HHS Public Access

Author manuscript

J Infect Dis. Author manuscript; available in PMC 2019 May 03.

Published in final edited form as:

J Infect Dis. 2018 September 08; 218(8): 1228–1237. doi:10.1093/infdis/jiy310.

Influenza Viral Shedding in a Prospective Cohort of HIV-Infected and Uninfected Children and Adults in 2 Provinces of South Africa, 2012–2014

Claire von Mollendorf[1,2], **Orienka Hellferscee**[1,3], **Ziyaad Valley-Omar**[1,6], **Florette K. Treurnicht**[1], **Sibongile Walaza**[1,2], **Neil A. Martinson**[4], **Limakatso Lebina**[4], **Katlego Mothlaoleng**[4], **Gethwana Mahlase**[7], **Ebrahim Variava**[5,8], **Adam L. Cohen**[9,10,a], **Marietjie Venter**[11], **Cheryl Cohen**[1,2], and **Stefano Tempia**[9,10]

[1]Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, University of the Witwatersrand, Johannesburg

[2]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

[3]School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

[4]Perinatal HIV Research Unit, Medical Research Council Soweto Matlosana Collaborating Centre for HIV/AIDS and TB, University of the Witwatersrand, Johannesburg

[5]School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

[6]Department of Pathology, Division of Medical Virology, University of Cape Town

[7]Pietermaritzburg Metropolitan, KwaZulu-Natal, Atlanta, Georgia

[8]Department of Medicine, Klerksdorp Tshepong Hospital, North West Province, Atlanta, Georgia

[9]Influenza Division, Centers for Disease Control and Prevention, Pretoria, Atlanta, Georgia

[10]Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia

[11]Department of Medical Virology, University of Pretoria, Pretoria, South Africa

Abstract

Background—Prolonged shedding of influenza viruses may be associated with increased transmissibility and resistance mutation acquisition due to therapy. We compared duration and magnitude of influenza shedding between human immunodeficiency virus (HIV)-infected and -uninfected individuals.

Methods—A prospective cohort study during 3 influenza seasons enrolled patients with influenza-like illness and a positive influenza rapid test. Influenza viruses were detected by real-

Correspondence: C. Cohen, MBBCh, PhD, Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases, 1 Modderfontein Road, Sandringham 2131, Gauteng, South Africa (cherylc@nicd.ac.za).

[a]Present affiliation: World Health Organization, Geneva, Switzerland.

time reverse transcription polymerase chain reaction. Weibull accelerated failure time regression models were used to describe influenza virus shedding. Mann-Whitney U tests explored initial influenza viral loads (VL).

Results—Influenza virus shedding duration was similar in 65 HIV-infected (6 days; interquartile range [IQR] 3–10) and 176 HIV-uninfected individuals (7 days; IQR 4–11; $P = .97$), as was initial influenza VL (HIV-uninfected 5.28 ± 1.33 $\log_{10}$ copies/mL, HIV-infected 4.73 ± 1.68 $\log_{10}$ copies/mL; $P = .08$). Adjusted for age, HIV-infected individuals with low CD4 counts shed influenza virus for longer than those with higher counts (adjusted hazard ratio 3.55; 95% confidence interval, 1.05–12.08).

Discussion—A longer duration of influenza virus shedding in HIV-infected individuals with low CD4 counts may suggest a possible increased risk for transmission or viral evolution in severely immunocompromised individuals. HIV-infected individuals should be prioritized for annual influenza immunization.

Keywords

influenza; shedding; HIV; South Africa

Quantifying influenza shedding duration, a possible predictor of infectivity and transmissibility, can inform infection prevention and control measures [1]. Several factors, such as age and to a lesser extent human immunodeficiency virus (HIV) status, have been found to impact shedding duration [2, 3]. Early studies suggested HIV-infected adults may shed influenza longer [2, 4], while more recent studies found that HIV-infected adults shed for a similar time to HIV-uninfected adults, possibly due to higher CD4 counts and virological suppression in the era of widespread antiretroviral therapy (ART) availability [5]. South Africa has a significant HIV epidemic, with an estimated 7.1 million children and adults living with HIV and 3.9 million individuals on ART in 2016 [6]. The mean CD4 count at time of first presentation and diagnosis in South Africa is approximately 250 cells/μL [7]. Differential shedding duration by HIV status and CD4 count is important to determine as prolonged influenza shedding may be associated with viral evolution and antigenic drift within a patient [8], the development of resistance to influenza antivirals following therapy [9], and an increased transmissibility.

Adult studies have reported a range of influenza virus shedding durations using mainly real-time reverse transcription polymerase chain reaction (rRT-PCR) assays, with a shorter duration reported in healthy versus hospitalized adults [1, 10]. A longer duration of shedding is often described in children and immunocompromised individuals [2]. In children the median duration of virus detection is usually 7–8 days, but may be longer [11].

Influenza virus shedding data from Africa, in particular countries with high HIV prevalence, are limited. One study from Kenya explored shedding patterns of influenza A(H1N1) pdm09 in 106 patients with low known HIV prevalence [12]. The median duration of influenza virus detection was 8 days using rRT-PCR.

There are minimal data regarding the impact of HIV infection on influenza virus shedding duration [5]. We aimed to evaluate whether seasonal influenza virus shedding duration and

viral load (VL) differed between HIV-infected and -uninfected individuals with influenza-like illness (ILI) in 2 provinces of South Africa in 2012–2014.

METHODS

Study Design and Setting

We conducted a prospective cohort study of individuals older than 6 months of age, during 3 consecutive influenza seasons (2012–2014). Patients who met the case definition for ILI, that is presenting to an outpatient clinic with sudden onset of fever (>38°C) and cough or sore throat in the absence of another diagnosis, and who had onset of symptoms in the last 3 days, were actively identified (active case ascertainment) at public primary health care clinics in 2 provinces (Edendale, KwaZulu Natal and Klerksdorp, North West Province). Respiratory samples from consenting eligible patients were tested with a rapid diagnostic test for influenza (BD Directigen EZ Flu A+B or BD Veritor [Becton, Dickinson and Company, MA]). Recruitment was restricted to patients presenting within 72 hours of symptom onset as influenza viral load usually peaks during the first 24–72 hours of illness.

