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Sero-epidemiology of rubella in the urban population of Addis Ababa, Ethiopia

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SUMMARY

We conducted a community-based cluster sample survey of rubella sero-epidemiology in Addis Ababa, Ethiopia in 1994. Among 4666 individuals for whom complete data were available, rubella antibody prevalence was 91% (95% confidence interval: 90, 92). On multivariable analysis, seroprevalence was lower among individuals who were resident in Addis Ababa for 1 year or less. Approx. 50% seroprevalence was attained by age 4 years, and the estimated average age at infection was 5·2 years. The highest age-specific force of infection was estimated to occur in 5- to 9-year-olds. The early age at infection corresponded with a low estimated incidence of congenital rubella syndrome (CRS) of 0·3 per 1000 live births, equivalent to nine cases of CRS in 1994. The predicted critical level of immunity for elimination of rubella via vaccination was 85–91%, requiring 89–96% coverage with a vaccine of 95% effectiveness. Unless very high coverage of rubella vaccine could be guaranteed, the introduction of childhood vaccination could increase the incidence of CRS in Addis Ababa.

INTRODUCTION

Rubella vaccine has been available for over 30 years, but as yet data on the burden of disease in developing countries have been considered insufficient to make global recommendations for its use [1]. The incidence of congenital rubella syndrome (CRS) in outbreaks in developing countries has been at least as high as that in industrialized countries prior to vaccination [2]. All countries which have documented large outbreaks of CRS now have national rubella vaccination policies [3]. The move in many countries towards accelerated measles control and measles elimination raises the question of the marginal cost of including rubella control strategies with this initiative [4, 5]. Collection of data on the burden of CRS in developing countries is thus a priority [2, 3]. In countries where surveillance of cases of CRS is poorly developed, serological surveys of the age-specific prevalence of antibody provide an alternative method of estimating the burden of CRS [6–8]. We report findings from a large community-based serosurvey of rubella immunity in urban Ethiopia.

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METHODS

A community-based cross-sectional survey was conducted in Addis Ababa in May–October 1994. Full details of the sample design and composition are given elsewhere [9], and only briefly outlined here. Addis

Ababa, the capital of Ethiopia, had an estimated population in 1994 of approx. 2·1 million inhabitants, having experienced high growth since the last census in 1984, when the estimated population was 1·5 million. Administratively, it is divided into five zones (ketenas), each of which is divided into woredas (28 in total), which are further divided into kebeles or urban dwellers' associations. Each kebele consists of approx. 2000 households, with an average of 5·2 persons per household [10]. A cluster sample survey was conducted, 20 clusters being selected by Probability Proportional to Estimated Size sampling [11], in each of two strata comprising the inner city and the outer city. In each kebele, a random sample of 35 house numbers was selected. The lists of houses and household numbers held at the kebele administrations were used as the sample frame in the inner city. In the outer city, where there is greater population migration, these lists were updated for the purpose of this survey. Within each house selected, each household head was listed, and one household selected randomly for registration in the study.

The sample size was based on the required precision in each age category, by single years up to 4 years of age, then 5 yearly age groups up to age 50, assuming a design effect of two in the absence of data on intracluster correlation of seropositivity [11]. A sample size of 150 per age class would give estimates with acceptable precision (7–12%) across the expected range of seroprevalence. This sample size would be obtained in a total sample of 1200 households in Addis Ababa, equally divided between strata, i.e. 20 kebeles of 30 households in each stratum. This was increased to 35 households per cluster to allow for up to 15% non-participation in the survey. We requested blood samples from all children aged under 5 years in every household; 1 in 2 children of school-age (5–14 years), selected randomly; and all adults aged 15–49 years.

All persons aged under 50 years who were part of the selected household were invited to participate in the study. Informed consent was obtained from the head of the household. Persons who were visiting the household and who slept in the selected house the night before the survey were included. If all family members were not present at the first visit, revisits were made until all members had been interviewed and samples obtained.

Two brief questionnaires were administered by trained surveyors to each household. The first contained information about each individual in the household (age, sex, ethnic group, length of residence in Addis Ababa, educational level, current school attendance, history of measles and measles vaccination). The second contained general information about the household (type of construction, number of rooms, type of roofing and floor material, source of water, type of sanitation, etc.).

Approval for the survey was given by the Ethical Committee of the Ethiopian Health and Nutrition Research Institute (EHNRI), and St Mary's Research Ethics Committee, London University, UK.

Specimen collection and processing

Where possible, 5 ml of venous blood were obtained from family members using sterile, disposable needles (21–23G) and Vacutainers (Beckton Dickinson, Banbury, England). Blood samples were transported in cold boxes daily to the laboratory at EHNRI. Serum samples were separated, aliquoted, labelled and stored frozen at -20 °C until analysis.

Laboratory assays

The laboratory was blind to the age of study participants. All serum samples were tested for rubella-specific IgG by radial haemolysis (RH) [7] and results expressed in international units per ml (IU/ml). As previously described [12], the detection limit of the assay under routine use was 8·6 IU/ml. Serum samples which were negative by RH ($< 8{\cdot}6$ IU/ml) for which sufficient residue was available were retested by ELISA (Enzygnost anti-Rubella virus IgG, Dade Behring, Milton Keynes, England); results also expressed in IU/ml. Serum samples which were negative by RH but with ELISA $> 8{\cdot}6$ IU/ml were tested by latex agglutination (LA) (Rubagen, Biokit S.A, Barcelona, Spain) with sensitivity level of 15 IU/ml, if serum residue remained. Consensus rubella antibody status based on agreement in two of these tests was established.

Statistical analysis

Accounting for survey design

In our analyses of seroprevalence account was taken of the survey design using STATA (V6·0) svy commands following the procedure described in detail elsewhere [9]. In brief, first, we applied probability weights to each measurement to account for the different population size in the inner and outer city strata, and the variation in the number of households

from each cluster (kebele) who participated, and second, variance estimation took into account the degree of inter- versus intra-cluster variability (i.e. the lack of independence of samples within clusters) for the first stage of cluster sampling where the kebele was the primary sampling unit.

