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Parasitic infections and pregnancy complications

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Summary

Infections of various aetiology during pregnancy present a serious risk factor that can lead to abortion, premature birth, stillbirth, birth of newborn with genetic developmental defects, or seemingly healthy infant that may develop symptoms later in life. A total of 530 sera of patients from gynaecology departments were examined for antibodies against *Toxoplasma gondii*, *Toxocara* spp. and *Listeria monocytogenes* using ELISA (IgG, IgM). In women with habitual abortions we determined up to 42.1 % seroprevalence of IgG antibodies against *T. gondii.* The prevalence of antibodies was statistically significant ($p < 0.0004$) when compared to control group (25.1 %). In the patients diagnosed with sterility the prevalence of antibodies (26.3 %) was similar to that of control group. The prevalence of antibodies against *Toxocara spp.* (5.5 %) and *Listeria monocytogenes* (2.3 %) in patients with habitual abortions were similar to those of women in the general population.

Keywords: human; pregnancy; *Listeria monocytogenes*; *Toxoplasma gondii*; *Toxocara* spp.; ELISA

Introduction

An infection of any aetiology could disturb the course of pregnancy and severely affect the foetus. Their consequences could manifest at later stages of pregnancy, causing habitual abortions. According to Krajčovičová *et al.* (2007), spontaneous abortion affects up to 15 – 20 % of recognized pregnancies.

The most common causative agents of intrauterine infections are parasites (*Toxoplasma gondii, Toxocara cati, Toxocara canis*), bacteria (*Escherichia coli*, *Listeria monocytogenes),* viruses (Rubella, Cytomegalovirus - CMV, Varicella - Zoster Virus, Human Immunodeficiency Virus - HIV, Human Papillomavirus - HPV) (Sterner G. *et al*., 1990). *T. gondii* has been included in the list of TORCH infections (Turbadkar *et al*., 2003). It is a group of maternally acquired communicable diseases of foetus and newborn that are rather similar in terms of clinical manifestation, a high risk of permanent damage and negative impact on pregnancy outcome (Ross *et al*., 2006).

Toxoplasmosis is one of the most common parasitic infections with a worldwide prevalence, caused by intracellular protozoan parasite *T. gondii*. Up to 50 % of the world's human population is estimated to carry a *Toxoplasma* infection, though in 9 out of 10 infected people the parasite rarely causes any symptoms. An acute *Toxoplasma* infection during pregnancy occurs even more rarely (0.2 – 1 %) (Wilson & Remington, 1987). In our conditions, the incidence of congenital toxoplasmosis has been estimated as 1 – 2 cases per 1000 live births. Newborns born to mothers with positive IgM antibodies against *T. gondii* had lower birth weight (Čatár *et al*., 1998). Number of children born with congenital Toxoplasma infection per year exceeds the number of children born with congenital rubella, syphilis and herpes simplex (Palička *et al*., 1998). A primary infection with *T. gondii* usually leads to formation of tissue cysts and the immunity, which persist for the lifetime of the host. Even though congenital infection occurs usually when the mother suffers a primary acute infection, it can follow from reactivation of latent infection, particularly in immunodeficient individuals (Wong *et al*., 1994), and even immunocompetent pregnant women (Silveira *et al*., 2003, Andrade *et al*., 2010). Thus toxoplasmosis has been listed as one of the serious opportunistic infections (Luft, 1992). Several authors have mentioned a link between latent toxoplasmosis and increased risk of abortions, or negative consequences to neonatal health (Remington *et al*., 1964; Hingorani *et al*., 1970; Decavalas *et al*., 1990; Al-Hamdani *et al*., 1997).

The cat serves as the host for both *T. gondii*, and *T. cati,* their infective stages both occur in the environment. Thus toxoplasmosis goes hand in had with toxocarosis. During serological examinations of the suspected patients, both infections are

often found accidentally. Clinical symptoms of toxocarosis in humans vary and result from mechanical damage to the tissues caused by the migrating larvae and depending on the intensity of infection and their immunosuppressive effect on human organism (Ondriska *et al*., 2002).

