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SEROPREVALENCE OF TOXOPLASMA GONDII, VARICELLA ZOSTER VIRUS AND HUMAN PARVOVIRUS B19 AMONG WOMEN IN THE BIAŁA PODLASKA DISTRICT OF EASTERN POLAND

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A – study design, **B** – data collection, **C** – statistical analysis, **D** – interpretation of data, **E** – manuscript preparation, **F** – literature review, **G** – sourcing of funding

ABSTRACT

Background: Infections in pregnant women or women planning pregnancy caused by the protozoan *Toxoplasma gondii* and the viruses *varicella zoster virus* (VZV) and human parvovirus B19 can be a danger to the fetus.

Aim of the study: The aim of the study was to determine the serological status of women of childbearing age in relation to *T. gondii,* VZV and human parvovirus B19 in a region of Eastern Poland (Biała Podlaska District).

Material and methods: The study group consisted of 174 women aged 19 to 35 (average 23, SD 3.68) from the Biała Podlaska District. Anti-*T. gondii* IgM/IgG antibodies, anti-VZV IgG and anti-human parvovirus B19 IgG were detected by ELISA.

Results: Serological screening revealed that the most common antibodies were anti-VZV and anti-parvovirus B19 (in 96% and 60.9% of women, respectively). Anti-*T. gondii* antibodies were found in 28.6%. No correlation was found between the presence of anti-*T. gondii*, human parvovirus B19, and VZV antibodies and the age of the examined women, their place of residence, and their education.

Conclusions: About 4%, 39% and 71.2% of women participating in this study were still susceptible to infection with VZV, human parvovirus B19, and *T. gondii,* respectively. It is therefore important to address health education primarily in women of childbearing age in order to help them undertake relevant measures for prevention of *T. gondii*, human parvovirus B19 and VZV infection.

KEYWORDS: human parvovirus B19, toxoplasma, herpesvirus 3, human, Poland, women

Background

Infections in pregnant women or women planning pregnancy caused by the protozoan *Toxoplasma gondii* and the viruses *varicella zoster virus* (VZV) and human parvovirus B19 can be a danger to the fetus. *Toxoplasma gondii* infections are mainly dangerous to pregnant women due to the potential transmission of the maternal infection through the placenta to the fetus, which leads to development of congenital toxoplasmosis in the fetus [1]. In rare cases, the fetus can be infected from a mother who was seropositive before pregnancy due to reinfection with a more virulent protozoan strain or recurrence of chronic infection, which can occur in immunosuppressed pregnant women [2]. In the case of primary infection, *T. gondii* exhibits affinity mainly to the central nervous system, eye, skeletal muscle, cardiac muscle, and placenta [3].

Varicella zoster virus (VZV), in accordance with the applicable nomenclature *Human alphaherpesvirus 3*, belongs of the *Herpesviridae* family and cause infections usually acquired during childhood. VZV is the etiological factor of chickenpox. However, the primary infection

of VZV in pregnant women can cause congenital varicella syndrome (CVS) [4], maternal varicella pneumonia, and neonatal varicella [5]. The symptoms of CVS in newborns are low birth weight, limb hypoplasia, eye disorders, neurological abnormality, and backwardness [6].

Human parvovirus B19 (*Parvoviridae* family) infects through the respiratory system, blood transfusion, and blood products, and vertical transmission from mother to fetus [7,8]. The percentage of people with anti-human parvovirus B19 antibodies increases with age but a lot of people become infected during their childhood and adolescence [7,9]. Most human parvovirus B19 infections are asymptomatic. However, in 3% of infected pregnant women it can cause severe congenital anomalies to the fetus, fetal anemia, non-immune hydrops fetalis, intrauterine fetal death and spontaneous abortion [8,10]. Virus infection can occur any time during the pregnancy period. However, the highest risk of fetal loss is during the second trimester [10].

