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Nationwide outbreak of Salmonella enterica serotype 4,12:i:- infections in France, linked to dried pork sausage, March-May 2010

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In May 2010, a nationwide excess of infections with the specific monophasic variant *Salmonella* enterica serotype 4,12:i:- was investigated in France. Subtyping with multilocus variable number of tandem repeats analysis revealed a distinct epidemic strain within this excess. Epidemiological investigations identified a dried pork sausage sold by a particular chain of supermarkets as the likely vehicle of transmission. The suspected batches have been withdrawn and recalled.

Introduction

On 7 May 2010, the National Reference Centre for *Salmonella* (NRC) alerted the French Institute of Public Health Surveillance (InVS) to a cluster of six cases of infection with *Salmonella enterica* subsp. *enterica* serotype 4,12:i:- in the area of Limoges, France, and to a nationwide increase of this specific monophasic serotype in comparison to previous years (Figure 1). At that time, 69 confirmed cases had been identified since the beginning of the year, compared with 37 in 2009 and eight in 2008 over the equivalent period of time. An epidemiological investigation was launched in order to determine the extent of the outbreak and identify the vehicle of transmission.

S. enterica serotype 4,12:i:- is one of a number of monophasic variants of the serovar Typhimurium, that have been emerging in Europe and elsewhere in recent years and are of increasing concern [1-3]. Information from the French Food Safety Agency (Agence francaise de securite sanitaire des aliments (AFSSA)) shows that this variant had been identified in a variety of foodstuffs, but most frequently in pork delicatessen.

Epidemiological and microbiological investigations

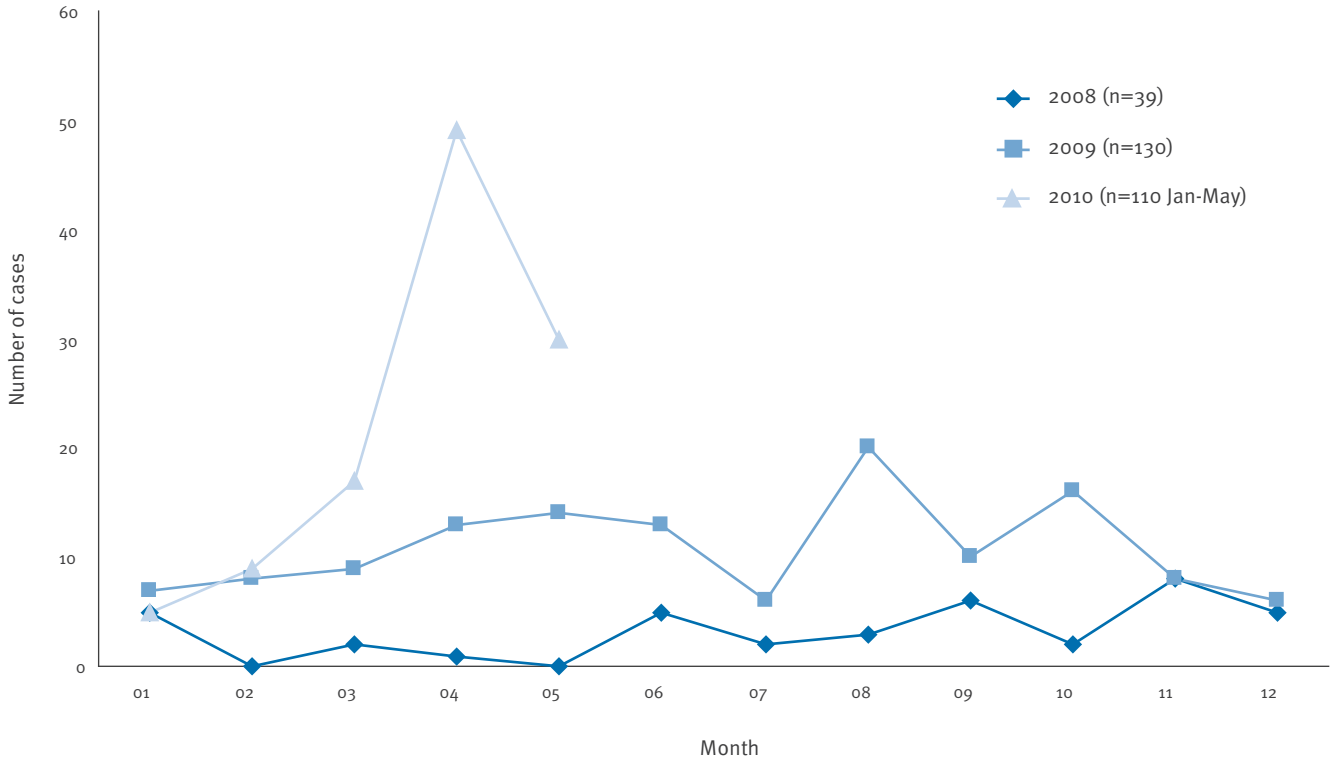
For this outbreak, a case was defined as a person resident in France with *S. enterica* serotype 4,12:i:- isolated from stool or blood in 2010, and with symptoms compatible with a *Salmonella* infection. The epidemic curve (by sample date, Figure 2) demonstrated an increase in the number of cases from week 12, with a peak in weeks 16 and 17. The investigation therefore focussed on the 90 (of 110) cases (as of 3 June) identified with a sample date from week 12 onwards. Among these cases, the median age was eight years (range 1–89 years), with a female:male sex ratio of 1.2. Cases were distributed throughout 49 of the 95 *départements* (administrative subdivisions) of mainland France, without any notable clustering (apart from the initial alert of six cases in Limoges).

As of 3 June 2010, 54 cases have been interviewed using a standardised semi-structured questionnaire exploring food consumption, travel history and other cases of diarrhoea in the household in the seven days before symptom onset. Dates of onset of symptoms for these cases ranged between 15 March and 16 May 2010. Twenty cases (37%) were hospitalised temporarily, with no deaths. Of these 54 cases, 53 (98%) reported buying pork delicatessen. Forty-two reported buying dried pork sausage (78%) and 33 reported shopping at supermarket chain A (61%). No other food types or activities were identified as likely sources of infection.

Multilocus variable number of tandem repeats analysis (MLVA) subtyping [4], using the latest nomenclature described by Larsson *et al.* [5], detected a major subtype, 3-13-15-NA-211, that allowed us to differentiate an epidemic strain from the sporadic cases. This profile differs from *S. enterica* serotype 4,12:i:- isolates from the beginning of 2010 and from 2007, as well as from other monophasic serotypes and serotype Typhimurium. To date, 53 of the 90 cases have been subtyped by MLVA, 32 of which had this specific subtype and have been retrospectively defined as 'epidemic cases'.

Figure 1

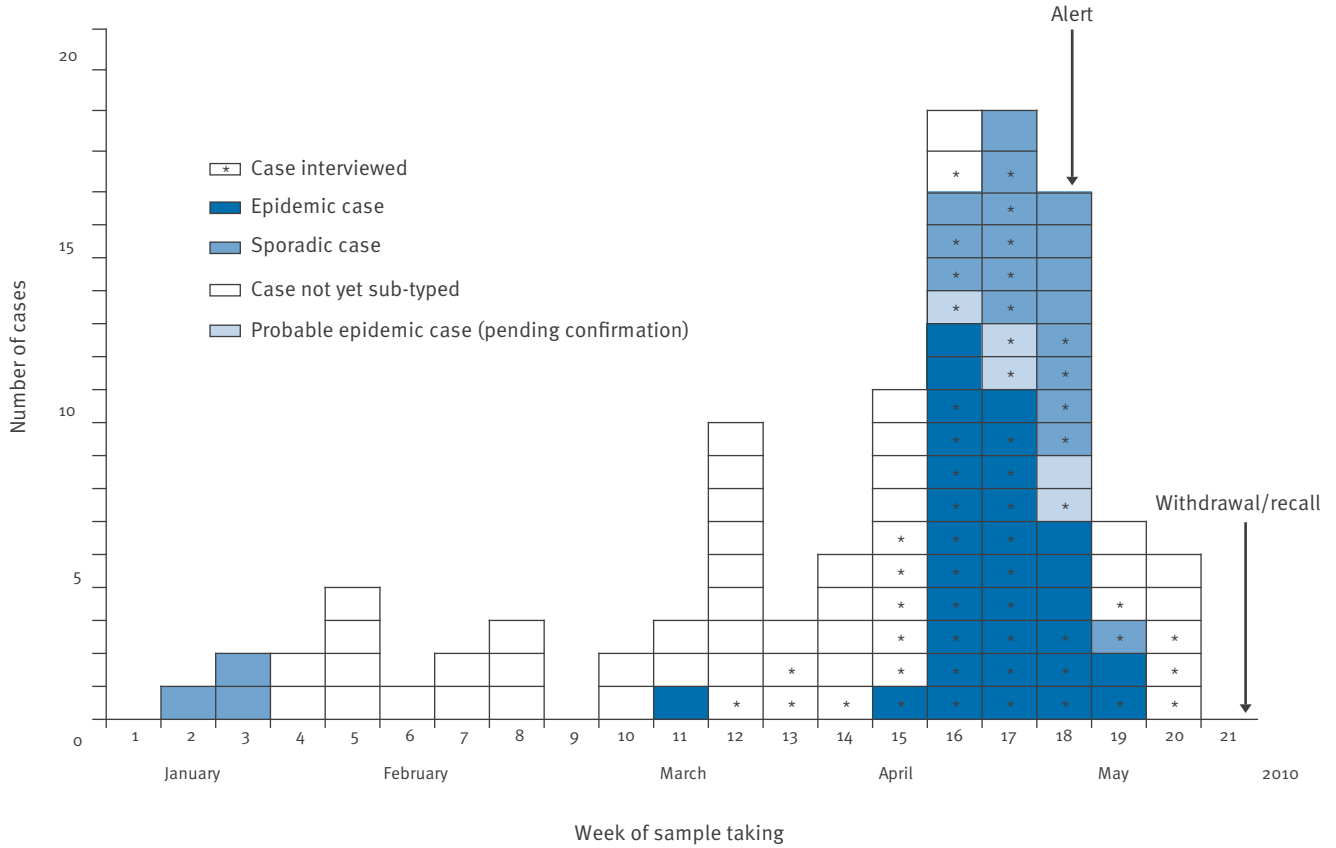
Number of *Salmonella enterica* serotype 4,12:i:- human isolates by month of sample collection, France, 2008-2010



Data as of 3 June 2010, National Reference Centre for *Salmonella*.

Figure 2

Number of human cases due to *Salmonella enterica* serotype 4,12:i:- by week of sample collection, France, 2010 (N=110)



Data reported by 3 June 2010.

Of the 53 subtyped cases, 36 have been interviewed. Of them, 24 (67%) were infected by the epidemic strain, two of whom were considered to be secondary cases and therefore excluded from further analysis. Twelve were considered to be sporadic. We noted that 20 of 24 epidemic cases shopped at a branch of supermarket chain A, compared with four of 12 sporadic cases (odds ratio 9.0, 95% confidence interval 1.41-61.7, p=0.0047). This reinforced the initial suspicions of an item purchased from supermarket chain A as the vehicle of transmission. Consumption of dried pork sausage was unusually high in both groups of cases (20 of 24 (82%) epidemic cases and nine of 12 (75%) sporadic cases), compared to previous outbreak investigations in France (range 33 of 67 (49%) to 21 of 33 (64%) in controls identified for outbreaks of *Salmonella* species linked to meat and cheese products since 2000 [6,7]).

Purchases of dried pork sausage made at branches of supermarket A in the three weeks prior to symptom onset were investigated by the French Directorate General for Food using data recorded through loyalty card numbers. Of the nine epidemic cases who used their card in the three weeks preceding symptom onset, all purchased the same type and brand of dried pork sausage produced by one manufacturer exclusively for supermarket A. *Salmonella* species had been isolated from a melee used to make a batch of this type and brand of sausage from this manufacturer in February 2010, but no failures in the production processes were identified. Later quality controls of this batch were negative for *Salmonella*. The isolate from the melee has been destroyed and is now unavailable for typing. Work is ongoing to identify any long term control measures to prevent future similar incidents.