Patients younger than 5 years of age had a nasopharyngeal aspirate taken while patients 5 years or older had a nasopharyngeal swab and oropharyngeal swab taken as per the routine surveillance program (described elsewhere) [13] that the shedding study was nested within. Specimens were submitted to the National Institute for Communicable Diseases (NICD) in Johannesburg in PrimeStore Molecular Transport Medium (Longhorn Vaccines and Diagnostics, Bethesda, MA) for multiplex rRT-PCR testing and confirmation of influenza infection. Patients whose enrolment specimen tested positive for influenza on rapid test, but negative on rRT-PCR, were excluded from the shedding study. Respiratory specimens were collected from patients whose enrolment sample tested positive for influenza by rRT-PCR every 2 days for the first 2 weeks (day 14) after enrolment and then at 21 days from the date of enrolment (influenza confirmation). A second rapid test was done on the day 21 respiratory specimen and if it tested positive another sample was taken on day 28. Patients were considered to be no longer shedding if specimens were negative for influenza by rRT-PCR at 2 consecutive visits. Symptom onset at the initial visit and the presence of symptoms at all study follow-up visits were recorded.

For HIV status, any documented or verbal confirmation was accepted for a positive status. For a negative status, if the patient had no recent documented proof of their status and they consented to a test, pretest counseling and testing for HIV was offered by study staff. If no consent for testing was given, the patient's HIV status was recorded as unknown. HIV-infected patients also had blood drawn for CD4 count.

Laboratory Diagnosis

A multiplex rRT-PCR assay detecting 10 respiratory viruses (influenza A and B viruses, para-influenza viruses 1–3, respiratory syncytial virus, enterovirus, human metapneumovirus, adenovirus, and rhinovirus) was used for confirmation of influenza virus infection [14]. Influenza A positive samples were subtyped using an assay developed by the Centers for Disease Control and Prevention (CDC), Atlanta [15], and influenza B viruses

were lineage typed by rRT-PCR assay [16]. Influenza A and B VLs were determined for positive specimens for all visits using the CDC rRT-PCR as a quantitative assay by including a series of standards of known copy numbers [17]. We considered a cycle threshold (Ct) value cutoff of ≤37 as a positive rRT-PCR result [18].

Durations of Symptoms and Shedding

We defined symptom duration as time from symptom onset to the first day on which the relevant symptom was resolved, based on participant report. The respiratory symptom category included sore throat, cough, and rhinorrhea; the all-symptom category included respiratory symptoms plus fever and myalgia; and fever alone was reported as a separate symptom category [19]. The duration of influenza viral shedding was considered as the number of days from onset of symptoms to last positive influenza rRT-PCR result.

Statistical Analysis

We compared baseline characteristics of enrolled HIV-infected and -uninfected participants using a multivariable logistic regression model. We started with all variables significant at $P < .20$ on univariate analysis, and dropped all nonsignificant factors ($P \geq .05$) with manual backward elimination. Patients with missing data for included variables were dropped from the model (data were >90% complete for all variables except CD4 count).

Time from symptom onset to (1) last day of influenza virus detection on rRT-PCR and (2) alleviation of symptoms was analyzed using Kaplan-Meier estimates and Weibull accelerated failure time regression models, which allow explanatory variables (eg, age, sex, HIV status, influenza virus type/subtype, underlying medical conditions, antibiotic use, study site, study year) to proportionally increase or decrease time to endpoint [20]. The Weibull accelerated failure time model allowed more flexibility than a Cox semiparametric model as the associated hazard rate was not constant with respect to time; the Weibull model was a better fit for our data. Hazard ratios measured the hazard of becoming PCR negative with a ratio <1 indicating a longer duration of shedding.

Influenza viral RNA load data (expressed in copies/mL) for the enrolment visit was $\log_{10}$ transformed. Mann-Whitney U tests were used to explore the difference in initial VLs between HIV-infected and -uninfected individuals. All statistical analyses were performed using STATA version 14.1 (StataCorp, College Station, TX).

Ethical Approval

The protocol was approved by the University of the Witwatersrand (M120129), University of KwaZulu-Natal (BF079/12) ethics committees, the relevant Provincial Departments of Health and the US Centers for Disease Control and Prevention. All study participants or parents/guardians (for children <18 years) were required to sign an informed consent form.

RESULTS

A total of 4214 patients were enrolled into the ILI surveillance program during 3 consecutive influenza seasons (May–October) (Figure 1). In 2012, study screening only

started in August in Edendale following ethics approval. Overall, 1152 patients were ineligible as they were younger than 6 months of age (n = 160) or reported presence of symptoms for more than 3 days prior to the visit (n = 992). Of the remaining 3062 (73%) ILI patients, 867 (28%) patients were not screened (lack of rapid tests or left clinic) and 23 patients (1%) refused consent. Only 2172 patients were screened with a rapid test and 1813 (83%) tested negative. Of the patients with a negative rapid test result, 16% (283/1813) were false negatives (positive for influenza on rRT-PCR). Of the 359 patients whose screening rapid test was positive, 26% (95/359) were subsequently reported to be negative for influenza on rRT-PCR, resulting in 264 patients enrolled in the study (Figure 1). Only 23 enrolled patients declined HIV testing in the absence of a documented HIV status.

The primary influenza type and subtype differed by year with influenza B predominating in 2012 (8/11, 73%), a similar proportion of A(H1N1)pdm09 (36/95, 38%), and A(H3N2) (42/95, 44%) in 2013, and a predominance of A(H3N2) (127/155, 82%) in 2014. Only 51 patients had other viral infections detected on PCR; 44 individuals had 1 additional virus detected (including 13 rhinovirus, 23 adenovirus, 5 human metapneumovirus, and 1 respiratory syncytial virus) and 7 had multiple other viruses detected.

Comparison of Patient Baseline Characteristics by HIV Status

Of the 264 patients, 241 (91%) had known HIV status and 65 (25%) were HIV infected. Of the infected individuals, 63% (41/65) were receiving HIV treatment at time of enrolment and of those with CD4 count results 88% (36/41) had a count of >200 cells/μL. On multivariate analysis (Table 1), compared with HIV-uninfected individuals HIV-infected patients were more likely to be older (25–44 years adjusted odds ratio [aOR] 33.1; 95% confidence interval [CI], 5.1–216.4; 45–64 years aOR 49.9; 95% CI, 6.7–373.1; compared with age <5 years), to be female (aOR 3.5; 95% CI, 1.5–8.5), and to have received treatment for tuberculosis in the last 12 months (aOR 36.9; 95% CI, 4.7–289.1), but were less likely to live in a crowded household with more than 3 people per room (aOR 0.2; 95% CI, 0.1–0.8). Only 1 patient (HIV-uninfected adult) was vaccinated for influenza in the last year. No patients received oseltamivir treatment or any other agents with anti-influenza activity as part of routine care.

