliminating the spores from the central nervous system of naturally infected rabbits was investigated. Fenbendazole (20 mg/kg bodyweight daily) was administered from seven days before until two or 21 days after rabbits had been infected orally with 10^6 spores of *E cuniculi*. Both regimens were effective in preventing the establishment of the parasites, as demonstrated by negative parasitic-specific serology and by the failure to isolate the parasite from brain tissue. In naturally infected, seropositive rabbits, parasites were successfully isolated from seven of nine untreated animals, but not from the brain tissue of eight animals treated with fenbendazole-medicated pellets for four weeks.

ENCEPHALITOZOONOSIS is of worldwide clinical significance in laboratory and pet rabbits. Infections with *Encephalitozoon cuniculi* can persist without clinical signs for years, with severe clinical disease occurring only sporadically, and apparently being unrelated to the age and sex of the affected animals, although outbreaks of encephalitozoonosis with 15 per cent morbidity have been reported in young rabbits. Spores of *E cuniculi* are excreted in the urine of infected rabbits and the disease is most often transmitted by the ingestion of the spores, although intrauterine infection also has been documented (Canning and Lom 1986).

The clinical signs include stunted growth, central nervous signs due to granulomatous encephalitis, for example, opisthotonus, torticollis, ataxia, hyperaesthesia and nystagmus, eye lesions, for example, cataract and granulomatous uveitis, and nephritis (Stiles and others 1997, Ewringmann and Göbel 1999). Differential diagnoses applicable to the neurological signs include pasteurellosis, toxoplasmosis, otitis media or interna due to ear mites (Kunstyr and Naumann 1985) and vitamin E and/or selenium deficiency. The putative clinical diagnosis can be corroborated by specific serology for *E cuniculi*, with the immunofluorescent antibody test (IFAT) or an ELISA.

In an uncontrolled study in pet rabbits, treatment with dexamethasone in combination with oxytetracyclines and vitamins resulted in a clinical cure of 10 of the 20 rabbits with neurological signs (Ewringmann and Göbel 1999). Apart from maintaining hygienic standards in units housing rabbits, no prophylactic strategies have been identified to prevent the introduction of encephalitozoonosis and a strict serology-based test and slaughter policy has been the only method for eliminating the parasite from rabbitries (Bywater and Kellett 1978).

A number of benzimidazole derivatives including fenbendazole are active against *Encephalitozoon* species in vitro (Kotler and Orenstein 1999). In rabbits, fenbendazole is used for anthelmintic treatment (Short and others 1988) and has no side-effects (Baeder and others 1974). This paper describes the results of an investigation to assess the prophylactic and therapeutic effect of fenbendazole in rabbits with experimental *E cuniculi* infections and in animals with naturally acquired encephalitozoonosis.

MATERIALS AND METHODS

Parasite cultivation and isolation from tissue

The *E cuniculi* isolate IPZ:CH-R5870 recovered from a rabbit and characterised as 'rabbit strain' (Mathis and others 1997) was propagated in primary human lung fibroblast cells (MRC-5) as described by Mathis and others (1996). Parasite spores were harvested twice a week from the culture supernatant. After centrifugation for 10 minutes at 700 *g* at 20°C, the spores were resuspended in phosphate buffered saline (PBS), counted in a Neubauer counting chamber and used immediately.

The spores were isolated from brain tissue as described by Deplazes and others (1996) and Mathis and others (1996). The cultures were examined for parasite foci at a magnification of 100 to 400. After two months of cultivation without detectable foci a culture was considered to be negative.

Indirect immunofluorescence antibody test (IFAT)

The IFAT followed the protocol described by Thomas and others (1997) and used fresh *E cuniculi* spores. All the sera were initially screened at a 1:40 dilution in PBS, and positive sera were retested in two-fold serial dilutions up to 1:5120.

