

**Mortality Associated with Seasonal and Pandemic Influenza and Respiratory
Syncytial Virus among Children less than 5 Years of Age in a High HIV-Prevalence
Setting – South Africa, 1998-2009**

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Abstract

Background. There are few published data describing the mortality burden associated with influenza and RSV infection in children in low- and middle-income countries and particularly from Africa and high HIV-prevalence settings.

Methods. We modeled the excess mortality attributable to influenza (seasonal and pandemic) and RSV infection by applying Poisson regression models to monthly all-respiratory and pneumonia and influenza deaths, using national influenza and RSV laboratory surveillance data as covariates. In addition, we estimated the seasonal influenza- and RSV-associated deaths among HIV-infected and -uninfected children using Poisson regression models that incorporated HIV prevalence and HAART coverage as covariates.

Results. In children <5 years of age, the mean annual number of seasonal influenza- and RSV-associated all-respiratory deaths were 452 (8 per 100,000 person-years) and 546 (10 per 100,000 person-years), respectively. Infants <1 year of age experienced higher mortality rates as compared to the 1-4 years age group for both influenza (22 vs. 5 per 100,000 person-years) and RSV (35 vs. 4 per 100,000 person years). HIV-infected as compared to HIV-uninfected children <5 years of age were at increased risk of death associated with influenza (age-adjusted relative risk (aRR): 11.5; 95% confidence interval (CI): 9.6-12.6) and RSV (aRR: 8.1; 95% CI: 6.9-9.3) infection. In 2009, we estimated 549 (11 per 100,000 person-year) all-respiratory influenza A(H1N1)pdm09-associated deaths among children aged <5 years.

Conclusions: Our findings support increased research efforts to guide and prioritize interventions such as influenza vaccination and HIV prevention in low- and middle-income countries with high HIV-prevalence such as South Africa.

Keywords

Influenza, respiratory syncytial virus, HIV, mortality, children, South Africa

INTRODUCTION

Globally, pneumonia is the leading cause of mortality in children [1] with the highest burden experienced in sub-Saharan Africa and Asia [2]. Influenza and respiratory syncytial virus (RSV) infections are common causes of pneumonia and are responsible for substantial global morbidity and mortality, in particular among individuals <5 and ≥ 65 years of age [3,4,5].

Recent studies estimated that worldwide there were between 13 and 32 million cases of influenza-associated pneumonia and as many as 110,000 deaths in children <5 years of age [6], while in the same age group RSV accounted for 33.8 million hospitalizations and between 66,000 and 199,000 deaths [7], 99% occurring in developing nations for both pathogens. While these studies suggest a greater burden in developing countries, available data are insufficient to prioritize strategies for influenza prevention and control especially in sub-Saharan Africa [8], highlighting the importance of generating disease burden estimates from developing nations.

The influenza season in South Africa is well defined and occurs during the southern hemisphere winter months (May to August) [9], while peak activity of RSV is observed from February to May [10,11]. In 2009, following the introduction of pandemic influenza A(H1N1)pdm09 in the country, South Africa experienced two distinct waves of influenza virus circulation. The first wave was dominated by influenza A(H3N2) followed by influenza A(H1N1)pdm09 [10].

Available data suggest that the burden of influenza and RSV infection may be higher among HIV-infected individuals [11,12,13,14,15,16,17,18,19,20,21] with nearly two-thirds of the 34 million people (3.5 million children) with HIV infection worldwide living in sub-Saharan Africa in 2010 [22]. In South Africa in 2009 there were approximately 210,000 HIV-infected children <5 years of age [23] and the HIV prevalence among the same age group increased from 1.4% in 1998 to 4.5% in 2006, slowly declining thereafter to 4% in 2009 [24]. The highly active antiretroviral treatment (HAART) uptake in the same age group slowly increased over time and reached a plateau of approximately 20% in 2009 [24].

Influenza vaccine is available in South Africa although uptake is low and RSV has been considered as an important future-target for vaccination [25]. Understanding the mortality burden of influenza and RSV in South Africa could assist to prioritize interventions; however, such information remains scanty especially among South African children. In South Africa and elsewhere, influenza and RSV infections are rarely confirmed by laboratory diagnosis and related deaths may be attributed to other comorbid conditions or secondary infections, making it difficult to assess the mortality burden associated with these pathogens. In recent years excess mortality models have become a popular statistical time series approach to estimate the mortality burden of respiratory pathogens [4,26,27,28,29,30]. We used excess mortality models to estimate national influenza- (seasonal and pandemic) and RSV-associated mortality among children <5 years of age from 1998 through 2009.

