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Title: Estimating the Burden of Rubella Virus Infection and Congenital Rubella Syndrome through a Rubella Immunity Assessment among Pregnant Women in the Democratic Republic of Congo: Potential Impact on Vaccination Policy

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Keywords: rubella; rubella antibody seroprevalence; rubella serosurvey; rubella IgG; rubella incidence; rubella transmission; Africa; Democratic Republic of the Congo; pregnant women; antenatal; congenital rubella syndrome

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Abstract: Rubella-containing vaccines (RCV) are not yet part of the Democratic Republic of the Congo's (DRC) vaccination program; however RCV introduction is planned before 2020. Because documentation of DRC's historical burden of rubella virus infection and congenital rubella syndrome (CRS) has been minimal, estimates of the burden of rubella virus infection and of CRS would help inform the country's strategy for RCV introduction.

A rubella antibody seroprevalence assessment was conducted using serum collected during 2008-2009 from 1,605 pregnant women aged 15-46 years attending 7 antenatal care sites in 3 of DRC's provinces. Estimates of age- and site-specific rubella antibody seroprevalence, population, and fertility rates were used in catalytic models to estimate the incidence of CRS per 100,000 live births and the number of CRS cases born in 2013 in DRC.

Overall 84% (95% CI 82, 86) of the women tested were estimated to be rubella antibody seropositive. The association between age and estimated antibody seroprevalence, adjusting for study site, was not significant (p=0.10). Differences in overall estimated seroprevalence by study site were observed indicating variation by geographical area (p<=0.03 for all). Estimated seroprevalence was similar for women declaring residence in urban (84%) versus rural (83%) settings (p=0.67). In 2013 for DRC nationally, the estimated incidence of CRS was 69/100,000 live births (95% CI 0, 186), corresponding to 2886 infants (95% CI 342, 6395) born with CRS.

In the 3 provinces, rubella virus transmission is endemic, and most viral exposure and seroconversion occurs before age 15 years. However, approximately 10%-20% of the women were susceptible to rubella virus

infection and thus at risk for having an infant with CRS. Per World Health Organization recommendations, introduction of RCV should be accompanied by a campaign targeting children 9 months to 14 years and vaccination of women of child bearing age through routine services.

Cover Letter

Global Immunization Division,
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21 October 2016

The Editor
Vaccine

Dear Sir or Madam:

On behalf of my co-authors, we thank *Vaccine* for the reviewers' comments received regarding the manuscript, "Estimating the Burden of Rubella Virus Infection and Congenital Rubella Syndrome through a Rubella Immunity Assessment among Pregnant Women in the Democratic Republic of Congo: Potential Impact on Vaccination Policy". We would also like to thank *Vaccine* for providing us with 2 additional weeks to prepare our revisions.

Our responses to the reviewers are contained in another document being submitted today.

Thank you for considering a revised version of the manuscript that is being submitted with this letter. Please feel free to contact me if you have any questions.

Sincerely,
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On behalf of my co-authors, we thank *Vaccine* for the reviewers' comments received regarding the manuscript, "Estimating the Burden of Rubella Virus Infection and Congenital Rubella Syndrome through a Rubella Immunity Assessment among Pregnant Women in the Democratic Republic of Congo: Potential Impact on Vaccination Policy". We would also like to thank *Vaccine* for providing us with 2 additional weeks to prepare our revisions.

Please find below our responses (in italics in blue below each reviewer's comments) to the comments and the details describing how the manuscript was revised to address each.

Reviewer #1: This manuscript provides estimates of Congenital Rubella Syndrome (CRS) for the Democratic Republic of Congo (DRC) using seroprevalence data obtained from existing blood specimens from antenatal clinics. It provides an idea of the occurrence of CRS in a country with little rubella data and that has not yet introduced a rubella-containing vaccine, thus shedding light on an issue that is relevant when discussing vaccine introduction and vaccination strategies. The paper is well written and the supplemental material useful to understand the models used.

Specific comments:

Abstract

1. What % of the population live in the selected provinces

Additional details regarding a number of elements of DRC's population have been incorporated into the Methods section of the manuscript (Please see lines 193-199, lines 206-211, and lines 252-257).

2. Clarify that the estimated CRS incidence and cases in 2013 is for all of DRC

The abstract has been revised to read "In 2013 for DRC nationally, the estimated incidence of CRS was 69/100,000 live births (95% CI 0, 186), corresponding to 2886 infants (95% CI 342, 6395) born with CRS."

Introduction

1. Consider changing deafness for hearing impairment

Lines 88-89 have been revised to replace the word "deafness" with "hearing impairment". The sentence now reads "CRS can result in hearing impairment, blindness, congenital heart disease, mental retardation, and/or other manifestations [1-2]."

2. Are the tentative plans for RCV intro in the country multiyear plan? Is this in any document from Gavi, the Vaccine Alliance? What is the source of this assertion?

The comprehensive Multi-Year Plan (cMYP) 2015-2019 prepared by DRC's Ministry of Public Health in November 2014 has, as one of its objectives, the introduction of rubella-containing vaccine via routine vaccination services. This document has been added to the reference list (Reference 10) and is now referenced in the text at the appropriate places.

Methods

1. How were the 7 sites in the 3 provinces selected?

The rubella serosurvey described in the manuscript was conducted using serum available after the conduct of a polio serosurvey in adults in the 7 ANC sites in the 3 provinces [Reference 15]. We hope that lines 128-133 more clearly explain this; a reference to a publication describing the polio serosurvey has been added [Reference 15]. The wording now states "Sera prepared from venous blood collected during 2008-2009, per WHO guidelines from 6,615 pregnant women aged 15-47 years from 7 ANC sites in Bandundu, Kinshasa, and Kasaï Occidental provinces for national HIV sentinel serosurveys in DRC, had been used for a polio serosurvey in adults and were available for additional testing [13-16]."

Details for how the national HIV sentinel ANC serosurvey selected the sites are described in lines 123 –125 in the Methods section [Reference 16].

2. What % of DRC's population live in those provinces? This could be even presented in the map.

See comments above.

3. Why were the equivocals classified as seronegative? This is exactly the opposite from what the same authors did for the PLoS 2016 paper by Vynnycky et al. It is a small number of samples, but it is curious.

The reviewer notes that "it is a small number of samples, but it is curious". Yes, that is correct. Only 14 of the women fell into the equivocal category. The 14 were distributed among 3 study sites among women whose ages are listed in parentheses after the study site name in the list that follows: Mikalayi (n=6, ages =17, 19, 22, 23, 37, 38), Vanga (n=1, age =34), Tshikapa (n=4, ages =17, 29, 34, 35) and Kinshasa (n=3, ages =17, 17, 18). These details have been added to the Methods section in lines 167-169.

The reviewer is correct about the classification of the equivocals in the 2016 PLoS article by Vynnycky et al. To address this we have conducted the analyses in both ways and have added the results with the equivocals classified as seropositive to the article supplement. Please see lines 251-257, 327-329 and the supplement Table S3.

4. Clarify in this section how the DRC country estimate for CRS is obtained. It is more apparent after seeing table 2 and the supplemental material, but it would be better if it were better explained in the main Methods section.

We are pleased that the reviewer found the supplemental materials of use in understanding the methodology behind the DRC country estimate for CRS. We hesitate to add more explanation of the methods to the main manuscript as we are already above Vaccine's word limit. In addition, we think that it would make the text very heavy. If the Editor feels differently, we proposing adding the entire supplement text to the main manuscript to provide the requested detail.

5. Table 2. Please confirm the 95% CI for estimated number of CRS cases in Kinshasa.

In reviewing our analyses for this manuscript revision, we realized that we needed to account for the differing population estimates for DRC that are found in United Nations documents versus those available from the Expanded Programme On Immunization-Ministry of Public Health. Moreover, we were able to find a source for the age distribution of the female population in urban and rural areas in the 2013/2014 DHS [Reference 17]. Please see lines 190-217 of the Methods section for a complete description. As a consequence, we have new estimates for the numbers of CRS cases born in DRC in 2013 in the various sites and settings, based on the two differing population sources. Please see Tables 2 and S3 for the detailed results.

Discussion
Are the authors proposing that a rubella vaccination policy should include catch-up of WCBA? Given the lack of differences seen in seroprevalence, this has important implications and could be better tackled.

These authors are not proposing catch-up campaigns for WCBA. The authors state that WCBA should be considered for rubella vaccination through routine services. Please see the Conclusion section of the abstract and lines 372-373 of the discussion.

In line 350, the authors indicate that DRC would have to establish nationwide CRS surveillance. Is this always so? Where is it recommended? Would CRS in sentinel sites plus "good" rubella surveillance be adequate to monitor CRS?

The sentence in lines 380-384 has been revised and hopefully will address the concern of Reviewers 1 and 4. The sentence now reads "Therefore, during the years before introducing RCV, DRC will need to a) focus efforts on improving the delivery of measles vaccination, thereby creating a successful platform on which to introduce rubella vaccination; b) establish an integrated nationwide measles-rubella surveillance system as well as, at least, sentinel sites for CRS surveillance; and c) use best practices from measles vaccination campaigns to assure a high-quality rubella wide-age campaign."

The 2013-2014 DHS cited here (ref 15) collected blood and did serology for measles among other pathogens, would that be a possibility to explore in the future to monitor rubella seroprevalence?

We appreciate the reviewer's comments regarding the serological studies conducted in DRC as part of the 2013-2014 DHS. We agree that such studies are a possibility for monitoring rubella seroprevalence. Unfortunately, the results of those serological studies conducted as part of the 2013-2014 DHS have not yet been published; thus, we could not include them as part of our discussion.

References
Ref 18. The 2015 revision is already available. Not to change anything, but just for information.

The authors thank the reviewer for this information.