Control measures

The batch of sausages ('use by' date up to 15 June) derived from this *Salmonella*-positive melee was subject to a national voluntary withdrawal and recall by the manufacturer on 27 May 2010, with a press release and posters in chain A supermarkets. A small proportion of the batch had been exported to Belgium, and the Belgian authorities were duly informed through the Rapid Alert System for Food and Feed (RASSF). Colleagues in other European countries were informed of this outbreak on 28 May via the Epidemic Intelligence Information System (EPIS) and Early Warning Response System (EWRS) of the European Centre of Disease Prevention and Control (ECDC). To date, no other European country has reported a current excess of cases of *S. enterica* serotype 4,12:i:-.

However, given that the suspected batch was delivered to supermarket A distribution platforms in the first two weeks of March, the relatively short turnover times at these platforms and at the supermarkets, and given that the last documented purchase from an epidemic case was made on 11 May (corresponding to a production date of 11 April at the latest), it is thought that the initial batch may not explain all the cases and that later batches may also have been contaminated. As a result, the French producer implemented a withdrawal and recall on 7 June of all batches available for purchase and produced before 12 April, accompanied by a press release from the authorities.

Conclusion

Epidemiological investigations identified one or more contaminated batches of dried pork sausage, produced by one manufacturer and supplied to branches of supermarket A, although *Salmonella* species were not isolated from a sample of the sausages. Incriminated batches have been withdrawn and recalled. Preliminary data suggest that the number of cases by week is decreasing. The investigation of this outbreak was assisted by the use of MLVA subtyping which was found to have an appropriate discriminatory power to identify a specific epidemic subtype. This outbreak of *S. enterica* serotype 4,12:i:- occurred on the background of the emergence of monophasic *Salmonella* strains in France and the rest of Europe, and future outbreaks due to this serotype are likely.

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2009 pandemic influenza A(H1N1) virus in Scotland: geographically variable immunity in Spring 2010, following the winter outbreak

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We determined the age- and location-specific seroprevalence of antibodies against 2009 pandemic influenza A(H1N1) virus in Scotland following the first two waves of infection. Serum samples collected following the winter outbreak were analysed by microneutralisation assay. The proportion of positive sera varied significantly between cities and, in the case of Inverness, between age groups (with younger adults more likely to be positive than older individuals). This study demonstrates that older people are no longer more likely to have antibodies against the virus than younger adults.

The pandemic influenza A(H1N1) virus has been spreading throughout the world since May 2009. The objective of this study was to determine the age- and location-specific seroprevalence of antibodies against 2009 pandemic influenza A(H1N1) virus in Scotland following the first two waves of the pandemic. Although population demographics and contact patterns will vary between countries, this information will assist European public health policy makers in planning for the 2010-11 influenza season.

Methods

Anonymised sera from leftover diagnostic samples taken in March 2010 (subsequently referred to as hospital/general practice (GP) samples) were obtained from biochemistry laboratories in four cities in Scotland: Aberdeen, Edinburgh, Glasgow and Inverness. These biochemistry laboratories receive material from a range of hospital departments as well as from general practices. For each site, samples were categorised by age of patient (20-29, 30-39, 40-49 and over 50 years) and 100 samples of each age group at each site were sent to the West of Scotland Specialist Virology Centre for analysis. The sample size was chosen to have a power of at least 80% to detect a difference of 10% points in seroprevalence between two age groups or between two sites, based upon 400 observations in each site and in each age group. In addition, 100 anonymised sera from leftover diagnostic samples taken in May 2010 in genito-urinary medicine (GUM) clinics in each of the four cities were collected (minimal influenza activity occurred in Scotland between March 2010 and May 2010 [1]), along with a further 128 routine hospital/GP samples from patients in Glasgow aged over 50. Antibody responses were detected by microneutralisation assays, according to standard methods [2]. The virus strain used was the NYMC X-179A reassortant derived from A/California/7/2009 (supplied by the National Institute for Biological Standards and Control, Potters Bar). Each serum sample was tested at a dilution of 1:40, since positivity at this dilution has previously been taken to indicate a significant antibody response [3]. Logistic regression analysis was used to estimate the effect of age group, location, and sample type on prevalence and 95% confidence intervals (CI) are used throughout. As a fixed sample size was used in each location, summary prevalences are based upon a weighted combination of the age and location specific prevalences for a stratified sample. The weights are derived from the population of Scotland in the age groups and catchment areas for the laboratories [4].

Results

Table 1 shows the percentage of hospital/GP samples that were found to be positive for antibodies against 2009 pandemic influenza A(H1N1) virus by age and location. Among these samples there is strong evidence from logistic regression modelling that the age seroprevalence profile is not the same at each site (p=0.0005).

In Aberdeen, Edinburgh and Glasgow there do not appear to be significant intra-site variations in percentage positivity with age. However, in comparison

with Aberdeen and Edinburgh, there appears to be lower overall positivity in Glasgow, particularly among younger age groups. To confirm the results for Glasgow, we tested a further 128 hospital/GP samples from patients aged over 50 years and found 41 to be positive (32.0%; 95% CI: 23.9-40.1), compared with 33.0% (95% CI: 23.8–42.2) from the first batch of samples.

The samples obtained from general practices and hospital departments cannot be considered a random sample from the general population as they are likely to have an overrepresentation among patients in groups more likely to receive an influenza vaccination. It is not likely that patients attending GUM clinics are over represented in such groups. The observation of similar levels of seroprevalence among 20-29 year-olds from GUM and hospital/GP samples at each of the four sites (p=0.19) is reassuring for the use of hospital/GP samples to estimate seroprevalence (Table 2).

Greater variation in percentage positivity was observed for Inverness, with a decrease in the percentage of positive samples with increased patient age. The geographical area served by the biochemistry laboratory in Inverness is larger, more rural, and considerably less densely populated than the catchment areas for the other biochemistry laboratories utilised in this study. A factor in the higher seroprevalence among 20-29 year-olds compared to those over 50 years old from this area might be mobility: compared with older patients from the same site, 20-29 year-old patients from the Inverness area might be more likely to have travelled,

Table 1

Hospital/general practice samples positive for antibodies against 2009 pandemic influenza A(H1N1) virus by age and location, Scotland, March 2010 (n=100 samples per age group in each location)

Location	Age group (years)							
	20-29		30-39		40-49		Over 50	
	Percentage of positive samples	95% confidence interval	Percentage of positive samples	95% confidence interval	Percentage of positive samples	95% confidence interval	Percentage of positive samples	95% confidence interval
Aberdeen	48%	38.2 - 57.8	51%	41.2 - 60.8	39%	29.4 - 48.6	39%	29.4 - 48.6
Edinburgh	40%	30.4 - 49.6	35%	25.7 - 44.3	28%	19.2 - 36.8	45%	35.2 - 54.8
Glasgow	22%	13.9 - 30.1	18%	10.5 - 25.5	26%	17.4 - 34.6	33%	23.8 - 42.2
Inverness	47%	37.2 - 56.8	29%	20.1 - 37.9	28%	19.2 - 36.8	19%	11.3 - 26.7

Table 2

Hospital/general practice and genito-urinary medicine clinical samples from patients aged 20-29 positive for antibodies against 2009 pandemic influenza A(H1N1) virus, Scotland, March 2010

Location	Hospital/GP[a] samples[b]		Genito-urinary medicine[c]	
	Percentage of positive samples	95% confidence interval	Percentage of positive samples	95% confidence interval
Aberdeen	48.0%	38.2 - 57.8	45.5%	35.7 - 55.3
Edinburgh	40.0%	30.4 - 49.6	46.3%	36.3 - 56.3
Glasgow	22.0%	13.9 - 30.1	30.3%	21.1 - 39.4
Inverness	47.0%	37.2 - 56.8	53.0%	43.2 - 62.8

[a] GP: general practice.

[b] n=100 samples per location.

[c] n= 99 samples in Aberdeen; 95 samples in Edinburgh; 99 samples in Glasgow; 100 samples in Inverness.

Figure

Age-adjusted estimates of seroprevalence by location and hospital admission rate per 100,000 population for the corresponding National Health Service board, Scotland, March 2010

Location	Estimated age-adjusted seroprevalence		Hospital admission rate as a result of infection with 2009 pandemic influenza A(H1N1) per 100,000 population
	Percentage	95% confidence interval	
Aberdeen	42.8%	38.1 - 47.6	40.3
Edinburgh	40.1%	33.5 - 46.7	35.5
Glasgow	27.7%	21.8 - 33.5	22.7
Inverness	28.8%	16.2 - 41.4	25.1

acquired infection from other parts of Scotland, and mixed more with other individuals in their age group.

The Figure shows age-adjusted estimates of seroprevalence by location. When the age-adjustment is taken into account, there are higher overall levels of seroprevalence in Aberdeen and Edinburgh than in Glasgow and Inverness. These results appear to correlate with the rates of hospital admission as a result of infection with the virus (calculated from data contained within Health Protection Scotland's Weekly Influenza Situation Reports [1]). Hospital admission rates as a result of infection with 2009 pandemic influenza A(H1N1) per 100,000 in Grampian (40.3) (the National Health Service board containing Aberdeen) and Lothian (35.5) (Edinburgh) were higher than those in Greater Glasgow and Clyde (22.7) (Glasgow) and Highland (25.1) (Inverness) (Figure). Health Protection Scotland have published influenza-like illness (ILI) and acute respiratory illness (ARI) consultation rates by National Health Service board [1], but these do not show major evidence of regional variation. From the Health Protection Scotland Sentinel Surveillance Scheme (unpublished) there is some evidence that the 2009 pandemic influenza A(H1N1) swab positivity rates in the East of Scotland (which includes Edinburgh) and the North of Scotland (Aberdeen and Inverness) are higher than in the West of Scotland (Glasgow).

Discussion

Since the outbreak of 2009 pandemic influenza A(H1N1) virus there have been several examinations of the frequency of antibodies against the virus [5 and references therein]. Taken together, these studies are contributing to our understanding of the spread of the virus and providing information that may help in planning future vaccination strategies. While hospital/GP samples cannot be considered to be a random sample from the general population, such samples have previously been used to estimate seroprevalence [3].

A goal of the work described here was to inform public health policy makers in planning for the 2010-11 influenza season. It was felt that this would be done most effectively by making our results available as quickly as possible and as a result the only serum dilution that we have tested to date is 1:40. Microneutralisation assays at this dilution are in line with several other recent analyses of 2009 pandemic influenza A(H1N1) seroprevalence [5].

Recent studies in England, Finland, and Italy examined pre-pandemic serum samples and found that the proportions of samples which contained significant levels of antibodies that are protective against 2009 pandemic influenza A(H1N1) virus increased with age [3,6,7]. During 2009, the burden of the virus was greatest among people aged under 30 years [8]. It has been suggested that older adults were affected less because they were more likely to have previously been exposed to strains with similarities to the new virus.

In Scotland, following the two waves of infection that have occurred so far, it appears that older people are no longer more likely to have significant levels of antibodies than young people. The seroprevalence among young people is, presumably, primarily due to exposure during the two waves of infection. While we currently do not have seroprevalence data for individuals aged under 20 years, the results presented here would suggest that during the 2010-11 influenza season, the burden of infection among adults in Scotland might be similar across age groups, with levels of infection among young adults more in line with those seen in older age groups during 2009.

A weakness of this study is that we do not have any information on the risk group and vaccination status of the patients as only aggregate data, which did not link to any patient characteristics, could be used. This means we are unable to separate out the effect of vaccination from infection or to adjust seroprevalence among the hospital samples for possible selection bias associated with risk groups.

The results presented here will have implications for public health policy in Scotland. Planning for the 2010-11 influenza season should include strategies to target risk groups as a significant proportion of the population remain susceptible to the virus. Glasgow and Inverness have lower overall levels of seroprevalence following the two waves of infection to date, and these cities might experience higher levels of influenza activity than Aberdeen or Edinburgh during the 2010-11 influenza season. The case fatality rate for 2009 pandemic influenza A(H1N1) among individuals aged over 65 years is greater than that observed for seasonal influenza [9]. If the majority of people aged over 50 years remain susceptible, targeting older individuals for vaccination should be a priority. This might be particularly desirable for the Inverness area, where our results indicate particularly high levels of susceptibility among older people.

