Article DOI: https://doi.org/10.37284/eajhs.6.1.1103

East African Journal of Health and Science
eajhs.eanso.org
Volume 6 Issue 1, 2023
Print ISSN: 2707-3912 | Online ISSN: 2707-3920
Title DOI: https://doi.org/10.37284/2707-3920

EAST AFRICAN NATURE & SCIENCE ORGANIZATION

Original Article

Immunity Against Measles in Urban Refugee Children, Adolescents, and Young Adults in Eastleigh, Kenya

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Article DOI: https://doi.org/10.37284/eajhs.6.1.1103

Date Published:
23 February 2023

Keywords:
Measles, Measles IgG, Refugees, Neutralisation Assay, Herd Immunity.

ABSTRACT

Background: Kenya has made progress towards the elimination of measles through routine and supplementary immunisation activities; however, sporadic outbreaks still occur. Studies on measles seroprevalence in Kenya are few and extensive analysis of refugee and migrant populations' immunity against measles is lacking. This cross-sectional study aimed at assessing the presence of immunity against measles in children, adolescents and young adults living in the high urban refugee area of Eastleigh Nairobi, Kenya. **Method**: A total of 384 samples serum samples were collected from consenting respondents and tested for measles IgG antibodies. Syncytium Inhibition Assay, a neutralisation assay was used to determine functionality. The establishment of herd immunity was calculated using the Plans- Rubio formulae. **Results**: Seropositive rate for all respondents were 84.38%, while equivocal and negative were 4.95% and 10.68%, respectively. None of the factors analysed was a significant predictor of positive measles antibodies. 87.76% of the total sera were neutralising, while 12.23 % were negative. There was a significant correlation between the neutralisation titres and ELISA values. **Conclusion**: This study highlights age-specific measles immunity gaps in an urban refugee population. Although measles antibodies are present in all age groups, none has established herd immunity. In order to avert outbreaks in the study population, it is necessary to direct immunisation interventions to increase the measles antibody prevalence to recommended levels.

APA CITATION

Maseghe, L. Z., Kariuki, D., Odari, E. O., Ochiel, D. & Achieng, C. M. (2023). Immunity Against Measles in Urban Refugee Children, Adolescents, and Young Adults in Eastleigh, Kenya *East African Journal of Health and Science*, *6*(1), 20-32. https://doi.org/10.37284/eajhs.6.1.1103.

CHICAGO CITATION

Maseghe Lilian Zighe, Daniel Kariuki, Eddy Okoth Odari, Daniel Ochiel and Calvin Mandela Achieng. 2023. "Immunity Against Measles in Urban Refugee Children, Adolescents, and Young Adults in Eastleigh, Kenya". *East African Journal of Health and Science* 6 (1), 20-32. https://doi.org/10.37284/eajhs.6.1.1103.

Article DOI: https://doi.org/10.37284/eajhs.6.1.1103

HARVARD CITATION

Maseghe, L. Z., Kariuki, D., Odari, E. O., Ochiel, D. & Achieng, C. M. (2023) "Immunity Against Measles in Urban Refugee Children, Adolescents, and Young Adults in Eastleigh, Kenya", *East African Journal of Health and Science*, 6(1), pp. 20-32. doi: 10.37284/eajhs.6.1.1103.

IEEE CITATION

L. Z. Maseghe, D. Kariuki, E. O. Odari, D. Ochiel & C. M. Achieng, "Immunity Against Measles in Urban Refugee Children, Adolescents, and Young Adults in Eastleigh, Kenya", *EAJHS*, vol. 6, no. 1, pp. 20-32, Feb. 2023.

MLA CITATION

Maseghe Lilian Zighe, Daniel Kariuki, Eddy Okoth Odari, Daniel Ochiel & Calvin Mandela Achieng. "Immunity Against Measles in Urban Refugee Children, Adolescents, and Young Adults in Eastleigh, Kenya". *East African Journal of Health and Science*, Vol. 6, no. 1, Feb. 2023, pp. 20-32, doi:10.37284/eajhs.6.1.1103.

