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Update on Age-Specific Rubella Seropositivity Rates among Pregnant Women 12 Years after Vaccine Introduction in Tunisia

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ABSTRACT

Background: Assessment of rubella immunity coverage relies on regular updates. This study aimed to determine the age-specific seropositivity rates among a large cohort of pregnant women approximately 12 years after vaccine introduction in Tunisia, where serosurveys are both old and scarce.
Materials & Methods: A prospective cohort study was conducted on pregnant women referring to the Maternity and Neonatology Center of Tunis in 2017. Eligible and consenting participants underwent blood sampling twice with a 15-day interval to detect and measure rubella-specific IgG and IgM antibodies. Demographic and obstetric data were also gathered.
Findings: A total of 800 participants with a mean age of 30.6±5 years (range: 17-48) were enrolled in this study. The overall seropositivity rate was 90.4% (n=723) (95%CI: 88.3-92.4). Also, 77 (9.6%) (95%CI: 7.6-11.7) participants were seronegative, among them 36 cases were in the first trimester of their pregnancy. The WHO minimum rubella immunization threshold of 95% was achieved for the first time in the 12-year-old vaccination program target population (96%) (95%CI: 92-99.8). No significant association was found between seropositivity rates and age, geographic origin, occupation, gestational age at the time of enrollment, parity, and abortion history ($p>0.05$), but a significant association was found with educational levels.
Conclusion: Pregnant women vaccinated at the age of 12 showed a high immunization rate. Next decades would witness the elimination of rubella virus circulation as well as congenital rubella syndrome.

Keywords: Rubella virus, Epidemiology, Congenital infection, Immunity/Immunization

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Introduction

Rubella, also known as German measles, is a highly contagious infection caused by rubella virus, a unique species belonging to the *Rubivirus* genus of the *Matonaviridae* family. Rubella virus is an enveloped, positive-sense, single-stranded RNA virus with a single serotype [1]. Typically, rubella is characterized by a maculopapular rash, lymphadenopathy, and fever in a mild clinical presentation [2]. Nevertheless, rubella could lead to rare but serious complications, such as congenital rubella syndrome (CRS) when maternal primary infection occurs in the early stages of gestation [3]. Indeed, the rubella virus is teratogenic, it infects the placenta and developing fetal tissues, resulting in infectious embryo-fetopathy [2]. CRS encompasses various fetal defects, including neurological, ophthalmic, auditory, and cardiac anomalies and even stillbirth in some severe cases. Although the risk of CRS decreases significantly after 12 weeks of gestation, the likelihood of sensorineural hearing deficit persists until 20 weeks [4].

CRS is a global public health concern, considering that more than 100,000 infants are born with CRS worldwide each year [5]. Its incidence is closely linked to high susceptibility to rubella virus among pregnant women and those of reproductive age [5]. Moreover, as the tip of the iceberg, CRS case detection often reveals high seronegative rates within populations. Given that there is currently no specific treatment for rubella or CRS, prevention remains as the key strategy. Since 2000, the World Health Organization (WHO) has strongly encouraged countries to improve immunization rates by implementing rubella vaccination programs. The WHO has also recommended the combination of measles and rubella control strategies and planning efforts. By 2019, 173 WHO member states implemented rubella vaccination programs [5]. This approach was highly effective in reducing the CRS burden in the Eastern Mediterranean Region (EMR), the Americas Region (AMR), and the European Region (EUR) from 2000 to 2010 as estimated by mathematical models based on seroprevalence data [6]. Currently, the WHO is striving to achieve the global elimination of rubella and CRS. In its global measles and rubella strategic plan 2012-2020, a rubella susceptibility threshold of 5% was set for women of reproductive age [7, 8].

In Tunisia, a WHO Eastern Mediterranean Region (EMR) country, a well-conducted national network designed in 1998 to monitor suspected measles/rubella cases (supervised by the Ministry of Health) highlighted the risks of rubella complications among the Tunisian population. This led to the introduction of a national rubella vaccination program (web site of the Tunisian Ministry of Health: http://www.santetunisie.rns.tn/) [9]. The program began in 2005 with the selective immunization of 12-year-old school girls. In parallel, a mass vaccination campaign for 13-18-year-old girls was conducted once. Subsequently, in 2014, vaccination was extended to both boys and girls at the ages of 12 months, 18 months, and 6 years. Since 2019, the rubella vaccine has been administered at 12 and 18 months of age. Additionally, since 2005, seronegative postpartum women have been systematically vaccinated against rubella immediately after childbirth (web site of the Tunisian Ministry of Health: http://www.santetunisie.rns.tn/).

