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Original Contribution

The Natural History of Respiratory Syncytial Virus in a Birth Cohort: The Influence of Age and Previous Infection on Reinfection and Disease

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This study aimed to quantify the effect of age, time since last infection, and infection history on the rate of respiratory syncytial virus infection and the effect of age and infection history on the risk of respiratory syncytial virus disease. A birth cohort of 635 children in Kilifi, Kenya, was monitored for respiratory syncytial virus infections from January 31, 2002, to April 22, 2005. Predictors of infection were examined by Cox regression and disease risk by binomial regression. A total of 598 respiratory syncytial virus infections were identified (411 primary, 187 repeat), with 409 determined by antigen assay and 189 by antibody alone (using a "most pragmatic" serologic definition). The incidence decreased by 70% following a primary infection (adjusted hazard ratio = 0.30, 95% confidence interval: 0.21, 0.42; $P < 0.001$) and by 59% following a secondary infection (hazard ratio = 0.41, 95% confidence interval: 0.22, 0.73; $P = 0.003$), for a period lasting 6 months. Relative to the age group <6 months, all ages exhibited a higher incidence of infection. A lower risk of severe disease following infection was independently associated with increasing age ($P < 0.001$) but not reinfection. In conclusion, observed respiratory syncytial virus incidence was lowest in the first 6 months of life, immunity to reinfection was partial and short lived, and disease risk was age related.

birth cohort; estimation; incidence; reinfection; respiratory syncytial virus; risk

Abbreviations: ADI, antigen-determined infection; CI, confidence interval; CYO, child-years of observation; HR, hazard ratio; $\log_{10}$(AU), logarithm (base 10)-transformed arbitrary units; LRTI, lower respiratory tract infection; SDI, serologically determined infection.

Respiratory syncytial virus is the major viral pathogen associated with lower respiratory tract infection (LRTI) worldwide (1, 2). The development of an effective vaccine and of strategies for intervention requires a full understanding of the virus-host interaction. Respiratory syncytial virus is characterized by recurrent epidemics (3–5), repeated reinfection throughout life (6–8), and age-related incidence of severe disease (6, 9–11). The epidemic nature of respiratory syncytial virus suggests the development of immunity to infection that constrains continued transmission. However, repeated reinfections argue for the absence or loss of immunity to reinfection. This apparent paradox can be reconciled by invoking combinations of partial and waning immunity to reinfection (12–14). Evidence suggests that primary infection results in the most severe disease and that reinfections have lower risk of disease (6), although some studies show no evidence for decrease in risk from second as opposed to further reinfections (7, 11). However, the effect of infection history is confounded by age as severity decreases with age (15–17) as reinfections occur, by definition, in older individuals. A high incidence of respiratory syncytial virus disease in infants (10, 11) occurs despite the passive transfer of maternal specific immunity (18).

These observations define a set of unresolved questions on the natural history of respiratory syncytial virus. First, to what degree does infection confer immunity to reinfection

and for how long? Second, in what way does the rate of infection vary with increasing age, and how does this influence the observed age-related pattern of disease occurrence? Third, how do the 2 factors of age and past exposure influence the risk of disease?

These questions are addressed by using data from a large birth cohort closely monitored for respiratory syncytial virus infections and associated disease (10, 18–20). The present analysis incorporates unpublished data on repeated measurements of respiratory syncytial virus-specific immunoglobulin G with commensurate increase in diagnostic sensitivity. The infant antibody response to respiratory syncytial virus is known to be inhibited to various degrees in the presence of maternal specific antibodies (21) and to decay following infection (22), making it problematic to determine infections serologically. Using a novel approach, we addressed this problem by defining a set of definitions by which to ascribe respiratory syncytial virus infections.

