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Temporal Trends in the Seroprevalence of *Toxoplasma gondii*, Rubella, and Cytomegalovirus Infections among Pregnant Women: An 11-Year Retrospective Study

Gebe Kadınlarda *Toxoplasma gondii*, Rubella ve Sitomegalovirüs Enfeksiyonlarının Seroprevalansındaki Zamansal Eğilimler: 11 Yıllık Retrospektif Bir Çalışma

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Abstract

Introduction: Maternal infection with *Toxoplasma gondii (T. gondii)*, rubella virus, and cytomegalovirus (CMV) during pregnancy may result in vertical transmission and adverse fetal or neonatal outcomes. Although these pathogens differ in their microbiology and prevention strategies, they are commonly evaluated together in antenatal care because serological testing plays a central role in assessing immunity, susceptibility, and potential recent infection. This study aimed to describe the seroprevalence of *T. gondii*, rubella virus, and CMV antibodies among pregnant women and evaluate temporal trends.

Materials and Methods: This retrospective study reviewed the antenatal records of pregnant women who attended a tertiary care obstetrics clinic in Türkiye between January 2012 and December 2022. Maternal age, gestational age at testing, and immunoglobulin G (IgG) and immunoglobulin M (IgM) serological results for *T. gondii*, rubella virus, and CMV were extracted from laboratory records. Annual seropositivity rates were calculated. Temporal trends in annual IgG seroprevalence were evaluated using binomial generalized linear models, with year treated as a continuous predictor.

Results: A total of 16,877 pregnant women were included (mean age, 28.72 ± 5.21 years; range, 15–49 years; mean gestational age at testing, 14.0 ± 5.5 weeks). Overall IgG seroprevalence was 31.5% for *T. gondii*, 94.8% for rubella, and 96.9% for CMV, whereas IgM positivity rates were 0.5%, 0.1%, and 0.1%, respectively. Annual IgG seroprevalence showed moderate year-to-year variation but no statistically significant linear trend over the 11-year study period for any of the pathogens (all $p > 0.05$). Analyses of IgM trends were limited because of the very low number of positive cases.

Conclusion: Over the 11-year study period, IgG seroprevalence remained high for rubella and CMV and moderate for *T. gondii*, whereas IgM positivity ranged from 0.1% to 0.5% among pregnant women. The absence of significant linear trends underscores the importance of sustained antenatal serological surveillance and preventive counseling to reduce the risk of congenital infections.

Keywords: Cytomegalovirus, pregnancy, rubella, seroprevalence, *Toxoplasma gondii*

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Öz

Giriş: Gebelik sırasında *Toxoplasma gondii (T. gondii)*, rubella virüsü ve sitomegalovirüs (CMV) ile gelişen maternal enfeksiyonlar, vertikal geçişe ve olumsuz fetal veya neonatal sonuçlara yol açabilir. Bu patojenler mikrobiyolojik özellikleri ve korunma stratejileri bakımından farklılık gösterse de, serolojinin bağışıklık, duyarlılık ve olası yeni enfeksiyonu değerlendirmedeki merkezi rolü nedeniyle antenatal bakımda yaygın olarak birlikte değerlendirilirler. Uzun vadeli seroepidemiyolojik veriler, antenatal tarama uygulamaları ve sürveyans stratejileri hakkında bilgi sağlamak açısından önemlidir.

Gereç ve Yöntemler: Bu retrospektif çalışmada, Ocak 2012 ile Aralık 2022 tarihleri arasında üçüncü basamak bir kadın doğum kliniğine başvuran gebe kadınların antenatal kayıtları incelenmiştir. Laboratuvar kayıtlarından maternal yaş, test sırasındaki gebelik haftası ile *T. gondii*, rubella virüsü ve CMV için immünoglobulün G (IgG) ve immünoglobulün M (IgM) serolojik sonuçları elde edilmiştir. Yıllık seropozitiflik oranları hesaplanmıştır. Yıllık IgG seroprevalansındaki zamansal eğilimler, yılın sürekli bir öngörücü değişken olarak kullanıldığı binomiyal genelleştirilmiş lineer modeller kullanılarak değerlendirilmiştir.