In 2015, 41 cases of congenital toxoplasmosis were reported in total in the European Union: in the Czech Republic, Germany, Hungary, Ireland, Lithuania, Poland, Slovenia, and the United Kingdom. France reported data with a 2-year delay and there were 216 confirmed congenital toxoplasmosis cases in 2014 [11]. As shown by the data from the National Institute of Public Health – National Institute of Hygiene, 19 cases of congenital toxoplasmosis were recorded in 2016 in Poland (incidence: 4.97 per 100 000 live births), which is 4 cases more than in 2015. Chickenpox was reported in 3419 women between the ages of 20 and 39 who were not vaccinated, and 1 case of congenital varicella was recorded in 2016 in Poland (incidence: 0.26 per 100 000 live births) [12]. As shown by data from the National Institute of Public Health, 69,357 vaccinations against varicella were made in 2016 in Poland, 1206 of which were performed on people over 20 years of age. Vaccination against VZV is recommended for women planning pregnancy who have not had chickenpox [13]. Regular recording of infections caused by human parvovirus B19 is not carried out.

Aim of the study

The aim of the study was to determine the serological status of women of childbearing age in relation to *Toxoplasma gondii*, VZV and human parvovirus B19 in a selected region of Eastern Poland.

Material and methods

Study design

The National Institute of Public Health – National Institute of Hygiene, Department of Epidemiology and Surveillance of Infectious Diseases registered cases of toxoplasmosis and congenital toxoplasmosis until 2008. In 2008, 19 cases of toxoplasmosis (incidence of 0.88 / 100,000) and 93 cases (incidence of 7.8 / 100,000) were found in the Lubelskie Voivodeship in the Podlaskie Voivodeship, respectively. It was a significant number of cases in the country. Since 2009, only cases of congenital toxoplasmosis have been recorded [14]. The exposure of women, especially of childbearing age, to *T. gondii* infections has not changed.

Setting

The Biała Podlaska region of Eastern Poland was selected as the research area due to its location on the border of the two mentioned provinces. Blood samples were collected in May 2015 by venipuncture. Sera were separated by centrifugation and stored at -20° C until analysis. Whole collected samples were tested by enzyme-linked immunosorbent assay (ELISA). The study was approved by the Bioethical Committee of the Medical University of Lublin, permission No. KE-0254/183/2014.

Participants

The study group consisted of 174 women aged 19 to 35 (average 23, SD 3.56) in the selected region of Eastern Poland (Lublin province, Biała Podlaska District): 95 women aged 19 to 34 (average 22, SD 2.93) lived in the countryside and 79 women aged 19 to 35 (average 23, SD 4.12) lived in the city of Biała Podlaska.

Variables

Anti-*T. gondii* IgM antibodies were detected by ELISA (Euroimmun, Germany). Results above or equal to 1.1 (ratio) were considered as positive, below 0.8 (ratio) as negative, whereas borderline results were ≥0.8 and <1.1 (ratio). The presence of anti-*T. gondii* IgG was detected by ELISA (Euroimmun, Germany). Results above or equal to 11 international units/ml (IU/ml) were considered as positive, below 8 IU/ml as negative, whereas borderline results were ≥8 and <11 IU/ml.

The presence of anti-VZV IgG was detected by ELISA (Euroimmun, Germany). Results above or equal to 110 IU/ml were considered as positive, below 80 IU/ml as negative, whereas borderline results were ≥80 and <110 IU/ml.

The presence of anti-parvovirus B19 IgG was detected by ELISA (Euroimmun, Germany). Results above or equal to 5.5 IU/ml were considered as positive, below 4 IU/ml as negative, whereas borderline results were ≥4 and <5.5 IU/ml. The tests were carried out and the results were interpreted according to the manufacturer's instructions.

Statistical methods

The data obtained were analyzed statistically using Statistica v.10 software (Chi-square test, Kruskal-Wallis test). The assumed level of significance was p=0.05.