All the aforementioned national and international vaccination efforts to interrupt rubella transmission need to be evaluated through seroprevalence studies on representative populations. To be informative, these studies should be conducted periodically and systematically, especially in the first decades following the

implementation of vaccination programs. In this context, we decided to assess the impact of the rubella vaccination program in Tunisia. Therefore, a prospective cohort study was conducted to determine age-specific rubella seropositivity rates among a large population of pregnant women referring to the Center of Maternity and Neonatology of Tunis (CMNT).

Objectives: The study period coincided with 12 years after the implementation of the national rubella vaccination program. Through this recent update, we intended to provide key characteristics of rubella-susceptible pregnant women to better focus preventive efforts on this target population.

Materials and Methods

Ethics statement: This study was conducted in accordance with the ethical standards set by the 1964 Helsinki Declaration and its later amendments. The samples used in this study were de-identified to maintain patient anonymity after obtaining the approval of the Bio-Medical Ethics Committee of the Center of Maternity and Neonatology of Tunis (CMNT) (REF: 2016/11).

Participant enrollment: This prospective cohort study was carried out in 2017 on 800 pregnant women referring to the Maternity and Neonatology Center of Tunis, a large tertiary maternity center with an annual delivery average rate of 10,000. These women were regularly followed up in the outpatient department for prenatal check-ups. All pregnant women not suffering from immune system-related diseases were eligible regardless of their age or gestational age. Inclusion criteria included willingness to participate in the study, ability to provide written informed consent, availability for blood sampling twice, and capacity to answer questions. Full participation included two visits with a 15-day interval: the first visit for obtaining written informed consent, conducting an interview, and collecting the first blood sample, and the second visit for collecting the second blood sample.

Data collection: Interviewers meticulously completed data collection sheets with demographic characteristics (age, geographic origin, highest level of education completed, occupation, and prenuptial visit), obstetric information about the current pregnancy (gestational age), as well as obstetric history (parity and abortion). Specific questions related to rubella, such as vaccination history and cultural factors, were also included.

Laboratory analysis: Laboratory analyses were performed in the microbiology laboratory of the CMNT. Blood samples were collected from the bend of the elbow vein in dry tubes. After centrifugation, sera were collected and stored at -80 °C. Both samples collected from each participant were tested on the same day within the same reaction. Detection and measurement of rubella-specific IgG and IgM antibodies were carried out using the Elecsys® Rubella IgG Roche® Cobas e-411 analyzer, a quantitative test, and the Elecsys® Rubella IgM Roche® Cobas e-411 analyzer, a qualitative test, respectively. Both are fully automated electrochemiluminescence immunoassays with excellent analytical performance (Elecsys® IgG test: sensitivity=100% and specificity=100%, Elecsys® IgM test: sensitivity=96% and specificity=97%). All procedures were performed strictly according to the manufacturers' instructions and result interpretation (detailed product flyers available on Roche Web site: https://diagnostics.roche.com/fr/fr/products/params/elecsys-rubella-igm.html for IgM and https://diagnostics.roche.com/fr/fr/products/params/elecsys-rubella-igg.html for IgG).

Initially, all sera were tested for the presence of IgG. A result of <10 IU/mL was considered

non-reactive, while a result of >10 IU/mL was considered positive for IgG [10]. Positive IgG indicated previous exposure to rubella virus through previous infection or vaccination. Next, the results of both samples of each participant were compared considering various scenarios:

1. Stable IgG titers: If rubella-specific IgG antibodies were detected with stable titers, the participant was likely previously infected and/or vaccinated. Therefore, the participant was considered seropositive and immunized against rubella.
2. Absence of IgG antibodies: If rubella-specific IgG antibodies were not detected in either sample, the participant was likely not immunized against the rubella virus and considered seronegative and susceptible to rubella.
3. Increased IgG titers (seroascension): If rubella-specific IgG antibody titers increased by a ratio of 2 or more within 15 days, a recent primary infection was suspected, and further investigations including testing for rubella-specific IgM antibodies were performed. If the IgM ratio exceeded 1, primary infection was confirmed, and the participant was urgently contacted to consult their doctor. If the IgM ratio was below 0.8, primary infection was excluded, suggesting asymptomatic reinfection. The participant was considered seropositive and immunized against rubella.
4. Seroconversion: If rubella-specific IgG antibodies were not detected in the first sample but detected in the second sample, acute primary infection was suspected, and the participant was urgently contacted to consult their doctor.