Duration of influenza shedding did not differ significantly ($P = .84$) between HIV-infected (median days 6, interquartile range [IQR] 3–11) and -uninfected individuals (median days 7, IQR 4–11) (Figure 2A). Symptom duration was similar between HIV-infected and -uninfected individuals for all symptoms (median days 16, IQR 7–21; and 15, IQR 9–18), respiratory symptoms (median days 16, IQR 10–18; and 15, IQR 11–18), and fever (median days 7, IQR 3–14; and 5, IQR 3–12). Fever duration was similar to viral shedding duration while respiratory symptom duration was generally longer (Table 1).

Factors Associated With Duration of Influenza Virus Detection by rRT-PCR

Overall, the median duration of influenza virus detection by rRT-PCR was 7 days (IQR 3–11 days). On multivariable analysis (Table 2), duration of detection of influenza virus by rRT-PCR differed by age group (Figure 2B), with longer detection in children aged <5 years compared with adults ≥45 years (adjusted hazard ratio [aHR] 0.53; 95% CI, 0.30–0.93); by

influenza subtype (Figure 2C), with longer detection in individuals with influenza A(H3N2) than individuals with influenza A(H1N1) pdm09 (aHR 0.57; 95% CI, 0.40–0.80); and longer detection with previous antibiotic use (aHR 0.43; 95% CI, 0.20–0.96). Duration of influenza virus detection did not differ significantly by HIV status in all participants (aHR 0.92; 95% CI, 0.64–1.33) or when we restricted our analysis to individuals aged ≥5 years (aHR 0.86; 95% CI, 0.60–1.24). We were unable to demonstrate a difference in virus detection by rRT-PCR with regards to influenza vaccination use as only 1 individual was vaccinated in our cohort.

When restricting to HIV-infected individuals (Table 3 and Figure 2D), on multivariable analysis influenza virus detection by rRT-PCR was significantly longer in individuals with CD4 counts ≤200 cells/μL compared to those with CD4 counts >200 cells/μL (aHR 3.55; 95% CI, 1.05–12.08) and older adults (aged ≥45 years) were more likely to shed for longer than children aged <5 years (aHR 26.75; 95% CI, 2.50–286.68). ART use was not significant in the final multivariable model. A subgroup analysis in individuals aged ≥5 years (data not shown), showed no difference in detection between the adult age groups, but the difference by CD4 count (longer in individuals with lower CD4 counts, aHR 3.45; 95% CI, 1.02–11.69) was retained.

Factors Associated With Duration of Symptoms

On multivariate analysis of factors associated with the duration of all ILI symptoms (Table 4), children <5 years (HR 0.53; 95% CI, 0.34–0.84), children 5–14 years (HR 0.52; 95% CI, 0.34–0.81), and adults aged 25–44 years (HR 0.57; 95% CI, 0.34–0.94) had a longer duration of symptoms than individuals aged 15–24 years. In addition, participants from Edendale reported ongoing symptoms for a shorter duration (aHR 1.65; 95% CI, 1.20–2.28) than those from Klerksdorp. On comparison, the 2 sites had similar characteristics except for a difference in the proportion of patients enrolled across the 3 years. There was no difference in duration of symptoms by HIV status (HR 0.84; 95% CI, 0.58–1.22) or CD4 count (not included in final model). Similar results were found when we explored factors associated with respiratory symptoms and fever separately.

Comparison of Initial Influenza Viral Loads

There were 112 HIV-uninfected and 35 HIV-infected individuals with initial influenza VL measurements. The VL for HIV-uninfected subjects at the initial visit was a mean (standard deviation) of 5.28 ± 1.33 $\log_{10}$ copies/mL, while for HIV-infected subjects it was 4.73 ± 1.68 $\log_{10}$ copies/mL ($P = .08$). When restricting to HIV-infected individuals no difference in initial influenza VL was noted by CD4 count levels ($P = 1.00$). Initial influenza VLs did, however, differ by influenza subtype: A(H1N1)pdm09 (n = 33, 5.67 ± 1.27 $\log_{10}$ copies/mL) and A(H3N2) (n = 93, 4.71 ± 1.39 $\log_{10}$ copies/mL, $P < .001$).

DISCUSSION

Our study found no overall difference in the duration and intensity at baseline of influenza virus shedding as detected by rRT-PCR in HIV-infected compared to HIV-uninfected individuals. We did, however, find some evidence that HIV-infected immunocompromised

individuals with lower CD4 counts may shed for longer than HIV-infected individuals with higher CD4 counts, although numbers were small. It is important to understand transmission dynamics to prevent transmission to these groups as the effectiveness of influenza vaccine in severely immunocompromised individuals has not been established and prolonged antiviral use may lead to the development of resistant mutants [21].

Amongst ILI patients with laboratory-confirmed influenza, when compared to HIV-uninfected individuals, HIV-infected individuals were older in age due to extremely low HIV-positivity rates in children aged <5 years (3%); this was likely due to improvements in prevention of mother-to-child transmission programs [22]. HIV-infected individuals were more likely to be female, which has been shown from other South African data, and is ascribed to biological vulnerability and sociobehavioral factors [23]. Lastly, HIV-infected individuals were more likely to have had tuberculosis in the last year than HIV-uninfected individuals, consistent with the known high rate of HIV/tuberculosis coinfection [24].

Most data regarding the duration of shedding in immunocompromised individuals have been obtained from patients with conditions other than HIV [2, 21, 25, 26]. Studies in cancer patients on chemotherapy and other immunocompromising conditions have shown prolonged shedding of influenza viruses, ranging from 2 weeks to 18 months, and a higher incidence of drug-resistant viruses than immunocompetent individuals [21]. HIV-infected individuals have been shown to develop more-severe influenza disease with higher mortality, especially in individuals with more-advanced HIV disease [27–29]. A study in 20 HIV-infected adults diagnosed with influenza reported a median duration of influenza detection by rRT-PCR of 10 days (IQR 6–15 days) and a median duration of any ILI symptoms of 14 days (IQR 12–26 days) [5]. The majority of these patients were virologically suppressed on ART and results did not differ from previous studies in HIV-uninfected individuals. Our study, which systematically enrolled ILI patients testing positive for influenza, showed a median duration of influenza detection by rRT-PCR in HIV-infected individuals of 6 days (IQR 3–11 days) and 16 days (IQR 7–21 days) for any ILI symptom; neither differed significantly from the HIV-uninfected group in our cohort.