Medicated pellets

A medicated feed was manufactured (Eberle Nafag; Gossau-SG) by the addition of 4 g/kg fenbendazole (Panacur; Hoechst Roussel) to a complete mixed feed for rabbits before it was pelleted. If it is assumed that the rabbits ate 5 g medicated feed per kg bodyweight per day, they would have received a daily dose of 20 mg fenbendazole/kg bodyweight.

Prophylactic study

Eight mixed-breed rabbits, four to seven months old and weighing 2·5 to 3·5 kg, were obtained from the *Encephalitozoon*-free colony of the Institute of Parasitology, and used in two separate experiments (A and B). In each experiment prophylactic doses of fenbendazole were administered daily to two rabbits, beginning seven days before they were infected orally with 10^6 spores of *E cuniculi* in 1 ml PBS. The viability of the parasites was established by the inoculation of an aliquot into a tissue culture. In experiment A, the rabbits received 0·1 ml/kg, twice daily, of a suspension of fenbendazole (Panacur 10 per cent suspension; Hoechst Roussel) until 21 days after infection; in experiment B, the medicated pellets were fed until two days after infection (5 g/kg daily). The other four rabbits were left untreated as controls. The four rabbits in each experiment were bled from an ear vein every other week and euthanased after six months.

Therapeutic study

The effect of fenbendazole on established infections was assessed by comparing the success of attempts to isolate the parasites from the brains of eight rabbits treated with 5 g medicated pellets daily for four weeks and from the brains of nine untreated rabbits. The rabbits were selected from a clinical study of rabbits with *E cuniculi*-specific antibody titres above 1:640. They were mixed-breed dwarf rabbits from 14

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FIG 1: Serological responses of rabbits infected experimentally with *Encephalitozoon cuniculi* after the prophylactic administration of fenbendazole. In two independent experiments (a and b) the specific antibody responses after oral infection with 10^6 spores of *E cuniculi* was measured by IFAT in two untreated and two medicated rabbits. In experiment (a) fenbendazole was administered from seven days before until 21 days after infection, and in experiment (b) from seven days before until two days after infection. The results of attempts to isolate the parasites from brain tissue and cultivate them in vitro are given at the end of each graph
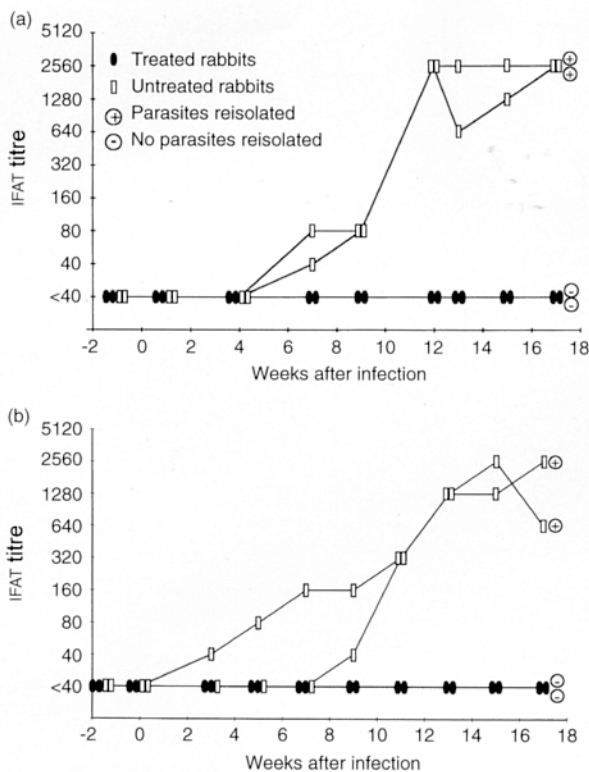
owners and were examined postmortem within one year after diagnosis or treatment. They were between four months and six years of age and weighed 0·5 to 2·5 kg. Ten of them were males and seven were females and most of them were housed in groups of two. Six of the eight treated rabbits died of causes unrelated to encephalitozoonosis (bacterial dysentery in two cases, stomach ulcers, a fungal infection, *Escherichia coli* sepsis, and ileus), and two were euthanased after four weeks of treatment because they showed persistent neurological signs consistent with encephalitozoonosis. Four of the nine untreated rabbits showed neurological signs which were attributed to encephalitozoonosis, and unrelated clinical pathology was observed in five cases, leukaemia, sudden death, ileus, dysentery, and a fractured limb.

