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SEROEPIDEMIOLOGIC EVALUATION OF
TOXOPLASMA GONDII, CYTOMEGALOVIRUS AND
RUBELLA VIRUS, AMONG WOMEN FROM
WESTERN ROMANIA

ABSTRACT

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SUMMARY

Infectious diseases in pregnancy are a major contributor to prenatal, perinatal, and postnatal morbidity and mortality and include TORCH complex. The causative organisms of these infections are *Toxoplasma gondii*, rubella virus, cytomegalovirus (CMV), herpes simplex virus (HSV 1 and 2), and others such as *Treponema pallidum*, hepatitis B virus, parvovirus, HIV, or varicella-zoster virus. Primary infection during pregnancy with one of these pathogens and intrauterine transmission can have serious effects on the fetus causing miscarriage, intrauterine fetal death, birth defects, intrauterine growth retardation, or prematurity.

Infections with TORCH pathogens are the most common causes of complications that affect the fetus like congenital abnormalities and birth defects. Maternal risk factors for a first infection with these pathogens during pregnancy include sexually transmitted infections, exposure to animals during pregnancy, and lack of immunity to some of these infections. Thus, maternal epidemiological and immunological history is a key point of investigation in the field to establish the risk of congenital infections.

Currently, in Romania, epidemiological studies on TORCH complex pathogens are few and on a small scale. Additionally, there are few data on the evolution of the rate of women of reproductive age who are immune to *Toxoplasma gondii*, CMV and Rubella infection during pregnancy.

In this paper, we will study seroepidemiological aspects of three pathogens of the TORCH complex, specifically *Toxoplasma gondii*, Cytomegalovirus, and Rubella virus. These three pathogens are of particular importance globally, with *Toxoplasma gondii* considered by CDC as the major cause of death attributed to foodborne illnesses in the United States, CMV being the most common intrauterine infection, and rubella still a major cause of birth defects such as deafness despite immunization programs.

This research aims to determine the evolution of the risk of primary infection with *Toxoplasma gondii* Cytomegalovirus and Rubella among women in the western area of Romania, and to identify groups at increased risk of primary infection according to demographic parameters, in order to determine the need for implementing effective screening programs, increasing prevention methods and establishing the targeting of vaccination programs.

Epidemiological arguments may lead to the development of health systems to intervene to increase screening and prevention of primary infection with these pathogens during pregnancy in women in high-risk groups.

This thesis is structured in two parts, namely the general part presenting the current state of knowledge and the special part dedicated to personal contributions.

Three cross-sectional studies were conducted on women of reproductive age or pregnant women who underwent TORCH screening at the Timisoara Municipal Emergency Hospital and the private medical laboratory BIOCLINICA SA.

Titers of IgG-anti and IgM-anti-*Toxoplasma gondii*, anti-CMV, and anti-Rubella antibodies were determined by chemiluminescence immunoassay (CLIA) and chemiluminescent microparticle immunoassay (CMIA). IgG titer results were classified into three categories according to the cut-off recommended by the manufacturer: negative titer, inconclusive titer and positive titer.

Participant data (place of origin, age, and year of birth) were collected from forms filled out by the participants at the time of sampling and centralized in Microsoft Office Excel (Microsoft Corporation, Redmond, WA, USA) and Astraia (Astraia Software GmbH, Munchen Germany) software.

GraphPad Prism 8.0.2 (GraphPad Software, Inc. 2365 Northside Dr. Suite 560, San Diego, CA, USA) and using SPSS 20.0 software (SPSS Inc,

Chicago, USA) were used for statistical analysis.

Continuous variables were tested for normality of distribution using the Shapiro-Wilk test and by visual assessment. Depending on the normality of the distribution, they were presented as means or medians and were compared using the t-test for independent samples or the Mann-Whitney U test. Categorical variables were presented as number and percentage and were compared using Fisher's exact test. $p < 0.05$ was considered statistically significant.

The following results were analyzed:

- Seroprevalence (IgG antibody titer above the cut-off value) of the three pathogens in women tested during 2008–2010.
- Seroprevalence of the three pathogens in women tested during 2015–2018
- Evolution of the seroprevalence of the three pathogens between the two periods, by statistical comparison
- Seroprevalence of the three pathogens according to women's place of origin and statistical difference between the two cohorts (urban areas vs. rural areas)
- Seroprevalence of the three pathogens according to women's age at the time of testing
- Seroprevalence of the three pathogens by year of birth of women

Study 1 was designed to determine the seroprevalence of IgG antibodies to *Toxoplasma gondii* among pregnant women in two different periods (2008-2010 and 2015-2018, respectively) to assess the evolution of the risk of primary infection, and to assess the correlation of *Toxoplasma gondii* seroprevalence with age, year of birth and place of origin. Thus, a cross-sectional study was conducted on 6889 Caucasian adult women. The target population was pregnant women residing in one of the 5 counties in the western area of Romania (Timis County, Caras-Severin County, Arad County, Hunedoara County, and Bihor County).