Statistical analysis: The statistical analysis was conducted using IBM SPSS Statistics software Version 26. Descriptive data of continuous and ordinal variables were presented as mean ± SD (standard deviation), while categorical variables were shown as numbers and percentages. Comparison of percentages on independent series was performed using Pearson's Chi-square test. All estimated parameters were reported with 95% confidence intervals estimated using the binomial distribution. For all statistical tests, the significance threshold (*p*-value) was set at 0.05. Additionally, graphical representations were created using Microsoft Excel 365 software.

Findings

Participant characteristics: A total of 800 pregnant women who were followed up for their pregnancies and prenatal check-ups at the CMNT were enrolled in this study. Demographic characteristics, obstetric information and history, as well as rubella vaccine history of the participants are presented in Table 1. The mean age of the study participants was 30.6 ± 5 years, ranging from 17 to 48 years. Participants in the age group of 17-24 years represented 12.3% (n=98) (95% CI: 10-14.5) of the cases, while the age groups of 25-30 and 31-35 years had the highest number of cases with 38.1% (n=305) (95% CI: 34.6-41.5) and 31.6% (n=253) (95% CI: 28.4-34.8), respectively. Participants came from all regions of the country, predominantly from the North, accounting for 78.9% (n=631) (95% CI: 76-81.7). In contrast, only 3.6% (95% CI: 2.3-4.9) were from the coastal region.

The vast majority of participants attended school (n=766, 95.7%) (95% CI: 94.4-97.1); however, 332 (41.5%) (95% CI: 36.9-43.6) participants completed only primary education. There were 34 unschooled participants (4.3%) (95% CI: 2.9-5.6). The quarter of the participants were employed (n=200, 25%) (95% CI: 22-28), with those working in the school and healthcare settings accounting for 3.5% (n=28) (95% CI: 2.2-4.8) and 2% (n=2) (95% CI: 1-3), respectively.

Regarding the gestational age at the time of

Table 1) Main characteristics of the whole study population, the seropositive and the seronegative populations (n: number, %: pourcentage)

	Study Population		Seropositive population		Seronegative population		*P*
	n	**%**	**n**	**%**	**n**	**%**	
Age							
≤24	98	12,3 [10% – 14.5%]	94	96 [92% – 99.8%]	4	4 [0.2% – 8%]	
25-30	305	38,1 [34.6% – 41.5%]	272	89,2 [85.7% – 92.7%]	33	10,8 [7.3% – 14.3%]	
31-35	253	31,6 [28.4% – 34.8%]	231	91,3 [87.8% – 94.8%]	22	8,7 [5.2% – 12.2%]	0,232
36-40	111	13,9 [11.5% – 16.3%]	97	87,4 [81.2% – 93.6%]	14	12,6 [6.4% – 18.8%]	
≥41	33	4,1 [2.7% – 5.5%]	29	87,8 [76.7% – 99%]	4	12,1 [1% – 23.3%]	
Age mean ± Standard Deviation		30,6±5		30,5±5		31,3±5	0.222
Geographic origin							
North	631	78,9 [76% – 81.7%]	566	89,7 [87.3% – 92.1%]	65	10,3 [7.9% – 12.7%]	
Center	114	14,3 [11.8% – 16.7%]	105	92 [87.2% – 97.1%]	9	8 [2.9% – 12.8%]	0.579
Coast	29	3,6 [2.3% – 4.9%]	27	93,1 [83.9% – 102.3%]	2	6,9 [-2.3% – 16.1%]	
South	26	3,2 [2% – 4.5%]	25	96,1 [88.8% – 103.5%]	1	3,9 [-3.5% – 11.2%]	
Highest level of education completed							
Unschooled	34	4,3 [2.9% – 5.6%]	26	76,5% [62.2% – 90.7%]	8	23,5% [9.3% – 37.8%]	0.005
Education level	766	95,7 [94.4% – 97.1%]	697	91 [89% – 93%]	69	9 [7% – 11%]	
Primary	332	41,5 [36.9% – 43.6%]	286	86,1% [82.4% – 89.9%]	46	13,9% [10.1% – 17.6%]	
Secondary	250	31,2 [29.1% – 35.6%]	235	94% [91.1% – 96.9%]	15	6% [3.1% – 8.9%]	0.0002
University	184	23 [20.1% – 25.9%]	176	95,7% [92.7% – 98.6%]	8	4,3% [1.4% – 7.3%]	
Occupation							
Unemployed	600	75 [72% – 78%]	537	89,5% [87% – 92%]	63	10,5% [8% – 13%]	0.146
Employed	200	25 [22% – 28%]	186	93 [89.5% – 96.5%]	14	7 [3.5% – 10.5%]	
School environment	28	3,5 [2.2% – 4.8%]	28	100%	0	0%	
Health environment	16	2 [1% – 3%]	14	87,5% [71.3% – 103.7%]	2	12,5% [28.7% – -3.7%]	0.227
Other	156	19,5 [16.5% – 22.2%]	144	92,3% [88.1% – 96.5%]	12	7,7% [3.5% – 11.9%]	