MATERIALS AND METHODS

Data source

The data are from a birth cohort study conducted in a rural district of coastal Kenya over the period 2002–2005 (10, 18, 19, 23–25). The study location experiences a tropical climate with twice yearly rains from April to July and from October to December. The population comprises predominantly subsistence farmers, has a demographic growth rate of 3.1% per annum, and ~18% are aged <5 years (26). The district hospital is located within Kilifi town, 60 km north of Kenya's main port of Mombasa. The birth cohort was recruited over 2 calendar years with 338 enrolled between January 31, 2002, and May 31, 2002, and 297 enrolled between December 3, 2002, and May 13, 2003. Study participants were recruited in the maternity ward or at the maternal and child health clinic within 2 weeks of birth, at Kilifi District Hospital, after written informed consent for participation was obtained from the mother. Cases of symptomatic respiratory syncytial virus were identified through home visits that were weekly during epidemics of this virus and monthly otherwise and through Kilifi District Hospital outpatient clinic attendance or inpatient admission. Nasopharyngeal washes were collected from children exhibiting mild-through-severe symptoms of acute respiratory infection. Children with acute cough or difficulty in breathing and fast breathing for age were diagnosed as having *mild LRTI*; those with acute cough or difficulty in breathing and one or more of lower chest wall indrawing, hypoxia (<90% pO_2), or impaired consciousness were diagnosed as having *severe LRTI*. Blood samples were collected at birth (cord blood) in the case of maternity ward deliveries and at approximately 3-month intervals for all recruits until the end of follow-up. Sera were stored at −80°C. Children were lost to follow-up through death, migration, refusal to continue, or end of the study. The study continued until children had experienced 3 respiratory syncytial virus epidemics, ending April 22, 2005. Ethics clearance was obtained from the Kenya National Ethics Review Board and from the Coventry Research Ethics Committee, United Kingdom.

Nasal specimens were screened for the presence of respiratory syncytial virus antigen by immunofluorescence antibody test. Sera were assayed for respiratory syncytial virus-specific immunoglobulin G by using crude respiratory syncytial virus A2 strain lysate as the solid phase in an indirect enzyme-linked immunosorbent assay, and the absorbance readings were quantified against a serially diluted high positive pool of sera and expressed as log (base 10)-transformed arbitrary units ($\log_{10}$(AU)) (20, 27, 28). Previous work established seropositive status as $\log_{10}(\text{AU}) \geq 1.5$ (18).

Definitions of respiratory syncytial virus infections

Two types of mutually exclusive infections were resolved. First, an antigen-determined infection (ADI) was defined as an immunofluorescence antibody test-positive nasal specimen. Second, a serologically determined infection (SDI) was defined by a change in serologic status between 2 serum samples that was independent of either an ADI or another SDI. The possible serologic changes and relation to ADI are schematically depicted in Figure 1 and defined in Table 1. Each consecutive pair of serum samples from the same individual collected at times s_1 and s_2 with antibody titers M_{s1} and M_{s2} were compared. An SDI was considered if there was a seroconversion (i.e., $M_{s1} < 1.5$ $\log_{10}$(AU) and $M_{s2} \geq 1.5$ $\log_{10}$(AU)) or if there was a sufficient boost to antibody titer (i.e., $M_{s2} - M_{s1} \geq Z$). There were 2 reasons why such changes might not be considered an SDI. First, the serum samples were taken too far apart, so that the existence and timing of an infection were vague, and consequently the criterion that $s_1 - s_2 < X$ was variously applied. Second, the antibody change might have been related to a known ADI, either if an ADI was bracketed by the samples or if the first sample was too close to the previous ADI such that the observed antibody boost should be attributed to that infection. If an ADI occurred in the interval ($s_1 - Y$, s_2), then the antibody change was ascribed to that ADI. If there was a sufficient change in antibody titer and the 2 additional criteria were passed, then an SDI was deemed to have occurred, and the time of an SDI for analysis was set at the date of s_2. The proportion of ADI detectable by serology was taken as a measure of the sensitivity of SDI detection.

Three groups of rules, termed "most liberal," "most pragmatic," and "most conservative" (Table 1), were selected to explore the effect of the uncertainty associated with defining SDI. The most conservative rules defined SDI with high specificity but low sensitivity. Consequently, samples had to be close ($<X = 100$ days), far from a previous ADI ($>Y = 200$ days, which generally put an ADI and subsequent SDI in separate epidemics), the rise in titer dramatic ($\geq Z = 10$ fold = 1.000), and seroconversions (without a boost of 10 fold or greater) were ignored. The most liberal rules defined SDI with low specificity but a high sensitivity. Consequently, there was no criterion on sample interval, time after the previous ADI was shorter ($>Y = 50$ days), the required antibody boost was small ($\geq Z = 2$ fold = 0.301), and