METHODS

Mortality data and population denominators

We obtained data on underlying causes of death for children <5 years of age from Statistics South Africa [31] from 1998-2009. We used the *International Classification of Diseases, Tenth Revision* (ICD-10) to compile age specific (<1 and 1-4 years) monthly mortality time series for all-respiratory (ICD-10: J00-J99) and pneumonia and influenza (P&I) (ICD-10: J10-J18 a subset of all-respiratory) deaths. To account for a systematic misclassification of cause of death due to coding practices that occurred between 1998 and 2005 in children 1-11 months of age we reallocated post-neonatal causes of death (i.e. ICD-10: P23 - congenital pneumonia) to more appropriate causes of infant death (i.e. ICD-10: J18 – pneumonia, organism unspecified) as recommended by Statistics South Africa [32]. In addition, we adjusted for underreporting of deaths from 1998 to 2006 using the year-specific estimates of proportion of underreported deaths provided by Darikwa and Dorrington [33]. According to these estimates data completeness increased from 55% in 1998 to 89% in 2006. From 2007 underreporting was estimated to be less than 5% [34]. Population denominators were obtained from Statistics South Africa [35], while age- and year-specific estimates of HIV prevalence in the population and HAART coverage among HIV-infected children were obtained from the Actuarial Society of South Africa AIDS and Demographic Model [24].

Influenza and RSV surveillance data

Prior to 2002, we obtained monthly influenza virus data by type and subtype from influenza-like-illness surveillance implemented by the National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Services (NHLS), South Africa [9] and from a cohort study for RSV [19]. From 2002 we acquired influenza and RSV virological data from a national database (the NHLS corporate data warehouse) that includes all patients tested by the NHLS laboratory network. We considered an influenza type or subtype to be dominant during the influenza season when it accounted for more than 50% of the circulating viruses.

Estimation of influenza- and RSV-associated mortality

To estimate the influenza- (seasonal and pandemic) and RSV-associated mortality, we fitted age-specific Poisson regression models to monthly deaths. The full model (model 1) included covariates for time trends and seasonal variation as well as viral circulation. The full model is provided in supplementary material.

To reduce possible bias associated with differences in specimen sampling and laboratory methods over time, we standardized the monthly numbers of specimens testing positive for influenza or RSV by the annual total of all specimens tested for the specific pathogen [5]. We estimated excess mortality associated with influenza and RSV by subtracting an expected baseline from the model 1 monthly mortality estimates. The baseline was obtained by setting the viral covariates to 0 and the annual excess mortality was obtained

as the sum of the monthly excess mortality estimates for each year. We obtained the 95% confidence interval (CI) for the estimated excess mortality using bootstrap resampling on blocks of calendar years (12 months block resampling with replacement) over 1000 replications [36]. Briefly, for each resampled dataset we refitted the Poisson regression model and the 95% CI were obtained from the 2.5 and 97.5 percentiles of the estimated influenza- and RSV-associated mortality from the 1000 resampled datasets.

In South Africa, the diagnosis of AIDS is rarely coded on the death certificate [32]. To assess changes in annual seasonal influenza and RSV excess mortality rates (as obtained from model 1) in relation to the HIV prevalence in the population and the HAART coverage over the years, we fitted separate multivariable Poisson regression models (model 2) for annual all-respiratory and P&I seasonal influenza- and RSV-associated mortality rates by age group. The full model is provided in supplementary material. We estimated the excess mortality associated with HIV-infection among influenza- and RSV-associated deaths by subtracting an expected baseline from the model 2 annual estimates. The baseline was obtained by setting the HIV and HAART covariates to 0. Mortality rates by HIV status were obtained by dividing the estimated excess deaths by the population at risk within each category. Age-specific and age-adjusted relative risk for influenza- and RSV-associated mortality related to HIV infection were estimated using log-binomial regression. The statistical analysis was implemented using STATA version 12 (StataCorp, Texas, USA).

