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Serological prevalence of E. intestinalis antibodies in sows in Eastern Slovakia*)

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Summary

The aim of the study was to examine the prevalence of antibodies against E. intestinalis in the blood serum of swine by the ELISA test and the possible effects of different states of the reproductive period on the occurrence of antibodies. The presence of specific anti-E. intestinalis antibodies was detected in 30 (56.6%) of 53 sows from the total count of examined sera. Each of the positive sera was reacted by the titre 1 : 200. Serological positivity to E. intestinalis was detected in 10 (58.8%) sows one week after birth of 17, in 6 (50%) sows one week before the birth of 12, in 7 (58.3%) sows one week after the weaning of 12 and in 7 (58.3%) sows one month after the birth of 12. The study did not detect any meaningful effect in the different state of the reproductive period of sows on the occurrence of antibodies.

Keywords: microsporidia, Encephalitozoon intestinalis, sows, ELISA

The term microsporidia is used, among others, as a general nomenclature for the obligate intracellular parasites belonging to the Phylum *Microsporidia*. To date, more than 1,200 species belonging to 143 genera have been described as parasites infecting a wide range of vertebrate and invertebrate hosts (1).

Microsporidia, are characterised by the production of resistant spores that vary in size, depending on the species. The microsporidia spores of species associated with human infection measure from 1 to 4 and that is a useful diagnostic feature (1). Two species of microsporidia in particular, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, are associated with gastrointestinal disease in human and animals (4, 8).

E. intestinalis was previously named *Septata intestinalis*; however, on account of its similarity at the morphologic, antigenic, and molecular levels to other species of this genus (1), it was reclassified.

The presence of species-specific antimicrosporidial antibodies has been confirmed by enzyme-linked immunosorbent assays (ELISA) or indirect fluorescent antibody tests (IFA). IFA tests require a compound microscope with a fluorescent light source, and ELISA tests require a plate reader to interpret results (7, 11).

The aim of this work was to study the occurrence of specific *E. intestinalis* antibodies in sows from different Slovak farms as potential sources of infection for the human population and possible effects of different states of different reproductive periods on the occurrence of antibodies.

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Material and methods

Altogether 53 randomly-selected clinical healthy sows (cross-breed Landrace × Slovak White) from three different Slovak farms were used in the experiment. All procedures involving the use of animals were approved by the Ethics committee for handling animals of the University of veterinary medicine, Košice, Slovak Republic.

Blood samples were withdrawn from the *vena cava cranialis* and blood serum were obtained by centrifugation at 3000 rpm.

Spores of *E. intestinalis* were used as antigens. They were grown in E6 (VERO green monkey kidney cells). The infected cells were cultivated in Roswell Park Memorial Institute 1640 medium, supplemented with 5% fetal calf serum and the addition of antibiotics and antimycotics. Spores were isolated from the medium by centrifugation and after rinsing in Percoll they were centrifuged again and stored at 4°C.

We used rabbit (anti-swine) immunoglobulin peroxidase conjugate (Sigma-Aldrich, Inc, USA) as a specific anti-species conjugate.

The prevalence of specific antibodies was determined in sera using ELISA (11). The animals whose sera reacted at a dilution of 1 : 200 were considered as positive.

Results and discussion

Animals whose sera reacted in a titre 1 : 200 and the average absorbancy of a sample was a minimum of 2.1 multiple of the average absorbancy of negative control were evaluated as positive. Specific antibodies against *E. intestinalis* were detected in 30 (56.6%) of 53 sows from the total count of examined serum.

Serological positivity to *E. intestinalis* was detected in 10 (58.8%) sows one week after birth, in 6 (50%) sows one week before birth, in 7 (58.3%) sows one week after weaning and in 7 (58.3%) sows one month before birth. Although the most frequent presence of species specific antibodies was detected in sows one month after birth (58.8%)

against other reproduction states, the difference was not meaningful, because the occurrence of antibodies in groups were from 50% to 58.8%.