Reviewer #3: This paper adds important information regarding the epidemiology of rubella in Africa, specifically in one of its largest countries, DRC. The information allows for better vaccination strategies. The strength of the paper is the large number of women tested for rubella antibodies, revealing substantial susceptibility to rubella, similar to the US before introduction of vaccination. It also shows that rubella circulation is mainly in children less than 15 years, who therefore must be the source of infection during pregnancy. The estimate made of congenital rubella cases suggests that CRS is an important public health problem in DRC. However, the paper has several weaknesses that should be addressed: the use of the ELISA test, which usually underestimates

protection, and in particular the estimate of CRS incidence, which is based on calculations of force of infection, not surveillance. The very wide confidence limits testify to the limitations of the estimate.

The reviewer is correct that a small percentage of individuals who are immune to rubella do not have ELISA titers indicating an IU/ml level above 10. However, in a population not selected for low IgG titers, this is a very small percentage of individuals who have been vaccinated or have had wild type rubella. For example, in a vaccination study in toddlers in Bangladesh, less than 0.5% did not respond with =>10 IU/ml after a single dose of RCV[*]. On the other hand, in a population selected for a low response, immunity determinations can be difficult[$].

For the population in question here, the small percentage of immune persons who do not have 10 IU/ml should not affect the results. A sentence has been added to the Methods section to reflect this; please see lines 169-170.

**J Infect Dis. 2016 Jun 1;213(11):1686-93. doi: 10.1093/infdis/jiw024. Epub 2016 Jan 27. Noninterference of Rotavirus Vaccine With Measles-Rubella Vaccine at 9 Months of Age and Improvements in Antirotavirus Immunity: A Randomized Trial.*

$J Clin Microbiol. 2016 Jul;54(7):1720-5. doi: 10.1128/JCM.00383-16. Epub 2016 May 4. Assessing Immunity to Rubella Virus: a Plea for Standardization of IgG (Immuno)assays.

Since there is currently no surveillance for CRS in DRC, CRS surveillance data cannot be used to estimate CRS incidence in DRC at this time. This is unfortunate. However, rubella antibody seroprevalence data were available and were paired with an established mathematical modeling methodology, which has been used to estimate the global burden of Congenital Rubella Syndrome. These authors acknowledge in Discussion lines 413-418 the wide confidence intervals, often approaching zero, associated with the CRS incidence and case number estimates. Similarly wide confidence intervals, with the lower limit approaching zero, have been found for estimates of the CRS incidence and burden for other countries [Reference 24]. However, we note that while the lower 95% confidence limit approaches zero for the numbers of cases of CRS in different parts of DRC, it does not approach zero for the national estimate of the burden of CRS. Our work therefore suggests that there is potentially a non-negligible burden of CRS, which should be investigated in further studies. The study is also the largest study of rubella seroprevalence in DRC to date and therefore provides a reference point for researchers wishing to estimate the burden of CRS in DRC in the future.

Reviewer #4: Congratulations to the authors for preparing a well written summary of an investigation using existing blood serum collected in 2008-2009 to test for HIV from women attending antenatal care clinics in the Democratic Republic of Congo. A sample of the stored sera was selected from seven clinics (three from Kinshasa) to estimate the percent of pregnant women with rubella-specific antibodies. The article contributes to knowledge about rubella immunity prior to introduction of rubella containing vaccine in a national immunization program.

I have the following specific comments:

1. Abstract results, lines 68-70: I'm not sure that I understand the rationale for showing a statistically significant result between one province when compared to four of five of the other sites. Why not include all sites? Alternatively, maybe it would be better to describe the differences of urban vs. rural, or Kinshasa vs other sites.

The results section of the abstract has been revised; please see the text in the following paragraph. The results regarding the differences in the various geographical areas are now presented in more general terms, and the results for rural versus urban settings have been added.

*"*Overall 84% (95% CI 82, 86) of the women tested were estimated to be rubella antibody seropositive. The association between age and estimated antibody seroprevalence, adjusting for study site, was not significant (p=0.10). Differences in overall estimated seroprevalence by study site were observed indicating variation by geographical area (p<=0.03 for all). Estimated seroprevalence was similar for women declaring residence in urban (84%) versus rural (83%) settings (p=0.67). In 2013 for DRC nationally, the estimated incidence of CRS was 69/100,000 live births (95% CI 0, 186), corresponding to 2886 infants (95% CI 342, 6395) born with CRS".

2. Abstract conclusions, lines 77-78: The recommendation to target women of child bearing age is not well described. I would suggest the recommendation be to conduct a wide age range campaign targeting all children 9 months to 14 years as well as vaccinating women of child bearing age through routine immunization services as described in lines 338-341.

The concluding statement of the abstract has been revised as suggested by the reviewer. The concluding statement now reads as follows: "Per World Health Organization recommendations, introduction of RCV should be accompanied by a campaign targeting all children 9 months to 14 years of age as well as vaccination of women of child bearing age through routine services."

3. Line 111: In my experience, ANC is usually used as the abbreviation for antenatal care rather than antenatal clinics.

Yes, the reviewer is correct. We appreciate the reviewer bringing this to our attention. The wording has been revised throughout the manuscript.

4. Line 186-187: Is there any estimates for the age distribution of women in rural areas vs urban areas? If not, then there is no other choice but to use national estimates, but it seems that rural vs urban may have very different distributions depending on migration patterns.

We appreciate this question which prompted us to look again for such data. We found that the 2013/2014 DRC Demographic and Health Survey [Reference 17] had an estimate of the age distribution of women in rural versus urban areas. The analyses were rerun using the respective age distributions, and the revised results are presented in Table 2 in the main manuscript and in Table S3 of the supplement.

5. Line 194: This sentence states "the remaining were considered rural". Since the remaining are only two sites, wouldn't it be more clear to state "the remaining two sites" or better yet list the names of the sites considered rural?

The sentence in lines 204-205 has been revised according to the reviewer's suggestion. It now reads "In these analyses, the Kinshasa, Kikwit and Tshikapa study sites were considered urban, and the Mikalayi and Vanga sites were considered rural [13, 14, 17]*."*

6. Lines 249-250: I would suggest to include the percentages of women in each of the population groups described.

The authors appreciate the reviewer's suggestion. We would prefer to leave those details out of the body of the text since their inclusion could render the text heavy and hard to read. All details are available in Table 1, and readers are guided to Table 1 in lines 274-275.

7. Lines 269-271: The sentence may imply that the results of statistical tests were displayed in Table 1. I would suggest to re-phrase as ". . . and the demographic variable categories described in Table 1 were observed."

The sentence in lines 295-297 has been revised according to the reviewer's suggestion. The sentence now reads "Moreover, no statistically significant associations between estimated rubella antibody seroprevalence and the demographic variable categories described in Table 1 were observed."

8. Lines 350-351: The surveillance recommendation to DRC seems too ambitious before 2020. I would suggest to re-phrase as "b) establish an integrated nationwide measles-rubella surveillance system as well as at least sentinel sites for CRS surveillance;"

The sentence in lines 380-384 has been revised according to the reviewer's suggestion. The sentence now reads "Therefore, during the years before introducing RCV, DRC will need to a) focus efforts on improving the delivery of measles vaccination, thereby creating a successful platform on which to introduce rubella vaccination; b) establish an integrated nationwide measles-rubella surveillance system as well as, at least, sentinel sites for CRS surveillance; and c) use best practices from measles vaccination campaigns to assure a high-quality rubella wide-age campaign."

9. Line 361: could "poorly fitted" be re-phrased as "was a poor fit for" the Kinshasa rubella antibody data?

The sentence in Lines 393-394 has been revised to read as follows: "We note that the selected catalytic model used to estimate the force of infection was a poor fit to the Kinshasa rubella antibody seroprevalence data."

Thank you for considering a revised version of the manuscript that is being submitted with this letter. Please feel free to contact me if you have any questions.

Sincerely,
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Estimating the Burden of Rubella Virus Infection and Congenital Rubella Syndrome through a Rubella Immunity Assessment among Pregnant Women in the Democratic Republic of Congo: Potential Impact on Vaccination Policy

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Conflict of Interest Statement: The authors declare no conflict of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the United States Centers for Disease Control and Prevention.

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Authors' Contributions: MMA, KAW, LP, JI, FF, SE, AM, KS, and SR designed the study, prepared the protocol, and played a role in acquiring the serum samples and other necessary data. LH, LP, and JI conducted the laboratory-based serological assays. MMA, KAW, and SR analyzed the rubella antibody seroprevalence data, including the associated statistical analyses. EV analyzed the data to obtain estimates for the force of rubella virus infection, the incidence of congenital rubella syndrome (CRS) in 2013 and the number of infants born with CRS in 2013 in the Democratic Republic of the Congo. All authors were involved in interpreting the data and in drafting the manuscript; moreover, all authors approved the manuscript's final version.

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ABSTRACT

Background: Rubella-containing vaccines (RCV) are not yet part of the Democratic Republic of the Congo's (DRC) vaccination program; however RCV introduction is planned before 2020. Because documentation of DRC's historical burden of rubella virus infection and congenital rubella syndrome (CRS) has been minimal, estimates of the burden of rubella virus infection and of CRS would help inform the country's strategy for RCV introduction.

Methods: A rubella antibody seroprevalence assessment was conducted using serum collected during 2008-2009 from 1,605 pregnant women aged 15-46 years attending 7 antenatal care sites in 3 of DRC's provinces. Estimates of age- and site-specific rubella antibody seroprevalence, population, and fertility rates were used in catalytic models to estimate the incidence of CRS per 100,000 live births and the number of CRS cases born in 2013 in DRC.

Results: Overall 84% (95% CI 82, 86) of the women tested were estimated to be rubella antibody seropositive. The association between age and estimated antibody seroprevalence, adjusting for study site, was not significant (p=0.10). Differences in overall estimated seroprevalence by study site were observed indicating variation by geographical area (p<=0.03 for all). Estimated seroprevalence was similar for women declaring residence in urban (84%) versus rural (83%) settings (p=0.67). In 2013 for DRC nationally, the estimated incidence of CRS was 69/100,000 live births (95% CI 0, 186), corresponding to 2886 infants (95% CI 342, 6395) born with CRS.