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Transmissibility of 2009 pandemic influenza A(H1N1) in New Zealand: effective reproduction number and influence of age, ethnicity and importations

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The first wave of pandemic influenza A(H1N1) has subsided in New Zealand as in other southern hemisphere countries. This study aimed to estimate the effective reproduction number (*R*) of 2009 pandemic influenza A(H1N1) taking into account imported cases. It also aimed to show the temporal variation of *R* throughout the New Zealand epidemic, changes in age- and ethnicity-specific cumulative incidence, and the effect of school holidays. Using a new modelling method to account for imported cases, we have calculated the peak *R* during the containment phase of the pandemic as 1.55 (95% confidence interval: 1.16 to 1.86). This value is less than previously estimated in the country early in the pandemic but in line with more recent estimates in other parts of the world. Results also indicated an increase in the proportion of notifications among school-age children after the school holiday (3–19 July 2009). This finding provides support for the potential effectiveness of timely school closures, although such disruptive interventions need to be balanced against the severity of the pandemic.

Introduction

The Federal Government of Mexico activated its national pandemic preparedness and response plan on 24 April 2009 in response to a severe outbreak of influenza-like illness, later identified as 2009 pandemic influenza A(H1N1) [1]. It has been estimated that around 23,000 people in Mexico were already infected with the emerging virus at that time [2].

The first cases of the pandemic influenza were imported into New Zealand on 25 April 2009, with a group of students returning from a school excursion to Mexico. The arrival of the students triggered the activation of New Zealand's influenza pandemic action plan and associated border containment measures. In the following week, on 30 April 2009, non-seasonal influenza A(H1N1) became a notifiable and quarantinable disease in the country. Evidence of community transmission was detected from the first week of June (week 23), with a rapid increase in notifications lasting until the epidemic peaked five weeks later [3]. New Zealand's sentinel general practitioner surveillance system and non-sentinel laboratory surveillance systems demonstrated that the pandemic virus had rapidly overtaken seasonal influenza viruses, becoming the predominant circulating strain of the 2009 influenza season within four weeks of the detection of community spread [4,5].

To determine the transmissibility and spread of a disease we need to know the *effective* reproduction number (*R*), and how it evolves over the duration of the pandemic. *R* is the average number of secondary cases generated by a single primary case in the actual population. It is always less than the *basic* reproduction number, R_o, which is calculated by assuming a hypothetical population with no prior immunity. If *R* is less than one, the disease will not persist but will manifest itself in outbreaks of varying size triggered by importations of the disease, whereas if *R* is greater than one there is a chance of a large outbreak. Over the course of an outbreak, *R* is influenced by, among other things, actions that slow the spread of the disease, such as behaviour change, social distancing [6,7] and antiviral drug use [8], as a result of interventions.

There was considerable interest in the transmission pattern of the pandemic virus during the 2009 influenza season. The *R* of the pandemic influenza was estimated in a variety of settings and at different stages of the 2009 pandemic. Previous estimates include: 1.4–1.6 in Mexico [2,9], 1.3–1.7 in the United States [10], 1.8–2.15 in New Zealand [11], 2.0–2.6 in Japan [12], 1.39–1.49 in Peru [13] and 2.1–2.6 (1.5–1.8 after allowing for bias in case ascertainment) in Victoria, Australia [14]. Differences in these estimates may be attributed to

sampling strategies, clustering due to heterogeneous mixing amongst teenagers, family and various cultural groups, changes in the diagnostic coverage of infected individuals, using data from only very early in an outbreak or not accounting for imported cases. Different countries also had varying public health interventions for reducing the transmission of the virus including school closures, quarantine and antiviral drug prophylaxis, which may have altered the transmission of the virus in certain settings.

This study aimed to recalculate *R* for disease transmission throughout the pandemic in New Zealand (June–September 2009), using a new method that takes into account imported cases, and to show how *R* changed through the course of the 2009 winter. In response to questions posed in an earlier paper by Baker *et al.* [3], we also aimed to describe the age-specific and ethnicity-specific cumulative incidence during the pandemic in the country and to examine the influence of school holidays.

Methods

Surveillance data

The New Zealand influenza pandemic action plan includes five stages: plan for it, keep it out, stamp it out, manage it and recover [15]. Border management and cluster control were the main interventions after the arrival of the student group from Mexico, as part of the keep it out and stamp it out strategies of the containment phase (25 April–21 June 2009). During this phase, data were collected under an enhanced surveillance regime in which all suspected cases who presented at a general practice or hospital were tested. A suspected case during the containment phase was defined as a person with an acute respiratory illness who had developed symptoms within seven days of travel to an area where there were confirmed cases and confirmed or suspected local transmission of the virus. A change in the case report form on 10 July 2009, focusing on collection of risk factor information, meant that information on recent travel abroad was no longer requested.

Case ascertainment

Confirmed cases of pandemic influenza A(H1N1) were identified by real-time polymerase chain reaction (PCR), viral culture or a four-fold rise in pandemic virus-specific neutralising antibodies.

To assist with ongoing surveillance and infection control responses during the change in phase from containment to management (on 22 June 2009), the case definition was updated on 19 June 2009. The suspected case category was removed and the probable category was updated from a person who met the suspected case definition and tested positive for influenza A to a person with symptoms of influenza-like illness who had strong epidemiological links to a confirmed case or a defined cluster but who lacked laboratory confirmation [16]. The definition of confirmed case remained the same.

The data for this study were extracted from EpiSurv, New Zealand's national notifiable disease surveillance system. Where symptom onset date was not available, the earliest date of hospitalisation, death or date reported was used [11]. Onset date was available for 1,644 cases (51%); for the remaining cases with missing onset date (n=1,610), report date was used for 1,289 (80%), while date hospitalised was used for 321 (20%). A short lag period from onset date to reporting date should not affect the overall estimation of *R* or the trend of the temporal variation. Including the data based on the date reported or hospitalised increased the power of the modelling results, outweighing any potential risk of inaccuracy of *R* estimates due to the lag.

Data analysis

The winter influenza season in New Zealand usually runs from weeks 18 to 40 (in 2009: 3 May – 4 October). In this study we analysed 3,254 pandemic influenza A(H1N1) cases reported in EpiSurv from 1 April to 1 November 2009 (weeks 17–44). Calculations of *R* used earliest date data from June to September 2009, while data for age- and ethnicity-specific incidence used June to August data, which encompass the highest incidence of cases at the peak of the epidemic. The change in response phase from containment to management meant that reported case numbers dropped considerably by September, so these low numbers were not included in the analysis. Data were analysed using Microsoft Excel and the numerical methods were developed using MATLAB (The Mathworks Inc.). Age-specific data from non-sentinel surveillance (EpiSurv) were compared with influenza-like illness data from the national sentinel general practitioner surveillance system [4] for trend comparison. Sentinel surveillance data are less prone to variation caused by changes in testing or response phase due to a consistent testing regime throughout the influenza season.

When calculating proportions of ethnic groups we used the New Zealand convention of prioritised ethnicity: where multiple ethnicities are recorded by individuals, Maori ethnicity takes precedence, followed by Pacific peoples, then Asian, with all remaining people included as Other or European [3]. For this study, because of small numbers, Asian people were combined with people in the Other ethnicity category. In determining the proportions of cases according to ethnicity, 2006 census population data were used as the denominator (all other proportions used mid-2008 population estimates).

Laboratory testing strategies

Laboratory testing strategies changed during the course of the pandemic. During the containment phase all suspected cases were tested to prevent transmission from imported cases to their close contacts and to contain transmission within small traceable clusters. During the management phase (22 June 2009 onwards) public health officials tried to mitigate the impact of sustained community transmission of the virus by

limiting sampling to those who had been hospitalised, had severe illness or were vulnerable to severe illness [4]. Swabs collected through sentinel general practitioner surveillance continued to be tested throughout the response.

Calculating the effective reproduction number

In recent years numerous methods have been published for determining the reproduction number of a disease from incidence data [17–20]. In our study, we adapted the method of Bettencourt and Ribeiro [18] and checked our calculations using the Wallinga and Teunis method [17]. The Bettencourt and Ribeiro method uses probabilistic Bayesian inference to determine a range of *R* values that best suit the data at any given point in time. This method is well suited to very stochastic data such as for emerging infectious diseases [21]. Due to the Bayesian nature of the method, successive case reports improve the estimation of *R* as time progresses. A probability distribution for *R* values is obtained and so confidence intervals of the mean *R* value are easily calculated. None of the methods mentioned above explicitly allows for multiple importations when determining *R*. Early in the New Zealand outbreak there were multiple imported cases and so the methods for calculating *R* need to be adjusted to allow for this. A common mechanism for this is to either remove imported cases from the calculations [11,12] or ignore their status as imported cases [14]. Both of these mechanisms overestimate *R*. This effect has been supported by simulation results in unpublished work by Mercer *et al.* Here we adapt the Bettencourt and Ribeiro [18] and Wallinga and Teunis [17] methods to include the imported cases in the transmission dynamics, but only use local cases when determining *R*. Details of the adaption can be found in Kelly *et al.* [22] on community transmission of pandemic influenza in Victoria, Australia, but are outlined here for completeness. Let *L*(t) be the number of locally acquired cases and *M*(t) the number of imported cases at day t. A standard susceptible, infective, recovered (SIR) model then gives the number of locally acquired cases at time t+τ as:

$$L(t+\tau) = b(R)(L(t)+M(t)) \;\; with \;\; b(R) = \exp(\tau\gamma(R-1))$$

where γ is the mean infectious period. Observed daily case numbers are highly variable so a probabilistic model is needed to determine the probability of the observed cases at time t+τ given the local and imported cases at time t and a given *R* distribution. Hence,

$$P\left[L(t+\tau) \leftarrow (L(t)+M(t))\middle|R\right] = P\left[b(R)(L(t)+M(t))\right] = P[\lambda]$$

where *P*[λ] is a chosen to be a Poisson distribution as this is the most general form if only averages are known, as is the case with an SIR model. We require the distribution of *R* given the data and so from Bayes' theorem:

$$P\left[R\middle|L(t+\tau) \leftarrow (L(t)+M(t))\right] = \frac{P\left[L(t+\tau) \leftarrow (L(t)+M(t))\middle|R\right]P[R]}{P\left[L(t+\tau) \leftarrow (L(t)+M(t))\right]}$$

The denominator is simply a scaling factor and need not be calculated. *P*[*R*] is the prior probability distribution of *R*, which reflects earlier calculated values of *R*. Initially *P*[*R*] is chosen to be an uninformative uniform distribution. The last above equation is iterated to obtain progressively better estimates for the probability distribution of *R* as time progresses and more data become available.

To calculate *R* values an estimate of the serial interval is needed. In this model, the serial interval is defined as the time between the onset of symptoms in case A to the onset of symptoms in case B, given that case A infects case B. Here we use the early New Zealand data of a mean serial interval of 2.8 days [11]. This is consistent with values used by other models ranging from 1.9 days [2] to 2.8 days [11–14] and 3.2 days [10].

Results

Descriptive epidemiology

A total of 3,254 cases of pandemic influenza A(H1N1) were notified in New Zealand from 1 April to 1 November 2009. Of these, 97.9% (n=3,186) met the confirmed case definition, with 2.1% (n=68) meeting the probable case definition. Known imported cases accounted for 2% (n=64). The highest notification rates were reported from the under one-year-old age group (223.2 per 100,000 population) followed by young adults 15 to 19 years (127.1 per 100,000 population) and 20 to 29 years (125.5 per 100,000 population) (Table 1). Of the total number of cases notified, 1,008 (31%) were hospitalised, with the highest rates reported in infants and preschool children (under 5 years) followed by young adults. Pandemic influenza A(H1N1) was identified as the primary cause of death in 19 cases; six of these were 30–39-year-olds (Table 1).

The highest rates of notifications and hospitalisations were reported in Pacific peoples, with rates that were more than double those of other ethnic groups (Table 2).

Epidemic curve and transmissibility of the pandemic virus

Figure 1 shows the temporal distribution of New Zealand's known imported cases and notifications of confirmed cases assumed to be autochthonous, by earliest date. The first observed non-imported cases experienced symptoms of illness in the first week in June 2009. Community transmission was detected by surveillance systems in the second week of June; notifications accelerated during June, reaching the peak of the epidemic in the second week of July (week 28).

