Impact factor 5.49

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 18 | Weekly issue 37 | 12 September 2013

Research articles

Meeting reports

News

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

The test-negative design: validity, accuracy and precision of vaccine efficacy estimates compared to the gold standard of randomised placebo-controlled clinical trials

G De Serres (gaston.deserres@inspq.qc.ca)[1], D M Skowronski[2], X W Wu[3], C S Ambrose[3]
1. Institut national de santé publique du Québec and Department of Social and Preventive Medicine, Laval University, Quebec, Canada
2. British Columbia Center for Disease Control, Vancouver Canada
3. MedImmune, Gaithersburg, MD, USA

Citation style for this article:
De Serres G, Skowronski DM, Wu XW, Ambrose CS. The test-negative design: validity, accuracy and precision of vaccine efficacy estimates compared to the gold standard of randomised placebo-controlled clinical trials . Euro Surveill. 2013;18(37):pii=20585. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20585

Article submitted on 09 April 2013 / published on 12 September 2013

The test-negative design (TND) is an efficient form of case–control study commonly applied to influenza vaccine effectiveness (VE) estimation. TND validity is predicated on the core assumption that the intervention (vaccine) has no effect on other non-targeted aetiologies resulting in similar illness/disease. Here we verify this core assumption and compare efficacy estimates derived by the TND versus classical per-protocol analysis of four datasets obtained from randomised placebo-controlled clinical trials (RCT) of the live attenuated influenza vaccine (LAIV) in children ≤7 years-old and the elderly ≥60 years-old. We further assess generalisability of the TND approach in two other RCT datasets to evaluate monoclonal antibody in the prevention of respiratory syncytial virus (RSV) hospitalisation. Efficacy estimates and their confidence intervals were virtually identical for per-protocol RCT versus TND analyses of LAIV and also for RSV monoclonal antibody. Neither LAIV nor monoclonal antibodies affected the risk of disease aetiologies that were not specifically targeted by the respective interventions (e.g. other respiratory viruses). This study validates the core assumption of the TND approach for influenza vaccine efficacy estimation and confirms the accuracy and precision of its estimates compared to the gold standard of classic per-protocol RCT analysis of the same data sets. The TND approach is generalisable for other conditions such as RSV for which the core assumption is also met. However, when used in observational studies, the TND, like all designs, still requires assessment for bias and confounding that may exist in the absence of randomised participation and blinded follow-up.

Introduction

The test-negative design (TND) was developed as an efficient approach to assess influenza vaccine effectiveness (VE) using available sentinel surveillance structures. First publications based on the TND for influenza VE estimation came from Canada in 2005 (for the pilot 2004/05 season) [1] and 2007 (for the subsequent 2005/06 season) [2]. In Canada this approach has been used within existing surveillance structures annually since [3-8]. Following a publication on the methodological validity of the TND [9], other investigators in Europe [10-13], the United States [14-16] and Australia [17,18] also began to publish VE findings based on the TND from 2009 onward.

The TND is a type of case–control design whereby vaccine status is compared between influenza test-positive cases versus test-negative controls who present to a clinician, generally with some standardised definition of influenza-like illness (ILI). While classical case–control studies require intense efforts to recruit non-diseased controls, the TND draws controls from the same source population as the cases, namely ill patients who are tested to identify a specific aetiology of interest for their illness or disease. It is therefore a convenient and relatively low-cost design that also has recently demonstrated its usefulness in rapidly gauging vaccine protection early in the influenza season [8,13,16].

The simplicity of the TND approach, however, has understandably raised concerns about its validity, including the misclassification of cases as controls due to imperfect test sensitivity. Theoretical work has previously shown that in fact test specificity rather than sensitivity is the most critical factor influencing VE estimation based on the TND [10]. Given high test specificity and prevalence of the targeted aetiology (i.e. influenza) equal to or lower than that of other aetiologies (i.e. other respiratory viruses) with similar clinical presentation (i.e. acute respiratory illness (ARI) or ILI), the TND performs comparably to the classical case–control

or cohort design, even with suboptimal test sensitivity [9]. Most published studies applying this method to influenza VE estimation have used highly specific diagnostic methods such as polymerase chain reaction (PCR) confirmation [1-8,10-18]. Detection of influenza by culture is nearly 100% specific [19]. As culture has historically been considered the gold standard, its sensitivity is very high especially in young children (<5 years-old) although PCR detects 2% to 13% more cases [20]. In elderly patients (>65 years-old), viral culture has a sensitivity between 21% and 51% compared to PCR [21].

The efficacy of a preventive intervention (e.g. vaccine) reflects proportionate reduction in the frequency of the targeted disease in those receiving the intervention (vaccine) compared to individuals who did not receive the intervention and is ideally assessed in the optimal conditions of suitably powered randomised placebo-controlled clinical trials (RCT). For vaccines, efficacy is calculated by comparing attack rates (ARs) in the vaccinated and unvaccinated through the relative risk (RR) and according to the following equation [22]:

$$\text{Vaccine efficacy} = \frac{\text{AR unvaccinated} - \text{AR vaccinated}}{\text{AR unvaccinated}} \times 100$$

$$= 1 - \text{RR vaccinated / unvaccinated} \times 100$$

Like efficacy, effectiveness also compares proportionate reduction of risk but this is estimated in field conditions through observational studies of the intervention, without randomisation to address other possible influences. Efficacy and effectiveness are calculated the same way. In RCT and in cohort studies where a census of the source population is available, ARs are calculated by dividing the number of vaccinated and unvaccinated cases of the disease in question by the total number of individuals belonging to their respective categories. In case–control studies where there is no census of the source population, effectiveness can be validly estimated by the odds ratio (OR) assuming that controls are a representative sample of that population and the exposure distribution (e.g. vaccine coverage) is the same as in the source population [23]. For this condition to be met and a control series to be valid, the sampling fraction (θ) must be the same in vaccinated and unvaccinated non-diseased controls. The θs then cancel out when calculating the OR which approximates the RR when the disease is rare (Table 1). For the TND approach, the condition of representative exposure distribution (e.g. vaccine coverage) in controls emerging from the same source population will apply if the intervention has no effect on other aetiologies manifesting similar clinical presentation as the target pathogen. For the TND as applied to influenza vaccine efficacy/effectiveness estimation, internal validity is therefore predicated on the core assumption that influenza vaccine has no effect on non-influenza causes (e.g. other respiratory pathogens such as parainfluenza, respiratory syncytial viruses (RSV)) of ARI or ILI. These non-influenza episodes would then be expected to occur at the same mean frequency (f) per vaccinated or unvaccinated individual (i.e. $f_{vaccinated} = f_{unvaccinated}$). Consequently, among individuals affected by these other aetiologies, the proportion who are vaccinated should be similar to the vaccine coverage in the source population (Table 1).

