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Containing Ebola virus infection in West Africa

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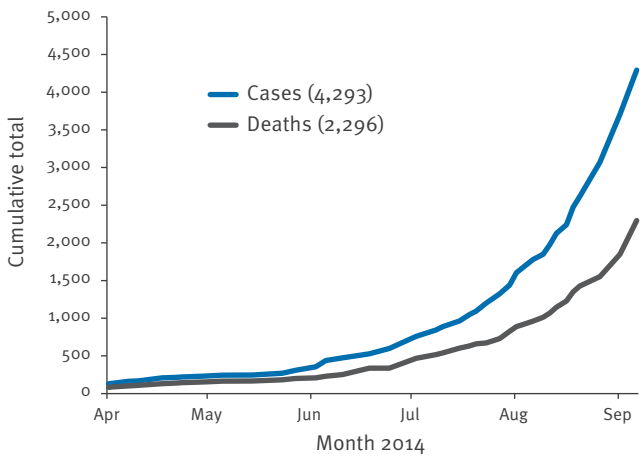
Ebola virus disease (EVD) is leaving a mark deeper and wider than ever before. The current outbreak now spans five countries in West Africa – Guinea, Liberia, Nigeria, Senegal and Sierra Leone – with over 4,200 cases and 2,200 deaths reported to the World Health Organization (WHO) as of 6 September 2014 (Figure 1) [1]. Unfortunately, with many cases either not reported or yet to show symptoms, the true number of infections is likely to be considerably higher. The first countries affected were among the world's poorest, areas where long periods of civil wars have battered health services and eroded public trust. As a result, the outbreak has spread to other countries, and continues to expand. What began as a local problem has turned into an international crisis.

Challenges for control in Africa

Past Ebola outbreaks have never risen beyond a few hundred reported cases, and even these events have been comparatively rare. When EVD spills over from its animal host into human populations, it typically generates dozens rather than hundreds of infections [2].

FIGURE

Cumulative number of Ebola virus disease cases and deaths in West Africa, April to 6 September 2014



Source: World Health Organization [1]

Chance events in the early stages of an outbreak can have a large impact on its final size. Infected individuals' movement patterns, social interactions, beliefs about disease causation and trust in authorities can all influence the extent of transmission, and hence the scale of control measures required to stop the infection.

In theory, Ebola is easily containable. It has a long incubation period – around a week on average – and cases are typically infectious only after displaying symptoms [3,4]. This means that isolation of symptomatic patients, contact tracing and follow-up surveillance of all contacts should be sufficient to stop transmission. Contrast this with pandemic influenza, which has a much shorter incubation period and can generate numerous cases who may be asymptomatic yet infectious [5]. For isolation to be effective during an Ebola outbreak, however, there must be rapid identification of cases and follow-up of contacts. Several factors can hinder this. In settings with limited testing facilities, cases that are not tested can be misdiagnosed. Not all EVD patients display distinctive hemorrhagic symptoms: the 1994 Ebola outbreak in Gabon was originally attributed to yellow fever [6], and early cases in the 1995 Kikwit outbreak were mistaken for dysentery and typhoid fever [7].

The exponential growth in case numbers during an outbreak also makes resource-intensive activities like contact tracing and surveillance increasingly difficult. Recent studies, including the one by Nishiura et al. in this issue, suggest that the reproduction number of Ebola (the average number of secondary cases generated by a typical case) is between 1.5–2 in some countries [8,9]. Based on the durations of incubation and infectiousness of EVD [3], it is plausible that the number of cases could therefore double every fortnight if the situation does not change. There are currently hundreds of new EVD cases reported each week; with the number of infections increasing exponentially, it could soon be thousands. Following up contacts and monitoring them for symptoms has already become unfeasible in areas where health authorities are stretched to the limit.

Disease control efforts in West Africa have been further hampered by cases not attending healthcare facilities, and instead remaining in the community. Fear and mistrust of health authorities has contributed to this problem, but increasingly it is also because isolation centres have reached capacity. As well as creating potential for further transmission, large numbers of untreated – and therefore unreported – cases make it difficult to measure the true spread of infection, and hence to plan and allocate resources. Even if patients are isolated, however, and their close contacts successfully traced, efforts can be undermined by unpredictable behavior. This was exemplified by the outbreak reported last week in Port Harcourt, Nigeria, which started after a contact of the index case in Lagos broke quarantine and left the capital [10].

Fear and mistrust are not unique to the current Ebola outbreak. During the 2000-1 outbreak in Uganda, health authorities faced similar challenges, including public protests, lack of co-operation from followed-up contacts, and shortages of staff willing to work in Ebola isolation units [11]. To control the infection, authorities needed to provide leadership and build trust. Interventions included education in various settings: in the community, educators strived to instill confidence, explaining how to avoid infection and recognise symptoms, while in hospitals, healthcare workers were provided with additional training, support and protection [12].

Education can also help address cultural practices that fuel outbreaks. The initial chain of Ebola virus transmission in Guinea in early 2014 included two funerals [13], and in May, another funeral introduced the epidemic to Sierra Leone [14]. Again, this is not just a feature of the present outbreak in West Africa. Funeral practices contributed to previous outbreaks in Central Africa too, but in many instances, it was possible to change people's behaviour. With support from health educators, communities altered the way burials were conducted, reducing transmission [12,15].

Need for an international response

Introducing control measures requires substantial resources, and there is a limit to what a local response can achieve alone. Yet as the current outbreak has grown, neighboring countries have closed borders and introduced travel restrictions. Similar actions were taken during past outbreaks, such as the one in Uganda in 2000-1 [16]. Such restrictions can hinder control efforts, making it harder to bring in personnel and resources.

Ebola cannot be ignored in the hope it will burn itself out. It is true that outbreaks of acute infections will generally decline once a large number people have been infected, because there are no longer enough susceptible individuals to sustain transmission. But if Ebola indeed has a reproduction number of 2 in some locations as described by Nishiura et al. [8], the susceptible pool – which likely includes most individuals – would have to shrink by at least half before the outbreak declined of its own accord [17]. Given the vast populations in affected areas and the disease's high fatality rate, this is clearly not an acceptable scenario.

Stopping transmission will instead require stronger control measures. On 28 August, the WHO issued a road map to provide a plan for the Ebola response [18]. It had three main objectives: (i) to achieve full coverage of control measures in countries with widespread transmission; (ii) to introduce emergency interventions in countries with an index case or small outbreak; and (iii) to strengthen Ebola preparedness in other countries, especially those connected to affected areas.

The scale of the current outbreak means an international response is needed. The threat to Europe and other continents remains low – in countries with strong health systems, an imported case should be straightforward to contain [19] – but without containment the devastation in West Africa will continue. Much of the damage is now coming from knock-on effects on basic healthcare. Not just EVD patients are affected by the outbreak; in cities like the Liberian capital Monrovia, the presence of the infection has led to the closure of most health facilities. As a result, untreated injuries and illnesses are leading to further loss of life.

In collaboration with affected countries, the international community must commit the resources required to control the outbreak. A week ago, Médecins Sans Frontières announced an urgent need for expertise and equipment [20]. As well as financial support, affected countries require experienced healthcare workers and specialists in biological disasters. The response must also include additional protective clothing and isolation units, and diagnostic tools and laboratory testing facilities. Health authorities will need food for those in quarantine too, plus vehicles to transport patients and trace their contacts, and air support to move resources between affected areas.

The scientific community can also support control efforts. Mathematical modelers can help quantify transmission in different areas, and provide short-term forecasts. Researchers are also working on potential drugs and vaccines. On 4 and 5 September 2014, WHO held a meeting to discuss what treatments are currently in development [21]. Testing of these experimental therapies and vaccines will soon start and must be fast-tracked to establish their safety and efficacy.

The effort required to control EVD will inevitably vary by country. In some locations, it has been suggested that the reproduction number could already be near 1; in others it could still be as high as 2 [8]. As pointed out above, the size of the transmission and the reproduction number will be influenced by multiple factors, including the level of public trust in authorities

and health services, as well as behaviours and beliefs shaped by social and cultural traditions. Transmission is also likely to be setting-specific. The reproduction number is an average value: some individuals and interactions will contribute more to transmission than others. The infection will be easier to control if it is possible to identify and target these crucial links in the transmission chain.

Over the past 38 years, there have been more than twenty Ebola outbreaks, and all of them have been successfully contained. Many of the issues currently facing West Africa – from lack of trust in health authorities to poor infection control – have surfaced before, and have been overcome. However, the current outbreak is unprecedented both in size and scale. It will require a response to match.

Conflict of interest

None declared.

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Early transmission dynamics of Ebola virus disease (EVD), West Africa, March to August 2014

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The effective reproduction number, R_t, of Ebola virus disease was estimated using country-specific data reported from Guinea, Liberia and Sierra Leone to the World Health Organization from March to August, 2014. R_t for the three countries lies consistently above 1.0 since June 2014. Country-specific R_t for Liberia and Sierra Leone have lied between 1.0 and 2.0. R_t<2 indicate that control could be attained by preventing over half of the secondary transmissions per primary case.

Introduction

The largest and first regional outbreak of Ebola virus disease (EVD) has been unfolding in West Africa since approximately December 2013, with the first cases traced back to southern Guinea [1]. However, the outbreak was not recognised until March 2014 [1], which facilitated the spread to neighbouring Sierra Leone and Liberia through porous borders as well as Nigeria via a commercial airplane on 20 July [2]. The World Health Organization (WHO) declared this EVD epidemic a Public Health Emergency of International Concern on 8 August 2014 [3]. According to phylogenetic analyses, the causative Ebola virus strain is closely related to a strain associated with past EVD outbreaks in Central Africa, and could have been circulating in West Africa for about a decade [4].

A total of 3,707 cases (including 2,106 confirmed, 1,003 probable and 598 suspected cases, respectively) and 1,848 deaths (concerning 1,050 confirmed and 557 probable cases, as well as 241 suspected cases and deaths, respectively) have been reported in Guinea, Sierra Leone, Liberia, Nigeria, and Senegal as of 31 August 2014 [5]. The total number of cases in Guinea, Sierra Leone, Liberia, Nigeria and Senegal have been 771, 1,216, 1,698, 21 and one, respectively. By contrast, the great majority of past outbreaks have been associated with small numbers of reported cases and have been confined to isolated rural areas in Central Africa. For reference, the largest outbreaks in Central Africa generated 315 cases in Congo in 1976 and 425 cases in Uganda in 2000 [6,7].