	Study Population		Seropositive population		Seronegative population		*P*
	n	%	n	%	n	%	
Pregnancy age							
Trimester 1	399	49,9 [46.4% – 53.3%]	363	91% [88.2% – 93.8%]	36	9% [6.2% – 11.8%]	0,07
Trimester 2	240	30 [26.8% – 33.2%]	213	88,8% [84.8% – 92.7%]	27	11,3% [7.3% – 15.2%]	
Trimester 3	120	15 [12.5% – 17.5%]	115	95,8% [92.3% – 99.4%]	5	4,2% [0.6% – 7.7%]	
Trimester 3	120	15 [12.5% – 17.5%]	115	95,8% [92.3% – 99.4%]	5	4,2% [0.6% – 7.7%]	
UNKNOWN	41	5,1 [3.6% – 6.7%]	32	78% [65.4% – 90.7%]	9	22% [9.3% – 34.6%]	
Parity							
nulliparus	376	47 [43.5% – 50.5%]	335	89,1% [85.9% – 92.2%]	41	10,9% [7.8% – 14.1%]	0.248
multiparus	424	53 [49.5% – 56.5%]	388	91,5 [88.9% – 94.2%]	36	8,5 [5.8% – 11.1%]	
1 parity	254	31,7 [28.5% – 35%]	228	89,8% [86% – 93.5%]	26	10,2% [6.5% – 14%]	0.276
2 parity	127	15,9 [13.3% – 18.4%]	120	94,5% [90.5% – 98.5%]	7	5,5% [1.5% – 9.5%]	
≥3 parity	43	5,4 [3.8% – 6.9%]	40	93% [85.4% – 100.6%]	3	7% [-0.6% – 14.6%]	
Abortion history							
No	618	77,2 [74.3% – 80.2%]	564	91,3 [89% – 93.5%]	54	8,7 [6.5% – 11%]	0,117
Yes	182	22,8 [19.8% – 25.7%]	159	87,4 [82.5% – 92.2%]	23	12,6 [7.8% – 17.5%]	
Rubella vaccine history							
Yes	112	14 [11.6% – 16.4%]	99	88,4% [82.5% – 94.3%]	13	11,6% [5.7% – 17.5%]	0.72
No	18	2,3 [2.2% – 3.3%]	16	88,9% [74.4% – 103.4%]	2	11,1% [25.6% – -3.4%]	
Unknown	670	83,7 [81.2% – 86.3%]	608	90,7 [88.6% – 92.9%]	62	9,3 [7.1% – 11.4%]	
Total	800	100	723	90,4 [88.3% – 92.4%]	77	9,6 [7.6% – 11.7%]	

enrollment, nearly half of the participants were in the first trimester of their pregnancy (n=399, 49.9%) (95% CI: 46.4-53.3). Also, 367 (47%) (95% CI: 43.5-50.5) participants had no previous pregnancies resulting in live births (i.e., nulliparous). Among the multiparous participants (n=424, 53%) (95% CI: 49.5-56.5), most of them had only one previous pregnancy (n=240, 31.7%) (95% CI: 28.5-35). Regarding abortion

history, 182 (22.8%) (95% CI: 19.8-25.7) participants had at least one previous abortion, which included spontaneous (14%), voluntary (2.4%), or medically-indicated abortions (6.2%) due to fetal malformation or intrauterine fetal demise. Furthermore, 560 (70%) participants had no prenuptial visits.