The calculated *R* values and 95% confidence interval are shown in Figure 2. Also shown are calculated *R* values when the imported cases are either removed from the data or their imported status is ignored: both methods give a higher estimate than the true *R* value. The decline and then rise of *R* around 22 June 2009 is due to the reduced testing regime as a result of the change in phase from containment to management. Due to the

short serial interval of influenza, these types of dramatic changes in ascertainment only impact on the calculated *R* value for a few days. The drop in *R* below one coincides with the peak of the epidemic in the week ending 12 July 2009 and remains consistently below one for the remainder of the 2009 winter influenza season.

Distribution by age group over time and effect of school holidays

Figure 3 shows the age-standardised proportions of cases as the outbreak progressed through the New Zealand 2009 winter. A proportion of one indicates that age group had case numbers in keeping with their proportion of the population; values greater than one mean they were overrepresented. Changes in these proportions indicate changing transmission dynamics in the population.

The proportion of school-age children (5–19 years) among the cases rose sharply in the early stages of observed community transmission during the school term before declining, probably due to the reduced testing regime as a result of the change to management phase. The decline continued into the school holiday period but was followed by a marked increase a week after most schools resumed. The 2009 winter school holiday lasted from Saturday 4 July to Sunday 19 July (weeks 28 and 29), with the exception of some private schools for which it continued until Sunday 26 July [23]. A similar pattern was seen in children aged under five years, with a slight decline in proportion during the school holiday, followed by an increase starting a week earlier than with the school-age children. The increase then dropped off around 26 July, when the proportion of school-age children increased. People under 40 years old were overrepresented proportion-

Table 1

Cumulative incidence of notified and hospitalised cases and number of fatal cases of pandemic influenza A(H1N1) by age group, New Zealand, 1 April – 1 November 2009 (n=3,254)

Age group (years)	Notified cases[a]		Hospitalised cases[a]		Fatal cases[b]
	Number	Cumulative incidence[c]	Number	Cumulative incidence[c]	
< 1	143	223	99	155	0
1–4	230	97	116	49	1
5–9	241	84	50	17	1
10–14	279	92	56	19	0
15–19	410	127	73	23	0
20–29	715	126	174	31	2
30–39	409	70	104	18	6
40–49	367	58	126	20	4
50–59	301	58	126	24	3
60–69	80	21	46	12	1
≥70	58	16	38	10	1
Unknown	21	–	0	–	0
Total	**3,254**	**76**	**1,008**	**24**	**19**

[a] All confirmed and probable cases notified from 1 April to 1 November 2009.
[b] Where 2009 pandemic influenza was determined as the primary cause of death.
[c] Cumulative incidence per 100,000 population, calculated using mid-2008 population estimates.

Table 2

Cumulative incidence of notified and hospitalised cases and number of fatal cases of pandemic influenza A(H1N1) by prioritised ethnicity, New Zealand, 1 April – 1 November 2009 (n=3,254)

Prioritised ethnicity	Notified cases[a]		Hospitalised cases[a]		Fatal cases[b]
	Number	Cumulative incidence[c]	Number	Cumulative incidence[c]	
Maori	687	122	273	48	2
Pacific peoples	534	304	229	101	5
European	1,323	49	372	14	8
Other	333	89	99	26	2
Unknown	377	225	35	21	2
Total	**3,254**	**81**	**1,008**	**25**	**19**

[a] All confirmed and probable cases notified from 1 April to 1 November 2009.
[b] Where 2009 pandemic influenza was determined as the primary cause of death.
[c] Cumulative incidence per 100,000 population, calculated using 2006 census population data.

ally throughout the epidemic; those aged 40 years and over were underrepresented.

Distribution by ethnicity over time and transmission dynamics

Early clustering of cases occurred in Pacific peoples: the proportion of notifications for this ethnic group was consistently high in the first few weeks of community transmission (Figure 4). The number of Maori cases increased in the last two weeks of June 2009. These increases, together with the early clustering in young children, are likely to have contributed to earlier higher estimates of *R* in New Zealand. By the later stages of the epidemic, towards the end of July, all ethnic groups were equally represented.

Discussion

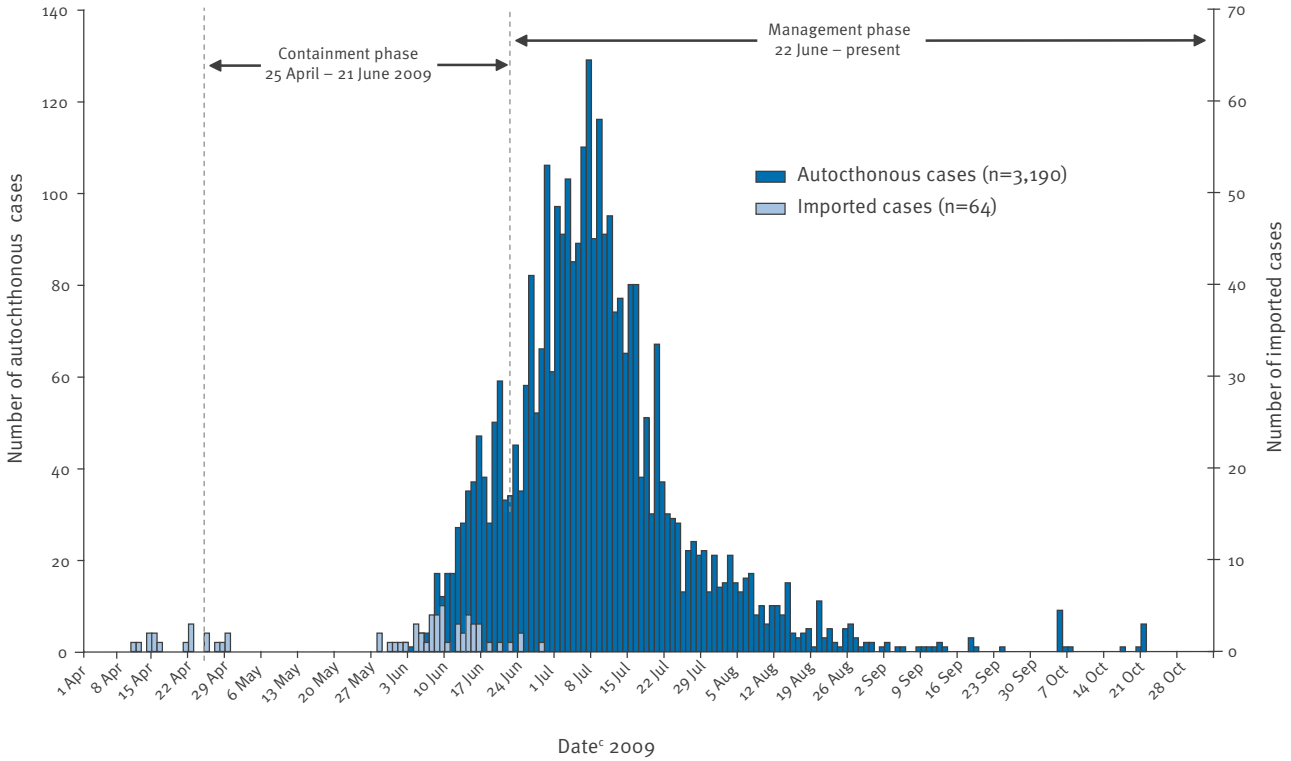
In this study, we found that the peak *R* was lower than previously calculated early in the New Zealand epidemic. There is also evidence of the seeding of pandemic influenza A(H1N1) cases from Australia in the weeks leading up to the detection of community transmission. When a pandemic of an emerging infectious disease arises, it can be considered advantageous to be isolated geographically from the threat. New Zealand, with its natural oceanic borders, has a high level of geographical isolation. The most likely way in which a new influenza virus could be introduced is the arrival of infected air travellers who are not detected by screening at the border [24]. Recent pandemic influenza activity highlights the susceptibility of New Zealand's population to incursion of a novel transmissible disease.

Short-term travel between Australia and New Zealand is increasingly common, with more than one million visitors arriving from Australia annually. In the month of June 2009, 298,267 people arrived by air from all countries including Australia into New Zealand [25,26], providing multiple opportunities for infectious travellers to arrive in the country undetected. Auckland, Wellington and Christchurch regions all have international airports that service flights between Australia and New Zealand: unsurprisingly these were the first regions to experience an observed escalation in community transmission. Early clustering in Pacific peoples' community was believed to have been seeded by a member of their community returning to Christchurch from Melbourne, Australia, with the virus in early June [27].

Australia's first observed community-acquired case of the pandemic virus had an onset of symptoms on 16 May 2009 and was reported in Victoria, although there is evidence to suggest that unobserved transmission was occurring in Australia before that time [14]. By 4

Figure 1

Confirmed and probable autochthonous and imported cases of pandemic influenza A(H1N1), New Zealand, 1 April – 1 November 2009 (n=3,254)[a,b]



[a] 3,186 confirmed cases, 68 probable cases.

[b] Cases assumed to be autochthonous and known imported cases.

[c] Cases are recorded according to earliest date of symptom onset, hospitalisation, death or date reported.

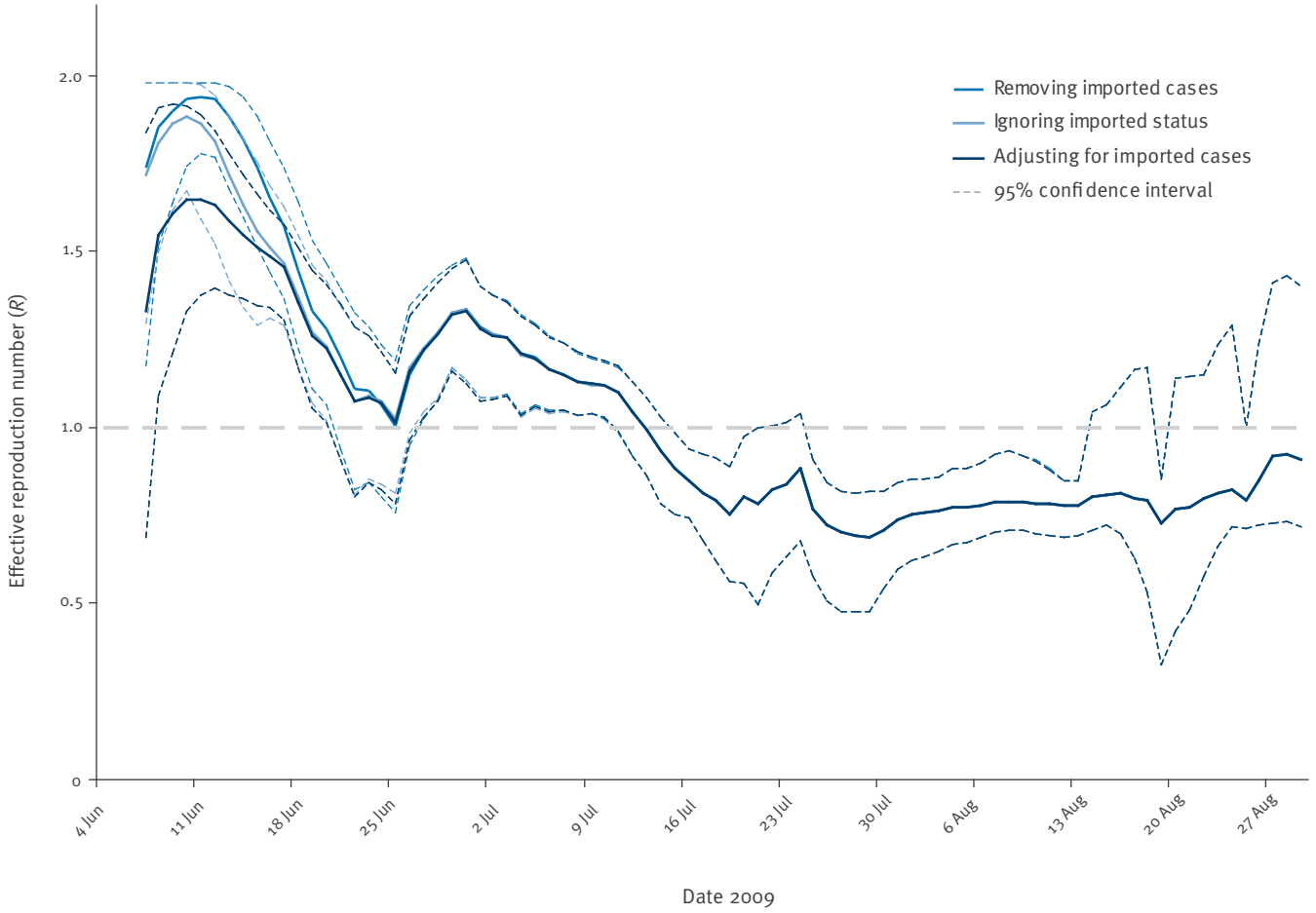
June the state of Victoria had reported a total of 977 confirmed cases of pandemic influenza to the Victorian Department of Health [28]. At that time, New Zealand's confirmed cases had been imported although unobserved transmission had begun; observed cases of community transmission appear to have been established by the second week of June.