Ethics

Since this analysis used only publicly-available mortality data and de-identified and aggregated laboratory data, the analysis was considered to be exempt from human subjects' ethics review.

RESULTS

Mortality data

South Africa had a population of approximately 5.1 million children <5 years of age in 2009; 1.1 (21%) and 4.0 million (79%) in the <1 year and 1-4 years age groups, respectively. A mean of 20,441 (range 14,560-23,320) all-respiratory deaths occurred annually among South African children <5 years of age over the study period (Table 1). Of these deaths, 76% occurred among children <1 year of age. The mean mortality rate for all-respiratory deaths was 12-fold higher in children <1 year as compared to children 1-4 years of age (1442 vs. 112 per 100,000 person-years). In children <5 years of age, the annual mortality rate per 100,000 person-years for all-respiratory deaths increased from 273 in 1998 to 373 in 2004, subsequently declining to 202 in 2009 (monthly trends reported in Figure 1B). A similar trend was observed for P&I and across age groups (results not shown).

Influenza and RSV laboratory surveillance

A mean of 3403 (range 227-15321) and 1810 (range 578-5247) samples was tested annually for influenza and RSV, respectively. The mean annual number of specimens testing positive was 937 (27%) for influenza virus and 356 (20%) for RSV. Over the study period, the influenza detection rates peaked between May and August with 10 of the 12 years experiencing peak activity in June-July (Figure 1A). In 2009, a first wave of influenza A(H3N2) peaked in June followed by a second wave of influenza A(H1N1)pdm09 that peaked in August. Conversely, RSV peak activity was observed between March and April in 8 of the 12 years with early or late peaks observed in February or May in the remaining years.

Influenza and RSV-associated mortality

Using model 1, we estimated that over the study period the annual number of all-respiratory seasonal influenza-associated deaths in children <5 years of age ranged between 284 and 667 (rate 6 to 13 per 100,000 person-years). In the same age group, the annual RSV-associated mortality for all-respiratory deaths ranged between 404 and 609 (rate 7 to 12 per 100,000 person-years) (Table 3).

Using model 2, we estimated that among children <5 years of age the mortality rate for all-respiratory cause of death was greater in HIV-infected as compared to HIV-uninfected individuals for both seasonal influenza (age-adjusted relative risk (aRR): 11.5; 95% CI:

9.6-12.6) and RSV (aRR: 8.1; 95% CI: 6.9-9.3) (Table 4). A similar trend was observed for P&I seasonal influenza- and RSV-associated deaths and across age groups.

Overall among HIV-uninfected children, RSV was associated with 1.2 times as many all-respiratory deaths as seasonal influenza; 1.6 and 0.8 among children <1 year and 1-4 years of age, respectively. Similar trends were observed among P&I deaths related to RSV and seasonal influenza virus infections.

In 2009, we estimated 549 (rate 10.9 per 100,000 person-year) all-respiratory influenza A(H1N1)pdm09-associated deaths among children aged <5 years; 311 (rate 27.0 per 100,000 person-years) and 238 (rate 5.9 per 100,000 person-years) among children <1 and 1-4 years of age, respectively. Among P&I deaths in children aged <5 years 470 (rate 9.2 per 100,000 person year) were associated with influenza A(H1N1)pdm09; 279 (rate 25.3 per 100,000 person-years) and 191 (rate 4.8 per 100,000 person-years) among children <1 and 1-4 years of age, respectively. In children <5 years of age all-respiratory influenza A(H1N1)pdm09-associated mortality rates (10.9 per 100,000 person-years) were 1.3-fold (95% CI: 1.2-1.5) higher than the mean annual seasonal influenza-associated mortality rates (8.1 per 100,000 person-years) over the study period and 1.8-fold (95% CI: 1.6-2.1) higher than seasonal influenza-associated mortality (5.9 per 100,000 person-years) in 2009.

DISCUSSION

To the best of our knowledge, this report is the first to provide estimates of influenza- and RSV-associated mortality in children <5 years of age in a high HIV-prevalence setting in Africa. The mortality burden was substantial for both influenza and RSV and the mortality rates were higher among HIV-infected as compared to HIV-uninfected children across age groups and underlying cause of death. While the mortality rates were greater among HIV-infected children, the burden remained considerable among HIV-uninfected individuals and was highest in infants as compared to older children. These findings support increased efforts for control of seasonal influenza in children in South Africa and other low- and middle-income countries. In addition, in countries with high HIV-prevalence such as South Africa, programs to prevent HIV infection and mortality, such as prevention of mother-to-child transmission and early antiretroviral therapy, will remain important interventions to reduce the mortality associated with RSV and influenza virus infection.