Large double-blind RCTs optimise the comparability of vaccinated and unvaccinated individuals with respect to eligibility criteria, follow-up, and disease ascertainment thereby minimising the influence of bias and confounding. As such, the RCT represents the ideal context to assess the validity of the TND core assumption and to verify the accuracy and precision of efficacy estimates derived in that way. Using four datasets from large double-blind RCTs of live attenuated influenza vaccine (LAIV) among children and the elderly we have therefore directly compared original RCT per-protocol efficacy estimates against those instead derived by TND analysis. To test the core assumption hypothesis that influenza vaccine has no effect on other ARI/ILI aetiologies we have also derived efficacy against non-influenza causes of illness. Finally, to illustrate the generalisability of the TND we applied it to two RCT datasets collected for the evaluation of humanised monoclonal antibody (palivizumab) in preventing RSV hospitalisation.

Methods

Four datasets from published, double-blind RCTs of LAIV (Flumist, MedImmune) among children and the elderly were used [24-27]. In these studies, after being administered vaccine or placebo, participants were actively followed throughout the winter season by phone calls or home visits and nasal/throat swabs were collected for each episode of ARI (Table 2). Respiratory specimens were tested by viral culture and the primary outcome was culture-confirmed influenza (test-positive) due to any strain regardless of antigenic similarity. Specimens with influenza-negative culture were not further tested to identify other specific causative pathogens. For the RSV studies, we analysed datasets from two published double-blind RCTs of palivizumab administered every 30 days during the RSV season to premature infants (≤35 weeks gestation) or infants with bronchopulmonary dysplasia [28] and to children with haemodynamically significant congenital heart disease [29]. The primary outcome was RSV-associated hospitalisation where diagnosis of RSV was confirmed by rapid antigen detection test (sensitivity: 82%, specificity: 95% [30]). No further testing was done to identify the aetiology in patients with RSV negative tests.

Per-protocol randomised cohort estimates of efficacy were calculated using ARs according to the above equation. Three approaches to TND analysis of LAIV protection were conducted. In participant-based analysis without censoring for influenza, controls included participants with any negative swabs without excluding

Table 1

Comparison of the randomised placebo-controlled clinical trial (RCT)/cohort, classical case–control and test-negative design (TND) case–control

	Influenza vaccine	No influenza vaccine	Relative risk (RR) or odds ratio (OR)
RCT or cohort design			
Influenza-confirmed cases	A	B	$\frac{A/(A+C)}{B/(B+D)} = RR$
All others in cohort	C	D	
Total	A+C	B+D	
Classical case–control design			
Influenza-confirmed cases	A	B	$\frac{A/\theta C}{B/\theta D} = \frac{A/C}{B/D} = OR \approx RR$
Number corresponding to fraction of all others in cohort	θC	θD	
TND case–control			
Influenza-confirmed cases	A	B	–
All others in cohort	C	D	–
			TND participant-based analysis without censoring for influenza
Participants with an episode of non-influenza illness, no censoring for influenza[a]	p(A+C)	p(B+D)	$\frac{A/p(A+C)}{B/p(B+D)} = \frac{A/(A+C)}{B/(B+D)} = RR$
			TND participant-based analysis with censoring for influenza
Participants with an episode of non-influenza illness, with censoring for influenza[b]	p(C)	p(D)	$\frac{A/pC}{B/pD} = \frac{A/C}{B/D} = OR \approx RR$
			TND specimen-based analysis
Non-influenza illness episodes[c]	f(A+C)	f(B+D)	$\frac{A/f(A+C)}{B/f(B+C)} = \frac{A/(A+C)}{B/(B+C)} = RR$

f: average number of non-influenza illness episodes during the follow-up period; p: probability of having an episode of non-influenza illness during the follow-up period (episodes past the first do not separately contribute); θ: sampling fraction of controls.

[a] Includes as controls participants with any negative swabs (i.e. without excluding those who tested positive for influenza at any other time during the study period).

[b] Includes as controls only participants with negative swabs who furthermore never had a test that was positive for influenza at any other time during the study period (i.e. excludes influenza positive participants).

[c] All non-influenza illness episodes count (without excluding those in participants who tested positive for influenza in another illness episode).

those who may have tested positive for influenza at another time within the study period (Table 1). In participant-based analysis with censoring for influenza, controls included only participants with negative swabs who furthermore never had a positive test for influenza at any other time during the study period (i.e. excludes those test-positive for influenza). In specimen-based analysis, cases were influenza-positive specimens and controls were influenza-negative specimens rather than individuals: this approach accounts for the multiple episodes of respiratory infections not attributable to influenza that an individual can sustain during the study period.

To estimate vaccine effects on other non-influenza ARI aetiologies, the AR of these infections was calculated by dividing the number of participants/specimens testing negative for influenza by the total enrolment in their respective categories of exposure (vaccine or placebo). Per above, this was conducted as participant-based (with/without censoring) and specimen-based analysis.

Each of the above was repeated for the RSV studies, modified for the intervention (palivizumab) and outcome of interest (laboratory-confirmed RSV hospitalisation).

Consistent with the expectation of large, randomised placebo-controlled participation in these RCT data sets, we assumed that risk factors for influenza were similarly distributed in both groups before vaccination and that blinding ensured comparable follow-up and case ascertainment, minimising bias and confounding. Consequently, only crude results are presented without further statistical adjustment. Point estimates of efficacy and their 95% confidence intervals (CI) for TND analyses were computed using Mantel–Haenszel method as (1-(OR or RR) X 100).

Table 2

Characteristics of participants in the live attenuated influenza vaccine (LAIV) and palivizumab randomised placebo-controlled clinical trials (RCT)

Author or study, year	Number of subjects	Intervention exposure (ratio)	Age mean	Health status	Type and frequency of follow-up	Specimen, diagnostic assay and clinical indication
Belshe, 1998 [24]	1,602	LAIV (2:1)	42 months	Healthy	Active weekly calls	Nasal swab, viral culture in case of ARI
Belshe, 2000 [25]	1,358	LAIV revaccination (2:1)	54 months	Healthy	Active weekly calls	Nasal swab, viral culture in case of ARI
Lum, 2010 [26]	1,150	LAIV co-administered with MMR (2:1)	14 months	Healthy	Active calls 2X/week	Nasal swab, viral culture in case of ARI
De Villiers, 2009 [27]	3,242	LAIV (1:1)	69 years	Elderly (≥60 years-old) with or without underlying medical conditions	Active weekly call or visit	Throat and nasal swab, viral culture in case of ARI
Impact study, 1998 [28]	1,502	Palivizumab (1:1)	6 months	Premature (≤35 weeks of gestation) or bronchopulmonary dysplasia	Monthly visit[a]	Nasal swab or wash, antigen test at ARI hospitalisation
Feltes, 2003 [29]	1,287	Palivizumab (1:1)	6 months	Congenital heart disease	Monthly visit[a]	Nasal swab or wash, antigen test at ARI hospitalisation

ARI: acute respiratory illness; MMR: measles, mumps, rubella vaccine.