In our study, for all individuals we showed longer influenza virus shedding in children aged <5 years compared with adults. However, in HIV-infected individuals only, adults were more likely to shed for longer when controlling for degree of immunosuppression. A systematic review [30] showed longer duration of viral shedding measured by rRT-PCR in children in 3 studies [19, 31, 32] but longer shedding in adults in 2 studies [33, 34]. A significant amount of diversity has been noted in estimates of duration of influenza viral shedding, especially in children [35]. Influenza severity and symptoms have been shown to differ by age group, with more-severe disease in young children and the elderly [36]; our study found that the duration of all symptoms differed by site and age group, with children having a longer duration of symptoms than young adults.

Other studies have demonstrated similar patterns of viral shedding between pandemic and seasonal influenza A [19] and different subtypes of seasonal influenza A viruses [35]. We demonstrated a difference in the initial VLs of seasonal influenza A as well as a difference in the duration of shedding.

Our study had a number of limitations. First, we used a case-ascertainment study design in which potential participants who met the ILI case definition were recruited from outpatient clinics and an influenza rapid test was used to detect patients testing positive for the influenza virus. Such recruitment may have led to bias in participant selection as individuals had illness that was severe enough to seek medical attention with a positive rapid test result, and may thus have had higher levels of viral shedding [37]. Second, it is unclear whether health-seeking behavior differed between the HIV-infected and -uninfected individuals identified with influenza in our study. We were not able to assess whether HIV is more likely to cause a clinical illness that warrants seeking care, nor were we able to exclude that there may be differential shedding among HIV-infected and -uninfected individuals ascertained in the community. We are currently conducting a community based study of influenza burden and transmission at 2 sites in South Africa, which may provide answers to some of these outstanding questions [38, 39]. Third, we had more false-positive rapid test results than previous studies [40]; this resulted in us having to follow-up more patients until their rRT-PCR results were available. We also tried to enroll patients who were rapid test negative and rRT-PCR positive, but this was only possible if the rRT-PCR results were available before the day 2 follow-up visit. We, however, only retained individuals in the study who tested positive on rRT-PCR. Fourth, we only measured the duration of viral shedding by rRT-PCR and not by virus isolation in cell cultures. This may have overestimated the duration of shedding in our study as culture only detects viable virus whereas rRT-PCR detects any viral particles present in the sample, thus detecting RNA remnants of past infection without active replication. The mean duration of viral shedding is reported as 1.5–6 days shorter when measured by culture compared with rRT-PCR and virus isolations are usually obtained from positive rRT-PCR specimens with a Ct value ≤30 [19, 41, 42]. Lastly, we had low numbers of HIV-infected individuals, especially young children, enrolled in the study, with available CD4 count results only in two-thirds as these bloods were processed in routine hospital laboratories. However, of those patients with known results nearly 90% had CD4 counts of more than 200 cells/μL and were seen with mild symptoms at general outpatient clinics; it is therefore likely that overall enrolled patients were mildly immunosuppressed accounting for the lack of difference in shedding duration between HIV-infected and -uninfected individuals.

Our study also had a number of strengths. We enrolled both HIV-infected and -uninfected individuals of all age groups from the same population; we enrolled participants over multiple seasons with different predominating influenza types; participants were actively followed up and swabbed every 2 days for 2 weeks and then weekly; and we assessed both quantitative and qualitative measures of viral shedding.

In conclusion, in the era of an established ART program we did not show any difference in influenza virus shedding as detected by rRT-PCR in HIV-infected and -uninfected individuals in outpatient clinics in 2 areas of South Africa. We did, however, show a potential difference in influenza virus shedding by CD4 count with individuals with lower counts shedding for longer. Even though the efficacy of the influenza vaccine in severely immunocompromised individuals is unclear, prior data from South Africa demonstrated that the trivalent influenza vaccine was efficacious in HIV-infected individuals with CD4 counts >100 cells/μL [43]. Influenza vaccination is recommended and offered as part of an annual

government-funded campaign for HIV-infected individuals in South Africa to reduce the incidence of severe disease; however, none of the HIV-infected individuals in our study had received the vaccine in the last 12 months. Although antiviral medication for influenza is recommended for high-risk groups it is not widely available in public health clinics in South Africa. In addition to influenza-specific prevention and treatment strategies, it is vital that severely immunocompromised HIV-infected individuals are given appropriate ART to prevent a potential increased risk of influenza transmission and resistant mutations.

Acknowledgments

We thank all the participants and their caregivers who kindly agreed to be included in this study; the surveillance officers for their efforts in enrolling and following up participants; laboratory staff at the NICD, especially Alexandra Moerdyk, for processing the samples and the NICD data team for data quality control and assurance.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, or the NICD/National Health Laboratory Service.

Financial support. This work was supported by the National Institute for Communicable Diseases of the National Health Laboratory Service and the Centers for Disease Control and Prevention (cooperative agreement no. 5U51IP000155).

Potential conflicts of interest. C. v. M., O. H., F. K. T., S. W., and C. C. report an institutional grant from the Centers for Disease Control and Prevention, USA during the conduct of the study; C. C. reports grants from Sanofi Pasteur and Parexel outside the submitted work; N. A. M. reports institutional grants from Pfizer and from manufacturers of novel TB diagnostics outside the submitted work. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Ryoo SM, Kim WY, Sohn CH, et al. Factors promoting the prolonged shedding of the pandemic (H1N1) 2009 influenza virus in patients treated with oseltamivir for 5 days. Influenza Other Respir Viruses 2013; 7:833–7. [PubMed: 23279949]
2. Weinstock DM, Gubareva LV, Zuccotti G. Prolonged shedding of multidrug-resistant influenza A virus in an immunocompromised patient. N Engl J Med 2003; 348:867–8. [PubMed: 12606750]
3. Sato M, Hosoya M, Kato K, Suzuki H. Viral shedding in children with influenza virus infections treated with neuraminidase inhibitors. Pediatr Infect Dis J 2005; 24:931–2. [PubMed: 16220098]
4. Ison MG, Gubareva LV, Atmar RL, Treanor J, Hayden FG. Recovery of drug-resistant influenza virus from immunocompromised patients: a case series. J Infect Dis 2006; 193:760–4. [PubMed: 16479508]
5. Patel P, Bush T, Kojic EM, et al. Duration of influenza virus shedding among HIV-infected adults in the cART Era, 2010–2011. AIDS Res Hum Retroviruses 2016; 32:1180–6. [PubMed: 27174191]
6. UNAIDS. Country factsheets HIV/AIDS estimates: South Africa. http://aidsinfo.unaids.org/. Accessed 6 June 2018.
7. Siedner MJ, Ng CK, Bassett IV, Katz IT, Bangsberg DR, Tsai AC. Trends in CD4 count at presentation to care and treatment initiation in sub-Saharan Africa, 2002–2013: a meta-analysis. Clin Infect Dis 2015; 60:1120–7. [PubMed: 25516189]
8. McMinn P, Carrello A, Cole C, Baker D, Hampson A. Antigenic drift of influenza A (H3N2) virus in a persistently infected immunocompromised host is similar to that occurring in the community. Clin Infect Dis 1999; 29:456–8. [PubMed: 10476769]
9. Memoli MJ, Hrabal RJ, Hassantoufighi A, Eichelberger MC, Taubenberger JK. Rapid selection of oseltamivir- and peramivir-resistant pandemic H1N1 virus during therapy in 2 immunocompromised hosts. Clin Infect Dis 2010; 50:1252–5. [PubMed: 20345239]