The all-respiratory seasonal influenza-associated mortality rate in children <5 years of age in South Africa (8 per 100,000 person-years) were elevated as compared to estimates obtained from similar studies in the United States and Europe (range 0.1-2 per 100,000 person-years for all respiratory or respiratory and circulatory influenza-associated deaths in equivalent age groups) [4,37,38,39]. South African estimates were closer to those of other middle income countries such as Mexico where rates in the range of 3.4-8.5 per 100,000 person-years (for all respiratory deaths among children <5 years of age) have

been reported [40]. The rates of seasonal influenza-associated mortality remained elevated even among HIV-uninfected South African children <5 years of age (6 per 100,000 person-years) as compared to more developed nations. A similar trend was observed for South African elderly individuals (≥ 65 years of age), an age group where the burden of HIV is lowest and where seasonal influenza-associated mortality rates were >4-fold higher than among seniors in the United States [41].

Similar to influenza, all-respiratory RSV-associated mortality rates among HIV-uninfected children in South Africa (28 per 100,000 person-years in children <1 year of age and 3 per 100,000 person-years in children 1-4 years of age) were elevated as compared to those reported in the United States and England (range 2.9-5.3 per 100,000 person-years and 0.2-0.3 per 100,000 person-years for all respiratory or respiratory and circulatory RSV-associated deaths in the <1 year and 1-4 years age groups, respectively) [4,37], indicating a heavy burden of both pathogens even among HIV-uninfected individuals in South Africa.

Recent meta-analyses have reported higher seasonal influenza- [6] and RSV-associated lower respiratory tract infection [7] case fatality rates in children from developing countries (2.9% for influenza and 2.1% for RSV) compared to more developed nations (0.2% for influenza and 0.3% for RSV). While the high HIV infection rate in developing countries [22] may partially explain the elevated burden of influenza and RSV infection, other factors such as poor nutritional status, poor access to health care as well as other

comorbidities and co-infections have been suggested as potential additional risk factors for poor outcome in developing nations [42].

We found that HIV-infected children experienced approximately a 10 times greater risk of death associated with seasonal influenza and RSV infection as compared to HIV-uninfected individuals. While the methodology differed from our present analysis, other studies have reported increased risk of death associated with HIV infection among seasonal influenza- and RSV-infected individuals [11,12,13,14,20,21].

In South African HIV-uninfected children RSV was associated with 1.6 and 0.8 times as many deaths as seasonal influenza in the <1 year and 1-4 years age groups, respectively. In the same age groups, reports from the United States of America indicated that RSV accounted for approximately 10 and 0.5 times the seasonal influenza mortality rate [4], while ratios of approximately 1.5 and 1 were reported in England [37]. While the South Africa estimates are closer to those from England, the observed wide range of the relative contribution of these two pathogens especially to infant mortality highlights the needs to implement additional studies in different countries and settings. In particular, ecological studies conducted in settings similar to ours, where influenza and RSV peak activities are not synchronous, may assist in better differentiating the relative burden of these two pathogens.

We found that in 2009 the influenza A(H1N1)pdm09-associated mortality among South African children <5 years of age was 1.3-fold higher compared to mean annual estimates

for seasonal influenza over the study period. The comparison of the mortality associated with influenza A(H1N1)pdm09 and seasonal influenza during previous seasons remains difficult in South Africa because of the changes in HIV prevalence and HAART coverage in the population that impact the annual seasonal influenza-associated mortality over the study period. However, the mortality associated with influenza A(H1N1)pdm09 was approximately 1.8-fold higher even when compared to those associated with influenza A(H3N2) in 2009. This suggests a moderately higher mortality burden of influenza A(H1N1)pdm09 in 2009 compared to seasonal influenza among South African children <5 years of age. Other studies have reported similar or higher mortality associated with influenza A(H1N1)pdm09 compared to seasonal influenza in children <5 years of age [26,39,40,43,44,45].