[a] Monthly visits were to administer monoclonal antibodies (palivizumab) or placebo at which time information about any hospitalisation since the last visit was collected.

Each of these RCTs had received prior Institutional Review Board/Ethics Committee approval. The current analysis involved only additional statistical analyses of de-identified data and thus no additional approvals were required.

Results

Live attenuated influenza vaccine (LAIV) studies

Three RCT datasets among children ≤7 years of age [24-26] and one among adults ≥60 years of age [27] were analysed. Together these trials included 6,077 participants each actively monitored for outcomes of interest accrued over the course of a single season. The first paediatric study included children who were vaccinated with LAIV and followed for one season. The second study recruited the same paediatric participants whose parents gave consent for revaccination and follow-up for a second season. For all LAIV trials, groups were shown in publication to be balanced with respect to baseline characteristics so that further adjustment for residual confounding was not required.

For all LAIV studies, the point estimates and surrounding 95% CIs for efficacy against ARI due to influenza were virtually identical in the classical per-protocol RCT and TND analyses with little variation using these datasets whether TND analysis was participant-based (with/without censoring) or specimen-based (Table 3). Good concordance between TND and RCT analysis approaches was observed both in paediatric studies with high efficacy and in the elderly study where protection was lower. LAIV had negligible effect on non-influenza aetiologies of ARI as shown by the zero or near-zero efficacy associated with test-negativity (Table 3).

To more closely represent surveillance-based TND approaches as extensively published [1-8,10-18], we also assessed the same parameters for medically-attended ARI. Again the point estimates and surrounding 95% CIs for efficacy were virtually identical in the classical per-protocol RCT and TND analyses (Figure).

Respiratory syncytial virus studies

For the two RSV trials, groups were shown in the original publications to be balanced on baseline characteristics except household smoking in one trial [28]; adjusted analysis did not influence efficacy estimates and we did not pursue adjustment here. In these studies, repeated hospitalisation during the study period was rare. Therefore all three TND analysis approaches gave similar results and we present only the participant-based analysis with censoring (Table 4 and Figure). Point estimates and 95% CI for palivizumab protection against RSV hospitalisation were virtually identical by the per-protocol RCT and the TND analysis. Palivizumab provided no protection against non-RSV causes of acute respiratory hospitalisation (RSV test-negative) as again shown by the null efficacy.

Table 3

Influenza vaccine efficacy against influenza and non-influenza illnesses estimated by per-protocol and various test-negative design (TND) analysis approaches in four randomised placebo-controlled clinical trials (RCT) of live attenuated influenza vaccine (LAIV)

Author / population	Participant-based analysis					Specimen-based analysis	
	Total	Test-positive[a] for influenza	Test-negative no censoring[b]	Test-negative with censoring[c]	No swab	Test-positive specimens	Test-negative specimens
Belshe Year 1 /children [24]							
LAIV (Number)	1,070	14	851	841	215	14	1,959
Placebo (Number)	532	94	424	345	93	98	939
		Classical RCT/cohort	**TND no censoring[b]**	**TND with censoring[c]**			**TND specimen-based**
Efficacy against influenza (95% CI)	NA	92.6 (86.9 to 96.1)	92.6 (86.8 to 95.8)	93.9 (89.1 to 96.6)	NA	NA	93.2 (87.9 to 96.1)
Efficacy against non-influenza (95% CI)	NA	NA	-14 (-21 to -7)	-21 (-30 to -13)	NA	NA	-8 (-11 to -5)
Belshe Year 2/children [25]							
LAIV (Number)	917	15	596	586	316	15	1,181
Placebo (Number)	441	56	298	266	119	56	575
		Classical RCT/cohort	**TND no censoring[b]**	**TND with censoring[c]**			**TND specimen-based**
Efficacy against influenza (95% CI)	NA	87.1 (76.9 to 93.2)	86.6 (75.9 to 92.6)	87.8 (78.1 to 93.2)	NA	NA	87.0 (76.7 to 92.7)
Efficacy against non-influenza (95% CI)	NA	NA	-2 (-11 to 6)	-6 (-16 to 3)	NA	NA	-2 (-7 to 3)
Lum (any strain)/children [26]							
LAIV (Number)	819	28	708	684	107	28	2,899
Placebo (Number)	413	39	372	333	41	39	1,566
		Classical RCT/cohort	**TND no censoring[b]**	**TND with censoring[c]**			**TND specimen-based**
Efficacy against influenza (95% CI)	NA	63.8 (39.6 to 78.5)	62.3 (37.7 to 77.2)	65.0 (42.2 to 78.9)	NA	NA	61.2 (36.7 to 76.2)
Efficacy against non-influenza (95% CI)	NA	NA	-2 (-7 to 3)	-4 (-10 to 2)	NA	NA	0 (-2 to 1)
De Villiers (any strain)/elderly [27]							
LAIV (Number)	1,620	71	944	894	655	73	1,847
Placebo (Number)	1,622	125	981	892	605	130	1,880
		Classical RCT/cohort	**TND no censoring[b]**	**TND with censoring[c]**			**TND specimen-based**
Efficacy against influenza (95% CI)	NA	43.1 (23.3 to 58.1)	41.0 (20.0 to 56.5)	43.3 (23.1 to 58.3)	NA	NA	42.8 (23.3 to 57.4)
Efficacy against non-influenza (95% CI)	NA	NA	1 (-5 to 7)	0 (-7 to 6)	NA	NA	0 (-3 to 4)

CI: confidence interval; NA: not applicable.