Classification of immunity to rubella

A positive RH test was taken as indicating immunity to rubella. Of the 754 serum samples negative by RH, 644 had sufficient sera for ELISA testing and 255 were positive. Of these, 249 could also be tested by LA and 229 were confirmed as positive and re-classified as such. The 20 RH-negative, ELISA-positive and LA-negative sera were classified as susceptible; all had low-level rubella antibody in the ELISA (mean 13 IU/ml). For analysis of factors associated with seropositivity, the 117 RH-negative samples that were not re-tested by ELISA and LA were excluded from the analysis. For analysis of the force of infection and incidence of maternal infection, we imputed the antibody status of RH negative samples not repeat tested by ELISA. For these samples, we assigned them as 'true' positive or negative based on the age-stratified proportion of samples which were negative by ELISA and LA among those tested by both assays.

Factors associated with seropositivity

Cross-tables were conducted of demographic and socio-economic variables against seropositivity and the χ^2 test used to assess significance. As this test does not take the cluster sampling design into account, we obtained an adjusted P value for factors showing an apparent significant association using the svymean command in STATA. Factors significant on univariate analysis were included in a logistic regression model using STATA svylogit command. A stepdown procedure was followed, dropping variables which did not contribute significantly to the model.

Age-specific incidence of rubella and incidence of maternal infection

Catalytic infection models were used to investigate age-dependence in the instantaneous incidence of rubella infection; also known as the force of infection [13–16]. The analysis is based upon the following assumptions: (i) the proportion serologically positive at age a, $p(a)$, defines the cumulative proportion infected by age a, and further assumes that no individual dies from post-natal rubella infection; (ii) seropositivity is non-reversible, i.e. individuals cannot revert from positive to negative; (iii) there has been no significant change in the force of infection over time in the population, such that differences in the force of infection identified in the age-profile reflect age-related rather than time-related changes in incidence. The form of age-dependence in the force of infection was identified in the data using graphical means [13, 17], in particular the cumulative per person incidence, $G(a) = -\ln(1-p(a))$, and by examination of point estimates of the force of infection calculated directly from the data using the expression $\lambda = -\ln(x(a+\Delta a)/x(a))/\Delta a$ [18], where $x(a) = 1-p(a)$ is the proportion seronegative at age a and Δa is the age difference over which the force of infection is being estimated. We subsequently applied two models to the data. First, a piece-wise constant (PWC) model [19] in which the force of infection, λ_i, in age class i, is constant, but can vary between age classes $i = 1, m$, hence the predicted proportion susceptible, $x'(a)$

$$x'(a) = x'(a_{i-1}) \exp[-\lambda_i(a-a_{i-1})] \quad a > D, \tag{1}$$

where i is such that $a_{i-1} \leqslant a \leqslant a_i$, and $\lambda_i \geqslant 0$. Thus the model describes an exponential decay in the proportion seronegative between age a_{i-1} and a where the rate of decay is dependent upon the force of infection applying in that age interval, λ_i. D is the average duration of maternal antibody protection, taken to be 3 months (unpublished data from Addis Ababa provided by Senait Kebede), and, for simplicity, $x(a) = 0$, $p(a) = 1$, for $D \leqslant 3$ months. The proportion seropositive, $p'(a)$ is simply $1-x'(a)$.

Secondly, we assumed the force of infection, $\lambda(a)$, is an exponentially damped linear (EDL) function of age, a, as described by Farrington [13], such that

$$\lambda(a) = (b_0 a - b_2) \exp(-b_1 a) + b_2 \tag{2}$$

and the proportion seronegative is

$$x'(a) = \exp\left[\frac{b_0}{b_1} a \cdot \exp(-b_1 a) + \frac{1}{b_1}\left(\frac{b_0}{b_1} - b_2\right)(\exp[-b_1 a] - 1) - b_2 a\right]. \tag{3}$$

Again, the proportion serologically positive is $p'(a) = 1-x'(a)$. The model describes a decay, with age, in proportion seronegative dependent upon a force of infection that has an initial near linear rise with age followed by a peak and subsequent exponential decline (the value of b_2 in (2) defining the long

term residual value of $\lambda(a)$). The duration of maternal antibody protection was implicitly modelled via the assumption that $\lambda(0) = 0$.

Although the PWC model allowed for flexibility in the form of age-dependence in the force of infection, and also enabled us to assess the significance of model fit to data as we increased the number of force of infection age classes, it did require us to define the age classes over which the force of infection applied (i.e. the limits of the age classes were not variables whose optimal solution was sought). The EDL model is not constrained by pre-defined age classes, and also has been shown to describe well the pattern of $\lambda(a)$ for a variety of childhood viral infections in various settings [13, 20].

Maximum-likelihood (ML) estimates (implicitly weighting for sample size) for parameters in each model were obtained using the procedure described by Nokes and colleagues [15] within Microsoft EXCEL using the Quasi-Newton search method. In deriving the 95% confidence intervals (CI) for each force of infection estimate in the PWC model we assumed the log-likelihoods approximate to a χ^2 distribution [21]. Having obtained a ML fit to the data, we allowed a single parameter estimate to vary (either increasing or decreasing to obtain upper or lower confidence limits) until the log-likelihood decreased by 1·92, keeping all other parameters constant. Fixing the estimate of the parameter of interest at this value we then allowed all other parameters to vary to obtain a maximum-likelihood fit to the data, and repeated the process iteratively until no further change in the log-likelihood occurred. We repeated this process to obtain 95% CI for each of the parameters in turn. Validity of the model fit to data was assessed graphically by the scatter of standardized residuals of the form,

$$s = \frac{p(a) - p'(a)}{\sqrt{([p'(a)(1-p'(a))]/n)}}, \tag{4}$$

where $p'(a)$ was the proportion seropositive by age a predicted by the model [equations (1) or (2)], and n was the sample size in age group a [15]. Comparison of goodness of fit to the data between models was made using the likelihood ratio test [21].