Conclusions: In the 3 provinces, rubella virus transmission is endemic, and most viral exposure and seroconversion occurs before age 15 years. However, approximately 10%-20% of the women were susceptible to rubella virus infection and thus at risk for having an infant with CRS. This analysis can guide plans for introduction of RCV in DRC. Per World Health Organization recommendations, introduction of RCV should be

79 accompanied by a campaign targeting all children 9 months to 14 years of age as well as vaccination of women

80 of child bearing age through routine services.

81

INTRODUCTION

Rubella is a vaccine-preventable disease with safe and effective vaccines available since 1969. In the absence of vaccination, infection with the rubella virus usually occurs in childhood and causes a mild, self-limited illness characterized by rash and fever. However, if rubella virus infection occurs in a susceptible woman during the first trimester of pregnancy, miscarriage, fetal death, or congenital rubella syndrome (CRS) in the surviving infant often occurs. CRS can result in hearing impairment, blindness, congenital heart disease, mental retardation, and/or other manifestations [1-2].

A single dose of the most common rubella vaccine, RA27/3, is highly efficacious in providing lifelong protection against disease. Prevention of congenital rubella virus infection, including CRS, is the primary goal of rubella vaccination. The preferred approach for prevention of rubella and CRS is for countries to introduce a rubella-containing vaccine (RCV) through a wide-age range campaign and then incorporate it into the national childhood vaccination schedule [2].

In recent years, several World Health Organization (WHO) regions have established rubella/CRS elimination or accelerated control goals [2-6]. In 2003 the WHO region of the Americas set a rubella/CRS elimination goal, achieved the goal in 2009, and in April 2015, was declared free of endemic rubella and CRS [2-5, 7]. The WHO European region set a 2015 rubella elimination goal [3, 4, 8]. In October 2014, a regional rubella elimination goal for the WHO Western Pacific Region was endorsed by its Regional Committee [6]. The WHO African region has not yet established a rubella elimination goal but recommends that countries document the burden of rubella virus infection/CRS and, when feasible, introduce RCVs [9].

RCVs have not been widely administered in the Democratic Republic of the Congo (DRC) nor introduced into the country's national vaccination program [10]. However, there are tentative plans for introduction into the

childhood vaccination schedule before 2020 [10]. Documentation of DRC's historical burden of rubella virus infection and CRS has been minimal [1, 11, 12]. Moreover, DRC has no surveillance system for either disease, but rubella virus transmission within the country has been documented by a rubella antibody seroprevalence assessment conducted in Kinshasa city in 1987-1988 and by measles case-based surveillance since 2005, with serological testing for rubella-specific immunoglobulin type M (IgM) when suspected measles cases are negative for measles IgM [1, 11, 12]. Considering the interest in rubella control/elimination in the WHO African region, estimates of the burden of rubella virus infection/CRS in DRC are urgently needed [8, 9]. We describe analyses of sera from 1,605 pregnant women aged 15-46 years from 3 provinces in DRC which were available from a human immunodeficiency virus (HIV) sentinel survey among women attending antenatal care (ANC) sites [13-15]. Estimates of age- and site-specific rubella antibody seroprevalence, population, and fertility rates were used in catalytic models to estimate the incidence of CRS per 100,000 live births and the number of CRS cases born in 2013 in DRC. These estimates will be valuable to DRC's Ministry of Public Health (MOPH) in planning for RCV introduction [10].

METHODS

Rubella antibody seroprevalence assessment

HIV sentinel surveys among pregnant women attending ANC sites are based on a convenience sample of sentinel sites chosen to capture women from a variety of geographical and socioeconomic backgrounds. Details on how sites are selected can be found here [16]. The 2008-2009 HIV sentinel surveys in DRC included 30 sentinel ANC sites [13, 14]. This study focuses on a subset of 7 ANC sites in 3 provinces.

Sera prepared from venous blood collected during 2008-2009, per WHO guidelines from 6,615 pregnant women aged 15-47 years from 7 ANC sites in Bandundu, Kinshasa, and Kasaï Occidental provinces for national HIV sentinel serosurveys in DRC, had been used for a polio serosurvey in adults and were available for additional testing [13-16]. Specifically, the 7 ANC sites were 1) Kikwit (urban) and 2) Vanga (rural) in Bandundu, 3) Binza-Meteo, 4) Boyambi, and 5) Kingasani (all urban) in Kinshasa, and 6) Mikalayi (rural) and 7) Tshikapa (urban) in Kasaï Occidental (Figure 1). The Demographic and Health Survey II (DHS II) conducted in 2013-2014 in DRC reported that, nationally, 88% of women aged 15-49 years participating in the survey who had a live birth in the 5 years preceding the survey had sought antenatal care during their pregnancy for their most recent live birth; the results were 90%, 89%, and 96% for women declaring residence in Bandundu, Kasai Occidental, and Kinshasa provinces, respectively, and were 94% and 86% for those declaring residence in urban and rural areas, respectively [17]. A survey conducted in 2009 in Kinshasa province among women at least 18 years of age who had been pregnant within the prior 3 years reported that 98% of women surveyed had attended ANC during their most recent pregnancy [18].

Sera from a randomly-sampled subset of the above-mentioned 6,615 women were quantitatively analyzed for rubella-specific immunoglobulin type G (IgG). Prior to random sampling of the women for the rubella antibody serosurvey described in this report, HIV-positive women were excluded since HIV infection may negatively impact serum IgG levels; HIV prevalence in the 7 above-mentioned ANC sites ranged from 1.8% - 5.1% in 2008 -

2009 [13, 14, 19]. Also prior to random sampling for the rubella serosurvey, women attending the 3 ANC sites in the densely populated urban area of Kinshasa city (Binza-Meteo, Boyambi, and Kingasani) were pooled. Kinshasa was thereafter considered a single study site (referred to as the "Kinshasa" study site); thus, there were 5 study sites for the serosurvey (Table 1). From 5,829 HIV-negative women from the original 6,615, 1,650 women (66 serum samples from each of 25 strata, i.e., 5 age groups from each of the 5 study sites) were randomly chosen. The sample size was determined based on the estimation of rubella antibody seroprevalence with a precision of +/-10% assuming true prevalence of >=80% and 5% unusable serum samples. Of the 1,650 sera, 45 (3%) had insufficient volume for IgG assessment: 16, 7, 8, 10, and 4 from the Kikwit, Kinshasa, Mikalayi, Tshikapa, and Vanga study sites, respectively. Demographic attributes (e.g., age at blood collection, age at first pregnancy, number of pregnancies, rural or urban residence, level of education, occupation, and civil status) were analyzed for associations with rubella antibody seropositivity [13, 14].

Sera were shipped by air from DRC to the Centers for Disease Control and Prevention (CDC-Atlanta) on dry ice and stored at -20^{o}C prior to rubella IgG assessments performed at CDC-Atlanta's Measles, Mumps, Rubella, and Herpesvirus Branch laboratory. Rubella-specific IgG antibody concentrations, expressed as International Units/millimeter (IU/ml), were determined using the Rubella IgG ELISA II system according to the manufacturer's instructions (Wampole Laboratories, Princeton, New Jersey). The optical density (OD) ratio was calculated by dividing the specimen OD by the cutoff value supplied by the manufacturer. Specimens with OD ratios >2.2 were diluted with kit dilution buffer, and rubella-specific IgG antibody concentrations were determined from the diluted serum. Sera with titers of >=10 IU/ml were considered seropositive for rubella antibody, whereas those with an equivocal determination (8.19 to 9.99 IU/ml) or with titers of <8.19 IU/ml were considered seronegative [2]. The 14 women with equivocal determination were distributed among 4 study sites as follows: Mikalayi (n=6, ages in years=17, 19, 22, 23, 37, 38), Vanga (n=1, age in years =34), Tshikapa (n=4, ages in years=17, 29, 34, 35), and Kinshasa (n=3, ages in years=17, 17, 18). Immune individuals with ELISA-determined IgG <10 IU/ml should be too small in number to affect the results presented in this report [20].

Site-specific rubella antibody seroprevalence was estimated overall and for each 5-year age group, accounting for the sampling probability in each stratum and treating the equivocals as seronegative. The rubella antibody seroprevalence estimates and associated confidence intervals (CIs) are representative of the study site assessment populations only and not of any DRC populations at large. The Pearson Chi-square test was used to assess differences in rubella antibody seroprevalence overall for the 5 study sites, across 5 age strata (overall and within each site), and across the other demographic attributes (Table 1); when statistically significant differences were observed, pairwise analyses were conducted using the Pearson Chi-square test. The Cochran-Mantel-Haenszel (CMH) Chi-square was used to test for statistically significant associations between rubella antibody seroprevalence and site controlling for age and between rubella antibody seroprevalence and age controlling for site. Tests were considered statistically significant at $p<0.05$.

Estimating CRS incidence and the number of CRS cases born in 2013

The age- and site-specific rubella antibody seroprevalence estimates were used in catalytic models to estimate the rate at which susceptible women were infected with rubella virus (i.e., force of rubella virus infection). The force of infection estimates were then used with estimated populations and fertility rates to obtain the CRS incidence/100,000 live births in 2013 and numbers of CRS cases born in 2013. Details follow.