10. Lee N, Chan PK, Hui DS, et al. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. J Infect Dis 2009; 200:492–500. [PubMed: 19591575]
11. World Health Organization Writing Group, Bell D, Nicoll A, et al. Non-pharmaceutical interventions for pandemic influenza, international measures. Emerg Infect Dis 2006; 12:81–7. [PubMed: 16494722]
12. Waiboci LW, Lebo E, Williamson JM, et al. Viral shedding in patients infected with pandemic influenza A (H1N1) virus in Kenya, 2009. PLoS One 2011; 6:e20320. [PubMed: 21695203]
13. Tempia S, Walaza S, Moyes J, et al. Attributable fraction of influenza virus detection to mild and severe respiratory illnesses in HIV-infected and HIV-uninfected patients, South Africa, 2012–2016. Emerg Infect Dis 2017; 23:1124–32. [PubMed: 28628462]
14. Pretorius MA, Madhi SA, Cohen C, et al. Respiratory viral coinfections identified by a 10-plex real-time reverse-transcription polymerase chain reaction assay in patients hospitalized with severe acute respiratory illness--South Africa, 2009–2010. J Infect Dis 2012; 206(Suppl 1):S159–65. [PubMed: 23169964]
15. Jernigan DB, Lindstrom SL, Johnson JR, et al. Detecting 2009 pandemic influenza A (H1N1) virus infection: availability of diagnostic testing led to rapid pandemic response. Clin Infect Dis 2011; 52(Suppl 1):S36–43. [PubMed: 21342897]
16. Radovanov J, Milosevic V, Cvjetkovic IH, et al. Influenza B viruses in the population of province of Vojvodina during the 2012/2013 season: differentiation of B/Yamagata and B/victoria lineages by real-time RT-PCR, antigenic and phylogenetic characterization. Srp Arh Celok Lek 2015; 143:429–37. [PubMed: 26506753]
17. Almansa R, Anton A, Ramirez P, et al. Direct association between pharyngeal viral secretion and host cytokine response in severe pandemic influenza. BMC Infect Dis 2011; 11:232. [PubMed: 21880131]
18. Seleka M, Treurnicht FK, Tempia S, et al. Epidemiology of influenza B/Yamagata and B/Victoria lineages in South Africa, 2005–2014. PLoS One 2017; 12:e0177655. [PubMed: 28542324]
19. Cowling BJ, Chan KH, Fang VJ, et al. Comparative epidemiology of pandemic and seasonal influenza A in households. N Engl J Med 2010; 362:2175–84. [PubMed: 20558368]
20. Collett D Modelling survival data in medical research. 3rd ed. Boca Raton: Chapman and Hall/CRC, 2015.
21. Boivin G, Goyette N, Bernatchez H. Prolonged excretion of amantadine-resistant influenza a virus quasi species after cessation of antiviral therapy in an immunocompromised patient. Clin Infect Dis 2002; 34:E23–5. [PubMed: 11807683]
22. Sherman GG, Lilian RR, Bhardwaj S, Candy S, Barron P. Laboratory information system data demonstrate successful implementation of the prevention of mother-to-child transmission programme in South Africa. S Afr Med J 2014; 104:235–8. [PubMed: 24893499]
23. Shisana O, Rehle T, Simbayi LC, et al. South African National HIV prevalence, incidence and behaviour survey, 2012. Cape Town: HSRC Press, 2014.
24. UNAIDS. Frequently Asked Questions about Tuberculosis and HIV. http://data.unaids.org/pub/factsheet/2006/tb_hiv_qa.pdf. Accessed 23 December 2016.
25. Gooskens J, Jonges M, Claas EC, Meijer A, Kroes AC. Prolonged influenza virus infection during lymphocytopenia and frequent detection of drug-resistant viruses. J Infect Dis 2009; 199:1435–41. [PubMed: 19392620]
26. Memoli MJ, Athota R, Reed S, et al. The natural history of influenza infection in the severely immunocompromised vs nonimmunocompromised hosts. Clin Infect Dis 2014; 58:214–24. [PubMed: 24186906]
27. Neuzil KM, Reed GW, Mitchel EF Jr, Griffin MR. Influenza- associated morbidity and mortality in young and middle-aged women. JAMA 1999; 281:901–7. [PubMed: 10078486]
28. Lin JC, Nichol KL. Excess mortality due to pneumonia or influenza during influenza seasons among persons with acquired immunodeficiency syndrome. Arch Intern Med 2001; 161:441–6. [PubMed: 11176770]
29. Ormsby CE, de la Rosa-Zamboni D, Vazquez-Perez J, et al. Severe 2009 pandemic influenza A (H1N1) infection and increased mortality in patients with late and advanced HIV disease. AIDS 2011; 25:435–9. [PubMed: 21139486]

30. Fielding JE, Kelly HA, Mercer GN, Glass K. Systematic review of influenza A(H1N1)pdm09 virus shedding: duration is affected by severity, but not age. Influenza Other Respir Viruses 2014; 8:142–50. [PubMed: 24299099]

31. Xiao H, Lu SH, Ou Q, Chen YY, Huang SP. Hospitalized patients with novel influenza A (H1N1) virus infection: Shanghai, June-July 2009. Chin Med J (Engl) 2010; 123:401–5. [PubMed: 20193477]

32. Hien TT, Boni MF, Bryant JE, et al. Early pandemic influenza (2009 H1N1) in Ho Chi Minh City, Vietnam: a clinical virological and epidemiological analysis. PLoS Med 2010; 7:e1000277. [PubMed: 20502525]

33. Suess T, Buchholz U, Dupke S, et al. Shedding and transmission of novel influenza virus A/H1N1 infection in house-holds--Germany, 2009. Am J Epidemiol 2010; 171:1157–64. [PubMed: 20439308]