If we take the average gestation period to be 40/52 years then to a good approximation the number of maternal infections per 1000 pregnancies can be estimated by [19]

$$M = \bar{x}[1 - \exp(40\lambda_{15\text{–}49}/52)] \times 1000, \tag{5}$$

where $\lambda_{15\text{–}49}$ was assumed to be a constant force of infection acting on susceptibles in the child-bearing age range 15–49 years, and $\bar{x}$ the average predicted proportion susceptible in this risk group. $\lambda_{15\text{–}49}$ was estimated from either the PWC [equation (1)] or EDL [equation (2)] model, whichever provided the best fit to the data, as the average force of infection over the at-risk age interval. Implicitly we assumed that seronegativity equates with susceptibility to infection. Since there is negligible probability of a fetal abnormality arising from primary rubella infection after the first 16 weeks of pregnancy [22], and given a weighted average probability of CRS over these 16 weeks of 0·65 [8], it follows that the estimated number of CRS cases per 1000 pregnancies is

$$\text{CRS} = 0{\cdot}65\bar{x}[1 - \exp(-16\lambda_{15\text{–}49}/52)] \times 1000. \tag{6}$$

Approximate 95% CI can be estimated by substituting into (5) or (6) the CIs for $\lambda_{15\text{–}49}$ [19].

Summary epidemiological parameters

The average (arithmetic mean) age of infection, A, across all ages at the time of a cross-sectional survey was defined as [23]

$$A = \frac{\int_0^\infty a\lambda(a)X(a)\,da}{\int_0^\infty \lambda X(a)\,da}. \tag{7}$$

$X(a) = x'(a)N(a)$ where $x'(a)$ was predicted using equations (1) or (3), and $N(a)$ was the number of individuals of age a in the urban population of Addis Ababa, 1994. The solution to equation (7) was approximated on EXCEL (Microsoft Corporation) using a discretized form with age interval of 0·1 year and upper age of 99 years.

Making the simplifying assumption of an average force of infection acting on susceptibles distributed across all ages, then the Basic Reproduction Number for rubella in Addis Ababa was approximated by the expression [24, 25]:

$$R_0 \approx 1 + \frac{B}{A - D}, \tag{8}$$

where B was the reciprocal of the crude birth rate (CBR) per 1000 population per year, and assumed a Type II mortality schedule (i.e. constant instantaneous death rate across all ages). Census data show that in 1994 the population of Addis Ababa was predominated by young individuals (32% $<$ 15 years old), which was an important determinant of the average age at infection, A. Therefore, for a given

Table 1. *Univariate analysis of potential factors associated with seropositivity to rubella, Addis Ababa, 1994 ($n = 4549$)*

Variable	Levels	*n*	% positive	*P* (unadjusted)*
Age	< 15	1750	81·6	0·0000
	15+	2799	96·9	
Sex	M	1813	90·6	0·7
	F	2736	91·3	
Sex, 15+ yrs	M	978	96·7	0·001
	F	1821	93·8	
Stratum	Inner city	2359	89·5	0·03
	Outer city	2190	87·5	
Length of residence	All life	2890	86·6	0·000
	> 1 year	1454	93·5	
	⩽ 1 year	205	81·5	
Ethnic group	1, Amhara	2184	90·7	0·8
	2, Oromo	789	90·6	
	3, Tigre	453	92·5	
	4, Gurage	830	91·2	
	5, Other	278	91·4	
Education	None	686	91·0	0·4
	1–6 years	985	89·5	
	7–12 years	1103	91·6	
	> 12 years	544	91·4	
Ownership of house	Rent/other	2655	91·1	0·9
	Own	1859	91·0	
Type of wall	Wood/bamboo/ canvass	4014	91·1	0·4
	Bricks/stone	509	90·0	
Type of roof	Corrugated iron	4415	91·1	0·3
	Stone/concrete/ tile	111	88·3	
Number of people in household	< 5	658	90·6	0·3
	5–9	2616	90·6	
	10+	1275	92·1	
Number of beds	0–1	912	89·4	
	2	1265	90·9	0·1
	3+	2343	91·7	
Electricity supply	Private meter	3161	91·6	0·04
	Other	1359	89·6	
Ownership of radio	No	717	89·5	0·1
	Yes	3803	91·3	
Source of water	Public source	593	89·9	0·6
	Shared tap	2028	91·2	
	Own tap	1898	91·1	
Number sleeping in same room	⩽ 2	1115	93·4	0·004
	3–5	2223	89·8	
	⩾ 6	1208	91·0	
Number of rooms	1	950	90·4	0·5
	2	1580	90·7	
	3+	1994	91·5	

* Adjusted *P* values as follows: Age; $P < 0{\cdot}0001$; sex (15+years) $P = 0{\cdot}001$; stratum, $P = 0{\cdot}02$; residence, $P < 0{\cdot}0001$, electricity supply, $P = 0{\cdot}08$; number sleeping in same room, $P = 0{\cdot}0001$, for comparison between 3·5, and < 3 people.