Demographic data

The total number of women of child-bearing age (WCBA) for 2013 in the zones de santé (health zones) in which the ANC sites were situated were extracted from DRC's Expanded Programme on Immunization (EPI)-MOPH population projections based on the 1984 census (the only official census ever conducted at the zone de santé level); health zones in DRC are the equivalent of districts in other countries [17]. Based upon those EPI-MOPH projections, in 2013 DRC's estimated total population was 86,508,633, and the estimated total population of Bandundu, Kinshasa, and Kasaï Occidental provinces was 8,350,279, 8,103,633, and 8,252,695, respectively;

196 estimates indicate that in 2013 the population of WCBA in each province was 21% of the province's estimated
197 total population. The 2013 estimated population of WCBA for the health zones in which the 7 ANC sites were
198 located were as follows: Boyambi (32,523), Binza Meteo (85,987), Kikwit (42,713), KIngasani (47,452), Mikalayi
199 (44,715), Tshikapa (73,249), and Vanga (57,273).
200
201 The age distribution of women in urban and rural areas was extracted from the 2013-2014 DRC Demographic
202 and Health Survey [17]. To calculate site-specific numbers of women in a given age group, the total number of
203 WCBA in the corresponding health zone was multiplied by the proportion of WCBA in the age of interest
204 according to whether or not the site was considered urban or rural. In these analyses, the Kinshasa, Kikwit and
205 Tshikapa study sites were considered urban, and the Mikalayi and Vanga sites were considered rural [13, 14, 17].
206 The total female population size (33,976,774 for 2013) and the proportion of DRC's population living in urban
207 and rural settings (35.4% and 64.6% respectively for 2013) were extracted from United Nations (UN) population
208 sources, and the two were multiplied together to obtain the number of females living in urban and rural areas in
209 DRC [20, 21]. These numbers were then scaled up by 28%, to account for a 28% difference between the
210 population size according to UN sources and that in the DRC EPI-MOPH projections for 2013 (67,513,677 vs
211 86,508,633, respectively). The number of women in each five year age group (15-19, 20-24, 25-29, 30-34, 35-39,
212 and 40-44 years) in urban and rural areas was calculated by multiplying the female population size in urban or
213 rural areas by the proportion of the female population in the given area that was in the age group of interest
214 [21]. Age-specific fertility rates for 2013-2014 for urban and rural settings were extracted from the 2013-2014
215 DRC Demographic and Health Survey [17]. The number of live births in each site or setting in DRC among
216 mothers in each 5-year age group was calculated by multiplying the corresponding age-specific fertility rates and
217 numbers of women in the site or area of interest.
218
219 *CRS incidence and CRS case estimations by site*

Following previous methods, four age-structured catalytic models were fitted to the observed age-stratified rubella antibody seroprevalence estimates from the different study-sites using maximum likelihood to estimate the force of rubella virus infection [23-25]. This was assumed to differ (models A and B) or be identical (models C and D) for the ages <15 and ≥15 years [23, 26]. The sensitivity of the rubella serological (antibody) assay was either estimated (models A and C) or assumed to be 100% (models B and D) [23, 26]. Models A-D are described in the article supplement (Table S1). The following equation gives the proportion of individuals of age *a* (*s(a)*) that are seronegative, where *p* is the sensitivity of the serological assay, and $\bar{\lambda}_y$ and $\bar{\lambda}_o$ are the average force of infection among younger and older individuals respectively.

$$s_n(a) = \begin{cases} 1 - p(1 - e^{-\bar{\lambda}_y(a-0.5)}) & a < 15 \, years \\ 1 - p(1 - e^{-14.5\bar{\lambda}_y} e^{-\bar{\lambda}_o(a-15)}) & a \geq 15 \, years \end{cases}$$

Subsequent estimates of the CRS incidence were based on models that were selected according to biological plausibility using criteria described elsewhere, with the additional criterion that model B was selected in preference to model A if all the other criteria were satisfied and the estimated sensitivity of the assay was 100% for model A, and the lower limit of the 95% confidence interval was implausibly low (less than 95%) [24]. If no model provided biologically-plausible estimates or if the model fitted the data from a given site poorly (passed through the confidence intervals of one or fewer datapoints), we excluded those data from estimates for urban or rural areas and from the whole of DRC. The article supplement provides details on the fitting.

For all sites, the best fitting value for the force of rubella virus infection was used to estimate the CRS incidence per 100,000 live births among women in 5 year age groups between 15-44 years using the following expression, where s(A) is the proportion of women in age group A that are susceptible.

$$s(A) \times 0.65 \times (1 - e^{-16\bar{\lambda}_o/52}) \times 100{,}000$$

As in previous analyses, the risk of a child being born with CRS was assumed to be 65% if the mother was infected during the first 16 weeks of pregnancy and zero thereafter [23, 24]. The weighted CRS incidence per 100,000 live births among women aged 15-44 years for each site was calculated as the average of the CRS

incidence per 100,000 live births in each 5 year maternal age group, weighted by the site-specific number of live births in each maternal age group in 2013. The number of CRS cases born in each site was calculated by multiplying the site-specific number of live births occurring in each 5 year maternal age group by the estimated CRS incidence for each site. CIs (95%) for the force of rubella virus infection and CRS incidence for each site and catalytic model were obtained by bootstrapping using 1,000 bootstrap datasets generated using the approach of Shkedy *et al.* [27]. These bootstrap-derived estimates were then used to compile the force of rubella virus infection, weighted CRS incidence per 100,000 live births in urban and rural areas and for the whole of DRC. Additional details are provided in the article supplement (Table S2). In sensitivity analyses and for consistency with previous analyses, we repeated the analyses, treating the equivocals as seropositive. In addition, given the discrepancy between the population size according to UN sources and that of DRC's EPI-MOPH projections for 2013, we calculated the number of CRS cases in urban and rural areas, and overall in DRC obtained by using female population size, as calculated according to UN sources [21, 22, 24]. The site-specific number of CRS cases consistent with the population size based on UN population sources were calculated by scaling down the estimates obtained using population data from DRC's EPI-MOPH projections for 2013 by 28%.

Data Analyses

Data analyses were conducted using SAS version 9.2 (SAS Institute, Cary, North Carolina), EPI-INFO version 7 (CDC, Atlanta, Georgia), and EXCEL version 2010 (Microsoft Corporation, Redmond, Washington). Figure 1 was created using ArcGIS version 10.1 (Environmental Systems Research Institute, Redlands, California). The catalytic modeling analyses were carried out using a program written in the "C" programming language [28]. The fitting used an algorithm based on the simplex method of Nelder and Mead [29].

Ethical Approval

The Human Subjects Research Coordinator of the Center for Global Health, CDC-Atlanta reviewed the protocol for the work described in this report. The work was determined to be research not involving human subjects,

because it involved using unlinked/anonymous specimens collected for another purpose, and was therefore exempt from institutional review board approval. The protocol was reviewed and approved by DRC's MOPH.

RESULTS

Sera from 1,605 HIV-negative, pregnant women, aged 15-46 years, who attended ANC in Bandundu, Kasaï Occidental, and Kinshasa provinces in DRC during 2008–2009, were analyzed for rubella-specific IgG. Relevant demographic attributes of these women are included in Table 1. Overall and at all sites, >80% of women had their first pregnancy before age 24 years, >=50% of women had been pregnant >=3 times, and >90% had been married at some time. The Kinshasa and Kikwit sites had the highest proportion of women having attended secondary school or higher education. In the rural sites (Mikalayi and Vanga), farming was the most common profession, as compared with housekeeping in the urban sites (Kikwit, Kinshasa, and Tshikapa).

Among the 1,605 women overall, 84% (95% CI 82, 86) were estimated to be seropositive for rubella IgG (Figure 2). Within the Kinshasa site, estimated rubella antibody seroprevalence was higher in the 20-24 year age group (89%) than the 15-19 (78%, p value =0.01) and 25-29 (75%, p value <0.001) year age groups. In contrast, no statistically significant trends or differences in estimated rubella antibody seroprevalence among age groups were found in the overall assessment population or within the Kikwit, Vanga, Mikalayi, or Tshikapa sites (Figure 2). The association between age and antibody seroprevalence, adjusting for study site, was not significant (CMH Chi-square p value =0.10).

The association between site and antibody seroprevalence, controlling for age, was statistically significant (CMH Chi-square p value =0.01). In pairwise comparisons, Kikwit (89%) and Vanga (88%) study sites (both in Bandundu province) each had higher estimated overall rubella antibody seroprevalence than the Mikalayi (80%) and Kinshasa (82%) sites (all p values <=0.03).

In the overall assessment population, estimated rubella antibody seroprevalence was similar for women declaring residence in urban (84%) versus rural (83%) settings (p value =0.67). Moreover, no statistically

296 significant associations between estimated rubella antibody seroprevalence and the demographic variable
297 categories described in Table 1 were observed.
298
299 Table 2 summarizes estimates from the selected models for the force of rubella virus infection per 1,000
300 susceptible individuals per year aged <15 and >=15 years, CRS incidence per 100,000 live births for women aged
301 15-44 years in 2013, and the number of CRS cases born in 2013; all estimates are shown by study site, by rural
302 and urban settings, and for DRC overall. The estimates based upon UN population and DRC EPI-MOPH
303 projections total population estimates are presented separately. The best-fitting estimates of the annual force
304 of rubella virus infection, the serological assay sensitivity, and the CRS incidence estimated from each of the 4
305 catalytic models are described in the article supplement. For Kikwit, Vanga, Mikalayi, and Tshikapa, the best-
306 fitting model (Model B) assumed that the force of rubella virus infection was different for persons aged <15
307 years and those aged >=15 years and was estimated, and the sensitivity of the assay was fixed at 100%
308 (Supplement Tables S1 and S2). The best-fitting model for Kinshasa fit the data poorly (Supplement Figure S1
309 and Table S2); thus Kinshasa's antibody seroprevalence data were excluded when generating estimates for
310 urban areas and the whole of DRC.
311
312 With the exception of the Kinshasa site, where the selected best-fit model assumed that the force of rubella
313 virus infection was identical for all age groups, the estimated force of rubella virus infection was higher for <15
314 year olds compared to ≥15 year olds for each site, setting, and the whole of DRC, e.g. 120 per 1000 per year
315 (95% CI 83, 159) vs 24 (95% CI 0, 73), respectively, for urban areas (Table 2). Moreover except for Kinshasa, the
316 forces of rubella virus infection for a given age group did not differ significantly between sites or for rural and
317 urban settings and overall for DRC (Table 2).
318
319 The estimated CRS incidence (CRS cases/100,000 live births) for 2013 ranged from 61 in both Kikwit (95% CI 0,
320 151) and Tshikapa (95% CI 0, 202), to 92 (95% CI 0, 246) in Mikalayi. In urban settings, the estimated CRS

incidence was 61 (95% CI 0, 186) per 100,000 live births, and the estimated number of CRS cases was 724 (95% CI 0, 2211). In rural settings, the estimated CRS incidence was 82 (95% CI 0, 218) per 100,000 live births, and the estimated number of CRS cases was 2037 (95% CI 0, 5397). The overall estimated CRS incidence for DRC for 2013 was 69 (95% CI 0, 186) per 100,000 live births, and the estimated number of CRS cases was 2886 (95% CI 342, 6395). When the population size was based on UN population sources, the estimated number of CRS cases in 2013 in urban areas, rural areas, and overall DRC decreased to 565 (95% CI 0, 1725), 1590 (95% CI 0, 4212) and 2253 (95% CI 267,4991) respectively. In general, including equivocal as seropositive did not greatly affect the estimates, with the confidence intervals overlapping with those obtained by treating equivocals as seronegative (Table S3 in the supplement).