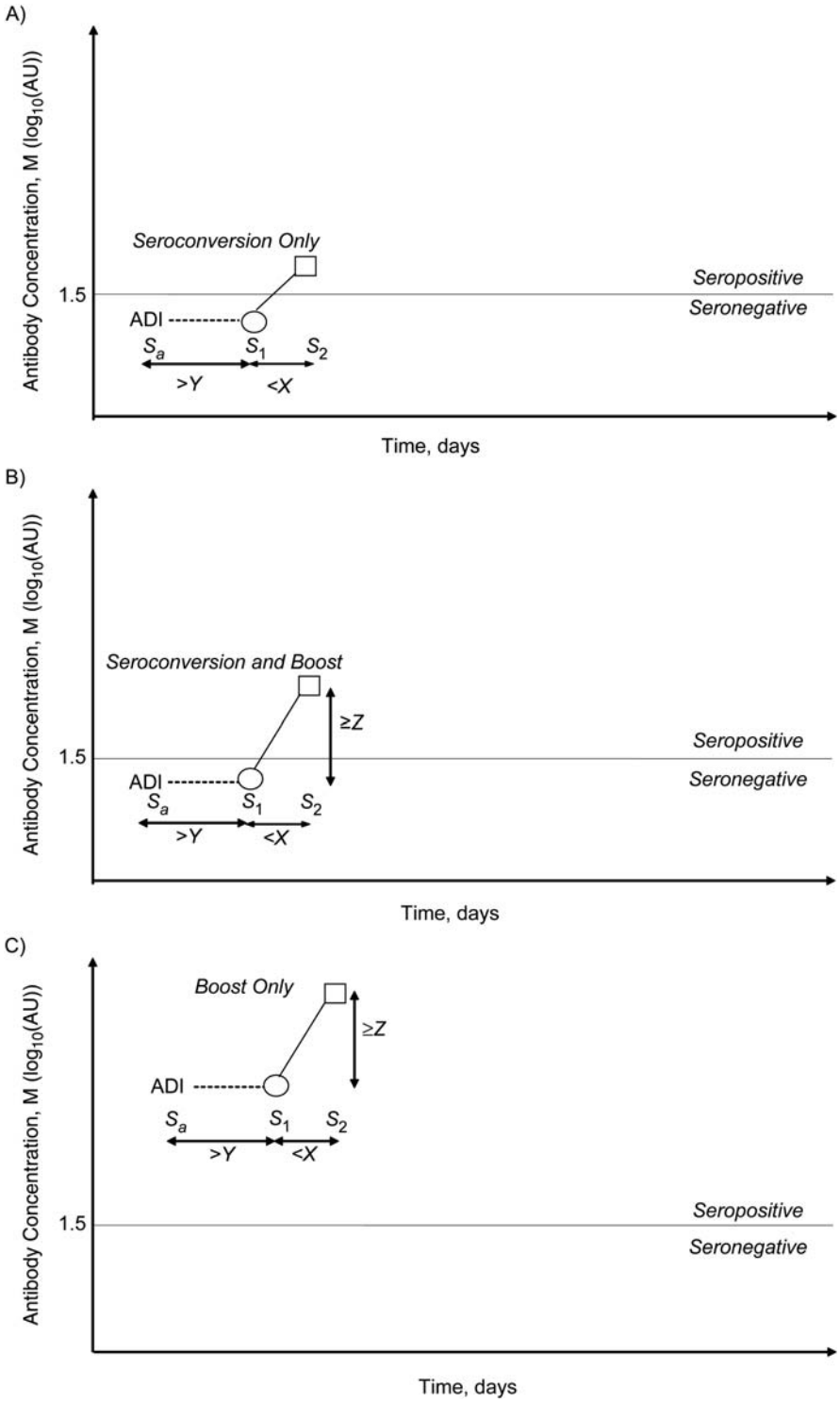

Figure 1. Schema representing criteria for defining a serologic change that forms the basis of serologically determined infections of a birth cohort of 635 children in Kilifi, Kenya, monitored over the period from January 31, 2002, to April 22, 2005. Three types of possibilities are described, each consisting of an "acute" (circle) and a "convalescent" (square) antibody measurement, occurring at time s_1 and s_2 days, respectively, and in relation to an antigen-determined infection (ADI) at a previous time s_a. The division between seropositive and seronegative antibody levels is shown as 1.5 $\log_{10}$(AU), arbitrary antibody units; X, threshold days between the dates of 2 sequential antibody results, $s_2 - s_1$; Z, threshold rise in antibody level between s_1 and s_2 ($M_{s2} - M_{s1}$); Y, threshold days among occurrence of ADI, s_a, and first sample date s_1; seroconversion, seronegative to seropositive among s_1 and s_2, $M_{s1} < 1.5 \log_{10}$(AU), and $M_{s2} \geq 1.5 \log_{10}$(AU) (A). Boost: minimal antibody level rise between s_1 and s_2: $M_{s2} - M_{s1} \geq Z$ (B and C). The serologically determined infection rules considered are defined as most conservative: $Z = 10$ fold and ($X = 100$ and $Y = 200$); most pragmatic: (seroconversion | $Z = 4$ fold) and ($X = 120$ and $Y = 50$); and most liberal: (seroconversion | $Z = 2$ fold and $Y = 50$).