INTRODUCTION

Measles is a highly transmissible viral infection caused by the measles virus a morbillivirus of the genus paramyxovirus. The infection produces strong measles virus-specific immune responses that clear the virus and confers lifelong immunity, slow recovery and post-measles complications can also occur (Gastañaduy *et al.*, 2021). Pre-vaccination era, measles infected over 95% of all children and was responsible for over 4 million deaths annually (Mina, 2017). Measles-containing vaccines (MCV) have greatly reduced the incidence and mortality rate of measles since they were first licenced in 1963; however, achieving elimination remains a significant public health challenge (Cutts *et al.*, 2013). Cases and outbreaks of measles still occur and it remains the fifth leading cause of morbidity for children less than 5 years (Strebel *et al.*, 2003). According to WHO Africa (2021), almost all measles-related deaths (more than 95%) occur in countries with low per capita income and poor health systems. Measles outbreaks are typically brought about by the measles virus finding immunity gaps in a population as well as travel-related importation (Gastañaduy et al., 2018). Other contributing factors to measles outbreaks, especially in the immunised population are vaccine failure (primary failure) and waning immunity (secondary failure) (Holzmann et al., 2016).

Kenya has been a strong follower of the World Health Assembly and Measles Elimination 2020 resolutions through Routine Immunisation and Supplementary Immunization activities. This has led to progress towards measles elimination over the years; however, there remains a risk of outbreaks with the stagnant MCV1 coverage with some periods of decline and the slow uptake in MCV2 (Kisangau et al., 2018). Another threat to the elimination of measles in Kenya is the influx of refugees with unknown immunisation status leading to outbreaks in refugee camps and informal settlements (Manakongtreecheep & Davis, 2017). Kenya is home to the largest refugee population and some of the oldest refugee camps in sub-Saharan Africa (IOM, 2015). Refugees and asylum seekers often arrive from countries with social and economic challenges and natural disasters. Some of these refugees migrate to urban areas and cities in search of better economic opportunities (Campbell, 2006). Although urban migrant communities are found throughout Nairobi, particularly within its informal settlements, Eastleigh region undeniably hosts the largest migrant community in the city (Campbell et al., 2011). Refugees and asylum seekers often experience unique health susceptibilities that are experienced by both the migrants and the communities with which they associate (Carballo & Nerukar, 2001). Evidence of health inequality among urban migrants residing in Eastleigh has been illustrated in tuberculosis, reproductive and maternal child health, malaria, measles and psychosocial (IOM, 2011).

Studies on measles in Kenya are few and a comprehensive analysis regarding the occurrence and extent of immunity against measles in refugees and asylum seekers in Kenya is lacking in published literature. To determine the presence of measles-specific antibodies in urban refugee children, adolescents, and young adults in the high urban refugee area of Eastleigh in Nairobi, Kenya, we undertook a cross-sectional study. Our main objective was to assess whether the study

Article DOI: https://doi.org/10.37284/eajhs.6.1.1103

population has immunity against measles. Specifically, we determined the presence of age-specific positive measles antibodies, established if the antibodies were neutralising and if the study population had established herd immunity. Our study findings will help provide useful data for the development of effective strategies aimed at eliminating the risk of measles in all population types.

MATERIALS AND METHODS

Study Design

The cross-sectional study was conducted targeting urban refugee children (5-10 years), adolescents (15-19 years) and young adults (20-25 years) from Eastleigh County Council Health Centre in Eastleigh North and Biafra/ Eastleigh Lions clinic in Eastleigh South who have resided in these locations for more than 2 years. Eastleigh which is to the east of Nairobi Central Business District was conveniently chosen as the study area because of its high urban refugee and immigrant population. The area is characterised by overcrowding in both residential and business areas, poor infrastructure, and poor sanitation.

Samples were collected from March 2022 to June 2022. A structured questionnaire was administered to all the approached respondents to determine if they met the criteria for recruitment. Informed consent was obtained from participants or from a parent or guardian in the case of minors.

Ethical Considerations and Approval

All ethical considerations concerning the use of human subjects were followed. A research permit authorising the study was issued by the National Commission of Science Technology and Innovation (NACOSTI) Kenya, reference number 764279. Ethics review and approval was acquired from Jomo Kenyatta University of Agriculture and Technology- Institutional Ethics Review Committee (JKUAT- IERC).

Sample Collection and Testing for the Presence of IgG Antibodies

Blood sample collection, collection and storage were done according to the WHO's recommended best practices (WHO., 2012). A total of 5 ml of whole blood was collected from the arm of the respondent by qualified personnel who had proper personal protective equipment. In order to the whole blood was allowed to clot by leaving it undisturbed at room temperature for about 15–30 minutes. The clot was removed by centrifuging at 1,000–2,000 x g for 10 minutes in a refrigerated centrifuge. The resulting supernatant is serum. The serum tubes were labelled, packaged, and stored at between -20° and -70 °C until analysed at Kenya Aids Vaccine Initiative- Institute of Clinical Research (KAVI- ICR).