Results

Outcome data

Among the participants of the investigations, 45.4% (79/174) lived in the city of Biała Podlaska and 54.6% (95/174) lived in the countryside.

Secondary school education was declared by 70.5% (67/95) of participants who lived in the countryside and 64.5% (51/79) of participants who lived in the city. Higher education was declared by 29.5% (28/95) who lived in the countryside and 35.4% (28/79) who lived in the city of Biała Podlaska.

Serological screening revealed that antibodies against VZV and human parvovirus B19 were the most common, being observed in 96% (167/174) and 60.9% (106/174) of participants, respectively. Positive results for anti-*T. gondii* antibodies only in the IgM or IgG class were found in 1.1% (2/174) and 23.5% (41/174) of the examined women, respectively. Simultaneous presence of anti-*T. gondii* IgM and IgG was reported in 4% (7/174) of participants.

Of participants who were positive for VZV antibodies, 27.6% (484/174) were positive for VZV only and 68.3% (119/174) were positive for human parvovirus B19 and/or *T gondii* as well. Human parvovirus B19 and VZV antibodies were detected in the same participants most frequently (39.1%, 68/174), whereas *T. gondii* and VZV antibodies were detected together less frequently (9.8%, 17/174). There were some samples (12.6%, 22/174) with concomitant *T. gondii,* human parvovirus B19, and VZV antibodies. Detailed results are shown in Table 1.

Table 1. Results of serological tests for *T. gondii*, VZV, and human parvovirus B19 – general screening.

anti-*T. gondii*	anti-*T. gondii*	anti-human parvovirus B19	anti-VZV	N(%) 174(100)
IgM	IgG			
-	-	+	+	68(39.1)
-	-	-	+	48(27.6)
-	+	+	+	22(12.6)
-	+	-	+	17(9.8)
-	-	+	-	6(3.4)
+/-	+	+	+	3(1.7)
-	-	+	+/-	2(1.1)
+	+	-	+	2(1.1)
+	+	+	+	2(1.1)
-	+	+	-	1(0.6)
-	+/-	+	+	1(0.6)
+	-	-	+	1(0.6)
+	-	+/-	+	1(0.6)
9(5.2)	48(27.6)	106(60.9)	167(96.0)	

+ positive result, +/- borderline result, - negative result

Main results

Results of serological tests in women living in the rural areas

Anti-*T. gondii* IgM antibodies were detected in 2.1% of IgG-free samples (2/95) whereas anti-*T. gondii* IgG antibodies found in 26.3% samples: 25.3% (24/95) were positive and 1.05% (1/95) were borderline. The simultaneous presence of anti-*T. gondii* IgM and IgG was reported in 2.1% of samples (2/95).

56.8% (54/95) of study subjects tested positive for human parvovirus B19 antibodies, 1.05% (1/95) were borderline and 42.1% (40/95) were seronegative.

VZV antibodies were detected in 98.9% of women: 97.9 % (93/95) were positive and 1.05% (1/95) were borderline. Detailed results are shown in Table 2.

Table 2. Results of serological tests for *T. gondii*, VZV, and human parvovirus B19 in women living in rural areas.

anti-*T. gondii*	anti-*T. gondii*	anti-human parvovirus B19	anti-VZV	N(%) 95(100)
IgM	IgG			
-	-	+	+	37(38.9)
-	-	-	+	27(28.4)
-	+	+	+	14(14.7)
-	+	-	+	10(10.5)
+	+	-	+	2(2.1)
-	+/-	+	+	1(1.05)
-	-	+	+/-	1(1.05)
-	-	+	-	1(1.05)
+	-	+/-	+	1(1.05)
+	-	-	+	1(1.05)
4(4.2)	27(28.4)	55(57.9)	94(98.9)	

+ positive result, +/- borderline result, - negative result

Results of serological tests in women living in the city

Positive results for anti-*T. gondii* antibodies only in the IgG class were found in 20.2% (16/79) of the examined women. Simultaneous presence of anti-*T. gondii* IgM and IgG was reported in 6.3% (5/79). Detailed results are shown in Table 3.