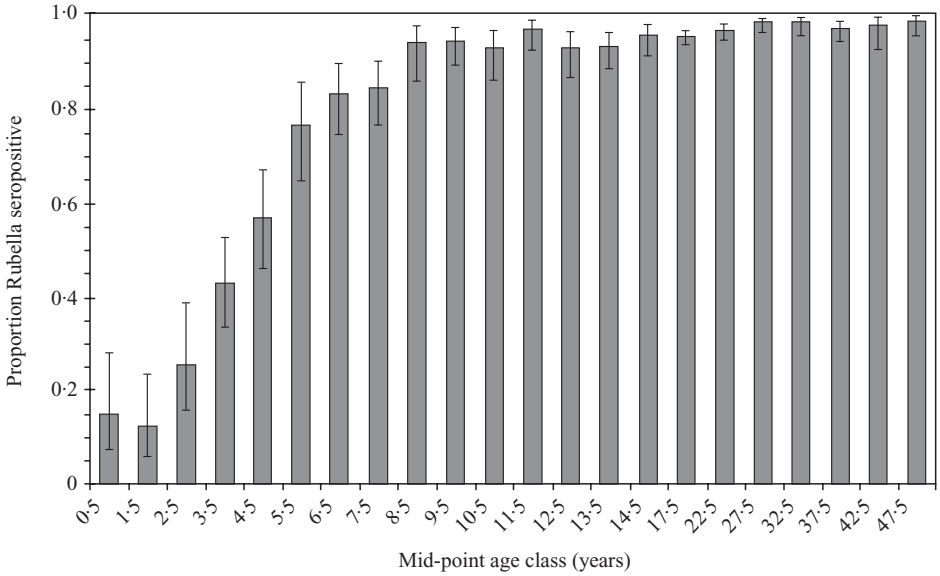

Fig. 1. Age-prevalence of rubella specific antibodies (95% confidence limits) in a representative sample of individuals ($n = 4666$) from Addis Ababa 1994. Age groups are yearly up to age 14, and 5-yearly thereafter.

reproductive capacity of the infection (R_0), the higher the proportion in the younger age classes, the lower the average age at infection. This explains why the term B is present in equation (8), such that a low average age at infection may not in itself reflect a high R_0, rather a low A may reflect low B (high CBR). However, census data over the past 30 years indicated the CBR in Addis Ababa to have fallen from 35·5 in 1967, to 32·2 in 1974, to 23·2 in 1984, to 15·5 in 1994. Hence to take account of this changing pattern we estimated R_0 using a range of values of B derived from average CBRs for the periods 1967–74, 1974–84, 1984–94, which are, 29·4, 37·0 and 53·8 years, respectively.

From equation (8) the critical level of immunity above which infection was predicted to be eliminated by vaccination (the herd immunity threshold), was defined as [26]

$$H = 1 - 1/R_0. \quad (9)$$

RESULTS

A total of 8638 persons were registered in the study. Of these, serum samples were obtained from 4777 but for 97 insufficient was available for analysis; a further 5 were excluded because the identification number did not match that on the questionnaire. Of the 4675 individuals with adequate serology and questionnaire information, 9 were excluded because of missing data on age. The characteristics of the sample in comparison to the census data for Addis Ababa have been presented in detail elsewhere [9]; in general, adult men were under-represented.

Factors associated with seropositivity

Of the 4666 serum samples 746 (16%) were negative by RH assay. However, a substantial number of these were false negatives, and after adjustment for results of repeat assays, 409 (9%) of 4549 sera were negative in at least two of the three assays. The unweighted proportion seropositive was thus 91%. The weighted seroprevalence, accounting for cluster size and stratum, was virtually unchanged at 90·9% (95% CI, 89·9, 91·9%). The estimated design effect (the ratio of the standard error in a cluster survey to that which would have been obtained with a simple random sample of the same size) was 1·42.

Table 1 presents results of univariate analysis of factors associated with seropositivity. Overall, there was no difference in seropositivity by sex. When adults over 14 years were examined, seroprevalence was slightly but significantly lower in women (93·8% *vs.* 96·7%, $P = 0{\cdot}0001$). Seroprevalence was higher in persons who had lived in Addis Ababa more than one year but less than lifelong. This relationship was confounded by age and sex, however, more adult males being in this group. Individuals who slept in rooms with more than two other persons were less likely to be seropositive on univariate analysis, but this was confounded by age as these were more likely to be children. Seroprevalence was slightly higher in the inner than outer city (89·5 *vs.* 87·5%, $P = 0{\cdot}03$).