RESULTS

Prophylactic study

The four rabbits which were infected experimentally during their treatment with fenbendazole (either suspension or medicated pellets) remained seronegative until 120 days after infection (Fig 1), and *E cuniculi* could not be isolated from their brain tissue. In contrast, all four untreated control rabbits seroconverted between day 23 and 40, and developed high titres (Fig 1). *E cuniculi* spores were isolated from the brain tissue of each of them and propagated in vitro.

Therapeutic study

Attempts to isolate *E cuniculi* from the brain tissue of the 17 naturally infected rabbits were successful in seven of the nine untreated animals but in none of the eight rabbits treated with fenbendazole.

DISCUSSION

This paper describes the potentially successful prophylactic and chemotherapeutic treatment of *E cuniculi* infections in

rabbits. The oral administration of fenbendazole before experimental infection was effective in blocking the establishment of *E cuniculi* in the rabbit host. The strategic application of fenbendazole may be a suitable method for controlling new *Encephalitozoon* infections in pet and commercial rabbits.

In the therapeutic study fenbendazole was administered for four weeks, on the hypothesis that resting spores of the parasite may not be affected by fenbendazole. The fact that the treatment eliminated tissue parasite stages was shown by the reduction of tissue spores to below the detection threshold in all the treated rabbits.

The only drug recommended for the treatment of disseminated *Encephalitozoon* species infections in human patients is albendazole (Kotler and Orenstein 1999). In a case report describing a rabbit with phacoclastic uveitis, treatment with oral albendazole in combination with topical 1 per cent prednisolone acetate for eight weeks resulted in the resolution of the intraocular granuloma (Stiles and others 1997). However, albendazole is embryotoxic and teratogenic in rats and rabbits (Kotler and Orenstein 1999). Like albendazole, fenbendazole is well absorbed and rapidly metabolised to oxfendazole. Both these compounds were active in vitro against *E cuniculi* and *E intestinalis* (Katiyar and Edlind 1997).

In a preliminary study (data not shown) 16 rabbits with neurological signs attributed to encephalitozoonosis, and differential diagnoses excluded, which had *E cuniculi*-specific antibody titres above 1:640 were treated with medicated pellets daily for four weeks. Eight animals were free of the neurological signs by the end of the treatment but three showed some residual signs. In two of these animals a slight head-tilt persisted throughout the study period of three months but the third rabbit became clear of signs by this time. Five rabbits responded poorly to the medication, and owing to the severity of the disease two were euthanased during the treatment period and three were euthanased at the end. In these animals, is seems most likely that the inflammatory lesions associated with the *E cuniculi* infection of the nervous system were too severe to be resolved or compensated for within the observation time. A similar 50 per cent initial recovery rate was also observed in response to glucocorticoid treatment of rabbits with central nervous encephalitozoonosis (Ewringmann and Göbel 1999). Glucocorticoids may alleviate the neurological signs through the suppression of the granulomatous inflammation. However, it is not clear whether this effect is lasting or has any effect on the parasite burden. In order to improve the clinical treatment of encephalitozoonosis in rabbits, the combination of fenbendazole and glucocorticoids could be valuable and should be examined in a controlled study.