Our study has limitations that warrant discussion. Firstly, the lack of weekly mortality statistics and the paucity of virological data prior to 2002 may have hindered the ability to accurately estimate the relative contribution of RSV and influenza virus on mortality. In addition, the limited information about influenza types and subtypes in the early years of our study hindered the ability to estimate subtype-specific associated mortality. Further, we did not have additional information on individual deaths. Secondly, because of poor recording of HIV infection in the death register in the early years of our study, we utilized indirect methods to assess the mortality burden among HIV-infected and -uninfected individuals. While the HIV epidemic in South Africa is considered to be a major factor responsible for the increased mortality rates observed over the years [32,34], the lack of time series data for other potential comorbidities/risk factors may have

resulted in overestimating the increased risk of death associated with HIV infection. In addition, we could not estimate the influenza A(H1N1)pdm09-associated mortality by HIV status because our method uses HIV prevalence data over several years. Thirdly, we did not include non-respiratory deaths in our study. While influenza-associated mortality has been reported among individuals with circulatory, diabetes or other chronic conditions, the prevalence of these conditions is low among the study population included in this study (children <5 years of age). Lastly, while we utilized methods suggested by Statistics South Africa to account for the systematic misclassification of cause of death in infants from 1998-2005 and deaths underreporting from 1998-2006, such adjustments may have introduced potential biases in the early years of our study.

In conclusion, we reported a substantial mortality burden associated with RSV and influenza virus infection in both HIV-infected and -uninfected children in South Africa. The effectiveness of RSV candidate vaccines is being evaluated [46] and vaccination remains the most effective method of preventing influenza virus infection and should be recommended for South African children.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Competing interests

No authors have any competing interests.

Financial disclosure

Since publicly available data were used no funding was received for this work.

Ethics

Since this analysis used only publicly-available mortality data and de-identified and aggregated laboratory data, the analysis was considered to be exempt from human subjects' ethics review.

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TABLES

Table 1: Mean annual respiratory deaths in children <5 years of age in South Africa, 1998-2009 [31]

Cause of death	Deaths ^a	
	Number Mean (Range)	Rate ^b Mean (Range)
All respiratory		
<1 year	15676 (11135-17973)	1442 (1070-1696)
1-4 years	4765 (3425-5875)	112 (83-138)
<5 years	20441 (14560-23320)	383 (284-439)
Pneumonia and influenza		
<1 year	13063 (7889-15651)	1199 (758-1415)
1-4 years	3701 (2497-4483)	87 (61-105)
<5 years	16764 (10387-20078)	314 (202-373)

^a Deaths adjusted for underreporting

^b Death rate per 100,000 person-years

Table 2: Estimated seasonal influenza-associated deaths in children <5 years of age by HIV status in South Africa, 1998-2009^a

Year	Predominant influenza type/subtype	Excess all respiratory deaths						Excess pneumonia & influenza deaths					
		Total		HIV+		HIV-		Total		HIV+		HIV-	
		Number	Rate ^b	Number	Rate ^b	Number	Rate ^b	Number	Rate ^b	Number	Rate ^b	Number	Rate ^b
1998	A(H3N2)	500	9	101	138	399	8	437	8	105	143	332	6
1999	B	363	6	87	86	276	5	317	6	86	85	231	4
2000	A(H1N1)	456	8	134	102	322	6	438	8	149	114	289	5
2001	A(H3N2)	580	11	190	118	389	7	548	10	206	128	342	6
2002	B	336	6	119	63	217	4	301	5	119	63	183	3
2003	A(H3N2)	533	10	198	95	334	6	479	9	201	96	278	5
2004	A(H3N2)	534	10	201	90	333	6	495	9	209	94	285	5
2005	A(H1N1)	452	8	169	73	282	5	415	8	172	75	242	5
2006	A(H3N2)	667	13	222	96	445	9	504	10	192	83	311	6
2007	A(H3N2)	368	7	121	53	247	4	315	6	113	50	201	4
2008	A(H1N1)	350	7	100	46	250	5	294	6	92	42	202	4
2009	A(H3N2)	284	6	65	31	219	4	238	5	54	26	184	4

^a Estimated from model 1 (overall deaths) and model 2 (deaths by HIV status)

