SYSTEMATIC REVIEW

Management of Hydrocephalus in Congenital Toxoplasmosis using Pyrimethamine and Sulfonamide: A Systematic Review

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Abstract

Background: Congenital toxoplasmosis is a serious disease that occurs when the foetus is infected with the parasite *Toxoplasma gondii*. A consequence of vertical transmission from mother to foetus is hydrocephalus. This is classified as an increase in intracranial pressure causing swelling of the brain. It is unknown whether the current gold standard of antibiotic treatment of pyrimethamine and sulfonamide is adequate. The objective of this review is to compare the efficacy of pyrimethamine and sulfonamide treatment duration in managing hydrocephalus induced by congenital toxoplasmosis.

Methods: A systematic review was conducted by two independent reviewers across several medical databases including Ovid MEDLINE, Cochrane Central and EMBASE. Seven articles including case reports, retrospective cohorts, randomised controlled trials, longitudinal studies, and systematic reviews met the inclusion criteria. Infants were classified from birth to 24 weeks old.

Results: There was a lack of conclusive evidence regarding the efficacy and safety of pyrimethamine and sulfonamide. Multiple studies revealed pyrimethamine and sulfonamide were effective in reducing infant deformities and neurological conditions, only when rapidly administered after birth. However, contradicting evidence revealed pyrimethamine and sulfonamide had no significant effect on hydrocephalus.

Conclusion: Novel pharmaceutical interventions for managing hydrocephalus caused by congenital toxoplasmosis are needed, as the existing treatments are inadequate. Since treatment options have dwindled in the last decade, toxoplasmosis is classified as a neglected parasitic infection. Renewed interest in conducting higher-quality trials is required to elucidate different therapeutic interventions for clinical use.

Keywords: Disease transmission, Hydrocephalus, Antibiotics, Congenital toxoplasmosis

Background

Toxoplasmosis is a zoonotic infection caused by the *Toxoplasma gondii* parasite[1]. Replication of the parasite occurs in the domestic cat. The cat sheds *Toxoplasma gondii* oocytes in its faeces into the environment. The oocytes can then be ingested by other warm-blooded animals or transmitted to humans through contaminated food, particularly undercooked meat, and soil. Toxoplasmosis is of critical public health importance, as it affects one-third of the global population with a seroprevalence in industrialised countries estimated to be between 10%–50% and around 80% in tropical areas with poor sanitation[2]. Toxoplasmosis can present with many non-specific symptoms, making it difficult to diagnose.

Toxoplasmosis can be vertically transmitted across the placental barrier, which can have severe health consequences for the developing foetus[1]. Early diagnosis of congenital toxoplasmosis through polymerase chain reaction (PCR), anti-*Toxoplasma* antibodies using enzyme-linked immunosorbent assay (ELISA), and IgG and IgM *Toxoplasma*-specific antibody detection can greatly improve the health of both mother and foetus, as interventions can be started immediately[3]. Symptoms of severe congenital toxoplasmosis in the neonate include blindness, intellectual disability, intracranial calcifications, and hydrocephalus. Hydrocephalus is an abnormal increase in intracranial pressure and expansion of the ventricles. This causes an increase in head circumference, irritability, vomiting, and sutural diastasis in the newborn[4].

Ventriculomegaly, a condition that can lead to hydrocephalus, can be observed on a prenatal ultrasound at 18–20 weeks' gestation[4]. Termination of pregnancy is recommended to mothers with a diagnosis of congenital toxoplasmosis before 26 weeks of gestation in France[5]. Antibiotic treatments for suspected cases of congenital toxoplasmosis include administration of pyrimethamine and sulfonamide (P/S), both of which inhibit parasitic folate metabolism[6]. Sulfonamide acts as an inhibitor to the dihydropteroate synthetase enzyme, whereas pyrimethamine inhibits the dihydrofolate reductase enzyme. The objective of this review is to compare the efficacy of P/S antibiotic duration of treatment in managing hydrocephalus induced by congenital toxoplasmosis. These results have the potential to aid in expanding the national guidelines in obstetrics and gynaecology for the Royal College of Physicians of Ireland.