Listeriosis is a bacterial infection caused by the opportunistic pathogen *L. monocytogenes* (Bálint, 2003). Even very mild *Listeria* infection in pregnant women may have a fatal outcome for foetus. Genital listeriosis may cause habitual abortions (Štork *et al*., 1981). Intrauterine infections can lead to spontaneous abortions, premature birth or stillbirth (Ross *et al*., 2006). Listeriosis in newborns can manifest as sepsis, meningitis, multiorgan abscesses, granulomas, affecting mucosa of the upper airways and gastrointestinal tract (Ellner, 1992).

Repeated infections in women lead to fertility problems. One of the reasons why the endometrium does not allow the fertilized egg to adhere to it is chronic inflammation of various aetiology (Řežábek, 2008). Chronic inflammation leads to functional intrauterine abnormalities and to reduction in endometrial receptivity, which negatively affects the process of embryo implantation and its early development (Hudeček *et al*., 2009). It is in particular the case of chronic infections - toxoplasmosis, listeriosis, brucellosis, rubella, cytomegalovirus, herpes virus. Up to 45 % of fertility problems are also due to the man (Citterbart *et al*., 2001) and at present, the number of childless couples varies between 15 – 20 % and is still increasing (Jirsová *et al*., 2009).

Material and methods

During 2007 – 2009 we examined 537 samples of the patients with pathological gynaecological anamnesis for the presence of specific anti-Toxoplasma, anti-Toxocara and anti-Listeria antibodies. All sera were collected at the gynaecology and infectious diseases departments located in Eastern Slovakia.

An average age of the patients was 30.2 ± 5.3 years and they were divided into three groups based on their diagnoses. The first group comprised 221 women diagnosed with Pregnancy Ending in Abortion (O 00-08); second consisted of 46 patients with Female Sterility (Infertility) (N 97); and the last group was formed by 179 women with a physiological course of pregnancy.

Sera samples, collected concurrently, were stored at the temperature of 2 – 8 °C in the refrigerator until further use, or at -20 °C.

Specific IgG and IgM antibodies against *T. gondii* were determined using a commercial diagnostic kit EIA Toxoplasma IgG and IgM EIA Toxoplasma (Test-Line, Canada). Methodologically, we follow the manufacturer's instructions. The absorbance-optical density (OD) was measured at 450 nm by spectrophotometer (ThermoLabsystems, Opsys MR, USA). Toxoplasmatic titer IgG and IgM antibodies present in the sample was determined quantitatively in international units IU/ml by reference to the calibration curve in the range of 6 – 240 IU/ml. Positivity threshold specified by the manufacturer is 6 IU/ml.

The data were evaluated based on the calculating the Positivity Index (IP):

IP = Sample Absorbance/Average Absorbance CUT-OFF

IP < 0.8 negative; IP 0.8 – 1.0 borderline; IP > 1.0 positive. Determination of specific antibodies was performed according to the manufacturer's recommendations and protocols.

In order to determine the specific antibodies against *T. cati*, ELISA test (Mačal, 1987) with excretory-secretory antigen (ES) was used. The data were analysed using spectrophotometric absorbance values measured at 490 nm (Thermo-Labsystems, Opsys MR, USA). The data were interpreted according to Table 1.

Table 1. Scheme for data interpretation

Absorbance	Titre
≤ 0.500	Negative
0.500 – 0.600	Borderline /1:100/
0.600 – 0.900	Low /1:200/
0.900 – 1.200	Moderate /1:400 – 1:800/
> 1.200	High /1:1600/
> 1.800	Very high /1:3200/

Sera were examined for the presence of specific antibodies against *L. monocytogenes* using complement fixation test in a co-operation with the Regional Authority of Public Health in Košice.

Statistical analysis

Mutual dependence of IgG antibodies against *T. gondii* in all three groups was analyzed using χ^2 test, calculating the OR (Odds Ratio) and CI (Confidence Interval). Comparison of the statistical significance in the observed differences between the values was performed using the Software GraphPad Prims 5.