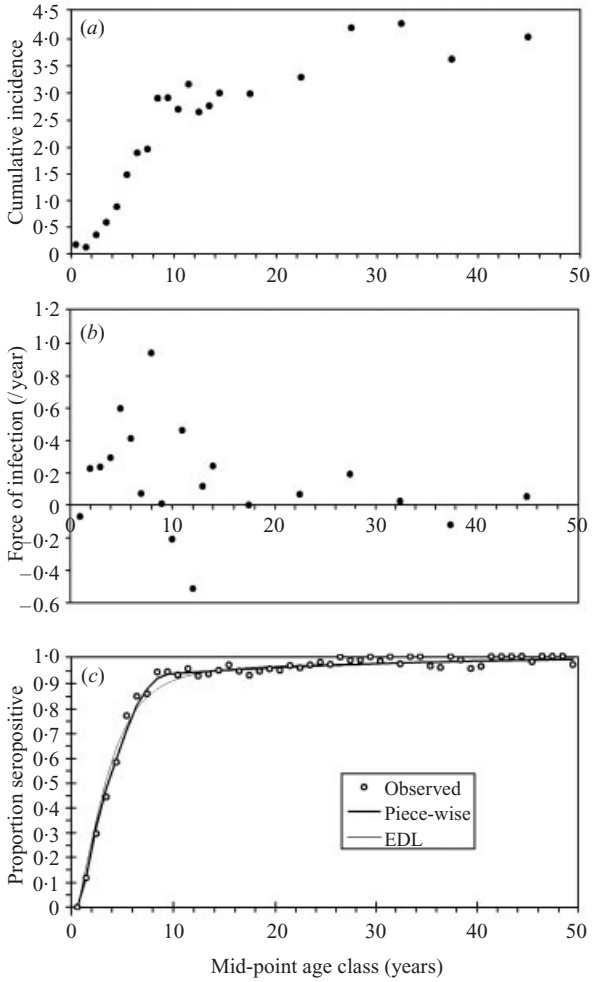

Fig. 2. Modelling age-prevalence of rubella. (*a*) Cumulative incidence of rubella as a function of age, $G(a)$. (*b*) Point estimates of age-specific forces of infection (/year). (*c*) Maximum-likelihood fit of a minimally specified (3 parameter) piece-wise constant (PCW) model [thick line, equation (1)] and an exponentially damped linear [EDL, equation (3)] model (thin line) to rubella age-prevalence data from Addis Ababa, 1994 (open markers). Sample sizes for each age class from 0, 49 years: 56, 68, 71, 97, 125, 109, 92, 141, 126, 145, 176, 116, 180, 155, 157, 134, 224, 156, 219, 153, 175, 90, 141, 97, 96, 136, 53, 75, 82, 42, 118, 28, 70, 32, 31, 133, 46, 51, 73, 21, 103, 16, 31, 23, 18, 91, 19, 15, 30, 30.

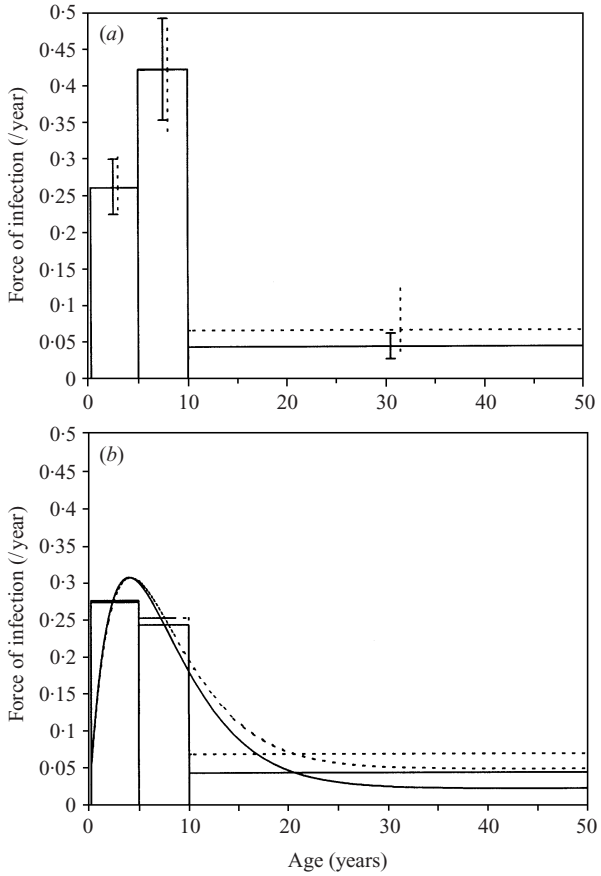

Fig. 3. Age-specific forces of infection, λ. (*a*) Estimates using a PWC model (with 95% CI) and (*b*) EDL model corresponding to the maximum-likelihood fitted models of Figure 2*c*. Full lines in each graph show results for raw data, and dashed lines for data adjusted for the estimated age-specific false negative probability (see Fig. 4*c*). Parameter estimates (95% CI) for the EDL model (*b*) are $b_0 = 0{\cdot}203$ (0·172, 0·243); $b_1 = 0{\cdot}254$ (0·218, 0·299); $b_2 = 0{\cdot}0207$ (0·0000, 0·0460), (raw data, full line) and $b_0 = 0{\cdot}191$ (0·161, 0·230); $b_1 = 0{\cdot}254$ (0·211, 0·308); $b_2 = 0{\cdot}0471$ (0·0000, 0·0810) (adjusted data, dashed line). In (*b*) average values of λ are shown for age classes corresponding to those in (*a*) for comparison.

Seroprevalence varied little and was not significantly associated with ethnic group or indicators of socio-economic status such as education, household size, type of housing or roof construction, number of rooms in the house, ownership of the house, number of beds owned, possession of a radio, or presence of piped water in the house.

On logistic regression, in addition to the interaction term for age (adult *vs.* child) and sex, only length of residence in Addis Ababa remained significantly associated with seropositivity. Adults were more likely to be seropositive than children (OR 14·4 for men, 6·2 for women ($P < 0{\cdot}0001$)) and individuals who had lived a year or less in Addis Ababa were less likely to be seropositive (OR 0·45, $P = 0{\cdot}0001$).

Age-specific incidence of rubella

Seroprevalence increased sharply up to age 8 years, when 94% were seropositive. After this age there was a very slow increase, and 98% of 45- to 49-year-olds were seropositive (Fig. 1).

Preliminary examination of the total (males and females) data using the cumulative incidence function $G(a)$ (Fig. 2*a*) and point estimates of $\lambda(a)$ (Fig. 2*b*)