Methods

A systematic review was conducted by two independent reviewers utilising Ovid MEDLINE, Cochrane Central, and EMBASE. The keywords "congenital toxoplasmosis," "pyrimethamine," "sulfadiazine," and "hydrocephalus" were used in the search. Eligibility assessment was performed by the independent reviewers and disagreements were resolved by consensus. A data extraction Excel sheet was developed and used to compile and summarise the relevant studies. The inclusion criteria were established in line with the study objective where relevant articles underwent data extraction and analysis. Full electronic search history can be found in the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) chart in **Figure 1**.

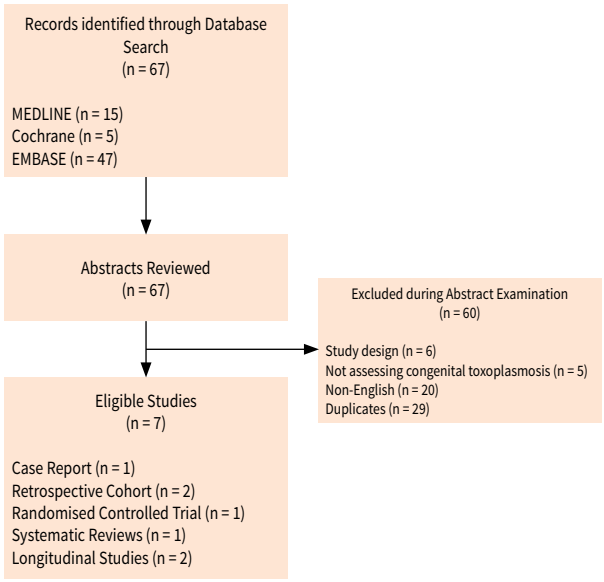
Inclusion and Exclusion Criteria

Qualitative, quantitative, cohort, and case studies written in English from January 1990 to November 2020 describing the use of antibiotic treatments for infants with hydrocephalus caused by congenital toxoplasmosis were included. The infant period considered was from birth to 24 months old. Mothers that were treated prenatally with P/S were also included in the review. The Population, Intervention, Comparison, and Outcome (PICO) tool was used to inform and guide the keywords and inclusion criteria used in the search. Studies published before January 1990 and after November 2020 were excluded. Foreign-language articles, and articles not assessing hydrocephalus or congenital toxoplasmosis as the primary focus were excluded.

Search Results

Relevant articles underwent data extraction and analysis. A search yielded 67 results (15 in Ovid Medline, 47 in Embase, 5 in Cochrane) that matched the predefined search parameters. Of the 67 articles identified in the search strategy, 7 articles met the final inclusion criteria and warranted analysis. 60 articles were excluded: 6 were commentary articles not adhering to the study design, 5 were not directly assessing congenital toxoplasmosis, 20 were non-English, and 29 were duplicates between the database searches. 7 articles met the inclusion criteria, including: 1 case report, 2 retrospective cohorts, 1 randomised controlled trial, 2 longitudinal studies, and 1 systematic review were included. Corroborative themes were identified and reported in the outcomes table in **Table 1**.

Figure 1. **Chart of Search History of Congenital Toxoplasmosis in Neonates with Hydrocephalus**



Results

The seven articles that were included in the final criteria revealed conflicting results on the efficacy of P/S in treating congenital toxoplasmosis. Hydrocephalus was examined as the main outcome.

Support of P/S Antibiotic Use

A longitudinal study conducted by McAuley et al. examined 44 children with congenital toxoplasmosis over ten years[12]. According to this study, developmental, neurological, and ophthalmological deformities including hydrocephalus were decreased with rapid administration of P/S, with a relatively low frequency of neutropenia being the only haematological toxicity observed. Dosage of pyrimethamine and sulfadiazine varied, given in milligrams for half the infants weight in kilograms[12]. After P/S was discontinued, 3 children developed retinal lesions and afebrile seizures, however, authors concluded that rapid P/S therapy was successful in treating neurological deformities, including hydrocephalus[12].