Table 3. Results of serological tests for T. gondii, VZV, and human parvovirus B19 in women living in the city.

anti-*T. gondii*	anti-*T. gondii*	anti-human parvovirus B19	anti-VZV	N(%) 79(100)
IgM	IgG			
-	-	+	+	31(39.2)
-	-	-	+	21(26.6)
-	+	+	+	8(10.1)
-	+	-	+	7(8.8)
-	-	+	-	5(6.3)
+/-	+	+	+	3(3.8)
+	+	+	+	2(2.5)
-	-	+	+/-	1(1.3)
-	+	+	-	1(1.3)
5(6.3)	21(26.6)	51(64.5)	72(91.1)	

+ positive result, +/- borderline result, - negative result

VZV antibodies were detected in 91.1% of women: 89.9% (71/79) were positive and 1.3% (1/79) were borderline. Anti-human parvovirus B19 IgG antibodies were found in 64.5% of samples (51/79).

Other analyses

No correlation was found between the presence of anti-*T. gondii*, human parvovirus B19, and VZV antibodies and the age of the examined women, their place of residence, or their education.

Discussion

Key results

The percentage of subjects seropositive for *T. gondii* varies within countries, regions, and communities within regions. The seroprevalence of *T. gondii* in the human population is low in North America and northern Europe (10–30%) and moderate in the countries of central and southern Europe (30–50%) [15]. In Poland, the seroprevalence of *T. gondii* is estimated at 36–66.9%, depending on the place and region of residence [1, 2, 16]. In accordance with the guidelines adopted in Poland, detection of specific antibodies is recommended in the case of native parasitic diseases, including toxoplasmosis. The basic tests include determination of the concentration of IgM, IgG, and IgA as well as IgG avidity assays. The recommendations define cases of primary *T. gondii* invasion. Confirmed cases are associated with seroconversion between two consecutive tests performed at a 2–3-week interval. In pregnant women, the infection is confirmed when both serum samples have been examined during pregnancy and probable when one of the samples was analyzed before pregnancy. Determination of the avidity of IgG antibodies is also recommended, as it reveals the character of the anti- *T. gondii* response and the time of infection acquisition [2,17,18]. This type of serological testing is particularly important in pregnant women or those planning pregnancy due to the potential of transmission of the infection to the fetus [19].

Zajkowska et al. reported that the *T. gondii* IgG antibodies were detected in 51% of pregnant women or women planning pregnancy; 32.7% were positive for IgG and IgM [7]. In this study, anti-T. *gondii* IgM/IgG antibodies were detected in 28.6% of women of childbearing age working or studying in Biała Podlaska (Lublin Province). 1.1% of these were anti-*T. gondii* IgM antibodies, indicating an early immune response. There was no correlation between the presence of anti-*T. gondii* IgM/IgG antibodies and the place of residence of the women. Similarly, the investigations conducted by Lewicka et al. did not demonstrate a statistically significant correlation between the place of residence of the analyzed pregnant women and the serological groups related to their immune status towards *T. gondii* [20].

The study showed that 71.2% of women had no contact with this protozoan. Since seronegative women are at a high risk of acquisition of *T. gondii* infection, education in prophylaxis is essential [1]. As shown by investigations, *T. gondii* was diagnosed in pregnant women who were seronegative before pregnancy, and the level of the infection was higher in patients living in rural areas (1.1%) than in cities (0.27%) [2]. Results of a study conducted among young people studying in Poland and Slovakia showed insufficient knowledge of the routes of *T. gondii* transmission. More than half of respondents were unaware of the routes of human *T. gondii* infection (56.5%) and the route of fetus infection with this protozoan (57.5%) [21].