Testing for the presence of measles IgG antibodies was done using the Measles IgG ELISA kit DEMEAG0330 (Demeditec Diagnostics GmbH). The assay is based on capturing virus-specific human IgG on the preparation of purified virus antigen immobilised on plastic wells. All reagents used were provided with the ELISA kit. Absorbance value cut-offs and interpretation of results were carried out according to the manufacturer's instructions.

All serum samples and kit reagents were brought to room temperature (20-25 °C) and mixed. The desired number of antigen-coated strips were placed into the holder. The negative control, positive control, and cut-off were ready to use. The samples dilution of 1:100 was prepared by adding 10 µL of the sample to 1000 µL of sample diluent and well mixed. 100 µL of diluted sera, cut-off, and controls were dispensed into the appropriate wells. For the substrate blank, 100 µL sample diluent was dispensed in the 1A well position. The holder was tapped to remove air bubbles from the liquid. Then incubated for 60 minutes ± 5 minutes at room temperature. The liquid was aspirated from all wells and then washed three times with 300 µL of 1X wash buffer using an automatic microplate washer machine. An absorbent paper towel was used to blot any residue. 100 µL of enzyme conjugate was

Article DOI: https://doi.org/10.37284/eajhs.6.1.1103

dispensed to each well except the substrate Blank well A1 and incubated for 30 minutes at room temperature away from direct light. Enzyme conjugate was removed from all wells and then washed three times with 300 µL of 1X wash buffer by the automatic microplate washer and blotted with an absorbent paper towel.

100 µL of TMB substrate was dispensed and incubated for 15 minutes at room temperature in the dark. 100 µL of stop solution was added immediately after. Measurement of absorbance was at 450 nm/620 nm using an ELISA reader within 30 minutes.

Syncytium Inhibition Assay for the Determination of Neutralizing Antibodies

A syncytium inhibition assay by Rabenau et al. (2007) was adopted for this study with minor adjustments. The Edmonton MV was obtained as the Rimevax vaccine (SmithKline Beecham, Madrid, Spain). The vaccine, which contains an MV titre of 1,000 50% tissue culture infective doses, will be resuspended in 0.6 ml of DMEM-5% FCS (Sigma- Aldrich) containing penicillin and streptomycin and was distributed in 30-µl aliquots in 96- well U-bottom plates (Deltalab S.A.). Two-fold serial dilutions (1:10 to 1:640) of serum prepared in DMEM-5% albumin will be incubated with the viral aliquots for 1 h at 37 ℃ in 5% CO_2

Vero cells (CCL-81) grown in DMEM-5% FCS and will be expanded the day before testing in 24- and 48-well plates. Cells at 70 to 80% confluence will be washed with DPBS, and the virus-antibody cocktail (40 µl) will be added to duplicate wells (24-well plates) or to four wells (48-well plates). The cells will be incubated with the virus for 1 h at 37 °C, the cocktail will be aspirated, and the cells will be washed with DPBS. The cells will then be incubated with 0.5 ml of DMEM-5% FCS-penicillin-streptomycin at 37 °C until syncytium formation could be determined by an inverted microscope. The end-point dilution was considered the first serum dilution that resulted in one or more syncytia.

Statistical Analysis

Descriptive and analytical analysis was carried out using Statistical package for social sciences SPSS® v25 (IBM Corp, Armonk, NY, USA). The statistical significance level was taken at the p-value of .05 at a 95% confidence interval (CI). Descriptive statistics were used to analyse the categorical variables, while chi-square was used to determine the association between variables of interest and positive measles IgG antibodies.

RESULTS

Basic Characteristics of the Study Participants

The study recruited a total of 384 participants. The study aimed at attaining an equal number of male and female participants; however, the number of female participants *Table 1*, was slightly higher (200, 52.08%) compared to males (184, 47.91%). Each age group had an equal number of respondents. The study divided the participants into two major groups those born in Kenya and those born elsewhere (outside Kenya); 267 (69.53%) participants were born in Kenya, while 117 (30.47%) were born outside Kenya *Table 1*.