Subsequently, Roizen et al. conducted a one-year longitudinal study that examined 36 infants from 1–13 months of age with congenital toxoplasmosis[11]. In this study, infants were treated with P/S for one month, one year, or not treated at all where dosage varied considerably between each infant depending on weight. The authors in this study found that children with hydrocephalus ex vacuo performed poorly on neurological and developmental tests. Neurologic and developmental outcomes were significantly reduced in children treated with P/S in comparison to those untreated or temporarily treated for less than a month ($p=0.001$) and children treated with P/S for the full year had improved neurological scores[11].

Foulon et al. expanded on the early neurological studies and examined the efficacy of P/S against the antibiotic spiramycin given antenatally to mothers[10]. In this study, prenatal doses of P/S ranged from 25–500 mg against 1 g of spiramycin. Multivariate analysis revealed that rapid prenatal treatment led to a significant reduction ($p=0.021$) in the severity of physical defects, including hydrocephalus and administration of P/S or spiramycin was predictive of the absence of sequelae ($p=0.026$)[10]. However, there was no difference in the efficacy between P/S compared to spiramycin[10].

Opposition of P/S Antibiotic Use

A large retrospective cohort study conducted by Gras

Table 1. **Outcomes of Pharmacological Interventions of Congenital Toxoplasmosis**

Study	Type	Population	Drug Used	Conclusion
Mandelbrot et al., 2018[7]	RCT	143 infants with congenital toxoplasmosis	Combination of P/S	Lower transmission of toxoplasmosis to the foetus when using P/S but did not reach statistical significance. No foetal cerebral toxoplasmosis lesions in the group.
Tamaru et al., 2011[8]	Case study	1 infant with severe congenital toxoplasmosis	Combination of P/S	An infant with severe hydrocephalus was treated seven days after birth with P/S. Side effects were observed, and treatment was terminated. New foetal drug therapies for treatment of congenital toxoplasmosis are suggested to reduce maternal and foetal risks.
Gras et al., 2001[9]	Retrospective cohort	181 liveborn infants with congenital toxoplasmosis	Combination of P/S against spiramycin or no treatment	No significant effects were found when using P/S on intracranial, pericranial, or ocular lesions by age 3.
Foulon et al., 1999[10]	Retrospective cohort	64 infants with congenital toxoplasmosis	Combination of P/S	Early treatment commencement resulted in a significant reduction (p=0.021) in the number of severely affected infants with hydrocephalus. P/S in combination did not have different effects from spiramycin.
Roizen et al., 1995[11]	Longitudinal study	36 infants followed to ten years of age with congenital toxoplasmosis	Combination of P/S	Neurologic and developmental outcomes were significantly reduced in children treated with P/S in comparison to those untreated or temporarily treated for less than a month (p=0.001).
McAuley et al., 1994[12]	Longitudinal study	44 infants followed to 1 year of age with congenital toxoplasmosis	Combination of P/S	P/S was concluded as a feasible treatment for infants under the age of 1. The toxicity of administered P/S was minimal and manageable. The relatively low frequency of neutropenia was the only significant form of haematologic toxicity observed.
Peyron et al., 1999[13]	Systematic review	594 children variable ages with congenital toxoplasmosis	Combination of P/S	Treatment of pregnant women with P/S showed inconclusive results in the vertical transmission rate of congenital toxoplasmosis.

et al. revealed no difference in the efficacy between P/S, spiramycin or no treatment when P/S was given antenatally in 181 new-borns[9]. In this study, mothers were prescribed 50 mg/day of pyrimethamine and 3 g/day of sulfadiazine after confirmation of infection with seroconversion of IgG and IgM antibodies and after birth neonates were immediately prescribed with pyrimethamine (3 mg/kg/3 days) and sulfadiazine (75 mg/kg/day) for three weeks. Results revealed no effect of P/S on intracranial, pericranial, or ocular lesions by three years[9].