The effective reproduction number, R_t, which measures the average number of secondary cases generated by a typical primary case at a given calendar time, can be helpful to understand the EVD transmission dynamics over time in affected countries as well as gauge the effect of control interventions [8]. Values of R_t<1 indicate that the epidemic is in a downward trend. By contrast, an epidemic is in an increasing trend if R_t>1. The mean reproduction number for EVD has been estimated at 1.83 for an outbreak in Congo in 1995 and 1.34 in Uganda in 2000 prior to the implementation of control interventions [9]. Here we sought to estimate the R_t, in real time in order to assess the current status of the evolving outbreak across countries affected in 2014. We also compare our estimates of the reproduction number for the current outbreak with those previously published for the largest outbreaks in Central Africa and discuss our findings from a public health perspective.

Methods

Case data

We analysed the cumulative case counts reported by the WHO [10] as of 26 August 2014. Case counts are classified into three categories, i.e. confirmed, probable and suspected cases. Confirmed cases are laboratory diagnosed by polymerase chain reaction (PCR), positive IgM antibody or viral isolation while suspected cases correspond to individuals presenting fever (≥38.5°C (101°F)) and no favourable response to treatment for usual causes of fever in the area, and at least one of the following clinical signs: bloody diarrhoea, bleeding from gums, bleeding into skin (purpura), bleeding into eyes and urine. Probable cases are suspected cases of EVD with an epidemiological link to a confirmed EVD case [11]. We analysed two different sets of grouped data, i.e. (i) confirmed plus probable

cases and (ii) the total number of reported cases (i.e. confirmed, probable and suspected cases).

Because case counts were reported in irregular time intervals, we estimated daily incidence curves of EVD cases in order to estimate R_t. For this purpose, we first fit a smoothing spline to country-specific cumulative curves of reported cases. Next we took the daily difference of the cumulative counts to obtain daily incidence time series. Of note, the cumulative case series reflects the diagnostic process (among suspected and probable cases) and sometimes declined as a function of time (e.g. 5 April and 12 July in Guinea and Sierra Leone, respectively). When the difference was negative, we replaced it by 0. The smoothing spline was chosen to obtain a coefficient of determination R^2 at 0.995. Data from Nigeria and Senegal have been omitted due to a limited number of cases recorded in these countries thus far.

Mathematical model

We employed mathematical modelling together with time- and country-specific incidence data to estimate the R_t. Thus, here we model the transmission dynamics of EVD using a country-specific next-generation matrix $\{k_{ij,t}\}$ representing the average number of secondary cases in country i at time τ generated by a single primary case in country j. Let g_τ represent the probability density function of the generation time of length t days for EVD. Hence, the expected value of EVD incidence in country i at time t is modelled as

$$E(c_{i,t}) = \sum_j k_{ij,t} \sum_{\tau=1}^{\infty} c_{j,t-\tau} g_\tau$$

The univariate version of Equation 1 has been employed by White and Pagano [12,13] in order to jointly estimate R_o and the generation time distribution of EVD. Assuming that EVD incidence follows a Poisson distribution, the likelihood to estimate $\{k_{ij,t}\}$ is

$$\prod_t \prod_i \frac{\left(\sum_j k_{ij,t} \sum_{\tau=1}^{\infty} r_{j,t-\tau} g_\tau\right)^{r_{i,t}} \exp\left[-\left(\sum_j k_{ij,t} \sum_{\tau=1}^{\infty} r_{j,t-\tau} g_\tau\right)\right]}{r_{i,t}!}$$

where $r_{i,t}$ is the estimated daily incidence in country i on day t derived from the difference of the smoothing spline fit to the cumulative data as explained above.

Each element of the next-generation matrix is interpreted as the average number of secondary cases generated by a single primary case at time t. We assume that the per-contact probability of infection and the average generation time do not differ by country. Thus, the contact matrix regulates the relative difference between each pair of entries of the next-generation matrix, and because the contact patterns within and between countries cannot be directly observed, we made a qualitative assumption for the matrix $\{k_{ij,t}\}$ to approximately capture the pattern of (domestic and transnational) transmission [14], i.e.

$$\mathrm{M}_t = \begin{pmatrix} k_{g,t} & \alpha & \alpha \\ \alpha & k_{s,t} & \alpha \\ \alpha & \alpha & k_{l,t} \end{pmatrix}$$

The matrix M_t qualitatively assumes that there are more frequent within-country transmissions (denoted by $k_{g,t}$, $k_{s,t}$ and $k_{l,t}$, where the subscripts g, s and l represent Guinea, Sierra Leone and Liberia, respectively) compared with transnational spread. The transnational spread is modelled by a single parameter a. We employed a piecewise constant model and change the parameters for the above-mentioned elements every seven days. Maximum likelihood estimates of the parameters were obtained by minimising the negative logarithm of Equation 2. Using the most recent incidence estimate i_o and the exponential growth rate r as calculated from $r=(R-1)/12$ (where R is the most recent reproduction number and 12 is the mean generation time), the expected number of additional cases in 2014 was calculated as

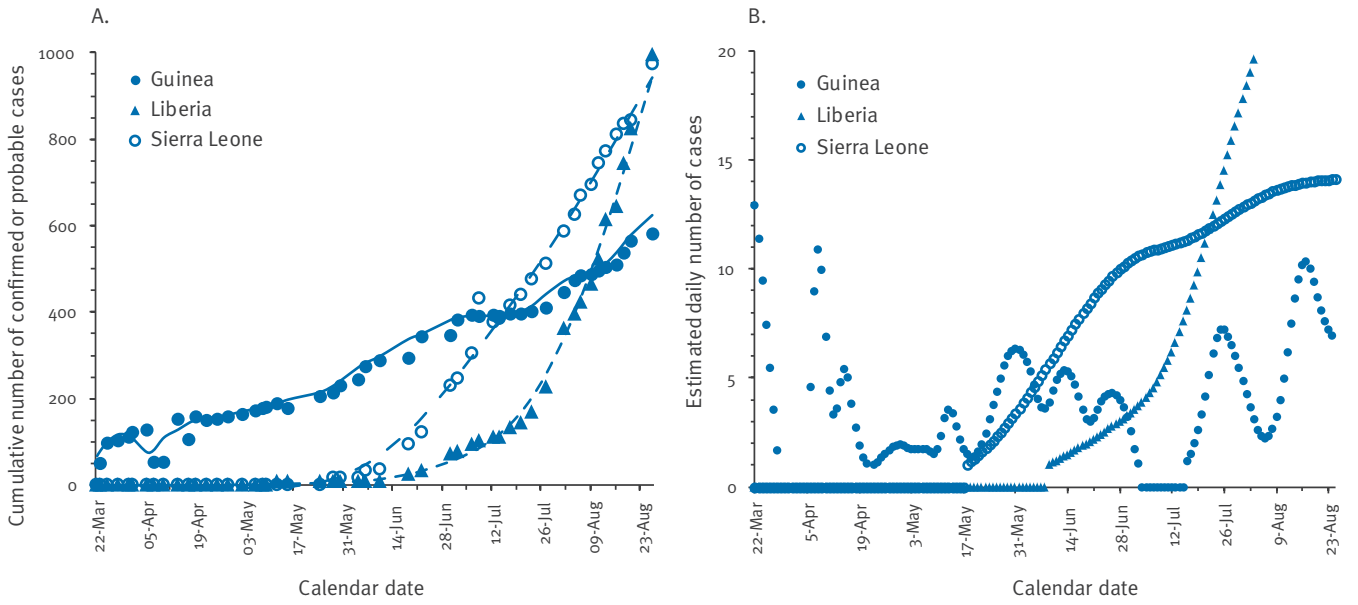
$$I = i_0 \int_0^{120} \exp(rt)\, dt$$

. The expected cases represent a 'worst-case' scenario based on the current situation by assuming a fixed reproduction number R for the remainder of the year (i.e. approximately 120 days remaining in 2014).

We also computed the R_t for all countries (hereafter referred to as the 'global' estimate of the reproduction number) by calculating the dominant eigenvalue of the estimated next-generation matrices. Moreover, we calculated column sums of the matrices to estimate the average number of secondary transmissions arising in and from a specific country and also extracted estimates of 2α, the value that governs the transnational spread generated by a single primary case. Although White and Pagano achieved the joint estimation of R_o and generation time distribution [12,13], we assumed that the generation time is known, because our analysis relies solely on the cumulative number of reported cases with irregular reporting intervals. The generation time was assumed to follow an exponential distribution with a mean of 12 days [15], which is known to be close to the mean incubation period [16]. Based on empirical data of the serial interval distribution [15], we also carried out a sensitivity analysis of reproduction numbers by varying the mean generation time between nine and 15 days. The 95% confidence intervals of the R_t can be computed via bootstrapping methods. However, our study focused on examining model uncertainty associated with the transnational mixing patterns and the mean generation time as model uncertainty in our study is likely more influential on R_t compared to uncertainty relating to measurement error. In sensitivity analyses, we also examined the impact of varying specified time interval on R_t. For this purpose, we also

Figure 1

Cumulative and daily epidemic curves of Ebola virus disease (EVD) in Guinea, Liberia, and Sierra Leone, 23 March–26 August 2014



A)Cumulative number of confirmed or probable cases of EVD reported to the World Health Organization [10]. Solid lines are the smoothing spline fits to cumulative curves for each country with a coefficient of variation R^2 at 0.995.

B) Estimated daily incidence curves based on the smoothing spline model. Data from Nigeria and Senegal have been omitted due to the limited number of cases recorded in these countries thus far.

analysed the piecewise constant model for every six and eight days instead of seven days.

