ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ - ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
ΠΡΟΓΡΑΜΜΑΜΕΤΑΠΤΥΧΙΑΚΩΝΣΠΟΥΔΩΝ
ΕΜΒΟΛΙΑ ΚΑΙ ΠΡΟΛΗΨΗΛΟΙΜΩΞΕΩΝ ΣΕ ΠΑΙΔΙΑ ΚΑΙ ΕΦΗΒΟΥΣ

POSTGRADUATE THESIS

SEROPREVALENCE OF PERINATAL INFECTIONS AMONG PREGNANT WOMEN IN CRETE

Nicolina Hilda Anagnostatou
Paediatrician-Neonatologist

Heraklion, February 2018

ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ - ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
ΠΡΟΓΡΑΜΜΑΜΕΤΑΠΤΥΧΙΑΚΩΝΣΠΟΥΔΩΝ
ΕΜΒΟΛΙΑ ΚΑΙ ΠΡΟΛΗΨΗΛΟΙΜΩΞΕΩΝ ΣΕ ΠΑΙΔΙΑ ΚΑΙ ΕΦΗΒΟΥΣ

ΜΕΤΑΠΤΥΧΙΑΚΗΕΡΓΑΣΙΑ

ΟΡΟΛΟΓΙΚΟΣ ΕΛΕΓΧΟΣ ΓΙΑ ΣΥΓΓΕΝΕΙΣ ΛΟΙΜΩΞΕΙΣ ΣΕ ΕΓΚΥΟΥΣ ΣΤΗΝ ΚΡΗΤΗ

Αναγνωστάτου Νικολίνα-Χίλντα

Παιδίατρος-Νεογνολόγος

Ηράκλειο, Φεβρουάριος 2018

Επιβλέποντες:
1. Εμμανουήλ Γαλανάκης, Καθηγητής Παιδιατρικής-ΛοιμωξιολογίαςΠαν.Κρήτης
2. Αντώνης Μακρυγιαννάκης, Καθηγητής Μαιευτικής-Γυναικολογίας Παν.Κρήτης
3. Βάνα Παπαευαγγέλου, Καθηγήτρια Παιδιατρικής-ΛοιμωξιολογίαςΕΚΠΑ

Πρόλογος - Ευχαριστίες

Οι συγγενείς λοιμώξεις αποτελούν κίνδυνο για κάθε αναπτυσσόμενο έμβρυο και ο προγεννητικός έλεγχος των εγκύων αποτελεί ακρογωνιαίο λίθο στην πρόληψή τους. Επιδημιολογικά στοιχεία αναφορικά με την επίπτωση των διαφόρων λοιμώξεων που μπορεί να προσβάλουν το έμβρυο υπάρχουν σε περιορισμένο βαθμό στην Ελλάδα. Το κενό αυτό είναι που θα προσπαθήσει να καλύψει μερικώς έστω η πτυχιακή αυτή εργασία, μελετώντας την οροεπίπτωση των διαφόρων λοιμώξεων στις εγκύους της Κρήτης κατά τη διάρκεια ενός έτους.

Θα ήθελα να εκφράσω τις ιδιαίτερες ευχαριστίες μου στον Καθηγητή Παιδιατρικής-Λοιμωξιολογίας του Πανεπιστημίου Κρήτης Εμμ. Γαλανάκη, για την ευκαιρία που μου έδωσε να συμμετάσχω σε αυτό το μεταπτυχιακό πρόγραμμα σπουδών, για τη συνεχή καθοδήγησή του και την εμπιστοσύνη που μου έδειξε. Παρομοίως, θα ήθελα να ευχαριστήσω και την Επικ.Καθηγήτρια της Παιδιατρικής του Πανεπιστημίου Κρήτης Χρ. Περδικογιάννη, για την πολύτιμη βοήθειά της κατά τη διάρκεια της φοίτησής μου.

Ευχαριστίες θα ήθελα να εκφράσω και στα υπόλοιπα μέλη της τριμελούς επιτροπής, και συγκεκριμένα στην Καθηγήτρια Παιδιατρικής-Λοιμωξιολογίας του ΕΚΠΑ, Β.Παπαευαγγέλου και τον Καθηγητή Γυναικολογίας-Μαιευτικής του Πανεπιστημίου Κρήτης, Α.Μακρυγιαννάκη, για τη βοήθειά τους στην πληρέστερη παρουσίαση της μεταπτυχιακής μου εργασίας.

Τέλος, δε θα μπορούσα να λησμονήσω να ευχαριστήσω τον άντρα μου και τα τρία παιδιά μου για την κατανόηση που έδειξαν κατά τη διάρκεια της φοίτησής μου και την πολύτιμη ψυχολογική βοήθεια που μου προσέφεραν.

TABLE OF CONTENTS

Περίληψη

Τίτλος εργασίας: Ορολογικός Έλεγχος για Συγγενείς Λοιμώξεις σε Εγκύους στην Κρήτη
Του/της: Αναγνωστάτου Νικολίνα-Χίλντα
Επιβλέποντες: Ε. Γαλανάκης, Α.Μακρυγιαννάκης, Β.Παπαευαγγέλου
Ημερομηνία: Φεβρουάριος 2018

Εισαγωγή: Είναι γνωστό ότι η κάθετη μετάδοση περιγεννητικώνλοιμώξεων κατά τη διάρκεια της εγκυμοσύνης μπορεί να έχει δυσμενείς συνέπειες στο αναπτυσσόμενο έμβρυο. Ο εστιασμένος ορολογικός έλεγχος και η κατάλληλη θεραπεία της εγκύου ή του νεογνού όπου αυτό χρειάζεται, κατέχουν εξέχουσα θέση στην προσπάθεια εξάλειψης των συγγενών αυτών λοιμώξεων. Επιπρόσθετα, η γνώση της ακριβούς οροεπίπτωσης κάθε συγκεκριμένου λοιμογόνου παράγοντα, με ειδικό ενδιαφέρον για ομάδες υψηλού κινδύνου, όπως οι μετανάστες, είναι ιδιαιτέρως σημαντική και απαραίτητη για τον προγραμματισμό της δημόσιας υγείας.

Μέθοδοι: Για το διάστημα από τον Ιανουάριο του 2017 μέχρι και το Δεκέμβριο του 2017 μελετήθηκαν οι φάκελοι των εγκύων που γέννησαν στα τρία μεγάλα δημόσια νοσοκομεία της Κρήτης (Πανεπιστημιακό Νοσοκομείο Ηρακλείου, Βενιζέλειο Γενικό Νοσοκομείο Ηρακλείου, Γενικό Νοσοκομείο Χανίων «Αγ.Γεώργιος»). Καταγράφηκαν δημογραφικά στοιχεία, όπως η ηλικία, η καταγωγή και ο τόπος διαμονής, καθώς και αναλυτικά τα αποτελέσματα του ορολογικού ελέγχου σχετικά με τις συγγενείς λοιμώξεις. Υπολογίσθηκε η οροεπίπτωση για την ηπατίτιδα Β, την ηπατίτιδα C, τον HIV, τη σύφιλη, τη λοίμωξη από Toxoplasmagondii και από τον CMV, καθώς και για την ερυθρά. Τα αποτελέσματα συγκρίθηκαν στη συνέχεια ανάλογα με την καταγωγή και με την ηλικιακή κατανομή των εγκύων.

Αποτελέσματα: Μελετήθηκαν 1912 ιατρικοί φάκελοι εγκύων, οι οποίοι διέθεταν προγεννητικό ορολογικό έλεγχο. Η μέση ηλικία των εγκύων ήταν 30,3 (± 6) έτη. Η καταγωγή τους ήταν από την Ελλάδα (77,2%), την Αλβανία (22,7%), τη Βουλγαρία (3,3%), τη Ρουμανία (1,7%), την Άπω Ανατολή (0,4%), πρόσφυγες από τη Συρία, το Μαρόκο και την Αίγυπτο (0,7%), από την πρώην Σοβιετική Ένωση (1,9%), από τον

πληθυσμό των Ρομά (2%), από την Κεντρική/Δυτική Ευρώπη (0,7%) και 1,1% από άγνωστη χώρα προέλευσης. Η μέση οροεπίπτωση του συνόλου του πληθυσμού ήταν 1,5% για την ηπατίτιδα Β, 0,43% για την ηπατίτιδα C, 0% για τον ιό HIV, 0,22% για τη σύφιλη, 21,7% για το τοξόπλασμα, 69,1% για τον CMV, και 84,2% για την ερυθρά. Η οροεπίπτωση των Ελληνίδων για την ηπατίτιδα Β ήταν 0,5%, ενώ οι Αλβανίδες, οι Βουλγάρες, οι Ρουμάνες και οι Ρομά, εμφάνιζανσημαντικά υψηλότερη επίπτωση, και συγκεκριμένα 4,3%, 5,7%, 2,8%, and 11,1% αντίστοιχα ($p<0.001$). Παρόμοια ήταν τα αποτελέσματα και για την ηπατίτιδα C, με την οροεπίπτωση των Ελληνίδων να είναι 0,1%, των Βουλγάρων 1,4%, των Ρομά 4,4% και των γυναικών από την πρώην Σοβιετική Ένωση 5,3%. Σύφιλη καταγράφηκε μόνο σε 2 εγκύους ρουμάνικης καταγωγής, ενώ καμία έγκυος δε βρέθηκε θετική για τον HIV. Η μέση οροεπίπτωση για το τοξόπλασμα ήταν 21,7%, κατατάσσοντας την Ελλάδα στις χώρες χαμηλής ενδημικότητας, ενώ η οροθετικότητα για τον CMVυπολογίστηκε στο 69,1%. Η συχνότητα ορομετατροπής για το τοξόπλασμα ήταν 4% και για τον CMV 3,4%. Ανοσία στην ερυθρά διαπιστώθηκε στο 84,2% των εγκύων. Η πλειονότητα των γυναικών ήταν επαρκώς ελεγμένες για την ηπατίτιδα Β, την ηπατίτιδα C και τον HIV, αλλά ο έλεγχος για σύφιλη ήταν ελλιπής σε >50% των περιπτώσεων. Η συντριπτική πλειοψηφία των εγκύων είχε ελεγχθεί επίσης για τοξόπλασμα και CMV, χωρίς να διαπιστωθεί κάποιο περιστατικό συγγενούς λοίμωξης.

Συμπέρασμα: Η οροεπίπτωση της ηπατίτιδα Β, της ηπατίτιδας C και της σύφιλης είναι σημαντικά χαμηλότερη στις Ελληνίδες σε σχέση με τις εγκύους με καταγωγή από την Ανατολική Ευρώπη ή την Άπω Ανατολή. Η οροεπίπτωση του τοξοπλάσματος είναι χαμηλή στην Κρήτη, γεγονός που σημαίνει ότι η πλειονότητα των εγκύων είναι ευάλωτες και διατρέχουν κίνδυνο πρωτολοίμωξης κατά την εγκυμοσύνη, ενώ αντιθέτως η περισσότερες έχουν ανοσία έναντι του CMV. Ο προγεννητικός έλεγχος των εγκύων στην Ελλάδα βρίσκεται σε πολύ ικανοποιητικό επίπεδο, εφάμιλλο των προηγμένων χωρών, προσφέροντας ορολογικό έλεγχο στην συντριπτική πλειοψηφία των περιπτώσεων για την ηπατίτιδα Β, την ηπατίτιδα C και τον HIV, αν και παρατηρείται κενό στον έλεγχο της σύφιλης. Όσον αφορά το τοξόπλασμα και τον CMV, αν και η συχνότητα ορομετατροπής είναι εξαιρετικά χαμηλή και δεν υπήρξε καμία διαπιστωμένη συγγενή λοίμωξη, ο έλεγχος που γίνεται από τους μαιευτήρες είναι σχεδόν καθολικός, πρακτική που επιφέρει μεγάλο οικονομικό κόστος.

Λέξεις κλειδιά: κάθετη μετάδοση, περιγεννητική λοίμωξη, συγγενής λοίμωξη, TORCH, οροεπίπτωση, επιδημιολογία, έγκυες γυναίκες, αναπαραγωγική ηλικία, ηπατίδια Β, ηπατίτιδα C, ιός HIV, σύφιλη, τοξόπλασμα, κυτταρομεγαλοιός, ερυθρά

Abstract

Title: Seroprevalence of perinatal infections among pregnant women in Crete
By:AnagnostatouNicolina-Hilda
Supervisors: Emmanuel Galanakis, AntoniosMakrygiannakis, VanaPapaevangelou
Date: February 2018

Background: Vertical transmission of infections during pregnancy can have deleterious consequences for the developing fetus. Focused prenatal screening and appropriate treatment of the pregnant woman or the neonate is of paramount importance in the struggle for the elimination of congenital infections. Exact knowledge of the seroprevalence of the infectious agents, with special attention to specific high-risk groups, such as immigrants, is necessary for proper public health planning.

Methods: Demographic and serologic data ofall pregnant women delivering from January 2017 to December 2017 in the three major public hospitals in Crete (University Hospital of Heraklion, Venizeleio General Hospital of Heraklion, and "Ag.Georgios" General Hospital of Chania) were collected. Seroprevalence was estimated for HBV, HCV, HIV, syphilis, Toxoplasma gondii, CMV, and rubella. Results were compared among ethnic groups and according to age stratification.