^b Death rates per 100,000 person-years

Table 3: Estimated respiratory syncytial virus-associated deaths in children <5 years of age by HIV status in South Africa, 1998-2009^a

Year	Excess all respiratory deaths						Excess pneumonia & influenza deaths					
	Total		HIV+		HIV-		Total		HIV+		HIV-	
	Number	Rate ^b	Number	Rate ^b	Number	Rate ^b	Number	Rate ^b	Number	Rate ^b	Number	Rate ^b
1998	510	9	85	116	424	8	456	8	91	124	365	7
1999	553	10	115	113	438	8	502	9	122	121	379	7
2000	588	11	143	109	444	8	540	10	153	116	387	7
2001	609	11	165	103	444	8	566	10	176	110	389	7
2002	599	11	176	93	422	8	559	10	187	99	372	7
2003	585	12	179	85	405	8	544	10	188	90	355	7
2004	577	11	177	79	399	8	525	10	181	81	344	7
2005	575	12	171	74	404	8	505	10	168	73	337	7
2006	565	11	154	66	410	8	480	9	146	62	333	7
2007	526	10	131	57	394	8	430	8	121	53	309	6
2008	467	9	101	46	366	7	371	7	91	41	279	6
2009	404	7	55	26	348	7	312	6	47	22	265	5

^a Estimated from model 1 (overall deaths) and model 2 (deaths by HIV status)

^b Death rates per 100,000 person-years

Table 4: Estimated seasonal influenza and respiratory syncytial virus mean annual associated deaths and relative risk for mortality due to HIV infection in children <5 years of age in South Africa, 1998-2009^a

Cause of death	Mean annual excess deaths							Relative Risk (HIV+ vs HIV-) (95% CI)
	Total		Percentage mortality over model baseline Mean (95% CI)	HIV+		HIV-		
	Number Mean (95% CI)	Rate ^b Mean (95% CI)		Number Mean (95% CI)	Rate ^b Mean (95% CI)	Number Mean (95% CI)	Rate ^b Mean (95% CI)	
Seasonal influenza virus								
All respiratory								
<1 year	240 (117-368)	22 (11-34)	4 (2-7)	72 (33-110)	162 (79-246)	168 (83-257)	16 (8-25)	10.1 (8.7-11.7)
1-4 years	212 (110-313)	5 (2-7)	9 (7-11)	71 (36-104)	52 (28-78)	141 (73-208)	3 (2-5)	15.4 (11.2-21.1)
<5 years	452 (227-681)	8 (4-13)	5 (4-8)	143 (69-214)	83 (42-123)	309 (157-466)	6 (3-9)	11.5 (9.6-12.6) ^c
Pneumonia and influenza								
<1 year	212 (106-330)	19 (10-30)	4 (3-6)	77 (37-119)	174 (87-267)	136 (69-209)	13 (6-20)	13.5 (11.4-15.8)
1-4 years	186 (97-279)	4 (2-6)	11 (8-13)	65 (33-96)	49 (26-73)	121 (64-180)	3 (2-4)	16.7 (11.8-23.4)
<5 years	398 (204-606)	7 (4-11)	6 (5-8)	142 (71-216)	83 (43-125)	256 (133-390)	5 (2-7)	14.0 (12.1-16.2) ^c
Respiratory syncytial virus								
All respiratory								
<1 year	384 (185-589)	35 (17-54)	6 (5-7)	90 (43-138)	204 (98-311)	294 (141-450)	28 (13-43)	7.2 (6.5-8.1)
1-4 years	162 (63-240)	4 (1-7)	5 (4-6)	47 (18-70)	35 (13-51)	114 (44-169)	3 (1-4)	12.5 (8.8-17.8)
<5 years	546 (248-829)	10 (5-15)	6 (5-7)	138 (62-208)	81 (36-122)	408 (186-619)	8 (4-12)	8.1 (6.9-9.3) ^c
Pneumonia and influenza								
<1 year	362 (198-568)	33 (18-52)	6 (5-7)	100 (54-156)	225 (123-351)	263 (144-412)	25 (14-39)	8.9 (7.9-10.1)
1-4 years	120 (47-191)	3 (1-5)	6 (4-8)	40 (15-63)	29 (11-46)	80 (31-127)	2 (1-3)	14.9 (9.8-22.6)
<5 years	482 (254-759)	9 (4-14)	6 (5-7)	139 (70-219)	83 (42-130)	343 (175-540)	7 (3-10)	9.4 (8.3-10.5) ^c