E. intestinalis is the second most prevalent microsporidian species. The microspodia infects some domestic animals and humans (4), suggesting the possibility that *E. intestinalis* infection is zoonotic in origin. The presence of *E. intestinalis* has been documented in tertiary sewage effluent, surface water, and groundwater (8).

E. intestinalis causes an enteritis with diarrhea, weight loss, and malabsorption dissemination to ocular, genitourinary and respiratory tracts. These parasites may infect the biliary tract and gallbladder, resulting in cholangitis and cholecystitis. Left untreated, small intestine infection with *E. intestinalis* can lead to perforation and peritonitis (5, 6, 9, 12).

In our study we detected serological positivity against *E. intestinalis* in 30 sows from several Slovak farms in different states of the reproductive period using ELISA. The animals are potential sources of infection for other animals and humans.

Bornay-Llinares et al. (4) was the first to describe *E. intestinalis* infection of domestic animals. For the diagnostics of *E. intestinalis* infection, microscopic molecular methods, as well as serological assay (IFA), were used.

Valencakova (14) determined serological positivity of sows by ELISA and presence of *E. intetinalis* was confirmed in feces by PCR. It was the first report of *E. intestinalis* infection of swine in Europe.

A similar serological presence of antibodies against microsporidia has been diagnosed by serological assays (IFA or ELISA) in many animals: rabbits, mice, cats, dogs, sheep, goats, cattle, and wild animals such as muflons and fallow deer (10, 11, 13).

Boot et al. (3) compared the sensitivities and specificities of IFA and ELISA. *E. cuniculi* infection, which is a genetically similar species to *E. intestinalis*, was diagnosed in 135 purpose-bred rabbits by histology and 75 rabbits without a history of illness. A few samples were positive using only one method, but no statistically-significant differences were calculated between the sensitivities and specificities of the two assays.

Another study examined sera from rabbits, dogs, mice and squirrel monkeys using the dot-ELISA test, and compared the results to IFA. A few samples were positive according to ELISA and negative according to IFA, but none were positive according to IFA and not ELISA (1).

In conclusion, although the possibility was not confirmed that *E. intestinalis* infection is zoonotic in origin, the relatively high seropositivity of sows could result in *E. intestinalis* infection of people who worked with seropositive animals.

Further studies are needed to conclusively determine the different ways of the transmission of *E. intestinalis* from animals to people, as well as from wild animals to domestic animals, and confirm the possibility that *E. intestinalis* infection is zoonotic in origin, thus elucidating the epidemiology of *E. intestinalis*.

Tab. 1. Presence of anti-*E. intestinalis* in examinated serum of sows from farm I

ELISA (titer)	Sows n	pos. %	Sows n	neg. %
Presence anti *E. intestinalis* antibodies in serum of sows one week after birth	3	50	3	50
Presence anti *E. intestinalis* antibodies in serum of sows one week before birth	3	50	3	50
Presence anti *E. intestinalis* antibodies in serum of sows one week after the wean	5	83.3	1	16.7
Presence anti *E. intestinalis* antibodies in serum of sows one month before the birth	5	83.3	1	16.7

Tab. 2. Presence of anti-*E. intestinalis* in examinated serum of sows from farm II

ELISA (titer)	Sows n	pos. %	Sows n	neg. %
Presence anti *E. intestinalis* antibodies in serum of sows one week after the birth	2	33.3	4	66.7
Presence anti *E. intestinalis* antibodies in serum of sows one week before the birth	3	50.0	3	50.0
Presence anti *E. intestinalis* antibodies in serum of sows one month before birth	2	33.3	4	66.7
Presence anti *E. intestinalis* antibodies in serum of sows one week after the wean	2	33.3	4	66.7

Tab. 3. Presence of anti-*E. intestinalis* in examinated serum of sows from farm III

ELISA (titer)	Sows n	pos. %	Sows n	neg. %
Presence anti *E. intestinalis* antibodies in the serum of sows one week after birth	5	100	0	0

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