Results: A total of 1912 medical records of pregnant women were studied based on their prenatal screening panel. The mean age of the participants was 30.38 (± 6) years. Their origin was Greek (77.2 %), Albanian (22.7%), Bulgarian (3.3%), Romanian (1.7%), East Asian (0.4%), refugees from Syria, Maroco, Egypt (0.7%), former Republics of Russia (1.9%), Roma population (2%), Central/Western Europe (0.7%)and 1.1% with unknown country of origin. The mean seroprevalence of the whole study population was 1.5% for HBV, 0.43% for HCV, 0% for HIV, 0.22% for syphilis, 21.7% for Toxoplasma, 69.1% for CMV, and 87.9% for rubella. The seroprevalence of Greek women for HBV was 0.5%, while Albanian women, Bulgarian, Romanian and Roma had a high seroprevalence of 4.3%, 5.7%, 2.8%, and

11.1% respectively ($p<0.001$). Similar results were found for HCV; seroprevalence in Greek women 0.1%, in Bulgarian 1.4%, Roma 4.4%, and former Soviet Union 5.3%. Syphilis was recorded only in 2 patients of Romanian origin. The mean seroprevalence for Toxoplasma was 21.7%, placing Greece in an intermediate endimicity category, while CMV seropositivty was estimated at 69.1%. The seroconversion rate for Toxoplasma was 4% and for CMV 3.4%. Rubella immunity was documented in 84.2% of pregnant women. Women were properly screened in the majority of cases against HBV, HCV, and HIV, but syphilis screening was omitted in >50% of cases. Screening for Toxoplasma and CMV was performed in the majority of cases, but no congenital infections were confirmed throughout the year.

Conclusions: The seroprevalence of HBV, HCV and syphilis is much lower in Greek women than in foreign women of east European or east Asian origin. Toxoplasma gondii seroprevalence is low in Crete, putting women at a high risk for being infected during pregnancy, while CMV seropositivity is much higher, conferring a level of protection to the population. Prenatal screening of pregnant women in Greece is performed at a good level, providing screening for HBV, HCV, and HIV to the majority of women, while syphilis screening needs to be optimized. CMV and toxoplasma are screened almost universally by obstetricians, placing a big economic burden on the health system, even though the seroconversion rates are low and no congenital infection was verified during the study period.

Key words: vertical transmission, perinatal infection, congenital infection, TORCH, seroprevalence, epidemiology, surveillance, pregnant women, childbearing age, HBV, HCV, HIV, Syphilis, Toxoplasma gondii, CMV, Rubella

Abbreviations

MTCT: mother-to-child-transmission, FMU: former Soviet Union, CRS: congenital rubella syndrome, HBV: hepatitis B virus, HCV: hepatitis C virus, HCDCP: Hellenic Center of Disease Control and Prevention

1. Introduction

1.1 Perinatal infections

Advances in perinatal medicine in the last decades have improved pregnancy outcomes and neonatal survival. Intrauterine or perinatal infections though, still pose a great threat to the developing fetus. Infections such as cytomegalovirus (CMV), rubella and toxoplasmosis may be completely asymptomatic in healthy adults. When contracted by a pregnant woman though, and depending on gestational age, transplacental fetus infection can cause a wide range of problems, from intrauterine death to long-term sequelae. Hepatitis B (HBV) and hepatitis C (HCV) virus, human immunodeficiency virus (HIV) and syphilis, can be vertically transmitted to the fetus, causing not only life-long infection to the newborn with its devastating consequences, but also perpetuation of the infectious disease reservoir. Knowledge of each pregnant woman's serological status, allows for proper guidance, prompt treatment during pregnancy if indicated, as in the case of *Treponemapallidum* infection, or appropriate treatment of the neonate in the immediate postnatal period, such as with hepatitis B immunoprophylaxis. Interestingly, universally accepted guidelines for prenatal screening exist only for some of the perinatally acquired infectious diseases, i.e. Hepatitis B, HIV. For the rest, i.e. *T.gondii* or CMV, a great debate exists among specialists, as to the best suitable screening approach. Special care should be paid to groups, like immigrants and Roma, which traditionally escape prenatal testing.

1.2HBV infection

HBV infection is characterized as "a major public health problem in need of an urgent response" in WHO's Global Hepatitis Report for 2017. In 2015 there were 257 million people living with chronic HBV infection, and approximately half of these were caused by mother-to-child transmission (MTCT). Since up to 90% of infants infected perinatally will develop chronic HBV infection, thus perpetuating the reservoir of chronically infected individuals globally, one can easily conclude that prenatal diagnosis and appropriate management is of paramount importance for the prevention of vertical transmission [1-4}. Vertical transmission during childbirth, by the HBV-infected body fluids or maternal blood entering fetal circulation during uterine contractions, remains the main route of infection. Additionally, intrauterine infection occurs in 13-44% of cases, by placental barrier disruption or even genetic vertical infection of the fetus from HBV-infected oocytes or sperm [2, 5]. Horizontal infection through daily contact or breastfeeding also occurs, but to a lesser extent, putting children of HBV-infected mothers in danger of acquiring HBV infection until their 5th birthday [6]. The risk for perinatal transmission of HBV is 70-90% for infants born to mothers who are both HBsAg and HBeAg(+), compared to 5-20% for infants born to HbeAg(-) mothers. The joint HBV standard passive-active immunopropylaxis with HBIG plus HBV vaccine in neonates within 12 hours after delivery, endorsed by WHO, the World Gastroenterology Organization and CDC, is very effective at reducing HBV transmission to 5-9%. The remaining percentage is mainly attributed to intrauterine infection, usually encountered with a high viral load (HBV DNA $>10^7$ IU/ml) and/or HBeAg positivity [2, 3, 5, 7]. In order to combat this problem, pregnant HBeAg(+) women or those with a high viral load, are now considered for treatment either with hepatitis B vaccine administration in an attempt to block placental transmission [2] or with nucleotide analogs, such as lamivudine and telbivudine, to reduce maternal viral load [2, 3, 5, 7]. Periodic administration of HBIG to the pregnant woman has also been investigated, but is not currently recommended [5]. Early and quick cleaning of the respiratory tract, mouth and skin of the newborns after delivery should be implemented, breastfeeding should be encouraged and HBV positivity should not be an indication for cesarean section, but invasive procedures that may damage the integrity of the fetal skin, like blood sampling, should be avoided [5]. With an estimated prevalence of 0.7-8.7% for hepatitis B infection in pregnant women in different parts of the world, a great number of neonates are in danger of perinatal transmission, if appropriate neonatal protocols are not

implemented. As prenatal risk factor-based screening will miss many chronic HBV infections among pregnant women, universal screening for hepatitis B infection during pregnancy with HbsAg has been recommended, as has repeat screening at delivery for women at high risk [3, 8].The Society for Maternal-Fetal Medicine (SMFM) also recommends that in pregnant women with HBV infection, HBV viral load testing be performed in the third trimester [3].

1.3HCV

Hepatitis C, affecting 71 million people with chronic disease worldwide, is also a major global health issue. Mother-to-child-transmission (MTCT) is the major source of HCV infection in children, occurring during pregnancy or in the perinatal period, with the exact timing and the ultimate mechanism remaining unknown [9, 10]. The rate of transmission is around 5% when the mother is positive for HCV RNA, with a virus load>615 copies/ml, dropping to <2% irrespective of HCV RNA status [10-12]. Prolonged duration of ruptured membranes, female gender of the neonate and the use of invasive fetal monitoring have been associated with increased perinatal transmission risk [11, 12]. Maternal co-infection with HIV has been associated with increased risk of perinatal transmission of HCV, with transmission rates between 10% and 20%. There is yet no consensus about whether cesarean section reduces this risk, but it is well known that breastfeeding is not associated with vertical transmission. Seroprevalence among pregnant women has been estimated at 1.3% in industrialized countries to 8.7% in underdeveloped ones [8, 10, 13]. Because HCV infection is mainly encountered in high-risk groups, such as intravenous drug abusers, transfusion recipients or people with multiple sex partners, routine screening of pregnant women is not advocated; targeted screening in high-risk women is recommended [8]. New therapeutic options with direct antiviral agents, which have proved efficacious for the treatment of chronic HCV, are not yet indicated for pregnant women. There is no single postnatal test to verify vertical transmission. Children born to mothers with HCV should be tested after 18 months of age for the presence of anti-HCV IgG; earlier testing with PCR can be done at 2 months of age and repeated after 12 months of age [10,13,14].

1.4HIV

Human immunodeficiency virus (HIV) infection in pregnant women, with a seroprevalence of 0.1% in USA and Western Europe and up to 12% in Southern Africa, is a major problem for perinatal medicine, since it carries a 22.6%-25.5% risk of vertical transmission, if appropriate measures are not taken [8, 15]. WHO has established that the minimum elimination of mother-to-child-transmission (EMTC) impact targets are fewer than 50 new pediatric infections per 100,000 live births and a transmission rate of either <5% in breastfeeding populations or <2% in non-breastfeeding populations [16]. The development and implementation of antenatal HIV testing programs and accordingly interventions to prevent transmission, has decreased the risk to 1%-2% in industrialized countries [8]. Cesarean delivery at 38 weeks' gestation, before onset of labor and before rupture of membranes, antiretroviral treatment during pregnancy, or at least perinatally, avoidance of breastfeeding and administration of antiviral medication to newborns for at least 6 weeks, are the basic guidelines for prevention of MTCT. Care should be taken for the newborn to be bathed and cleaned of maternal secretions as soon as possible [15]. HIV testing in all pregnancies at the first prenatal visit is recommended by most authorities, but an opt-out approach often exists. Alternatively, neonatal screening can be offered immediately postnatally, when the mother's status is unknown. Infants born to HIV (+) mothers should have diagnostic testing with HIV DNA or RNA assays performed at 48 hours of age, 14-21 days, 1-2 months and again at 4-6 months. Proper antiretroviral therapy should be immediately initiated pending the results. Antibody assays can be safely used for diagnosis only after 24 months of age [15].

1.5Syphilis

Syphilis is a sexually transmitted infection caused by the spirochete *Treponemapallidum*, and unlike other congenital infections, it is fully treatable in the pregnant woman, enabling prevention of vertical transmission [8]. It is estimated that approximately 1.36 million pregnant women have syphilis worldwide [17]. If left undiagnosed or untreated, 50% of infants born to mothers with untreated early syphilis and 35% with latent syphilis will be affected; 25% of pregnancies may result in stillbirth, miscarriage, or other adverse pregnancy outcomes [18]. Infection can occur in all stages and in all trimesters. In addition, even after treatment, a significantly higher risk of pregnancy adverse outcome remains [18]. The economic and reproductive costs are enormous, and even though it is estimated that for every case of syphilis identified and treated, around 2800 women need to be screened, it nonetheless remains cost-effective [17]. In 2007, WHO launched an initiative to eliminate MTCT of syphilis by 2015, setting fewer than 50 cases of congenital syphilis/100,000 live births as a goal and underscoring the importance of testing >95% of gravidas for syphilis and treating >95% of seropositive gravidas [16-18]. Syphilis testing is recommended at the first prenatal care visit in most countries, and if risk factors exist, repeat testing might be offered at 28-32 weeks gestation and at delivery. Non-treponemal assays (RPR or VDRL) are most commonly used for initial screening, and positive tests are further verified with a specific treponemal test, although none of these tests can exclude early primary syphilis [18, 19}. No newborn infant should be discharged from the hospital without determination of the mother's serologic status for syphilis at least once during pregnancy [19]. When the neonate is at danger of congenital syphilis because of inadequate maternal treatment, penicillin G should be administered, even if physical examination and laboratory tests are normal [19]. Late or limited prenatal care and failure of health care providers to follow maternal syphilis screening recommendations are the limiting factors towards elimination of congenital syphilis.

1.6*Toxoplasma gondii* infection

Toxoplasma gondii is an obligate intracellular protozoan able to infect different species, but *Toxoplasma gondii's* importance for humans refers mainly either to infection in the immunocompromised or primary infection during pregnancy, resulting in abortion/stillbirth or congenital toxoplasmosis. Congenital infection mainly results from the transplacental transmission of *T.gondii* during pregnancy or shortly before conception (<3 months) [8]. The risk of transmission increases respectively to the gestational age, from 15% in the first trimester to greater than 70% in the last month, but the risk of congenital infection in inversely decreased [8]. There is a wide variation in seroprevalence of Toxoplasma infection throughout the world, depending among other factors on the climate and the cultural eating habits. Countries such as Brazil show a prevalence >60%, others like Australia, Italy and Spain around 20-40% and some like USA, Canada and the Scandinavian countries below 10% [8,20]. It is evident that the pregnant women most at danger are seronegative women of childbearing age in high seroprevalence areas.

Due to the large variability of the seroprevalence, lack of standardized serologic assays outside of reference laboratories, high cost, and the fact that treatment in pregnancy has not conclusively proven to be of benefit for the prevention of congenital infection or the amelioration of long-term sequelae, there is no universal consensus on the screening protocol that should be endorsed in pregnancy [20-22]. Diverse policies including no screening, neonatal screening, and prenatal screening with monthly or 3-monthly re-testing schedules exist. An even stricter protocol endorsed by the American College of Obstetrics and Gynecology is that of a risk-factor based approach; serological screening is performed only in women with suspicious findings on prenatal ultrasonography (ie hydrocephalus) or immunocomprimised pregnant women or co-infected with HIV [8,20]. Serologic tests are the primary means of diagnosing primary and latent infection, while PCR testing of body fluids, and specifically amniotic fluid, confirm the diagnosis. Avidity testing of IgG antibodies helps differentiate recent from past infection, with a high-avidity IgG indicating infection that occurred at least 12-16 weeks prior to testing. Treatment of primary infection in pregnancy is recommended, and spiramycin is used <18 weeks gestational age, in an attempt to prevent vertical transmission. Once fetal infection is confirmed and once midpregnancy has been achieved, consideration should be given to starting therapy with pyrimethamine and sulfadiazine. In the infant, congenital infection is usually confirmed serologically by persistently positive

IgG titers beyond the first 12 months of life, or earlier by a positive PCR test result. Seronegative pregnant women should be cautioned to avoid activities that potentially expose them to cat feces, abstain from consumption of raw or undercooked meat and be meticulous at washing raw vegetables and fruit [23].

1.7CMV

CMV usually causes a mild and self-resolving disease, but primary infection during pregnancy, as well as reinfection, can lead to congenital infection, with a prevalence of 0.4-2.3% of all newborns, remaining the commonest cause of infective neurological handicap [24]. Cytomegalovirus (CMV) infection seropositivity in women of childbearing age has a great variability in the different parts of the world. Seroprevalence ranges from 30% in Western Europe and the United States up to nearly 100% in South America, Africa and Asia [8,25]. It is estimated that 1-2% of the seronegative women will undergo seroconversion during pregnancy, with this percentage being even higher in specific high risk groups, such as women working in day-care settings or caring for young children [24].