Table 1. Parameters and Rules for Defining Classes of Serologically Defined Infection of a Birth Cohort of 635 Children in Kilifi, Kenya, Monitored Over the Period From January 31, 2002, to April 22, 2005

	Definition
Symbol	
X	Threshold days between dates of 2 sequential antibody results: $s_2 - s_1$
Z	Threshold rise in antibody level between s_1 and s_2: $M_{s2} - M_{s1}$
Y	Threshold days among occurrence of ADI, s_a, and first sample date s_1
Serologic changes	
Seroconversion	Seroconversion (seronegative to seropositive) between s_1 and s_2: $M_{s1} < 1.5 \log_{10}$(AU) and $M_{s2} \geq 1.5 \log_{10}$(AU)
Boost	Minimal antibody level boost between s_1 and s_2: $M_{s2} - M_{s1} \geq Z$
SDI rule	
Most conservative	$Z = 10$ fold and ($X = 100$ and $Y = 200$)
Most pragmatic	(Seroconversion | $Z = 4$ fold) and ($X = 120$ and $Y = 50$)
Most liberal	(Seroconversion | $Z = 2$ fold and $Y = 50$)

Abbreviations: ADI, antigen determined infection; AU, arbitrary antibody units; SDI, serologically determined infection.

seroconversions were included. The most pragmatic rules defined SDI on the basis of the intermediate sample interval ($<X = 120$ days), the same spacing after the previous ADI as the most liberal definition ($>Y = 50$ days), the conventional antibody boost for defining infection ($\geq Z = 4$ fold $= 0.602$), and inclusion of seroconversion.

The choices of X, Y, and Z were made for the following reasons. Antibody data were collected at intervals of between 90 and 120 days, giving approximate limits to the values of X chosen. Values of Y of <50 days between the last ADI and the first antibody measurement in any seroevent were unreliable, because antibody titers were commonly seen to continue to rise for several weeks after an infection. A 4-fold increase in antibody titer applied to the most pragmatic rule is conventional for the antibody change defining infection, and 2-fold and 10-fold increases were chosen as representing extreme definitions.

Measures of the performance of each of the 3 classes of SDI are shown in Table 2. The most conservative definition was of low sensitivity (21%) when compared with the ADI (i.e., "gold standard" definition of a respiratory syncytial virus infection). The most liberal definition captured most ADI (92% sensitivity). The most pragmatic classification gave intermediate sensitivity (52%). The results presented are based primarily on the use of the most pragmatic rule, and data derived from the most conservative rules and the most liberal rules are used to check the robustness of results.

Statistical analysis

Rates of infection were modeled by Cox regression (29). A "failure" was defined as respiratory syncytial virus infection indicated by an ADI or an SDI initially using the most pragmatic definition. The "observation time" for each child was days from the date of recruitment to the last visit of the study (right censored because of the end of the study) or loss to follow-up or death. The Efron approximation was used to account for several infections occurring on the same day (30). To allow for dependence between repeat infections in the same individual, robust variance estimates (Huber-White sandwich estimator) are reported (30). To account for the time-dependent incidence of respiratory syncytial virus, calendar time was adopted as analysis time, which enabled modeling the baseline hazard of infection as a nonparametric function of calendar time (31). The model was assessed for any violation of the proportional hazards assumption by using the Schoenfeld test. The relation between disease risk and age class, by infection history, was assessed by using the χ^2 test for trend (age categories: 0–5 months, 6–11 months, 12–17 months, 18–23 months, and ≥24 months). Adjusted risk ratios were obtained by binomial regression with robust standard errors. All data

Table 2. Summary Table of Outcomes for Each Class of Infection (ADI or SDI) of a Birth Cohort of 635 Children in Kilifi, Kenya, Monitored Over the Period From January 31, 2002, to April 22, 2005[a]

Rule	No. of ADIs Detected by Serology	No. of ADIs Detectable	Sensitivity of SDI Detection, %	No. of SDIs	Total Infections (ADI + SDI)	No. of Children Infected at Least Once
No serology (ADI only)	N/A	N/A	N/A	N/A	409	326
Most conservative	73	341	21.4	67	476	355
Most liberal	332	363	91.5	382	791	464
Most pragmatic	189	363	52.1	189	598	411

Abbreviations: ADI, antigen-determined infection; AU, arbitrary antibody units; N/A, not applicable; SDI, serologically determined infection.