DISCUSSION

This is the first documentation of rubella antibody seroprevalence among WCBA in geographic areas outside of DRC's capital, Kinshasa [11]. Availability of sera previously obtained from pregnant women attending ANC in three provinces made the study feasible [13-15]. The results indicate an overall estimated rubella antibody seroprevalence of 84% in the assessment population with a range of 80%-89% among the 5 study sites. Rubella virus transmission is endemic in DRC, and the results in this report suggest that the majority of women are exposed to rubella virus and subsequently seroconvert before age 15 years. No trends or differences in the estimated rubella antibody seroprevalence were observed between the age groups in the overall assessment population.

A previous serosurvey in Kinshasa, conducted in 1987 among 106 women aged 16-45 years having just given birth reported high, age-independent rubella antibody seroprevalence (93%), suggesting a high level of viral transmission [11]. Other publications confirm more recent rubella virus transmission in DRC and report the majority of cases being aged <15 years with some cases among WCBA [1, 12]. Our finding of lower antibody seroprevalence in Kinshasa and lower overall antibody seroprevalence than previously documented may be explained by conducting the studies at different points in the epidemic cycle of rubella, differences in laboratory methodologies (e.g., haemagglutination inhibition versus ELISA), or differences in the age distribution of the populations [11, 30].

Our observations are generally consistent with trends in overall rubella IgG seroprevalence described among pregnant women from other countries in the WHO African region before the introduction of RCV [1, 31-52]. Our finding of no statistically significant increases in rubella antibody seroprevalence with increasing age (after approximately 15 years of age) has been observed in a number of the above-mentioned serosurveys and others [34, 35, 39, 42-46, 48, 49, 53, 54]. Moreover, in agreement with published observations from other African countries, for the assessment population in DRC overall, rubella antibody seroprevalence was similar among

356 women declaring residence in urban versus rural settings; however, differences in antibody seroprevalence were
357 observed between different geographic areas in the country [31, 34, 38, 41, 43, 44, 46, 51]. Last and consistent
358 with reports from other African countries, age at first pregnancy, number of pregnancies, civil status,
359 educational level, and occupation were not associated with rubella antibody seroprevalence in DRC [34, 38, 41,
360 44-46, 53].
361
362 The estimate for CRS incidence of 69 per 100,000 live births for 2013 in DRC overall is consistent with estimates
363 for other African countries and the African region as a whole; more specifically, estimates of CRS incidence in
364 2010 for 13 African countries ranged from 19 to 283 per 100,000 live births [24]. Additionally, for the African
365 region overall in 2010, CRS incidence was estimated to be 116 per 100,000 live births (95% CI 56, 235) [24]. DRC
366 was among 7 African countries estimated to have >1,000 CRS cases born in 2010 [24].
367
368 To date, 8 of 47 countries in the WHO African region (Burkina Faso, Cape Verde, Ghana, Mauritius, Rwanda,
369 Senegal, Seychelles, and Tanzania) have introduced RCV into routine vaccination schedules, given at age 9
370 months simultaneously with measles containing vaccine [2]. In most of these countries, introduction of RCV was
371 accompanied by a wide age range campaign targeting all children aged 9 months to 14 years (catch-up
372 campaigns) [2, 8]. Moreover, vaccination of girls not eligible for catch-up campaigns and of WCBA is
373 recommended through routine immunization service delivery [2].
374
375 RCV introduction is planned in DRC before 2020 [10]. It is recommended that countries introducing rubella
376 vaccination be able to maintain rubella vaccination coverage of at least 80% with at least one dose nationally
377 either through routine immunization services or through campaigns [2, 3, 8]. A proxy indicator for being able to
378 achieve this recommendation is a country's experience and success with the delivery of routine measles
379 vaccination. Available reports indicate that DRC has had challenges with achieving national and sub-national
380 annual measles vaccination coverage of >=80% [17, 55-57]. Therefore, during the years before introducing RCV,

DRC will need to a) focus efforts on improving the delivery of measles vaccination, thereby creating a successful platform on which to introduce rubella vaccination; b) establish an integrated nationwide measles-rubella surveillance system as well as, at least, sentinel sites for CRS surveillance; and c) use best practices from measles vaccination campaigns to assure a high-quality rubella wide-age campaign. Moreover, as found in two Nigerian studies, awareness of rubella virus infection in DRC is probably low; therefore, increased public awareness of CRS should accompany RCV introduction [44, 46]. According to this report, a significant proportion of WCBA in DRC (including adolescent girls in whom pregnancies at age 12 years are recorded in DRC's ANC site data) are susceptible to rubella virus infection and must be considered in the country's RCV introduction. Studies measuring rubella-specific IgM in pregnant women and measles case-based surveillance data from the WHO African region provide evidence that new rubella virus infections occur in adult women in Africa [1, 12, 33, 42, 46, 50, 51, 54, 58].

We note that the selected catalytic model used to estimate the force of infection was a poor fit to the Kinshasa rubella antibody seroprevalence data. This poor fit resulted from the fact that rubella antibody seroprevalence for older women remained similar to that for the youngest women, whereas catalytic models assume that the proportion of women that are susceptible decreases with increasing age, if the force of infection is non-zero and that the average force of infection is constant over time. The similar rubella antibody seroprevalence for younger and older women in Kinshasa could have resulted from several factors which remain unclear. For example, it could have occurred if there was much migration of either younger or older women from high or low transmission settings, respectively, into Kinshasa; if the force of infection increased disproportionately for younger people, or if, the women attending ANC were not representative of others in their age group.

The analysis had limitations. Because the ANC sites selected for the HIV sentinel serosurveys were a convenience sample of all ANC sites in the various provinces in DRC, the population of pregnant women was not designed to be representative of all pregnant women/WCBA in the health zones, provinces, or in DRC as a whole

406 [13, 14, 16]. Data regarding the lifetime residential history of the women in the assessment population were
407 unavailable; thus, it was not possible to hypothesize on why higher rubella antibody seroprevalence was
408 observed in the Kikwit and Vanga sites versus the Kinshasa and Mikalayi sites or among the 20-24 age group at
409 the Kinshasa site. Because the catalytic model used to estimate the force of rubella virus infection poorly fitted
410 the Kinshasa rubella antibody seroprevalence data, the Kinshasa data were excluded when calculating the
411 estimates for CRS incidence in urban areas, and then it was assumed that the estimated CRS incidence could be
412 applied to Kinshasa. The latter assumption would have led to an overestimate in the overall CRS incidence in
413 DRC if the true force of infection in Kinshasa was so high that most women had been infected in childhood. We
414 acknowledge the wide confidence intervals associated with the CRS incidence and CRS case estimates and that
415 many of the lower confidence intervals for the site and urban/rural estimates approach zero; nonetheless, the
416 estimates provide information for DRC, beyond what is currently available in the absence of specific rubella or
417 CRS surveillance. However, it should be noted that the lower 95% confidence limits do not approach zero for
418 the national estimates of the burden of CRS.
419
420 CONCLUSIONS
421 As the WHO African Region begins discussions about rubella and CRS elimination, data is needed to document
422 the burden of rubella virus transmission/infection and of CRS prior to introducing RCV [8, 9]. In the absence of
423 formal surveillance for rubella/CRS, the historical and current burden of both in DRC are largely unknown;
424 however, there is evidence for rubella virus transmission [this report, 1, 11, 12]. The use of sera from HIV
425 sentinel surveys among pregnant women attending ANC provided a unique opportunity to estimate the burden
426 of rubella virus infection and CRS. The results reported here can add to other available data to guide plans for
427 introduction of RCV in DRC and will provide a background from which the impact of vaccination can be assessed.