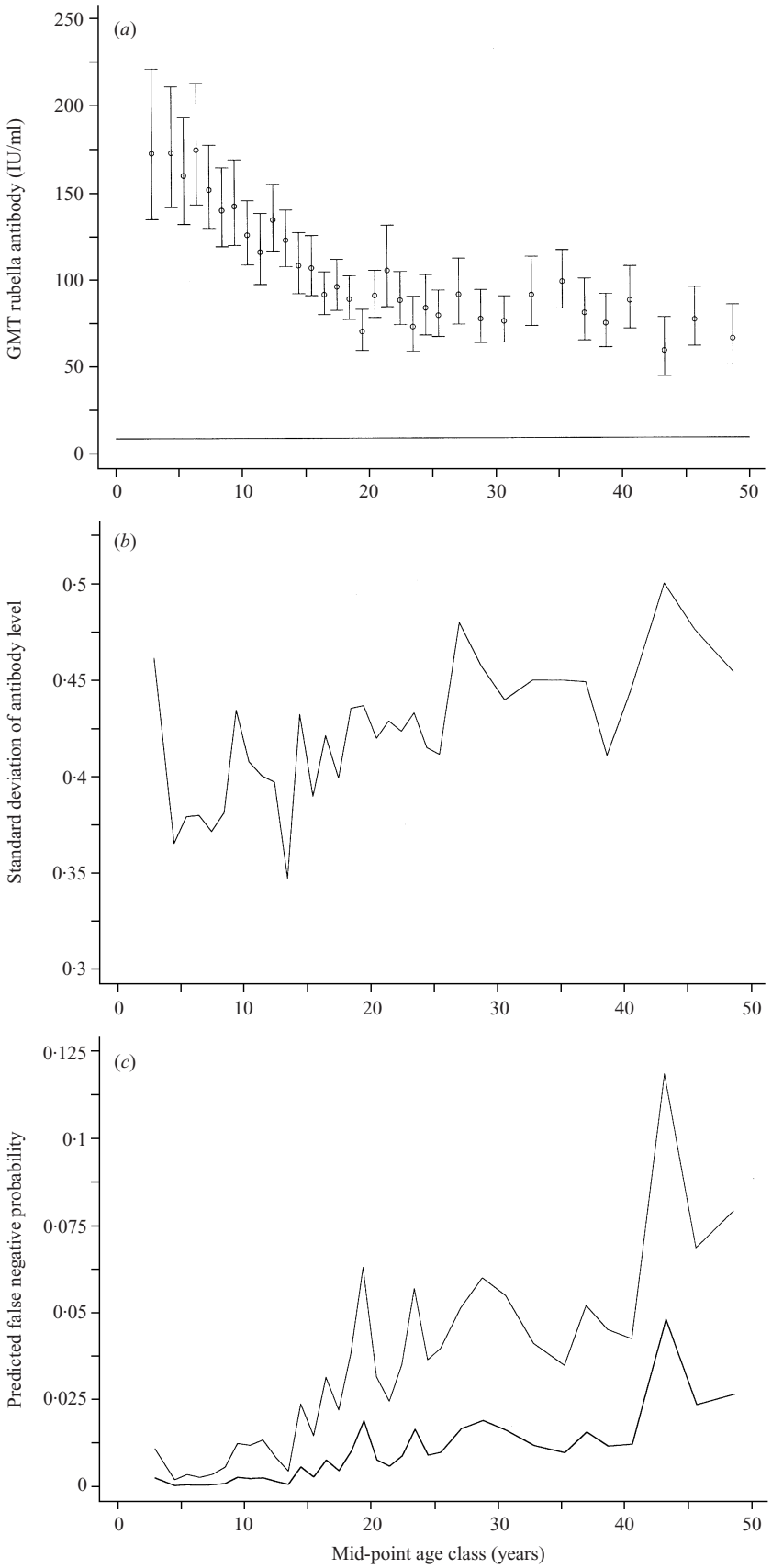

Fig. 4. Changes in rubella-specific antibody levels with age. Graphs show the changes with age in (*a*) GMT (95% CI) (horizontal line indicates threshold of 8·6 IU/ml; (*b*) standard deviation of $\log_{10}$-transformed antibody level, and (*c*) the

suggests three phases in the force of infection by age namely, low and rising in early childhood (0·25–4 years), highest in age range 5–9 years, and decreasing to low level in the age class 10–49 years. Using these age groups as a baseline we explored the data using the PWC model, and assessed the improvement in model fit for fewer age classes or for an increase in classes using the following range: $i = 0{\cdot}25$ to < 1, 1, 2, 3, 4, 5–9, 10–14, 15–19, 20–29, 30–49 years. Figure 2*c* shows the maximum-likelihood fit of the minimally specified PWC model (thick line) to the raw data with three force of infection age classes (0·25–4, 5–9, 10–49 years). Reduction, or addition, of further age classes showed no statistical improvement in log-likelihood ratio. The maximum-likelihood fit of the EDL model to the data is shown by the thinner line in Figure 2*c*. The corresponding scatter of standardized residuals for each model fitted to the data suggests a better overall explanation of the data by the PWC model compared with the EDL model (not shown), and this is supported by a significantly lower log-likelihood value for the PWC model ($-2 \times$log-like PWC 2050.29; EDL 2066.71; both models have three parameters and hence are comparable using log-likelihoods).

The corresponding maximum-likelihood estimates of the age-dependent forces of infection (/susceptible/year) for rubella are given in Figure 3 (solid lines). Figure 3*a* shows the λ_i estimates for the PWC model (with 95% CI), and Figure 3*b* shows $\lambda(a)$ for the EDL model (with bars showing averages over the same age ranges as in graph *a*; 95% CI for each parameter are given in the figure legend). The relationship with age is similar for both models, with markedly higher instantaneous incidence in children (< 10 years) relative to older ages, although the peak force of infection of 0·42/susceptible person per year for the PWC model in the age class 5–9 years is higher, and in an older age class, than the peak of 0·31 at age 4 years for the EDL model.

Antibody levels (GMT) showed a decline with age primarily over the age range 1–20 years, with little evidence of further decline in the age group 20–49 years (Fig. 4*a*), although the variation in antibody levels (log-transformed, Fig. 4*b*) continued to rise throughout the age range. Log-transformed antibody levels showed a near normal distribution for each age class (not illustrated), and on this basis we estimated the proportion of individuals with previously detectable antibody levels expected to have levels which have decayed below a defined threshold (8·6 or 15 IU/ml). Figure 4*c* shows that this proportion rises throughout the age range, such that for adults (> 20 years) around 1–2% are predicted to have levels less than 8·6 IU/ml (thick line) and 3–5% less than 15 IU/ml (thin line).

Using the data shown in Figure 4*c* with threshold 8·6 IU/ml, we computed the expected number of false negatives for the sample of positives in each age class and adjusted the observed numbers accordingly. Revised estimates of the age-specific forces of infection are shown as dotted lines in Figures 3*a*, *b*, and reveal the most significant change in the older age class, with over 50% increase in the estimated force of infection.

