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Epidemiol. Mikrobiol. Imunol. 60, 2011, č. 4, s. 156–160

The Occurrence of Microsporidial Infections and Toxoplasmosis in Slovak Women

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SUMMARY

Aim of the study: **To determine the prevalence of antibodies to intracellular pathogens (*Toxoplasma gondii*, *Encephalitozoon cuniculi*, and *Encephalitozoon intestinalis*) in sera of Slovak women.**
Material and methods: **Enzyme-linked immunosorbent assay (ELISA) was used for the detection of specific IgG antibodies to *Toxoplasma gondii*, *Encephalitozoon cuniculi*, and *Encephalitozoon intestinalis* in 118 sera samples from Slovak women with different diagnoses.**
Results: **Women were divided into three groups based on the age. In group 1 (n = 26, age > 51), nine women tested positive for *T. gondii*, two for *E. cuniculi*, and one for *E. intestinalis*. The respective numbers in group 2 (n = 53, age range 36–50) were 18, three, and seven, and in group 3 (n = 39, age range 20–35) 13, two, and four. In an attempt to find the relationship between the clinical diagnosis and the prevalence of the three pathogens, we divided the study subjects into four groups based on the condition (diseases of the nervous, respiratory, and immune systems and pregnancy). A statistically significant relationship between the diagnosis and the prevalence of a pathogen was found (p < 0.001). In women with diseases of the respiratory system, antibodies against Toxoplasma gondii were most often detected, while infection with *E. intestinalis* was most commonly associated with nervous system diseases and the highest seroprevalence of *E. cuniculi* was found in women diagnosed with immune system disorders.**
Conclusions: **In our study, the seroprevalence rates of the three infections in pregnant women were similar, showing no significant difference. Women with diseases of the respiratory system were most often seropositive for *T. gondii*. Our result is consistent with the known fact that in humans, toxoplasmosis may affect various organs of the body, causing diverse clinical signs. However, the respiratory system is commonly involved and pneumonia may result. The most common finding is a mild, flu-like illness that lasts a few days. The women with different types of immunodeficiency disorders had the highest seropositivity rates for *E. cuniculi* and the second most common infection in this group was toxoplasmosis. Both of these parasites are opportunistic and can cause serious problems in immunocompromised individuals (HIV-positives, organ transplant recipients, chemotherapy patients). The women diagnosed with the nervous system disorders were most commonly infected with *E. intestinalis*. The relationship between the disease and this pathogen is not clear, but *E. intestinalis* is responsible for various pathologies; it causes mainly disseminated infections and damage to the digestive tract. *E. intestinalis* is a parasite very close to *E. cuniculi* affecting either the nervous system or the respiratory tract depending on the type of host.**
Keywords: **enzyme-linked immunosorbent assay – microsporidiosis – seroprevalence, toxoplasmosis**

SÚHRN

Luptáková L., Petrovová E.: Výskyt protilátok proti mikrosporidióznym infekciam a toxoplazmóze u slovenských žien

Cieľ práce: **Zistiť výskyt protilátok proti intracelulárnym patogénom (*Toxoplasma gondii*, *Encephalitozoon cuniculi* a *Encephalitozoon intestinalis*) u slovenských žien.**
Materiál a metodika: **Na detekciu špecifických IgG protilátok proti Toxoplasma gondii, *Encephalitozoon cuniculi* a *Encephalitozoon intestinalis* bola použitá imunoenzýmová analýza. Špecifické protilátky IgG boli detegované v krvných sérach 118 slovenských žien s rozličnou diagnózou pomocou ELISA.**
Výsledky: **Ženy pochádzajúce zo Slovenska boli rozdelené do troch skupín na základe veku. V skupine 1 (n = 26, vek > 51 rokov) bolo pozitívnych 9 žien na prítomnosť špecifických protilátok proti *T. gondii*, 2 na *E. cuniculi* a 1 na *E. intestinalis*. V skupine 2 (n = 53, vek 36–50 rokov) bolo 18 žien pozitívnych na *T. gondii*, 3 na *E. cuniculi* a 7 na *E. intestinalis*. V skupine 3 (n = 39, vek 20–35 rokov) bolo 13 žien pozitívnych na prítomnosť protilátok proti *T. gondii*, 2 na *E. cuniculi* a 4 na *E. intestinalis*. V rámci tejto štúdie sme sa pokúsili zistiť súvislosť medzi klinickou diagnózou**