Young children and Pacific peoples contributed disproportionately to early propagation of the pandemic in the country. It is possible that the school holidays were associated with a lowering of the age-specific incidence rate in school-age children. All schools had resumed classes by 27 July 2009, which coincided with an increase in notifications lasting around a week before declining in August as the epidemic tailed off. The increase in confirmed cases among young children could suggest an increased risk of exposure to the virus through infected older siblings or that this age group is more likely to be presented at their general practice for consultation. Data from the sentinel general practice surveillance system were consistent with the proportions from the notification data. Proportions of cases seen in the over 60-year-old age group were consistently much lower than expected. The high proportions in the younger age distributions are consistent with a lack of pre-existing antibodies to the pandemic virus in children and young adults. Recent studies suggest older adults (over 60 years) have some degree of pre-existing immunity and this may have contributed to lower numbers in this age group [29].

When importations are removed our method is in agreement with the early results from New Zealand of R=1.96 [11]. Early in the outbreak our adjusted R value was 1.55 (95% confidence interval: 1.16 to 1.86). As the epidemic progressed the calculated R value declined to around 1.0 (95% confidence interval: 0.83 to 1.25) until the management phase was implemented on 22 June 2009. This change in phase impacted on the case ascertainment, probably affecting the adjusted value of R. An increase in R in late June to around 1.25 (95% confidence interval: 1.08 to 1.37) is consistent with ongoing work on Australian data (unpublished) but is on the lower end of other estimations [9,13].

Figure 2

Effective reproduction number (R) calculated from confirmed and probable pandemic influenza A(H1N1) cases, New Zealand, 6 June – 1 September 2009 (n=3,197)[a]

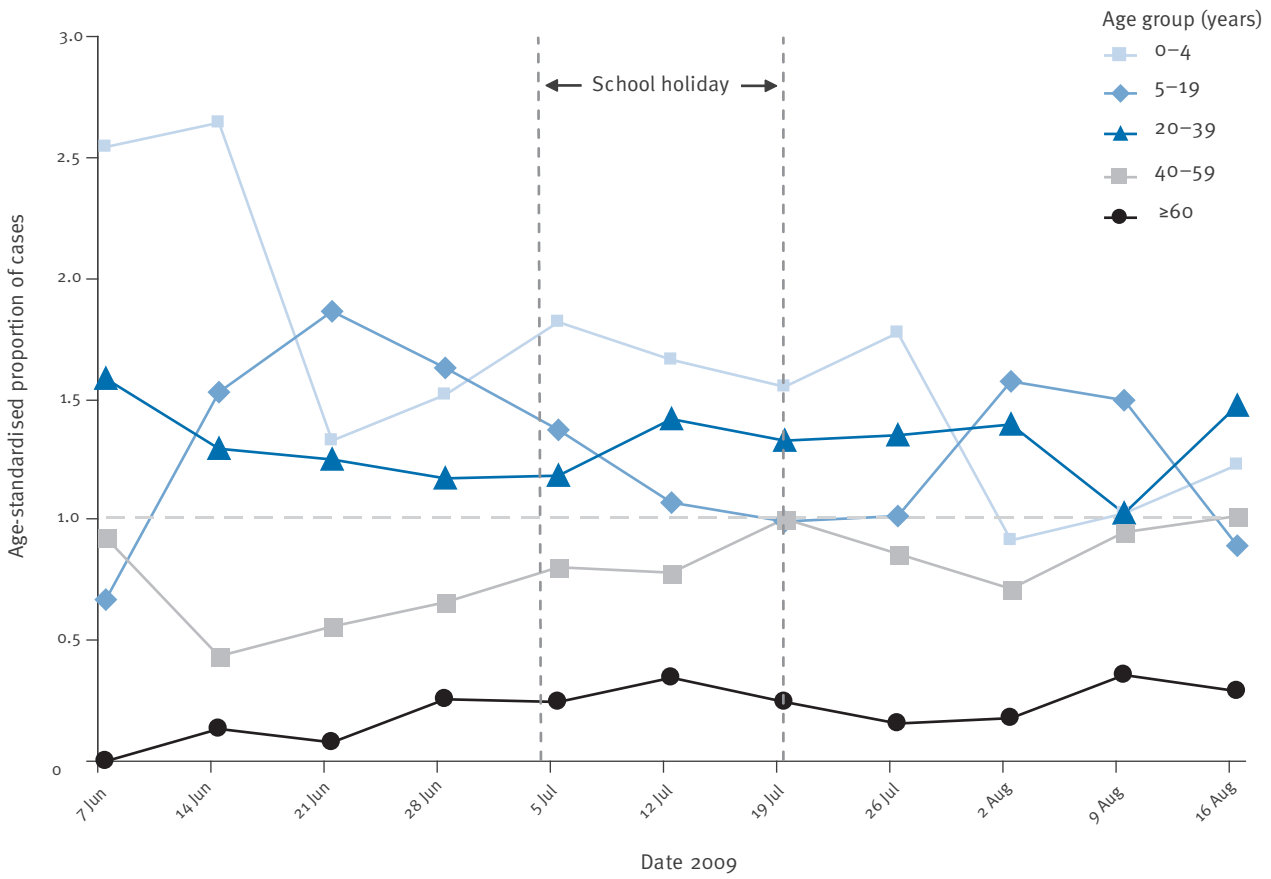


[a] 3,136 confirmed cases, 61 probable cases.

If R is less than one, the disease will not persist but will manifest itself in outbreaks of varying size triggered by importations of the disease, whereas if R is greater than one there is a chance of a large outbreak.

Figure 3

Age-standardised proportions of pandemic influenza A(H1N1) cases, New Zealand, 7 June – 16 August 2009 (n=3,126)[a]

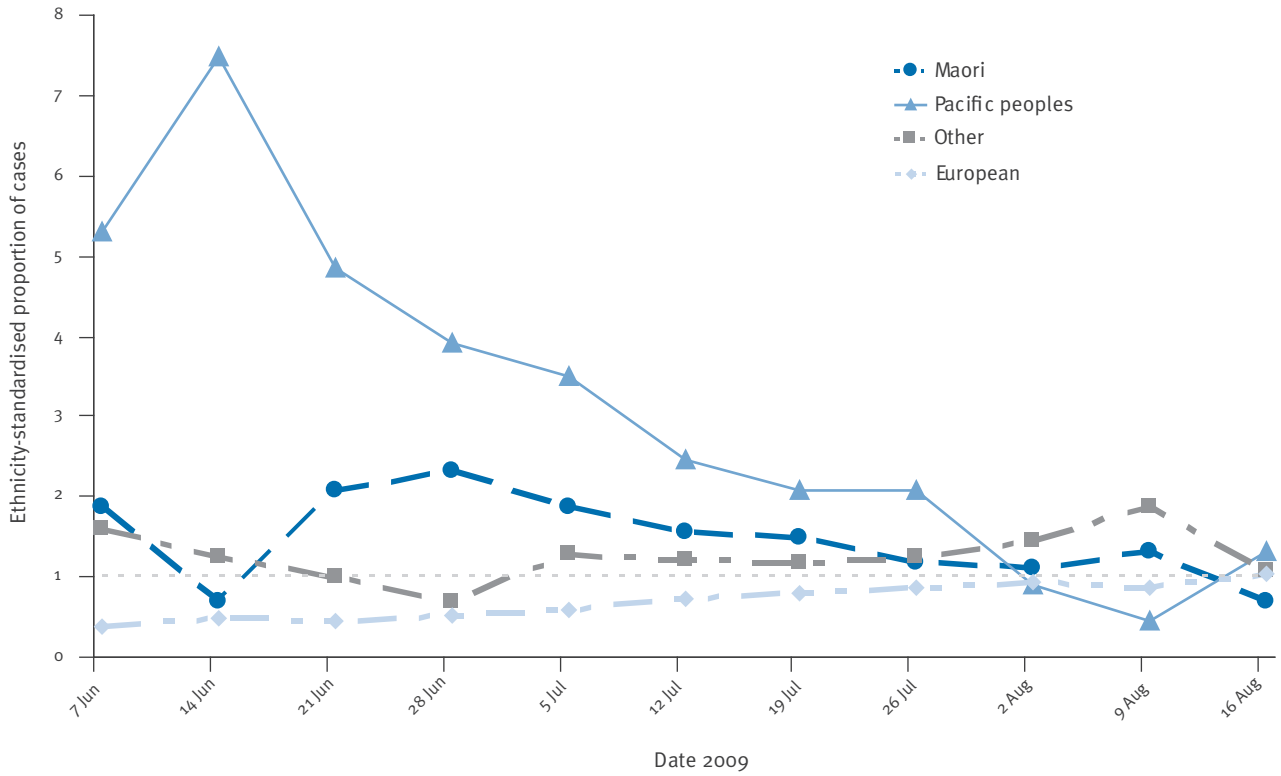


[a] 3,067 confirmed cases, 59 probable cases.

A proportion of one indicates that age group had case numbers in keeping with their proportion of the population; values greater than one mean they were overrepresented.

Figure 4

Prioritised ethnicity-standardised proportions of pandemic influenza A(H1N1) cases, New Zealand, 7 June – 16 August 2009 (n=3,126)[a]



[a] 3,067 confirmed cases, 59 probable cases.

A proportion of one indicates that ethnic group had case numbers in keeping with their proportion of the population; values greater than one mean they were overrepresented.

The reduction in *R* below one around 12 July 2009 coincided with reduced testing after implementation of the management phase. Public health measures in place at the time and other factors such as voluntary social distancing may have also contributed to the reduction in transmission of the virus.

Other countries have reported high transmission rates in schools that have contributed to high measures of *R* [12,14]. In New Zealand the highest numbers and rates were reported in the under one-year-old and 15–29-year-old age groups, indicating that there were high levels of transmission occurring outside school settings. Reasons for this may relate to the communities that were initially affected. Heterogeneous mixing of imported cases into susceptible populations who interact in close proximity – such as cultural and religious communities and people in childcare centres, work places and schools – could have contributed to case clustering and rapid transmission of the virus within those groups.

Limitations and need for further research

Complete ascertainment of imported cases was not possible during the pandemic as travel information was only collected during the containment phase during an active surveillance period. A change in the case report form on 10 July 2009 meant the question was no longer asked.

There are difficulties in comparing values of *R* from this study with those from other countries as differences in generation time and modelling methodologies vary across studies. Methods for modelling pandemic influenza are evolving rapidly. Our analysis suggests it is important to consider imported cases, particularly in the early stages of an epidemic. Consequently, surveillance systems should attempt to record such status on all cases at least until local transmission is dominant.

The data collected before the management phase commenced were influenced by case clustering in Pacific peoples, which could have overestimated the community-wide *R* value. Calculations towards the end of August 2009 are less reliable as low numbers of cases were reported.

Conclusions

Using a new modelling method to account for imported cases, we have calculated the peak *R* during the growth phase of the pandemic as 1.55 (95% CI: 1.16 to 1.86). This value was less than previously estimated for New Zealand early in the pandemic but is in line with more recent estimates in other parts of the world.