A total number of 188 participants (about 49%) had resided in Eastleigh for more than ten years when the study was being conducted. Thirty-six percent of the participants had lived in Eastleigh for 8-10 years a total of 139 participants. The number of participants that had stayed in Eastleigh for 5-7 years was 47 (12.23%), and only 10 participants (2.60%) had lived in Eastleigh for 2-4 years.

Prevalence of Measles IgG Antibodies

The overall prevalence of positive measles IgG antibodies in the samples was 324/384 (84.38%), while 19/384 (4.95%) of all respondents had equivocal, and 41/384 (10.68%) were negative. The proportion of measles IgG-positive samples differed by age and was highest in children 5-10 years (114/12, 89.06%), followed by young adults (109/128, 85.16%) and lowest in adolescents (101/128, 78.90%).

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Male respondents had a slightly higher proportion of positive measles IgG antibodies (156/184, 84.78%) compared to females (168/200, 84%). Respondents born outside Kenya who tested positive for measles IgG antibodies were 101/117 (86.32%) compared to those born in Kenya 223/267 (83.52%). Those who had lived in Eastleigh for between 5-7 years had 37/47 (78.72%) seropositive, with those who lived for between 8-10 years (121/139) and more than ten years (157/188) having a seropositive rate of 87.05% and 83.51% respectively.

The possible association between the prevalence of measles IgG antibodies and the categorical variables was analysed by Chi-square.

Table 1: Association between study population characteristics history seroprevalence of measles antibodies (measles IgG titre)

Characteristic		Positive		Equivocal		Negative		Total	x^2	P-value
		n	%	n	%	n	%	n		
Total		324	84.38	19	4.95	41	10.68	384	-	-
Age	5-10 Years	114	89.0	4	3.1	10	7.8	128	7.466	0.113
	15-19 Years	101	78.91	11	8.59	16	12.50	128		
	20-25 Years	109	85.16	4	3.13	15	11.72	128		
Gender	Male	156	84.78	10	5.44	18	9.78	184	0.441	0.802
	Female	168	84.00	9	4.50	23	11.50	200		
Country of birth	Kenya	223	83.52	17	6.37	27	10.11	267	3.904	0.142
	Outside Kenya	101	86.33	2	1.71	14	11.97	117		
Years in Eastleigh	2-4 Years	9	90.00	0	0.00	1	10.00	10	6.229	0.398
	5-7 Years	37	78.72	2	4.25	8	17.02	47		
	8-10 Years	121	87.05	9	6.48	9	6.48	139		
	>10 Years	157	83.51	8	4.26	23	12.23	188		

Presence of Neutralizing Antibodies

The conventional neutralising antibody titre was considered to be 1:16 for 100% inhibition of CPE. Syncytium Inhibition Assay (SIA), a neutralisation Assay, was done in all 384 serum samples, out of which 337 (87.76%) were positive and 47 (12.23%) were negative.

Table 2: Correlation of measles NT and ELISA-IgG values of 384 tested respondents' sera

ELISA units	Titres								
	<1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	Total
0-9	25	16	7	0	0	0	0	0	48
10-19	5	57	33	13	0	0	0	0	108
20-29	1	11	71	29	11	0	0	0	123
30-39	0	1	6	26	26	12	7	0	78
40-49	0	0	2	3	5	9	5	3	27
Total	31	85	119	71	42	21	12	3	384

A regression analysis was conducted to examine how well neutralisation titre can predict ELISA results in units. The overall correlation between the Neutralization Assay result and ELISA results was statistically significant [r= 0.870; 95%CI (0.843, 0.892) *P*<.001] (see *Table 2*).