[a] X: threshold days between dates of 2 sequential antibody results: $s_2 - s_1$; Z: threshold rise in antibody level between s_1 and s_2: $M_{s2} - M_{s1}$; Y: threshold days among occurrence of ADI, s_a, and first sample date s_1; seroconversion (seronegative to seropositive) between s_1 and s_2: $M_{s1} < 1.5 \log_{10}$(AU) and $M_{s2} \geq 1.5 \log_{10}$(AU) (Figure 1A). Boost: minimal antibody level boost between s_1 and s_2: $M_{s2} - M_{s1} \geq Z$ (Figure 1, B and C). The SDI rules considered are defined as 1) most conservative: $Z = 10$ fold and ($X = 100$ and $Y = 200$); 2) most pragmatic: (seroconversion | $Z = 4$ fold) and ($X = 120$ and $Y = 50$); and 3) most liberal (seroconversion | $Z = 2$ fold) and ($Y = 50$).

Table 3. Multivariable Cox Regression Analysis of Factors Associated With Respiratory Syncytial Virus Incidence of a Birth Cohort of 635 Children in Kilifi, Kenya, Monitored Over the Period From January 31, 2002, to April 22, 2005, for the Most Pragmatic Rule After Experiencing Primary and Secondary Infections

Variable	Most Pragmatic Rule After Experiencing Primary Infection				Most Pragmatic Rule After Experiencing Secondary Infection			
	No. of Infections	Adjusted Hazard Ratio	95% Confidence Interval	*P* Value	No. of Infections	Adjusted Hazard Ratio	95% Confidence Interval	*P* Value
Sex								
Female	319	1	Referent		109	1	Referent	
Male	279	0.94	0.80, 1.11	0.48	78	0.77	0.58, 1.02	0.07
Time since last infection, months	411	1	Referent		152	1	Referent	
0–5	35	0.30	0.21, 0.42	<0.001	15	0.41	0.22, 0.73	0.003
6–11	63	0.81	0.66, 1.43	0.88	14	1.57	0.90, 2.74	0.12
12–17	32	0.97	0.63, 1.79	0.82	5	0.99	0.40, 2.45	0.98
≥18	22	0.88	0.56, 1.39	0.59	1	0.92	0.12, 7.41	0.94
Age group, months								
0–5	104	1	Referent		6	1	Referent	
6–11	151	2.54	1.60, 4.03	<0.001	21	3.84	1.05, 14.08	0.042
12–17	118	2.35	1.42, 3.90	0.001	26	3.76	0.96, 14.75	0.057
18–23	142	2.24	1.30, 3.86	0.004	83	6.69	1.61, 27.90	0.009
24–30	83	1.59	0.91, 2.78	0.1	51	5.22	1.28, 21.35	0.021

analysis was undertaken by using Stata, version 11, software (StataCorp LP, College Station, Texas).

RESULTS

Descriptive analysis of the cohort

There were 635 children in the cohort (50.6% female) with a total observation time of 1,208 child-years and a median number of contacts per child of 65 (interquartile range: 45–75; range: 1–111). A total of 409 ADIs were detected in 326 children. There were 3,855 antibody measurements in total from 606 children. No antibody measurements were available for 29 children. The number of SDIs identified in the 3 classes (most conservative, most pragmatic, and most liberal) was 67, 189, and 382, respectively. In the most pragmatic class, the 189 SDIs arose in 162 children, of whom 136 had 1 SDI, 25 had 2 SDIs, and 1 had 3 SDIs. Pooling SDI and ADI results, we found that there were 598 infections and that these occurred in a total of 411 children (64.7%). Of these 411 children, 259 had 1 infection, 124 had 2 infections, 23 had 3 infections, 3 had 4 infections, and 2 had 5 infections, yielding a total of 187 reinfections. The proportion of children seropositive was 97% in the age group <2 months, 63% at 3–5 months, 39% at 6–8 months, 50% at 9–11 months, 66% at 12–17 months, 73% at 18–23 months, and 88% at 24–30 months.

Respiratory syncytial virus incidence

The crude estimate of respiratory syncytial virus incidence was 495 cases per 1,000 child-years of observation (CYO), ranging from 369/1,000 CYO in children less than 6 months of age to 649/1,000 CYO in children aged 24–30 months. Respiratory syncytial virus incidence was 552/1,000 CYO for primary infections and 404/1,000 CYO for reinfections. Web Table 1 (available at http://aje.oxfordjournals.org/) summarizes incidence data across a range of factors using SDI data defined by most pragmatic, most conservative, and most liberal definitions, respectively. There is no change in the patterns, although incidences are generally much higher for most liberal compared with most pragmatic and much lower for most conservative compared with most pragmatic.