An increase in the proportion of notifications among school-age children could be associated with the children returning to school after the July holiday. Although this relationship should be analysed further, this preliminary evidence could support the effectiveness of timely school or selected classroom closures as a potential public health intervention for future influenza pandemics. It should be noted that it is likely that it was the importation of the virus through infected travellers from abroad that fuelled the influenza A(H1N1) pandemic early in the 2009 New Zealand winter. Future resources might be better focused on containing the spread of infection caused through exposure to infected travellers returning to or arriving in New Zealand, in particular, through early case and cluster detection, effective contract tracing systems and the timely use of prophylaxis.

The overrepresentation of Pacific peoples and to a lesser extent people of Maori ethnicity was observed during the containment phase and was probably due to case clustering, The high proportions initially observed in these groups decreased towards the end of July 2009 when all ethnic groups were more evenly represented.

Descriptive epidemiology and modelling are useful for understanding how a novel pandemic influenza virus affects a newly exposed population. They can help measure how successful public health interventions have been in mitigating the severity of the pandemic and estimate the potential effectiveness of interventions before they are implemented. Northern hemisphere countries yet to experience the peak of their 2009–10 winter influenza season can benefit from lessons learnt in the south. During a pandemic of moderate severity, where most infected individuals experience self-limiting illness, governments could consider public health actions that target groups most at risk of infection and that cause the least disruption to society. Applying mitigating measures of limited benefit that have a disruptive effect on society, such as reactive school closures in a relatively moderate pandemic, should be carefully scrutinised before being implemented.

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Using tests for recent infection to estimate incidence: problems and prospects for HIV

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Tests for recent infection (TRIs), such as the BED assay, provide a convenient way to estimate HIV incidence rates from cross-sectional survey data. Controversy has arisen over how the imperfect performance of a TRI should be characterised and taken into account. Recent theoretical work is providing a unified framework within which to work with a variety of TRI- and epidemic-specific assumptions in order to estimate incidence using imperfect TRIs, but suggests that larger survey sample sizes will be required than previously thought. This paper reviews the framework qualitatively and provides examples of estimator performance, identifying the characteristics required by a TRI to estimate incidence reliably that should guide the future development of TRIs.

Introduction

When monitoring HIV epidemics it is vital to estimate incidence in order to plan and evaluate HIV programmes [1]. Prospective cohort studies are the most direct way to achieve this. They are, however, expensive, prone to recruitment and retention bias, and potentially rendered unrepresentative by ethical obligations. The use of prevalence data in conjunction with mathematical modelling is an alternative approach [2,3], but is indirect and requires accurate knowledge of mortality and migration. The disadvantages of these methods have focused attention on estimating incidence from cross-sectional surveys [4-8], with the result that a number of assays and algorithms that test for recent infection have been developed [9,10]. In the context of HIV, such an assay or algorithm has sometimes been termed a STARHS (Serological Testing Algorithm for Recent HIV Seroconversion) [9,10], but we prefer to use the generic term 'test for recent infection' (TRI), because it does not specify a particular disease and method of testing. Recently, the World Health Organization (WHO) Technical Working Group on Statistical Approaches for Development, Validation and Use of HIV Incidence Assays has proposed using the term 'recent infection testing algorithm' (RITA). The term has not, however, gained universal acceptance.

TRIs identify HIV-positive individuals who have been infected recently. By using a TRI in a serosurvey, incidence (I) can be estimated by applying the epidemiological relationship (based on 'Prevalence = Incidence x Duration'):

$$I = \frac{R}{SD}$$

where R and S are the counts of 'recently infected' and 'susceptible' (HIV-uninfected) individuals observed in the cross-sectional survey and D is the mean duration spent in the 'recently infected' state, often called the (mean) window period. This incidence estimate is an average of the instantaneous incidence over a period of approximately D prior to the survey. The problem of incidence estimation then reduces to measuring the prevalence of 'recent infection', given knowledge of its duration.

TRIs usually discriminate recent from established infections by measuring specific aspects of the immune system which evolve during the course of initial infection. For HIV, this is typically the antibody response, with the titre, proportion of HIV-specific IgG, or antibody avidity (or a combination of these) providing quantitative output [10]. Laboratory-defined thresholds are chosen to convert these outputs into categorical results. These results may be augmented with other clinical information, such as CD_4 lymphocyte counts and antiretroviral therapy (ART) status, to classify individuals as either TRI-positive (P i.e. recent) or TRI-negative (N i.e. non-recent). Positive and negative in this context should not be confused with HIV-positive and HIV-negative.

The interaction between the virus and the immune system is complex, and individuals vary in their response to infection as assessed by a particular TRI. Modest

variation is not intrinsically problematic, but serious complications arise if, in some individuals, the immune response is such that they remain indefinitely classified as TRI-positive or if individuals revert back to a TRI-positive classification as a result of advanced disease or in the presence of antiretroviral therapy. Unfortunately, both these complications arise for TRIs currently in use. This not only limits the applicability of the simple incidence estimator above, but also makes it difficult to define and estimate the mean duration spent in the recently infected state (i.e. to evaluate *D*). Methods for 'adjusting' estimates of incidence have been proposed [7,8] and adopted by the United States Centers for Disease Control and Prevention [11] but are currently under debate [12-15]. Recently, a formally rigorous framework has been developed [16,17]. We provide a summary of the framework and explore its implications for the analysis of surveys and development of new TRIs.

Theoretical framework

We now briefly describe the theoretical framework and how it can be generalised. The key results that emerge from the analysis are:

- A TRI is ideal if *all* individuals eventually progress permanently out of the TRI-positive state before there is any disease-related mortality. In this case, the TRI-positive category directly corresponds to a useful definition of 'recently infected' [16,18], which means that an estimate for the number of recent infections is:

$$R=P$$

- For a non-ideal TRI (i.e. when some individuals never progress out of the TRI-positive state), it is in principle still possible to estimate the number of individuals in a well-defined 'recently infected' state, even though this state is not directly observable in all individuals. If P_{np} is the *proportion of the HIV-positive individuals who never progress on the TRI under consideration*, then an estimate for the number of recent infections is [16]:

$$R = P - \frac{P_{np}}{1 - P_{np}} N \qquad (1)$$

  When the TRI is ideal, then P_{np} = 0, and this formula reduces to the previous expression.
- For *all* applications (including determination of a trend without regard to the absolute level of incidence), an estimate of P_{np} is required.
- To determine the *absolute level* of incidence, it is also necessary to estimate the *mean time spent TRI-positive in the subset of individuals who eventually do progress to become TRI-negative*. This quantity, which we denote by ω, is analogous to the duration *D* in the simple estimator, but differs in the requirement that it should be estimated in the subset of individuals that progress on the TRI.
- As P_{np} increases (i.e. a larger fraction of individuals fail to progress on the TRI) and as ω decreases (i.e. individuals spend less time in the TRI-positive state) statistical power is lost. This means that estimates of incidence will have more uncertainty (i.e. wider confidence intervals), and it is less likely that a true change in incidence will be detected.

Previous work by McDougal *et al.* [7] used terminology usually employed to characterise the performance of diagnostic tests, such as sensitivity and specificity, to characterise TRI performance. 'Recent infection' was defined as being infected for less than a particular time (chosen to be the mean window period). A sensitivity and two specificity parameters were introduced to characterise imperfect classification. No procedure incorporating the effect of parameter uncertainty has thus far been proposed to estimate statistical error or power for the McDougal approach. It has recently been shown that use of sensitivity and specificity parameters is a redundant description of the TRI characteristics [17,19]. In contrast, the new framework defines the condition of being 'recently infected' directly in terms of the TRI result. This approach is applicable under less restrictive assumptions, is less prone to bias, and admits an equally informative description of TRI performance using only ω and P_{np} [17].

In deriving the results outlined above, two assumptions were made. Firstly, it was assumed that individuals who do not progress on the TRI have the same survival outcomes as TRI progressors. There is, however, evidence for some TRIs that individuals that fail to progress on the test have a survival advantage. For example, in Baltimore, USA, 60% of elite suppressors (individuals with naturally suppressed virus below 50 copies per ml) failed to progress on the BED assay [20], and elite suppressors have been observed to survive for longer than others [21]. Secondly, it was assumed that TRI progressors never regress back to the TRI-positive state, but there are indications that this is not true for some TRIs. For example, the rate of misclassification by the BED assay is observed to be higher in individuals with advanced infection [22] and individuals on ART [22-24]. When these assumptions are true, P_{np} is always equal to the proportion of non-recently infected individuals who are classified TRI-positive. When the assumptions are violated, this proportion, or false-recent rate, denoted by ε, varies according to the historic trajectory of the epidemic [17,25]. This would be consistent with the apparently higher BED assay false-recent rate in Uganda [26] (an older, declining epidemic) than in South Africa [27] (a younger, growing epidemic) [25]. It is, however, still possible to estimate the number of recent infections by replacing P_{np}, in the expression (1) above, with an estimate of ε applicable to the time and place of an incidence survey [27] (see eAppendix http://wwwo.sun.ac.za/sacema/publications/eAppendix.pdf for justification). The incidence estimator can then be written as:

$$I = \frac{P - \frac{\varepsilon}{1-\varepsilon} N}{\omega S} \qquad (2)$$

The inputs to this estimator are of two types: survey counts (P, N and S), which need to be estimated in every incidence survey, and parameters that describe the characteristics of the TRI (ω and ε), which ideally are estimated in a smaller number of parameter estimation studies.

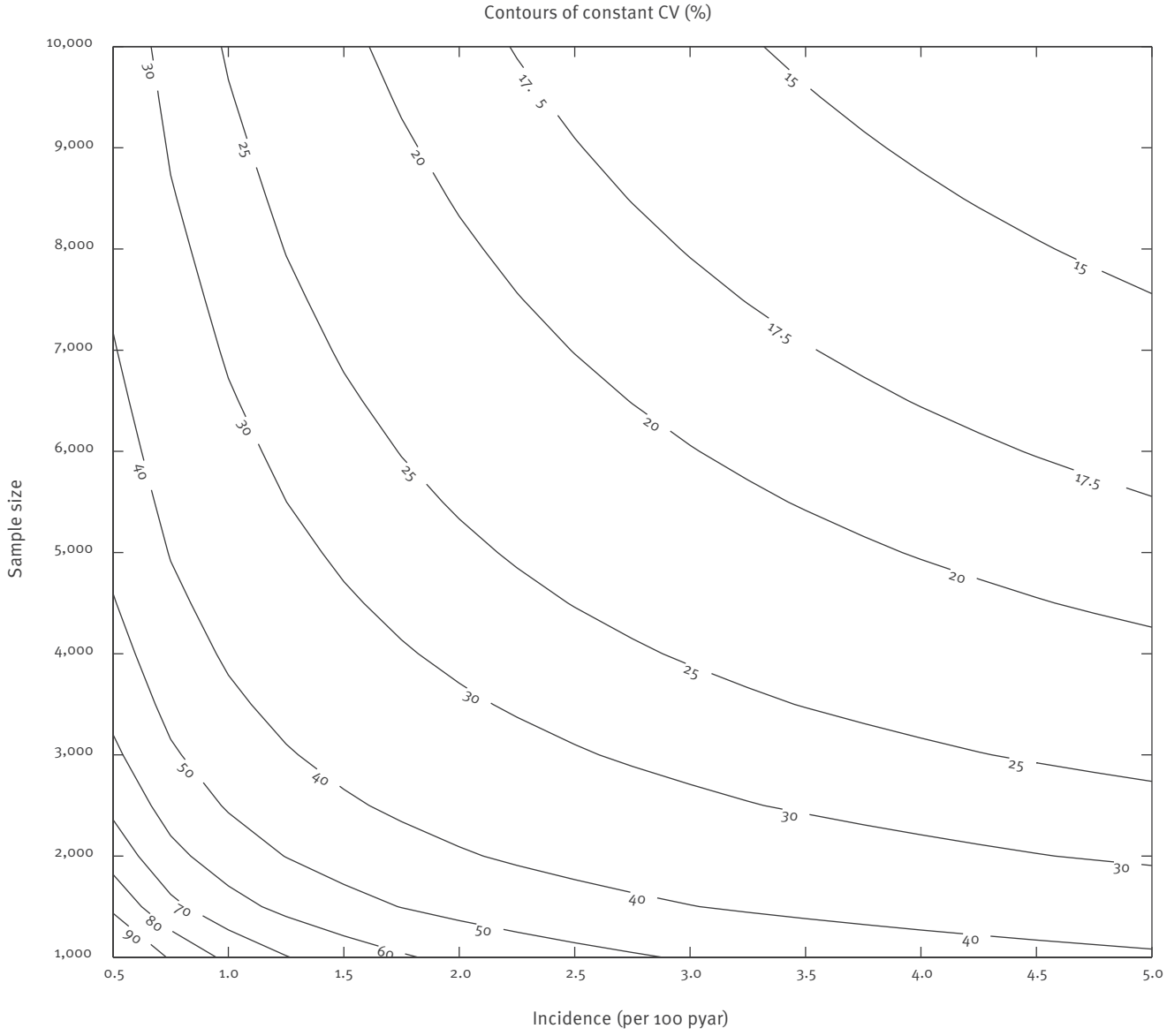
When ε and ω are known with sufficient accuracy, there are no theoretical reasons why an imperfect TRI should not allow the accurate estimation of incidence. However, two distinct types of practical problems arise – counting error and TRI parameter error. An important component of recent developments is the first consistent analysis of incidence uncertainty accounting for both counting and parameter error (see eAppendix http://wwwo.sun.ac.za/sacema/publications/eAppendix.pdf for a description of the uncertainty expression). We now illustrate this uncertainty with a somewhat idealised model of the BED assay, which has received much attention and application [28].

