

PREVALENCE OF ANTIBODIES TO *ENCEPHALITOZOON CUNICULI* IN HORSES IN ISRAEL

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Levkutová M, Hipíková V, Faitelzon S, Benath G, Paulík Š, Levkut M: Prevalence of antibodies to *Encephalitozoon cuniculi* in horses in the Israel. *Ann Agric Environ Med* 2004, **11**, 265–267.

Abstract: Infection with the intracellular microsporidium *Encephalitozoon cuniculi* can cause a serious disease - encephalitozoonosis in various animals and people. Several species of mammals, including the horse, were seem to be potential sources of encephalitozoonosis for animal as well as human hosts. The disease diagnosis is based on clinical signs, pathological findings, and the detection of *E. cuniculi* or circulating antibodies directed against the parasite. This study investigates the seroconversion to *E. cuniculi* in horses admitted to the Veterinary Teaching Hospital of the Hebrew University of Jerusalem and 3 different private horse-riding farms across Israel. Antibodies to *E. cuniculi* were determined using the IFA test in the sera from 102 horses. Of 72 asymptomatic horses, 60% were seropositive and 19% of the positive samples showed a titer of 1:512. Of 30 horses with various clinical signs, 80% were seropositive and 68% of the positive samples showed a titer of 1:512. High titers were associated with colic and neurological signs. This could prove to be interesting if the high percentages of prevalence of antibodies level in horses are an indication of health risk in humans.

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Key words: horses, clinical signs, *Encephalitozoon cuniculi*, positivity.

INTRODUCTION

The importance of microsporidian infections in mammals has risen dramatically in the last two decades, mainly due to the occurrence of the infection among relatively healthy populations of responsive hosts [17]. *Encephalitozoon cuniculi* infections (microsporidiosis) have been recognized in a wide variety of mammalian species [2, 8], including laboratory mice, rabbits, guinea pigs, golden hamsters and rats [4, 6, 11], arctic fox, blue fox, wild dogs [1, 15, 19], domestic dogs and cats [13, 16,

18], and farm animals, such as goats, cattle and horses [3, 7, 21]. In some hosts, such as rabbit and carnivores, infection can cause disease [10, 12]. *Encephalitozoon cuniculi*, together with other microsporidia species, has emerged as an opportunistic infection in immunocompromised patients, i.e. persons suffering from AIDS [8, 21, 22]. The development of clinical disease in different species is highly influenced by natural innate species susceptibility, and by the immune status of the individual. Infection in animals is usually subclinical, but severe neurological signs can occur in rabbits, dogs, and blue fox

(*Alopex lagopus*) because of granulomatous encephalitis [1, 12]. The blue fox is one of the species most susceptible to encephalitozoonosis. Nothing is known about the spread of the *E. cuniculi* in horses.

Our screening results indicated that the examination of horse's sera for the presence of anti-*E. cuniculi* antibodies is of great importance, especially in horses with health problems. The IFA test is a greatly sensitive method for detection of early microsporidian infection.

MATERIALS AND METHODS

Serum samples. Ten ml blood samples were taken into evacuated tubes from the jugular vein of horses. Serum was removed from the clotted blood samples by centrifugation at 4,000 rpm for 10 minutes. Sera obtained were maintained at -20°C until testing.

Animals. The 102 horses examined comprised 2 groups. The first consisted of 30 animals (admitted to the Veterinary Teaching Hospital of the Hebrew University of Jerusalem, Israel) showing a variety of clinical manifestations. The second consisted of 72 asymptomatic horse from 3 different private horse riding farms across Israel, aimed to indicate the prevalence of antibodies to *E. cuniculi* in a healthy population (control group).

***E. cuniculi* organisms.** The entire cell corpuscular antigen of the *Encephalitozoon cuniculi* was used. The agent spores of murine and dog origin [5] were grown within E6 cells (Vero green monkey kidney cell), cultivated in modified RPMI 1640 medium, supplemented with 5% foetal calf serum and the addition of antibiotic and antimycotics (penicillin, streptomycin and amphotericin B).

Spore isolation. After bursting of the infected cells, mature spores were released into the media. The organisms were collected from culture supernatants after centrifugation at 450 xg for 20 minutes. Sediment was resuspended in non-buffered Percol (density 1.30 g/ml, pH 8.8), and following centrifugation at 750 xg for 20 minutes, separated from the spores. Subsequently, the organisms were processed according to Koudela *et al.* (1993) [10].