Results

Figure 1 illustrates the process of deriving daily EVD incidence curves by country from cumulative curves of reported cases. Multiple fluctuations are evident from the incidence curve for Guinea (Figure 1). In Liberia, the early transmission phase did not appear to exhibit sustained growth and was probably driven by case importations during first epidemic month. Exponential growth was subsequently seen, reflecting self-sustaining transmission. Similarly, the incidence curve for Sierra Leone also displayed steady growth since early June. Most recent EVD incidence data for Guinea also showed an increasing pattern.

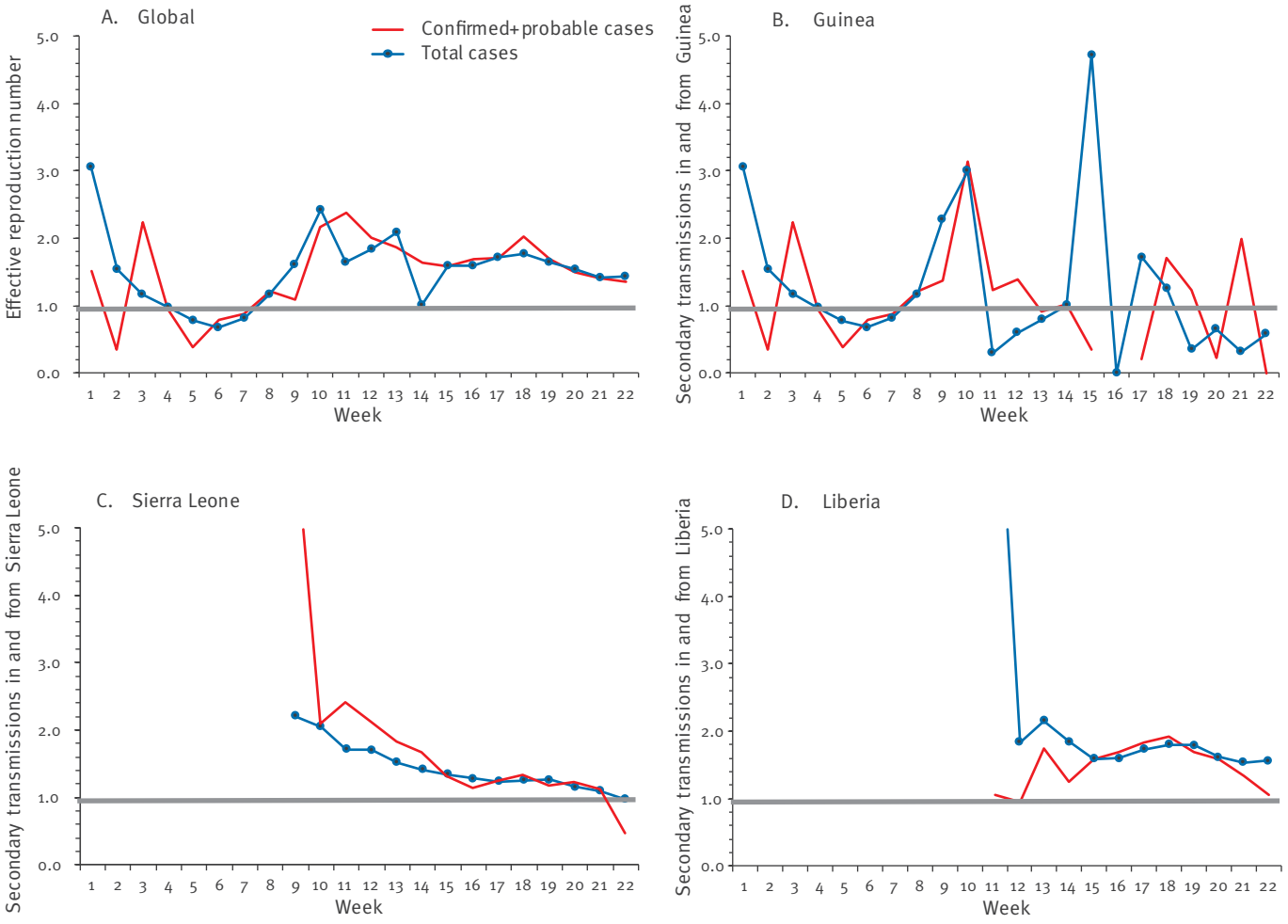
Our weekly maximum likelihood estimates of the R_t for each affected country and for the global system in West Africa are displayed in Figure 2. Results indicate that the reproduction number for all countries reached levels below unity in April and May, but has appeared to be continuously above one since early June (Figure 2A). This pattern was robust when using two different datasets (including and excluding suspected cases). Estimates of R_t using total case reports from June to July 2014, a period during which exponential growth of cases has been observed in Sierra Leone and Liberia, ranged from 1.4 to 1.7, respectively. In the hypothetical worst-case scenario that the current situation with an estimated reproduction number R ranging from 1.4 to 1.7 continues for the remainder of the year, we would expect to observe a total of 77,181 to 277,124 additional cases within 2014.

Maximum likelihood estimates of R_t in Guinea appeared to have fluctuated around 1.0 (Figure 2B), which reflects the observed variation in the corresponding incidence curve. Importantly, R_t in this country has not been continuously below 1.0, which supports the view that in this country the outbreak is not yet under control. Estimates of R_t in Sierra Leone and Liberia appeared to be consistently above 1.0 up to week 22 (i.e. the week starting on 18 August) (Figure 2C and 2D). Although R_t in Sierra Leone has been declining with the highest estimates obtained for early June, R_t has not been consistently below 1.0 in this country, including estimates for the latest reporting week (Figure 2). The pattern of R_t in Liberia shows values well above 1.0 since July 2014. In this country, the estimates of R_t reaching values up to 2.0 indicate that the outbreak could only be brought under control if more than half of secondary transmissions per primary case were prevented.

Figure 3A shows the estimated average number of transnational transmissions per single primary case as a function of time (calculated by 2α). α has been high in early June, but has declined dramatically since late June. Nevertheless, most recent model estimates still suggest a non-negligible number of cross-border

Figure 2

Effective reproduction number of Ebola virus disease (EVD) estimated for Guinea, Sierra Leone, Liberia, and for the global system in West Africa, 23 March–26 August 2014



A) Global (maximum likelihood) estimates of the effective reproduction number of EVD based on data from all affected countries (Guinea, Sierra Leone and Liberia) were derived from the dominant eigenvalue of the next generation matrix.

B-D) The average number of secondary transmissions arising from Guinea, Sierra Leone and Liberia, was calculated from the corresponding column sum of the next generation matrix. The horizontal grey solid line indicates the reproduction number at 1.0 for reference, below which the epidemic follows a declining trend. Estimates were derived using either confirmed cases plus probable cases or the total reported case counts (confirmed, probable plus suspected cases). Data from Nigeria and Senegal have been omitted due to limited number of cases recorded thus far. Epidemic week 0 corresponds to the week that includes 22 March 2014.

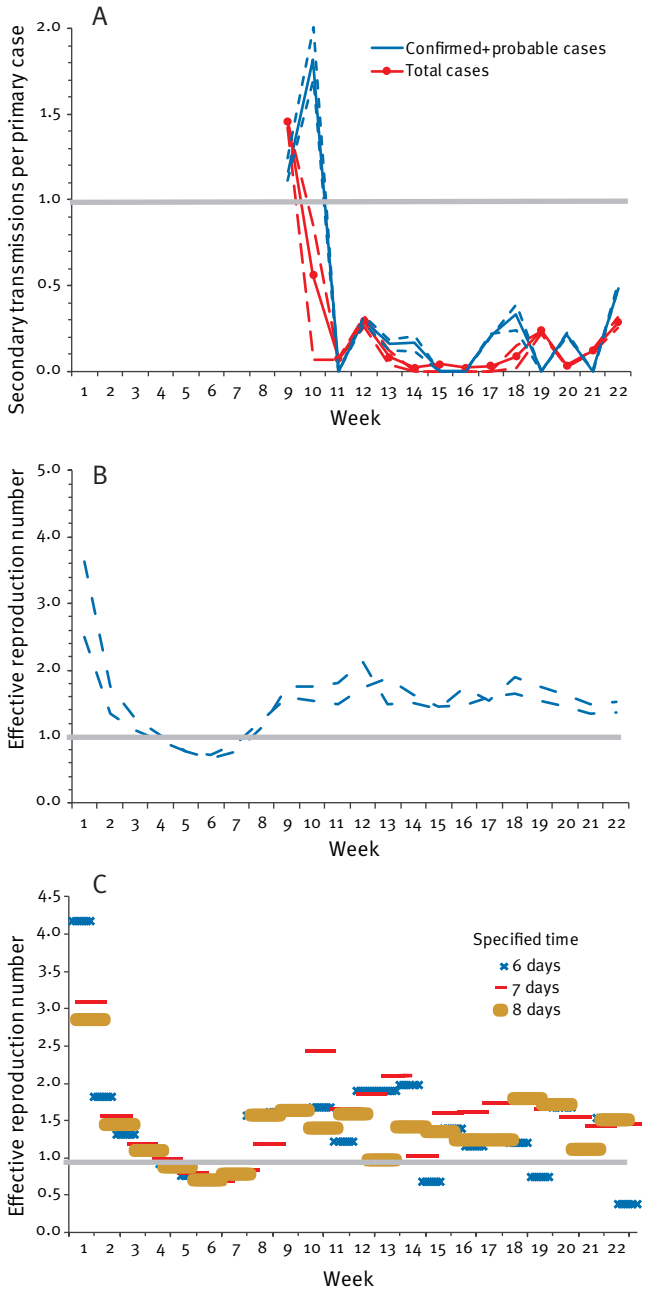
transmissions. Figure 3B examines the sensitivity of R_t for all countries to changes in the mean generation time. Although the absolute values of R_t are positively correlated with the mean generation time, the above-mentioned qualitative patterns of R_t are preserved, which indicates that the ongoing EVD epidemic has yet to be brought under control. Figure 3C examines the sensitivity of R_t to a specified time interval of the piecewise constant model. Perhaps not surprisingly, as the interval is shortened, fluctuations in R_t tend to increase, perhaps due to stochastic effects. Nevertheless, all models roughly provide qualitatively similar patterns in R_t.