Bulgular: Çalışmaya toplam 16.877 gebe kadın dahil edilmiştir (ortalama yaş 28,7 ± 5,2 yıl; test sırasındaki ortalama gebelik haftası 14,0 ± 5,5 hafta). Genel IgG seroprevalansı *T. gondii* için %31,5, rubella için %94,8 ve CMV için %96,9 olarak saptanırken; IgM pozitifliği her üç enfeksiyon için de düşük kalmıştır (sırasıyla %0,5, %0,1 ve %0,1). Yıllık IgG seroprevalansı yıldan yıla orta dereceli varyasyonlar göstermiş, ancak hiçbir patojen için 11 yıllık dönem boyunca istatistiksel olarak anlamlı bir lineer eğilim göstermemiştir (tümü için $p > 0,05$). IgM eğilim analizleri, vaka sayılarının çok düşük olması nedeniyle sınırlı kalmıştır.

Sonuç: On bir yıllık dönem boyunca gebe kadınlar arasında IgG seroprevalansı rubella ve CMV için yüksek, *T. gondii* için orta düzeyde seyretmiş; IgM pozitifliği ise tutarlı bir şekilde düşük kalmıştır. Anlamlı lineer eğilimlerin olmaması, konjenital enfeksiyon risklerini yönetmek için sürdürülebilir antenatal serolojik sürveyansın ve koruyucu danışmanlığın önemini desteklemektedir.

Anahtar Kelimeler: Sitomegalovirüs, gebelik, rubella, seroprevalans, *Toxoplasma gondii*

Introduction

Maternal infections during pregnancy remain a major concern because primary infection or reactivation can result in vertical transmission and adverse fetal or neonatal outcomes. In this context, *Toxoplasma gondii* (*T. gondii*), rubella virus, and cytomegalovirus (CMV) are of particular clinical relevance, as maternal infection is often asymptomatic, whereas congenital infection may lead to miscarriage, stillbirth, structural anomalies, or long-term neurodevelopmental sequelae[1-3].

Global seroprevalence patterns vary across geographic regions and populations. The global seroprevalence of latent *T. gondii* infection among pregnant women has been estimated at approximately 30%, whereas the seroprevalence of rubella and CMV is influenced by vaccination coverage and population exposure patterns[4-7]. In Türkiye, recent regional cohort studies have reported *T. gondii* immunoglobulin G (IgG) seropositivity rates of 23.0% in İstanbul, 28.3% in Konya, and 35.2% in Diyarbakır, with corresponding rubella IgG seropositivity rates of 89.1%, 98.13%, and 91.2% and CMV IgG seropositivity rates of 98.7%, 99.78%, and 98.7%, respectively[8-10].

T. gondii infection is typically acquired through the ingestion of tissue cysts in undercooked meat or exposure to oocysts in contaminated environments. Congenital toxoplasmosis can result in neurological and ophthalmologic complications[11-13]. Rubella infection during pregnancy may cause congenital rubella syndrome, which is characterized by hearing loss, cardiac defects, ocular abnormalities, and developmental delay; population immunity is strongly influenced by vaccination coverage[3,5,14]. CMV is the most common cause of congenital viral infection worldwide. Although maternal disease is often mild or asymptomatic, congenital CMV infection can result in permanent sequelae, including sensorineural hearing loss and neurodevelopmental impairment, and no licensed vaccine is currently available[15,16].

Seroprevalence studies provide valuable epidemiological information by quantifying immunity and susceptibility patterns among pregnant women, thereby supporting risk communication, preventive counseling, and surveillance strategies. Antenatal testing practices vary across countries and healthcare settings; where available, serological testing may be used to support clinical assessment and public health surveillance of infections associated with congenital disease. Therefore, this study aimed to describe the seroprevalence of *T. gondii*, rubella virus, and CMV antibodies among pregnant women and to examine temporal changes in their distribution over the study period, thereby providing long-term data to support antenatal infectious disease surveillance and prevention.

Materials and Methods

This retrospective study reviewed the antenatal records of pregnant women who attended the outpatient obstetrics clinics of a tertiary care hospital in Türkiye between January 2012 and December 2022.