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a výskytom jednotlivých typov infekcií. Ženy boli preto rozdelené do štyroch skupín na základe diagnóz (diagnózy súvisiace s nervovým, respiratórnym a imunitným systémom a gravidné ženy). V našej štúdii bol potvrdený štatisticky významný vzťah medzi diagnózou a výskytom infekcie ($p < 0.001$). U žien s výskytom ochorení respiratórneho systému bola detegované v najväčšej miere toxoplazmóza. U žien s ochoreniami nervového systému sa najčastejšie vyskytovala infekcia s *E. intestinalis* a u žien s ochoreniami imunitného systému sa vyskytovala prevažne infekcia *E. cuniculi*.

***Záver:* V rámci našej štúdie sme zistili, že prevalencia infekcií u gravidných žien a jednotlivých infekcií bola veľmi podobná. V skupine žien s respiratórnymi ochoreniami bola najčastejšia infekcia s *T. gondii*. Naše výsledky potvrdzujú skutočnosť, že u ľudí môže toxoplazmóza postihnúť rozdielne orgánové systémy a môže byť spojená s rozličnými klinickými príznakmi. Respiratórny systém býva v prípade toxoplazmózy postihnutý veľmi často a môže vyústiť až do pneumónie. Medzi veľmi často sa vyskytujúce klinické príznaky patria príznaky podobné chrípke. V skupine s diagnózami, ktoré súviseli s imunitným systémom, sa najčastejšie vyskytovala infekcia s *E. cuniculi* a druhou najčastejšou infekciou bola toxoplazmóza. Oba patogény patria medzi oportúnne patogény, ktoré môžu spôsobiť vážne zdravotné problémy v dôsledku imunodeficiencií (HIV, transplantácia, chemoterapia). V skupine s diagnózami spojenými s nervovým systémom sa najčastejšie vyskytovala infekcia s *E. intestinalis*. V prípade *E. intestinalis* nie je úplne jasné, ktoré patologické zmeny môže spôsobiť. Infekcia veľmi často prebieha diseminovane a veľmi často postihuje tráviaci trakt. Ale keďže je *E. intestinalis* veľmi blízky s *E. cuniculi*, ktorý postihuje nervový systém, je možné, že *E. intestinalis* môže spôsobiť poškodenie nervového systému.**

***Kľúčové slová:* imunoenzýmová analýza – mikrosporidióza – séroprevalencia – toxoplazmóza**

Introduction

Toxoplasma gondii (T. gondii) is a facultatively, heteroxenous, polyxenous protozoon that has developed several potential routes of transmission within and between different host species. It is a causative agent of toxoplasmosis, the one of the more common parasitic zoonoses world-wide [17]. In humans, *T. gondii* does not usually cause any symptoms and in most cases a person does not realise they have caught infection. It can cause symptoms like flu or sometimes an unpleasant illness similar glandular fever. But toxoplasmosis is a common latent infection, which can be dangerous to humans if their immune system is underdeveloped or compromises, as in the case of an unborn baby, somebody with HIV/AIDS or in immune-suppressant drugs. In such cases, the immune system is unable to restrict the spread of the parasite, which can then cause damage. If the first contact with *T. gondii* is during the pregnancy, *T. gondii* may be transmitted vertically by tachyzoites that are passes to the foetus via the placenta. The result of this transmission can be abortion or serious damage to the brain and eyes of an unborn child [4].