Knowledge and awareness of rubella: When asked about rubella infection, only 278 (34.7%) participants were familiar with it; however, they were rarely familiar with its common symptoms, fetal risks, and prevention methods. Similarly, the majority of participants were unaware of their rubella vaccination status (n=670, 83.7%) (95% CI: 81.2-86.3). Confirmed vaccination was found in only 112 participants (14%) (95% CI: 11.6-16.4).

Rubella seroprevalence: The serology results of both patient samples are categorized and illustrated in Figure 1. The seropositive population, either with stable or increasing IgG titers without increasing IgM, constituted the majority of participants (n=723, 90.4%) (95% CI: 88.3-92.4). No cases with primary infection were found. The seronegative population included 77 participants (9.6%) (95% CI: 7.6-11.7). The characteristics of both populations are summarized in Table 1. The mean ages of both groups were similar (30.5 ± 5 years for the seropositive group and 31.3 ± 5 years for the seronegative group, p=0.222). Participants under 24 years of age had the highest seropositivity rate (96%) (95% CI: 92-99.8) and thus the lowest susceptibility to rubella virus compared to participants over 36 years of age, who were mostly susceptible to rubella.

The immunization rate was found to be slightly higher in the 12-year-old vaccination program target population than in the mass vaccination campaign target population (96% in the <24 age group, 95% CI: 92-99.8 versus 89.2% in the 25-30 age group, 95% CI: 85.7-92.7). Although younger participants seemed to be more immunized against rubella virus, and susceptibility increased slightly from 36 years of age, this difference was statistically insignificant (p>0.05).

Association of seropositivity with demographic and obstetric characteristics: Regarding the association of seropositivity with demographic and obstetric characteristics, the results showed no statistically significant

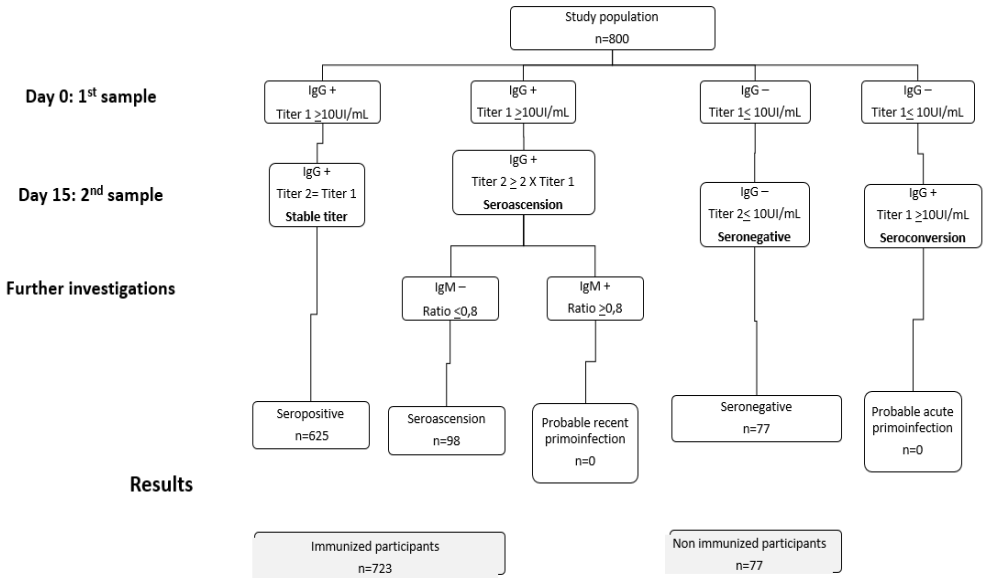

Figure 1) Flow chart of anti-rubella serology analysis and obtained results

associations between seropositivity rates and geographic origin, occupation, parity, gestational age, and abortion history. However, a significant association was found with education levels. The findings revealed that the higher the education level of pregnant women, the higher the likelihood of being immunized against rubella (p<0.05). Specifically, participants who completed only primary education had the lowest seropositivity rate (86.1%) (95% CI: 82.4-89.9) compared to those with higher education levels.