34. Suryaprasad A, Morgan OW, Peebles P, et al. Virus detection and duration of illness among patients with 2009 pandemic influenza A (H1N1) virus infection in Texas. Clin Infect Dis 2011; 52(Suppl 1):S109–15. [PubMed: 21342881]

35. Lau LL, Ip DK, Nishiura H, et al. Heterogeneity in viral shedding among individuals with medically attended influenza A virus infection. J Infect Dis 2013; 207:1281–5. [PubMed: 23382573]

36. Chaves SS, Aragon D, Bennett N, et al. Patients hospitalized with laboratory-confirmed influenza during the 2010–2011 influenza season: exploring disease severity by virus type and subtype. J Infect Dis 2013; 208:1305–14. [PubMed: 23863950]

37. Tsang TK, Cowling BJ, Fang VJ, et al. Influenza A Virus shedding and infectivity in households. J Infect Dis 2015; 212:1420–8. [PubMed: 25883385]

38. Cohen C, Moyes J, Mkhencele T, et al. The attack rate of respiratory syncytial virus in two South-African communities with high HIV prevalence, 2016 - preliminary results. Presented at: Transmission of respiratory viruses: from basic science to evidence based options for control, 19–21 June, 2017; Hong Kong https://transmission2017.med.hku.hk/ Accessed 6 June 2018.

39. Tempia S, Moyes J, Mkhencele T, et al. The attack rate of influenza virus in two South African communities with high HIV-prevalence, 2016 – preliminary results. Presented at: Transmission of respiratory viruses: from basic science to evidence based options for control, 19–21 June, 2017; Hong Kong https://transmission2017.med.hku.hk/. Accessed 6 June 2018.

40. Chartrand C, Leeflang MM, Minion J, Brewer T, Pai M. Accuracy of rapid influenza diagnostic tests: a meta-analysis. Ann Intern Med 2012; 156:500–11. [PubMed: 22371850]

41. Leung YH, Lim WL, Wong MH, Chuang SK. Delayed oseltamivir treatment is associated with longer viral shedding of pandemic (H1N1) 2009 virus. Epidemiol Infect 2012; 140:814–7. [PubMed: 21801465]

42. Bhattarai A, Villanueva J, Palekar RS, et al. Viral shedding duration of pandemic influenza A H1N1 virus during an elementary school outbreak-Pennsylvania, May-June 2009. Clin Infect Dis 2011; 52(Suppl 1):S102–8. [PubMed: 21342880]

43. Madhi SA, Maskew M, Koen A, et al. Trivalent inactivated influenza vaccine in African adults infected with human immunodeficient virus: double blind, randomized clinical trial of efficacy, immunogenicity, and safety. Clin Infect Dis 2011; 52:128–37. [PubMed: 21148531]

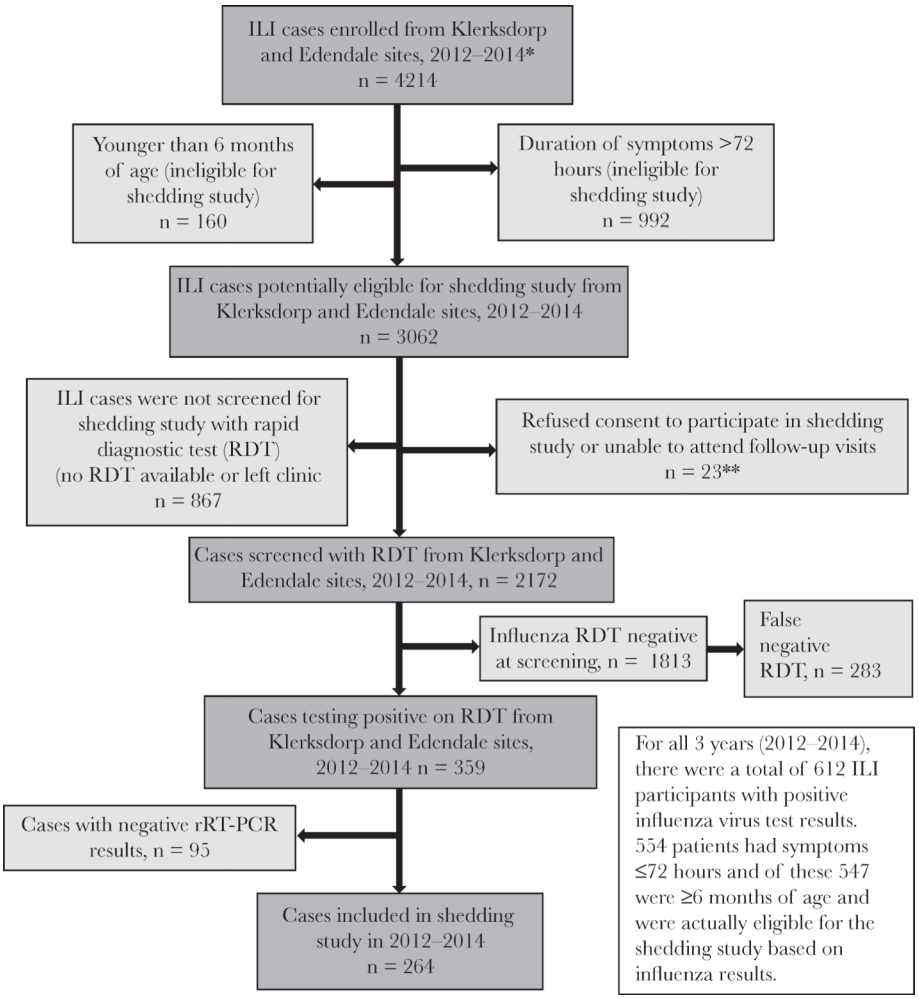
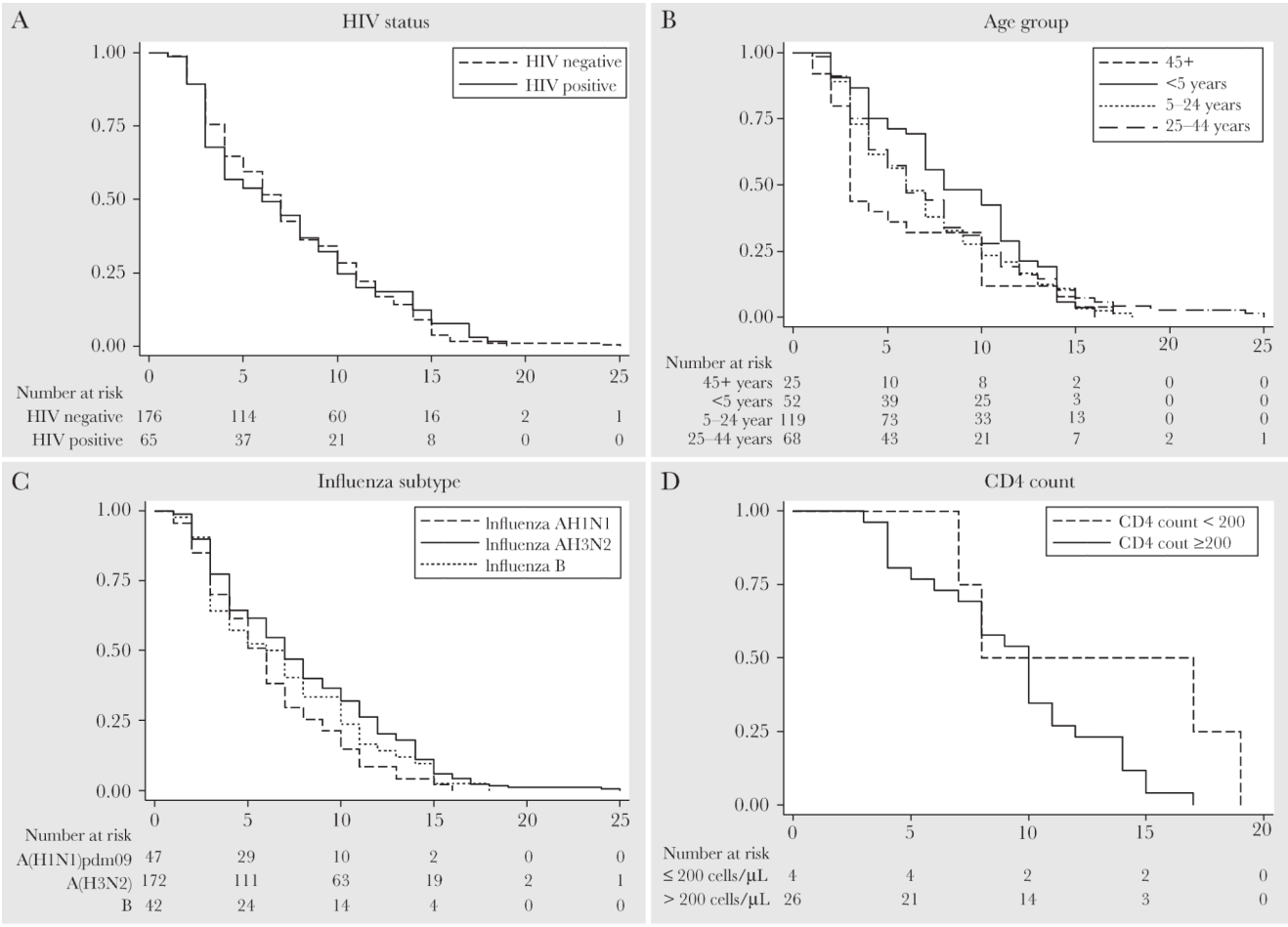