There was a decreasing trend in *Toxoplasma gondii* seroprevalence in fertile women from western Romania. In the first testing period, the seroprevalence was observed to be 43.79% compared to 38.81% in the second testing period (OR=1.22, 95%CI=1.09- 1.38; $P < 0.001$).

Among women tested in 2008-2010, the seroprevalence of *Toxoplasma gondii* is statistically significantly lower in urban compared to rural areas (40.53% vs. 52.22%; $p < 0.0001$). Similarly, among pregnant women tested in 2015-2018, *Toxoplasma gondii* seroprevalence is statistically significantly lower in urban compared to rural settings (34.85% vs. 46.22%; $p < 0.0001$) (Table 3). Comparing the two groups (2008-2010 vs. 2015-2018), to assess the evolution of *Toxoplasma gondii* seroprevalence, in urban and rural environments respectively, a statistically significant decrease in seroprevalence can be observed, between the two periods, in both: urban 40.53% vs. 34.85% ($p < 0.001$) and rural 51.6% vs. 45.8% ($p = 0.02$).

According to participant's age, we found an increasing tendency of seroprevalence with the age of pregnant women. The difference was observed in both groups, however a significant value was found only in the second group ($P = 0.035$), while in the first group the significance was borderline ($P = 0.074$).

There was no statistically significant correlation between year of birth and *Toxoplasma gondii* seroprevalence among women included in group 1, $r_s = -0.403$, $p = 0.051$. On the contrary, among the participants included in group 2, there is a statistically significant correlation of seroprevalence with the year of birth of women. $r_s = -0.461$, $p = 0.009$.

A binomial logistic regression was designed to determine the effects of age, year of birth and environment of origin on the likelihood of participants to have a positive *Toxoplasma gondii* IgG titer. In this model, women from rural areas had 1.72 times higher odds to have *Toxoplasma gondii* IgG positive result than women from urban areas. Also, increased year of birth was associated with a reduced likelihood of a positive IgG *Toxoplasma gondii* result.

Study 2 was designed to determine the seroprevalence of IgG antibodies to CMV among pregnant women in two different periods (2008-2010 and 2015-2018, respectively) to assess the evolution of the risk of primary infection, and to assess the correlation of CMV seroprevalence with age, year of birth and place of origin. Thus, a cross-sectional study was performed on 8951 consecutive pregnant women in Timisoara, Romania, during two successive time intervals: 2008-2010 (1,466 pregnant women tested at the

Emergency Clinical City Hospital Timișoara) and 2015-2018 (7,485 pregnant women tested at SC Bioclinica srl, Timisoara, Romania). The target population was pregnant women residing in one of the 5 counties in the western area of Romania (Timis County, Caras-Severin County, Arad County, Hunedoara County, and Bihor County).

The area of residence of the first group patients was urban for 71.90% of participants, rural 27.76% of participants and not specified for 0.34% of cases, while out of the women that were included in the second group 47.69% were originated from urban areas, 25.78% from rural areas and 26.51% did not specify their origin. The comparison between group showed that women included in the first group were younger at the time of sampling as compared with the women included in the second group: $p < 0.0001$.

The seroprevalence of Cytomegalovirus IgG among group 1 participants (tested between 2008-2010) was 94.68%, while among women included in group 2 (tested during 2008-2010) the seroprevalence of Cytomegalovirus IgG was 91.80%. The results show that the seroprevalence of Cytomegalovirus IgG antibodies among pregnant/reproductive age women in the western area of Romania decreased statistically significantly between 2008-2010 and 2015-2018: $p < 0.0001$; OR=1.59; 95% CI= 1.25- 2.02).

The stratification of participants included in the first group according to the area of residence showed no significant difference for the CMV seroprevalence: urban vs. rural (94.5% vs. 95.33%; OR=0.8409; $P=0.602$; 95% CI, 0.4966-1.423) (Fig.12).

Among women included in group 2 (tested 2015-2018) a CMV seroprevalence of 89.08% in urban areas and 94.92% in rural areas was observed (OR=0.43; $P < 0.0001$).