The risk of fetal infection following primary CMV infection is estimated at 32%-47% overall, increasing accordingly to the gestational age, to a high of 73% in the third trimester [26,27]. The risk of long-term complications, on the other hand, is inversely related to gestational age. Overall, 10-15% of infected infants will have symptoms at birth, and from the remaining asymptomatic ones, about 10-15% will develop symptoms later [24].

CMV primary infection is usually diagnosed by the presence of IgM and avidity testing of IgG, which can differentiate false positive IgM or a past infection, since IgM can persist for months to years [24]. Fetal infection is confirmed by a positive PCR for CMV on amniotic fluid attained by amniocentesis, which should not be performed earlier than 7 weeks after maternal seroconversion or 20 weeks gestation, in order to be valid. Even though the negative predictive value of amniocentesis is estimated to be 85-95%, all newborns whose mothers seroconverted during pregnancy should be tested with urine PCR within the first 3 weeks, and further evaluated and treated if needed [24,28]. Women who seroconvert during pregnancy might benefit from CMV hyperimmune globulin (CMV HIG), with some studies reporting a reduction in vertical transmission from 40% to 16%, but conclusive results haven't been reached [29,30].

A universal screening program for CMV infection during pregnancy has not been implemented worldwide for several reasons: 1) maternal immunity does not eliminate the possibility of fetal infection given that up to 75% of congenital CMV infections worldwide may be due to reactivation of latent virus or reinfection with a new viral strain [20,28,31]. 2) The lack of a proven treatment to prevent congenital

transmission further diminishes the potential benefit of universal screening [20,24,28] and 3) only a minority of infected newborns develop neurologic sequelae [28].

Screening for CMV takes different forms around the world: a) universal screening of all women prior to or early in pregnancy, allowing for proper guidance of seronegative women on how to minimize their chance of becoming infected and frequent retesting for possible seroconversion, B) screening only women at increased risk, such as those with prolonged contact with children under the age of three, young children at home or work in day-care setting, c) performing "once off" serology, including avidity at around 20 weeks gestation , and d)according to the 2015 ACOG guidelines, offering maternal serology only to those with abnormal findings suggesting of CMV on the midtrimester morphology ultrasound [24].

1.8.Rubella

Rubella is an acute, contagious viral infection, usually causing a mild fever and rash in children and adults. Infection during pregnancy, especially during the first trimester, can result in miscarriage, fetal death, stillbirth, or infants with congenital malformations, known as congenital rubella syndrome (CRS). Congenital malformations may be present at birth with children suffering from hearing impairments, eye and heart defects, or may develop later, ie. type I diabetes, deafness, subacute encephalitis and autism [32-35]. In 2016, 22,361 rubella cases in 165 countries and 367 cases of CRS were reported [34].

The highest risk of CRS is in countries where women of childbearing age do not have immunity to the disease (either through vaccination or from natural disease) [32]. Moreover, over time, vaccine efficacy against rubella may decline, and as much as 10% of children may be seronegative 12 years after the 2nd dose. The frequency and severity of the congenital syndrome are inversely related to gestational age; maternal exposure to rubella during the first trimester results in rubella congenital syndrome in 85% of cases, but exposure after midpregnancy actually carries no risk [33].

Routine prenatal screening for rubella immunity should be undertaken. If a woman is found to be non-immune, rubella vaccine should be administered during the immediate postpartum period, preferably before discharge. In addition, she is instructed to minimize exposure by frequent hand-washing, avoid contact with sick people and avoid travel to endemic countries. Cocooning in the family should also be implemented. Vaccinated women of childbearing age whose serum IgG concentrations are not clearly positive, even though proof for 1or 2 doses of vaccine exists, should receive an additional dose of MMR [33,35]. Immunoglobulin is not routinely recommended for post-exposure prophylaxis in non-immune pregnant women, and should only be offered if termination of pregnancy is not considered. Diagnosis of congenital infection is usually made by detection of rubella-specific IgM antibody in a newborn, or by stable or increasing serum concentrations of IgG over the first 7-11 months of life [35].

1.9 Aim of study

The importance of proper prenatal testing of pregnant women cannot be overstressed. It allows not only for timely treatment of the pregnant woman in an attempt to minimize vertical transmission of the offending agent, but also offers valuable information to the neonatologist, for further evaluation and treatment of the newborn immediately after birth. The fact that in Greece official national guidelines for prenatal infectious disease screening do not exist, allows for big discrepancies among practices of different specialists. In addition, the gap that exists in the knowledge of the exact seroprevalence of these infections, in the general Greek population, and especially in pregnant women, only maximizes this problem. To make things worse, in the last years, Greece has been experiencing an ever enlarging mass of refugees and economically unprivileged people who often escape medical care during pregnancy. Taking into consideration that these same groups of people traditionally have high seroprevalence rates for infectious diseases like HBV and HCV, proper screening of all pregnant women is transformed into an almost unachievable task.

Although quite a few studies have taken place on the seroprevalence of congenital infections in pregnant women in different countries, data available from Greek studies is very scarce. Four surveys exist on the prevalence of HBV infection in pregnant women residing in Athens, one study concerning seroprevalence of rubella in the general population has taken place in Northern Greece and one more study exists on Toxopasma seroprevalence in pregnant women in Crete. Studies on the seroprevalence of other infections, such as HCV of syphilis, do not exist. It can easily be concluded that a large gap in the knowledge of seroprevalence of these infections exists in Greece.

The aim of this study is the estimation of individual seroprevalence for each of HBV, HCV, HIV, Syphilis, *Toxoplasma gondii*, CMV, and Rubella in pregnant women in Crete. Special consideration will be given to particular groups, ie. Roma, refugees, and immigrants, for which prenatal testing is usually missing or scarce. The results will also reflect the true seroprevalence of these diseases in all of Greece and in the whole adult population, since pregnant women can be considered a representative part of healthy adults. Additionally, information on the screening practices of obstetricians in Crete will be analyzed. Findings will be extrapolated to the practices specialists follow in Greece, comparing them to the guidelines that exist worldwide for perinatal screening of infectious diseases. All the above will allow for better programming of perinatal care offered to women in Greece in the following years.

2. Patients and methods

2.1 Study population

All of the pregnant women who gave birth in the Obstetric Clinics of the three major public hospitals of Crete, Greece, (University Hospital of Heraklion, Venizeleio General Hospital of Heraklion and General Hospital of Chania) during the period from January 2017 to December 2017 were included. Preliminary results from a similar survey that took place during December 2016 at the University Hospital of Heraklion were also included. Women experiencing miscarriage or stillbirth were excluded from the survey. After getting permission from the Ethic Committees of each hospital and the Directors of the individual clinics, we reviewed the medical records. Specifically, we searched for the results of prenatal serological testing for HBV, HCV, HIV, syphilis, *Toxoplasma gondii*, CMV, and Rubella. Medical records that were either not found or had missing data were crosschecked with the electronic database of the hospitals, where it was available. In women with no prenatal screening documented, serologic tests for HBV, HCV, HIV and syphilis were ordered on an emergency basis in the majority of cases. Records of women who were HBsAg(+) were further checked, to verify whether appropriate immunoprophylaxis against HBV had been offered to the newborn. In addition, in women who had evidence of CMV or *Toxoplasma gondii* infection during pregnancy, further testing that had been performed and was available was recorded, such as avidity testing, prenatal ultrasound findings, amniocentesis and newborn screening results. Data that was also collected from the medical records included the ethnic origin of the participating women, their age and their residence (urban or rural).

Initially, mean seroprevalence was calculated for the total of the study sample for each test recorded. Then, the estimated seroprevalence was further analyzed according to the nationality, age distribution and residence of the participating women. Analysis was also done among women of Eastern Crete (Heraklion) and Western Crete (Chania) for CMV and *Toxoplasma gondii*infections.

Due to the great sample size and geographic distribution of the patients, the serologic tests analyzed had been performed in a diversity of laboratories; laboratories of the three hospitals and private laboratories. Positive tests had usually been verified at least once at a different laboratory.

2.2 Statistical analysis

An excel database was created initially for each hospital and at the end collectively for the whole study population. Data analysis was performed using Vassarstats and GraphPrism software. Specific prevalence was calculated in each ethnic group of mothers, and compared to the mean seroprevalence and that of the Greek women. Chi-square test was used to compare qualitative values, whenever appropriate. P values<0.05 were considered statistically significant.

3. Results

3.1 General results

Our study included a total of 2438 women who gave birth from January 2017 to December of 2017 in Crete. Results on some or the total of the seroprevalence markers were available for 1912 women (78.4%). The medical records of the remaining parturients were either not found or the specific data concerning the seroprevalence was missing. The mean age of the participants was 30.38 (± 6) years. Their origin was Greek (77.2 %), Albanian (10.8%), Bulgarian (3.3%), Romanian (1.7%), East Asian (0.4%), refugees from Syria, Maroco, Egypt (0.7%), former Republics of Russia (1.9%), Roma population (2%), Central/Western Europe (0.7%)and 1.1% with unknown country of origin (graph 1).

Their residence was urban in 58.7% (1350/2300), rural in 26.3% (605/2300), and semiurban in 15% (345/2300).

There were 65/1948 (3.34%) women under 20 years old, 789/1948 (40.5%) 20-29 years old, 959/1948 (49.23%) 30-39 years old, and 135/1948 (6.93%) over 40 years old.

The seroprevalence rate of the whole study population and of different nationalities for each of the serologic tests was estimated (table 1, table 2, graph 2, graph 3). The mean seroprevalence for HBsAg (+) was 1.5% (28 /1846) (95% CI 1-2.2), for HCV 0.43% (8/1851) (95% CI 0.2-0.9), for HIV 0% (95% CI 0-0.26)(0/1843), and for syphilis 0.22% (2/894) (95%CI 0.04-0.89). Seropositivity for CMV existed in 1115/1750 (63.7%, 95% CI 61.4-65.9), for *Toxoplasma gondii* in 391/1802 (21.7%, 95% CI 19.8-23.7), and 1517/1726 for Rubella (87.9%, 95% CI 86.3-89.3).

Negative prenatal tests for HBV, HCV, HIV and syphilis were usually not repeated during the pregnancy or at birth, even for women at high risk. Women who proved immune to CMV, Toxo and Rubella at the first visit were also not tested again. On the contrary, women nonimmune for CMV, Toxo and Rubella, were tested monthly during the pregnancy in the majority of cases.

3.2 Hepatitis B

There were 1846 medical records that enclosed data about the HBV status of the mother. The remainder records were either not found or specific data concerning HBV infection was missing, in which case it was postulated that serological tests had been done on a private basis, but not recorded in the medical file. In any case, these missing results were most possibly negative, since HBsAg positivity would have most probably been recorded.

The seroprevalence rate of the study population for HBsAg (+) was 28 /1846 (1.5%, 95% CI 1-2.2). The highest seroprevalence of HbsAg was in women of Far East origin (37.5%, 95% CI 10-74) and in Roma women (11.1%, 95% CI 4.8-23.5). Bulgarian women had a seroprevalence of 5.7% (95% CI 2.2-14), Albanian 4.3% (95% CI 2-8.5) and Romanian 2.8% (95% CI 0.5-14). (graph 4) There were no HbsAg(+) women from the former Soviet Union (FSU), Central and Northern Europe and refugee group, but the number tested was small, so safe conclusions cannot be made. Greek women showed a low seroprevalence of 0.5% (95% CI 0.2-1), and the difference compared to the mean seroprevalence or the whole population was statistically significant ($p < 0.01$). Among the seropositive women, 28.6% were Albanian, 25% Greek, 17.85% Roma, 14.2% Bulgarian, 10.7% from Far East, and 3.6% Romanian. The women who tested positive for HBsAg were stratified according to age: there was no case under 20 years old, 32.14% were between 20-29 years old, 60.71% between 30-39 years old and only 7.14% above 40 years old. This difference was statistically significant ($p<0.001$). According to the medical records, appropriate passive and active immunoprophylaxis was given to all 28 newborns whose mothers were HBsAg(+).Due to the fact that HbeAg and HBV viral load were not documented in the records, calculations on the exact seroprevalence of HbeAg(+) women or women with a high viral load cannot be made.

3.3 Hepatitis C

Among the 1851 women who had serologic tests for HCV, 8 (0.43%, 95% CI 0.2-0.9) were found positive. In only one case was the viral load recorded (7.6×10^5). All of the 8 cases were negative for HBV or HIV. According to ethnic origin, women that were HCV(+) were 25% from Greece, Former Republics of Russia and Roma population respectively and 12,5% from Albania and Bulgaria accordingly. Both of the Greek women were in high risk populations (one was a drug-addict and the other one was the spouse of a drug abuser). There were no recorded risk factors for the remainder of the cases. The specific seroprevalence for each nationality was 0.1% for Greeks (95% CI 0.04-0.5), 0.5% for Albanians (95% CI 0.09-2.9), 1.4% for Bulgarians (95% CI 0.2-7.6), 4.4% for Romas (95% CI 1.2-14.8), and 5.3% for women from the Former Democracies of Russia (95% CI 1.4-17.2). The differences between the different ethnic groups compared to the mean seroprevalence of the whole population were statistically significant ($p<0.0001$). The risk ratio among Russian, Roma, Bulgarian and Albanian women compared to Greeks was RR=37.4, RR=31.6, RR=10 and RR=3.8 respectively.

3.4 HIV

In the present study, 1842 women had evidence of HIV serological testing during their current pregnancy. No woman was found seropositive, so the rate was 0% (95% CI 0-0.26).