REFERENCES

1. Goodson JL, Masresha B, Dosseh A, et al. Rubella Epidemiology in Africa in the prevaccine era, 2002-2009. J Infect Dis 2011; 204 (Suppl 1):S215-S225.
2. World Health Organization. Rubella vaccines: WHO position paper. Wkly Epidemiol Rec 2011; 86:301-316.
3. Centers for Disease Control and Prevention. Progress toward control of rubella and prevention of congenital rubella syndrome-worldwide, 2009. MMWR Morb Mortal Wkly Rep 2010; 59:1307-1310.
4. Centers for Disease Control and Prevention. Rubella and congenital rubella syndrome control and elimination-Global Progress, 2000-2012. MMWR Morb Mortal Wkly Rep 2013; 62:983-986.
5. Castillo-Solorzano C, Reef SE, Morice A, et al. Guidelines for the documentation and verification of measles, rubella, and congenital rubella syndrome elimination in the region of the Americas. J Infect Dis 2011; 204(suppl 2):S683-S689.
6. World Health Organization Western Pacific Region. Report of the 24th Meeting of the Technical Advisory Group on Immunization and Vaccine-Preventable Diseases in the Western Pacific Region, 9-12 June 2015, Manilla. Available from the Western Pacific Regional World Health Organization Office.
7. Pan American Health Organization. Elimination of rubella and congenital rubella syndrome in the Americas, Fact Sheet 2015, 29 April 2015. Available at http://www.paho.org/hq/index.php?option=com_content&view=article&id=10798%3A2015-americas-free-of-rubella&Itemid=1926&lang=en Accessed 26 July 2016.
8. Burki T. GAVI Alliance to roll our rubella vaccine. Lancet Infect Dis 2012; 12:15-16.
9. World Health Organization. Consensus points from the African regional consultation on measles and rubella/CRS elimination, 27-29 November 2013. Available at http://www.sabin.org/updates/events/african-regional-consultation-measles-rubellacrs-elimination Accessed 26 July 2016.
10. Ministry of Public Health-Democratic Republic of the Congo. Plan Pluri Annuel Complet du PEV de la République Démocratique du Congo, 2015-2019, novembre 2014, Programme Elargi de Vaccination, Ministère de la Santé Publique, Kinshasa, République Démocratique du Congo. (Comprehensive Multi-Year

Plan of the EPI of the Democratic Republic of the Congo, 2015 – 2019, November 2014, Expanded Programme on Immunization, Ministry of Public Health, Kinshasa, Democratic Republic of the Congo). Available from the Expanded Programme on Immunization, Ministry of Public Health, Democratic Republic of the Congo.

11. Omanga U, Goussard B, Kapepela K, et al. Séroprévalence de la rubéole à Kinshasa (Zaïre) [Seroprevalence of rubella in Kinshasa (Zaïre)]. Bull Soc Path Ex 1991; 84: 994-1001.
12. Nsambu MN, Coulibaly T, Donnen P, et al. Fréquence de la rubéole à Kinshasa de 2010 – 2012, République Democratic du Congo (RDC): données issues du système de surveillance de la rougeole [Incidence of rubella in 2010-2012 in Kinshasa, Democratic Republic of the Congo (DRC)]: data from the measles case-based surveillance system). Santé Publique 2014; 26:393-397.
13. Ministry of Public Health-Democratic Republic of the Congo. Rapport épidémiologique de surveillance du VIH chez les femmes enceintes fréquentant les structures de CPN 2008 (Epidemiological report of HIV surveillance in pregnant women attending prenatal consultations 2008). Available from the National HIV/AIDS Program, Ministry of Public Health-Democratic Republic of the Congo.
14. Ministry of Public Health-Democratic Republic of the Congo. Rapport épidémiologique de surveillance du VIH/SIDA chez les femmes enceintes fréquentant les structures de CPN 2009 (Epidemiological report of HIV/AIDS surveillance in pregnant women attending prenatal consultations 2009). Available from the National HIV/AIDS Program, Ministry of Public Health-Democratic Republic of the Congo.
15. Alleman MM, Wannemuehler KA, Weldon WC, et al. Factors Contributing to Outbreaks of Wild Poliovirus Type 1 Infection Involving Persons Aged >= 15 Years in the Democratic Republic of the Congo, 2010-2011, Informed by a Pre-Outbreak Poliovirus Immunity Assessment. J Infect Dis 2014; 210 (Suppl 1):S62-S73.
16. UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance. Guidelines for conducting HIV Sentinel Serosurveys among Pregnant Women and Other Groups, 2003. Available at http://www.who.int/hiv/pub/surveillance/anc_guidelines/en/ Accessed 26 July 2016.

17. Ministry of Planning and Monitoring of the Implementation of the Revolution of Modernity, Ministry of Public Health, Democratic Republic of the Congo and MEASURE DHS. Ministère du Plan et Suivi de la Mise en Œuvre de la Révolution de la Modernité, Ministère de la Santé Publique, Kinshasa, République Démocratique du Congo, et MEASURE DHS, ICF International, Rockville, Maryland, USA. République Démocratique du Congo Enquête Démographique et de Santé, 2013-14 (Ministry of Planning and Monitoring of the Implementation of the Revolution of Modernity, Ministry of Public Health, Kinshasa, Democratic Republic of the Congo and MEASURE DHS, ICF International, Rockville, Maryland, USA. Democratic Republic of the Congo Demographic and Health Survey, 2013-14). Available at http://dhsprogram.com/publications/publication-FR300-DHS-Final-Reports.cfm Accessed 26 July 2016.
18. Feinstein L, Dimomfu B, Mupenda B, et al. Antenatal and delivery services in Kinshasa, Democratic Republic of Congo: care-seeking and experiences reported by women in household-based survey. Trop Med Int Health 2013; 18:1211-1221.
19. Singh H, Chiu Y, Wilkin T. Measles, Mumps, and Rubella Serostatus and Response to MMR Vaccination Among HIV-Infected Adults. AIDS Patient Care STDS. 2015 Sep;29(9):461-4. doi: 10.1089/apc.2015.0050. Epub 2015 Jul 8.
20. Zaman K, Fleming JA, Victor JC, et al. Noninterference of Rotavirus Vaccine With Measles-Rubella Vaccine at 9 Months of Age and Improvements in Antirotavirus Immunity: A Randomized Trial. J Infect Dis 2016; 213:1686-1693.
21. UN Statistics Division, United Nations Population Division, World Population Prospects. 2012 revision.
22. United Nations Department of Economic and Social Affairs. World Statistics Pocketbook, 2014 World Edition. New York, New York. Available at http://unstats.un.org/unsd/pocketbook/ Accessed 26 July 2016.
23. Cutts FT, Vynnycky E. Modelling the incidence of congenital rubella syndrome in developing countries. Int J Epidemiol 1999; 28:1176-1184.

24. Vynnycky E, Adams EJ, Cutts FT, et al. Using seroprevalence and immunisation coverage data to estimate the global burden of Congenital Rubella Syndrome, 1996-2010. Plos One 2016. doi 10.1371/journal.pone.0149160.
25. Mao B, Chheng K, Wannemuehler K, et al. Immunity to polio, measles and rubella in women of child-bearing age and estimated congenital rubella syndrome incidence, Cambodia, 2012. Epidemiol Infect 2015; 143:1858-1867.
26. Muench H. Catalytic models in epidemiology. Cambridge: Harvard University Press, 1959.
27. Shkedy Z, Aerts M, Molenberghs G, et al. Modelling age-dependent force of infection from prevalence data using fractional polynomials. Stat Med 2006; 25:1577-1591.
28. Press WH, Teukolsky SA, Vetterling WT, et al. Numerical Recipes in C: The art of scientific computing, Second Edition. New York: Cambridge University Press, 1992.
29. Nelder, JA, Mead R. A simplex method for function minimization. Computer Journal 1965; 7:308–313. doi:10.1093/comjnl/7.4.308.
30. Horstmann DM. Rubella: the challenge of its control. J Infect Dis 1971; 123:640-54.
31. Gomwalk NE, Ahmad AA. Prevalence of rubella antibodies on the African Continent. Rev Infect Dis 1989; 11:116-121.
32. Rodier MH, Berthonneau J, Bourgoin A, et al. Seroprevalences of toxoplasmosis, malaria, rubella, cytomegalovirus, HIV and treponemal infections among pregnant women in Cotonou, Republic of Benin. Acta Trop 1995; 59:271-277.
33. Linguissi LS, Nagalo BM, Bisseye C, et al. Seroprevalence of toxoplasmosis and rubella in pregnant women attending antenatal private clinic at Ouagadougou, Burkina Faso. Asian Pac J Trop Med 2012; 5:810-813.
34. Tahita MC, Hübschen JM, Tarnagda Z, et al. Rubella seroprevalence among pregnant women in Burkina Faso. BMC Infect Dis 2013; 13: 164 doi: 10.1186/1471-2334-13-164.
35. Fokunang CN, Chia J, Ndumbe P, et al. Clinical studies on seroprevalence of rubella virus in pregnant women of Cameroon regions. Afr J Cln Exper Microbiol 2010; 11:79-94.

36. Ndumbe PM, Andela A, Nkemnkeng-Asong J, et al. Prevalence of infections affecting the child among pregnant women in Yaoundé, Cameroon. Med Microbiol Immunol 1992; 181:127-130.
37. Sandow D, Okubagzhi GS, Arnold U, et al. Seroepidemiological study in rubella in pregnant women in Gondar Region, northern Ethiopia. Ethiop Med J 1982; 20:173-178.
38. Lawn JE, Reef S, Baffoe-Bonnie B, et al. Unseen blindness, unheard deafness, and unrecorded death and disability: congenital rubella in Kumasi, Ghana. Am J Public Health 2000; 90:1555-1561.
39. Faye-Kette YH, Sylla-Koko DJ, Akoua-Koffi GC, et al. Séroprévalence de la rubéole chez 461 femmes enceintes à Abidjan (Côte d'Ivoire). [Seroprevalence of rubella in 461 pregnant women in Abidjan (Ivory Coast)]. Bull Soc Pathol Exot 1993; 86:185-7.
40. Vrinat M, Dutertre J, Helies H, et al. Prévalence sérologique de la rubéole chez la femme enceinte à Abidjan (A serological survey of rubella among pregnant women in Abidjan). Med Trop 1978; 38:53-57.
41. Kombich, JJ, Muchai PC, Borus, PK. Seroprevalence of natural rubella antibodies among antenatal attendees at Moi Teaching and Referral Hospital, Eldoret, Kenya. J Immunol Tech Infect Dis 2012; 1:1 doi:10.4172/2329-9541.1000102.
42. Dromigny JA, Pécarrère JL, Ollivier GL, et al. Séroprévalence de la rubéole chez la femme enceinte à Antananarivo, Etude Effectuée à l'Institut Pasteur de Madagascar sur 853 sérums (Rubella seroprevalence among pregnant women at Antananarivo, study conducted by the Pasteur Institute of Madagascar on 853 sera). Arch Inst Pasteur Madagascar 1996; 63:53-55.
43. Barreto J, Sacramento I, Robertson SE, et al. Antenatal rubella serosurvey in Maputo, Mozambique. Trop Med Int Health 2006; 11:559-564.
44. Amina MD, Oladapo S, Habib S, et al. Prevalence of rubella IgG antibodies among pregnant women in Zaria, Nigeria. Int Health 2010; 2:156-159 doi: 10.1016/j.inhe.2010.03.004.
45. Kolawole OM, Anjorin EO, Adekanle DA, et al. Seroprevalence of rubella IgG antibody in pregnant women in Osogbo, Nigeria. Int J Prev Med 2014; 5:287-292.