A case study of a seven-day old neonate with congenital toxoplasmosis was administered a combination of P/S postnatally in addition to antenatal treatment[8]. In this study, during the mother's pregnancy, she was given oral administration of azithromycin in addition to P/S and acetylspiramycin starting at 23 weeks gestation. Postnatally, Tamaru et al. observed serious side effects including hepatosplenomegaly, intracranial calcifications, meningitis, and ascites[8]. The authors of this study hypothesised that these side effects could have been caused by the teratogenic effects of pyrimethamine during the first trimester of pregnancy.

A systematic review conducted by Peyron et al. examined different treatments for congenital toxoplasmosis including P/S, spiramycin, azithromycin, or no treatment[13]. The authors of this study concluded that there was a lack of sufficient evidence that antenatal P/S had any positive effects on the foetus. Recent research has shed further conflicting evidence on the use of P/S. A novel randomised controlled trial (RCT) conducted by Mandelbrot et al. examined the antenatal treatment for mothers with P/S in comparison to spiramycin[7]. In this study, there was a lower transmission rate observed with P/S treatment, however, these results did not reach statistical significance and there were no foetal cerebral toxoplasmosis lesions when treated with P/S.

From the results obtained, there was a lack of conclusive evidence regarding the efficacy and safety of P/S treatment for infants diagnosed with hydrocephalus caused by congenital toxoplasmosis. Multiple studies using P/S were shown to be effective in reducing infant deformities and neurological conditions when rapidly administered after birth[10–12]. However, contradicting results elucidated that combination therapy did not affect intracranial lesions or deformities caused by congenital toxoplasmosis[9]. This could be due to the reduced efficacy of the drug itself or the timing of the treatment. The lack of conclusive evidence against using P/S as a treatment for hydrocephalus can be attributed to the different methods used in various studies. Many of the studies used direct imaging techniques to visualise the progression of hydrocephalus such as ultrasonography, radiography, computed tomography, and magnetic resonance imaging[8–10]. However, in the studies conducted by Roizen et al.[11] and McAuley et al.[12] used indirect methods such as neurological and blood serum tests were used to measure the effects of hydrocephalus. The Health Service Executive (HSE) Ireland's guidelines to diagnose hydrocephalus requires an occipitofrontal circumference measurement greater than 38 cm followed by an immediate cranial ultrasound[14]. The inconsistent measurement of hydrocephalus between these studies contributes to the inability to draw meaningful and concrete conclusions from the data.

Reducing the transmission rates of congenital toxoplasmosis with P/S was also investigated. Data collected by Peyron et al.[13] recognised that there was inconclusive evidence on whether the rate of transmission of congenital toxoplasmosis was lower when pregnant

women were treated with P/S[13]. Furthermore, the only high quality RCT conducted by Mandelbrot et al.[7] showed a reduction in the vertical transmission from mother to foetus, however, the results were not statistically significant[7]. Due to conflicting results, it remains unknown whether treatment with P/S during pregnancy reduces the transmission of congenital toxoplasmosis.

The safety of P/S treatment was examined and revealed conflicting results. The P/S treatment on both mother and infant was concluded to be safe in managing the infection and had few minor adverse effects[12]. Neutropenia was a side effect, however, the authors noted that this could easily be treated with other drugs, and therefore was not considered a serious adverse reaction[12]. In contrast, a recent case study by Tamaru et al.[8] revealed adverse side effects from treatment in both mother and foetus. Side effects of this study included: bone marrow suppression, hepatotoxicity, meningitis, intracranial calcifications, and ascites. Therefore, in this study, P/S was not recommended as a treatment[8]. The results of these studies prompt further investigation into the non-neurological P/S side effects.

