MISJ-International Journal of Medical Research and Allied Sciences
Available Online at http://ijmraas.misj.net Volume 01|Issue 03 (September-December)|2023 Page: 112-120
Original Research Paper

Seroprevalence of Antibodies Against To RCH Infectious Pathogens (*Toxoplasma gondii*, Rubella and Cytomegalovirus) Among Pregnant Women in El-Beida City

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Article Received: 02-August-2023 Revised: 18-August-2023 Accepted: 08-September-2023

ABSTRACT:
An infections of pregnant women by *Toxoplasma gondii*, *Rubella* and Cytomegalovirus (CMV) can lead to intrauterine infection that leads to congenital anomalies in the fetus during pregnancy. These complications range from early pregnancy miscarriage to fetal abnormalities such as physical deformities, poor vision, and delayed neurological, hearing, and mobility. This study was conducted to examine the seroprevalence of antibodies to *T. gondii*, CMV, and Rubella in Al-Beida city. 71 pregnant women was tested using an enzyme linked immunosorbent assay method ELISA to reveal of presence of antibodies (IgG and IgM) *T. gondii*, Rubella virus and cytomegalovirus (CMV) in blood serum. Data from serum samples showed that CMV seroprevalence was the highest followed by rubella, then *T. gondii* that was found to be CMV seropositive (IgG/IgM) 94%, Rubella (IgG/ IgM) 90% and *T. gondii* (IgG/ IgM) 60%. the highest level of IgG in all age groups was found in CMV 90% with a mean (2.845), then in Rubella virus 87% with a mean (1.896) and the low level reported in *T. gondii* IgG 57.7% with a mean (0.831). These results may give a better understanding of which rubella, toxoplasma and cytomegalovirus infections was more common and more effected on pregnancy leading to fetal loss.

Keywords: T. gondi, Cytomegalovirus and Rubella virus.

INTRODUCTION:

Toxoplasma gondii (*T. gondii*) causes Toxoplasmosis. This disease is common in parts of the world, for example the United States, where *T. gondii* infects about 1.1 million people annually (Jones et al., 2010), with 789 deaths associated with toxoplasmosis Between 2000 and 2010 (Cummings et al., 2010). The symptoms of infection are represented in fever and high temperature, feeling tired and exhausted, headaches, and health problems and birth defects in the newborn from the infected mother (Mahmoud, 2012). Ordinarily Toxoplasmosis is diagnosed by detecting antibodies. In acute infections, increased IgG and IgM antibodies usually appear during the first week or two of infection (Villard et al., 2016). High levels of specific IgG antibodies indicate that the individual was been infected previously. However, these antibodies do not distinguish a recent infection from one acquired long before the detection of specific IgM antibodies can help determine if the infection is recent (Reshika, et al., 2015).

Additionally, these antibodies can persist for months or even years after acute infection (Mahmoud, 2012). A negative IgM test result of a pregnant woman in the first 24 weeks of pregnancy with a low IgG test titer (i.e., DT <1024) essentially establishes the acquisition of infection before pregnancy (Sumeeta and Batra 2016)..

CMV is a common virus in the herpesvirus family. Peak infection occurs in children under two years of age and during adolescence. Once a person is infected, the virus is still alive but is usually inactive (latent) within that person's body for life. Reactivation can occur during pregnancy in previously infected women, with a very small risk of transmission of CMV to the fetus. CMV is a major cause of congenital diseases and disabilities, including hearing loss and mental retardation (Khee-Siang et al., 2014). A positive test for CMV IgG indicates that a person was infected with CMV at some time during their life but does not indicate when a person was infected. Measurement of CMV IgG in paired samples taken one to three months apart can be used to diagnose primary infection; seroconversion (1st sample IgG negative, 2nd sample IgG positive) is clear evidence for recent primary infection. The presence of CMV IgM cannot be used by itself to diagnose primary CMV infection because IgM can also be present during secondary CMV infection. IgM positive results in combination with low IgG avidity results are considered reliable evidence for primary infection (NCCLS, 2004).