46. Olajide OM, Aminu M, Randawa AJ, et al. Seroprevalence of rubella-specific IgM and IgG antibodies among pregnant women seen in a tertiary hospital in Nigeria. Int J Women's Health 2015; 7:75-83.
47. Onyenekwe CC, Kehinde-Agbeyangi TA, Ofor US, et al. Prevalence of rubella IgG antibody in women of childbearing age in Lagos, Nigeria. West Afr J Med 2000; 19:23-26.
48. Dromigny JA, Nabeth P, Perrier Gros Claude JD. Evaluation of the seroprevalence of rubella in the region of Dakar (Senegal). Trop Med Int Health 2003; 8:740-3.
49. Corcoran C, Hardie DR. Seroprevalence of rubella antibodies among antenatal patients in the Western Cape. S Afr Med J 2005; 95:688-90.
50. Maselle SY, Haukenes G, Rutahindurwa A. Preliminary observations on rubella infection in Tanzania and the challenge for its control. East Afr Med J 1988; 65:319-24.
51. Mwambe B, Mirambo MM, Mshana SE, et al. Sero-positivity rate of rubella and associated factors among pregnant women attending antenatal care in Mwanza, Tanzania. BMC Pregnancy Childb 2014; 14:95. doi: 10.1186/1471-2393-14-95.
52. Bracken PM, Stanfield, JP. Rubella antibodies in Uganda. East Afr Med J. 1971; 48:176-181.
53. Bamgboye AE, Afolabi KA, Esumeh FI, et al. Prevalence of rubella antibody in pregnant women in Ibadan, Nigeria. West Afr J Med 2004; 23:245-248.
54. Onakewhor JU, Chiwuzie J. Seroprevalence survey of rubella infection in pregnancy at the University of Benin Teaching Hospital, Benin City, Nigeria. Niger J Clin Pract 2011; 14:140-145. doi: 10.4103/1119-3077.84002.
55. World Health Organization. WHO/UNICEF estimates of measles vaccination coverage. Available at http://www.who.int/immunization/monitoring_surveillance/data/en/ Accessed 26 July 2016.
56. Ministère du Plan avec la collaboration du Ministère de la Santé Kinshasa, République Démocratique du Congo and Macro International Inc. Calverton, Maryland, USA. République Démocratique du Congo Enquête Démographique de la Santé, 2007 (Ministry of Planning in collaboration with the Ministry of Health, Kinshasa, Democratic Republic of the Congo and Macro International Inc. Calverton, Maryland, USA.

Democratic Republic of the Congo Demographic and Health Survey, 2007). Available at http://dhsprogram.com/publications/publication-FR208-DHS-Final-Reports.cfm. Accessed 26 July 2016.

57. United Nations Children's Fund, National Institute of Statistics of the Democratic Republic of Congo. Democratic Republic of the Congo Multiple Indicator Cluster Survey, 2010. Available at http://www.agrodep.org/dataset/democratic-republic-congo-multiple-indicator-cluster-survey-mics-2010-0 Accessed 26 July 2016.
58. Pennap G, Amauche G, Ajoge H, et al. Serologic survey of specific rubella virus IgM in the sera of pregnant women in Makurdi, Benue State, Nigeria. Afr J Reprod Health 2009; 13:69-73.

FIGURES, FIGURE LEGENDS, AND TABLES

Figure 1 Legend: Approximate location of antenatal care (ANC) sites where blood specimens were collected in 2008-2009 from pregnant women in the rubella antibody seroprevalence assessment population, by zone de santé (health zone) and province, Democratic Republic of the Congo.

Figure 2 Legend: Estimated seroprevalence (%) of rubella IgG in the assessment population, by age group and study site in Bandundu, Kasaï Occidental, and Kinshasa provinces, Democratic Republic of the Congo.

Figure 2 Footnote: For each age group and overall, the number of serum samples analyzed by ELISA is noted. Within the Kinshasa site, the 20-24 year age group had a higher antibody seroprevalence than the 15-19 (p value =0.01) and 25-29 (p value <0.001) year age groups. Kikwit (89%) and Vanga (88%) study sites had higher overall rubella antibody seroprevalence than the Mikalayi (80%) and Kinshasa (82%) sites (all p values <=0.03).

Table 1: Demographic characteristics of the rubella antibody seroprevalence assessment population, overall and by 5 study sites in Bandundu, Kasaï Occidental, and Kinshasa Provinces, Democratic Republic of the Congo.

Characteristic	Overall (n=1,605) No. (% of n)	Kikwit (n=314, urban) No. (% of n)	Vanga (n=326, rural) No. (% of n)	Kinshasa (n=323, urban) No. (% of n)	Tshikapa (n=320, urban) No. (% of n)	Mikalayi (n=322, rural) No. (% of n)
Residence						
Town	914 (57.0)	275 (87.6)	3 (0.9)	322 (99.7)	309 (96.6)	5 (1.6)
Village	690 (43.0)	39 (12.4)	323 (99.1)	0 (0)	11 (3.4)	317 (98.5)
Unknown	1 (0.1)			1 (0.003)		
Age at time of serum collection (years)	Range: 15-46 years	Range: 15-46 years	Range: 15-44 years	Range: 15-46 years	Range: 15-46 years	Range: 15-46 years
15-19	321 (20.0)	64 (20.4)	65 (19.9)	64 (19.8)	62 (19.4)	66 (20.5)
20-24	322 (20.1)	65 (20.7)	65 (19.9)	64 (19.8)	64 (20.0)	64 (19.9)
25-29	318 (19.8)	60 (19.1)	66 (20.3)	63 (19.5)	66 (20.6)	63 (19.6)
30-34	320 (19.9)	63 (20.1)	65 (19.9)	66 (20.4)	62 (19.4)	64 (19.9)
>=35	324 (20.2)	62 (19.8)	65 (19.9)	66 (20.4)	66 (20.6)	65 (20.2)
Age at first pregnancy (years)	Range: 12-37 years	Range: 12-33 years	Range: 12-33 years	Range: 13-36 years	Range: 13-37 years	Range: 13-28 years
12-17	664 (41.4)	80 (25.5)	117 (35.9)	78 (24.2)	178 (55.6)	211 (65.5)
18-23	769 (47.9)	176 (56.1)	178 (54.6)	186 (57.6)	120 (37.5)	109 (33.9)
>=24	172 (10.7)	58 (18.5)	31 (9.5)	59 (18.3)	22 (6.9)	2 (0.6)
Number of times pregnant including current	Range: 1-15	Range: 1-10	Range: 1-15	Range: 1-12	Range: 1-14	Range: 1-12
1 time	367 (22.9)	95 (30.3)	70 (21.5)	94 (29.1)	55 (17.2)	53 (16.5)
2 times	254 (15.8)	56 (17.8)	57 (17.5)	69 (21.4)	34 (10.6)	38 (11.8)
>=3 times	984 (61.3)	163 (51.9)	199 (61.0)	160 (49.5)	231 (72.2)	231 (71.7)
Civil Status						
Married*	1528 (95.2)	289 (92.0)	309 (94.8)	302 (93.5)	311 (97.2)	317 (98.5)
Not-married	77 (4.8)	25 (8.0)	17 (5.2)	21 (6.5)	9 (2.8)	5 (1.6)
Educational level						
None/1^{o} completed^{+}	627 (39.1)	40 (12.7)	191 (58.6)	31 (9.6)	123 (38.4)	242 (75.2)
>=2^{o} attended	978 (60.9)	274 (87.3)	135 (41.4)	292 (90.4)	197 (61.6)	80 (24.8)
Occupation						
Housekeeper	638 (40.0)	150 (47.8)	38 (11.7)	168 (53.0)	247 (77.4)	35 (10.9)
Farmer	558 (35.0)	43 (13.7)	244 (74.8)	0 (0)	14 (4.4)	257 (79.8)
Other$^{‡, §}$	400 (25.0)	119 (38.1)	44 (13.5)	149 (47.0)	58 (18.2)	30 (9.3)

*Married (refers to those married monogamous, married polygamous, in a free-union, separated, divorced, and widowed), Not-married (refers to those single)

$^{+}$None/1^{o} completed (no education, primary school attended, or primary school completed), 2^{o} (secondary school attended, secondary school completed, graduate, or license obtained)

‡Other (refers to student, no occupation, government worker, business person, and other).

§Nine responses were excluded from the analysis as their meaning could not be interpreted; the 9 were distributed as follows: 2 in Kikwit, 6 in Kinshasa, and 1 in Tshikapa.

Table 2. Estimated force of rubella virus infection among susceptible individuals <15 and >=15 years of age/year, CRS incidence/100,000 live births among women aged 15-44 years in 2013, and estimated number of CRS cases born in 2013 by 5 study sites in Bandundu, Kasaï Occidental, and Kinshasa provinces, rural and urban settings, and overall in the Democratic Republic of the Congo. Equivocals were classified as seronegative in these analyses.