Discussion

There is an apparent lack of conclusive evidence regarding the safety and efficacy of P/S on the prevention and treatment of hydrocephalus caused by congenital toxoplasmosis. The results of various study types cannot be accurately compared to each other to draw results from this collection of studies. Therefore, no formal conclusions can be made from the data.

Toxoplasmosis is classified as a neglected parasitic infection in the United States[15]. Therefore, there is a limitation on the volume and quality of current research. Most investigations on *Toxoplasma gondii* were conducted in the 1990s when the disease was newly discovered. Since then, there has been a decrease in research and interest on this topic, which has led to a lack of epidemiological data. Hence, the conclusions that can be drawn from this information are based on limited amounts of research. Furthermore, this lack of research has diminished progress in the development of novel pharmaceutical therapies to treat congenital toxoplasmosis. Consequently, patients are still being treated with P/S, which has remained the gold standard for decades.

Another limitation of this review is that most articles had a small sample size, which affects the generalisability of the studies. The results are relevant for the sample, however, may not be able to be extrapolated to an entire population. The studies included in this systematic review are also limited, as they are mainly longitudinal and retrospective in their design, rather than RCTs. RCTs are the gold standard in evidence-based medicine due to the control for bias by randomisation and should be the goal in determining treatment efficacy[16]. Additionally, the P/S therapy was not compared to a single dose of each independent drug. Therefore, the efficacy of pyrimethamine and sulfadiazine on their own in comparison to the combination of P/S is unknown. A final limitation of this review is that the studies all used the same combination of drugs but did not compare the treatment efficacy to the same control group. For example, Foulon et al.[10] compared the efficacy of P/S to another pharmacological treatment of spiramycin, whereas Roizen et al.[11] compared the efficacy of the drug combination to untreated patients.

Conclusion

Further research on novel pharmaceutical interventions for hydrocephalus caused by congenital toxoplasmosis is warranted. An increased interest in conducting RCTs for various combinations of treatments and antiparasitic drugs are required to obtain objective data for clinical use. Some examples of alternative antiparasitic drugs that can be included in combination therapy are spiramycin and clindamycin, both of which are bacteriostatic agents[7,17]. As pyrimethamine has shown teratogenicity in the first trimester of pregnancy, the safety and efficacy of novel drugs should be researched during this time[18]. The development and use of antiparasitic drugs pose challenges for various parts of the world. Therefore, it is required to investigate which pharmaceutical interventions are the most appropriate options for populations of various socioeconomic and geographic backgrounds.

A critical effort must be made to increase public awareness of congenital toxoplasmosis in both endemic and non-endemic areas. This not only includes education for pregnant women, but also for women of childbearing age. An 2008 article written by Elsheika suggests multiple approaches should be taken to prevent congenital toxoplasmosis[19]. This includes informing and educating local health officials so accurate information can be passed onto pregnant women[19]. This is especially critical in endemic and resource-poor regions, as it is a low-cost intervention. Another preventative measure suggested in this paper is the implementation of screening programmes for pregnant mothers so interventions can be started immediately if they are infected with the parasite.

Countries in Africa and South America do not routinely screen for possible congenital toxoplasmosis infections, as it can be costly for these populations of lower socioeconomic status[20]. However, this is a global obstacle, as countries in Europe and North America do not currently have long-term routine national screening programmes. There have, however, been temporary screening programmes. In Ireland, a two-year pilot newborn screening programme was initiated in 2005, which involved a two-step IgM dried heel test 72–120 hours after birth[21]. The programme found early signs of visual and neurodevelopmental signs for postnatal infants. As a result, screening allowed immediate treatment that was faster. Currently, in France, there is a national screening programme called *ToxoSurv*, which can diagnose antenatal and post neonatal toxoplasmosis infection up to one year of age. ToxoSurv and previous programmes dating back to 1995 have been associated with a decrease in seroprevalence of toxoplasmosis[22].