3.5 Syphilis

Syphilis was only tested in 895 women from the whole cohort (46.8%). In all cases NTT were used (VDRL or RPR). Only two women tested positive (0.22%, 95% CI 0.06-0.81), and in these cases the results were verified with specific treponemal tests. Both of the positive women were from Romania, producing a specific seroprevalence for Romanians of 13.3% (95% CI 3.7-37.9) and they were both habitants of adjacent villages in the center of Heraklion prefecture. One was diagnosed during the third trimester and proper treatment was initiated and the other one was diagnosed postnatally, so treatment was offered to the neonate. Ultrasound findings of the neonates were insignificant, but both neonates were lost to follow-up.

3.6 *Toxoplasma gondii*

Medical records reviewed revealed data for 1808 women of childbearing age. Only 393 of these women were immune to *Toxoplasma gondii* infection, a percentage of 21.8% (95% CI 19.9-23.7). According to nationality, the specific seroprevalence was as follows: Greeks 22.7% (95% CI 20.3-25.2), Albanians 33.7% (95% CI 27.7-40.9), Bulgarians 41% (95% CI 29.5-53.5), Rumanians 42.8% (95% CI 26.5-60.9), Roma 21.4% (95% CI 11.7-35.94), FSU 37.8% (95% CI 24.1-53.9), East Asian 14.3% (95% CI 2.6-51.3), West European 16.7% (95% CI 4.7-44.8), and among refugees 27.3% (95% CI 9.7-56.6) (graph 5). The seroprevalence among Greek pregnant women is almost identical to the mean seroprevalence of the whole study population, but it is much smaller than that of Albanians, Bulgarians, Romanians and Russians. On the contrary, Roma population has an almost identical seroprevalence and women from Far East and from West Europe have a lower seroprevalence.

The age stratification is shown in table 3. There is statistical significance between < 29 years old and over 30 years ($p<0.001$) with a risk ratio of 1.37 for ages > 30 years. (table 3)

Seroprevalence was estimated for women living in Eastern (20.24%, 95% CI 18-22.7) and Western Crete (24.5%, 95% CI 21.4-27.9). The difference in seroprevalence was statistically significant ($p<0.05$) with living in Western Crete having a risk ratio of 1.21 (RR 1.21). Furthermore, 2 of the 4 women who seroconverted during pregnancy, were from Western Crete (seroconversion rate of 0.4%, 95% CI 0.1-1.45), with a RR=1.85.

Women were also stratified according to their residence, in urban, semiurban and rural areas. Seropositivity was reported in 209/1066 (19.6%, 95% CI 17.3-22.1) of women in urban areas, 42/150 (28%, 95% CI 21.4-35.7) in semiurban, and 120/393 (23.4%, 95% CI 19.9-27.2) in rural areas. There was a trend for less seroprevalence in urban areas compared to rural (RR=0.84), but the difference did not reach statistical significance.

For the 5 women who seroconverted at the beginning or during pregnancy, avidity test results were available only for 4. In 3/4 of the cases, the avidity was high, abnormal prenatal ultrasounds were not present and no further diagnostic invasive tests were done. In the case with a low avidity, amniocentesis was performed, with negative results.

3.7 CMV

Among the 1783 women who had CMV serologic tests in their records, 69.1% were immune (95% CI 66.9-71.2). The seroprevalence of CMV in Greek women was 62.1% (95% CI 59.5-64.6), in Albanians 95.5% (95% CI 91.4-97.7), in Bulgarians 96.9% (95% CI 89.5-99.2), in Romanians 89.3% (95% CI 72.8-96.3), in Roma 95,1% (95% CI 84-99), in women from the Former Soviet Republics 82.9% (95% CI 67.3-91.9), 100% for women from China and the Fillipines (95% CI 64.6-1), 75% for women from western Europe (95% CI 46.8-91.1), and 100% for refugees (95% CI 74.1-100) (graph 5). There was statistical significance in the difference of seroprevalence among Greeks and Albanians, Romanians, Bulgarians, women from the former Soviet Union, Roma and refugees ($p<0.001$).

Women immune for CMV were stratified according to age groups, but statistical significance was not achieved. Specifically, the seroprevalence for the different age groups are as follows: < 20 years 78.3%, 20-29 years 71.6%, 30-39 years 69.2%, and > 40 years 68%. Women immune for CMV were also compared as to where they lived, ie. East or West Crete, and no difference with statistical value was found.

Of the 551 who were nonimmune, 26 seroconverted during pregnancy (4.7%, 95% CI 3.2-6.8). There was a great difference ($p<0.0001$) for seroconverting in women of foreign origin (33.3%) compared to Greek women (3.45), with a RR=9.7. If stratified according to Western and Eastern Crete, nonimmune women from Chania had a 5.5% chance of seroconverting, while women from Heraklion had a 4.2%. The difference was of no statistical significance. In 8/26 cases avidity test results were available; 2/8 had a low avidity, while the other 6/8 had a high avidity. In both cases with a low avidity, only detailed prenatal ultrasound was performed (normal findings), and no amniocentesis was recorded in the medical records. All neonates were tested with PCR for CMV in the urine during the first 2 weeks and no positive results were found.

3.8 Rubella

Pregnant women's rubella status could be evaluated in 1726 cases. Seropositivity was attained in 1517/1726 (87.9%, 95% CI 86.3-89.3), while 209/1726 were susceptible (12.1%, 95% CI 10.7-13.7). In two cases, IgM rubella antibodies were identified at the first prenatal visit, but due to preexisting IgG antibodies and consistently low IgM titers, no further testing was considered necessary.

Among Greek women, rubella status was stratified according to age: 84% were seropositive < 20years, 89.1% 20-29years, 87.6% 30-39 years, and 88.9% > 40 years. These differences were not statistically significant.

According to ethnic group, 88.2% Greek women were immune for rubella, 93.2% of the Albanians, 75.8% of the Bulgarians, 92.3% of the Romanians, 71.4% of the Roma population, 80% of the women former Soviet Union, 100% or the Central/West Europeans and East Asians, and 44.4% of the refugees.

4.Discussion

Prenatal screening is usually advocated on a cost-effect basis if the condition which is screened for has a low seroprevalence, has a precise method of laboratory diagnosis, and treatment during pregnancy or immediately postnatally can alter the prognosis of the congenital infection. Knowing the exact seroprevalence of the most frequently encountered congenital infections in pregnant women at a local level, enables specialists to better plan the screening strategy that will be implemented, especially for diseases for which consensus has not been reached (i.e. CMV) or in countries, like Greece, where official guidelines do not exist for all perinatally transmitted infections.

4.1 Hepatitis B

Proper screening of pregnant women for Hepatitis B virus (HBV) infection is of paramount importance and consensus exists as for the necessity of such screening.

Vertical transmission occurs in up to 70% of double positive HBsAg/HBeAgwomen, and 90% of the perinatally infected infants will develop chronic HBV infection, progressing to chronic liver disease, cirrhosis and hepatocellular cancer. The only method to combat this catastrophy is to screen all pregnant women, regardless of risk factors. HBsAg(+) women could be offered antiviral medication to reduce MTCT transplacentaly, and standing immunoprophylaxis protocols will be implemented to the newborn, in an attempt to lower the risk of MTCT of HBV to 5-9% [4].

In Greece, a national prevention program for Hepatitis B with universal screening of pregnant women is in effect since January 1998. Greece is considered a country of low endimicity, with the prevalence of HBV carriers in native Greeks being below 1%. The influx of refugees from countries with high endimicity, however, poses a threat to the increase of the total number of people living with chronic hepatitis B in Greece [6].

It is evident that compliance to screening guidelines is extremely important in preventing vertical transmission, but underprivileged groups often do not seek medical advice during their pregnancy. In a study by Papaevangelou, et al, that took place in Athens, prenatal screening for HBV had taken place in 91,3% of the women, much improved than in another study by the same author, where only 63,1% had been tested [6,36]. What was of most interest however is that in two different studies, women that escaped prenatal testing, had a much higher prevalence [6,37];in total 5,3%, Greeks 2,8% , Albanians 7,4% and Roma 4,3% [6,37]. These findings

emphasize that specific populations should be targeted for prenatal testing and vaccination coverage [37]. Our study did not record the percentage of women with regular prenatal follow-up versus those who only sought medical advice at term, so conclusions on this topic cannot be made.

Seroprevalence of HBsAg(+) in Greece has been previously studied in a couple of surveys. Prevalence of HbsAg(+) in women delivering in Greece during a two-week study period in 2003 was 2.89%. Women of Greek origin had the lowest prevalence of 1.7%, Albanians the highest with 9.8%, immigrants from other countries a prevalence of 5.7% and gipsy women 3.6 % [6]. Elefsiniotis et al, estimated the seroprevalence of HBsAg in 26,746 women at reproductive age in Greece from 2003-2005 and found that 1.53% of women were HBsAg positive. The mean prevalence of HBsAg in Albanians was 4.9%, in Asians 5.57%, and in women from East Europe 1.29%. Greek women had the lowest prevalence of 0.57% [38]. The results are similar in a more recent survey by Karatapanis et al, that took place form 2007-2009, where the total HBsAg(+) prevalence is 1,2%, that of Greek women is 0,6% and that of the Albanians 5,4% [37].

In our study, the mean seroprevalence of the study population for HBsAg (+) was 1.5% (95% CI 1-2.2), and the Greek women showed a low seroprevalence of 0.5% (95% CI 0.2-1), similar to the most recent surveys by Elefsiniotis et al. and Karatapanis et al., reporting 0.57% and 0.6% respectively[37,38]. The 1.7% seroprevalence in Greek pregnant women that Papaevangelou et al. reported in an older study, probably highlights the constantly falling prevalence of HBV in the Greek population.

Seroprevalence in Albanian pregnant women living in Greece has always been in excess of that reported for Greeks, reflecting the higher burden of HBV in Albania. In the general Albanian population, the seroprevalence of HBV is 9.5%, which means that Albania remains a country of high endimicity [39]. In a study by Malamitsi-Puchner et al, published in 1996,HbsAg(+) prevalence of 13,4% was found among Albanian pregnant women [40]. More recent studies show a gradual decrease to 4.9-5.4% [37,38], very similar to our findings of 4.3% (95% CI 2-8.5).This trend can be explained by the ever improving hygiene conditions among Albanian families, as well as the increasing percentage of immunization in this population.

Our study is the first to our knowledge to report the seroprevalence of Bulgarian and Romanian women separately, and not as a whole, like the former studies. In our study, Bulgarian women had a seroprevalence of 5.7% (95% CI 2.2-14), which is much

bigger than that reported in a study by Tsankova et al, where the seroprevalence in Bulgaria for pregnant women is estimated at 2.26%, with residence in rural areas and belonging to an ethnic minority important risk factors [41]. This discrepancy could be explained by the fact that most of the Bulgarian immigrants to Greece belong to the Roma population and are not indigenous Bulgarians. On the contrary, Romanian pregnant women living in Greece had a seroprevalence of 2.8% (95% CI 0.5-14) for HBsAg(+) in our study, slightly lower than the 4.4% reported for the whole Romanian population in 2013 [42].

The Roma population in Greece, although Greek in nationality, often behaves as a separate group with discrete characteristics, often nonadherent to national guidelines and immunization practices. A large proportion does not seek medical advice, so the exact prevalence of HBV, as well as other diseases, is actually unknown. In our study, 11.1 % (95% CI 4.8-23.5) of Roma women were HBsAg(+), but the overall number of Roma participants (N=45) is not big enough to extract safe conclusions. Papaevangelou et al, had found a seroprevalence of 3.6% (95% CI 0.8-10.2) among the same group [6], while in a study of hepatitis B among Roma children in Athens, 22% were identified with evidence of past infection (anti-HBc(+)), among whom five (4% of the total) were chronic carriers (HBsAg(+)) [43].

The highest seroprevalence of HbsAg(+) was in women of Far East origin (37.5%, 95% CI 10-74). Only Elefsiniotis et al. reported separately on these women, and he found a seroprevalence of 5.57% in 251 women, being the highest in his study too. In China, HBV infection in women of childbearing age is at a high level, with a prevalence of 7.18% [5]. The extremely high rate found in our study, is definitely misleading and due to the very small size of the sample, but nonetheless, women from China should be treated as high risk and every effort should be made to screen them prenatally.

There were no HbsAg(+) women from Central and Northern Europe, as was the case in the study by Elefsiniotis et al, the former Soviet Union (FSU), and refugee group, but the number tested was small, so safe conclusions cannot be made.

The Society of Maternal and Fetal Medicine (SMFM) also recommend that in pregnant women with HBV infection, HBeAg should be tested and HBV viral load should be performed in the third trimester[3]. Elefsiniotis et al, found only 2,67% of HBsAg(+) women were HBeAg(+) and from the women whose blood was tested for viral load, only 12.7% exhibited extremely high serum HBV-DNA levels of $>10^7$ copies/ml, suggesting that only a slight proportion of HBsAg positive women in

Greece pose a great risk for vertical transmission to their offspring [38]. Accordingly, in HEPNET study in Greece, an ongoing nationwide retrospective–prospective study initiated in 1997, it was shown that the prevalent form of hepatitis B among the Greek patients was HBeAg negative, in comparison with immigrants who were positive in 16,6% of the cases and had a higher DNA load [44]. Malamitsi et al, reported a HBeAg positivity of 7.5% in Albanians living in Greece [40]. The medical records reviewed in our study did not provide any information either on HBeAg or on HBV viral load, so conclusions cannot be made.