Predicted incidence of maternal rubella and CRS

Separate analyses for males (dark grey) and females (light grey) using the PWC model with three age classes yield estimates of λ_i shown in Figure 5 for raw data (solid bars) and data adjusted for false negatives (dashed bars). Based upon equations (5) and (6) we predicted the number of cases of primary maternal infection to be about 1/1000 pregnancies or 33–35 in total in Addis Ababa 1994 (assuming 32237 births in Addis Ababa in 1994 reported in the 1994 census), resulting in an estimated 9 (95% CI: 5, 13) cases of CRS in 1994 (Table 2). There was little difference in the results using the raw data or that adjusted for false negatives [the higher force of infection from adjusted data is balanced by a smaller fraction susceptible (Table 2)]. Note that these estimates based upon the adjusted data make the assumption that individuals whose antibody levels have waned below the level of detection remain immune to re-infection.

Summary epidemiological parameters

Approx. 50% of individuals have evidence of past infection by age 4 years (Fig. 1, 2*c*). The estimated average age at infection from equation (7) is 5·2 years. From equation (8) and adopting a range of values of B (1/CBR), the estimated basic reproduction number, R_0, lies within the range 6·9 and 11·8 (the latter assuming more recent lower CBR, thus higher B), with corresponding estimates of the critical level of

proportion of previously seropositive individuals predicted to be falsely antibody-negative given a threshold antibody level of 8·6 (thick lower line) or 15 (thin upper line) IU/ml. Analysis excludes individuals with antibody levels $< 8{\cdot}6$ IU/ml (i.e. seronegative), and age classes merged where sample size was lower than 50.

Table 2. *Predicted incidence of primary maternal rubella infection per 1000 pregnancies and of CRS per 1000 live births in Addis Ababa, Ethiopia* [*see equations* (5) *and* (6) *in text*]

Data	$\lambda_{15\text{–}49}$ (/year) (95% CI)	$\bar{x}$	Maternal infection per 1000 pregnancies (95% CI)	Cases of maternal infection, Addis Ababa, 1994*	Incidence of CRS per 1000 live births (95% CI)	Cases of CRS, Addis Ababa, 1994*
Raw	0·0402	0·0332	1·01	33	0·265	9
	(0·0605, 0·0212)		(1·52, 0·537)	(49, 19)	(0·398, 0·141)	(13, 5)
Adjusted	0·0601	0·0240	1·085	35	0·286	9
	(0·0845, 0·0377)		(1·51, 0·686)	(49, 22)	(0·400, 0·180)	(13, 6)

* In 1994 there were 32237 live births (census 1994).

effective immunity through vaccination for elimination, H, of 85–91%.

DISCUSSION

In our large community-based study of rubella seroepidemiology, immunity to rubella was acquired early in life, with 94% of 8 year old children having detectable antibodies. The low estimated average age at infection of around 5 years reflects in part the high λ estimates and in part the demographic characteristics of this population, where 32% are under 15 years old. The average age at infection is higher than that of 2–3 years estimated in The Gambia in 1976 [23], but lower than other published estimates of 6 years in Brazil [27], 8 years in Mexico [28] or 9–12 years in England and Wales, Germany and the USA in the pre-vaccination era [18]. Correspondingly, the basic reproductive rate, R_0, estimated from our study (6·9–11·8) is somewhat higher than the 6–7 estimated previously for England and Wales and Germany, but lower than that in The Gambia [23]. Estimates of R_0 reported here are very approximate as they do not account for age-heterogeneity in transmission [23] and because of the changing demographic pattern; assumptions about the timing of the decline in birth rate having relatively large effects on the average age of infection and R_0. However, our approximate estimates of R_0 between 7 and 12 suggest a level of immunity for elimination of around 85–91%. Rubella vaccine is highly immunogenic, most studies showing at least 95% seroconversion [3]. Given a vaccine of 95% effectiveness, the level of coverage required would be 89–96%. This compares to the current reported level of measles vaccination coverage in Addis Ababa of 80–85% and in Ethiopia as a whole of 52% (WHO, unpublished data, 1997).

Data on the seroprevalence of rubella in Addis Ababa, which is a very densely populated city, cannot be extrapolated to the whole of Ethiopia. A WHO collaborative study in the Americas in 1967–8 showed urban–rural differences in susceptibility to rubella of 43 *vs.* 51% in Jamaica, 38 *vs.* 65% in Panama, and 22 *vs.* 40% in Peru [29]. In contrast, results from the same collaborative study in Argentina, Brazil, Chile, Trinidad and Tobago, and Uruguay showed few urban–rural differences, and Gomwalk and colleagues [30] found no urban–rural differential in Imo State, Nigeria. Preliminary data from a study in one rural area of Ethiopia show that while rubella seropositivity increases slightly more slowly than in Addis Ababa,