The indirect immunofluorescence antibody assay (IFA). The indirect immunofluorescence antibody test was used as the diagnostic method to determine specific anti-*E. cuniculi* antibodies. The test was conducted as described in detail by Chalupský *et al.* [9]. A fresh suspension of *E. cuniculi* from the tissue culture was placed in each well of a slide. The slide was air-dried for 24 h, then fixed in absolute acetone for 15 min and air dried. Horse sera tested were serially diluted, beginning at 1:2 and ending at 1:512. Each of the wells on the slide was covered with 10 μl diluted serum, and slides were incubated for 30 min at 37°C within a moist chamber. The

Table 1. Antibodies to *Encephalitozoon cuniculi* in horses.

Group	Antibody titer					No. of examined animals
	< 32	64	128	256	512	
Asymptomatic horses	29	16	3	16	8	72
Symptomatic horses	5	2	3	3	17	30
Clinical signs						
colic	1	0	0	1	9	11
neurological signs	1	0	0	0	5	6
fever with unknown origin	0	1	1	1	1	4
lameness	1	0	1	0	0	2
reproductive disturbance	1	0	1	0	2	4
pruritus	1	0	0	0	0	1
sinusitis	0	1	0	1	0	2

slides were then washed twice in distilled water and PBS at 10-min intervals. Following air-drying, the wells were covered with 10 μl of rabbit anti-horse IgG marked fluorescein isothiocyanate conjugate (FITC, SIGMA) of 1:160 dilution. After 30 min at 37°C the slides were washed and air-dried. Then they were counter-stained with Evans blue and cover slips mounted with buffered glycine. The reaction was examined using a fluorescence microscope (Olympus) at $\times 200$ magnification using 510 nm coloured light, excitation filters of 405-409 nm and barrier filter of 550 nm. Both a positive and negative serum were included in this test as control. In the case of a positive immunological reaction, spores were observed as oval fluorescent formations of 1.5-2.5 μm in size. As the majority of the asymptomatic horses showed activity in dilutions $\leq 1:32$, sera that reacted at a dilution $\geq 1:64$ were considered positive.

RESULTS

Of 72 asymptomatic horses, 43 (60%) were seropositive and 19% of the positive samples showed a titer of 1:512 (Tab. 1). Of 30 horses with various clinical signs, 25 (80%) were seropositive and 68% of the positive samples showed a titer of 1:512. High titers were associated with colic and neurological signs (Tab. 1).

DISCUSSION

The results of serological examination showed the occurrence of antibodies to *Encephalitozoon cuniculi* in horses in Israel. The sera showing positivity at a dilution of 1:64 and higher were chosen on the basis of selection of our positive samples, which were relevant with Chalupský *et al.* [9] previous experience. Titer of antibodies to *E. cuniculi* was shifted to the highest titer in horses with clinical signs compared to clinically healthy horses. Increasing the titer to follow microsporidia suggests multiplication of the antigen and a more severe

immune response against it. The pathological changes seen in the horses could be responsible for increasing the titer. Colic, neurological signs, and fever of unknown origin prevailed in the horses seropositive to *E. cuniculi*. These signs demonstrate a systemic affliction of the different organs. The question arises: could this be connected with the infection of the *E. cuniculi* with other microsporidia? Several works indicate that there is a cross-reaction of antibodies to *E. cuniculi* [5, 14]. Similarly, it is suggested that *E. intestinalis*, *E. hellem*, apart from infecting humans, also infects other mammals. In spite of the residence of *E. intestinalis* in the intestine, there is no report on this parasite playing a certain role in the arising of colic. Microsporidia as *E. intestinalis* could be one of the initiators of colic. Many neurological signs observed in horses were consistent with the changes seen in other animals infected with *E. cuniculi*. It is known that in rabbits infected with *E. cuniculi* there is a predominance of neurological signs [12].

To date, the only study by Van Rensburg *et al.* [20] has described morphological lesions due to *E. cuniculi* in horses. This report deals with an isolated case of a still born foal, showing renal lesions in the form of diffuse intestinal lymph plasmatic infiltrate and perivascular cuffing. Similar changes can be observed in *E. cuniculi* infection of the brain.

In conclusion, examination of seropositivity to *E. cuniculi* showed that prevalence of the highest titer of antibodies was detected in horses with clinical signs. Colic, neurological signs, and fever of unknown origin were mainly seen in seropositive horses. It is not clear to what degree microsporidia could cause clinically manifested changes. Cross-reaction of the antibody used with other mammals microsporidia can include *Encephalitozoon* species in participation in the course of the mentioned diseases [21]. It could prove to be interesting if the high percentages of prevalence of antibodies level in horses are an indication of health risk in humans.

We can only speculate as to the initial source of infection with *E. cuniculi* at these horses.

Further studies are needed to isolate and compare strains isolated from different hosts coming from this area.

Acknowledgments

We would like to express our sincere appreciation to Amir Steinman from the Hebrew University of Jerusalem for supplying the horse serum, data and professional advice. The presented work was performed within the frames of grant projects VEGA No. 1/0571/03 and 1/0580/03 of the Slovak Ministry of Education.

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