As detailed in Table 1, 112 participants with confirmed vaccination were predominantly seropositive (88.4%) (95% CI: 82.5-94.3). Intriguingly, 13 of them (11.6%) (95% CI: 5.7-17.5) were negative for rubella-specific IgG antibodies. Conversely, 16 out of 18 confirmed unvaccinated participants were seropositive (88.9%) (95% CI: 74.4-103.4). No significant association was found between the previous rubella vaccine administration and the seropositivity rate.

Focusing on the 77 seronegative participants revealed that they were predominantly from the North (65 of 77) and mostly unemployed (63 of 77), and none of the employees worked in the school environment. Regarding education, the majority of seronegative participants were educated (69 of 77), but most of them completed only primary school (46 of 69). Some of the seronegative participants were nulliparous (41 of 77), and among the multiparous participants, almost all had only one previous pregnancy (26 of 36). No abortion history was found for the majority of pregnant women (54 of 77), and almost half of them were in the first trimester of their pregnancy (36 of 77).

Discussion

In this study, 800 pregnant women referring to a large capital-based maternity center were investigated via serology testing to determine seropositivity rates. Notably, the seropositivity rate among the participants targeted by the well-conducted 12-year-old vaccination program was the highest 96% (95% CI: 92-99.8) compared to other age groups as well as other results reported in Tunisia. For the first time, the WHO minimum rubella immunization threshold of 95% was achieved among the vaccination program target population. Given that this population becomes increasingly dominant, the findings raise hopes of achieving optimal immunization coverage in the near future. However, this study also revealed a non-negligible number of first-trimester seronegative participants across all age groups. Rubella infection during this period would expose their fetuses to the maximal risk of congenital rubella syndrome (CRS). These findings emphasize the urgent need to allocate special preventive care to rubella-susceptible pregnant women based on a detailed examination of their demographic and obstetric characteristics.

The novelty of this study lies not only in its large cohort, which covered different age groups, geographic origins, education levels, occupational status, gestational ages, and parity, but also, and most importantly, in its timing. This update coincided with 1) 12 years after the implementation of the national rubella vaccination program and 2) a decade after the publication of the only national seroprevalence study targeting the vaccinated population (Figure 2) [17].

Decline in rubella virus circulation and epidemic outbreaks is closely associated with improved hygiene standards [2, 5]. In Tunisia, the gradual improvement in socio-economic status has impacted rubella immunity among the general population, especially women of reproductive age (15-49 years, as defined by the WHO) [11]. The age of primary infection has been reported to shift to older ages [12]. Before the introduction of vaccination, national studies