Counting error

Even in the largest HIV epidemics, infection events are relatively rare (about 2% of the population per year) and 'recent' infections (infections in the last 155 days or so, for the BED assay [7,29]) are even less common

Figure 1

Uncertainty of incidence point estimates as a result of sample size and background incidence



CV: coefficient of variation; pyar: person years at risk; TRI: test for recent infection.

The coefficient of variation of estimates of incidence using a TRI depends on the sample size of the survey and the true incidence rate. Note that a sample size of 10,000 approximates to the typical size of household-based surveys in Sub-Saharan Africa, and that incidence in South Africa (where there is one of the largest epidemics) is estimated to be about two per 100 pyar).

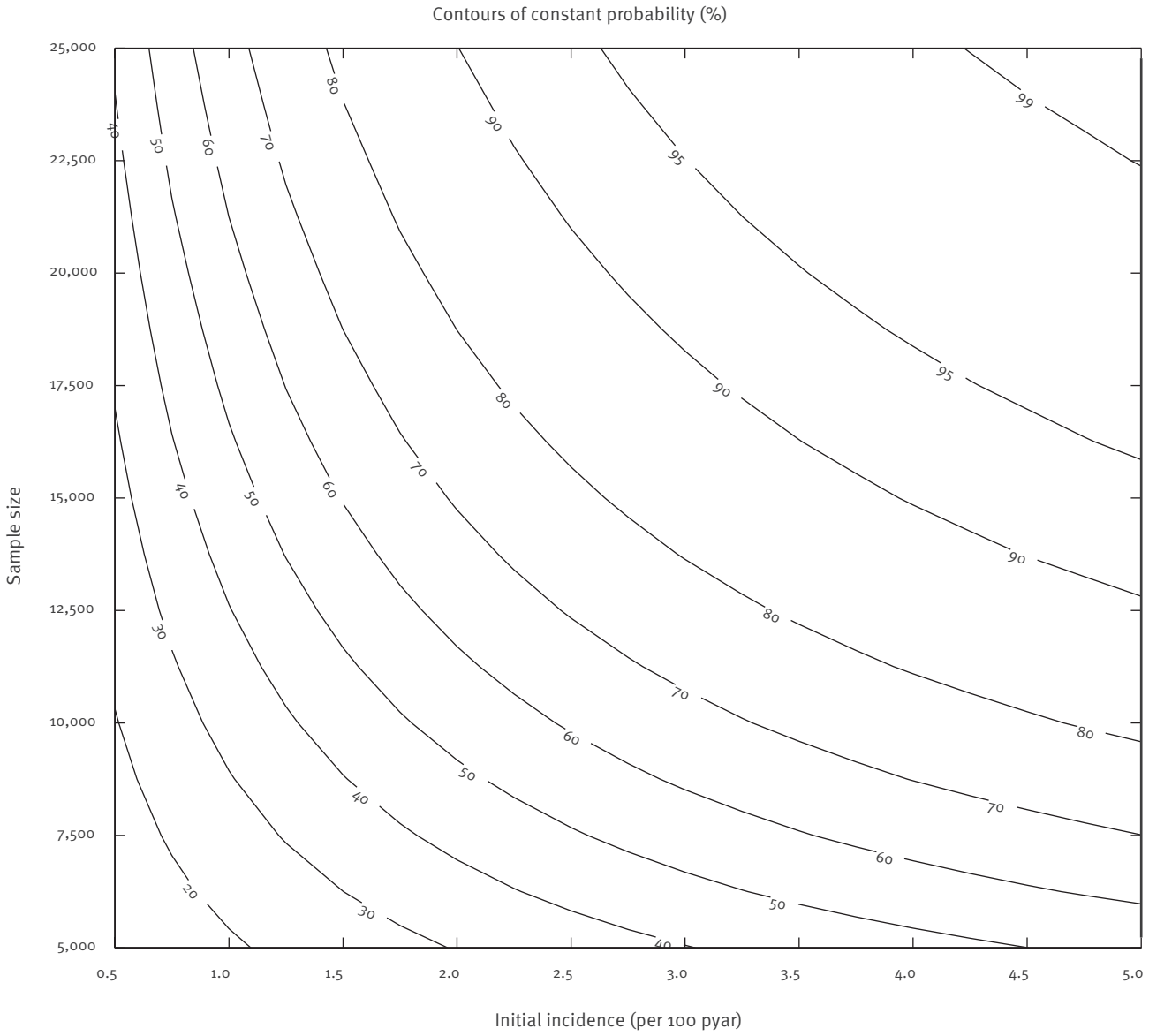
Assumptions: ω= 155 days; ε=0.05; no TRI parameter uncertainty; steady-state epidemic conditions; mean survival with HIV: 11 years [31-33].

(about 0.85% in a cross-section of the population). Thus, estimates of incidence are associated with substantial uncertainty since there are few recent infections to be counted. Figure 1 shows the coefficient of variation (CV, which is the ratio of the standard deviation to the estimate) for the estimator (2) calculated under various survey sample sizes and steady-state HIV incidence rates (see eAppendix http://wwwo.sun.ac.za/sacema/publications/eAppendix.pdf for a description of the uncertainty and steady-state calculations). The TRI parameters (ω and ε) are assumed to be known with absolute certainty. Low values of CV are desirable and indicate that estimates of incidence have small confidence bounds, while high values indicate that incidence estimates will be less certain. For example, in a cross-sectional survey of 5,000 individuals from a population with a steady-state incidence of 2.0 per 100 person years at risk (pyar) the CV is 25.8% – i.e. the 95% likelihood interval for an incidence estimate is 1.0 to 3.0 per 100 pyar.

To explore the ability to detect a change in incidence, a substantial reduction (halving) in incidence is simulated (initially in a steady-state epidemic, with prevalence remaining constant between the two surveys), and a two-tailed test of the null hypothesis that incidence is the same in the two surveys is performed. The possible outcomes are: sustaining the null hypothesis, or concluding that incidence has either increased or decreased. Figure 2 shows the probability of correctly

Figure 2

Probability of correctly inferring a reduction in incidence



pyar: person years at risk; TRI: test for recent infection.

The probability of detecting a reduction in incidence between two surveys, when incidence has actually been reduced by half, as a function of the sample size of the surveys (both assumed to be the same) and the baseline incidence rate.

Assumptions: ω=155 days; ε=0.05; no TRI parameter uncertainty; significance α=5%; steady-state epidemic conditions at first survey, with equal prevalence at second survey; mean survival with HIV: 11 years [31,33].

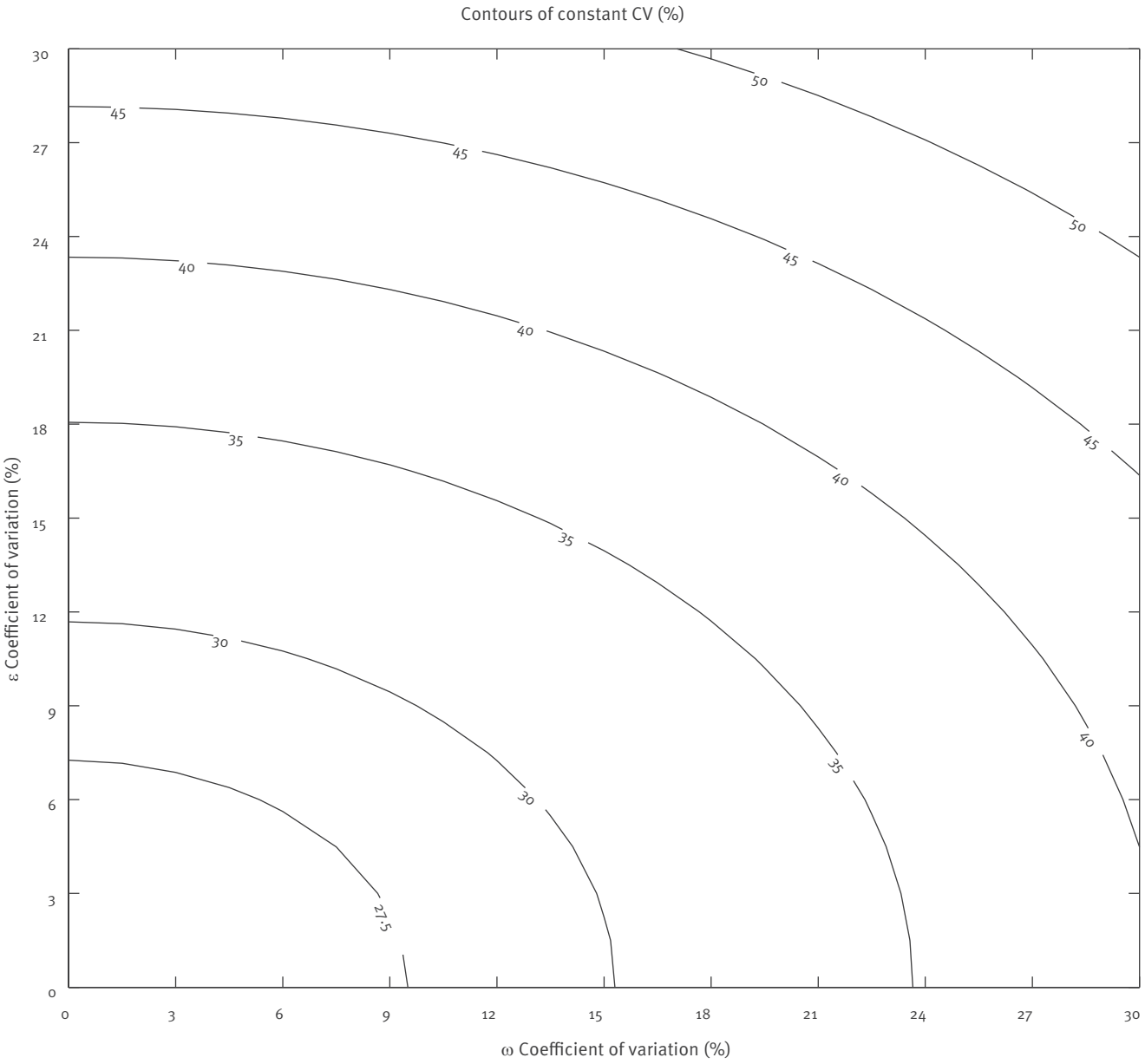
inferring a reduction in incidence, when testing the null hypothesis at a significance level of a=5%. A probability close to 100% indicates that reductions in incidence will be reliably detected, with a probability of less than 90% indicating that results will be unreliable. The South African National Strategic plan for HIV AIDS [30] has ambitiously set a target of halving incidence between 2007 and 2012. Our calculations suggest that the sample size of each of two surveys (in 2007 and 2012) required to reliably conclude that incidence has decreased, at the 5% significance level, is approximately 25,000.