Discussion

We have derived global and country-specific estimates of the R_t of EVD for the ongoing outbreak in West Africa. Our global estimates of the R_t appear to be continuously above one since early June, indicating that the epidemic has been steadily growing and has not been brought under control as of 26 August 2014. The country-specific estimates for Sierra Leone and Liberia were also above one, perhaps reflecting the increasing trend in cases in these countries since June. Our estimated reproduction numbers, broadly ranging from one to two, are consistent with published estimates from prior outbreaks in Central Africa [9,17]. Our estimates of R_t<2 indicate that the outbreak could

Figure 3

Sensitivity analysis of the effective reproduction number of Ebola virus disease (EVD), West Africa, 23 March–26 August 2014



A) The estimated average number of secondary cases per single primary case arising from transnational spread. Solid lines represents estimates derived from the mean generation time of 12 days, while dashed lines correspond to estimates derived using nine and 15 days as the mean generation time.

B) Upper and lower bounds of the effective reproduction number (R_t)for the global dynamics in West Africa are shown assuming a mean generation time of EVD ranging from nine to 15 days. The horizontal grey line is shown as a reference for the reproduction number at 1.0 below which the epidemic follows a declining trend.

C) Sensitivity of R_t to varying specified time intervals of the piecewise constant model. Estimates in B and C were derived using the total number of reported EVD cases (confirmed, probable plus suspected cases). Epidemic week 0 corresponds to 22 March 2014. Of note, estimates overlap at week 9 as these were derived from epidemiological data for a single country (i.e. Guinea).

be brought under control if more than half of secondary transmissions per primary case are prevented.

Our statistical analysis of the reproduction number of EVD in West Africa has demonstrated that the continuous growth of cases from June to August 2014 signalled a major epidemic, which is in line with estimates of the R_t above 1.0. Moreover, the timing of R_t reaching levels above one is in line with a concomitant surge in cases in Sierra Leone and Liberia. In a worst-case hypothetical scenario, should the outbreak continue with recent trends, the case burden could gain an additional 77,181 to 277,124 cases by the end of 2014. Although such numbers must be interpreted with caution (as they rest on an assumption of continued exponential growth within 2014, which is unlikely), our study supports the notion that the ongoing EVD epidemic must be regarded as a Public Health Emergency of International Concern [3]. This finding also implies that transnational spread of EVD might have hindered control efforts, suggesting that preparedness plans for potential case introductions is critical particularly for countries at high risk of EVD case importations [18] with suboptimal public health systems. The transnational spread per person appears to have been reduced over time, but our most recent model estimates still suggest a non-negligible number of secondary cases arising from transnational spread. Uncontrolled cross-border transmission could fuel a major epidemic to take off in new geographical areas (e.g. as seen in Liberia). Unaffected countries at risk of transnational spread should be on high alert for potential EVD introductions and be ready to launch comprehensive and timely containment responses to avert outbreaks.

Our analysis is not exempted of limitations. First, the epidemic is ongoing in multiple geographical locations, and no simple mixing matrix can capture the complex geographical patterns of spread in the region. Second, cases may be under-ascertained, and hence reported cases may represent only a portion of the total number of infected individuals. However, our estimates of the reproduction number are not affected whenever the diagnosis and reporting rates have not dramatically changed over time. Third, the reporting delays are known to induce a downward bias in incidence in the latest observation, which can complicate real-time analyses. Several studies have successfully addressed this bias [19-22], but we were unable to incorporate this delay into our analyses due to a lack of empirical data to characterise the reporting delay distribution.

Despite the above-mentioned limitations, we believe that our findings are useful to demonstrate that the cases have been steadily growing in the last three months with an R_t above one. Close monitoring of this evolving epidemic should continue in order to assess the status of the outbreak in real time and guide control interventions in the region. Reviewing possible countermeasures for countries at risk of transnational

spread [18] would be of utmost importance to confront the ongoing propagation of cases over time and space.

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Conflict of interest

None declared.

Author contributions

HN conceived mathematical modeling method and analyzed the data. HN and GC drafted and revised the manuscript.

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Does the length of specimen storage affect influenza testing results by real-time reverse transcription-polymerase chain reaction? An analysis of influenza surveillance specimens, 2008 to 2010

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In some influenza surveillance systems, timely transport to laboratories for reverse transcription-polymerase chain reaction (RT-PCR) testing is challenging. Guidelines suggest that samples can be stored at 4°C for up to 96 hours but the effect of longer storage times has not been systematically evaluated. We collected nasopharyngeal and oropharyngeal specimens from patients in Kenya and stored them in viral transport medium at 2 to 8°C before testing for influenza A and B using real-time RT-PCR. From April 2008 to November 2010, we collected 7,833 samples; 940 (12%) were positive for influenza. In multivariable analysis, specimens stored for six days were less likely to be influenza-positive compared to specimens stored between zero and one day (adjusted odds ratio (aOR): 0.49, 95% confidence interval (CI): 0.27–0.93). There was no statistically significant difference in influenza positivity of specimens stored for five days compared to zero to one day. There was no statistically significant relationship between days in refrigeration and cycle threshold (C_t) values for positive samples (p=0.31). We found that samples could remain in storage for at least five days without affecting the proportion-positive of samples, potentially increasing the feasibility of including influenza surveillance sites in remote areas.

Introduction

Worldwide, an estimated 28,000 to 111,500 deaths attributable to influenza-associated acute lower respiratory infections occurred in children under five years-old in 2008 [1]. In recent years, with increased concerns about detecting and responding to an influenza pandemic, influenza surveillance has expanded globally. Many surveillance sites in resource-poor countries are remote and lack onsite diagnostic capacity, requiring that samples be transported far distances to a central laboratory [2,3]. In influenza surveillance systems throughout the world, real-time reverse transcription-polymerase chain reaction (rRT-PCR) is increasingly used as the test of choice to confirm influenza virus infection [4]. However, little is known about the optimal time that specimens can be stored in a refrigerator before being tested by rRT-PCR and recommendations regarding the maximum length of storage time of respiratory specimens before such assays vary among institutions. The World Health Organization (WHO) and the United States (US) Centers for Disease Control and Prevention (CDC) recommend that a sample be stored for no more than four days at 4°C before freezing or diagnostic testing [5,6] but neither guideline cites evidence as the basis for this recommendation, and a literature search revealed no published manuscripts on this topic.

In Kenya, as in many other countries in Africa, surveillance for influenza is conducted in healthcare facilities that are far from the laboratory that processes the specimens, and timely transport of specimens is often challenging. As a result, specimens sometimes may be stored at 4°C for longer than 96 hours. In order to address the question of whether prolonged refrigeration might lead to virus deterioration, which in turn would be associated with lower rates of positivity and lower overall viral loads, we evaluated two-and-a-half years of influenza surveillance data from Kenya to determine the relationship between the number of days a specimen was kept in storage and detection of influenza positivity by molecular testing. In addition, we evaluated the relationship between the number of storage days and the cycle threshold (C_t) values of influenza-positive samples.

Methods

Data collection

Influenza sentinel surveillance system

In 2007, the Kenya Ministry of Public Health and Sanitation (MoPHS) and the Kenya Medical Research Institute/Centers for Disease Control and Prevention-Kenya (KEMRI/CDC) established a national influenza sentinel surveillance system in Kenya in order to better understand the seasonality, burden, and epidemiology of influenza in the country and to detect new influenza virus strains with pandemic potential and for possible use in new vaccine formulations. At each of the sentinel healthcare facilities, a trained surveillance officer collects nasopharyngeal (NP) and oropharyngeal (OP) samples from all hospitalised patients with severe acute respiratory illness (SARI) and from up to three outpatients a day with influenza-like illness (ILI). For this study verbal consent was obtained from all patients before questionnaires were administered and specimens were collected. For children, verbal consent was obtained from guardians. Case definitions for SARI and ILI and the procedure for specimen collection have been previously described [7].

Specimen storage and transport

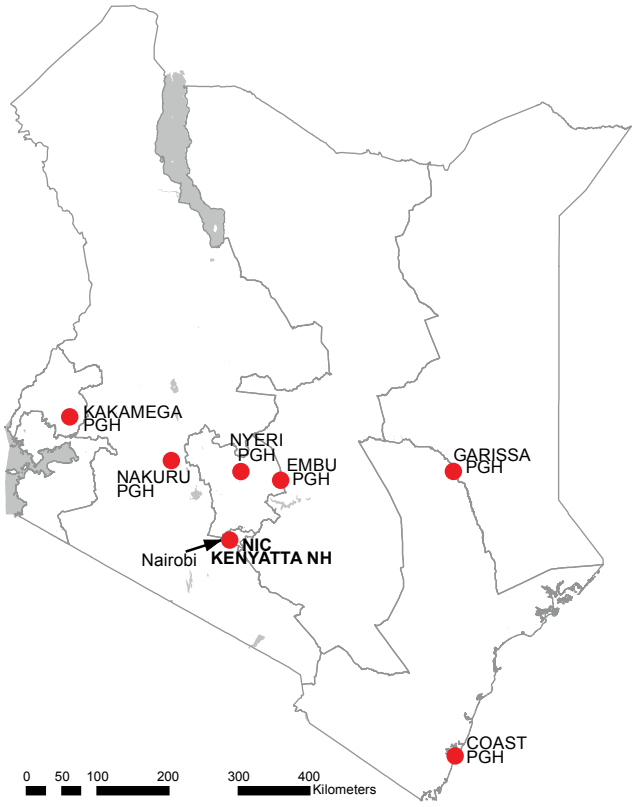
NP and OP specimens from each patient were placed in the same cryovial with viral transport medium (VTM). VTM was prepared centrally at the KEMRI/CDC laboratory using a WHO protocol that includes bovine serum albumin and veal infusion broth supplemented with amphotericin B [8]. Briefly, 10 g veal infusion broth, 2 g of bovine albumin fraction V and 3.2 ml of fungizone (250 µg/ml amphotericin) were weighed and 400 ml of distilled water added and the contents allowed to dissolve by gentle stirring. The media was allowed to stand for one hour at 4°C, sterilised by filtration, and using aseptic techniques, 1 ml aliquots dispensed into sterile 1.8 ml propylene cryovials. Quality control steps were included at all steps in the VTM preparation. The VTM was shipped, at 2 to 8°C, to surveillance sites and refrigerated prior to and after insertion of the swab specimens. VTM was used for up to three months after preparation. After collection, specimens in VTM were immediately placed in refrigeration at 2 to 8°C. All specimens were transported in cool boxes by road to the National Influenza Center (NIC) in Nairobi and were tested for influenza by rRT-PCR at KEMRI/CDC laboratory in Nairobi. The cool boxes were kept at refrigeration temperature with ice packs. Upon arrival at the laboratory all specimens were frozen at -80°C.