4.2 Hepatitis C

Transmission from mother-to-child is the major source of HCV infection in children, occurring during pregnancy or in the perinatal period, at a rate of 2-5%, depending on the mother's viral load [12]. Screening only pregnant women in high-risk groups is usually advocated, since seroprevalence in the general population is very low and no treatment exists for newborns infected vertically. The current prevalence of hepatitis C in Greece ranges from 0.5%-2% [9,45], but in Crete a slightly greater seroprevalence was found byDrositis et al, estimated at 2.2% [46]. Immigrants who live in Greece have a seropositivity of 2.3%-4.82% for HCV, which mainly concerns formerSoviet Union and African refugees, and to a lesser extent Albanians [9,47]. This is the first study to our knowledge on seroprevalence of HCV among pregnant women in Greece. A mean seroprevalence of 0.43% (95% CI 0.2-0.9) was found. According to ethnic origin, the seroprevalence was 0.1% for Greeks (95% CI 0.04-0.5), 0.5% for Albanians (95% CI 0.09-2.9), 1.4% for Bulgarians (95% CI 0.2-7.6), 4.4% for Romas (95% CI 1.2-14.8), and 5.3% for women from the former Soviet Union (95% CI 1.4-17.2). Greeks' low seroprevalence matches hepatitis C prevalence in pregnant women in England in 2012 (0.095%) [48]. Malamitsi-Puchner et al, reported a similar prevalence of 0.6% among Albanian pregnant immigrants [40]. In Bulgaria, estimated prevalence of hepatitis C is 1.3% [49], very similar to our results. In the FSU, anti-HCV was detected in only 1.3% of pregnant women in 2011–2012 [50], much rarer than in our study, probably due to our small sample size. In our study, no Romanians were found HCV (+), but the 95% CI (0-9.6%) enclose the reported seroprevalence of the general population in Romania, being 3.50% (CI 3.10–3.92%) [51]. Even though the sample size is small, the seroprevalence found among Roma is alarming (4.4%). Data on Roma have not been published elsewhere and perinatal specialists should be aware of the increased danger for HCV in this group; screening should definitely be offered to these women. Both of the Greek women were in high risk populations (one was a drug-addict and the other one was the spouse of a drug abuser), which underscores the importance of screening specific high-risk populations. On the contrary, it is surprising to note that in 1846 out of 1912 medical records with relevant data, screening for HCV had been performed, without high-risk conditions being present, a practice that has great cost and should definitely be discussed among specialists.

4.3 HIV

Detection of human immunodeficiency virus (HIV) infection in pregnant women is of paramount importance, since it carries a 22.6%-25.5% risk of vertical transmission [8,15]. The development and implementation of antenatal HIV testing programs and accordingly interventions to prevent transmission, has decreased the risk to 1%-2% in industrialized countries [8]. HIV testing in all pregnancies at the first prenatal visit is recommended by most authorities, but an opt-out approach often exists in some countries.

In the present study, 1842 women had evidence of HIV serological testing during their current pregnancy, proving that obstetricians abide by the official guidelines and screen all women, irrespective of risk-factors. Data on whether consent on the part of the pregnant mother existed was lacking, but from personal experience, in GreeceHIV testing is usually ordered by physicians without informing the woman. Repeat screening at term was not documented, which might be inappropriate for specific high-risk groups. No woman was found seropositive, so the rate was 0% (95% CI 0-0.26). Nonetheless, universal screening should continue. In a retrospective study covering 14 years (2000-2013) in Crete, 8 neonates were born to 7 seropositive mothers, 75% of which were Greek and 62.5% of which had been on antiretroviral therapy before pregnancy. After following the official guidelines for prevention of MTCT, no neonate was infected (100% prevention) [52]. In Greece, a total of 134 seropositive children < 13 years at the time of diagnosis have been reported and 55.7% of them have been infected vertically. In 2016, 3 new MTCT cases were reported [53]. In Europe < 3% of new cases are in children 0-19 years old and the WHO target of fewer than 50 new MCTC cases/100,000 live births/year has been achieved.

4.4 Syphilis

Syphilis is a sexually transmitted disease that can cause vertical infection of the developing fetus in up to 50% of cases with detrimental consequences. Infection can occur in all stages and in all gestational ages and clinical findings may be evident at birth of many years later. Additionally, syphilis is a completely treatable disease, and on these grounds, syphilis testing is recommended at the first prenatal care visit in all women; if risk factors exist, repeat testing should be done at 28-32 weeks gestation and at delivery.

In our study, only 894(46.7%) out of the 1912 pregnant women that had prenatal testing documented, had been tested for syphilis. This is probably due to the fact that specific guidelines form the Hellenic Society of Obstetrics and Gynecology do not exist and a majority of specialists consider syphilis to be a well-forgotten disease. What is even more interesting is that even among women of East European origin, the rate of screening remained under 50%. In 2007, WHO launched an initiative to eliminate congenital syphilis by 2015, the goal set was <50 cases of CS per 100.000 live births and one of the measures necessary was testing >95% of gravidas for syphilis and treating >95% of seropositive gravidas [16-18]. Accordingly, screening is routinely offered and recommended to all pregnant women in England, with an uptake of over 97% [17].

In 2014, 69 congenital syphilis cases were reported in 23 EU/EEA Member States, an overall rate of 2.3 cases per 100,000 live births. The trend for reported congenital syphilis cases has remained stable in recent years, and the majority of cases were reported from Bulgaria (24 cases) and Poland (17 cases). The number of reported cases are decreasing in Bulgaria, but have increased in Portugal, Romania and Spain compared with 2013 [53]. The percentage of women testing positive for syphilis had nearly doubled in the UK from 1999 to 2007, with 10 cases of congenital infection being reported annually. 54% of confirmed cases were in women born in Europe, of whom 39% were born in Eastern Europe [17]. In 2014, 0.14% of pregnant women in England tested positive and only 21 cases of congenital syphilis have occurred between February 2011 and January 2017, usually born to mothers socially marginalized and encountering barriers to antenatal care. Interestingly, in 4 of these cases, the mothers had tested negative at the beginning of the pregnancy and no risk factors were identified to warrant repeat testing [54]. In Greece, only congenital syphilis is a notifiable disease and there have been 12 cases reported during the period from 2007-2013 [55]. Data on syphilis seroprevalence among pregnant women in

Greece does not exist. In our study, only two women tested positive (0.22%, 95% CI 0.06-0.81), and in these cases the results were verified with specific treponemal tests. Both of the positive women were from Romania, producing a specific seroprevalence for Romanians of 13.3% (95% CI 3.7-37.9) and they were both habitants of adjacent villages in the center of Heraklion prefecture. One was diagnosed during the third trimester and proper treatment was initiated and the other one was diagnosed postnatally, so treatment was offered to the neonate. Ultrasound findings of the neonates were insignificant, but both neonates were lost to follow-up. As mentioned earlier, Romania is one of the countries encountering a rising rate of syphilis congenital infections, with the mean annual incidence of syphilis being 25.2 ± 15 cases per 100,000 inhabitants form 1980-2009 [56]. The fact that under 50% of our study population was screened for syphilis could mean that at least 2 cases of congenital infection might have been missed.

4.5 *Toxoplasma gondii*

Toxoplasma gondii's importance for humans refers mainly to primary infection during pregnancy, resulting in abortion/stillbirth or congenital toxoplasmosis. There is a wide variation in seroprevalence of Toxoplasma infection throughout the world, which might play a role in planning public health policies. It is evident that the pregnant women most at danger are seronegative women of childbearing age in high seroprevalence areas. Countries such as Brazil show a prevalence >60%, others like Australia, Italy and Spain around 20-40% and some like USA, Canada and the Scandinavian countries below 10% [8,20]. Specifically for Greece, different studies on the seroprevalence of toxoplasma infection show results from 20.1% to 36.4% in women of childbearing age or pregnant women [65]. Antoniou et al, estimated the incidence of toxoplasmosis in 5532 pregnant women in Crete over a period of five years (1998-2003). A percentage of 29.45% of pregnant women were found seropositive and 3.34% fulfilled the criteria for primary *T.gondii* infection [66].

In our study, a mean seroprevalence of 21.7% (95% CI 19.9-23.7%) was estimated for the whole study population and positive IgM was found in 0.3% of women. Similar results come from reports on *Toxoplasma gondii* infection in women of childbearing age in the last 30 years in the Balkans [67]. Stratified according to nationality, Greek and Roma pregnant women had a similar seroprevalence of 22.7% and 21.4% respectively. The higher seroprevalence found in women from Eastern Europe, and namely Albania, Bulgaria, Romania and the former Soviet Union (33.7% - 42.8%), coincide with the increased prevalence encountered in less developed countries. On the other hand, women from Far East and West Europe had, as expected, the lowest seroprevalence (14.3% and 16.7% respectively).

Interestingly, in the other Mediterranean countries, the seropositivity rates are greater than in our study. In Turkey, the seroprevalence of Toxoplasma in women attending the Obstetric Clinic was estimated at 31% [60]. Munoz et al, in a study in Spain on 3547 pregnant women showed that the seroprevalence of toxoplasmosis was 39.5% and acute toxoplasmosis occurred in 1.2% of the seronegativewomen [68]. In Italy, on the other hand, in 2010 only 19.4% of women of childbearing age were found to be positive for Toxoplasma, in comparison with 48.5% in 1995. Different seroprevalence rates among native and non-native women were noted, as in our study, with the women living in Italy but coming from Africa, Eastern Europe and South America showing a toxoplasma seroprevalence as high as those documented in their country of origin and much higher of that found among Italian women. Interestingly, a

significant discrepancy between immigrant and native mothers was observed: despite immigrant women being less susceptible to infection, given the higher percentage of previous infection among them, their RR for toxoplasma primary infection during pregnancy was 5-fold higher than that of Italian women [69].

In our study, a statistical significant difference was found in the seroprevalence among women under and over 30 years old, with a risk ratio of 1.37 for ages > 30 years. To our knowledge such a finding has not been mentioned elsewhere, although a plausible explanation exists, since as women age their chances of contracting *Toxoplasma gondii* infection increase. In addition, the trend towards a smaller seroprevalence in women living in urban compared to rural areas (RR=0.84), without the difference reaching statistical significance, is biologically explainable, since women in rural areas are more prone to come in contact with cats, animals in general and raw vegetables. Similar findings were presented by Apostolaki et al [63].

Diagnosis of maternal infection is made by positive IgM antibodies combined with low avidity IgG assays and further verified by PCR on the amniotic fluid. Congenital toxoplasmosis has been a notifiabledisesase in Greece since 2004 and subject to continuous data collection by the HCDCP (Hellenic Center of Disease Control and Prevention). Since 2004, only 4 cases have been reported (2 in 2004, 1 in 2010 and 1 in 2013).In our study, for the 5 women who seroconverted at the beginning or during pregnancy, avidity test results were available only for 4. In 3/4 of the cases, the avidity was high, abnormal prenatal ultrasounds were not present and no further diagnostic invasive tests were done. In the case with a low avidity, amniocentesis was performed, with negative results. In all 5 cases, infants were followed-up serologically for the first year of life and no congenital infection was confirmed.

As far as screening protocols are concerned, just as with CMV infection, great controversy exists among specialists, since seroprevalence shows great variability among different parts of the world and treatment of the pregnant woman doesn’t conclusively reduce long-term sequelae [22]. The ACOG in 2015 suggested screening only pregnant women who are immunocomprimised or HIV-positive, while others entertain also screening women with abnormal fetal ultrasound findings. In our study, 94.5% of women whose medical records were retrieved had serologic tests performed for toxoplasma, proving that obstetricians in Greece consider this type of screening extremely important. Taking into account though that 78.3% of women tested negative, and that the majority of these underwent monthly serologic reevaluations, one should consider if the cost is justifiable, especially if one considers

that only 4 cases of congenital toxoplasmosis have been reported in Greece in 10 years.

4.6 CMV

Cytomegalovirus (CMV) infection remains the commonest cause of infective neurological handicap with a birth prevalence of congenital CMV of 0.4-2.3% [24]. The risk of fetal infection following primary CMV infection is estimated at 32%-47% overall and the risk of long-term complications is inversely related to gestational age [24,26,27]. In a literature review by Cannon et al, CMV infection was relatively common among women of reproductive age, with seroprevalence ranging from 45 to 100%. CMV seroprevalence tended to be highest in South America, Africa and Asia and lowest in Western Europe and United States. Worldwide, seroprevalence among non-whites tended to be 20-30 percentage points higher than that of whites and people of lower socioeconomic status were more likely to be CMV positive [25]. A large scale survey on 5714 women of reproductive age in the USA revealed that 70% were IgG(+) and 2.8% was CMV IgM(+) [59]. In France, the overall point estimate of CMV infection seroprevalence for women aged 15–49 years was 45.6%, and people born in a non-Western country were more likely to be CMV seropositive than those born in France or in another Western country (93.7% vs. 37.7%) [57]. In a cohort of pregnant women in Canada from 2010-2013, 1938 women were tested and 40.4% were seropositive for CMV. Risk factors for seropositivity were day—care educator, lower education, lower income, having had children, first language other than English or French, and being born outside Canada or the USA [58]. In contrast, countries with higher IgG prevalence, such as Turkey and Korea (>98%), report a much lower seroprevalence of IgM(+) of 0.2-0.5% and 1.3% respectively [59-61]. In China, the CMV seroprevalence is 96.2% [62]. In our study, the mean seropositivity rate of the whole population was 69.1%, and positive IgM was detected in 1.5% of the population, lying somewhere in between industrialized and underdeveloped countries. The seroprevalence of CMV in Greek women was lower than the mean (62.1%, 95% CI 59.5-64.6), but in immigrants from other countries, and especially Eastern European countries, the seroprevalence was much higher, achieving a great statistical significance ($p<0.001$): Albanians 95.5% (95% CI 91.4-97.7), Bulgarians 96.9% (95% CI 89.5-99.2), Romanians 89.3% (95% CI 72.8-96.3), Roma population 95,1% (95% CI 84-99), women from FSU 82.9% (95% CI 67.3-91.9), and 100% for women from China and the Fillipines (95% CI 64.6-1). Women from Western Europe were CMV positive in 75% of cases (95% CI 46.8-91.1), a rate higher than that expected in developed countries, as was the case in the studies previously mentioned.

It is estimated that 1-2% of the seronegative women will undergo seroconversion during pregnancy, and in specific high risk groups, this rate might be bigger (8% / year in day care workers and 24% in parents of children shedding CMV) [24]. In the study from France, 2.3% of the women seroconverted during pregnancy [58]. In our study, 4.7% of seronegative women seroconverted during pregnancy. Data on possible high-risk characteristics of these women were not recorded in this study. What was of great surprise though, was the difference in the seroconversion rate in women of foreign origin (33.3%) compared to Greek women (3.45), with a RR=9.7 and a statistical significance of $p<0.0001$. Similar results were presented at the 5th Panhellenic Neonatal Conference in Athens in 2014 from a survey in pregnant women in Chania: foreign citizens had 11 times bigger chance of being seropositive, compared to Greek women (95% CI 7.246 – 17.241) [63]. To our knowledge, such a finding has not been previously reported and probably reflects the poor hygiene standards that characterize many of the immigrants in combination with the extremely high prevalence of CMV among them.