Rubella (German measles) is a common mild disease characterized by a skin rash. When the rubella virus infects exposed women early in pregnancy, it may be transmitted to the fetus and may cause birth defects. Therefore, an accurate diagnosis is crucial during pregnancy (Alexander et al., 2019). The primary symptom of rubella virus infection is the appearance of a rash (rash) on the face that spreads to the trunk and extremities and usually fades after three days. Other symptoms of low-grade fever are swollen glands (suboccipital and posterior lymphadenopathy), joint pain, headache, and conjunctivitis (Alexander et al., 2019). Rubella usually causes Posterior cervical lymphadenopathy (Alexander et al., 2019).

Rubella IgM antibodies develop directly after primary infection. Four days later, Rubella IgG become detectable and reach peak levels in the following one to two weeks. While rubella IgM persists for up to three months, rubella IgG can last a lifetime (Alexander et al., 2019).

This study aims to investigate the seroprevalence of antibodies to *T. gondii*, Cytomegalovirus and Rubella virus among available various age groups of pregnant women at EL-Beida City.

MATERIAL AND METHODS:

Sampling & Study Area:

This work was carried out to investigate the seroprevalence of antibodies to *T. gondii*, Cytomegalovirus, and Rubella virus among pregnant women at El-Beyda City.

All sera samples of 71 pregnant women were tested for the TORCH infections by using sandwich and capture ELISA based commercial kits (Biotech Lab. Ltd, UK and Omega Diagnostics, UK) and the BioTek ELx50 biochemistry analyzer (Winooski, Vermont, U.S.A) in the Serology Section of the Department of Parasitology. All the sera were assayed according to the manufacturer's instructions and the results were calculated on the basis of the cut off Activity Index (AI) (Raveendran and Kesavan, 2010).

Blood samples were collected from the cases aseptically in plain vials. After half an hour, they were centrifuged and serum was separated. using sandwich and capture ELISA based commercial kits (Biotech Lab. Ltd, UK and Omega Diagnostics, UK) and the BioTek ELx50 biochemistry analyser (Winooski, Vermont, U.S.A) in the Serology Section of the Department of Parasitology.

A volume of 10µl of test serum was diluted using 1ml of sample diluent and the diluted specimen incubated in microplate wells coated with rabbit antibodies anti-human IgM or IgG. The wells were then washed using washing solution to remove residual test sample, and 100µl of antigen labelled with peroxidase was added. The plates were then washed again using washing solution to eliminate unbound material. Furthermore, 100µl of solution of enzyme substrate and chromogen were added. This solution developed a blue colour if the sample contained anti- IgM or IgG. The blue colour changed to yellow after blocking the reaction with sulphuric acid. The plates were then read using an ELISA reader at 450 nm wavelength. The concentration of antibodies in the sample was then determined by means of a calibration curve. The cut-off value of the assay was calculated and results were expressed in an index by dividing sample absorbance by the cut-off value.

Statistical Analysis:

SPSS 21.0 was used for data analysis (IBM Corporation. Chicago, United States). TORCH IgM & IgG infection differences among age groups were calculated by ANOVA; $P < .05$ was considered significant level of statistical analysis.

RESULTS:

The current work was carried out on 71 patients to examine the seroprevalence of antibodies to *T. gondii*, Cytomegalovirus and Rubella virus among pregnant women at El-Beida City was found the seroprevalence of CMV was the highest followed by Rubella then *T.gondii* were found to be seropositive for CMV (IgG/ IgM) 94%, Rubella (IgG/ IgM) 90% and *T.gondii* (IgG/ IgM) 60%. Table (1) illustrates the ANOVA results of levels of Torch's IgG & IGM among age groups were examined.