The CMV seroprevalence of the women screened in the two periods showed a decrease in the urban area ($P < 0.0001$).

In both groups, the Cochran-Armitage test of trend did not show a statistically significant linear trend between age and the proportion of women with CMV-IgG positive result ($p = 0.581$ and $p = 0.10$).

Among women in the first group (tested between 2008 and 2010), the highest risk of primary CMV infection during pregnancy is among women younger than 20 years old, who come from rural areas, with a seroprevalence of anti-CMV IgG antibodies of 92.5%, while among women tested between 2015 and 2018, the highest risk of primary CMV infection during pregnancy was also found in women from 26-30 years subgroup, from urban areas, with a seroprevalence of anti-CMV IgG of 88.0%.

In the urban areas there was a decreasing tendency of seroprevalence with age, but this difference was not significant, while in the rural areas an increasing, but non-significant trend, of CMV seroprevalence with age was found in both groups.

Study 3 was designed to determine the seroprevalence of IgG antibodies to Rubella among pregnant women in two different periods (2008-2010 and 2015-2018, respectively) to assess the evolution of the risk of primary infection, and to assess the correlation of Rubella seroprevalence with year of birth (eligibility to immunization programs) and place of origin.

A cross-sectional study was performed on 6914 Caucasian fertile women (14–45 years old). The target population was pregnant women residing in one of the 5 counties in the western area of Romania (Timis County, Caras-Severin County, Arad County, Hunedoara County, and Bihor County).

Of the 1452 participants included in Group 1 (2008–2010), 72.0% come from urban areas and 28.0% from rural areas. Of the 5462 women included in Group 2 (2015–2018), 64.9% come from urban areas and 35.1% from rural areas. Distributions of the participants age were similar, as assessed by visual inspection. Median age was statistically significantly different between group 1 and group 2, $U = 4.538$, $z = 8.48$, $p < 0.001$.

Seroprevalence of rubella was 94.1% among women included in the first group and 91.4% in women in the second group, respectively.

The Rubella seroprevalence among fertile women from western Romania decreased significantly statistic from 2008–2010 to 2015–2018, $p = 0.0007$; OR = 0.67; 95% CI = 0.47– 0.85 (Fig. 18).

No statistically significant difference was found between seroprevalence in the two backgrounds (urban/rural): $p = 0.80$, OR = 0.94 (95% CI = 0.58-1.50) in group 1 and $p = 0.44$, OR = 0.92 (95% CI = 0.50-1.12) in group 2 (Table 16). When comparing the two groups (2008-2010 vs. 2015-2018), to assess the evolution of Rubella seroprevalence, in urban and rural

areas respectively, we observe a statistically significant decrease in seroprevalence, between the two periods, in the urban area: 94.2 vs. 91.6% ($p=0.007$; OR = 0.67, 95%CI = 0.50- 0.90). However in rural areas the difference in seroprevalence between the two testing periods (93.8% vs. 91.0%) is not statistically significant: $p=0.07$; OR = 0.66, 95%CI = 0.43- 1.02) (Table 12).

By stratifying the participants of both groups (tested 2008-20101 respectively 2015-2018) according to age groups it was noted that Rubella seroprevalence was stable in each age subgroup.

When analysed independently of place of residence, the highest rubella seroprevalence was found in women born between 1989-1994 who were eligible for the monovalent vaccine through the school-based programme, followed by women born before 1989, women born in 1995-1996 (not eligible for vaccination) and women born between 1997 and 2004 who were eligible for MMR vaccination carried out through their family physician. A similar trend was found among rural women. In urban areas, both cohorts of women ineligible for the national vaccination programme, born before 1989 and in 1995-1996, had similar seroprevalence rates.

A binomial logistic regression was designed to determine the effects of age, year of birth and environment of origin on the likelihood of participants to have a positive Rubella IgG titer. All data (2008-2010 respectively 2015-2018) have been merged into single analysis.

Women born between 1990 and 1994 had 2.39 times higher odds to had Rubella IgG positive than women born before 1990. Contrary, women born between 1995-1996 and born after 1997 had a lower odds to had Rubella IgG positive than women born before 1989.

The studies presented in this thesis are the largest epidemiological investigations carried out so far in Romania.

The results in this thesis highlight that such interventions are needed, and can be used by relevant institutions to implement public health intervention programmes. In addition, assessment of rubella virus seroprevalence shows the need to increase the effectiveness of the national MMR vaccination programme.