CMV seroprevalence is reported to increase from 56% between the ages of 30-34 years to 79% between the ages of 35-39 years [24]. In a study by Antona et al. in France, CMV seropositivity depended on the age group; <30% of them were CMV positive before 25 years of age, but nearly 50% above this age, putting young seronegative women (>70%) at higher risk of primary CMV infection during pregnancy [64]. In our study on the contrary, women immune for CMV were stratified according to age groups, but statistical significance was not achieved, possibly reflecting an early in childhood acquisition of CMV.

Maternal infection is diagnosed by the combination of positive IgM and low avidiyIgG antibodies, and fetal infection is confirmed by a positive PCR for CMV on amniotic fluid attained by amniocentesis. Following birth, even neonates with negative amniocentesis results, should be tested with urine PCR within the first 3 weeks and treated accordingly [24,28]. In our study, only in 8/26 cases were avidity test results available; 2/8 had a low avidity, while the other 6/8 had a high avidity, and were considered past infections. In both cases with a low avidity, only detailed prenatal ultrasound was performed (with normal findings), and no amniocentesis for PCR analysis was recorded in the medical records. Nonetheless, all neonates were tested with PCR for CMV in the urine during the first 2 weeks and no positive results were found. In the presentation by Apostolaki et al, during the years 2011-2013, 2 congenital CMV infections were identified in the area of Chania [63].

A great debate exists as to what type of screening is best for pregnant women. In the majority of countries, universal screening of pregnant women is not advocated, since, among other reasons, effective treatment cannot be offered to infected pregnant women. Additionally, a great proportion of congenital CMV infections occur in women previously immune undergoing a reinfection, so positive testing at the beginning of the pregnancy might convey a false sense of safety. In the USA, CMV testing is recommended only when there is clinical suspicion or fetal abnormalities on fetal scanning [28]. In Greece, official guidelines do not exist. Based on our study though, one can conclude that at least 72.1% of the pregnant women have had CMV serologic testing performed. Moreover, the majority of 40% of the women who are susceptible to CMV, have been repeatedly tested (usually once monthly) throughout the pregnancy. Based on the fact that no congenital infection was recorded, this strategy bears an enormous cost to the insurance system, with no apparent benefit.

4.7 Rubella

Rubella is an acute, contagious viral infection, which can have devastating consequences when contracted during pregnancy, especially during the first trimester. The highest risk of CRS is in countries where women of childbearing age do not have immunity to the disease (either through vaccination or from natural disease). In the USA, the majority of CRS cases reported the last 20 years are among infants born to foreign-born mothers, mainly from Latin America [33]. During early prenatal care it is standard of care in the USA and in most parts of the world, to ascertain the seroconversion rate of rubella using serological assays. If the pregnant woman is non-immune, proper guidance is offered to minimize the risk of contracting rubella, and immunization with rubella vaccine is programed immediately after birth [33]. In a study in Northern Greece on the seroprevalence of rubella, among women of reproductive age (16-40 years), 13.9% were susceptible to rubella [70]. Karacan et al, determined the prevalence of IgG antibodies against rubella in Turkey in pregnant women from 2009 to 2013 to be 95%. In other studies, seropositivity for rubella is stated as 87%-91% in the USA, 85.8% in Italy and 98% in Spain, 94.4% in Norway and around 96% in England [71,72]. In a large study assessing rubella susceptibility among pregnant women in Spain, that was conducted from 2008-2013 and included a total of 22,681 women, the proportion of women susceptible to rubella was 5.9%, with immigrants being more susceptible (7.6%), especially those from Asian countries (10.8%). Susceptibility to rubella declined with increasing age (perhaps due to natural immunity in women > 40 years), multiparity and being native Spanish [72]. In a survey on seroprevalence of rubella in Japanese women, there was a difference between primiparous women (3%) vs multiparous women (5%), and the teenage pregnant women were consistently more susceptible to rubella (20%) [73]. Ina study by Pejcic et al. significant high seropositivity was observed in mothers >30 years as compared to mothers < 29 years [74]. In our study, rubella status was stratified according to age in Greek women: 84% were seropositive < 20years, 89.1% at 20-29years, 87.6% at 30-39 years, and 88.9% > 40 years. These differences were not statistically significant, in contrast to the two previous studies. According to ethnic groups, differences did exist, but extrapolations are difficult to make, since rubella is the only vaccine-preventable perinatal infection, and the exact vaccination coverage of each immigrant population is unknown. Greeks have been privileged with a robust national immunization schedule, encompassing rubella vaccination since 1989 with a 2-dose schedule. The seroprevalence found in our study was 88.2% in Greek women,

similar to that found in Northern Greece as well as most of the developed countries. Effort was made to inform the susceptible to rubella pregnant women for the necessity of immediate postnatal immunization. The 11.8% of seronegative women found might be due to incomplete or failure of vaccination, or might have to do with the fact that over time, vaccine efficacy against rubella may decline, and as much as 10% of children may be seronegative 12 years after the 2nd dose [75].

In Europe, up to October 2017, a total of 754 cases of congenital rubella infection had been reported, with an average of 7-23 per year. In 2012 there were 55 cases reported only in Romania, experiencing at that point of time a huge rubella outbreak. In Greece, no CRS cases have been filed from 2003-2014. Nevertheless, rubella certainly hasn't been eliminated from Europe, and screening of all pregnant women should be implemented. In our study, 90.2% of the women were tested for rubella at least once in pregnancy, and care should be taken for the percentage to reach 100%. Focus should be given on immunizing susceptible women immediately after giving birth and before hospital discharge, not omitting those whose IgG results were in the intermediate zone. Special attention should also be given to the refugees, who showed the lowest seroprevalence (44.4%), since overcrowding conditions often encountered among them and missed immunization opportunities can put them in great danger of contracting rubella. In our study, there was no register in the medical records of whether rubella immunization was offered to susceptible women prior to their discharge. Based on personal experience, there seem to be quite a few obstacles concerning both the gynecologists' inexperience with immunizations and technical difficulties that have to do with the economical coverage of the vaccine.

4.8 Limitations of the study

One of the weaknesses of this study was that the population studied was pregnant women giving birth at one of the three major public hospitals in Crete. Women who gave birth at the private hospitals, might have slightly different seroprevalence rates, as they tend to belong to a higher income category as a whole, and would thus be expected to have better living standards. Additionally, public hospitals tend to have an overrepresentation of minority groups, such as immigrants and Roma, which could adversely affect the mean seroprevalence estimated.

Data was not collected concerning the parity of women, their occupation, their educational background and the place they grew up(rural or urban), all being characteristics that have been studied in seroprevalence surveys. In addition, information related to high-risk categories for the different infections was not recorded, ie drug abuse or multiple sex partners for HCV, HIV and HBV or close contact with young infants or toddlers for CMV.

Moreover, there was no information on how long the foreign women had been living in Greece, or whether they had been born and immunized in our country. Whether the women of foreign origin had regular prenatal testing during pregnancy or only at term on an emergency basis, was also not recorded. Higher HBV disease burden and low vaccination-induced protection are characteristic in pregnant women nonadherent to HBsAg prenatal testing. More intense surveillance and implementation of immunization programs should be applied in these populations.

As far as HBV infection is concerned, testing for HBeAg and HBV viral load was not performed by obstetricians, so conclusions about the prevalence of HBeAg/viral load cannot be made, even though the risk of vertical transmission is mainly based on these parameters.

4.9 Future research

Future studies should focus on identifying the appropriate screening protocol that should be implemented for each of the diseases. Specific national prenatal testing guidelines should be constructed, depending on the seroprevalence of these infections in each country. Some infections might profit from universal screening, ie HBV, others from high-risk group screening, ie HCV, while infections like Toxoplasma or CMV might not be worth testing in the first place. Cost-effective studies should also be done, to help in the abovementioned decision planning.

In addition, a prospective study could be implemented, for newborns born to mothers with HBV, HCV, syphilis, Toxoplasma and CMV, which would yield interesting data on the final prevalence of congenital diseases in Greece and the breaches in prenatal and postnatal treatment, when implemented. The results from such a study would also highlight the need for prenatal testing according to each disease.

At last, future research could study the seroprevalence of all pregnant women in the first trimester, including those who experienced a miscarriage or a stillbirth, since these women might have a higher seroprevalence of infections, such as syphilis or CMV and *Toxoplasma gondii* infection.

4.10 Conclusions

Vertical transmission of infections during pregnancy can have deleterious consequences for the developing fetus. Focused prenatal screening and appropriate treatment of the pregnant woman or the neonate is of paramount importance in the struggle for the elimination of congenital infections. Exact knowledge of the seroprevalence of the infectious agents, with special attention to specific high-risk groups, such as immigrants, is necessary for proper public health planning. In the present study, we estimated the seroprevalence for HBV, HCV, HIV, syphilis, *Toxoplasma gondii* infection, CMV, and rubella in a cohort of pregnant woman who gave birth in the three major public hospitals of Crete during 2017. Differences between Greek women and women of foreign origin were found in most of the cases. Specifically, for HBV, HCV, syphilis and rubella, seroprevalence in Greeks was comparable to that found in industrialized countries, while women of east European origin mainly, and Roma, show a much higher prevalence of these infections. Women were properly screened in the majority of cases against HBVand HIV, but syphilis screening was omitted in >50% of cases. Screening for HCV, Toxoplasma and CMV was performed in almost all the women, opposed to more stringent guidelines present in Western countries. No congenital CMV or Toxoplasma infections were verified in the whole study period, a fact that challenges the decision planning for universal screening of all pregnant women, due to the high cost. It might be more prudent to spend money on educating pregnant women on prevention of *Toxoplasma gondii* and CMV infection, by avoiding consumption of raw meet, improperly washed vegetables, cat exposure and caution in dealing with toddlers' urine and saliva respectively, than performing monthly reevaluations of womens' seropositivity.

The results of this study could be generalized to the whole adult population in Greece, since pregnant women consist a healthy part of each country's population. Seroprevalence of all the infectious diseases studied reflects the prevalence of these diseases in the society and can help formulate public health prevention programs. It is also a ground on which perinatal specialists can discuss on topics that are still under debate, like universal or only high-risk screening of pregnant women, and accordingly shape national guidelines for Greece.

Sponsoring

This study was done without sponsoring from any source.

Approvals

The ethics committee of each participating hospital approved this study, and the directors of each obstetrics clinic had also consented, prior to the beginning of the study.

Confidentiality

Personal data gathered from all the participating women were reviewed only by the main researcher and were in no way accessible by others.

Acknowledgments

I would like to thank Professor of Gynecology and Obstetrics of the University of Crete and Director of the Gynecology and Obstetrics Clinic of the University Hospital of Crete, A.Makrygiannakis, for his consent to this study and permission to use the data of his patients. I would also like to thankProfessor of Gynecology and Obstetrics of the University of Crete and Director of the Gynecology and Obstetrics Clinic of the Venizeleio General Hospital of Heraklion, I.Matalliotakis, for his approval and help in gathering data from the patients of his clinic. I am also very grateful to G. Daskalakis, Director of the Gynecology and Obstetrics Clinic of the General Hospital of Chania. I would like to thank separately, Dr.EmmanouilKaravitakis, Pediatrician at the Neonatal Clinic of the General Hospital of Chania for his immense help in gathering data from the Chania Hospital. Moreover, I am grateful to all the staff (midwives and doctors) of the three obstetric clinics for their patience and help, during all the time I spent running through the medical records.

References

1. WHO Executive Summary. Global Hepatitis Report, 2017
2. Ma L, Alla NR, et al. Mother-to-child transmission of HBV: review of current clinical management and prevention strategies. Rev. Med. Virol. 2014; 24: 396–406
3. Dionne-Odom J, Alan TN, et al. Society for Maternal-Fetal Medicine (SMFM): Hepatitis B in pregnancy screening, treatment, and prevention of vertical transmission. Am J ObstetGynecol 2016;214(1):6-14
4. Red Book 2015. American Academy of Pediatrics. 30th Ed. Chapter 3, Hepatitis B, p.400
5. Chen P.R., Huang Y., et al. Management of mother-to-child transmission of hepatitis B virus: Propositions and challenges. Journal of Clinical Virology 2016; 77:32-39
6. Papaevangelou V., Hadjichristodoulou C., et al. Adherence to the screening program for HBV infection in pregnant women delivering in Greece. BMC Infectious Diseases 2006;6: 84
7. Papaevangelou G, Farmaki G, et al. Hepatitis B maternal-fetal transmission in Southern Europe.Intervirology 1998; 41:197-200
8. Neu N, Duchon J, et al. TORCH infections. Clin Perinatol 2015;42:77-103
9. Triantos C, Konstantakis C, et al. Epidemiology of hepatitis C in Greece. World J Gastroenterol 2016;22(36):8094-102
10. Red Book 2015. American Academy of Pediatrics. 30th Ed. Chapter 3, Hepatitis C, p.430
11. Garcia-Tejedor A, Maiques-Montesinos V, et al. Risk factors for vertical transmission of hepatitis C virus: a single center experience with 710 HCV-infected mothers. Eur J ObstetGynecolReprod Biol. 2015;194:173-7
12. Indolfi G, Azzari C, et al. Perinatal transmission of hepatitis C virus.J Pediatr. 2013; 163(6):1549-1552
13. Mast EE, Hwang LY, et al. Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. J Infect Dis. 2005; 192(11):1880-9
14. Syriopoulou V, Nikolopoulou G, et al. Mother to child transmission of hepatitis C virus: rate of infection and risk factors. Scand J Infect Dis. 2005; 37(5):350-3