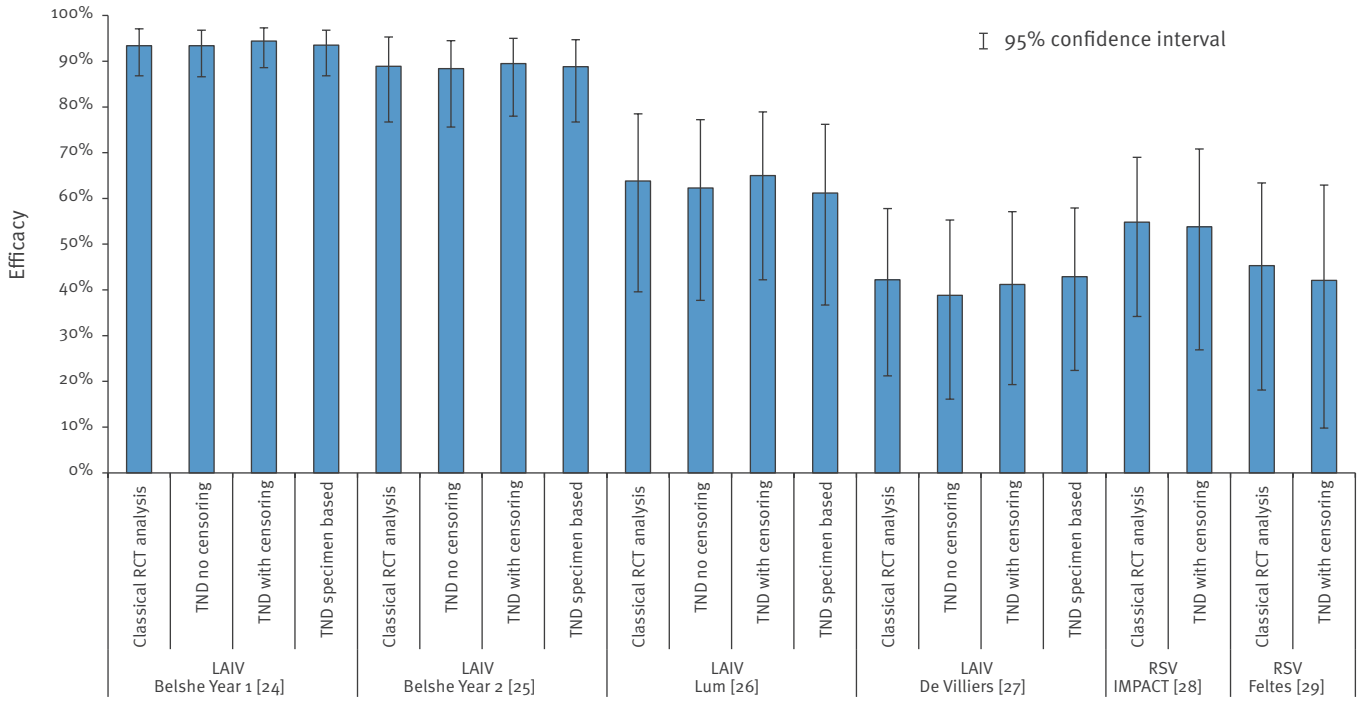
[a] Positivity is defined by the detection of the target virus (influenza) by culture thus labelled as test-positive.

[b] Includes as controls, participants with any negative swabs (i.e. without excluding those who also tested positive for influenza at any other time during the study period).

[c] Includes as controls, only participants with negative swabs who furthermore never had a test that was positive for influenza at any other time during the study period (i.e. excludes influenza positive participants).

Figure

Efficacy (% of disease prevented) of live attenuated influenza vaccine (LAIV) against medically-attended influenza ARI estimated by per-protocol and various test-negative design (TND) analysis approaches in four randomised placebo-controlled clinical trials (RCT) and efficacy (% of RSV hospitalisation prevented) by palivizumab in two RCTs



ARI: Acute respiratory illness RSV: respiratory syncytial virus.

As information on medical consultation was not collected in Lum's study [26], results presented are those from Table 3.

Discussion

In this analysis, we confirm that estimates of efficacy and their 95% CIs were similar when derived according to the classical per-protocol RCT analysis or various TND approaches and this was observed in children and the elderly at respectively high or reduced efficacy values. When applied to data for an unrelated passive immunising agent targeting RSV, the TND gave similar results, thereby demonstrating its generalisability beyond influenza and to endpoints representing more severe disease outcomes such as hospitalisation.

To obtain valid case–control study results, controls must be representative of the source population [23]. As such, they should belong to the same source population from which cases were identified and should be individuals who theoretically would have been identified as cases had they acquired the targeted aetiology of interest. The actual source population from which clinic or hospital cases emerge is often undefined. In classic case–control studies, controls are usually recruited among patients consulting or admitted to the same facility as cases but the disease for which they consult may be distinct from that which the targeted aetiology typically manifests. In contrast, test-negative controls derived from among patients presenting with similar clinical illness (e.g. ARI or ILI) and tested for diagnosis provide some inherent reassurance that they emerge from the same source population as cases, would have consulted and would have been considered cases had their aetiology been the targeted pathogen rather than otherwise. More than thirty years ago, Broome et al. applied this sort of approach in using patients infected by non-vaccine type invasive pneumococcal infection to serve as controls in their analysis of pneumococcal VE [31]. The TND as now applied to influenza VE is an extension of the same logic. The main advantage of the TND is its ease of access to a series of controls representative of the source population. Here we have shown that this simplicity does not necessarily come at the cost of validity.

We explicitly presented three TND analysis approaches. While in this paper all three approaches performed similarly this may not always be true. Censored/uncensored participant-based and specimen-based approaches are not intrinsically the same and the choice of one approach over another must be consciously expressed. As displayed in Table 1, the uncensored and the specimen-based analyses directly derive the RR of influenza in vaccinated versus unvaccinated individuals. Conversely the analysis censoring for influenza positive participants shifts the effect measure to an OR that is necessarily sensitive to the rare disease

Table 4

Efficacy of palivizumab to prevent RSV and non-RSV hospitalisation by per-protocol and test-negative design (TND) participant-based analysis with censoring in two randomised placebo-controlled clinical trials (RCT) conducted in children.

Author or study name	Total	RSV-positive[a] hospitalisation	RSV-negative hospitalisation with censoring[b]	No swab
IMPACT [28]				
Palivizumab (Number)	1,002	48	196	758
Placebo (Number)	500	53	100	347
		Classical RCT/cohort	TND with censoring[b]	
Efficacy against RSV hospitalisation (95% CI)	NA	54.8 (34.2 to 69.0)	53.8 (26.9 to 70.8)	NA
Efficacy against non-RSV hospitalisation (95% CI)	NA	NA	2 (-21 to 21)	NA
Feltes [29]				
Palivizumab (Number)	639	34	318	287
Placebo (Number)	648	63	341	244
		Classical RCT/cohort	TND with censoring[b]	
Efficacy against RSV hospitalisation (95% CI)	NA	45.3 (18.1 to 63.4)	42.1 (9.8 to 62.9)	NA
Efficacy against non-RSV hospitalisation (95% CI)	NA	NA	5 (-5 to 15)	NA

CI: confidence interval; RSV: respiratory syncytial virus; NA: not applicable.

[a] Positivity is defined by the detection of the target virus (RSV) by antigen detection. This is therefore referred as RSV-positive.

[b] Includes participants with negative swabs who furthermore never had a test that was positive for RSV at any other time during the study period (i.e. excludes RSV positive participants).

assumption. Even if the vaccine does not actually influence the risk of non-influenza aetiologies, unvaccinated individuals should more often be infected by influenza during the study period assuming that vaccine is protective against influenza. As such, censoring participants based on influenza positivity eliminates more participants with non-influenza aetiologies in the unvaccinated than the vaccinated group thereby introducing a bias that skews findings toward suggesting the vaccinated are at increased risk of these other aetiologies. This bias will increase with greater prevalence of influenza compared to other respiratory infections in the study population. These concerns related to censoring will apply not only to TND, but also to other fixed cohort follow-up or case–control analyses, requiring authors to be explicit in the approach taken when interpreting their results.