Laboratory testing

An aliquot of each respiratory specimen was tested by rRT-PCR for influenza A and influenza B after one freeze-thaw cycle. Specimens positive for influenza A were subtyped for seasonal H1 and H3 as well as for H5 and A(H1N1)pdm09 by rRT-PCR [6]. Samples were aliquoted and total RNA was extracted from 100 µl aliquots of each sample using QIAamp viral RNA minikit

Figure 1

Sentinel influenza surveillance sites in Kenya, 2008–2010 (n=7)



NH: national hospital; NIC: National Influenza Center; PGH: provincial general hospital.

(Qiagen inc, Valencia CA, USA), according to manufacturer's instructions. One step rRT-PCR was carried out using AgPath kits (Applied Biosystems, California USA). The primers, probes, and positive controls for all influenza viruses were provided by CDC-Atlanta [9]. Following the reverse transcription step, a typical 45 cycle PCR reaction was run and fluorescence was read at the annealing/extension step at 55°C, and recorded at each cycle [10]. Appropriate negative and positive control specimens were run alongside each reaction. The results were recorded as cross-over C_t values. A C_t value ≤39.9 was regarded as positive, whereas C_t values ≥40.0 were considered negative in the analysis. Samples with no C_t values were regarded as negative [9].

Data analysis and statistical methods

Samples

Samples collected from seven sentinel surveillance sites between 10 April 2008 and 8 November 2010 with available storage, demographic, and laboratory data were included in the analysis. The seven sites were located from 2 km to 487 km from the laboratory in Nairobi (Figure 1). We determined the number of storage days that samples were refrigerated by subtracting

the date the sample was collected from the date the sample was received at the NIC laboratory. Transport time was included in the refrigeration period. Samples that were in refrigeration for duration of zero to 10 days were included in the analysis.

Statistical analysis

Storage days were analysed as individual days. Because a relatively small number of samples were stored for only one day and there was no difference in influenza positivity between zero and one storage days ($p>0.05$), we combined zero and one storage days into one category to use as a reference group. We used the Cochran–Armitage trend test to assess the relationship between storage days and positivity of influenza samples. We then modeled influenza positivity with storage day as a categorical predictor (0–1 vs 2, 3, 4, 5, 6, 7, 8, 9, 10) using multivariable logistic regression, controlling for the following variables: patient age, days since illness onset, surveillance site, and syndrome (ILI or SARI) classification. If the variable was found to be associated with influenza positivity at $p<0.2$ in the bivariate analysis it was considered a potential confounder and included in the multivariable analysis. We included days since illness onset in the model because the quantity of viral shedding decreases after three to five days following illness onset [11]. We then fit the logistic regression model for ILI and SARI cases (controlling for patient age, days since illness onset, surveillance site). We used logistic regression rather than linear regression because we felt that logistic regression was the best way to assess the effect of the length of specimen storage on influenza positivity; in our analysis, using logistic regression allowed us to target the outcome variable (influenza positivity), which was dichotomous and categorical.

In order to determine the relationship between storage days and C_t values of influenza samples we performed multivariable generalised ordinal logit analysis for influenza-positive samples. We excluded the 39 specimens that had influenza A/B co-infections because including two C_t values for an individual sample would have made it impossible to draw a single conclusion about the relationship between storage time and C_t value for the sample. C_t values were not normally distributed; therefore we created quartiles for C_t values, and a multivariable generalised ordinal logit model was fit using zero to one as a reference group. We also analysed the mean C_t values for influenza-positive samples by storage day. We stratified the positive results by C_t values using the categories ≤29, 30–≤37, and 38–<40 [12]. Data analysis was done using SAS version 9.1 (SAS Institute Inc, Cary, North Carolina, USA) and findings were considered statistically significant if the p-value was <0.05.

Ethical considerations

The Kenyan Ministry of Health determined that the surveillance system was part of routine national disease surveillance and did not represent research and did not require ethical review.

Results

Demographics

Of 12,541 samples collected during the study period in the seven sites, 7,833 (62%) had storage, demographic, and testing data available and were included in the analysis. Of the 7,833 samples, 940 (12%) were positive for influenza; 718 (9%) were influenza A only, 183 (2%) were influenza B only, and 39 (<1%) were positive for both A and B. Of the 528 influenza A-positive samples that were subtyped, 95 (18%) were seasonal H1, 222 (42%) were seasonal H3, and 211 (40%) were A(H1N1)pdm09; 4,311 samples (55%) were from male patients. The mean age was 2.4 years, and the majority of samples (5,095; 65%) were from patients <2 years-old. Of the 4,708 samples that were not included because storage and/or testing data were not available, 3,568 (76%) had age data and, 3,624 (77%) had

Table 1

Demographic characteristics and influenza positivity among influenza-like illness and severe acute respiratory illness patients tested for influenza infections, Kenya, 2008–2010 (n=7,833)

Variable	Number tested (%)	Number positive for Influenza (%)
Age group in years		
<2	5,095 (65)	493 (10)
2–4	2,147 (27)	331 (15)
5–17	454 (6)	102 (22)
≥18	137 (2)	14 (10)
Total	**7,833 (100)**	**940 (12)**
Sex		
Male	4,311 (55)	511 (12)
Female	3,522 (45)	429 (12)
Total	**7,833 (100)**	**940 (12)**
Sentinel site		
Embu	474 (6)	41 (9)
Garissa	383 (5)	49 (13)
Kakamega	2,383 (30)	264 (11)
Kenyatta	478 (6)	33 (7)
Coast	840 (11)	73 (9)
Nakuru	1,433 (18)	196 (14)
Nyeri	1,842 (24)	281 (15)
Total	**7,833 (100)**	**940 (12)**
Case type		
ILI	3,813 (48)	561 (15)
SARI	4,012 (51)	378 (9)
Total	**7,825[a] (100)**	**939[a] (12)**

ILI: influenza-like illness; SARI: severe acute respiratory illness.

[a] Eight samples were missing data on SARI and ILI categorisation. One of the samples with missing data was positive for influenza.

Table 2

Association between duration of storage of respiratory samples and percentage of influenza A and B-positive using zero to one storage days as a reference, Kenya, 2008–2010

Storage time in days	n/N (% positive)	Bivariate analysis	Multivariable analysis[a]
		OR (95% CI)	aOR (95% CI)
0–1	462/3,969 (12)	REF	REF
2	240/1,899 (13)	1.10 (0.93–1.30)	0.98 (0.83–1.17)
3	79/617 (13)	1.12 (0.86–1.44)	0.96 (0.74–1.25)
4	103/673 (15)	1.37 (1.09–1.73)[b]	1.26 (1.00–1.61)
5	26/222 (12)	1.01 (0.66–1.53)	0.98 (0.66–1.54)
6	11/180 (6)	0.49 (0.27–0.92)[b]	0.49 (0.27–0.93)[b]
7	8/119 (7)	0.55 (0.27–1.13)	0.50 (0.25–1.07)
8	7/67 (10)	0.89(0.40–1.95)	0.88 (0.40–1.94)
9	2/47 (4)	0.34 (0.08–1.40)	0.33 (0.08–1.35)
10	2/40 (5)	0.4 (0.10–1.66)	0.36 (0.09–1.53)

aOR: adjusted odds ratio; CI: confidence interval; OR: odds ratio; REF: reference category.

[a] Logistic regression model controlling for patient age, days since illness onset, surveillance site, and syndrome classification (influenza-like illness vs severe acute respiratory illness); 7,792 samples were used In the multivariate analysis; eight samples had missing syndrome classification and 33 had missing data for days since illness onset.

[b] Statistically significant.

data on sex. For these samples, the mean age was 2.8 years and 2,036 (56%) were from males. There was no significant difference in patient age and sex distribution between the analysed and non-analysed samples. Of the 7,833 tested samples, the majority were received from the following sentinel sites: Kakamega (n=2,383; 30%), Nyeri (n=1,842; 24%), and Nakuru (n=1,433; 18%). Nearly half (n=3,813; 49%) of the 7,825 samples that had clinical data were from ILI cases (Table 1).

Influenza positivity and storage time

Overall, 3,969 (51%) of specimens included in the analysis were stored for zero to one day; 3,411 (44%) of specimens analysed were stored for two to five days; and 453 (6%) were stored for six to 10 days.

In the bivariate analysis, the per cent positivity of samples stored for zero to one day (12%) was not significantly different from that of two, three, four, and five days (13%, 13%, 15%, and 12%, Table 2). In the bivariate analysis, there was a statistically significant difference in the per cent-positivity of samples stored for zero to one day compared with samples stored for six days (12% vs 6%, p=0.03). The per cent positive of samples stored for zero to one day (12%) was higher than the per cent positive of samples stored for seven, eight, nine, and 10 days (7%, 10%, 4%, and 5%, respectively) but these differences were not statistically significant (Table 2). Overall, the Cochran–Armitage trend test showed that the positivity of influenza decreased as the storage days increased (p<0.05).