Figure 1.

Patients with influenza-like illness (ILI) enrolled into the influenza shedding study, Klerksdorp and Edendale, South Africa, 2012–2014. *In 2012 participants were enrolled in Klerksdorp from May to October, while in Edendale enrolment only started in August following ethics approval. In 2013 and 2014, enrolment was from May to October at both sites. **Does not include patients who refused to participate in the ILI surveillance program or left before they were screened. Abbreviation: rRT-PCR, reverse transcription polymerase chain reaction.



Log-rank test for equality of survivor functions: HIV status $P = .86$; Age group $P = .17$; Influenza subtype $P = .02$; CD4 count $P = .14$.

Figure 2.

Kaplan-Meyer plots showing the probability of reverse transcription polymerase chain reaction-positive Influenza virus result by day after shedding onset by (*A*) human immunodeficiency virus (HIV) status, (*B*) age group, (*C*) influenza subtype, and (*D*) CD4 count.

Table 1.

Comparison of Baseline Characteristics, Based on HIV Status, in Individuals Enrolled into the Influenza Virus Shedding Study, Klerksdorp and Edendale, South Africa, 2012–2014

	All Individuals no./No. (%)	HIV-Infected no./No. (%)	HIV-Uninfected no./No. (%)	Univariate Analysis Odds Ratio[a] (95% CI)	*P* value	Multivariable Analysis Adjusted Odds Ratio[a] (95% CI)	*P* Value
Age group, years							
<5	52/264 (20)	2/65 (3)	47/176 (27)	Reference		Reference	
5–24	119/264 (45)	11/65 (17)	94/176 (53)	2.8 (0.6–2.9)	.20	3.0 (0.5–9.9)	.25
25–44	68/264 (26)	37/65 (57)	26/176 (15)	33.4 (75–50.1)	<.001	**33.1 (5.1–16.4)**	**<.001**
45+	25/264 (9)	15/65 (23)	9/176 (5)	39.2 (76–01.7)	<.001	**49.9 (6.7–73.1)**	**<.001**
Sex							
Male	104/256 (41)	10/63 (16)	88/176 (50)	Reference		Reference	
Female	152/256 (59)	53/63 (84)	88/176 (50)	5.3 (2.5–11.1)	<.001	**3.5 (1.5–8.5)**	**.005**
Crowding, ratio people/room							
≤3	20½55 (79)	57/62 (92)	134/176 (76)	Reference		Reference	
>3	54/255 (21)	5/62 (8)	42/176 (24)	0.3 (0.1–0.7)	.01	**0.2 (0.1–0.8)**	**.03**
Received tuberculosis treatment[b]							
No	245/252 (97)	56/61 (92)	172/174 (99)	Reference	.02	Reference	
Yes	7/252 (3)	5/61 (8)	2/174 (1)	77 (1.4–40.7)		**36.9 (4.7–289.1)**	**.001**
Duration of influenza virus positivity on rRT-PCR, days median (range)	7 (3–11)	6 (3–11)	7 (4–11)		.84		
Duration of all symptoms, days median (range)	15 (8–18)	16 (7–21)	15 (9–18)		.30		
Duration of respiratory symptoms, days median (range)	15 (10–18)	16 (10–18)	15 (11–18)		.45		
Duration of fever, days median (range)	6 (3–12)	7 (3–14)	5 (3–12)		.23		

Only parameters significant on multivariable analysis are shown in the table. Bold indicates significant variables. Median duration of influenza shedding and symptoms are also shown. The following additional variables were evaluated but not found to be significant on univariate analysis: study year, study site, race, underlying medical conditions, and influenza subtype. Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; rRT-PCR, reverse transcription polymerase chain reaction.