Pregnant women who attended the antenatal clinic at least once during pregnancy and whose antenatal records contained the required serological test results were included. Records were linked using unique patient identifiers within the hospital information system, enabling the identification and removal

of duplicate entries before screening and analysis. A total of 18,176 de-duplicated antenatal records were initially screened. Of these, 1,299 were excluded because of incomplete key data, including inconsistent maternal age information that could not be reliably verified from hospital records (n = 12), missing gestational age at testing (n = 556), and missing serological results (n = 731). Records with missing serological results were classified under that category even when additional variables were missing. The final analytical cohort consisted of 16,877 pregnant women.

From the eligible records, we extracted maternal age, gestational age at testing, and serological laboratory results for IgG and immunoglobulin M (IgM) antibodies against *T. gondii*, rubella virus, and CMV. The data were entered into a secure study database and checked for completeness and internal consistency.

Serum samples were tested for IgG and IgM antibodies against *T. gondii*, rubella virus, and CMV using routine commercial assays employed by the hospital laboratory. During the earlier years of the study, serological analyses were performed using the Abbott ARCHITECT platforms (i1000SR/i2000SR; Abbott, USA) with the corresponding ARCHITECT Toxo IgG/IgM, Rubella IgG/IgM, and CMV IgG/IgM assays. From 2021 onward, testing was performed using the Roche cobas e801 platform (Roche Diagnostics, Germany) with the corresponding Elecsys Toxo IgG/IgM, Rubella IgG/IgM, and CMV IgG/IgM assays. All assays were performed according to the manufacturers' instructions and interpreted using the contemporaneous kit-specific cutoff values.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Clinical Research Ethics Committee of Kartal Dr. Lütfi Kırdar Training and Research Hospital (approval no.: 2020/514/177/38; date: 13.05.2020). Because this study was a retrospective review of existing records, individual informed consent was not required, as confirmed by the approving ethics committee. All extracted data were de-identified before analysis, stored securely, and accessed only by the study investigators.

Statistical Analysis

Annual seropositivity rates were calculated by dividing the number of positive test results by the total number of tests with available results for each year and are reported as percentages. Descriptive statistics were used, and temporal trends in seropositivity were evaluated using binomial generalized linear models with year treated as a continuous predictor. For the pathogen-specific IgG models, yearly aggregated counts were analyzed, with the number of positive test results relative to the total number of tests performed in each calendar year specified as the dependent outcome. Results are reported as odds ratios (ORs) per one-calendar-year increase. Because of the extremely low number of IgM-positive events, formal trend modeling was not performed for IgM markers, and these results were analyzed descriptively.

Categorical variables are presented as frequencies and percentages, whereas continuous variables are presented as means ± standard deviations or medians, as appropriate. All statistical analyses were performed using IBM SPSS Statistics software (version 24.0; IBM Corp., Armonk, NY, USA).

Results

Following data extraction, the final study dataset comprised records from 16,877 pregnant women. The participants had a mean age of 28.72 ± 5.21 years (range, 15–49 years), and the mean gestational age at testing was 14.0 ± 5.5 weeks (range, 6–28 weeks).

Between 2012 and 2022, laboratory records identified 82 IgM-positive and 5,323 IgG-positive results for anti-*T. gondii*, 28 IgM-positive and 16,003 IgG-positive results for anti-rubella, and 20 IgM-positive and 16,362 IgG-positive results for anti-CMV. The corresponding overall seropositivity rates were 0.5% (IgM) and 31.5% (IgG) for *T. gondii*, 0.1% (IgM) and 94.8% (IgG) for rubella, and 0.1% (IgM) and 96.9% (IgG) for CMV (Table 1).

Table 1. IgG and IgM seroprevalence of anti-*T. gondii*, anti-CMV, and anti-rubella antibodies by year.