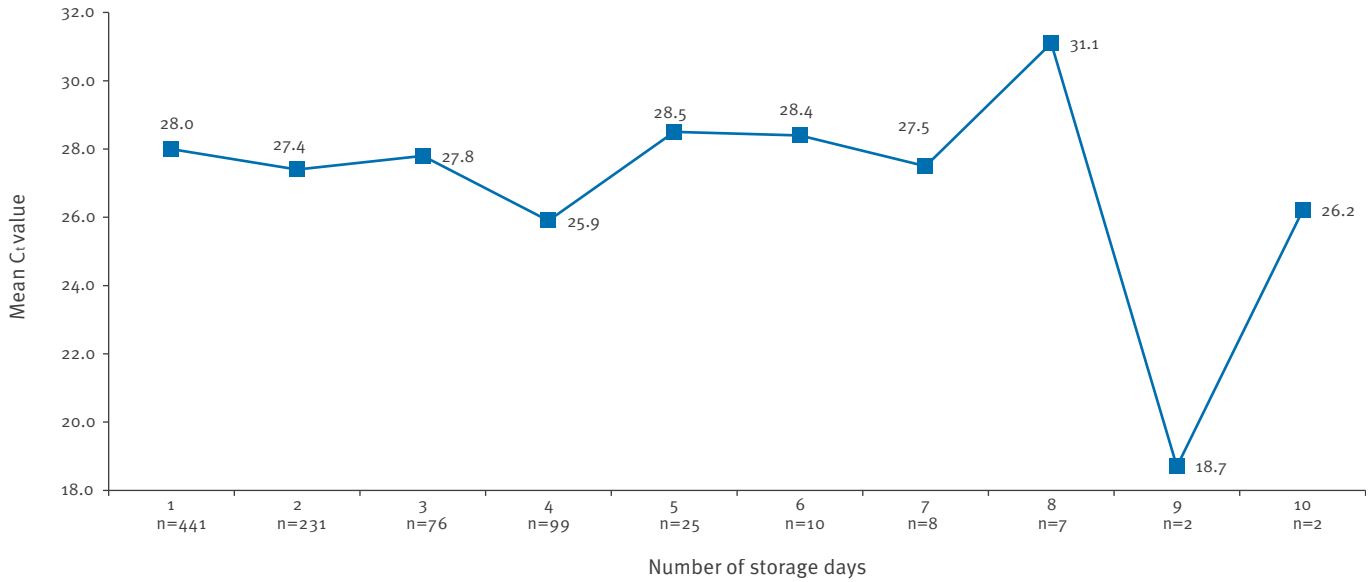
In the multivariable model, the positivity of samples stored for five days did not differ from that of zero to one day (12% vs 12%; adjusted odds ratio (aOR): 0.98; 95% confidence interval (CI): 0.66–1.54). Samples stored for six days were significantly less likely to be positive compared with samples stored for zero to one day (6% vs 12%; aOR: 0.49; 95% CI: 0.27-0.93). Samples stored for seven days were less likely to be positive as well, but this finding did not reach statistical significance (7% vs 12%; aOR: 0.50; 95% CI: 0.25–1.07). Samples stored for eight, nine, and 10 days respectively were less likely to be positive than those samples stored for zero to one day, but these findings were not statistically significant and had wide confidence intervals due to the small sample size (Table 2). We compared specimens stored for zero to one day with specimens stored for eight to 10 days using a multivariable model, and samples stored for eight to 10 days were less likely to be positive for influenza than those in storage for zero to one day (7% vs 12%; aOR:0.56; 95% CI: 0.03–1.05). Additionally, in the multivariable model, samples stored for six to 10 days were 49% less likely to be positive than those stored for zero to five days (7% vs 12%; aOR: 0.51; 95% CI: 0.35–0.75).

In a multivariable analysis comparing storage time and positivity rates of influenza A, we found no statistically significant difference in the positivity rates of specimens stored for two, three, four and five days compared to specimens stored for zero to one day. However, specimens stored for six days were less likely to be positive for influenza A compared to specimens stored for zero to one day (8/180 (4%) vs 353/3,969 (9%); aOR: 0.48; 95% CI: 0.23–0.99). There was no difference in the positivity rates between specimens stored for seven, eight, nine and 10 days and those stored for zero to one day, but only 17 specimens stored for seven to 10 days were positive for influenza A. The trend test showed no trend between storage days and positivity rates for influenza A (p>0.05).

We conducted a multivariable analysis comparing storage time and positivity rates of the influenza A subtypes, and we found similar trends in positivity rates, although the analysis was limited by the small sample size. For H1, compared to specimens stored for zero to one days, there was no statistically significant difference in the positivity rates of samples stored for three, four, five, six, and seven to 10 days, but samples stored for two days were twice as likely to be negative (17/1,899 (1%) vs 60/3,969 (2%); aOR: 0.50; 95% CI: 0.30–0.90). For H3, we found no statistically significant difference in the positivity rates of specimens stored for >1 day compared to specimens stored for zero to one day. Finally, for A(H1N1)pdm09, the positivity rates of specimens stored for >1day were similar to the positivity rates of specimens stored for zero to one day with one exception: samples stored for four days were

Figure 2

Mean cycle threshold (C_t) values of influenza positive samples relative to the number of storage days, Kenya, 2008–2010 (n=901)[a]



The number of samples (n) stored for the respective amount of storage days is indicated under the X axis values.

[a] Does not include 39 co-infections.

twice as likely to be positive compared to those stored at zero to one day (33/673 (5%) vs 86/3,969 (2%); aOR: 2.33; 95% CI: 1.52–3.56). Overall, the Cochran–Armitage Trend test showed that the positivity of influenza decreased as the storage days increased ($p<0.05$) for H1 and A(H1N1)pdm09, but not for H3.

In a multivariable analysis of storage time and influenza B, there were no statistically significant differences in positivity rates by storage time. However, numbers were small; only 12 specimens stored for five to 10 days were positive for influenza B. There was no significant trend between storage days and the positivity rates for influenza B ($p>0.05$). When we analysed specimens from ILI and SARI cases separately, in the multivariable analysis, there was no significant decrease in the proportion-positive of samples stored for any number of storage days compared with those stored for zero to one day.

Cycle threshold value and storage time

We analysed 901/940 (96%) of all positive samples. We excluded 39 positive samples because they had influenza A and influenza B co-infections. The C_t values ranged from 12.66 to 39.99. In the multivariable generalised ordinal logit analysis, C_t values from influenza-positive specimens stored for >1 day were not significantly higher than influenza-positive specimens stored for zero to one day ($p>0.05$, Figure 2). Stratifying the positive results by C_t values showed that 599 (66%) of the 901 positive samples analysed had C_t results of ≤29, 178 (20%) had C_t results in the range of 30 to ≤37, and 124 (14%) specimens had C_t results in the range 38 to <40. The distribution of the C_t values of the positive samples was relatively consistent for each day of storage.

Discussion

While existing guidelines recommend that samples can be stored at 4°C for up to 96 hours before being tested influenza by rRT-PCR, our results suggest that maintaining samples at refrigeration temperature for up to five days after collection is unlikely to compromise results by rRT-PCR. The implications of our study are potentially relevant for influenza diagnostic testing throughout Kenya and other countries where surveillance systems have adopted the use of rRT-PCR as the diagnostic of choice for influenza viruses. In influenza surveillance systems that use rRT-PCR and collect samples during weekdays only, weekly transport of specimens on Friday – if samples could arrive at the laboratory the same day or the following day – would not compromise specimen integrity. This flexibility in the frequency of transport may be especially useful in rural areas, where frequent transport of samples to central laboratories can be challenging and costly.

Our analysis included nearly 8,000 specimens, and we controlled for confounding variables. The per cent-positivity of samples was similar through five days. Samples that were stored for more than five days had reduced odds of testing positive for influenza relative to the reference group of zero to one day. There were few samples stored for eight to 10 days, making

it difficult to assess these longer periods of storage. Likewise, our sub-analyses were limited by small sample size; although we found consistent positivity trends in our sub-analysis of H1 and A(H1N1)pdm09, the trend was not statistically significant for H3, influenza A, or influenza B.

Influenza-positive specimens with higher C_t values by rRT-PCR present more difficulty for influenza virus isolation. We have previously shown that the rates of isolation for PCR-positive samples are lower for C_t values >35 for influenza A and C_t values >30 for influenza B [13]. In our surveillance system in Kenya, culture is attempted only for samples with a C_t value ≤35. In our analysis, although the mean C_t values of influenza-positive samples increased after four days of storage with the exception of the mean C_t value for samples stored for nine days, there was no statistically significant association between these C_t values and storage day. However, there were a relatively small number of influenza samples stored for five to 10 days. Because we did not evaluate isolation rates by storage time, we were unable to draw conclusions about the relationship of storage time and viral isolation. In a previous study conducted in the US that tested respiratory samples for influenza using virus isolation, there was no difference in the percentage of influenza-positive samples by storage days when samples were stored for as long as five days at 4°C [14]. Our findings that C_t values of influenza samples did not vary by days of storage were also similar to those of a previous study of influenza A in wild birds; in that study, C_t values of influenza-positive samples tested by rRT-PCR were similar in samples maintained at 4°C for up to three weeks [15].

For remote surveillance sites where specimen transport is challenging, there are storage options other than traditional VTM that could be considered, particularly when culturing specimens is not a main objective. Samples could be divided into two aliquots at the site. One aliquot could be placed in VTM and the other could be placed in lysis buffer before transport to the laboratory, which would reduce the need for prompt transport to the laboratory for PCR detection. If specimens will not ultimately be cultured, which is currently the situation in some countries in Africa, they can be stored in ethanol at room temperature without reducing the yield by PCR [16] or collected dry or placed in saline and stored in 4°C or ambient temperature [17]. In our surveillance system, these alternative storage methods were not a suitable option; if we had employed any of these methods we would not have been able to culture PCR-positive specimens.

The findings from this analysis are subject to limitations. First, because data associated with specimen collection and testing were incomplete, 4,708 (37%) specimens could not be used in the analysis. However, we included nearly 8,000 samples in our evaluation, and the mean age and sex distribution were similar between those samples included and those excluded. Second, it is possible that samples were stored and transported under conditions outside the recommended storage temperature of 2 to 8°C. However, even if this occurred, it is unlikely that this variability affected samples stored for different periods of time differently, because samples of different collection dates were placed in the same cool box for transport. In addition, based on the schedule followed for each site, transport time from site to laboratory was consistent for every site throughout the study. In addition, we only evaluated specimen positivity by rRT-PCR, so our findings may not be applicable where other testing methods are used. However, our findings of consistent C_t values across storage times, particularly for specimens stored for zero to five days, of which there were many, lead us to believe that isolation rates would not be affected by up to five days of storage. In addition, while our sample was large, we compared different samples rather than testing the same samples over multiple days, which would be the ideal way to evaluate variability of test results according to refrigeration time. Finally, we only tested for influenza, and therefore our results are not generalisable to other pathogens.