With increased research on congenital toxoplasmosis, the ultimate goal is the development of a vaccine, which would decrease transmission of the parasite.

Currently, there are no human vaccine candidates for *Toxoplasma gondii*, as it shows a large degree of variability and antigenic polymorphism[23]. Since there is no imperative for a vaccine at this current time, it is important to elucidate successful pharmacological alternatives. Due to the lack of evidence supporting P/S as a treatment for hydrocephalus caused by congenital toxoplasmosis, future research is required to discover novel interventions. ◄

Declarations

The authors declare no conflicts of interest. Sarah Waicus holds the position of production director on the editorial board of the TSMJ Volume 21. This article was anonymised following submission and subsequently reviewed and accepted by an independent team of editors and peer reviewers as per the TSMJ's peer review and article acceptance protocol.

References

1. Ybañez RHD, Ybañez AP, Nishikawa Y. Review on the Current Trends of Toxoplasmosis Serodiagnosis in Humans. *Front Cell Infect Microbiol*. 2020 May 8;10:204. doi: 10.3389/fcimb.2020.00204.
2. Ahmed M, Sood A, Gupta J. Toxoplasmosis in pregnancy. *Eur J Obstet Gynecol Reprod Biol*. 2020 Dec;255:44-50. doi: 10.1016/j.ejogrb.2020.10.003.
3. Khan K, Khan W. Congenital toxoplasmosis: An overview of the neurological and ocular manifestations. *Parasitol Int*. 2018 Dec;67(6):715-721. doi: 10.1016/j.parint.2018.07.004.
4. Kahle KT, Kulkarni AV, Limbrick DD Jr, Warf BC. Hydrocephalus in children. *Lancet*. 2016 Feb 20;387(10020):788-99. doi: 10.1016/S0140-6736(15)60694-8.
5. Berrebi A, Kobuch WE, Bessieres MH, Bloom MC, Rolland M, Sarramon MF, Roques C, Fournié A. Termination of pregnancy for maternal toxoplasmosis. *Lancet*. 1994 Jul 2;344(8914):36-9. doi: 10.1016/s0140-6736(94)91054-5.
6. van der Ven AJ, Schoondermark-van de Ven EM, Camps W, Melchers WJ, Koopmans PP, van der Meer JW, Galama JM. Anti-toxoplasma effect of pyrimethamine, trimethoprim and sulphonamides alone and in combination: implications for therapy. *J Antimicrob Chemother*. 1996 Jul;38(1):75-80. doi: 10.1093/jac/38.1.75
7. 7. Mandelbrot L, Kieffer F, Sitta R, Laurichesse-Delmas H, Winer N, Mesnard L, Berrebi A, Le Bouar G, Bory JP, Cordier AG, Ville Y, Perrotin F, Jouannic JM, Biquard F, d'Ercole C, Houfflin-Debarge V, Villena I, Thiébaut R; TOXOGEST Study Group. Prenatal therapy with pyrimethamine and sulfadiazine vs spiramycin to reduce placental transmission of toxoplasmosis: a multicenter, randomized trial. *Am J Obstet Gynecol*. 2018 Oct;219(4):386.e1-386.e9. doi: 10.1016/j.ajog.2018.05.031.
8. Tamaru S, Kikuchi A, Takagi K, Wakamatsu M, Horikoshi T, Ogiso Y. Fetal therapy of severe symptomatic toxoplasmosis using azithromycin. *J Obstet Gynaecol Res*. 2011 Jul;37(7):953-7. doi: 10.1111/j.1447-0756.2010.01459.x.
9. Gras L, Gilbert RE, Ades AE, Dunn DT. Effect of prenatal treatment on the risk of intracranial and ocular lesions in children with congenital toxoplasmosis. *Int J Epidemiol*. 2001 Dec;30(6):1309-13. doi: 10.1093/ije/30.6.1309.