Microsporidia are obligate intracellular pathogens with a direct life cycle. They have a very extensive intervertebrate and vertebrate host range. In recent years they have emerged as important opportunistic pathogens of immunocompromised humans and several genera and species including *Encephalitozoon cuniculi (E. cuniculi)* and *Encephalitozoon intestinalis (E. intestinalis)* have been described as causing disease in man. Long-lasting subclinical infections usually develop in immunocompetent adult hosts infected with microsporidia, while in immunocopromised hosts, such as patients with an acquired immunodeficiency syndrome or those with organ transplants, clinically significant and potentially lethal infections may occur. *E. intestinalis* is associated with diarrhea, disseminated infection and superficial keratoconjuctivitis. *E. cuniculi* was the first microsporidium to be recognized as a parasite of mammals [15]. The first mammalian microsporidial infection was reported in 1922 by Wright and Craighead [20] in rabbits and this microsporidium was named by Levaditi et al. in 1923 [10]. In humans, the first case was described by Magarinos Torres in 1927 [18], but generally microsporidia were not routinely diagnosed. *E. cuniculi* has been associated with hepatitis, encephalitis and disseminated disease. All *Encephalitozoon* spp. develop within parasitophorous vacuole.

Material and methods

Samples

Serum samples were collected from 118 Slovak women during the period of January to December 2006 for a survey of the occurrence of two microsporidial infections and toxoplasmosis. After coagulation and subsequent centrifugation, the acquired sera were stored at the temperature of -20 °C until their use in serological tests.

Groups of women

In our study we tried to find the relationship between the age of examined women or clinical diagnosis and the occurrence of individual infection. Therefore, in the first case women were divided into three groups based on the age: group

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1 – women older than 51 years (n = 26), group 2 – women in the age 36–50 years (n = 53), and group 3 – women in the age 20–35 years (n = 39).

In the second case women were divided into four group based on the diagnosis. In the first group were women with diagnosis bound to nervous system. In the second group were women with diagnosis bound to respiratory system and in the third groups were diagnoses bound to immune system. The last group contains women during pregnancy.

Serological analysis

Toxoplasma gondii: An immuno-enzymatic test (ELISA) was carried out for the detection of IgG antibodies targeted against *T. gondii* according to the manufacturer's instructions (Test-Line, Czech Republic). The reaction is based on the principle of an indirect enzymatic reaction. Serum were diluted 1 : 100 or 1 : 200. For each sample, Index of positivity (IP) was calculated according to the scheme provided by the manufacturer: IP = sample absorbance/average absorbance of cut-off serum (cut-off serum is serum sample which contains antibodies to *T. gondii* in limiting concentration). Samples with IP > 0.8 were considered to be negative, samples with IP between 0.8–1.0 were considered to be dubious and samples with IP > 1.0 were considered to be positive.

***Encephalitozoon* spp.:** Mature spores of microsporidia (*E. cuniculi* and *E. intestinalis*) grown in monolayer cell cultures of rabbit kidney cells (RK 13) in vitro were used as antigens in the serological assays [19]. Permanently infected cells were cultivated in the modified RPMI 1640 medium supplemented with 5% foetal bovine serum and with addition of antibiotics (streptomycin/gentamicin). After centrifugation (4 000g, 20 minutes, 20 °C), spores were isolated from the supernatant, rinsed in PBS, counted in a haemocytometer and finally re-suspended in PBS to the required concentration (106 spores/mL). The IgG antibodies against *E. cuniculi* and *E. intestinalis* were detected using a modified ELISA described by Hollister and Canning in 1987 [7]. Briefly, 100 µL of *E. cuniculi* or *E. intestinalis* spores (10^6 spores/mL) in coating buffer (Na_2CO_3 – Sodium carbonate and $NaHCO_3$ – sodium bicarbonate) were put into each well of ELISA plates and after incubation overnight at 4 °C, the plates were dried and fixed with 1 : 1 mixture of acetone and methanol for 10 min. After blocking the unbound sites with 5 % new-born calf serum (NBCS) in PBS for one hour at 37 °C, the plates were washed with 0.05 % Tween 20 in PBS (T-PBS) three times. Each serum diluted to 1 : 200 and 1 : 400 in 2% NBCS in PBS (0.1 mL) was added into the well and after incubation (37 °C, 1 hour), plates were washed with T-PBS and incubated with rabbit anti-swine immunoglobulin peroxidase conjugate (Sigma, Germany) for one hour at 37 °C. After washing, an enzymatic colour reaction was generated using orthophenylenediamine substrate (OPD). The reaction was stopped with 2 M H_2SO_4. Sample absorbance was measured by a spectrophotometer Dynex Elisa Reader (Dynex Technologies, USA) at the wavelength of 490 nm. Sera for which the absorbance was at least 2.1 times higher than the absorbance of a negative control serum were considered positive.