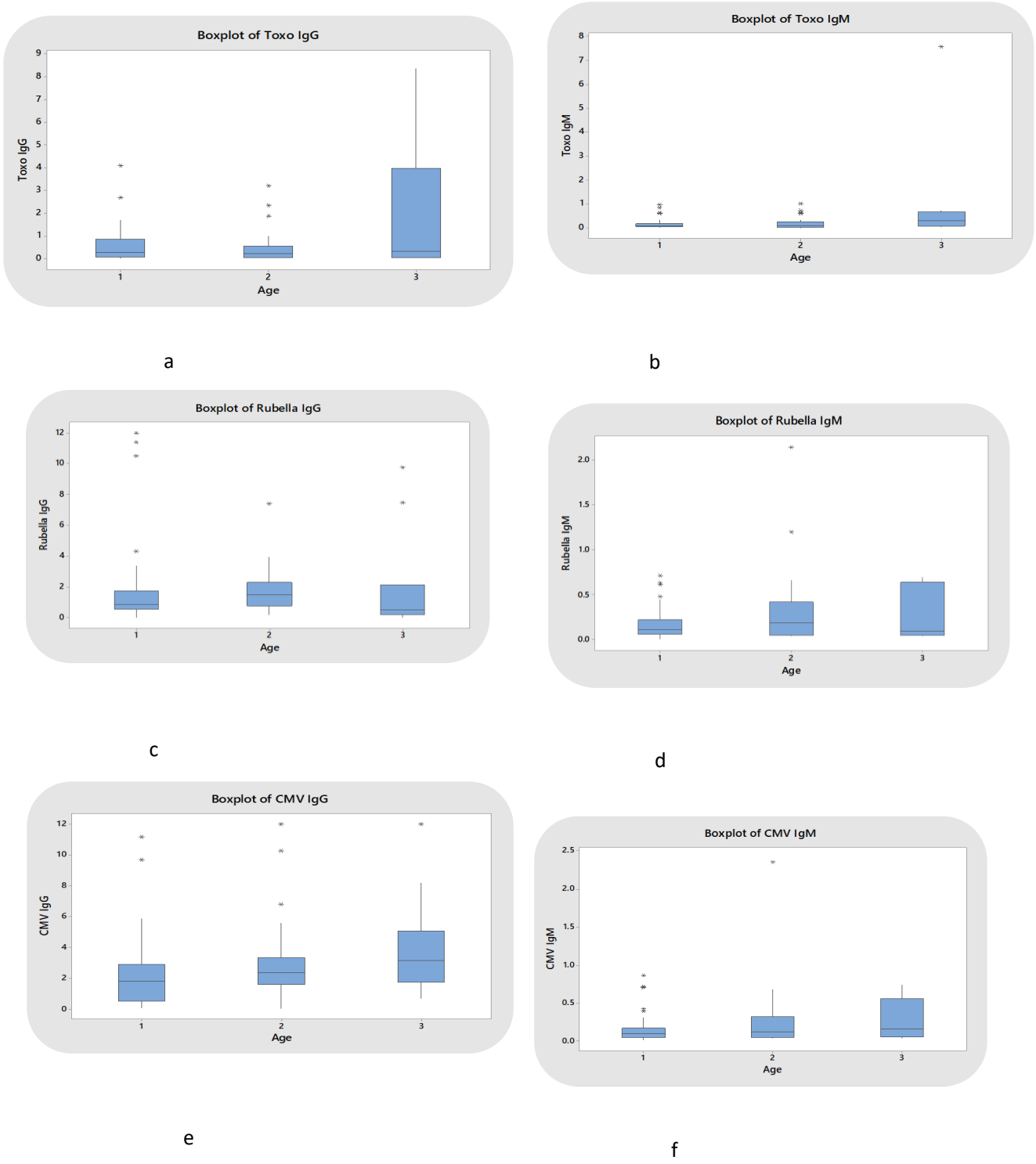

Fig. (1). Grouped boxplots demonstrating the levels of Torch Immunoglobulins (gG & IGM) among age groups

Table (1): The levels of Torch IgG & IGM among age groups

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Immunoglobulins	Age groups	Mean	F statistic	p-value
T. gondii IgG	20 – 30 31 – 40 More than 40	0.69 b 0.53 b 1.97 a	4.99	0.010
T. gondii IgM	20 – 30 31 – 40 More than 40	0.18 b 0.22 b 0.99 a	3.81	0.027
Rubella IgG	20 – 30 31 – 40 More than 40	1.95 a 1.74 a 2.05 a	0.07	0.937
Rubella IgM	20 – 30 31 – 40 More than 40	0.18 a 0.34 a 0.26 a	1.77	0.178
CMV IgG	20 – 30 31 – 40 More than 40	2.30 a 3.11 a 4.05 a	1.80	0.174
CMV IgM	20 – 30 31 – 40 More than 40	0.18 a 0.27 a 0.27 a	0.72	0.491

The levels of T. gondii IgG in three age groups:
According to results were illustrated in table (1) and Figure (1a), the level of *T.gondii* IgG in the ages of (20-30) with a mean (0.69), while in the group aged (31-40) with a mean (0.53) and last group aged (41-50) with a

mean (1.97). There are significant differences were found in the three groups (F = 4.99, p-value = 0.010).