	Toxoplasma gondii			**Rubella virus**			**CMV**		
	IgM	IgG	IgM + IgG*	IgM	IgG	IgM + IgG*	IgM	IgG	IgM + IgG*
2012	20 (1.7%)	395 (33.2%)	4 (0.3%)	5 (0.4%)	1151 (96.6%)	4 (0.3%)	4 (0.3%)	1147 (96.3%)	0
2013	6 (0.7%)	277 (30.1%)	1 (0.3%)	3 (0.3%)	911 (99.1%)	1 (0.1%)	1 (0.1%)	879 (95.6%)	0
2014	6 (0.4%)	348 (25.8%)	2 (0.3%)	2 (0.1%)	1319 (97.6%)	0	0	1348 (99.8%)	2 (0.1%)
2015	8 (0.7%)	394 (32.7%)	0	2 (0.2%)	1155 (95.8%)	0	0	1190 (98.7%)	0
2016	2 (0.2%)	271 (25.6%)	0	0	1024 (96.7%)	0	3 (0.3%)	1022 (96.5%)	0
2017	2 (0.2%)	370 (28.3%)	0	0	1285 (98.2%)	0	3 (0.2%)	1261 (96.3%)	0
2018	8 (0.4%)	702 (33.9%)	4 (0.2%)	4 (0.2%)	1935 (93.5%)	3 (0.1%)	3 (0.1%)	2001 (96.7%)	1 (0.0%)
2019	16 (0.8%)	676 (34.9%)	7 (0.4%)	7 (0.4%)	1750 (90.3%)	2 (0.1%)	2 (0.1%)	1808 (93.3%)	0

Table 1. Continued.

	Toxoplasma gondii			Rubella virus			CMV		
	IgM	IgG	IgM + IgG*	IgM	IgG	IgM + IgG*	IgM	IgG	IgM + IgG*
2020	4 (0.3%)	531 (35.1%)	0	2 (0.1%)	1405 (92.8%)	0	2 (0.1%)	1475 (97.4%)	0
2021	4 (0.2%)	683 (32.5%)	0	2 (0.1%)	1988 (94.6%)	0	0	2073 (98.6%)	0
2022	6 (0.3%)	676 (30.5%)	1 (0.0%)	1 (0.0%)	2080 (93.8%)	0	2 (0.1%)	2158 (97.3%)	0
All	82 (0.5%)	5323 (31.5%)	19 (0.1%)	28 (0.1%)	16003 (94.8%)	10 (0.0%)	20 (0.1%)	16362 (96.9%)	3 (0.0%)

*: Concurrent positivity of IgG- and IgM-, values as counts and percentages. *T. gondii, Toxoplasma gondii*; IgG, immunoglobulin G; IgM, immunoglobulin M; CMV, cytomegalovirus.

Annual seroprevalence estimates ranged from 25.6–35.1% for *Toxoplasma* IgG, 90.3–99.1% for rubella IgG, and 93.3–99.8% for CMV IgG over the study period. Annual IgM positivity remained consistently low for all three pathogens, ranging from 0.2–1.7% for *Toxoplasma*, 0.0–0.4% for rubella, and 0.0–0.3% for CMV.

Trend analysis using binomial generalized linear models with year treated as a continuous predictor yielded the following results for the IgG markers: *Toxoplasma* IgG, OR per year = 1.01 [95% confidence interval (CI), 0.99–1.03; $p = 0.25$]; rubella IgG, OR per year = 0.99 (95% CI, 0.97–1.01; $p = 0.18$); and CMV IgG, OR per year = 1.00 (95% CI, 0.99–1.02; $p = 0.62$). These findings indicate that there were no statistically significant linear trends in annual IgG seroprevalence for any of the three pathogens during the 2012–2022 study period.

Yearly trends in IgG seroprevalence for *T. gondii*, rubella virus, and CMV are illustrated in Figure 1. Year-specific IgG and IgM seroprevalence estimates with 95% CIs are provided in Supplementary Tables 1 and 2.

Discussion

This 11-year retrospective study of 16,877 pregnant women provides a comprehensive overview of the seroprevalence trends of three clinically important pathogens: *T. gondii*, rubella virus, and CMV. Our findings showed that although rubella and CMV IgG seroprevalence remained consistently high (94.8% and 96.9%, respectively), *T. gondii* IgG seroprevalence was moderate at 31.5%. Formal trend modeling demonstrated stable IgG seroprevalence throughout the 2012–2022 study period, with no statistically significant linear changes for any of the three pathogens. Furthermore, the low frequency of IgM positivity across all pathogens suggests a low burden of acute maternal infections in this population during the study period.