Univariable analysis of factors associated with respiratory syncytial virus incidence

The rate of respiratory syncytial virus infection was reduced by 70% (hazard ratio (HR) = 0.30, 95% confidence interval (CI): 0.22, 0.41) during the first 6 months after an infection. There were a 40% (HR = 0.60, 95% CI: 0.50, 0.73) reduction in the rate of secondary infection compared with the rate of primary infection and a 55% (HR = 0.45, 95% CI: 0.30, 0.66) reduction for tertiary or more infections, when compared with primary infections. The rate of infection was lowest in the youngest age group (0–5 months) and was 1.4–2.1 fold higher for children in older age groups relative to children 0–5 months of age (Web Table 2). These most pragmatic results differ for other SDI rules only in the age-group effect, where the significant increase extends to the group aged 24–30 months for the most liberal class and is reduced to the group aged only 6–11 months for the most conservative class (Web Table 2).

Table 4. Age-dependent Risk of Disease Following Respiratory Syncytial Virus Infection of a Birth Cohort of 635 Children in Kilifi, Kenya, Monitored Over the Period From January 31, 2002, to April 22, 2005

Age, months	No. of Infections[a]	Mild LRTI[b]		Severe LRTI[b]		LRTI[b]	
		No.	%	No.	%	No.	%
Primary infections							
0–2	77	10	13.0	24	31.2	34	44.2
3–5	21	3	14.3	6	28.6	9	42.9
6–8	30	1	3.3	6	20.0	7	23.3
9–11	100	9	9.0	13	13.0	22	22.0
12–17	92	15	16.3	7	7.6	22	23.9
18–23	59	10	16.9	1	1.7	11	18.6
≥24	32	5	15.6	1	3.1	6	18.8
Total	411	53	12.9	58	14.1	111	27.0
Repeat infections							
0–11	27	3	11.1	2	7.4	5	18.5
12–23	109	13	11.9	3	2.8	16	14.7
≥24	51	6	11.8	4	7.8	10	19.6
Total	187	22	11.8	9	4.8	31	16.6

Abbreviations: LRTI, lower respiratory tract infection; pO_2, partial pressure of oxygen.

[a] Denominator includes antigen-defined infections (ADIs) and the "most pragmatic" class of serologically determined infections (SDI), that is, (SDI + ADI).

[b] Mild LRTI was defined as children with acute cough or difficulty in breathing and fast breathing for age; severe LRTI was defined as children with acute cough or difficulty in breathing and one or more of lower chest wall indrawing, hypoxia (<90% pO_2), or impaired consciousness; LRTI includes mild LRTI and severe LRTI.

Multivariable analysis of factors associated with respiratory syncytial virus incidence

The final multivariate model included sex, age, number of previous infections, and time since previous infection and is separately presented for primary respiratory syncytial virus infection and for secondary respiratory syncytial virus infection (Table 3), which avoids the problem of collinearity. Relative to primary incidence, the rate of secondary infection was 70% lower during the first 6 months following first infection and no different in any time periods beyond (Table 3). Relative to the rate of secondary infections, the rate of tertiary infection was 59% lower during the first 6 months following a secondary infection and not beyond (Table 3). Relative to primary respiratory syncytial virus incidence in the age group 0–5 months, primary incidence was over 2 times greater in all other age groups up to 18–23 months (Table 3). Relative to secondary respiratory syncytial virus incidence in the age group 0–5 months, the rate of tertiary respiratory syncytial virus infection was 3–7 fold higher in older age groups (Table 3). No sex differential in the rates of infection was observed in the cohort. There was no evidence that the model violated the proportional hazards assumption either in general or for each fitted variable (Schoenfeld global test, $P = 0.99$). These results are based on the most pragmatic rule for SDI but are robust to different definitions for SDI (Web Tables 3 and 4) except that the increased hazard of infections in older age groups relative to young infants is less pronounced for the most conservative class (for which specificity of infection diagnosis is presumed less) and more pronounced for the most liberal class (high specificity) (Web Tables 3 and 4). Analysis using ADI alone indicated partial protection from primary infection of 76% (95% CI: 60, 86) in the period 0–5 months and 44% (15%–63%) in the period 6–11 months, after infection, whereas the increased incidence in older children relative to children 0–5 months of age was no longer significant.

Risk of respiratory syncytial virus-associated disease

There were 142 respiratory syncytial virus-associated LRTI (mild and severe) cases identified in the cohort with 67 (47.2%) being severe LRTI. The risk of LRTI following infection was 23.7% (142/598): 12.5% (75/598) for mild LRTI and 11.2% (67/598) for severe LRTI. Following primary infection, the risk of LRTI was 27% (111/411) and of severe LRTI was 14.1% (58/411) (Table 4).