The levels of T.gondii IgM in three age groups:
Likewise, table (1) and Figure (1b) shows that the level of *T.gondii* IgM in the ages of (20-30) with a mean (0.18), while in the group age of (31-40) with a mean (0.22) and the last group age of (41-50) with a mean (0.99). There are significant differences were found in the three groups (F = 4.99, p-value = 0.027).

The levels of Rubella IgG in three age groups:
Furthermore, table (1) and Figure (1c) demonstrates that the level of Rubella IgG at age of (20-30) with a mean (1.95), while the group aged (31-40) with a mean (1.74) and the last group aged (41-50) with a mean (2.05). ANOVA results reported that there are no significant differences among three groups (F = 0.07, p-value = 0.937).

The levels of Rubella IgM in three age groups:
Additionally, table (1) and Figure (1d) illustrates that the level of Rubella IgM at age of (20-30) with a mean (0.18), while the group aged (31-40) with a mean (0.34) and the last group aged (41-50) with a mean (0.26). ANOVA results reported that there are no significant differences among three groups (F = 1.77, p-value = 0.178).

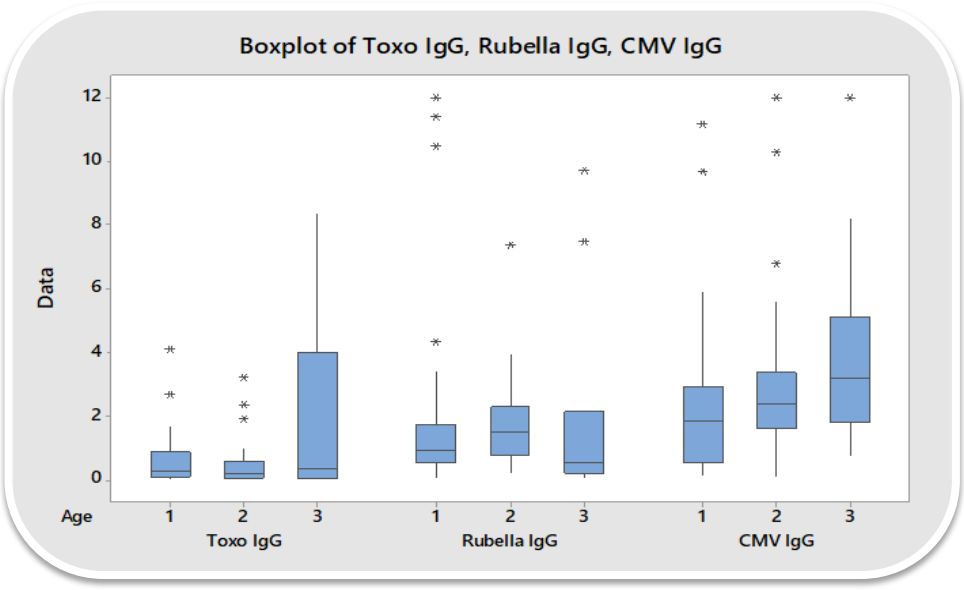
The levels of CMV IgG in three age groups:
Moreover, table (1) and Figure (1e) shows that the level of Rubella IgG at age of (20-30) with a mean (2.30), while the group aged (31-40) with a mean (3.11) and the last group aged (41-50) with a mean (4.05). ANOVA results reported that there are no significant differences among three groups (F = 1.80, p-value = 0.174).

The levels of CMV IgM in three age groups:
lastly, table (1) and Figure (1f) demonstrates that the level of CMV IgM in the ages of (20-30) with a mean (0.18), while in the group age of (31-40) with a mean (0.27) and the last group age of (41-50) with a mean (0.27). ANOVA results reported that there are no significant differences among three groups (F = 0.72, p-value = 0.491).

On the other hand, table (2) and Figure (2a), illustrates the results of comparing among the levels of Immunoglobulins throughout *T.gondii*, Rubella and CMV. The results reveal that the highest level of IgG in all age groups found in CMV at 90% with a mean (2.86), then in Rubella virus at 87% with a mean (1.99) and the low level reported in *T.gondii* IgG at 57.7% with a mean (0.83). ANOVA results reported that there are high significant differences among Immunoglobulins (F = 1.85, p-value = 0.000). Whereas the highest level of IgM in all age groups found in Rubella 32.3% with a mean (0.25), then in *T. gondii* 29.5% with a mean (0.32) and the low level reported in CMV IgM at (28.1%) with a mean (0.22). ANOVA results suggeste that there are no significant differences among Immunoglobulins (F = 0.51, p-value = 0.601).