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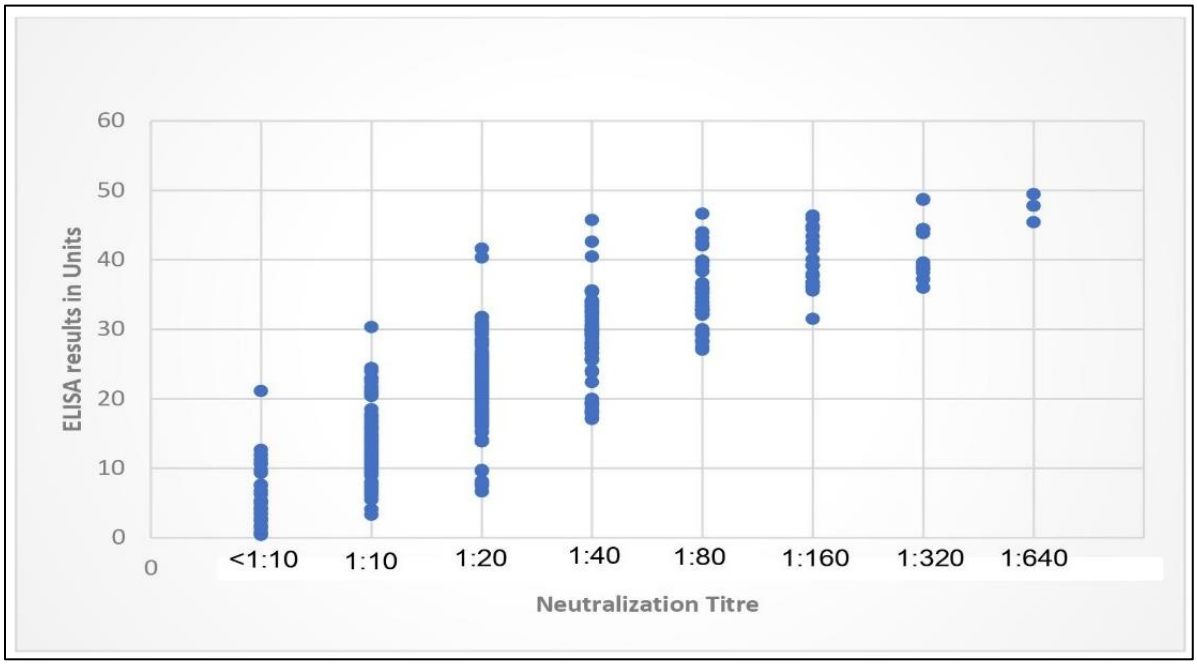
Figure 1: Pearson regression analysis of the 384 respondent sera with the measles ELISA-IgG and the corresponding NT titres



The regression equation for the prediction of ELISA results units using neutralisation titre was $\hat{y}=23.091+6.581x$. The R^2 for this equation was 0.757; that is, 75.7% of the variance in ELISA units was predictable from neutralisation titres can be predicted by this model. This is a moderately strong relationship.

Table 3: Relevant statistical data on regression analysis

Model	R	R^2	Adjusted R^2	RMSE
H_0	0.000	0.000	0.000	11.319
H_1	0.870	0.757	0.756	5.591

ANOVA

	Model	Sum of Squares	df	Mean Square	F	P
H_1	Regression	37131.895	1	37131.895	1188.052	< .001
	Residual	11939.191	382	31.254		
	Total	49071.086	383			

Note. The intercept model is omitted, as no meaningful information can be shown.

Coefficients

	Model	Unstandardised	Std Error	Standardised	t	p	95% CI Lower	95% CI Upper
H_0	(Intercept)	23.091	0.578		39.97	< .001	21.95	24.22
H_1	(Intercept)	1.050	0.700		1.499	0.135	-0.327	2.42
	Titres	6.581	0.191	0.870	34.46	< .001	6.206	6.95

Establishment of Herd Immunity

The Hazlina *et al.* (2016) adaptation of the Plans-Rubio formulae of herd immunity was used in the determination of the critical prevalence of antibodies (pc) associated with herd immunity in order to evaluate if a population has established herd immunity. The herd immunity threshold (Ic) will be 92.5%, the mean of the recommended Plan-Rubio threshold of 91%-94%. We shall first determine the PPV, the missing value of the formulae: pc=IcSe / PPV

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A. Calculation of Positive Predictive Value of Serological Result

Formulae

$$PPV = \frac{(pSe)}{[(pSe)+(1-p)(1-Sp)]}$$

Where: p is prevalence, Se is sensitivity, Sp is specificity

B. Calculation of critical prevalence (pc):

$$pc = \frac{IcSe}{PPV}$$

Where Ic = Herd immunity threshold equals 92.5% based on Plans' indicator, Ic=Ro- 1/ Ro=1 - 1/ Ro, Ic=Ro- 1/ Ro=1 - 1/ Ro, I_c is 91-94%, of which the mean was 92.5% based on Plans with R_o, the basic reproductive number for measles of 11 – 18.

- Worst scenario Assuming all equivocal results are negative
- Best scenario: Assuming all equivocal results are positive
- Absolute scenario: Equivocal results are not presented

(p) Seroprevalence of positive measles antibodies; (pc) Critical prevalence of antibodies associated with herd immunity (HI) Herd Immunity; + Herd Immunity Established (p > mean pc); - Herd Immunity Not Established (p < mean pc) Calculated using Critical Prevalence of Protected Individuals, Ic (%) = 92.5 (91–94).