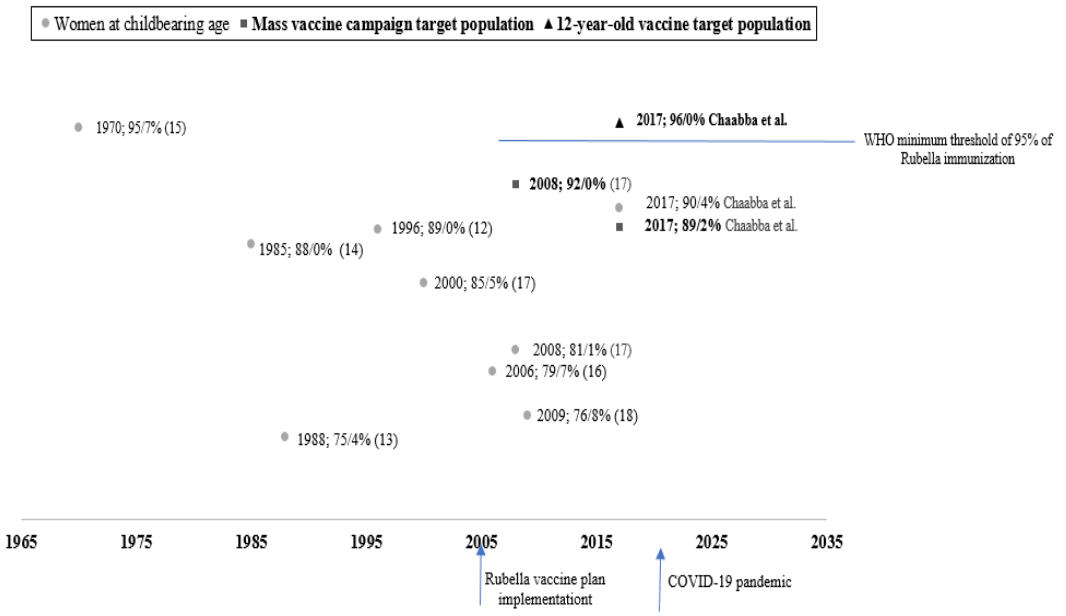

Figure 2) Seropositivity rates among pregnant women and women of reproductive age in Tunisia over the years following rubella vaccine introduction. Each point represents the seropositivity rate in percent (%), followed by the reference.

indicated that seroprevalence among women of reproductive age reached 95.7% in 1970, but this rate fluctuated significantly thereafter, reaching the lowest rate of 75.4% in 1988 [9, 13-18] (Figure 2). Although the design of these studies varied, making direct comparisons difficult, this fluctuation could likely be attributed to small rubella epidemic outbreaks [9]. Consequently, the population of pregnant women, which relied almost entirely on natural immunization, became increasingly seronegative and susceptible to rubella virus (Figure 2). Thus, the rise in CRS cases was inevitable and frequently reported on a national scale. For example, 15 CRS cases were reported during the 2011-2012 rubella national epidemic outbreak [9, 19], with some sporadic cases reported occasionally [20, 21]. It is worth noting that no acute rubella infection was detected during this study period as no IgM-positive cases were detected.

Over time, high rubella seropositivity rates have been reported again in the vaccination era. In a study by Chaabouni et al. in 2008, the seropositivity rate was reported to be 81.8% in the general population and 92% among the vaccination program target population only two years after the vaccine introduction [17] (Figure 2). A decade later, the present study revealed that the seropositivity rate among the entire cohort was 90.4% (95% CI: 88.4-92.4), which is higher than that reported in Tunisia for almost half a century. These findings clearly indicate a global improvement in immunization coverage. Furthermore, a deeper analysis of different age groups revealed even more promising results. The seropositivity rate among the 12-year-old vaccination program target group (<24 age group) was higher (96%) (95% CI: 92-99.8) compared to other age groups, exceeding the WHO's 95% threshold for the first time in the rubella vaccine era in Tunisia (Figure 2). A lower seropositivity rate (89.2%) (95% CI: 85.7-92.7) was found among the mass vaccination campaign target group (25-30 age group). This slight difference may be attributed to the limitations of mass vaccination campaigns, which may not be able to cover all target groups or may be encountered with population hesitancy

or resistance.

Compared with studies conducted in Eastern Mediterranean Region (EMR) countries adopting similar vaccination strategies, this study results closely align with those of other studies conducted in Morocco. Rubella vaccination was introduced in Morocco in 2003 for infants aged 9-18 months, which was followed by subsequent revisions to include girls of reproductive age in 2008 and 2013. Alaoui et al. (2023) reported a seropositivity rate of 92.5% among pregnant women aged 17-24 years in 2021, which was higher than the overall rate of 85.9% among all age groups and rates previously reported before or immediately after vaccine introduction [22]. In the current study, immunization coverage was found to be higher in Tunisia than in other countries in different WHO regions, such as Burkina Faso (84.6% in 2006), Peru (87.2% in 2003), and Italy (88.6% in 2015) [23-25]. Nevertheless, Tunisia has not yet reached the levels of some developing countries [7]. Although comparing the results of different studies was complicated due to differences in study design, timing of local epidemic outbreaks, and local and regional vaccination strategies, this study results shed light on Tunisia's progress in reducing rubella susceptibility among the CRS high-risk population.