Table (2): The comparing among the levels of Immunoglobulins throughout *T.gondii*, Rubella and CMV

Immunoglobulins	ToRCH		F statistic	p-value
IgG	Toxo IgG Rubella IgG CMV IgG	0.83 c 1.99 b 2.86 a	12.85	0.000
IgM	Toxo IgG Rubella IgG CMV IgG	0.32 a 0.25 a 0.22 a	0.51	0.60



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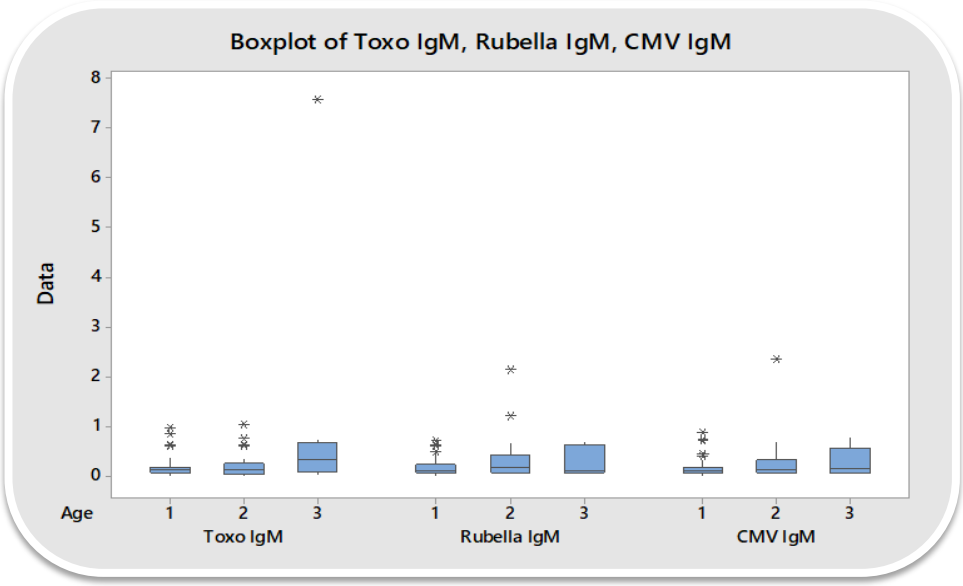

b

Fig. (2). Grouped boxplots illustrating the levels of ToRCH Immunoglobulins (gG & IGM) among ToRCH pathogens (T. gondii, Rubella, CMV).

DISCUSSION:

This study was conducted on 71 pregnant women to investigate the seroprevalence of antibodies to *T. gondii*, Cytomegalovirus and Rubella among pregnant women in Al-Bayda city. The topic of prevalence of antibodies to acute and chronic infections in pregnant women

including *T. gondii*, CMV, and rubella virus (Dhumne et al., 2007). Also, the CMV seroprevalence rate was the highest, followed by rubella. Then *T. gondii* was found to be CMV seropositive (IgG/IgM) with 94%, rubella (IgG/IgM) with 90% and *T. gondii* (IgG/IgM). 60%. Moreover, the highest level of IgG in all age groups was found in CMV at 90% with an average (2.85), then in rubella virus at 87% with an average (1.99) and the lowest level recorded in *T. gondii* IgG at 57.7% with an average (0.83). Also the highest level of IgM was found in all age groups in rubella 32.3% with a mean (0.25), then in *T. gondii* virus 29.5% with a mean (0.32) and the lowest level was reported in CMV IgM. 28.1% with an average (0.22), and the highest level of *T. gondii* IgG was recorded at the age (41-50) with an average (1.97), while in the age group (20-30) with an average (0.69) and the lowest level was recorded in the last age group (31-40) with an average of (0.53). Also, the highest level of *T. gondii* IgM was recorded in ages (41-50) with an average of (0.99), while in the age group (31-40) with an average of (0.22) the low level was reported. In the last age group (20-30) with an average of (0.18). Also, the highest level of rubella IgG was recorded at the age (41-50) with an average of (2.05), while in the group aged (20-30) with an average of (1.95) a low level was reported in the last age group (31-40). with an average of (1.74).