In our analysis, there was a statistically significant but slight increased risk of non-influenza respiratory episodes during the first year of the Belshe study but this was not observed in the three other datasets for which efficacy against other respiratory viruses even more closely approximated the null. It may be argued that the same may not extend to inactivated or other influenza vaccine formulations. While another small trial from Cowling et al. involving 115 participants followed during one season reported an increased risk of non-influenza viruses among recipients of inactivated influenza vaccine [32] a much larger study covering six seasons and including more than 3,000 patients found no such association [33]. It could be argued that our findings of null vaccine effects against other respiratory viruses are explained by the fact that live virus vaccine is itself predicated on replicating virus and may thereby also directly contribute to broadly cross-protective innate immune mechanisms that are precluded by effective inactivated vaccine (a theory proposed to explain Cowling's findings [32]). Although active follow-up of thousands of participants included in our own datasets resulted in one to 3.5 specimens on average per participant (≤10% positive for influenza), the specific non-influenza cause of ARI was not sought in any of the RCTs we used so that we cannot directly address possible vaccine effects on individual pathogens. However, temporary innate immunity is short-lasting, in the range of several weeks and in each of these trials LAIV was administered well before the winter period. LAIV-induced innate immunity is thus unlikely to have substantially altered the overall risk of other respiratory viruses through the full follow-up period. On that basis we believe that our findings supporting the core assumption of the TND for LAIV can also be extended to inactivated formulations although we encourage direct assessment of that through other similarly available RCT data sets. If vaccine nevertheless truly does increase the likelihood of other non-influenza infections by whatever mechanism, this would generally tend to over-estimate efficacy/effectiveness against influenza suggesting TND findings are optimistic representations of vaccine performance.

There are other issues and limitations worth considering in our analysis. RCT estimates and cohort studies provide absolute measures (attack rates) whereas under the usual conditions of TND application there is

no census of the source population and analysis can only provide relative measures. We have validated the TND approach for influenza and RSV but this does not imply universal validity in the evaluation of all infectious diseases or their interventions. Before extending the TND to other vaccines or interventions, it is necessary to confirm earlier specified pre-conditions related to test characteristics and the mix of target/non-target disease aetiologies [9] as well as the core assumption of no effect on non-targeted aetiologies of diseases with similar symptomatology. In the influenza vaccine studies we used, influenza was the targeted aetiology of ARI and positive viral culture for influenza was the main outcome. The sensitivity of viral culture to detect influenza in specimens collected early after the onset of disease is expected to be high in young children but lower in the elderly [11]. Despite that, the TND performed comparably well to De Villiers' RCT analysis in the elderly. The current analysis took advantage of existing large clinical trial databases where participants had been randomised and followed in blinded fashion to identify the outcome with stringent approaches applied equally to immunised and unimmunised participants. This most likely minimised differences between the two groups, eliminated confounding and provided efficacy estimates. Effectiveness derived from observational studies, in contrast, is susceptible to these additional methodological concerns. As such, the TND approach is valid but cannot compensate for other methodological flaws. Indiscriminate use of the TND in observational studies can lead to errors of interpretation, particularly if testing was applied differentially and varied with the likelihood of immunisation, exposure and/or test-positivity. TND should be considered as a variation on the case–control design and as for all observational designs, one needs to begin from the premise that bias and confounding may be operating. Intense scrutiny of the methods and data set for signals of bias (e.g. selection, information, confounding bias) remains a requirement.

In conclusion, the TND approach appears valid not only for influenza vaccine efficacy and effectiveness assessment but also for other diseases and interventions provided that the core assumption requiring no effect of the preventive intervention on non-targeted aetiologies is fulfilled and that bias as a potential concern for all observational designs is adequately addressed.

Acknowledgements

Funding: Costs related to professional time were covered by the institutions/organisations of the individual investigators. No additional financial support for this analysis was provided.

Conflict of interest

GDS received research grants from GSK and Sanofi Pasteur and participated to an adhoc GSK advisory board meeting for which travel expenses were reimbursed. CSA and XWW are MedImmune employees.

Authors' contributions

The study was conceived and led by Gaston De Serres and Danuta M Skowronski. Christopher Ambrose identified and provided access to the appropriate clinical trial data, and Xionghua Wilson Wu conducted the statistical analysis. All authors contributed to the analysis of the data, the writing of the manuscript and its intellectual content.

References

1. Skowronski DM, Gilbert M, Tweed SA, Petric M, Li Y, Mak A, et al. Effectiveness of vaccine against medical consultation due to laboratory-confirmed influenza: results from a sentinel physician pilot project in British Columbia, 2004-2005. Can Commun Dis Rep. 2005;31(18):181-91.
2. Skowronski DM, Masaro C, Kwindt TL, Mak A, Petric M, Li Y, et al. Estimating vaccine effectiveness against laboratory-confirmed influenza using a sentinel physician network: results from the 2005-2006 season of dual A and B vaccine mismatch in Canada. Vaccine. 2007;25(15):2842-51. http://dx.doi.org/10.1016/j.vaccine.2006.10.002 PMid:17081662
3. Skowronski DM, De Serres G, Dickinson J, Petric M, Mak A, Fonseca K et al. Component-specific effectiveness of trivalent influenza vaccine as monitored through a sentinel surveillance network in Canada, 2006-2007. J Infect Dis. 2009;199(2):168-79. http://dx.doi.org/10.1086/595862 PMid:19086914
4. Janjua NZ, Skowronski DM, De Serres G, Dickinson J, Crowcroft NS, Taylor M, et al. Estimates of influenza vaccine effectiveness for 2007-2008 from Canada's sentinel surveillance system: Cross-protection against major and minor variants. J Infect Dis. 2012;205(12):1858-68. http://dx.doi.org/10.1093/infdis/jis283 PMid:22492921
5. Skowronski DM, De Serres G, Crowcroft NS, Janjua NZ, Boulianne N, Hottes TS, et al. Association between the 2008-09 seasonal influenza vaccine and pandemic H1N1 illness during Spring-Summer 2009: four observational studies from Canada. PLoS Med. 2010;7(4):e1000258. http://dx.doi.org/10.1371/journal.pmed.1000258 PMid:20386731 PMCid:PMC2850386
6. Skowronski DM, Janjua NZ, De Serres G, Hottes TS, Dickinson JA, Crowcroft N, et al. Effectiveness of AS03-adjuvanted pandemic H1N1 vaccine: case-control evaluation based on the sentinel surveillance system in Canada, autumn 2009. BMJ. 2011;342: c7297. http://dx.doi.org/10.1136/bmj.c7297 PMid:21292718 PMCid:PMC3033439
7. Skowronski DM, Janjua NZ, De Serres G, Winter A-L, Dickinson JA, Gardy JL, et al. A Sentinel Platform to Evaluate Influenza Vaccine Effectiveness and New Variant Circulation, Canada 2010-2011 Season. Clin Infect Dis. 2012;55(3):332-42. http://dx.doi.org/10.1093/cid/cis431 PMid:22539661
8. Skowronski DM, Janjua NZ, De Serres G, Dickinson JA, Winter AL, Mahmud SM, et al. Interim estimates of influenza vaccine effectiveness in 2012/13 from Canada's sentinel surveillance network, January 2013 Euro Surveill. 2013;18(5):pii=20394. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20394
9. Orenstein EW, De Serres G, Haber MJ, Shay D K, Bridges CB, Gargiullo P et al. Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. Int J Epidemiol. 2007;36(3): 623-31. http://dx.doi.org/10.1093/ije/dym021 PMid:17403908
10. Kissling E, Valenciano M, Falcao J, Larrauri A, Widgren K, Pitigoi D, et al. I-Move towards monitoring seasonal and pandemic influenza vaccine effectiveness: lessons learnt from a pilot multi-centric case-control study in Europe 2008-9. Euro Surveill. 2009;14(44):pii=19388. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19388
11. Kissling E, Valenciano M, Larrauri A, Oroszi B, Cohen JM, Nunes B et al. Low and decreasing vaccine effectiveness against influenza A(H3) in 2011/12 among vaccination target groups in Europe: results from the I-MOVE multicentre case–control study. Euro Surveill. 2013;18(5):pii=20390. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20390
12. Pebody RG, Andrews N, McMenamin J, Durnall H, Ellis J, Thompson CI et al. Vaccine effectiveness of 2011/12 trivalent seasonal influenza vaccine in preventing