Although this study demonstrated a reduced risk of CRS, the global seronegativity rate (9.6%) (95% CI: 7.6-11.7) may still promote the incidence of CRS cases. More concerning, out of 77 seronegative pregnant women, 36 were in the first trimester, and 27 were in the second trimester of their pregnancy. Rubella infection during this period exposes their fetuses to a significant risk of CRS, although the severity decreases after 12 weeks of gestation [4]. Moreover, rubella infection is subclinical in 20 to 50% of cases, which may lead to delays in receiving medical care. Hence, there is an urgent need for pre-pregnancy desensitization and immediate postpartum vaccination. Unfortunately, the majority of the participants did not complete their prenuptial visits (70%) and were unaware of their vaccination status (83.7%), and only 34.7% were familiar with rubella and its congenital risks.

Postpartum vaccination for seronegative pregnant women has been implemented since 2005. In theory, every seronegative participant who delivered after 2005 must have been vaccinated. However, 36 out of 77 seronegative women were multiparous but remained seronegative, which may be related to the absence of serology testing at the time of delivery, emergency conditions, or limited resources in some hospitals where previous deliveries took place. No significant association was found between seropositivity rates and parity and number of previous pregnancies, aligning with other national and international findings [16, 26, 27]. In addition to age and parity, this study analyzed various other demographic characteristics. Seropositivity rates showed no statistically significant association with geographic origin, occupation, gestational age at the time of enrollment, and abortion history. However, a significant association was found with education levels. Notably, considering that vaccines were primarily administered at schools, unschooled girls were susceptible to missing vaccination, which may explain the presence of seronegative participants in the younger age groups. This study found that the overall school enrollment rate (95.7%) was close to the most recent national data (95.8%), suggesting that these results may be extrapolated to a larger scale (web site of the National Institute of Statistics of Tunisia: https://www.ins.tn/). Moreover, a significant difference in seropositivity rates was observed between non-educated and educated participants, which was

significantly higher among those with higher levels of education. These findings emphasize the crucial role of schools in the success of vaccination programs, consistent with other national and international studies [16, 28].

When examining work environments, it was found that none of the seronegative participants worked in school environments, and only two worked in healthcare environments. These findings may highlight the role of contact with infants and patients in the spread of the virus and the acquisition of natural immunity or awareness of rubella vaccine administration.

Rubella vaccine is a low-cost and effective live-attenuated vaccine available either in monovalent formulation or in combination with other vaccine antigens such as measles or mumps [5].

In the private sector, it has always been possible to vaccinate infants with rubella-measles-mumps vaccines. This practice may partly explain the seropositivity rates among vaccine non-target participants, i.e., those older than 31 years. However, rubella vaccine immunogenicity depends on factors such as the number of doses, age at the time of vaccine administration, and in some cases, the strains [5]. For example, Shih et al. (2016) reported higher seroprevalence rates among girls vaccinated at age 15 years compared to those vaccinated at preschool and 15 months of age in Taiwan [28]. These intrinsic characteristics of the vaccine may explain why some vaccinated participants in our cohort were seronegative.

This study had some limitations, such as its duration, representativeness of the entire Tunisian pregnant women and women of reproductive age, absence of follow-up of seronegative participants until delivery, and lack of exploration of the causes of rubella-specific IgG antibody negativity among declared vaccinated participants. However, in this large capital-based center of maternity, 800 participants sufficiently covered various age groups. Although participants aged <24 years should have ideally been more present, Tunisian women typically become pregnant at older ages. Finally, to the best of our knowledge, no other national seroprevalence studies have been conducted and published since our study period to date. During this gap, the COVID-19 pandemic occurred. We would suggest to conduct post-COVID-19 pandemic studies to assess the impact of disruptions in vaccination strategies and to detect potential immunity gaps. This study may serve as a reference for the pre-COVID-19 context.

Conclusion

Despite the improvement of rubella vaccine coverage 12 years after rubella vaccine introduction in Tunisia, this paper revealed that there were high proportions of seronegative pregnant women among the vaccine target and non-target populations, highlighting the need for urgent actions based on a comprehensive understanding of their individual characteristics.

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Authors' contributions: YC: conceptualization, data curation, formal analysis, methodolo-

gy, supervision, validation, writing the original draft. MG: methodology, writing, reviewing, and editing the final draft. HE: methodology, statistical analysis. IJ: investigation, data curation, formal analysis, writing the original draft. AM: conceptualization, methodology, writing, reviewing. All authors read and approved the final manuscript.

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