^a Estimated from model 1 (overall deaths) and model 2 (deaths by HIV status)

^b Death rates per 100,000 person-years

^c Age-adjusted relative risk

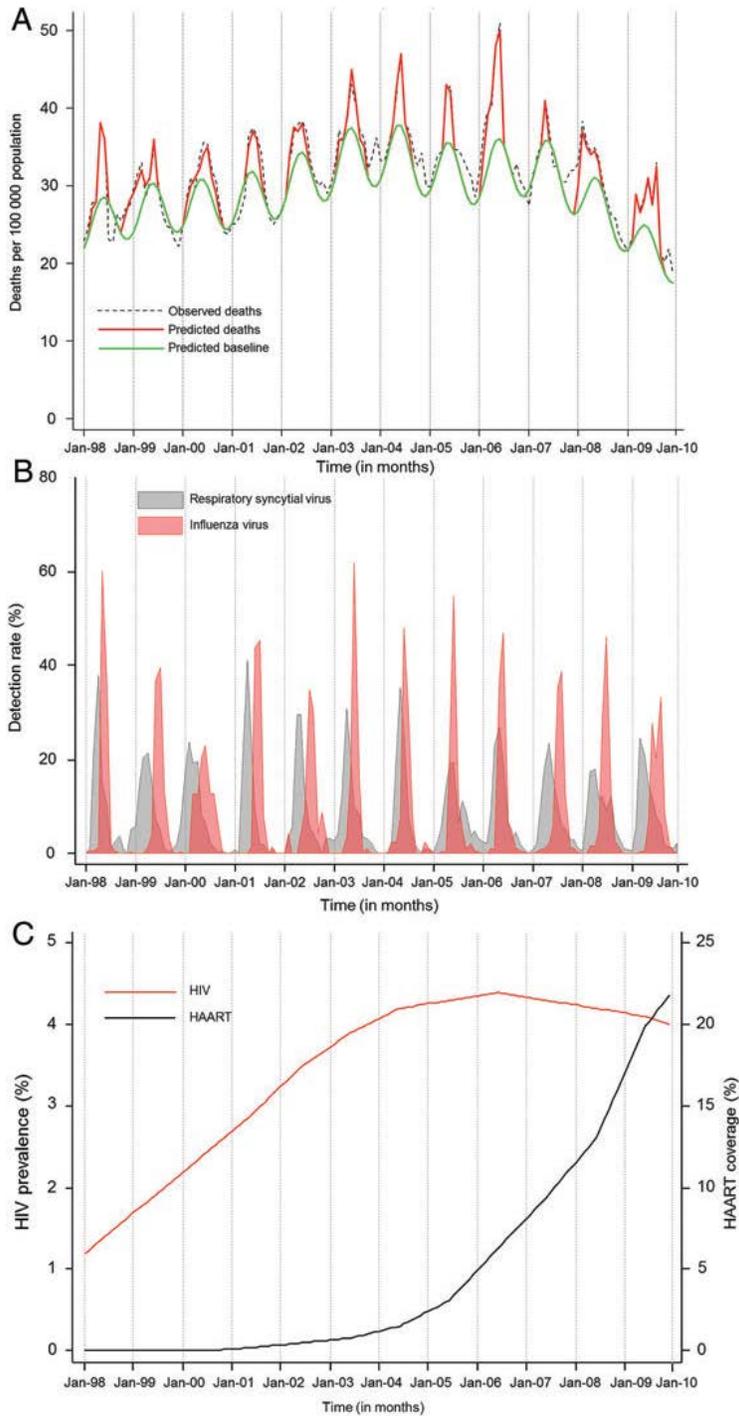


Figure 1: Detection of influenza and respiratory syncytial virus, mortality rate, HIV prevalence and HAART coverage in children <5 years of age in South Africa, 1998-2009. **A**: Monthly detection rate (i.e., monthly number of positive specimens divided by total specimens) of influenza and respiratory syncytial virus (all ages). **B**: Observed all respiratory deaths, predicted deaths and predicted baseline by month (Poisson model 1). **C**: Annual HIV prevalence and HAART coverage obtained from the Actuarial Society of South Africa AIDS and Demographic Model [24]. Observed deaths are adjusted using estimates of death underreporting from 1998-2006 [33].