laboratory-confirmed influenza in primary care in the United Kingdom: evidence of waning intra-seasonal protection. Euro Surveill. 2013;18(5):pii=20389. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20389

13. McMenamin J, Andrews N, Robertson C, Fleming D, Durnall H, von Wissmann B et al. Effectiveness of seasonal 2012/13 vaccine in preventing laboratory-confirmed influenza infection in primary care in the United Kingdom: mid-season analysis 2012/13. Euro Surveill. 2013;18(5):pii: 20393. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20393
14. Belongia EA, Kieke BA, Donahue JG, Greenlee RT, Balish A, Foust A et al. Effectiveness of inactivated influenza vaccines varied substantially with antigenic match from 2004-2005 to the 2006-2007 season. J Infect Dis. 2009;199(2):159-67. http://dx.doi.org/10.1086/595861 PMid:19086915
15. Treanor JJ, Talbot HK, Ohmit SE, Coleman LA, Thompson MG, Cheng PY et al. Effectiveness of seasonal influenza vaccines in the United States during a season with circulation of all three vaccine strains. Clin Infect Dis. 2012;55(7):951-9. http://dx.doi.org/10.1093/cid/cis574 PMid:22843783
16. Centers for Disease Control and Prevention (CDC). Early estimates of seasonal influenza vaccine effectiveness-United States, January 2013. MMWR Morb Mortal Wkly Rep. 2013;62(2):32-5. PMid:23325354
17. Kelly H, Carville K, Grant K, Jacoby P, Tran T, Barr I. Estimation of the influenza vaccine effectiveness from routine surveillance data. PLoS One. 2009;4(3):e5079. http://dx.doi.org/10.1371/journal.pone.0005079 PMid:19333374 PMCid:PMC2658741
18. Fielding JE, Grant KA, Tran T, Kelly HA. Moderate influenza vaccine effectiveness in Victoria, Australia, 2011. Euro Surveill. 2012;17(11): pii=20115. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20115 PMid:22449867
19. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. J Infect Dis. 2006;194 Suppl 2:S98-110. http://dx.doi.org/10.1086/507554 PMid:17163396
20. World Health Organization (WHO).WHO recommendations on the use of rapid testing for influenza diagnosis. Geneva: WHO. [Accessed 16 Aug 2013]. Available from http://www.who.int/influenza/resources/documents/RapidTestInfluenza_WebVersion.pdf
21. Talbot HK, Falsey AR. The diagnosis of viral respiratory disease in older adults. Clin Infect Dis. 2010;50(5):747-51. PMid:20121411 PMCid:PMC2826599
22. Orenstein WA, Bernier RH, Hinman AR. Assessing vaccine efficacy in the field. Further observations. Epidemiol Rev. 1988;10:212-41. PMid:3066628
23. Rothman KJ, Greenland S, Lash TL, editors. Case-control studies. Modern Epidemiology 3rd Edition. Philadelphia: Lippincott Williams & Wilkins; 2008, p111-128. PMid:17999090
24. Belshe RB, Mendelman PM, Treanor J, King J, Gruber WC, Piedra P et al. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. N Engl J Med. 1998;338(20):1405-12. http://dx.doi.org/10.1056/NEJM199805143382002 PMid:9580647
25. Belshe RB, Gruber WC, Mendelman PM, Cho I, Reisinger K, Block SL et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. J Pediatr. 2000;136(2):168-75. http://dx.doi.org/10.1016/S0022-3476(00)70097-7
26. Lum LC, Borja-Tabora CF, Breiman RF, Vesikari T, Sablan BP, Chay OM et al Influenza vaccine concurrently administered with a combination measles, mumps, and rubella vaccine to young children. Vaccine. 2010;28(6):1566-74. http://dx.doi.org/10.1016/j.vaccine.2009.11.054 PMid:20003918
27. De Villiers PJ, Steele AD, Hiemstra LA, Rappaport R, Dunning AJ, Gruber WC et al. Efficacy and safety of a live attenuated influenza vaccine in adults 60 years of age and older Vaccine. 2009;28(1):228-34. http://dx.doi.org/10.1016/j.vaccine.2009.09.092 PMid:19796721
28. Impact-RSV study group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics. 1998;102(3 Pt 1):531-7.
29. Feltes TF, Cabalka AK, Meissner HC, Piazza FM, Carlin DA, Top FH Jr et al Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. J Pediatr 2003;143(4):532-40. http://dx.doi.org/10.1067/S0022-3476(03)00454-2
30. Boivin G, Côté S, Déry P, De Serres G, Bergeron MG. Multiplex real-time PCR assay for detection of influenza and human respiratory syncytial viruses. J Clin Microbiol. 2004;42(1):45-51. http://dx.doi.org/10.1128/JCM.42.1.45-51.2004 PMid:14715730 PMCid:PMC321675
31. Broome CV, Facklam RR, Fraser DW. Pneumococcal disease after pneumococcal vaccination: an alternative method to estimate the efficacy of pneumococcal vaccine. N Engl J Med. 1980;303(10):549-52. http://dx.doi.org/10.1056/NEJM198009043031003 PMid:6995835
32. Cowling BJ, Fang VJ, Nishiura H, Chan KH, Ng S, Chiu SS et al. Increased Risk of Noninfluenza Respiratory Virus Infections Associated With Receipt of Inactivated Influenza Vaccine. Clin Infect Dis. 2012;54(12):1778-82. http://dx.doi.org/10.1093/cid/cis307 PMid:22423139 PMCid:PMC3404712
33. Sundaram ME, McClure DL, VanWormer JJ, Friedrich TC, Meece JK, Belongia EA. Influenza vaccination is not associated with detection of non-influenza respiratory viruses in seasonal studies of influenza vaccine effectiveness. Clin Infect Dis. 2013 Epub June 6 2013.