TRI parameter error

In the previous section, it was assumed that the correct TRI parameters were known with certainty. The incidence estimates are very sensitive to changes in the values of ω and ε, however, and small differences between the values used in the calculation and the *true* values can lead to large errors. These parameters have to be estimated in separate studies, usually using cohorts of individuals whose infection time is known approximately. Such cohorts are rare, however, and the numbers of individuals in them are typically small, resulting in substantial uncertainty for the values of ω and ε. In Figure 3 we explore the uncertainty of the estimator (expressed as a CV), as a function of the uncertainty in the TRI parameters. For example, when

Figure 3

Uncertainty of incidence point estimates as a result of TRI parameter uncertainty



CV: coefficient of variation; pyar: person years at risk; TRI: test for recent infection.

Coefficient of variation of incidence estimator, using a BED-like assay on a sample size of 5,000, in a population exposed to an incidence of two per 100 pyar, as a function of the uncertainty in the TRI parameters, assumed to be normally distributed.

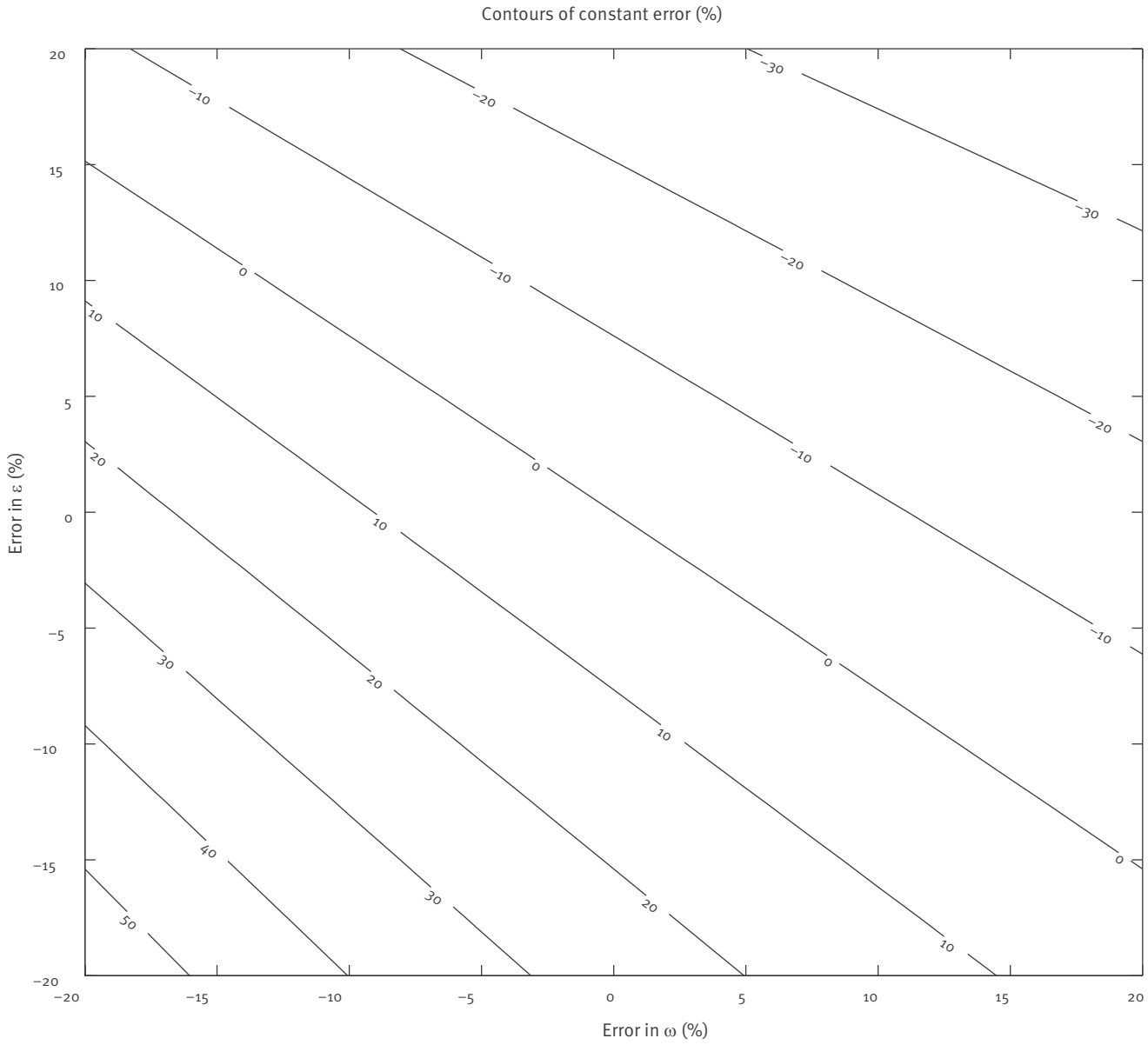
Assumptions: ω= 155 days; ε=0.05; steady-state epidemic conditions; mean survival with HIV: 11 years [31,33].

the BED-like parameters are known with a CV of only 15.0% (reference to a CV for ω relates to the uncertainty of the estimate of ω, not the variation associated with progression times), at a sample size of 5,000 and a steady-state incidence of 2.0 per 100 pyar the CV, as a result of both counting error and parameter uncertainty, is 35.7% – i.e. the 95% likelihood interval for an incidence estimate is 0.6 to 3.4 per 100 pyar.

Since the TRI parameter estimation study may be conducted in a separate population, it is possible to introduce systematic bias if the true values of the TRI parameters vary between populations or over time. The few estimates of ε that have been published vary widely. For example, the false-recent rate is estimated at 1.7% in a South African survey [27] and 26.7% in Rwanda and Zambia [26] presumably due to population differences in the historic courses of the epidemics, viral subtypes, host immune profiles, and uptake of antiretroviral therapy. This undermines confidence in the ability to use an estimate for ε obtained in a different population to the one in which incidence is to be estimated, and could contribute to the apparently inflated estimates of incidence reported recently [34,35]. There is also currently no general theoretically unbiased procedure for estimating ε – work on this problem is in progress [36]. In Figure 4 we explore the systematic error in the incidence estimate, expressed as a percentage of the correct value, introduced by systematic errors in the TRI parameters, also expressed

Figure 4

Systematic error in incidence point estimates as a result of systematic error in TRI parameters



pyar: person years at risk; TRI: test for recent infection.

Systematic error expressed as a percentage of the correct estimate, excluding counting error, observed in the incidence estimator, using a BED-like assay, as a function of a precisely known systematic error in the TRI parameters.

Assumptions: ω=155 days; ε=0.05; steady-state epidemic conditions; mean survival with HIV: 11 years [31,33].

as percentages. There is a region in which bias may be small due to cancellation of systematic errors (see the zero error contour).

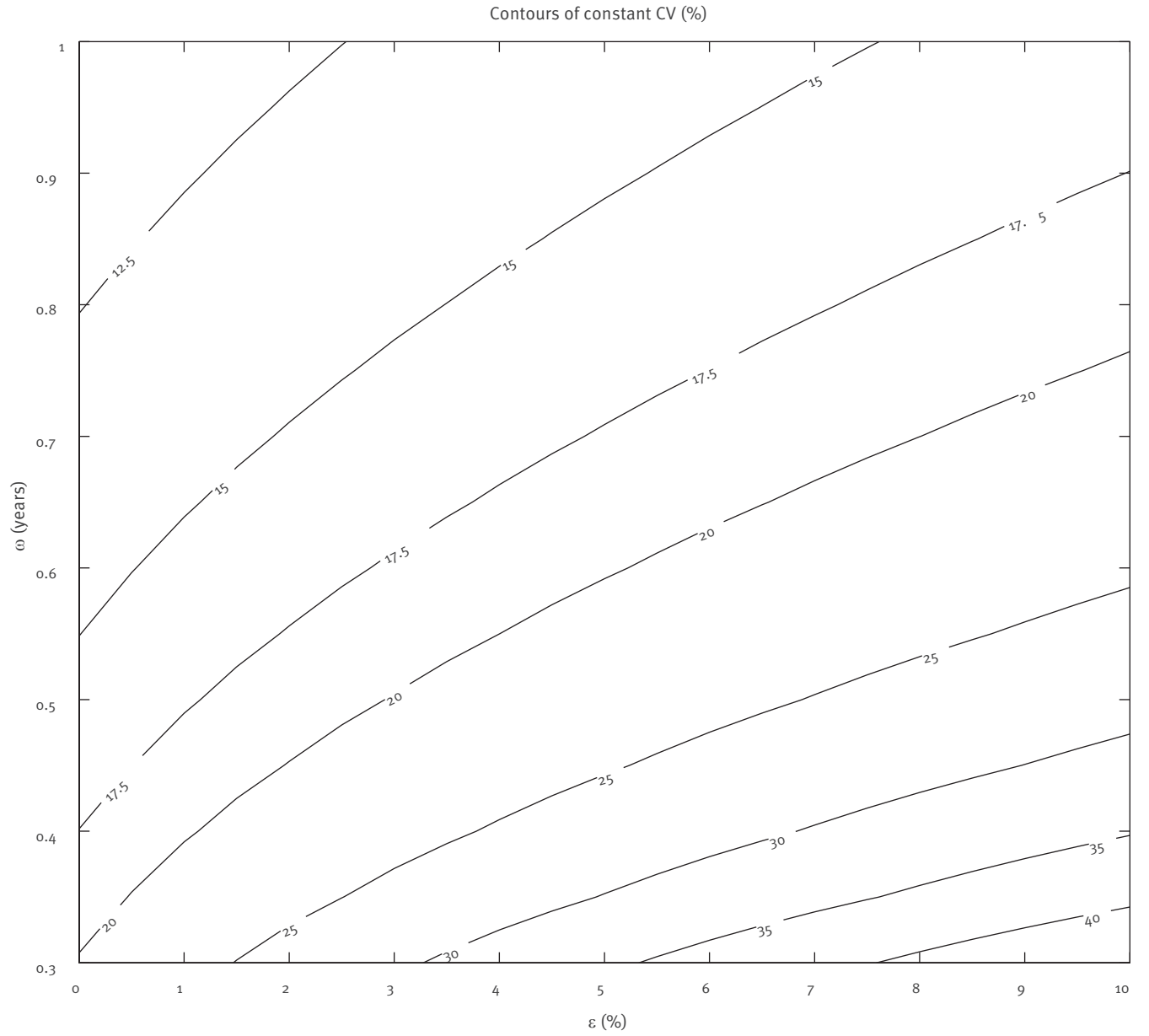
Conclusion

In the short term, reports from early studies using BED should be interpreted with caution [28], given the substantial uncertainties identified above. Analysis of TRI data should be performed within a more general theoretical framework [16,17], rather than using earlier methods. Most importantly, incidence surveillance should not currently rely on any single methodology, but make use of multiple methods for estimating incidence [37], such as interpretation of prevalence trends and epidemiological and demographic modelling [3,38].

The search for robust means of estimating incidence from cross-sectional surveys is at a crucial juncture. Although an imperfect TRI can be used to estimate HIV incidence reliably, the reliance on having accurate and precise values of two key aspects of TRI performance (ω and ε) can undermine the use of this technology. The effect of ω and ε on statistical power is shown in Figure 5. While larger values of ω provide sufficient numbers of TRI-positive individuals to ensure statistical power, ω should not be so large that the estimated incidence is not representative of the recent past. On this basis,

Figure 5

Uncertainty of incidence point estimates as a result of TRI performance



CV: coefficient of variation; pyar: person years at risk; TRI: test for recent infection.

Coefficient of variation of incidence estimator, on a sample size of 5,000, in a population exposed to an incidence of two per 100 pyar, as a function of the TRI parameters.

Assumptions: no TRI parameter uncertainty; steady-state epidemic conditions; mean survival with HIV: 11 years [31,33].

a value of approximately six months to a year is desirable. It is also essential that ε be small (progress in this regard is being made, for instance using TRIs consisting of an assay in combination with clinical information [39]). Ideally, to ensure that the fraction of misclassifications is independent of time and epidemic state, inter-individual variability in TRI progression should be unrelated to survival outcomes, and there should be no regression to the TRI-positive state. These form the core requirements for the development of new TRI assays and algorithms used to estimate incidence.

In the next phase of TRI development, it will be essential to be guided by these insights into the key determinants of test performance, and to focus on characterising the performance of the test within a systematic framework.

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