Also, through the results, it was shown that the highest level of Rubella IgM was recorded at the age (31-40) with an average of (0.340), while in the group aged (41-50) with an average of (0.26), the lowest level was recorded in the last age group (20- 30) with an average of (0.18). In addition, the highest level of CMV IgG was reported in ages (41-50) with an average of (4.05), while in the age group (31-40) with an average of (3.15) a low level was reported in the last age group (20-30). with an average of (2.30). Also, the highest level of CMV IgM was recorded in the ages (31-40) and (41-50) with an average of (0.27), while in the age group (20-30) with an average of (0.18). In a previous study it was found that the prevalence of *T. gondii* (IgG/IgM) was highest followed by rubella (IgG/IgM) and then CMV (IgG/IgM) (Mewara et al., 2004).

On the other hand, a previous study found that the prevalence of TORCH infection varies greatly from region to region, depending on various factors such as climatic conditions, socioeconomic status, including personal hygiene, cultural beliefs, dietary habits, and other human factors (Sirin et al., 2017). Estimating the regional seroprevalence of TORCH factors from time to time is of great help in formulating strategies for prenatal screening and in guiding clinicians in making screening decisions. It is even more significant in countries (Deka et al., 2021). Also, this study reported that a significant difference was observed between *T. gondii* IgG levels in three age groups. Also, this study indicated the significant difference was observed between *T. gondii* IgM levels in three age groups. age groups. Also, no significant difference was observed between rubella IgG levels in three age groups, and no significant difference was observed between rubella levels in three age groups. Moreover, this study reported that no significant difference was observed between CMV IgG levels in three age groups, and that no significant difference was observed between CMV IgM levels in three age groups. In another previous study it was found that the IgM positivity rate for *T. gondii* was 1.9%, for rubella 1.2% and for CMV 1.5%. While the IgG positivity rate was 32.3% for *T. gondii*, 93.5% for rubella and 98.9% for CMV. Seropositivity rates were expressed with 95% confidence intervals (Deka et al., 2021).

RECOMMENDATIONS:

Through the results obtained, the researcher recommends avoiding direct contact with patients, washing hands frequently, and not sharing drinks or utensils with other people. Also, avoid traveling to countries where such infectious diseases are common. The researcher also recommends that meat and eggs be cooked well so that the sources of infection are eliminated. Paying attention to cleaning litter boxes well during pregnancy, while following medical instructions.

Conflict of Interest: The authors declare that there are no conflicts of interest.

REFERENCES:

1. Jones, J.L.; Holland, G.N. Annual burden of ocular toxoplasmosis in the US. Am. J. Trop. Med. Hyg. 2010, 82, 464–465. [CrossRef] [PubMed]
2. **Antona, D., et al. "Seroprevalence of cytomegalovirus infection in France in 2010." Epidemiology & Infection 145.7 (2017): 1471-1478.**
3. Berger, F.; Goulet, V.; Le Strat, Y.; Desenclos, J.-C. Toxoplasmosis among pregnant women in France: Risk factors and change of prevalence between 1995 and 2003. Rev. D'épidémiologie St. Publique 2009, 57, 241–248. [CrossRef] [PubMed].
4. **Chan, Khee-Siang, et al. "Cytomegalovirus colitis in intensive care unit patients: difficulties in clinical diagnosis." Journal of Critical Care 29.3 (2014): 474-e1.**