Laboratory-confirmed invasive meningococcal disease: effect of the Hajj vaccination policy, Saudi Arabia, 1995 to 2011

Z Memish (zmemish@yahoo.com)[1,2], R Al Hakeem[1], O Al Neel[1], K Danis[3,4], A Jasir[5], D Eibach[5,6]

1. Public Health Directorate, WHO Collaborating Center for Mass Gathering Medicine, Ministry of Health, Riyadh, Kingdom of Saudi Arabia
2. College of Medicine, Alfaisal University, Riyadh, Kingdom of Saudi Arabia
3. European Programme Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
4. Institut de Veille Sanitaire, Paris, France
5. European Public Health Microbiology Training Programme (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
6. Centre de Biologie et Pathologie Est, Hospices Civils de Lyon, University of Lyon, Lyon, France

Citation style for this article:
Memish Z, Al Hakeem R, Al Neel O, Danis K, Jasir A, Eibach D. Laboratory-confirmed invasive meningococcal disease: effect of the Hajj vaccination policy, Saudi Arabia, 1995 to 2011. Euro Surveill. 2013;18(37):pii=20581. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20581

Article submitted on 15 May 2013 / published on 12 September 2013

Saudi Arabia (SA) experienced two large invasive meningococcal disease (IMD) outbreaks during the 2000 and 2001 Hajj pilgrimages. In 2002, polysaccharide quadrivalent ACWY vaccines became mandatory for Mecca and Medina pilgrims/residents older than two years. This study aimed to analyse IMD surveillance data among citizens, residents and pilgrims in SA from 1995 to 2011, focusing on changes before and after the new vaccination policy. For all laboratory-confirmed IMD cases in the national surveillance database from 1995 to 2011, serogroup and age were retrieved. The cases' seasonal distribution as well as the case fatality ratios (CFR) were obtained. For Saudi citizens/residents and Hajj pilgrims, annual rates were calculated using mid-year population estimates. The Student's t-test was used to compare means between the pre-epidemic (1995–1999) and post-epidemic (2002–2011) periods, excluding outbreak years. From 1995 to 2011, laboratories notified 1,103 cases. Between the pre- and post-epidemic periods, mean annual IMD rates decreased from 0.20 (standard deviation (SD): 0.1) to 0.06 cases/100,000 (SD: 0.06; p=0.02), mean numbers of Hajj-related cases from 13 (SD: 9.3) to 2 cases/year (SD: 2.3; p=0.02) and the mean age from 31 (SD: 1.3) to 18 years (SD: 1.4; p<0.01). The CFR in Saudi citizens (10.4) was lower than among foreign pilgrims (28.9) and decreased from 19.3% (SD: 1.8) in the pre-epidemic to 11.4% (SD: 7.0; p=0.04) in the post-epidemic phase. The decrease of annual IMD rates, CFR and Hajj-related cases between the pre- and post- vaccine era suggests a possible positive effect of the mandatory ACWY vaccination for pilgrims/residents in Mecca and Medina. Regular surveillance with an annual data analysis is necessary to monitor trends and circulating serotypes and to implement appropriate public health measures to avoid new IMD epidemics during upcoming Hajj seasons.

Introduction

Invasive meningococcal disease (IMD) is caused by the gram-negative coccoid bacterium *Neisseria meningitidis* [1,2]. Transmission via respiratory droplets can lead to bacterial meningitis and septicaemia in the persons who get infected. Six serogroups (A, B, C, W135, X and Y) differentiated by their polysaccharide capsule, account for the majority of IMD cases [3]. IMD primarily affects children below five years of age, causing an estimated 500,000 cases and 50,000 deaths annually worldwide [4]. Polysaccharide and conjugated vaccines are available for serogroups A, C, W135 and Y [5]. One vaccine for serogroup B has been licensed in Europe in January 2013 and other serogroup B vaccines have been used in New Zealand, Cuba and France to control epidemics [6].

In Saudi Arabia (SA), the annual Hajj and the year-round Umrah pilgrimage lead to a particular high risk of outbreaks with invasive *N. meningitidis*. The Hajj pilgrimage is held on every 12th month of the Islamic calendar (2011 attendance: 2.9 million pilgrims, including 1.8 million from >140 foreign countries), making it one of the largest mass gathering events worldwide [7-9]. About 45,000 pilgrims arrive from the European Union each year [7]. Compared to the Hajj, the Umrah pilgrimage includes slightly different rituals and can be undertaken at any time of the year. Foreign pilgrims generally arrive by air in the city of Jeddah and continue to the pilgrimage sites in Mecca, Mina, Mount Arafat and Muzdalifah. Many end the journey with a visit to the holy sites in Medina [7]. Extreme congestion with populations from diverse geographical areas appears to promote a high prevalence of asymptomatic *N. meningitidis* carriage, with up to 80% reported for Mecca pilgrims [10,11]. In comparison carriage prevalence between 3% and 30% has been shown for the

African meningitis belt and in a Norwegian randomised study, 9.6% of Norwegian volunteers harboured *N. meningitidis* [12,13]. The United States (US) Centers for Disease Control reported five to 10% of adults as asymptomatic nasopharyngeal carriers [14].

Following an outbreak of serogroup A IMD among pilgrims in 1987, Saudi Arabian health authorities implemented three interventions: (i) the compulsory vaccination before entering SA with bivalent AC vaccine for all Hajj pilgrims, (ii) annual vaccination campaigns for all residents in the proximity of pilgrimage sites and (iii) compulsory oral ciprofloxacin upon entering SA to pilgrims from sub-Saharan Africa to eradicate nasal carriage [11,15,16].

In response to two large IMD outbreaks caused by *N. meningitidis* serogroup W135 in the 2000 and 2001 Hajj seasons [15,17], the SA Ministry of Health (MoH) adjusted their vaccination policy. In 2002, they required a polysaccharide quadrivalent non-conjugated ACWY meningococcal vaccine for (i) children and adults aged above two years living in Mecca and Medina, (ii) Hajj pilgrims aged above two years from within and outside of SA, (iii) healthcare workers in SA and (iv) government personnel serving the pilgrims [18]. Since 2010, a conjugated polysaccharide quadrivalent ACWY meningococcal vaccine is given to the same target groups aged from above two to 55 years. The vaccines are administered during annual vaccination campaigns in Mecca and Medina in a single dose with boosters every three years. As of 2013 no meningococcal vaccines are included in the SA national childhood immunisation schedule (NCIS). This study aims to describe the epidemiology of IMD in Saudi Arabia for the years 1995 to 2011, with a focus on changes in incidence and case fatality ratio (CFR) after the introduction of the polysaccharide quadrivalent ACWY vaccine in 2002, in order to evaluate the effect of this Hajj vaccination policy change.