When interpreted alongside findings from recent regional cohorts in Türkiye, our results suggest regional heterogeneity rather than a uniform national seroepidemiological profile. Compared with the Konya enzyme-linked fluorescent assay (ELFA)-based cohort reported by Ezer et al.[8], which found *T. gondii*, rubella, and CMV IgG seroprevalence rates of 28.3%, 98.13%, and 99.78%, respectively, our cohort showed slightly higher *T. gondii* IgG seroprevalence but somewhat lower rubella and CMV IgG seroprevalence. Conversely, our study demonstrated lower *T. gondii* and CMV IgG seroprevalence but higher rubella IgG seroprevalence than an 8-year enzyme-linked immunosorbent assay (ELISA)-based study conducted in Diyarbakır[9]. An İstanbul cohort using chemiluminescent microparticle immunoassay (CMIA) also highlighted this regional variation, reporting *T. gondii*, rubella, and CMV IgG seroprevalence rates of 23.0%, 89.1%, and 98.7%, respectively[10].

These differences among studies likely reflect a combination of geographic, dietary, and sociodemographic and behavioral factors, as well as important methodological differences. In particular, variation in the analytical sensitivity and specificity of ELISA, ELFA, and CMIA platforms, together with differences in antigen preparations and kit-specific cutoff values, may contribute to discordant serological classifications, particularly for borderline results[8-10]. Our 11-year study provides valuable longitudinal context for these observations. Despite moderate year-to-year variation, no significant linear trends in IgG seroprevalence were identified within our center, indicating a stable seroepidemiological profile over the past decade.

Regarding rubella, the high IgG seroprevalence observed in our study is consistent with previous reports and supports the effectiveness of vaccination programs[3,5,17]. From a public health perspective, persistently high rubella IgG seroprevalence is consistent with sustained population immunity, although periodic fluctuations in vaccine uptake or outbreak dynamics may still occur[18-20]. In our cohort, the absence of a statistically significant temporal trend in rubella IgG seroprevalence suggests a stable immunity profile throughout the study period, which is reassuring for the prevention of congenital rubella.

For CMV, our cohort demonstrated very high IgG seroprevalence, consistent with widespread prior exposure. Although CMV IgG seroprevalence varies among populations, higher rates are commonly reported in settings with greater early-life exposure. Differences in sociodemographic

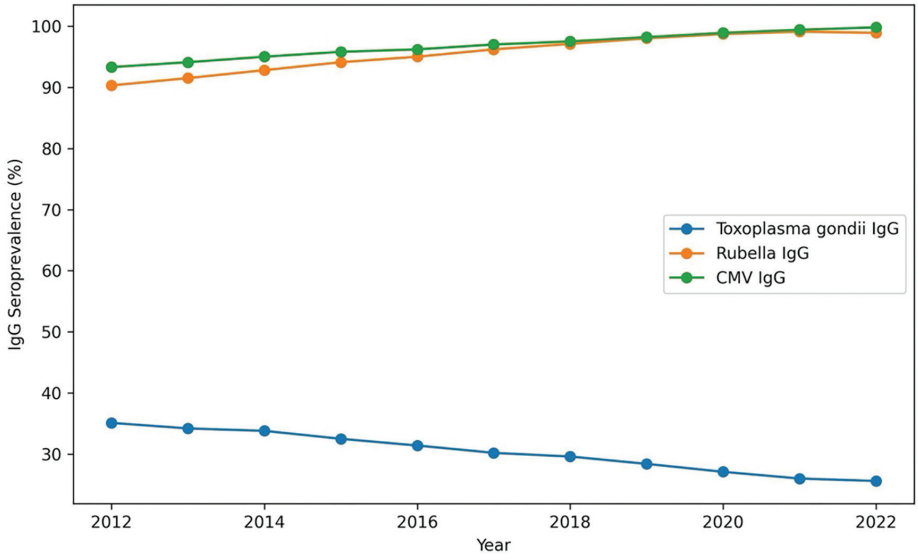

Figure 1. Yearly trends in IgG seroprevalence of *T. gondii*, rubella virus, and CMV among pregnant women between 2012 and 2022. Points represent the annual seroprevalence estimates, and the lines illustrate the overall temporal patterns across the study period. IgG, immunoglobulin G; *T. gondii, Toxoplasma gondii*; CMV, cytomegalovirus.

characteristics and exposure patterns may partially explain the variability observed across studies[6]. CMV IgM positivity remained rare in our cohort, whereas studies from Europe, North America, and Japan have reported higher IgM seroprevalence rates of 1.0–4.6%, 2.3–4.5%, and 0.8%, respectively, potentially reflecting differences in population risk profiles, testing strategies, and assay performance[7]. The combination of very high IgG seroprevalence and low IgM positivity in our cohort is consistent with high background immunity and a low frequency of suspected recent infection during routine antenatal screening.