Our results suggest that respiratory samples can be stored at 2 to 8°C for up to five days after collection before reaching the laboratory; this finding could ease the burden of specimen transport in surveillance systems where sampling sites are far from the laboratory or budget for specimen transport is limited. Further studies should be conducted to better understand the association between duration of specimen storage prior to rRT-PCR testing of influenza and other viruses and bacteria as well as the effect of refrigeration storage time on virus isolation rates.

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Conflict of interest

None declared.

Authors' contributions

D. Caselton contributed to the overall design of the analysis, analysed the data, and wrote the manuscript. G. Arunga coordinated the data entry, analysed the data, and reviewed the manuscript. G. Emulke supervised data entry and analysis. L. Waibochi, L. Mayieka, and A. Kosgey were responsible for all the PCR-testing and reviewed the manuscript. R. Ochola contributed to the data collection and reviewed the manuscript. J. Mott contributed to the study design and reviewed the manuscript. D. Feiken and R. Brieman contributed to the

overall design of the influenza sentinel surveillance system, the design of the analysis, and reviewed the manuscript. M. Katz contributed to the overall design of the influenza sentinel surveillance system, the study design, the design of the analysis, and reviewed the manuscript.

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Did narcolepsy occur following administration of AS03-adjuvanted A(H1N1) pandemic vaccine in Ontario, Canada? A review of post-marketing safety surveillance data

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A vaccine safety signal and association between new onset of narcolepsy and AS03-adjuvanted pandemic influenza A(H1N1) vaccine (Pandemrix, GlaxoSmithKline) in children and young adults has been reported in several European countries. In Ontario, Canada, AS03-adjuvanted pandemic A(H1N1) vaccine (Arepanrix, GlaxoSmithKline) was the primary vaccine administered in 2009/10, with 4.8 million doses distributed. We assessed post-marketing safety surveillance data by extracting adverse events following immunisation (AEFIs) associated with this vaccine from the integrated Public Health Information System. Reports were screened for key terms related to narcolepsy and further limited to children and young adults four to 29 years of age. Of 1,604 AEFIs reported in Ontario, 53 reports met the search criteria. Individual assessment by a nurse consultant for additional context suggestive of narcolepsy yielded five reports for secondary medical review. None of the five reports proved consistent with a possible narcolepsy diagnosis based on the available information. We present the first post-marketing assessment from Canada of narcolepsy reports following receipt of Arepanix. Continued investigation of differences between Arepanrix and Pandemrix and subsequent risk of narcolepsy is indicated. In light of the limitations of passive surveillance to detect a signal in this instance, validation using other data sources is prudent.

Introduction

Narcolepsy is a chronic neurological disorder characterised by excessive daytime sleepiness and sudden daytime sleep attacks, cataplexy, hypnagogic hallucination and sleep paralysis [1]. The prevalence is estimated to be between 25 and 50 per 100,000 [2]. Onset can occur at any age; however, peak onset has been observed in those aged 10 to 19 years [3]. Narcolepsy has been associated with a strong genetic predisposition, specifically with the human leukocyte antigen (HLA) DQB1*0602, an allele that is approximately twice as common in northern as in southern Europe [4].

A vaccine safety signal involving new onset of narcolepsy associated with AS03-adjuvanted influenza A(H1N1) pandemic vaccine Pandemrix (GlaxoSmithKline, Rixensart, Belgium) was first reported by Sweden and Finland in August 2010 [5,6]. Subsequent post-marketing safety assessments in these and other European countries have reported an increased risk of narcolepsy among children and young adults following receipt of this vaccine [4,7-9].

The Global Advisory Committee on Vaccine Safety (GACVS) reviewed the available evidence in December 2012. At that time, an association between abrupt juvenile narcolepsy and Pandemrix had been confirmed in four countries with high vaccine uptake among children and adolescents: Finland, Ireland, Norway and Sweden. The GACVS noted that while absolute risk was low, the relative risk was significantly raised, ranging from 6.6 per 100,000 (95% confidence interval (CI): 3.1–14.5) in Sweden to 13.0 per 100,000 (95% CI: 4.8–34.7) in Ireland [10].

In February 2013, a similar association in England was found by Miller et al. who reported an odds ratio of 14.4 (95% CI: 4.3–48.5) for vaccination with AS03-adjuvanted pandemic vaccine at any time before onset of narcolepsy among four to 18 year-olds [11], reinforcing the signal detected in the other countries [4,7-11]. An updated GACVS review in June 2013 acknowledges the findings suggesting a possible risk of narcolepsy among young adults and reiterates the urgency of continued research given the threat of emergence of new

pandemics and the expected future need for pandemic vaccines [12].

In Canada, the AS03-adjuvanted influenza A(H1N1) pandemic vaccine Arepanrix (GlaxoSmithKline Inc.) was authorised for use in October 2009 and was the primary vaccine administered during the influenza A(H1N1) pandemic of 2009/10 in addition to a limited quantity of unadjuvanted influenza A(H1N1) pandemic vaccine (Panvax) for pregnant women. In Ontario, Canada's largest province (13.2 million population in 2010), approximately 4.8 million doses of AS03-adjuvanted pandemic vaccine were distributed between October 2009 and March 2010 (T. Scott, Ontario Ministry of Health and Long-Term Care, personal communication, July 2014). The entire population older than six months was eligible for vaccination; however, the date the vaccine was made available varied by risk group and age [13].

Pandemrix and Arepanrix are manufactured at different locations. The products contain the same adjuvant (AS03) but the antigen is produced using different manufacturing steps, resulting in several differences between the vaccines. An assessment by the European Medicines Agency (EMA) notes that the biological mechanism for the association between Pandemrix and narcolepsy is not yet known and should continue to be evaluated [14]. A difference in the immune response to Pandemrix and Arepanrix has been hypothesised; however, an assessment by the EMA has indicated that there is not at present any evidence of this [7,14].

In Canada, a possible signal of narcolepsy was initially observed in 2010 by Montplaisir et al. at the Sleep Disorder Centre (Sacré-Coeur Hospital) in Montreal, Canada [15], and an evaluation of the risk of narcolepsy following administration of Arepanrix in the province of Quebec has been completed but not yet published [16]. To date there has been no signal of narcolepsy reported by the Public Health Agency of Canada (PHAC) from adverse events following immunisation (AEFIs) reported by the provinces and territories to the Canadian Adverse Event Surveillance System (CAEFISS). The objective of this report is to summarise a review of passive vaccine safety surveillance data for possible reports of narcolepsy following administration of Arepanrix in Ontario, Canada.

Methods

In Ontario, reporting of AEFIs by immunisers (physicians, registered nurses and pharmacists) is mandated by provincial public health legislation; however, vaccine recipients or their parents may also voluntarily report an AEFI. Initial reports of AEFIs are received by the local public health unit where they are reviewed and investigated; recommendations may be made to the vaccine recipient or provider by the local Medical Officer of Health (MOH) regarding additional follow-up and receipt of further doses of vaccine. AEFI reports are entered into the integrated Public Health Information System (iPHIS), the passive electronic reporting system for reportable diseases and AEFIs in Ontario. Provincially reported AEFIs are not further validated or assessed using any other source of information beyond what is available in the iPHIS application.

For this review, we included all AEFI reports associated with administration of AS03-adjuvanted A(H1N1) pandemic vaccine (Arepanrix) and reported in iPHIS starting October 2009. Data were extracted from iPHIS on 25 April 2013.

Narcolepsy was not specifically described in provincial AEFI reporting criteria during the reporting period. Although this review is not limited to specific types of events, it is assumed that reports which included possible signs and symptoms of narcolepsy would probably have been classified as 'Other severe/unusual events' which was defined during this reporting period as 'any adverse event believed to be temporally related to immunisation that does not fit any of the categories listed above and for which no other cause is clearly established. Report events of clinical interest which require medical attention, and particularly events that are (i) fatal, (ii) life-threatening, (iii) require hospitalisation, or (iv) result in residual disability' [17].

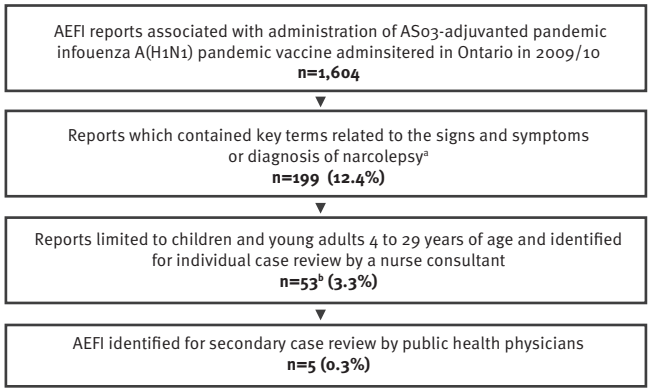
In order to further identify AEFI reports for review we executed a search on key all text fields within the data output that contained narrative case notes. We used key terms related to the signs and symptoms or to the diagnosis of narcolepsy including: cataplexy, muscle weakness, muscle tone, slurred, slurring (speech), sleepiness, sleepy, sleep disturbance(s), sleep paralysis, hallucination(s), dream(s), night terror(s), neurology and neurologist [18]. Reports were then further limited to children and young adults four to 29 years of age, which is consistent with the association previously noted in the literature. The identified reports were individually assessed by a nurse consultant at Public Health Ontario (PHO) for additional context suggestive of signs and symptoms of narcolepsy and, based upon this assessment, identified for secondary medical review. Secondary medical review was completed by two public health physicians at PHO who independently assessed reported AEFI case information. No specific case definition was applied to AEFI reports for this assessment.

Results

We identified a total of 1,604 AEFI reports associated with administration of Arepanrix in 2009 and 2010 in Ontario (no Arepanrix was administered after 2010). The Figure summarises the results of the sequential review process to identify possible reports of narcolepsy. There were 53 reports which contained one or more key terms possibly related to the signs and symptoms or diagnosis of narcolepsy and were within the pre-specified age range (4–29 years of age).