Supplementary material

METHODS

Estimation of influenza- and RSV-associated mortality

Different modeling approaches have been used to estimate influenza associated mortality, including (i) peri- and summer-season rate-difference models [1,2], (ii) Serfling cyclical regression models, which do not incorporate influenza viral surveillance data [3,4], (iii) autoregressive integrated moving average (ARIMA) models, which do not incorporate influenza viral surveillance data [5,6] and (iv) regression models (Poisson, Negative-Binomial or linear), which do incorporate influenza surveillance data [7,8,9]. A study comparing the four methods found comparable estimates of influenza associated mortality over 31 influenza seasons in the United States except for estimates from the summer-season rate-difference model, which were consistently higher [10]. Regression models that incorporate viral covariates have been suggested as the preferred method when at least 5 years of mortality and viral surveillance data are available and the mortality associated with multiple pathogens is estimated [10].

In our study, we fitted age-specific Poisson regression models (with an identity link) to monthly deaths. The identity link was selected because it is considered the most biologically plausible link to model the impact of pathogen circulation on mortality [11,12,13,14]. Indeed, an identity link assumes additive (rather than multiplicative) effects of different pathogens on mortality. The full model (model 1) included covariates for time trends and seasonal variation as well as viral circulation as follows:

$$E(Y_i) = \beta_0 + \beta_1[t_i] + \beta_2[t_i^2] + \beta_3[t_i^3] + \beta_4[t_i^4] + \beta_5[\sin(2t_i\pi/12)] + \beta_6[\cos(2t_i\pi/12)] \\ + \beta_7[Seasonal_Influenza_i] + \beta_8[A(H1N1)pdm09_i] + \beta_9[RSV_i] + \varepsilon_i \quad (1)$$

$E(Y_i)$ represents the age-specific number of deaths during a particular month i , β_0 is the model constant, β_1 to β_4 are coefficients associated with time trends (linear to quartic polynomial terms) included to account for annual variation of number of deaths, β_5 and β_6 are coefficients associated with harmonic terms included to account for seasonal variations, β_7 to β_9 are

coefficients associated with the proportion of specimens testing positive for respiratory viruses (seasonal influenza: A(H1N1), A(H3N2) and B; pandemic influenza: A(H1N1)pdm09; and RSV) and ε_i is the error term. Model selection procedures included the assessment of model fit considering the inclusion of polynomial (1st to 6th degree) and harmonic terms. The final model (model 1) was that for which the Akaike value was minimized, that is, the model that provided best fit to the data whilst maintaining parsimony. We also considered b-spline instead of polynomial terms but polynomial terms provided the best fit to the South African data.

In South Africa, the diagnosis of AIDS is rarely coded on the death certificate [15]. To assess changes in annual seasonal influenza excess mortality rates (as obtained from model 1) in relation to the HIV prevalence in the population and the HAART coverage among HIV-infected individuals over the years, we fitted separate multivariable Poisson regression models (model 2) for annual all-respiratory and P&I seasonal influenza-associated mortality rates by age group. The following model was used:

$$E(Y_i / N_i) = \beta_0 + \beta_1[t_i] + \beta_2[t_i^2] + \beta_3[Influenza_i] + \beta_4[HIV_i] + \beta_5[HAART_i] + \varepsilon_i \quad (2)$$

$E(Y_i/N_i)$ represents the age-specific influenza-associated mortality rate during a particular year i , β_0 is the model constant, β_1 and β_2 are coefficients associated with time trends (linear and quadratic) included to account for potential variations of health indicators not associated with HIV or HAART, β_3 is the coefficient associated with dominant seasonal influenza type/subtype each year (A(H3N2), A(H1N1) and B; treated as categorical variable with A(H3N2)-dominant years as reference group), β_4 is the coefficient associated with age- and year-specific HIV prevalence in the population, β_5 is the coefficients associated with age- and year-specific HAART coverage among HIV-infected individuals and ε_i is the error term. Similar models, with the exclusion of the dominant influenza types/subtypes covariate, were used for RSV-associated mortality rates.

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