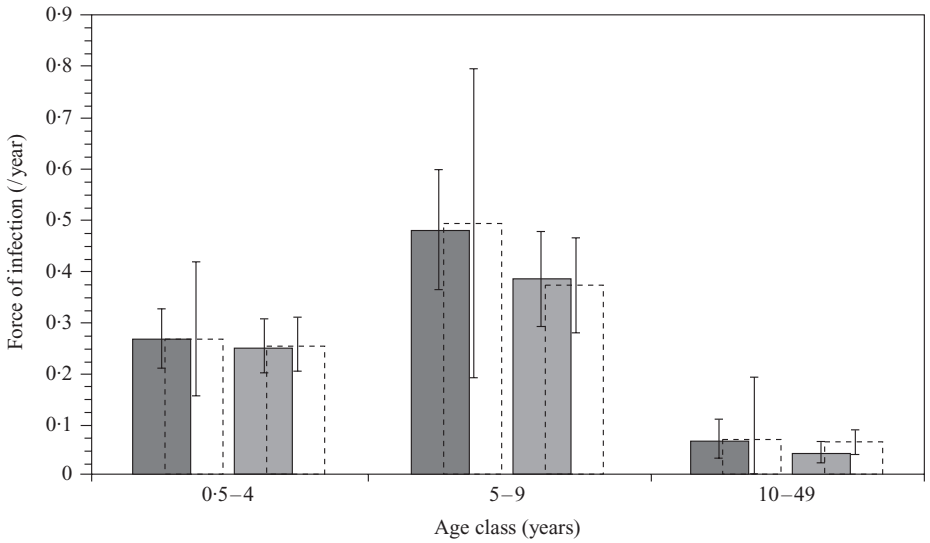

Fig. 5. Age- and sex-specific forces of rubella infection (95% CI) in Addis Ababa, 1994, estimated using a PWC catalytic infection model [equation (1)]. Males (dark bars), females (light bars), raw data (full bars), adjusted data (dashes).

> 90% of 10- to 19-year-olds are immune [12]. Our finding of a significant association between residence for more than one year in Addis Ababa and seropositivity supports the likelihood of a higher force of infection in Addis Ababa than elsewhere in Ethiopia.

The significantly lower proportion seropositive in adult women than men is difficult to explain, as it remained significant after including length of residence in the model. Repeated exposure to rubella via contact with children would normally be higher in women, but in the crowded urban environment exposure was likely to be high for everyone. The absolute difference in seroprevalence was small (2%). If our estimates for the age-specific probability of false negatives are correct (1–2% in individuals 20–49 years) then, after adjustment, this difference becomes negligible, with the result that there is no difference in the force of infection for adults between the sexes (see Fig. 5). The estimated force of infection in adult women was low [$\lambda = 0{\cdot}04$/year (raw data), 0·06 (adjusted)] and only 3·3% (2·4%) of women were considered susceptible to rubella, with a correspondingly low estimated incidence of maternal infection and an estimated total of nine CRS cases in Addis Ababa in 1994. This is similar to the estimate of maternal infection of 1·3/1000 in Sao Paulo, Brazil and 1·2 in Cordoba State, Mexico but lower than that in other African countries (Côte d'Ivoire, Gabon, Nigeria and Zambia) for which thes estimates were available [8]. While some cases of CRS will be expected, the public health burden is relatively low, and it would be hard to improve on the immunity profile of adults acquired via natural infection through a vaccination programme in this low income country. Results from the analysis of Anderson and May [18, 23], bearing in mind various simplifying assumptions they make (age-independent mixing and mortality), suggest that with an $A = 3$–6, infant vaccination will (in the long term) worsen the pre-vaccination incidence of CRS, potentially many-fold, until effective vaccination coverage approaches the critical level for elimination (of around 90% in Addis Ababa).

There are constraints on the use of data from a cross-sectional survey to estimate the transmission dynamics of rubella. Epidemics of rubella occur at varying intervals, usually of 4–6 years [2], causing variation in the age-specific prevalence of rubella antibody over time. However, since seroprevalence reflects cumulative exposure over time/age, the impact of the epidemic nature of infection will be observable predominantly in the younger ages. Thus, the periodicity of epidemics is unlikely to change the finding that most persons acquire infection before childbearing age in Addis Ababa. We used the radial haemolysis test to quantify rubella antibody levels in sera. This is an inexpensive test that was easy to establish in Ethiopia, and has been well evaluated in previous studies [7]. We have found that the RH assay tends to give negative results in adults who are sero-positive by ELISA and presumably have relatively low antibody levels reflecting waning immunity [12]. Hence we confirmed negative samples using an ELISA and latex agglutination. The use of a cut-off of 8·6 IU/ml is

comparable to most commercial ELISA methods, and is similar to the threshold for rubella immunity of 10 IU/ml suggested by others [20, 31].

The implications for rates of transmission and CRS incidence of an increase in the probability of false-negative individuals with increasing age, estimated from this study, are dependent upon our assumptions about immunity in these individuals. Although reinfection has been documented in individuals with low antibody levels, it appears to result in a low-grade viraemia, with a much reduced risk of transmission to contacts or to a fetus [32, 33]. Rare CRS cases have nonetheless been documented in vaccinated women known to have been seropositive on at least one previous occasion [34].

Age-dependence in the force of infection has not previously been so thoroughly investigated in a developing country setting. Elucidation of age differences requires large sample sizes over a wide age range and fine age stratification, ideally from representative samples of the population under study. Such studies are few [7, 20, 35]. A functional form for age-dependence in λ put forward by Farrington [13] and shown to fit data from widely different locations of England and Wales and Sao Paulo, Brazil, was unable to describe data from Addis Ababa adequately. There was no statistical evidence for a rise in λ over the 0–4 years age group, or for any differences in λ in 10+ years ages. The clear distinction in λ between 1- to 4-year-olds and 5- to 9-year-olds may reflect school attendance, and the decrease into teenages and adulthood reflect fewer infectious contacts within these age groups due to the dwindling supply of susceptibles.

In the past, rubella has not been considered a public health priority in developing countries, and this is still appropriate in the poorest countries that have high infant, child and maternal mortality and poor infrastructure for health and education services. However, developing countries span a wide range of economies and demographic structures. Outbreaks of CRS are highly distressing to parents and health professionals. As more countries introduce rubella vaccine into their routine immunization programme, there will be increasing pressure to include rubella vaccine in the global Expanded Programme on Immunization. It would be difficult and expensive to obtain data on the current burden of CRS in every country, on which a policy decision about vaccination could be made at the local level. It is more likely that recommendations will follow regional or global priorities and, should a global measles eradication goal be declared, the inclusion of rubella vaccine would be attractive. Nonetheless, our results demonstrate that it is essential that any country planning to introduce rubella vaccine must have the economic, logistical and technical capacities and commitment to sustain and monitor an effective programme.

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