The risk of disease associated with primary infection was greatest in children less than 2 months of age, that is, 44.2% (34/77) for LRTI and 31.2% (24/77) for severe LRTI, and this declined with age to 18.8% and 3.1% in children aged at least 24 months for LRTI and severe LRTI, respectively ($P_{trend} < 0.001$) (Table 4). Following reinfection, the risk of LRTI was 16.6% and did not significantly differ by age group (Fisher's exact test, $P = 0.36$), and the risk of severe LRTI was 4.8% with no significant difference by age group (Fisher's exact test, $P = 0.14$).

The risk of LRTI was lower for reinfection relative to primary infection, in particular for severe LRTI (Table 5). With adjustment for age, the decrease in disease risk in secondary infections was not statistically significant (Table 5). The risk of LRTI declined with increasing age at infection, most markedly for severe LRTI (Table 6). With adjustment for infection history, the risk of LRTI and of severe LRTI (not mild LRTI) was reduced in children 6 months and older relative to children under 6 months of age (Table 6). There was a significant trend (adjusted for infection history) for decline with increasing age in the risks of LRTI ($P = 0.01$) and severe LRTI ($P < 0.001$) but not of mild LRTI ($P = 0.11$).

DISCUSSION

An analysis is presented of data collected from a large and intensively monitored birth cohort with the aim of elucidating key features of the natural history of respiratory syncytial virus infection. The present study differs from previous cohort studies (6, 8) in its examination of the relative roles of age and infection history on respiratory syncytial virus reinfection rates, which have confounded earlier work, and the investigation of the relative importance of age and prior exposure on the risk of disease following infection.

Table 5. Risk of Respiratory Syncytial Virus-associated Disease by Infection History (Primary vs. Repeat Infections) for the Birth Cohort of 635 Children in Kilifi, Kenya, Monitored Over the Period From January 31, 2002, to April 22, 2005, With Risk Ratios Adjusted for Age Class

Disease Severity[a]	No. of Primary RSV Infections ($n = 411$)[b]	Risk, %	No. of All Repeat RSV Infections ($n = 187$)[b]	Risk, %	Adjusted RR[c]	95% Confidence Interval	*P* Value
Mild LRTI	53	12.9	22	11.8	0.78	0.49, 1.25	0.31
Severe LRTI	58	14.1	9	4.8	0.58	0.29, 1.18	0.14
LRTI	111	27	31	16.6	0.72	0.50, 1.03	0.07

Abbreviations: LRTI, lower respiratory tract infection; pO_2, partial pressure of oxygen; RR, risk ratio; RSV, respiratory syncytial virus.

[a] Mild LRTI was defined as children with acute cough or difficulty in breathing and fast breathing for age; severe LRTI was defined as children with acute cough or difficulty in breathing and one or more of lower chest wall indrawing, hypoxia (<90% pO_2), or impaired consciousness; LRTI includes mild LRTI and severe LRTI.

[b] Denominator includes antigen-defined infections (ADIs) and the "most pragmatic" class of serologically determined infections (SDI), that is, (SDI + ADI).

[c] Risk ratio adjusted for age class (0–11 months, 12–23 months, and ≥24 months), comparing repeat infections with primary infections, with 95% confidence interval.

There are 3 principal results from the analysis not reported for any previous study of respiratory syncytial virus. First, following infection, there is a reduced rate (60%–70%) of reinfection that is temporary (approximately of 6-month duration). Second, there is observed to be age dependence in the rate of infection, with the incidence lowest in children <6 months of age. Third, the principal factor independently associated with risk of LRTI and severe LRTI, following infection, is age, suggesting that the physiological changes associated with increasing age are most important in disease risk, rather than previous exposure.

The study was not able to provide evidence for an independent association between the incidence of respiratory syncytial virus infection and history of infection; that is, it did not identify secondary respiratory syncytial virus incidence to be lower than primary incidence as previously reported (6). Furthermore, the evidence for a role for past exposure (i.e., immunological in origin) in a reduced respiratory syncytial virus-associated LRTI risk (shown elsewhere (6, 8)) was not strong enough to be confirmed statistically in the presence of the dominant age effect.