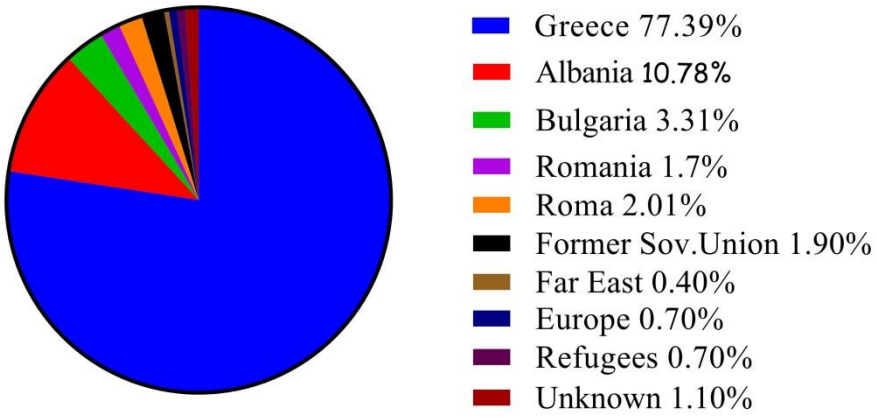
15. Red Book 2015. American Academy of Pediatrics. 30th Ed. Chapter 3, Human Immunodeficiency Virus, p.453
16. World Health Organization. Global guidance on criteria and processes for validation: elimination of mother-to-child transmission (EMTCT) of HIV and syphilis. Geneva: World Health Organization, 2013.
17. Townsend CL, Francis K, et al. Syphilis screening in pregnancy in the United Kingdom, 2010-2011: a national surveillance study. BJOG. 2017; 124(1):79-86
18. Rac MW, Revell PA, et al. Syphilis during pregnancy: a preventable threat to maternal-fetal health. Am J Obstet Gynecol. 2017; 216(4):352-363
19. Red Book 2015. American Academy of Pediatrics. 30th Ed. Chapter 3, Syphilis, p.775
20. American College of Obstetricians and Gynecologists. Practice bulletin no. 151: Cytomegalovirus, parvovirus B19, varicella zoster, and toxoplasmosis in pregnancy. Obstet Gynecol. 2015;125(6):1510
21. European Centre for Disease Prevention and Control. Annual Epidemiological Report 2016 –Toxoplasmosis.Stockholm: ECDC; 2016
22. Thiébaut R, Leproust S, et al. SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group1. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. Lancet. 2007;369(9556):115-22.
23. Red Book 2015. American Academy of Pediatrics. 30th Ed. Chapter 3, Toxoplasma gondii infection, p.787
24. Walker SP, Palma-Dias R, et al. Cytomegalovirus in pregnancy: to screen or not to screen. BMC Pregnancy Childbirth 2013;13:96
25. Cannon MJ, Schmid DS, et al. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. RevMed Virol. 2010; 20(4):202-13.
26. Bodéus M, Zech F, et al. Human cytomegalovirus in utero transmission: Follow-up of 524 maternal seroconversions. J ClinVirol 2010;47:201–202
27. Enders G, Daiminger A, et al: Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational age. J ClinVirol 2011; 52(3):244–246
28. Saldan A, Forner G, et al. Testing for Cytomegalovirus in Pregnancy. J ClinMicrobiol. 2017; 55(3): 693–702

29. Nigro G, Adler SP, et al: Congenital Cytomegalovirus Collaborating Group. Passive immunization during pregnancy for congenital cytomegalovirus infection. N Engl J Med 2005; 353(13):1350–1362
30. Revello MG, Lazzarotto T, et al. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus.N Engl J Med. 2014; 370(14):1316-26
31. de Vries JJ, van Zwet EW, et al. The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: a population-based prediction model. Rev Med Virol. 2013;23(4):241-9.
32. WHO: Report on Rubella, Nov.2017
33. White SJ, Boldt KL, et al. Measles, mumps, and rubella.ClinObstet Gynecol. 2012; 55(2):550-
34. Grant GB, Reef SE, et al. Progress in Rubella and Congenital Rubella Syndrome Control and Elimination - Worldwide, 2000-2016. MMWR Morb Mortal Wkly Rep. 2017;66(45):1256-1260
35. Red Book 2015. American Academy of Pediatrics. 30th Ed. Chapter 3, Rubella, p.730
36. Papaevangelou G, Farmaki G, et al.Hepatitis B maternal-fetal transmission in Southern Europe. Intervirology 1998; 41:197-200
37. Karatapanis S, Skorda L, et al. Higher rates of chronic hepatitis B infection and low vaccination-induced protection rates among parturients escaping HBsAg prenatal testing in Greece: a 2-year prospective study. EJGH 2012;24:878-883
38. ElefsiniotisI.S. et al. Serological and virological profile of chronic HBV infected women at reproductive age in Greece. European Journal of Obstetrics & Gynecology and Reproductive Biology 2007; 132: 200–203
39. Resuli B, Prifti S, et al. Epidemiology of hepatitis B virus infection in Albania. World J Gastroenterol 2009;15(7):849-852
40. Malamitsi-Puchner A, Papacharitonos S, et al. Prevalence study of different hepatitis markers among pregnant Albanian refugees in Greece.Eur J Epidemiol 1996; 12:297-301
41. TsankovaG.S., KostadinovaT., et al. Seroprevalence of hepatitis B among pregnant women in Varna Region, Bulgaria. J Med Virol. 2016;88(11):2012-5
42. Gheorghe L, Csiki IE, et al. The prevalence and risk factors of hepatitis B virus infection in an adult population in Romania: a nationwide survey. Eur J GastroenterolHepatol. 2013;25(1):56-64.

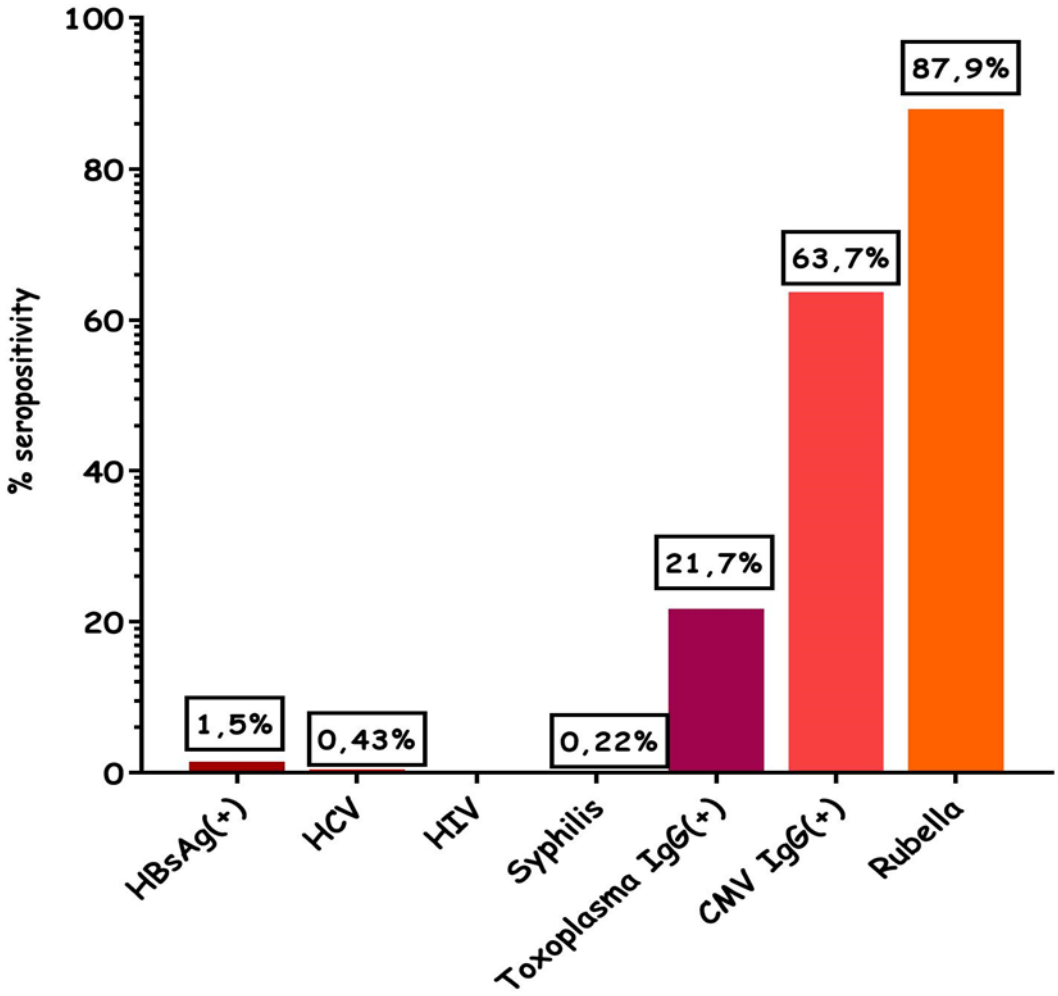
43. Michos A, Terzidis A, et al. Seroprevalence and risk factors for hepatitis A,B and C among Roma and non-Roma children in a deprived area of Athens, Greece. J Med Virol.2008;80(5):791-7
44. Raptopoulou M, Papatheodorakis G, et al. Epidemiology, course and disease burden of chronic hepatitis B virus infection. HEPNET study for chronic hepatitis B: a multicenter Greek study. J Viral Hepat. 2009;16(3):195-202
45. "ΙογενείςΗπατίτιδες." HCDCP, 2012.www2.keelpno.gr/blog/?p=1635
46. Drositis I, Bertsias A, et al. Epidemiology and molecular analysis of hepatitis A, B and C in a semi-urban and rural area of Crete.Eur J Intern Med. 2013; 24(8):839-45.
47. Raptopoulou-Gigi M, Orphanou E, et al. Prevalence of hepatitis C virus infection in a cohort of pregnant women in northern Greece and transmission of HCV from mother to child. Eur J Epidemiol. 2001; 17(3):263-6.
48. Cortina-Borja M, Williams D, et al. Hepatitis C virus seroprevalence in pregnant women delivering live-born infants in North Thames, England in 2012.Epidemiol Infect. 2016;144(3):627-34
49. European Centre for Disease Prevention and Control. Technical missions: HIV, STI and viral hepatitis in Bulgaria. Stockholm: ECDC; 2016
50. Mukomolov S, Trifonova G, et al. Hepatitis C in the Russian Federation: challenges and future directions. Hepat Med. 2016; 8:51-60.
51. Liana G, Speranta J. Prevalence of hepatitis C in Romania: Different from European rates? Journal of Hepatology 2008; 49(4):661–662
52. Michopoulou O, et al. Outcome of neonates born to HIV seropositive mothers in Crete 2000-2013. Poster at the 5th Panhellenic Neonatal Conference, Athens, 2014Perinatal Medicine and Neonatology.2014;9 (1):39
53. Επιδημιολογική επιτήρηση της HIV/AIDS λοίμωξης στην Ελλάδα. Δηλωθέντα στοιχεία έως 31.12.2016. ΚΕΕΛΠΝΟ
54. Furegato M, Fifer H, et al. Factors associated with four atypical cases of congenital syphilis in England, 2016 to 2017: an ecological analysis. Euro Surveill.2017; 22(49).
55. European Centre for Disease Prevention and Control.Congenital syphilis - Annual Epidemiological Report 2016[2014 data]. Stockholm: ECDC; 2016
56. Neghina R, NeghinaAM. Epidemiology of syphilis, gonorrhea and chlamydia in Romania, 1980-2009.J Infect. 2011;63(1):54-9

57. Antona D, Lepoutre A, et al. Seroprevalence of cytomegalovirus infection in France in 2010.Epidemiol Infect. 2017;145(7):1471-1478
58. Lamarre V, Gilbert NL, et al. Seroconversion for cytomegalovirus infection in a cohort of pregnant women in Québec, 2010-2013.Epidemiol Infect. 2016; 144(8):1701-9
59. Wang C, Dollard SC, et al. Cytomegalovirus IgM Seroprevalence among Women of Reproductive Age in the United States.PLoS One 2016;11(3):e0151996
60. Numan O, Vural F, et al. TORCH seroprevalence among patients attending Obstetric Care Clinic of Haydarpasa Training and Research Hospital affiliated to Association of Istanbul Northern Anatolia Public Hospitals. North ClinIstanb. 2015; 2(3):203-209.
61. Uysal A, Taner CE, et al. Cytomegalovirus and rubella seroprevalence in pregnant women in Izmir/Turkey: follow-up and results of pregnancy outcome. Arch Gynecol Obstet. 2012;286 (3):605-8.
62. Wang S, Wang T, et al. Cohort study on maternal cytomegalovirus seroprevalence and prevalence and clinical manifestations of congenital infection in China. Medicine (Baltimore). 2017;96(5):e6007
63. Apostolaki et al. Seroprevalence for Rubella, CMV, and Toxoplasma infection during pregnancy in the Chania area. Poster at the 5th Panhellenic Neonatal Conference 2014, Athens
64. Antona D, Lepoutre A, et al. Seroprevalence of cytomegalovirus infection in France in 2010. Epidemiol Infect. 2017;145(7):1471-1478
65. Pappas G, Roussos N, et al. Toxoplasmosis snapshots: Global status of Toxoplasma gondii seroprevalenceand implications for pregnancy and congenital toxoplasmosis. International Journal for Parasitology 2009;39:1385–1394
66. Antoniou M, Tzouvali H, et al. Incidence of toxoplasmosis in 5532 pregnant women in Crete, Greece: management of 185 cases at risk. Eur J ObstetGynecolReprod Biol. 2004;117(2):138-43
67. Bobić B, Nikolić A, et al. Kinetics of Toxoplasma infection in the Balkans. Wien KlinWochenschr. 2011 Oct;123Suppl 1:2-6
68. Muñoz C, Izquierdo C, et al. Recommendation for prenatal screening for congenital toxoplasmosis. Eur J Clin Microbiol Infect Dis. 2000;19(4):324-5