Statistical analysis

The significance of the differences in prevalence of toxoplasmosis and encephalitozoonosis according to the age of women and the significance of clinical diagnosis according to the occurrence of infection was evaluated by Chi Quadrate test and was considered as positive when p value was less than 0.05.

Results

In our study the prevalence of antibodies to *T. gondii, E. cuniculi* and *E. intestinalis* was examined in women serum samples by ELISA.

In the first case the prevalence of these three infections was found out in three aged groups of women. In the group 1 (n=26, > 51) 9 women were tested positive for T. gondii, 2 for *E. cuniculi* and 1 for *E. intestinalis*. In the group 2 (n = 53, age 36–50) 18 were tested positive for *T. gondii*, 3 for *E. cuniculi* and 7 for *E. intestinalis*. In the group 3 (n = 39, age 20–35) 13 were tested positive for *T. gondii*, 2 for *E. cuniculi* and 4 for *E. intestinalis*. By statistical analysis no statistical significance was proved (Table 1).

Table 1. The seroprevalence of three opportunistic pathogens in the individual aged group of women

Groups of women	Infection with	Positive samples	%
Group 1 n = 26	*T. gondii*	9	34.6
	E. cuniculi	2	7.7
	E. intestinalis	1	3.8
Group 2 n = 53	*T. gondii*	18	34.0
	E. cuniculi	3	5.7
	E. intestinalis	7	13.2
Group 3 n = 39	*T. gondii*	13	33.3
	E. cuniculi	2	5.1
	E. intestinalis	4	10.3

n – Number of examined women

Table 2. The relationship between the occurrence of individual infection and clinical diagnoses of examined women

Diagnosis	Infection with		
	T. gondii	*E. cuniculi*	*E. intestinalis*
Respiratory system n=35	22	1	0
Immune system n = 30	4	5*	1
Nervous system n = 25	1	1	10*
Pregnancy n = 28	13	0	1

*($p < 0.001$)

In the second case the relationship between the occurrence of individual infection and the clinical diagnosis was examined. The diagnoses bound to respiratory system were mainly found with toxoplasmosis. Infection with E. cuniculi was mainly found in women with diagnoses bound to immune system and infection with E. intestinalis was mainly found in women with diagnoses bound to nervous system (Table 2). The relation between

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the occurrence of individual infection and the diagnoses was statistically significant ($p < 0.001$).

Discussion

In human medicine opportunistic pathogens (microsporidia, *T. gondii*) were first known as casual pathogens for immunosupressed patients mainly after organ transplantation, but cases of clinical diseases were rare. Most current data about toxoplasmosis and microsporidial infections were obtained after as much as knowledge of spectrum of potential pathogens in context of discovery of AIDS. During opportunistic infections caused by microsporidia or *T. gondii* three main types of relationship between pathogens and hosts are present. In the first type are immunocompetent hosts. In those persistent subclinical infections are developing. The second type of relationship includes immunosupressed hosts. In those clinical significant infections with organ damage are manifesting. In the third group there are hosts after transplacental infections. The results of these infections could be acute or lethal disease.