Study site or Setting	Force of Rubella Virus Infection per 1,000 susceptible individuals/year (95% Confidence Intervals)*		Estimated weighted CRS Incidence/ 100,000 live births among women aged 15-44 years in 2013[+] (95% Confidence Intervals)*	Estimated number of CRS cases born in 2013 (95% Confidence Intervals)*, with the population size obtained from:	
	<15 years of age	>=15 years of age		Democratic Republic of the Congo Expanded Programme on Immunization-Ministry of Public Health Population projections based on the 1984 census	UN population sources [21]
Kikwit	129 (98, 164)	27 (0, 69)	61 (0, 151)	5 (0, 11)	3 (0, 8)
Vanga	118 (88, 155)	32 (0, 72)	81 (0, 184)	11 (0, 24)	8 (0, 18)
Mikalayi	92 (64, 120)	23 (0, 68)	92 (0, 246)	10 (0, 26)	7 (0, 18)
Tshikapa	113 (80, 146)	20 (0, 73)	61 (0, 202)	8 (0, 26)	6 (0, 18)
Kinshasa	66 (59, 72)	66 (59, 72)	252 (238, 264)	73 (68, 76)	52 (49, 55)
Urban[‡]	120 (83, 159)	24 (0, 73)	61 (0, 186)	724 (0, 2211)	565 (0, 1725)
Rural[§]	104 (70, 149)	28 (0, 71)	82 (0, 218)	2037 (0, 5397)	1590 (0, 4212)
Overall[‖]	110 (71, 152)	27 (0, 72)	69 (0, 186)	2886 (342, 6395)	2253 (267, 4991)

***Confidence intervals were obtained by bootstrapping.**

+Weighted by the number of live births occurring among women in different maternal age groups.

[‡]Compiled using estimates derived from rubella antibody seroprevalence data from Kikwit and Tshikapa. As explained in the Methods, antibody seroprevalence data from Kinshasa were excluded from these estimations.

§Compiled using estimates derived from rubella antibody seroprevalence data from Mikalayi and Vanga.

[‖]Compiled using estimates from the urban and rural settings

CRS, Congenital Rubella Syndrome

Figure 1

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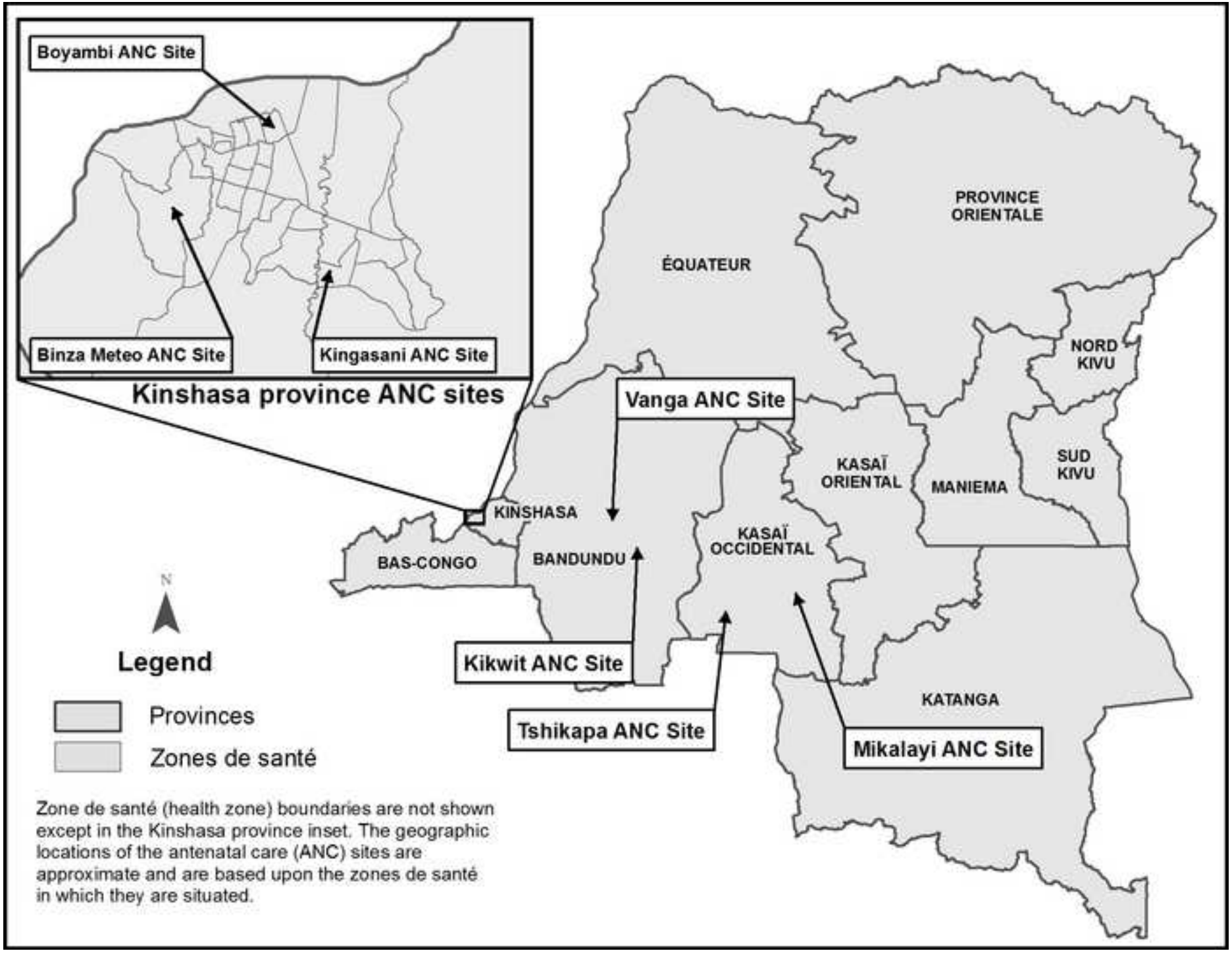

Figure 2

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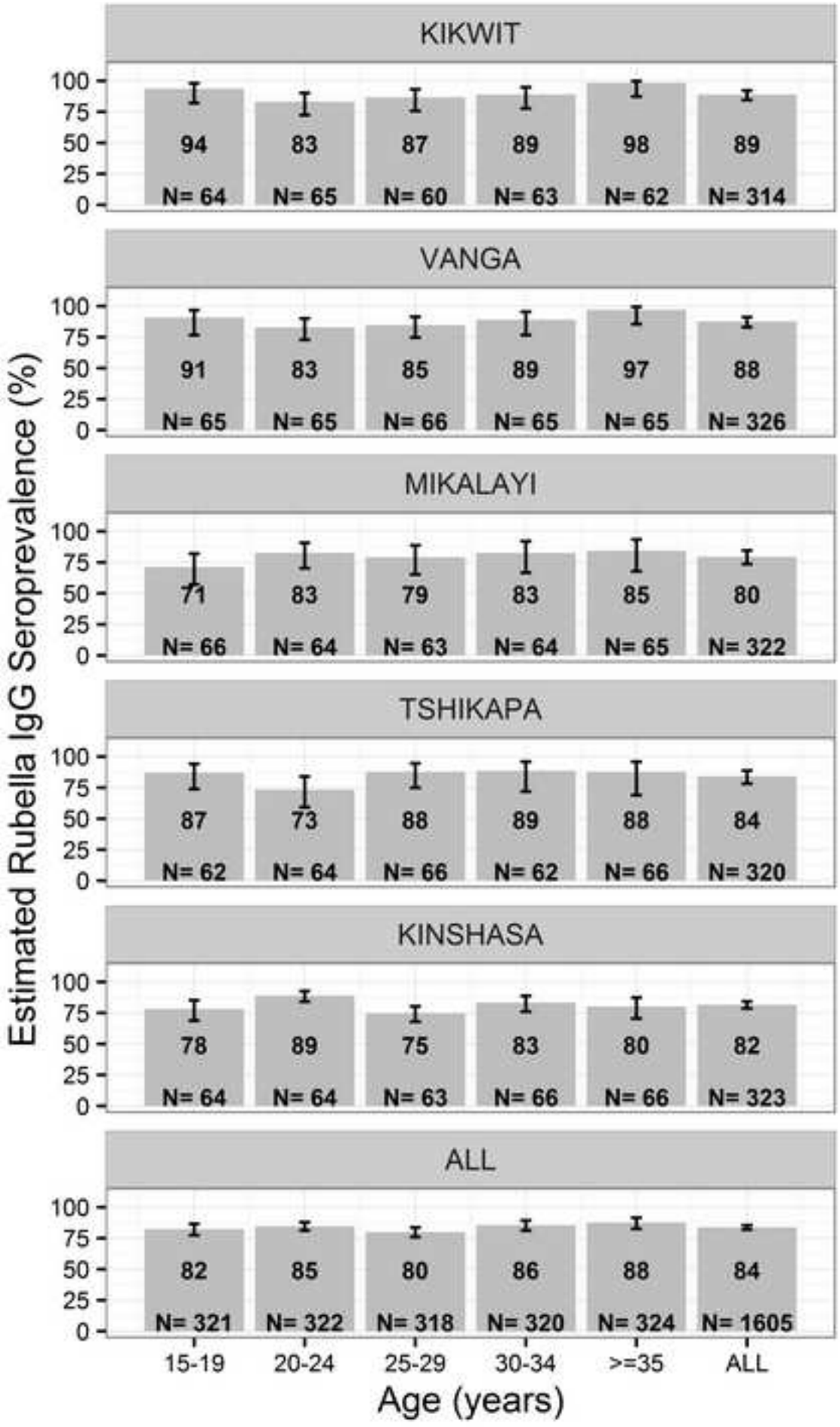

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