[a]Odds ratio represents odds of being HIV positive.

[b] Received tuberculosis treatment in the last year.

Table 2.

Accelerated Weibull Regression for Duration of Influenza Virus Detection by rRT-PCR by Patient Baseline Characteristics, for All Participants (HIV-Infected and -Uninfected), Klerksdorp and Edendale, South Africa, 2012–2014

		Duration of Shedding, Days	Univariate Analysis		Multivariable Analysis	
Case Numbers Included in Each Category in Survival Analysis		Median (IQR)	Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value
Overall	264	7 (3–11)				
Age group, years						
<5	52	8 (5–12)	0.61 (0.38–0.98)	.04	**0.53 (0.30–0.93)**	**.03**
5–24	119	6 (3–10)	0.78 (0.51–1.20)	.26	0.69 (0.41–1.14)	.15
25–44	68	6 (4–11)	0.68 (0.43–1.07)	.10	0.68 (0.42–1.10)	.11
45+	25	3 (3–10)	Reference		Reference	
HIV status						
Uninfected	176	7 (4–11)	Reference		Reference	
Infected	65	6 (3–10)	1.01 (0.75–1.34)	.97	0.92 (0.64–1.33)	.67
CD4 count in HIV-infected participants						
≤200 cells/μL	4	13 (8–18)	Reference			
>200 cells/μL	26	10 (6–12)	2.26 (0.77–6.64)	.14		
Use of antibiotics						
No	248	6 (3–11)	Reference		Reference	
Yes	7	11 (6–15)	0.60 (0.28–1.25)	.17	**0.43 (0.20–0.96)**	**.04**
Influenza subtype						
A(H1N1)pdm09	47	6 (3–9)	Reference		Reference	
A(H3N2)	172	7 (4–12)	0.64 (0.46–0.89)	.008	**0.57 (0.40–0.80)**	**.001**
B	42	7 (3–10)	0.79 (0.52–1.20)	.27	0.76 (0.49–1.18)	.22

Only parameters significant on multivariable analysis and other key variables (such as HIV status and CD4 count) are shown in the table. Bold indicates significant variables. The following additional variables were evaluated but not found to be significant on univariate analysis: study year, study site, sex, race, crowding, housing type, underlying medical conditions, asthma, malnutrition, alcohol use, smoking, working in a mine, received tuberculosis treatment, and other viral coinfections.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; IQR, interquartile range; rRT-PCR, reverse transcription polymerase chain reaction.

Table 3.

Accelerated Weibull Regression for Duration of Influenza Virus Detection by rRT-PCR by Patient Baseline Characteristics, for HIV-infected Participants, Klerksdorp and Edendale, South Africa, 2012–2014

		Duration of Shedding, Days	Univariate Analysis		Multivariable Analysis	
	Case Numbers Included in Each Category in Survival Analysis	Median (IQR)	Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value
Overall	65	6 (3–10)				
Age group, years						
<5	2	3 (2–4)	4.60 (1.00–21.12)	.05	**26.75 (2.50–286.68)**	**.007**
5–24	11	8 (3–15)	0.69 (0.32–1.51)	.35	2.97 (0.80–10.97)	.10
25–44	37	7 (4–11)	0.90 (0.49–1.63)	.72	1.42 (0.52–3.84)	.49
45+	15	3 (2–10)	Reference		Reference	
CD4 count in HIV-infected group						
≤200 cells/μL	4	13 (8–18)	Reference		Reference	
>200 cells/μL	26	10 (6–12)	2.26 (0.77–6.64)	.14	**3.55 (1.05–12.08)**	**.04**
Received tuberculosis treatment[a]						
No	56	6 (3–11)	Reference			
Yes	5	3 (3–4)	3.35 (1.29–8.70)	.01		
On HIV treatment						
No	15	8 (4–14)	Reference			
Yes	42	4 (3–10)	1.78 (0.98–3.24)	.06		

Only parameters significant on multivariable analysis and other key variables (such as receipt of tuberculosis treatment or HIV treatment) are shown in the table. Bold indicates significant variables. The following additional variables were evaluated but not found to be significant on univariate analysis: study year, study site, sex, race, crowding, housing type, underlying medical conditions, asthma, alcohol use, smoking, use of antibiotics, other viral coinfections, and influenza subtypes.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; IQR, interquartile range; rRT-PCR, reverse transcription polymerase chain reaction.

[a]Received tuberculosis treatment in the last year.

Table 4.

Accelerated Weibull Regression for Factors Affecting Duration of All Symptoms[a] for All Participants (HIV-Infected and -Uninfected), Klerksdorp and Edendale, South Africa, 2012–2014

	Case Numbers Included in Each Category in Survival Analysis	Duration of Symptoms, Days Median (IQR)	Hazard Ratio (95% CI)	*P* Value	Adjusted Hazard Ratio (95% CI)	*P* Value
Overall	264	15 (8–18)				
Study site						
Klerksdorp	65	16 (12–22)	Reference		Reference	
Edendale	197	14 (6–17)	1.48 (1.12–1.97)	.007	**1.65 (1.20–2.28)**	**.002**
Age group, years						
<5	52	16 (13–23)	0.64 (0.42–0.98)	.04	**0.53 (0.34–0.84)**	**.007**
5–14	73	17 (14–22)	0.63 (0.42–0.95)	.03	**0.52 (0.34–0.81)**	**.003**
15–24	46	13 (9–17)	Reference		Reference	
25–44	66	16 (12–17)	0.72 (0.48–1.09)	.12	**0.57 (0.34–0.94)**	**.03**
45–64	25	16 (12–22)	0.66 (0.38–1.14)	.14	0.61 (0.32–1.17)	.14
HIV status						
Uninfected	176	15 (9–18)	Reference		Reference	
Infected	65	16 (7–21)	0.90 (0.68–1.20)	.48	0.86 (0.57–1.32)	.50

Only parameters significant on multivariable analysis and other key variables (such as HIV status) are shown in the table. Bold indicates significant variables. The following additional variables were evaluated but not found to be significant on univariate analysis: study year, sex, race, CD4 count, crowding, housing type, underlying medical conditions, asthma, malnutrition, alcohol use, smoking, working in a mine, received tuberculosis treatment, use of antibiotics, other viral coinfections, and influenza subtypes.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; IQR, interquartile range.

[a]All symptoms included respiratory symptoms (sore throat, cough, and rhinorrhea) plus fever and myalgia.

J Infect Dis. Author manuscript; available in PMC 2019 May 03.