Results

Comparison of the occurrence of specific anti-Toxoplasma antibodies in selected women groups are given in Table 2. In the group of 221 women with habitual abortions we determined 42.1 % seropositivity of IgG anti-Toxoplasma antibodies, with the patients average age 31.3 ± 5,6 years. Out of the set, IgM antibodies were determined in 1.8 % (4/221) examined patients. In the group of 179 women with physiological pregnancy and an average age 29.4 ± 5.6 years, the seropositivity was 25.1 %. The difference in the seropositivity values was statistically significant (χ^2 12.5615; $p < 0.0004$, OR - 2.16, 95 % CI - 1.4066 – 3.3276). In the sterile group of women we determined 26.3 % seropositivity of IgG antibodies, while IgM antibodies were determined only in a single case. An average age of the group was 30.09 ± 5.8 years. Our data on the occur-

Table 2. Results of ELISA test for toxoplasmosis in different groups of subjects

	1 - normal pregnancy group		2 - habitual abortion group		3 - sterility group	
Toxoplasmosis	No.	%	No.	%	No.	%
Seropositive	45	25.1	93	42.1	36	26.3
Seronegative	134	74.9	128	57.9	101	73.7
Total	179	100	221	100	137	100

1-2 χ^2 12.56; $p < 0.0004$; OR - 2.16, 95 % CI - 1.4066 - 3.3276
1-3 χ^2 0.05; $p = 0.8231$; OR - 1.06; 95 % CI - 0.6382 – 1.7653

rence of specific anti-Toxoplasma antibodies among women diagnosed with sterility and women with physiological course of pregnancy were not statistically different (χ^2 0.05, p = 0.8231, OR - 1.06, 95 % CI - 0.6382 – 1.7653).
When comparing the age-adjusted occurrence of IgG antibodies (Fig. 1) we found the highest seropositivity in the age group of 37 – 44 years. It is interesting to note the high

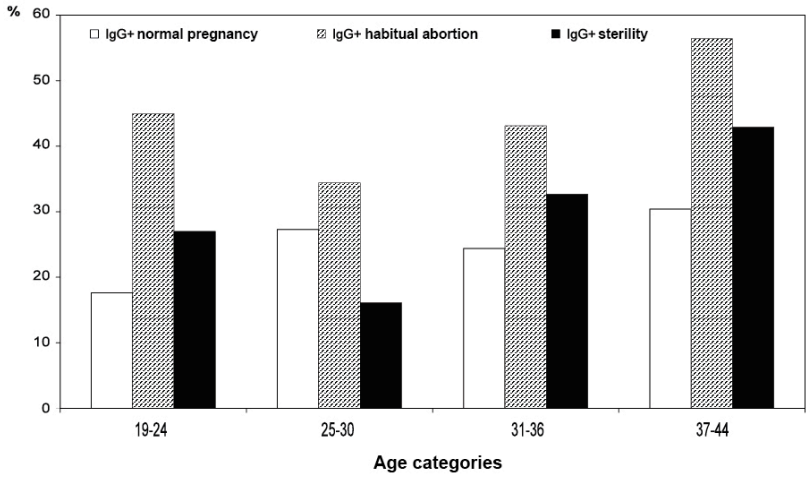

Figure 1. Seroprevalence of IgG antibodies against *T. gondii* among women of different age

seropositivity values were determined in the youngest age group (19 – 24 years) with a history of habitual abortions.
In the group of 221 women (Table 3) with habitual abortions we also focused on the detection of other pathogens that can severely affect the course of pregnancy. The sera were examined for the presence of anti-Toxocara and anti-Listeria antibodies. We determined the low seropositivity for anti-Toxocara and anti-Listeria antibodies, 5.5 % (12/221) and 2.3 % (5/221), respectively.

Table 3. Seroprevalence of antibodies against *Toxocara* spp. and *L. monocytogenes* among pregnant women with habitual abortions

	Habitual abortion group				
	No of samples	Negative		Positive	
		n	%	n	%
Toxocarosis	221	209	94.5	12	5.5
Listeriosis	221	216	97.7	5	2.3

Discussion

Toxoplasmosis is a serious zoonosis affecting both humans and warm blooded animals. In immunocompetent patients it can manifest as asymptomatic infection or it can be accompanied by non-specific clinical symptoms and its detection is often accidental (Bobić *et al*., 1998).