Figure

Identification of possible reports of narcolepsy through sequential review of all reports on adverse events following immunisation associated with administration of AS03-adjuvanted influenza A(H1N1) pandemic vaccine (Arepanrix) in Ontario, Canada, 2009/10 (n=1,064)



AEFI: adverse event following immunisation.

[a] Key terms related to the signs and symptoms / diagnosis of narcolepsy included: cataplexy, muscle weakness, muscle tone, slurred, slurring (speech), sleepiness, sleepy, sleep disturbance(s), sleep paralysis, hallucination(s), dream(s), night terror(s), neurology and neurologist.

[b] Two reports could not be further reviewed due to lack of further information in the original report.

Individual assessment of reports by a nurse consultant yielded five reports for secondary medical review (Table). Upon this review, it was determined that none of the five reports were consistent with a possible narcolepsy diagnosis based on the available information.

Discussion

This review process did not identify any potential reports of narcolepsy in individuals 29 years and younger following administration of Arepanrix and thus, no safety signal was noted in passively reported AEFI surveillance data in Ontario, Canada. Of note, subsequent to this review, one case of narcolepsy associated with Arepanrix was reported through the AEFI reporting system in Ontario. However, this case was older than the pre-specified age range of four to 29 years of age for this review and subsequent investigation determined that onset of symptoms pre-dated receipt of the vaccine (data not shown).

Spontaneously reported narcolepsy following AS03-adjuvanted influenza A(H1N1) pandemic vaccine among four to 19 year-olds from seven countries (Canada, Finland, Germany, Iceland, Norway, Sweden, United Kingdom) varied widely [19]. The highest incidences, that also exceeded expected background rates, were seen in Iceland, Sweden and Finland (4.9–9.4 per 100,000 vaccinated cases), whereas Canada reported the lowest incidence (0.1 per 100,000 vaccinated cases), which did not exceed the expected background [19].

In order to further evaluate our findings from a local perspective, we estimated the expected background number of narcolepsy cases in the population of four to 29 year-olds in Ontario using published estimates of the population-based incidence rate from the United States of 0.79 per 100,000 per year (all ages), as Canadian data were not available [3]. Between October 2009 and December 2010, we would have expected 44 new cases of narcolepsy, yet there were no reports to the passive AEFI reporting system during the same

Table

Reports identified for secondary medical review for possible narcolepsy associated with administration of AS03-adjuvanted A(H1N1) pandemic vaccine (Arepanrix) in four to 29 year-olds in Ontario, Canada, 2009/10 (n=5)

Age range (years)	Reported adverse event category	Signs and symptoms	Time to onset / duration	Outcome
15–19	Other severe/unusual events	Fatigue, disorientation, low grade fever, paraesthesia in lower extremities	1 day/ unresolved as of day 3 following immunisation	Outcome unknown
10–14	Other severe/unusual events	Auditory hallucinations for three nights following receipt of vaccine	6 hours/ 3 days	Symptoms spontaneously resolved, no recurrence as of two months following receipt of vaccine
10–14	Other severe/unusual events	Immediately fell asleep and unable to rouse, unresponsive to pain	15 minutes/ 15 minutes	Blood tests and EEG normal, no recurrence after initial episode
4–9	Encephalopathy/ encephalitis: depressed level of consciousness	Confusion, disorientation, shortness of breath, headache, dizziness, malaise	1 day/ 2 hours	Outcome unknown
4–9	Other severe/unusual events	Daytime sleepiness, night-time hallucinations	<1 day / 1 day	Normal medical examination, spontaneous resolution of symptoms; no recurrence as of two months following receipt of vaccine

AEFI: adverse event following immunization; EEG: electroencephalography.

time period in which the influenza A(H1N1) pandemic vaccine campaign also occurred. This number, based on the incidence across all ages, is likely to be an underestimate since peak onset is among the adolescent and young adults.

Expected cases notwithstanding, the lack of signal detected by our passive vaccine safety surveillance system may still not be surprising given a number of factors including the rarity of the disease, the lack of previous association between narcolepsy and vaccine, the delay from onset of symptoms to diagnosis and the decentralised nature of narcolepsy diagnosis in Ontario. In general, reports to the provincial surveillance system of neurological adverse events following any vaccine are rare with 3.5 reports per 1 million doses distributed [20]. With respect to the diagnosis of narcolepsy, referral to a sleep clinic is a common component of the diagnostic workup in Ontario; however, most clinics operate as independent health facilities which are regulated but not coordinated provincially. Within this decentralised model of care an overall increase in reports of narcolepsy may not necessarily be observed at the clinic level. Furthermore, health professionals involved in the diagnosis and treatment of narcolepsy are not routinely involved in the assessment and management of AEFIs and therefore may not necessarily recognise and report an adverse event, particularly one that has not been previously associated with any particular vaccine.

Other limitations of this assessment include those which are shared with other passive AEFI surveillance systems including under-reporting, inconsistent quality and completeness of AEFI reports and reporting bias [21]. In particular, the lack of outcome information was a key limitation to the identification of possible cases of narcolepsy. AEFI reports in iPHIS generally contain descriptions of signs and symptoms temporally associated with receipt of a vaccine, but not necessarily the results of specialist consultation and subsequent diagnosis which for narcolepsy can take several weeks to months following onset of symptoms. In addition, while the Brighton definition of narcolepsy [22] was used to inform this assessment, it was not formally used to classify reports due to the lack of detailed information available in provincial AEFI surveillance reports.

The limitations of passive reporting underscore the need for strengthened capacity and better systems to actively search large administrative databases, coupled with efficient international communication and rapid response when new signals emerge. The use of keyword searching (also referred to as 'text mining' or 'natural language processing') for signal generation has the potential to improve vaccine safety surveillance particularly for emerging or previously unrecognised events. However, subsequent evaluation including clinical case review can be labour-intensive depending on the number of signals generated and the frequency of the event assessed using this approach [23-25].

In addition to the already established association between Pandemrix and narcolepsy, the absence of a safety signal from passive surveillance of Arepanrix requires further study. The United Kingdom for example was not initially a country where a signal was identified; however, subsequent assessment demonstrated an increased risk of narcolepsy [9,11]. To this end, Ontario is also participating in an international study led by the Brighton Collaboration assessing the relationship between AS03-adjuvanted pandemic vaccine and narcolepsy in jurisdictions using Arepanrix compared with previous similar assessments of Pandemrix [26]. In addition, signals that have meanwhile been detected in older adults present a limitation of this current assessment which was limited to children and young adults four to 29 years of age [9,27].

Conclusions

This report represents the first published post-marketing assessment from Canada of reports to a passive AEFI surveillance system on narcolepsy following receipt of the AS03-adjuvanted influenza A(H1N1) pandemic vaccine Arepanrix. No reports of narcolepsy were identified. Given the lack of safety signal to date from Arepanrix, continued investigation of differences between Arepanrix and Pandemrix and subsequent risk of narcolepsy appears to be indicated. However, in light of the limitations of passive surveillance to detect a signal in this instance, validation using other data sources is prudent.

Conflict of interest

None declared.

Author contributions

TMH designed the study, conducted case-level review, contributed to the analysis of the data and drafted the manuscript. KW contributed to the design of the study, analysed the data and commented on the final version of the manuscript. LS conducted case-level review and critically commented on the manuscript. JF contributed to the design of the study and critically commented on the manuscript. NC contributed to the design of the study, conducted case-level review and critically commented on the manuscript. SLD supervised the conduct and report of the study, conducted case-level review and critically commented on the manuscript. TMH and KW had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors participated in interpretation of the results and approved the final version of a manuscript.

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ESCAIDE 'late-breaker' abstract call open

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The 2014 European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) now welcomes abstracts on recent infectious disease outbreaks and emerging findings to support disease control. A so-called 'late breaker' session will be organised during the 5-7 November 2014 ESCAIDE. The call to submit abstracts for this session is open from 8 to 21 September.

For more information on eligibility criteria for abstract submission, visit the conference website at www.escaide.eu.

Programme details and conference registration instructions are available on the ESCAIDE website. For further information, contact: escaide.conference@ecdc.europa.eu

Hepatitis A outbreak: Report on results from food trace-back investigation

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The European Food Safety Authority (EFSA) published a report with results from the trace-back investigation of food items connected with a multinational outbreak of hepatitis A in European Union/European Economic Area (EU/EEA) countries coordinated by EFSA, on 8 September 2014.

The trace-back investigations were done by a working group composed of microbiologists, public health and food safety experts from France, Ireland, Italy, Norway, the Netherlands, Poland and Sweden, specialists on tracing analysis from the German Federal Institute for Risk Assessment and experts on food-borne outbreak investigations from the European Centre for Disease Prevention and Control (ECDC).

Laboratory testing of food items and interviews with affected people identified consumption of mixed frozen berries as the source of the outbreak. Bulgarian blackberries and Polish red currants have been identified as the most common ingredient in the contaminated lots and in the food consumed by affected people. No single point source of contamination could be identified but 12 food operators were identified that were linked to cases and lots in five of the affected countries. Further investigations at the local level are needed to identify where the suspect berries were harvested and the conditions at these harvest or production sites.

As contaminated berries could still be circulating in the food chain, the report recommends enhanced surveillance, risk communication, vaccination and further research in the area of public health. The EFSA also recommends good hygiene, manufacturing and agricultural practices in berry producing countries.

The trace back investigation followed the detection of the outbreak for which since January 2013, more than 1,440 hepatitis A cases have been reported to the ECDC, the agency responsible for monitoring the occurrence of hepatitis A infections in humans in the EU/EEA. Cases were reported from 12 European countries, with 331 cases confirmed by genotyping.

The published scientific report follows several Rapid Outbreak Assessments on the status of hepatitis A published by EFSA and ECDC.

Read more:

- European Food Safety Authority (EFSA). Tracing of food items in connection to the multinational hepatitis A virus outbreak in Europe. Parma: EFSA; 2014. EFSA Journal 2014;12(9):3821 [186 pp.]. Available from: http://www.efsa.europa.eu/en/efsajournal/pub/3821.htm
- Rapid Outbreak Assessments on the outbreak of hepatitis A in EU countries: Available from: http://www.ecdc.europa.eu/en/healthtopics/hepatitis_A/risk-assessment/Pages/default.aspx