Parvovirus B19 in adults and children causes mild infections; however, fetal infection may have serious consequences. Infection spreads through the droplet route and most pregnant women get infected from young children. Laboratory diagnostics of parvovirus B19 infection in pregnant women is performed in suspected B19V infection in case of exposure to the infection; suspected symptomatic B19V infection in pregnancy (clinical symptoms in the pregnant or fetus); or miscarriage of unknown cause. Specific laboratory diagnostics for parvovirus B19 infection are based on the identification of anti-B19V IgM/IgG antibodies in ELISA and Western blot tests. In diagnostic tests using molecular biology methods, viral DNA is detected [22]. Chickenpox is diagnosed on the basis of clinical features and epidemiological history; however, in doubtful cases laboratory methods are used: serological or PCR. Anti-VZV antibodies are determined using fluorescence test for antibodies against membrane antigen, latex agglutination and ELISA [23]. In the course of chickenpox, the diagnostic tests show the production of IgA, IgM (to 2 days after the onset of the rash) and the IgG antibody that can survive to the end of life of the patient [24]. The highest concentration of anti-VZV antibodies occurs 4–8 weeks after infection. VZV infection can be confirmed by at least a 4-fold increase in antibody titers in serum samples taken during the acute period of the disease and during the recovery period. Persistence of anti-VZV antibodies in infants over 8 months old suggests intrauterine chickenpox infection. Skin scraping, alveolar fluid, airway secretions, and cerebrospinal fluid by PCR are also useful in diagnosing VZV infection [23]. Chickenpox vaccination is recommended for those who have not had chickenpox and have not been vaccinated, and women who have not had chickenpox and are planning to become pregnant [25]. It is estimated that after contact with VZV, 90% of adult Americans and Europeans have protective antibodies [26]. Similarly, anti-parvovirus B19 IgG antibodies indicate previous infection, which leads to lifelong immunity [8]. In the research conducted by Pembrey et al. the differences were shown in the seroprevalence of VZV among pregnant women according to ethnic group and country of birth (women born in the United Kingdom: white British 94.8% and South Asian 95; women born in South Asia 89.6%) [4]. Talukder et al. estimated seropositivity for VZV among white

British women at 93.1% [27]. A study conducted by van Rijckevorsel et al. showed a high seroprevalence of VZV IgG antibodies in the Amsterdam population by age categories: 18–34 years 92%, 35–44 years 95%, 45–54 years 93% [28]. Our investigation showed a very similar level of seroprevalence for VZV among women (96%). There was no correlation between the presence of anti-VZV IgG antibodies and the place of residence of the examined women and their age and education. Studies published by Cieślik-Tarkota et al. showed that seroprevalence for anti-VZV IgG in pregnant women from Śląskie Voivodeship (Poland) in the years 2011–2015 was 69.2% [24].

Serological screening revealed that the IgG antibodies were recorded against parvovirus B19 in 60.9% of participants. These results are similar to the results of Siennicka's research conducted on a similar age group (prevalence of 50–80%) [29]. Similar results were reported by others: 69.1% [8] and 52.6% [7].

Limitations

We did not analyze the issues related to having children and the contact of women with pets, especially cats. Analysis of these data in connection with knowledge of toxoplasmosis may be relevant. This issue will be considered in future studies.

Interpretation

About 4%, 39% and 71.2% of women participating in the study are still susceptible to infection with VZV, human parvovirus B19, and *T. gondii,* respectively.

Generalizability

It is therefore important to address health education primarily in women of childbearing age in order to help them undertake relevant measures for prevention of *T. gondii*, human parvovirus B19 and VZV infection.

Conclusions

About 4%, 39% and 71.2% of women participating in the study are still susceptible to infection with VZV, human parvovirus B19, and *T. gondii,* respectively. It is therefore important to address health education primarily in women of childbearing age in order to help them undertake relevant measures for prevention of *T. gondii*, human parvovirus B19 and VZV infection.

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