Combined, the results above have important implications for the design of vaccination programs. In particular, the introduction of a vaccine that would increase the average age at infection in those unvaccinated (i.e., through reduced wild-type virus circulation) would have positive benefits on the risk of disease in those unvaccinated, because disease is more strongly associated with age than with previous

Table 6. Risk of Respiratory Syncytial Virus-associated Disease of a Birth Cohort of 635 Children in Kilifi, Kenya, Monitored Over the Period From January 31, 2002, to April 22, 2005, by Age Class With Risk Ratios Adjusted for Infection History

Age, months	Total No. of Infections[a]	Severe LRTI[b]					LRTI[b]				
		No. of Infections	Risk, %	Adjusted RR[c]	95% Confidence Interval	*P* Value	No. of Infections	Risk, %	Adjusted RR[d]	95% Confidence Interval	*P* Value
0–5	104	31	29.8	1	Referent		47	45.2	1	Referent	
6–11	151	20	13.2	0.54	0.33, 0.86	0.01	30	19.9	0.53	0.38, 0.75	<0.001
12–17	118	7	5.9	0.27	0.13, 0.58	0.001	25	21.2	0.64	0.45, 0.91	0.014
18–23	142	4	2.8	0.12	0.04, 0.35	<0.001	24	16.9	0.46	0.30, 0.70	<0.001
24–30	83	5	6	0.34	0.13, 0.87	0.02	16	19.3	0.69	0.43, 1.09	0.11
Overall	598	67	11.2	0.92	0.89, 0.95	<0.001	142	23.7	0.97	0.95, 0.98	<0.001

Abbreviations: LRTI, lower respiratory tract infection; pO_2, partial pressure of oxygen; RR, risk ratio.

[a] Includes antigen-defined infections and the "most pragmatic" class of serologically determined infections.

[b] Mild LRTI was defined as children with acute cough or difficulty in breathing and fast breathing for age; severe LRTI was defined as children with acute cough or difficulty in breathing and one or more of lower chest wall indrawing, hypoxia (<90% pO_2), or impaired consciousness; LRTI includes mild LRTI and severe LRTI.

[c] Risk ratios adjusted for infection history, comparing the risk of severe LRTI disease by age class, with 95% confidence interval.

[d] Risk ratios adjusted for infection history, comparing the risk of LRTI disease by age class.

infection. For example, vaccination of older children that induced partial short-lived immunity (e.g., 70% for 6 months) in a significant fraction could be beneficial in reducing disease in early infants by providing indirect protection through their first respiratory syncytial virus epidemic.

Serologic information has been used in previous studies of respiratory syncytial virus infection (6, 8). Interpretation of serologic data for diagnosis of respiratory syncytial virus infections is complicated by 2 factors, both of which reduce the sensitivity to detect infection. First, the presence of maternally derived specific antibodies during the first few months of life has been shown to shroud the acquired humoral response in a proportion of cases (28, 32–35). Age dependence in the sensitivity of serology to detect infection could lead to bias in our results, rendering the observed relatively low incidence of respiratory syncytial virus in children 0–5 months of age an artifact. Potentially offsetting this effect is the observed inverse relation between level of viral shedding and increasing age at respiratory syncytial virus infection (36): Higher level shedding in early infants might equate to increased sensitivity of the antigen assay. Second, the acquired humoral response can be transient and significantly decay over a period of a few months, particularly following primary infection (34). Consequently, a critical factor in such interpretation is the relation between sampling interval and rates of antibody change, both of which have been explored in the present analysis. Three definitions for a serologically defined infection were compared with the intention of quantifying the uncertainty in the data analysis due to antibody dynamics. Despite the widely differing incidence estimates using most pragmatic, most conservative, and most liberal rules (due to the different sample sizes generated), these definitions did not greatly influence the interpretation of the data, suggesting no introduction of systematic bias.

The observation of the lowest incidence rate in infants <6 months of age is supportive of other evidence for a protective effect of maternal specific antibody in young children (37). The low incidence might also be due to low mixing rates in this group. However, there may be a methodological reason resulting from a difficulty in diagnosis of infection in children in which maternal antibody is at levels obscuring or inhibiting an immune response from infection. This effect may be offset by the observation that, in infants, respiratory syncytial virus infection is almost invariably symptomatic (8), which would have resulted in the collection of a nasal washing for antigen detection.

The decline in risk of respiratory syncytial virus-associated LRTI with increasing age has conventionally been attributed to accumulated immunity following previous respiratory syncytial virus exposure, ontogeny of the immune system, and physiological changes, such as larger airways. The results presented here suggest that factors directly associated with the host, such as increasing size and immunologic maturity, dominate this effect, rather than immunologic changes resulting from pathogen exposure. The study does not preclude an effect of exposure, only that it is less dominant than the age effect within the age range and number of exposures under study.

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