In our study we had a group of pregnant women. Here is important one question. Is the immune system during pregnancy lowered? Recently we can find studies with two main explanations to this question. The one explanation is that the pregnancy decreases the immune response to infectious diseases. Immune system is suppressed during pregnancy otherwise the baby might be rejected by mother's body as a foreign object. This can make a pregnant woman more prone to infection and diseases which have implications on her own health as well as for the developing fetus [11]. Pregnant women are considered immunocompetent during pregnancy; however, the maternal immune response may be modulated away from cellular immunity, towards humoral immunity. It is speculated that selective aspects of cell-mediated immunity are also depressed in pregnancy. Therefore parasitic infections, against which the host defenses are primarily cell-mediated, are likely to be more virulent during pregnancy [1, 16]. On the other side we can find studies that contradict to the theory described above. This study explains why pregnant women respond differently to the presence of microorganisms or its products. Therefore, pregnancy should not imply more susceptibility to infectious diseases; instead there is a modulation of the immune system which leads to differential responses depending not only on the microorganisms, but also on the stages of pregnancy [14]. In our study the seroprevalence of pregnant women to individual infections was similar. Seroprevalence of no examined infection was significantly higher. In the other group, women with diseases of respiratory system were included. In this group the most often infection was infection with *T. gondii*. Our result establishes fact, that in humans, toxoplasmosis may affect many different organs of the body, causing many different types of clinical signs. However, the respiratory system is commonly involved and pneumonia may result. The most common finding is a mild, flu-like illness that lasts a few days [13]. The next group included women with some kinds of immunodeficiency. In this group infection of *E. cuniculi* was found in the most of cases, and the second most often infection was toxoplasmosis. Both of these parasites are opportunistic and they can cause serious problem when the immune system is decreased (by HIV, organ transplantation, chemotherapy). In the last group women with diagnoses bound to nervous system were included. In this group infection with *E. intestinalis* was most common. It is not so clear because E. intestinalis is responsible for various pathologies; it causes mainly disseminated infections and damage to digestive tract. However, *E. intestinalis* is parasite very closed to *E. cuniculi* affecting the nervous system as well as the respiratory tract depending on the type of host.

The prevalence of microsporidial infection (*E. cuniculi* and *E. intestinalis*) in 215 Slovak humans was examined by Malcekova et al. [12]. The presence of antibodies to *E. cuniculi* was proven in 2 (0.9%) and to *E. intestinalis* in 12 patients (6%). In this study patients were divided into three groups based on the type of infection (bacterial, viral and gynaecological). The prevalence of anti-*E. intestinalis* antibodies was the highest in gynaecological patients and anti-*E. cuniculi* antibodies was the highest in patients with bacterial infections. Other studies in various parts of the world monitored seroprevalence of microsporidial infections. Based on these studies, Bryan and Schwartz in 1999 [2] estimated an overall prevalence of microsporidiosis in AIDS patients of 15%. In individuals not infected with HIV, seroprevalence rates ranged from 1.3 to 22% among blood donors, pregnant women, slaughterhouse workers, and persons with unknown causes of diarrhea, similar to the earlier seroprevalence data [3, 8, 9]. Cislakova and Halanova [5] examined 120 patients with some secondary immunodeficiency and 198 immunocompetent individuals by ELISA for the detection of anti-*E. intestinalis* antibodies. 29 (24.16%) patients out of 120 patients with secondary immunodefiecieny were tested positively and 20 (10.1%) out of 198 immuno-

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competent individuals were tested positively. Halánová et al. [6] also examined an antibody immune response to the presence of antibodies against E. cuniculi using indirect immunofluorescence antibody test (IFAT). Of the total number of 113 sera from immunologically compromised patients examined, 5 were positive at the titre 1:32, 5 at the titre 1:64, and 6 at the titre 1:256. From total number of positive respondent patients were 12 children and 4 adults.

The serological positivity recorded in our study within the chosen group of women confirmed that it is more than advisable to accomplish screening examination in human population and, consequently, to decrease spreading of these opportunistic infections mostly from infected animals as well as from environment.

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Acknowledgement: The study was financed by Grant VEGA Nos. 1/0271/11 and 1/0108/10 of the Ministry of Education and Science of the Slovak Republic.

Do redakce došlo dne 6. 5. 2011.

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