The absence of statistically significant linear trends in IgG seroprevalence should be interpreted with caution. Rather than indicating a lack of epidemiological significance, these stable estimates may reflect consistently high baseline immunity or persistent exposure patterns that are not expected to change linearly over time. In addition, assay variability and population heterogeneity may have obscured subtle temporal changes. Importantly, these findings highlight the need for continued surveillance, as non-linear fluctuations or cohort-specific changes may still emerge over time.

The moderate year-to-year variation observed in IgG seroprevalence should also be interpreted in the context of assay-related factors. Because different commercial immunoassay platforms were used during the study period, assay-specific cutoff values, borderline classification thresholds, and analytical performance characteristics may have differed across years. Consequently, a small number of samples with results close to the decision thresholds may have been classified differently depending on the assay used. Therefore, some of the observed annual variation in IgG seroprevalence may reflect inter-assay variability in addition to true epidemiological variation.

Study Limitations

The retrospective study design and reliance on IgG and IgM serological testing may not fully capture the timing of infection or the dynamics of the immune response, and pregnancy outcome data were unavailable to correlate serological patterns with clinical outcomes. In addition, different commercial immunoassay platforms were used during the 11-year study period. Although all assays were performed according to the manufacturers' instructions and interpreted using the assay-specific cutoff values applicable at the time of testing, differences in analytical performance among platforms—including variations in sensitivity, specificity, and cutoff definitions—may have contributed to the modest year-to-year variation observed in seroprevalence estimates. Consequently, small annual fluctuations, particularly in IgG seroprevalence, should be interpreted cautiously and should not be assumed to reflect epidemiological changes alone. Other TORCH pathogens were not evaluated; therefore, a more comprehensive assessment of congenital infection profiles was not possible. Despite these limitations, the large sample size, extended study period, and consistent laboratory setting are important strengths that enhance the reliability of the findings.

Future research should prioritize prospective study designs using harmonized assays, more comprehensive clinical outcome data, and, where feasible, confirmatory testing strategies (e.g., avidity testing for *T. gondii* when clinically indicated) to better characterize incident infections and the risk of congenital transmission. Longitudinal follow-up studies could further clarify the relationship between serological screening results, maternal infection status, and pregnancy outcomes, as well as evaluate the effectiveness of targeted preventive counseling and vaccination strategies.

Conclusion

This large antenatal screening study provides long-term seroepidemiological data on *T. gondii*, rubella virus, and CMV infections during pregnancy. IgG seroprevalence remained high for rubella and CMV and moderate for *T. gondii*, whereas IgM positivity remained rare throughout the study period. Formal trend analyses demonstrated no statistically significant linear changes in annual IgG seroprevalence between 2012 and 2022. These findings support the continued value of antenatal serological surveillance, preventive counseling, and vaccination programs where applicable, and underscore the importance of sustained surveillance to inform strategies for the prevention of maternal and congenital infections.

Ethics

Ethics Committee Approval: The study protocol was approved by the Clinical Research Ethics Committee of Kartal Dr. Lütfi Kırdar Training and Research Hospital (approval no.: 2020/514/177/38; date: 13.05.2020).

Informed Consent: Because this study was a retrospective review of existing records, individual informed consent was not required, as confirmed by the approving ethics committee.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.Ö., K.T., Design: K.T., Data Collection or Processing: B.Ö., Analysis or Interpretation: K.T., Literature Search: B.Ö., K.T., Writing: B.Ö., K.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Tables 1 and 2: https://d2v96fxpocvxx.cloudfront.net/4458d962-0fb2-48a3-a23a-e35265f70f9b/content-images/f1800fe4-5c37-4e3f-bd08-127934f57797.pdf

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