5. Cummings, P.L.; Sorvillo, F.; Kuo, T.; Javanbakht, M. Trends, Productivity Losses, and Associated Medical Conditions Among Toxoplasmosis Deaths in the United States, 2000–2010. Am. J. Trop. Med. Hyg. 2014, 91, 959–964. [CrossRef] [PubMed].
6. **Dabritz, H. A., and P. A. Conrad. "Cats and Toxoplasma: implications for public health." Zoonoses and public health 57.1 (2010): 34-52.**
7. **Deka S, Kalita D, Gupta P, Mathuria YP: A contemporary insight into the sero-epidemiology of *Toxoplasma gondii* infection in the foot-hills of Himalayas: a cross-sectional study from a tertiary care center in Northern India. Nepal J Epidemiol. 2021, 11:937-48. 10.3126/nje.v11i1.34228.**
8. **Dhakal Reshika, et al. "Significance of a positive Toxoplasma immunoglobulin M test result in the United States." Journal of clinical microbiology 53.11 (2015): 3601-3605.**
9. **Dhumne M, Sengupta C, Kadival G, Rathinaswamy A, Velumani A: National seroprevalence of *Toxoplasma gondii* in India. J Parasitol. 2007, 93:1520-1. 10.1645/GE-1159.1**
10. **Edlich RF, Winters KL, Long WB, Gubler KD (2005). "Rubella and congenital rubella (German measles)".**
11. **F. Leslé, F. Touafek, A. Fekkar, D. Mazier & L. Paris European Journal of Clinical Microbiology & Infectious Diseases volume 30, pages1207–1212 (2011)**
12. **Jones, J. L., and J. P. Dubey. "Waterborne toxoplasmosis–recent developments." Experimental parasitology 124.1 (2010): 10-25.**
13. Jones, J.L.; Price, C.; Wilkins, P.P.; Kruszon-Moran, D.; Rivera, H.N. Toxoplasma gondii Seroprevalence in the United States 2009–2010 and Comparison with the Past Two Decades. Am. J. Trop. Med. Hyg. 2014, 90, 1135–1139. [CrossRef] [PubMed].
14. **Khurana, Sumeeta, and Nitya Batra. "Toxoplasmosis in organ transplant recipients: Evaluation, implication, and prevention." Tropical Parasitology 6.2 (2016): 123.**
15. **Leung, Alexander KC, K. L. Hon, and K. F. Leong. "Rubella (German measles) revisited." Hong Kong Medical Journal 25.2 (2019): 134.**
16. **McAuley, James B. "Congenital toxoplasmosis." Journal of the Pediatric Infectious Diseases Society 3.suppl_1 (2014): S30-S35**
17. **Mewara A, Singh S, Khurana S, Gupta P, Sehgal R: Seroprevalence of toxoplasmosis at a tertiary care centre in North India from 2004 to 2014. Indian J Med Microbiol. 2019, 37:351 -7 . 10 .4103 /ijmm .IJMM_19_327**
18. **Ouermi D, Simpore J, Belem AM, Sanou DS, Karou DS, Ilboudo D, Bisseye C, Onadja SM, Pietra V, Pignatelli S, Gnoula C. Co-infection of *Toxoplasma gondii* with HBV in HIVinfected and uninfected pregnant women in Burkina Faso. Pakistan journal of biological sciences: PJBS. 2009 Sep;12(17):1188-93.**
19. **Parasuraman, S., R. Raveendran, and R. Kesavan. "Blood sample collection in small laboratory animals." Journal of pharmacology & pharmacotherapeutics 1.2 (2010): 87.**
20. **Rahdar, Mahmoud, ZADEH ALI REZA SAMARBAF, and Leila Arab. "Evaluating the prevalence of *Toxoplasma gondii* in meat and meat products in Ahvaz by PCR method." (2012): 570-573.**
21. **Sarfraz-ur-Rahman; Akbar, H.; Shabbir, M.Z.; Ullah, U.; Rashid, M.I. Development of Human Toxo IgG ELISA Kit, and False-Positivity of Latex Agglutination Test for the Diagnosis of Toxoplasmosis. Pathogens 2021, 10, 1111.**

Sirin MC, Agus N, Yilmaz N, et al.: Seroprevalence of *Toxoplasma gondii*, rubella virus and cytomegalovirus among pregnant women and the importance of avidity assays. Saudi Med J. 2017, 38:727-32. 10.15537/smj.2017.7.18182

22. **Villard, O., et al. "Serological diagnosis of *Toxoplasma gondii* infection: recommendations from the French National Reference Center for Toxoplasmosis." Diagnostic Microbiology and Infectious Disease 84.1 (2016): 22-33.**
23. NCCLS. Clinical Use and Interpretation of Serologic Tests for *Toxoplasma gondii*; Approved Guideline. NCCLS document M36-A [ISBN 1-56238-523-2]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.
24. Bouthry E, Perillaud-Dubois C, Lebraud P, Soutiere MP, Grangeot-Keros L, Vauloup-Fellous C. Positive predictive value of seroconversion or positive rubella IgM in diagnosis of maternal rubella infection: Seven-years review of French National Reference Laboratory for Rubella. J Clin Virol. 2021;134:104708.