In the control group with the physiological course of pregnancy, 25.1 % seroprevalence of IgG antibodies against *T. gondii* was determined, while IgM antibodies were detected only in 2.8 % of the patients. Low percentage of IgM indicating an acute infection can be due to the fact that the group comprised a healthy population of pregnant women. On the other hand the presence of the IgG antibodies refers to the fact that up to one third of a healthy women population has been exposed to *Toxoplasma* infection and has developed antibodies (Ondriska *et al*., 2003).
Our data on seropositivity of IgG anti-Toxoplasma antibodies correlate with the data of other authors where the seroprevalence ranged between 7 – 51.3 % and in women with a history of abortions and pregnancy failures varied from 17.5 to 52.3 % (Singh, 1998). Our data are also similar to those from the Czech Republic, which rather resembles Slovakia in terms of geographical, social and economic conditions. Svobodová and Literák (1998) report 32.1 % seroprevalence of IgG anti-Toxoplasma antibodies in the healthy population of the Czech Republic. Hejlíček *et al*. (1999) report prevalence of 25 – 35 % in pregnant

women, depending on a technique used for examination of *T. gondii*. In the healthy population in Slovakia, seroprevalence of 24.2 % (Studeničová *et al*., 2006) and 31.3 % (Strhársky *et al*., 2009) was determined. In the countries with an increased exposure to risk factors, such as Saudi Arabia, Brazil and France, the prevalence of toxoplasmosis varies between 35 – 80 % (Studeničová *et al*., 2006).

For the pregnant women diagnosed with Dg. O00-08 Pregnancy Ending in Abortion, *T. gondii* is highly pathogenic. In this group, 42.1 % seropositivity of IgG antibodies was detected that is almost double when compared to a group of women with physiological course of pregnancy.

Data resulting from our study indicate the link between chronic toxoplasmosis and habitual or repeated sporadic abortions. Similar data were reported in the studies of several authors (Langer, 1963; Hingorani *et al*., 1970; Sharf *et al*., 1973; Mahajan *et al*., 1976; Sahwi *et al*., 1995; Al-Hamdani, 1997). Turbadkar *et al*. (2003) also reported 42.1 % prevalence of IgG antitoxoplasma antibodies in a group of women with BOH (Bad Obstetric History). On the other hand such data do not correlate with other studies, which do not support the link between *Toxoplasma* infection and abortions (Southern, 1972; Stray-Pedersen, 1977; Golledge, 1990).

The highest IgG seropositivity of anti-Toxoplasma antibodies was recorded in an age group of 37 – 44 years in all studied groups (Fig. 1) with an increasing trend of seroprevalence. This may be due to the fact that humans are repeatedly exposed to the parasite and thus seroprevalence in the population increases with age. A high seroprevalence of IgG antibodies in a group of women aged 19 – 24 with repeated abortions may be due to a small number of examined sera. Our data on seroprevalence increasing with age correlate with the data of other authors (Studeničová *et al*., 2006; Strhársky *et al*., 2009).

Despite the fact that *T. gondii* and *Toxocara* spp. are distinctively different parasites, they have a similar mode of transmission to humans. In the group with repeated abortions we determined rather low percentage of anti-Toxocara antibodies that correlates with the 7.0 % prevalence of anti-Toxocara antibodies in pregnant women (Schlosserová *et al*., 2007). In comparison to the data of other authors, our values are slightly lower, i.e. 11.9 % (Reiterová *et al*., 2003), 12 % (Ondriska *et al*., 2002) as pregnant women represent a healthy population sample.

According to Tomolová *et al*. (2004) the population comprises 5 % of asymptomatic carriers of *Listeria.* Pregnant women are though more sensitive to *L. monocytogens* infection and as much as 30 % intrauterine infected foetuses are stillborn (Mašata, 2004). In our group of pregnant women with a history of habitual abortions the seropositivity of anti-Listeria antibodies was rather low (2.3 %). Even though there are no reports concerning similar patient group, several authors report serious effect of *Listeria* infection on repeated abortions, or severe threat for the foetus or newborn (Rappaport *et al*., 1960; Benshushan *et al*., 2002).

At present, infertility is still regarded as a serious problem that needs to be treated. In a group of women struggling with fertility problems the seroprevalence of anti-Toxoplasma antibodies reached 26.3 %. Such results, however, did not significantly differ from those of the control group of healthy pregnant women. Similar seropositivity of 27 % in the patients diagnosed with sterility was reported by Huang *et al*. (1998). It is interesting to note that *Toxoplasma* and *Toxocara* infections have been identified as risk factors affecting male infertility due to malfunctions in spermiogenesis (Qi *et al*., 2005; Terpsidis *et al*., 2009).

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