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SHORT COMMUNICATIONS

Equine Borna disease in Japan

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BORNA disease is a fatal disease of the central nervous system (CNS) in horses and sheep that was originally recognised in Borna, Saxony, Germany (Durrwald and Ludwig 1997). The disease is caused by infection with Borna disease virus (BDV), and is a non-suppurative encephalomyelitis with a predilection to involve the grey matter of the cerebral hemispheres and the brainstem (Stitz and others 1993, Gonzalez-Dunia and others 1997). BDV belongs to a neurotropic, non-segmented negative-sense, single-stranded RNA virus, and is the prototype of a new family of the Bornaviridae in the order Mononegavirales (Gonzalez-Dunia and others 1997). Cases of spontaneous Borna disease in the horse have been noted only in Germany, Switzerland and Austria (Lange and others 1987, Weissenbock and others 1998b). A similar syndrome (called staggering disease) was recently described in cats (Lundgren and others 1995, Nakamura and others 1999), dogs (Weissenbock and others 1998a) and ostriches (Malkinson and others 1995). Furthermore, serological studies indicate that human BDV infections also exist (Ludwig and others 1988). Recently, the relationship between BDV infection and psychiatric disorders, such as schizophrenia, mood disorder and chronic fatigue syndrome, was investigated (Rott and others 1985, de la Torre and others 1996).

Serum anti-BDV antibodies and BDV RNA in peripheral blood mononuclear cells (PBMCs) have been demonstrated in horses that were either clinically normal or pathologically diagnosed with a disease unrelated to Borna disease in the USA, Japan, Iran and Sweden (Nakamura and others 1995, Bahmani and others 1996, Hagiwara and others 1997). However, the authors have not previously seen evidence of clinical Borna disease in horses in Japan. This short communication describes the results of the histopathological examination performed on the CNS of two racing horses exhibiting pharyngeal paralysis, flaccid paresis and motor incoordination.

A seven-year-old thoroughbred mare (horse 1) showed a sudden onset of pharyngeal paresis and hyperaesthesia in May 1999. Two months later, the horse gradually showed paralysis of the tongue and severe posterior motor incoordination. The mare was euthanased because of severe cachexia, and a postmortem examination was performed.

A three-year-old thoroughbred male (horse 2) suddenly developed flaccid paresis in November 1999. Three months later the horse showed a severe posterior motor incoordination and a cervical vertebral malformation accompanied by stenosis of the spinal canal at C6 to C7 as demonstrated by radiographic examination. The animal was euthanased because of neural paralysis, and submitted for postmortem examination.

Serum and cerebrospinal fluid (CSF) antibodies to BDV p40 protein were detected in both animals by immunoblotting as described by Nakamura and others (1999). A 1:100 dilution of sera and native CSF showed a clear positive reaction with 40 kDa BDV p40 antigen. BDV p24 RNA in the brain tissue was detected by reverse transcriptase (RT)-nested PCR (Nakamura and others 1999). BDV p24 RNA-positive signals were detected in the frontal lobe, mesencephalon and medulla oblongata of horse 1 (Fig 1), and the corpus striatum, hippocampus, and mesencephalon of horse 2. Detection of equine herpesvirus 1 (EHV-1) DNA by nested PCR was also performed for both animals. The primers for the glycoprotein B (gB) gene of EHV-1 and conditions for PCR were as described by Kirisawa and others (1993). EHV-1 DNA-positive signals were not detected in sample tissues collected from the corpus striatum, parietal lobe, hippocampus, mesencephalon, pons, cerebellum or spinal cord.

Thin paraffin sections (4 μm) of the brain tissues fixed in 4 per cent paraformaldehyde solution were stained with haematoxylin and eosin for histological and immunohistochemical evaluation by light microscopy. Neuropathological lesions were characterised by a non-suppurative encephalitis dominated by perivascular cuffs consisting of lymphocytes, macrophages and plasma cells (Fig 2), the presence of inflammatory cell infiltrates in the neural parenchyma, neuronophagia, focal gliosis, and scattered axonal degeneration. Severe lymphocytic cuffs in small vessels were occasionally associated with a surrounding neural necrosis and an infiltration of fat granule cells and lymphocytes. Large pyramidal cells

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