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Table 4: Seroprevalence of positive measles antibodies (p), critical prevalence of antibodies associated with herd immunity (pc) and establishment of herd immunity (HI) - comparing worst, best and absolute case scenario

Variable		Worst (n=384)				Absolute (n =365)				Best case (n=384)			
		P (%)	CP (%)	95% CI		P (%)	CP (%)	95% CI		P (%)	CP (%)	95% CI	
Total		84.375	0.899895	88.88	91.08	88.767	89.7183	88.59	90.847	89.32	89.69	88.59	90.79
Age	5-10 years	89.063	89.701	87.795	91.607	91.935	89.5387	87.603	91.475	92.18	89.53	87.62	91.43
	15-19 years	78.906	90.3694	88.464	92.275	86.325	89.8657	87.873	91.859	87.50	89.79	87.88	91.69
	20-25 years	85.156	89.9392	88.034	91.845	87.903	89.7695	87.833	91.706	88.28	89.75	87.84	91.65
Gender	Male	84.784	89.963	88.374	91.552	89.655	89.6667	88.033	91.301	90.22	89.63	88.04	91.21
	Female	84	90.014	88.49	91.538	87.958	89.7662	88.206	91.326	88.50	89.73	88.20	91.25
Country of Birth	Kenya	83.521	90.0455	88.726	91.365	89.2	89.693	88.329	91.057	89.89	89.65	88.33	90.96
	Outside Kenya	86.325	89.8657	87.873	91.859	87.826	89.7741	87.764	91.784	88.03	89.76	87.76	91.75
Years in Eastleigh	2-4 years	90	89.6469	82.829	96.465	90.0	89.6469	82.829	96.465	90	89.64	82.83	96.46
	5-7 years	78.723	90.383	87.238	93.528	82.222	90.133	86.919	93.347	82.98	90.08	86.93	93.22
	8-10 years	87.00	89.8241	87.995	91.653	93.077	89.477	87.586	91.368	93.52	89.45	87.62	91.27
	>10 years	83.511	90.0462	88.474	91.619	87.222	89.8106	88.204	91.418	89.77	89.78	88.20	91.35

Note: P = Prevalence (%); CP = Critical prevalence (%); CI = Confidence Interval

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DISCUSSION

In this study, we assessed measles-specific IgG among a subpopulation of urban refugee children, adolescents and young adults and further explored the potential influence of gender, age, place of birth and years of residency in the study area. The positive seroprevalence of measles IgG antibodies in this study was 84%. This is higher than the mean percentage prevalence of individuals in the African region of 77% (Plans-Rubió, 2021). This is consistent with a study by Bernett et al. (2002) of migrants between 0-20 years in the United States that found that 82% of the migrant had antibodies to measles. Children 5-10 years had the measles IgG prevalence of 89.06%, comparatively a study carried out by Kanga et al. (2017) in Narok, Kwale and Lamu looking at the seroprevalence of measles IgG in children 9 months and 59 months was lower at 83%. The differences in seroprevalence could be attributed to the differences in the period of study, the area of study and differences in vaccination coverage. The difference in prevalence between the two studies could further be explained by the Measles and Rubella SIA that was carried out from late June to Early July 2021, targeting 4 million children between 9 months and years in 22 counties including Nairobi. This provided a second opportunity for measles immunisation for the children who had not been immunised during routine immunisation or those who did not seroconvert. Very high seroconversion is usually seen after measles SIAs (Strebel et al., 2003). The high seroprevalence of children 5-10 years indicates urban refugees adhere to immunisation schedules and avail their children of immunisation during campaigns.

In this study, teenagers (15-19) had a lower positive measles antibodies seroprevalence and higher equivocal compared to young adults (20-25) at 78.9% and 85 %, respectively. These results are unique to this study, as a prospective cohort study by LeBaron *et al.* (2007) showed a progressive decline in measles antibodies 10 years after the second vaccine dose of measles; this decline in measles antibodies is due to secondary vaccine failure. The higher measles-specific antibodies in the young adults in this study could be due to previous natural measles infections by some of the respondents in the age group. Waning measles antibodies is less rapid in those who experience natural measles infection than those who were vaccinated (Hickman et al., 2011). Natural immunity to measles is both robust and more enduring to vaccination; however, natural infections frequently have serious complications and are better avoided through vaccination.