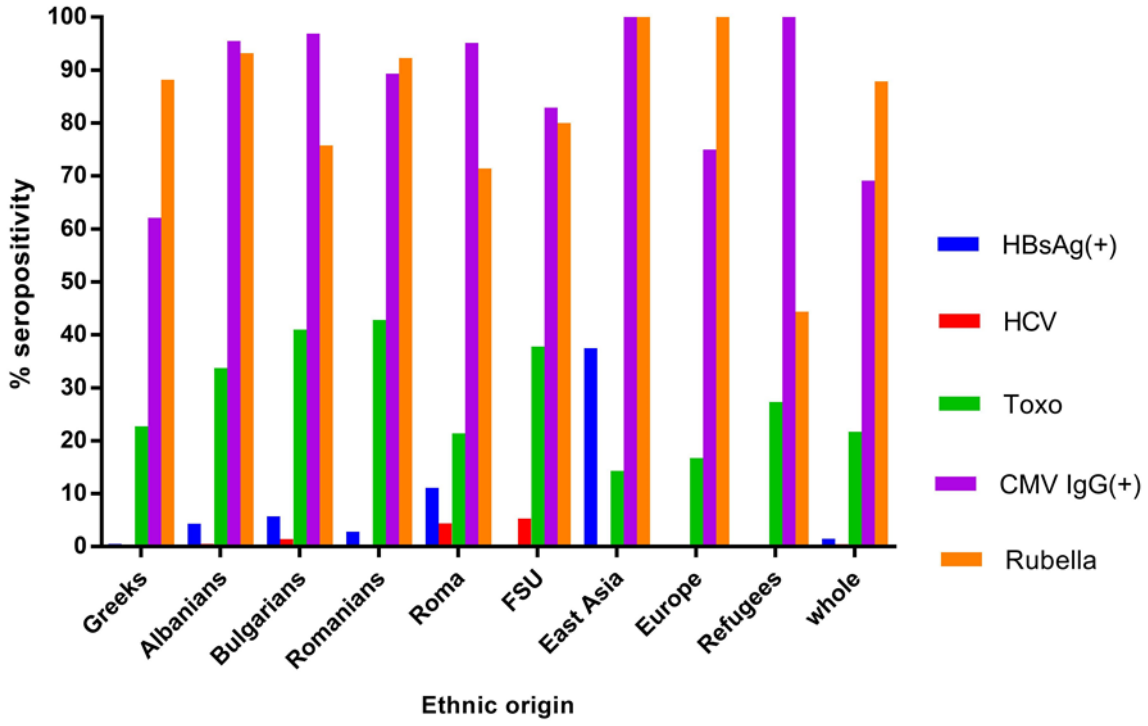
69. Capretti MG, De Angelis M, et al. Toxoplasmosis in pregnancy in an area with low seroprevalence: is prenatal screening still worthwhile? Pediatr Infect Dis J. 2014; 33(1):5-10.
70. Gioula G, Fylaktou A, et al. Rubella immunity and vaccination coverage of the population of northern Greece in 2006.Euro Surveill. 2007;12(11):E9-10
71. Karacan M, Batukan M, et al. Screening cytomegalovirus, rubella and toxoplasma infections in pregnant women with unknown pre-pregnancy serological status. Arch Gynecol Obstet. 2014;290(6):1115-20
72. Vilajeliu A, García-Basteiro AL, et al. Rubella susceptibility in pregnant women and results of a postpartum immunization strategy in Catalonia, Spain. Vaccine 2015;33(15):1767-72
73. Yamada T, Mochizuki J, et al. Effects of campaign for postpartum vaccination on seronegative rate against rubella among Japanese women. BMC Infect Dis. 2014; 14:152
74. Pejcic I, Rankovic JM, et al. Rubella immune status of neonates - a window towards seroprevalence among childbearing women. BMC Public Health. 2016; 16(1):838
75. LeBaron CW, Forghani B, et al. Persistence of rubella antibodies after 2 doses of measles-mumps-rubella vaccine. J Infect Dis. 2009; 200:888–899



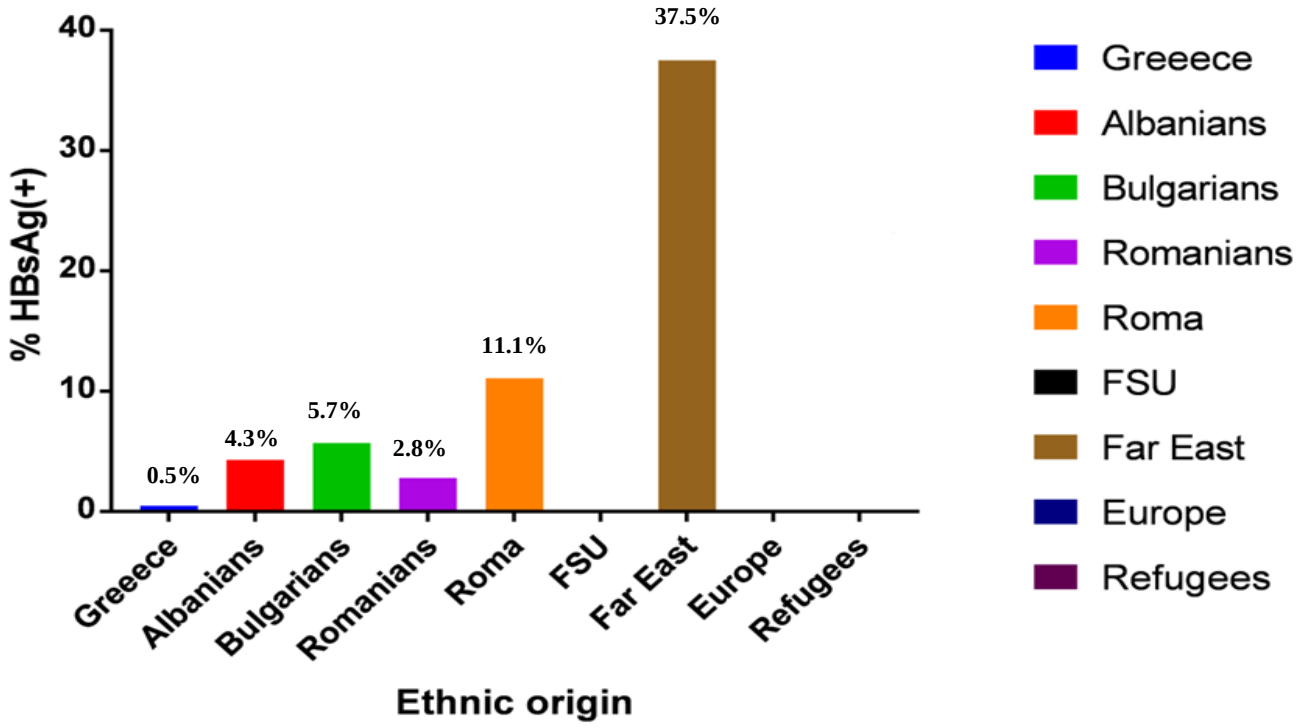
Graph 1. Study population according to nationality (total population N=2,438)



Graph 2. Seroprevalence of HBV, HCV, HIV, Syphilis, Toxoplasma, CMV, and Rubella in the whole population



Graph 3. Seroprevalence of HBV, HCV, Toxoplasma, CMV, and Rubella according to ethnic group



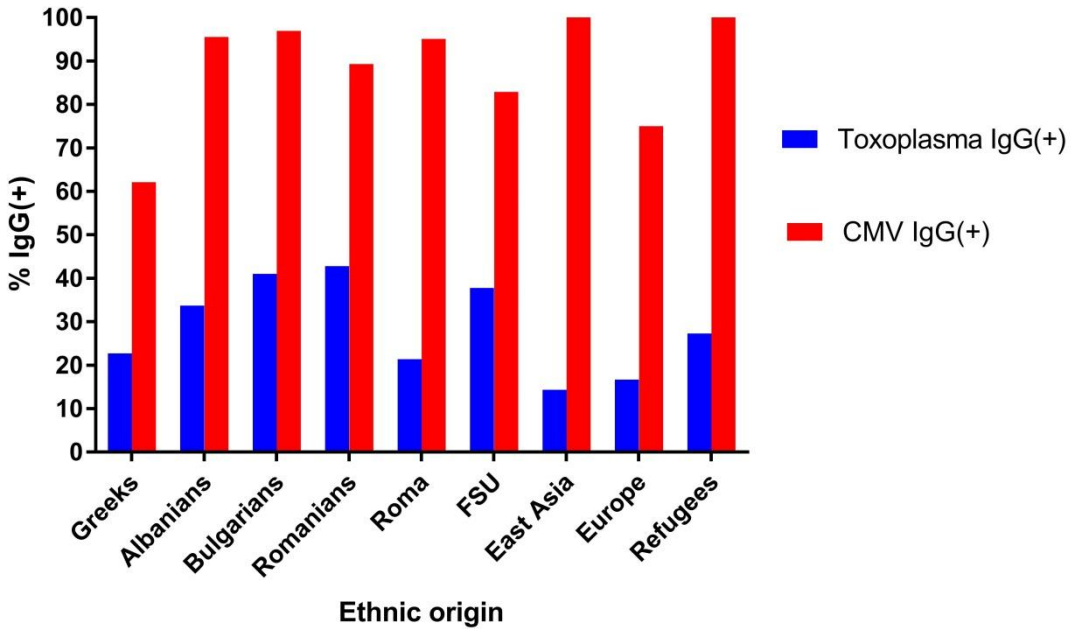
Graph 4.HBsAg(+) seroprevalence according to ethnic group (Total population N= 1,846)

	HBsAg(+)			HCV			HIV			Syphilis		
	N tested	N(+)	Rate % (95% CI)	N tested	N(+)	Rate % (95% CI)	N tested	N(+)	Rate % (95% CI)	N tested	N(+)	Rate % (95% CI)
Greek	1417	7	0.5 (0.2-1.1)	1421	2	0.1 (0.4-0.5)	1416	0	0 (0-0.3)	709	0	0 (0-0.5)
Albanian	188	8	4.3 (2-8.5)	187	1	0.5 (0.09-2.9)	188	0	0 (0-2)	70	0	0 (0-5.2)
Bulgarian	70	4	5.7 (2.2-14)	70	1	1.4 (0.2-7.6)	69	0	0 (0-5.3)	29	0	0 (0-11.7)
Romanian	36	1	2.8 (0.5-14)	36	0	0 (0-9.6)	35	0	0 (0-9.9)	15	2	13.3 (3.7-37.9)
Roma	45	5	11.1 (4.8-23.5)	45	2	4.4 (1.2-14.8)	44	0	0 (0-8)	27	0	0 (0-12.5)
FSU	38	0	0 (0-9.2)	38	2	5.3 (1.4-17.2)	36	0	0 (0-9.6)	16	0	0 (0-19.4)
East Asia	8	3	37.5 (10-74)	5	0	0 (0-43.4)	8	0	0 (0-32.4)	5	0	0 (0-43.5)
Europe	13	0	0 (0-23)	13	0	0 (0-22.8)	12	0	0 (0-24.2)	5	0	0 (0-43.5)
Refugees	10	0	0 (0-28)	10	0	0 (0-27.7)	11	0	0 (0-25.9)	5	0	0 (0-43.5)
Whole population	1846	28	1.5 (1-2.2)	1851	8	0.43 (0.2-0.9)	1842	0	0 (0-0.26)	894	2	0.22 (0.06-0.8)

Table 1. Seroprevalence of HBV, HCV, HIV, and syphilis for the study population and each nationality separately

	Toxoplasma				CMV					Rubella		
	N tested	N(+)	Rate % (95% CI)	seroconverion	N tested	N(+)	Rate % (95% CI)	seroconverion	Rate of seroconversion	N tested	N(+)	Rate % (95% CI)
Greek	1407	260	22.7 (20.3-25.2)	4	1385	860	62.1 (59.9-64.6)	18/525	3.4	1335	1178	88.2 (86.4-89.9)
Albanian	178	60	33.7 (27.7-40.9)	1	178	170	95.5 (91.4-97.7)	3/8	33.3	177	165	93.2 (88.5-96.1)
Bulgarian	66	25	41 (29.5-53.5)	0	65	63	96.9 (89.5-99.2)	0/2	0	177	165	93.2 (88.5-96.1)
Romanian	28	12	42.8 (26.5-60.9)	0	28	25	89.3 (72.8-96.3)	2/3	33.3	26	24	92.3 (75.9-97.9)
Roma	42	9	21.4 (11.7-35.9)	0	41	39	95.1 (84-99)	0/2	0	42	30	71.4 (56.4-82.8)
FSU	37	14	37.8 (24.1-53.9)	0	35	29	82.9 (67.3-91.9)	2/6	33.3	35	28	80 (64.1-90
East Asia	7	1	14.3 (2.6-51.3)	0	7	7	100 (64.6-100)	0/0	0	7	7	100 (64.6-100)
Europe	12	2	16.7 (4.7-44.8)	0	12	9	75 (46.8-91.1)	1/3	33.3	12	12	100 (76-100)
Refugees	11	3	27.3 (9.7-56.6)	0	11	11	100 (74-100)	0/0	0	9	4	44.4 (18.9-73.3)
Whole population	1808	393	21.7 (19.9-23.7)	5	1783	1232	69.1 (66.9-71.2)	26/549	4.7	1726	1517	87.9 (86.3-89.3)

Table 2. Seroprevalence of Toxoplasma, CMV, and Rubella for the study population and each nationality separately



Graph 5. Toxoplasma and CMV seropositivity according to ethnic group

Age	Toxoplasma IgG (+) N(number)	Toxoplasma IgG (-) N(number)	Rate % (95% CI)	Total number
< 20	7	55	**11.3** (5.6-21.5)	62
20-29	135	591	**18.6**(16-21.6)	724
30-39	194	619	**23.9 (** 21.1-26.9)	813
>40	32	69	**31.7**(23.4-41.3)	101

Table 3. Toxoplasma seroprevalence according to age stratification