10. Foulon W, Villena I, Stray-Pedersen B, Decoster A, Lappalainen M, Pinon JM, Jenum PA, Hedman K, Naessens A. Treatment of toxoplasmosis during pregnancy: a multicenter study of impact on fetal transmission and children's sequelae at age 1 year. *Am J Obstet Gynecol*. 1999 Feb;180:410-5. doi: 10.1016/s0002-9378(99)70224-3.
11. Roizen N, Swisher CN, Stein MA, Hopkins J, Boyer KM, Holfels E, Mets MB, Stein L, Patel D, Meier P, et al. Neurologic and developmental outcome in treated congenital toxoplasmosis. *Pediatrics*. 1995 Jan;95(1):11-20.
12. McAuley J, Boyer KM, Patel D, Mets M, Swisher C, Roizen N, Wolters C, Stein L, Stein M, Schey W, et al. Early and longitudinal evaluations of treated infants and children and untreated historical patients with congenital toxoplasmosis: the Chicago Collaborative Treatment Trial. *Clin Infect Dis*. 1994 Jan;18(1):38-72. doi: 10.1093/clinids/18.1.38.
13. Peyron F, Wallon M, Liou C, Garner P. Treatments for toxoplasmosis in pregnancy. *Cochrane Database Syst Rev*. 2000;(2):CD001684. doi: 10.1002/14651858.CD001684.
14. Murphy J, Jennings P. The Newborn Clinical Examination Handbook [Internet]. Ireland's Health Services (HSE); 2020;72. Available from: https://www.hse.ie/eng/about/who/healthwellbeing/our-priority-programmes/child-health-and-wellbeing/newborn%20exam.pdf
15. Hotez PJ. Neglected parasitic infections and poverty in the United States. *PLoS Negl Trop Dis*. 2014 Sep 4;8(9):e3012. doi: 10.1371/journal.pntd.0003012.
16. Burns PB, Rohrich RJ, Chung KC. The levels of evidence and their role in evidence-based medicine. *Plast Reconstr Surg*. 2011 Jul;128(1):305-310. doi: 10.1097/PRS.0b013e318219c171.
17. Dubey JP. The history of Toxoplasma gondii--the first 100 years. *J Eukaryot Microbiol*. 2008 Nov-Dec;55(6):467-75. doi: 10.1111/j.1550-7408.2008.00345.x.
18. Peters PJ, Thigpen MC, Parise ME, Newman RD. Safety and toxicity of sulfadoxine/pyrimethamine: implications for malaria prevention in pregnancy using intermittent preventive treatment. *Drug Saf*. 2007;30(6):481-501. doi: 10.2165/00002018-200730060-00003.
19. Elsheikha HM. Congenital toxoplasmosis: priorities for further health promotion action. *Public Health*. 2008 Apr;122(4):335-53. doi: 10.1016/j.puhe.2007.08.009.
20. Torgerson PR, Mastroiacovo P. The global burden of congenital toxoplasmosis: a systematic review. *Bull World Health Organ*. 2013 Jul 1;91(7):501-8. doi: 10.2471/BLT.12.111732.
21. Ferguson W. Newborn screening for congenital toxoplasmosis in Ireland [Internet]. Royal College of Surgeons in Ireland; 2016. Available from: https://doi.org/10.25419/rcsi.10817450.v1
22. Robinson E, de Valk H, Villena I, Le Strat Y, Tourdjman M. National perinatal survey demonstrates a decreasing seroprevalence of Toxoplasma gondii infection among pregnant women in France, 1995 to 2016: impact for screening policy. *Euro Surveill*. 2021 Feb;26(5):1900710. doi: 10.2807/1560-7917.ES.2021.26.5.1900710.
23. Liu Q, Singla LD, Zhou H. Vaccines against Toxoplasma gondii: status, challenges and future directions. *Hum Vaccin Immunother*. 2012 Sep;8(9):1305-8. doi: 10.4161/hv.21006.