The categorical variables that were explored among all respondents in this study included age, gender, country of birth, and years of residence in the study area. Other factors that may contribute to the failure to be immune or get immunisation such as the history of medical illness and medication history, were not investigated.

Different studies have demonstrated that there are gender differences in measles seroprevalence (Domínguez *et al.*, 2006; Poethko-Müller & Mankertz, 2012; Kostinov *et al.*, 2021; Mathew *et al.*, 2022). Nevertheless, it is noteworthy that while some studies observed that male participants were more likely to have immunity against measles (Kostinov *et al.*, 2021; Mathew *et al.*, 2022), others found male participants more likely to be seronegative (Poethko-Müller& Mankertz, 2012). In this study, while the proportion of male respondents seropositive to measles was higher than females, an analysis of differences in gender seroprevalence was not demonstrated, in line with other measles seroprevalence studies (Hazlina *et al.*, 2016; Levine et al., 2015

This study observed that there was no significant difference in measles seroprevalence between respondents that were born in Kenya and those born outside Kenya. A review of the literature on the effect of place of birth as a predictor for positive measles antibodies is inconclusive. McQuillan *et al.*

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(2007) study concluded that the place of birth was a predictor for positive measles antibodies. United States-born Mexican Americans had higher seropositivity for measles antibodies than foreign-born Mexican Americans. This is supported by another study by Poethko-Müller and Mankertz (2012), where foreign-born children in Germany also had a higher risk of susceptibility to measles. However, Hazlina *et al.* (2016) did not find any association between positive measles antibodies titre and place of birth (either in or outside Seremban) or rural or urban locality of birth.

A comparison of ELISA results and neutralisation assay shows that an increase in neutralisation titre leads to increased ELISA values; this is in line with different studies (Ratnam *et al*., 1995; van den Hof *et al*., 2003: Hong et al., 2019; Rabenau *et al*., 2007). The number of positive samples using the Measles IgG ELISA kit was 326, while the 338 samples were positive using the neutralising assay, therefore the sensitivity (true positive) of the ELISA kit with neutralising assay as the standard is 324/337 (96.14%). The specificity (true negative) is 41/47(87.23%). The manufacturer reported sensitivity and specificity were 97.01% and 100%, respectively. The reason for the differences in the specificity and sensitivity between the Measles IgG ELISA kit and neutralising assay could be that while the neutralisation assay detects all isotypes of measles-specific antibodies, the ELISA kit is specific to measles IgG antibodies (Hong et al., 2019; Rabenau *et al.*, 2007). The difference in the sensitivity could be due to the difference in the dilution of sera and differences in the conditions of the assays. Similar to other reports, there is a correlation between the neutralisation test and ELISA; this could be attributed to the wider spectrum and heterogeneity of the involved or measured measles antibodies (Rabenau et al., 2007). Measles ELISA is therefore, a practical alternative to neutralisation tests.

The seroprevalence of the study in all three groups (n=384) was 84%, while the critical prevalence (pc) associated with herd immunity, as calculated using the Plans-Rubio formulae, was 89.98%. The study population has not established herd immunity. Lack of herd immunity has been demonstrated to increase susceptibility to measles in a population. An analysis of the distribution of measles cases during an outbreak of 2006-2007 by Plans-Rubio *et al.* (2014) in Catalonia, Spain, showed that measles cases were more frequent in age groups without herd immunity than those with herd immunity. Another study of the measles outbreak in the Republic of Marshall Islands by Hyde *et al.* (2006) showed that a lack of circulating measles provided false confidence in vaccination programmes that were inadequate, and the result was detrimental when measles was imported into the population. They concluded that the lack of endemic transmission should be supported by maintaining recommended population immunity through high vaccination coverage.

CONCLUSION

All the age groups had antibodies against measles. The critical prevalence, as calculated by Plans-Rubio formulae was higher than the prevalence. Therefore, herd immunity necessary to control or eliminate measles has not been reached in all age - groups; this represents a risk that should be addressed. Adolescents had the highest percentage of negative and equivocal antibody levels among the age groups; hence future supplemental Immunization should include adolescents living in high refugee areas. All the variables tested in this study had no significant association with the development of measles antibodies. Continued high rates of routine two-dose vaccination and supplemental immunisation and periodic seroepidemiological monitoring aimed at assessing risk should be encouraged.

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