Methods

A confirmed IMD case was defined as either isolation of *N. meningitidis* from cerebrospinal fluid (CSF) or blood or detection of capsular antigen in CSF by latex agglutination assay [19]. An IMD surveillance system was started in 1994 in SA based on recommendations of the World Health Organization (WHO) [20]. Since then, the Preventive Medicine Directorate at the MoH requires laboratories from all 20 health regions in SA to anonymously report confirmed IMD cases. The case-based reporting form collects information on age, sex, nationality, SA residency status, vaccination status, date of onset of symptoms (by Gregorian and Islamic calendar), clinical status and place of laboratory confirmation (health region). Information on the capsular groups, determined by latex agglutination, is also collected on the reporting form.

For the purposes of this study, all IMD cases in the surveillance database from 1 January 1995 to 31 December 2011 were extracted. This included cases among Saudi citizens and residents, foreign pilgrims and illegal immigrants. A citizen was considered a person in possession of the Saudi Arabian nationality, and a resident a person originating from outside SA but residing and working in SA. A foreign pilgrim was defined as a person holding a special visa for the Hajj or Umrah pilgrimage, whereas an illegal immigrant was an unregistered person devoid of any valid entry permit for SA. Hajj-related cases were specified as IMD cases with dates of disease onset during the Hajj season in the cities of Mecca or Medina.

Statistical analysis

Age group-, sex- and region-specific annual and cumulative disease incidences were calculated for IMD cases among Saudi citizens or residents. Age group- (0–4; 5–14; 15–64; >65 years of age) and year-specific population denominators were obtained from the United Nations Development Programme (UNDP) website [21]. To calculate the cumulative incidence over several years, mid-period population estimates were used as a denominator. The Ministry of Hajj, Saudi Arabia [22] provided the numbers of foreign and domestic Hajj pilgrims. For foreign Hajj pilgrims, no age or sex specific incidences have been calculated, as no age or sex specific Hajj pilgrim numbers were available. In the absence of a population register to calculate rates, illegal immigrants were excluded from any incidence calculations.

From all cases, regardless of citizenship and nationality, numbers of Hajj-related IMD cases as well as year, age and region specific CFRs were calculated. Relative risks for death from IMD were identified by calculating ratios of case-fatalities and their 95% confidence intervals (95% CIs) for the residence status, seasons, age groups and sex. The Student's t-test was used to compare means (age, CFRs and number of cases) between the pre-epidemic (1995–1999) and post-epidemic (2002–2011) periods, excluding the outbreak years (years 2000, 2001), and linear regression models were fitted to describe trends. A p-value <0.05 was considered statistically significant. Surveillance data were computerised using Excel programme (Microsoft, USA) and statistical analysis was performed with Stata 12 (Statacorp, Texas, USA) software.

Results

Study population

During the study period, the population of SA rose from 18,491,845 in 1995 to 27,448,000 persons in 2011, the latter of which comprises 68% SA citizens and 32% residents of foreign origin [21]. In 2011, children less than five years-old and adults above 65 years of age accounted for 10%, and 3% of the population, respectively. The largest population increase has been recorded for the 15 to 64 years age group, which comprised 66.7% of the population in 2011 (compared to 56.1 in 1995). Forty-five percent of the population were

Table 1

Confirmed invasive meningococcal disease cases by residency status in Saudi Arabia, 1995–2011 (n=1,103)

Confirmed IMD cases[a]	Residency status					
	Citizen	Resident	Visa-holding Hajj pilgrim	Visa-holding Umrah pilgrim	Illegal	Unknown
Male/female	227/149	181/88	136/163	54/19	78/4	3/1
Mean age in years (SD)	9.6 (0.8)	14.4 (1.0)	48.9 (0.9)	51.4 (2.1)	29.5 (1.2)	35 (2.9)
Number of deaths	39	28	111	14	6	0
Time period						
1995–1999	78	61	60	46	18	2
2000–2001	193	179	223	26	31	2
2002–2011	105	29	16	1	33	0
Serogroup						
Serogroup A	36	45	63	30	18	1
Serogroup B	33	7	22	2	2	0
Serogroup C	5	2	0	2	1	0
Serogroup W135	153	99	110	4	9	0
Other serogroups	11	1	0	0	0	0
Unknown serogroups	138	115	104	35	52	3
Total confirmed IMD cases	**376**	**269**	**299**	**73**	**82**	**4**
Mid-period population estimates (1995–2011)[b]	22,652,297		1,452,978	Unknown	Unknown	Unknown

IMD: invasive meningococcal disease; SD: standard deviation.

[a] Unless otherwise specified.

[b] For the period considered, only the number of citizens and residents combined are available. No reliable numbers for Umrah pilgrims and persons with illegal residency status are available.

female. There were 1,936,124 and 1,858,490 persons registered in the health districts of Mecca and Medina in 2011 respectively, representing 14% of the population in SA. During the study period the number of Hajj pilgrims increased by 57% from 1,865,234 (1,080,465 from outside SA) in 1995 to 2,927,717 (1,828,195 from outside SA) in 2011 [22].

Annual and cumulative invasive meningococcal disease incidences

Between 1995 and 2011, 1,103 cases of IMD were reported to the MoH in SA (Table 1). Of those, 645 cases were Saudi citizens/residents and 299 were foreign Hajj pilgrims. Of the remaining cases, 82 were illegal immigrants (not included in incidence calculations), 73 were foreign Umrah pilgrims and four had unknown identity (combined cumulative incidence of IMD among citizens, residents and foreign Hajj pilgrims: 3.92/100,000 population). Between 1995 and 1999, the mean annual incidence was 0.20/100,000, ranging from 0.25/100,000 in 1995 to 0.06/100,000 in 1999 (Figure 1A). In the two outbreak years of 2000 and 2001, the annual incidence increased to 1.42 and 1.32/100,000, respectively. In the post-epidemic period, the mean annual incidence did not exceed 0.06/100,000, ranging from 0.21/100,000 in 2002 to 0.01/100,000 in 2010, a significant decrease compared to the pre-epidemic period (p=0.02) (Figure 1A).

In an analysis restricted to SA citizens and residents, the cumulative incidence between 1995 and 2011 (2.85 cases/100,000) was lower compared to rates including Hajj pilgrims. Outside the outbreak periods the annual incidence followed the same trend described above (Figure 1A).

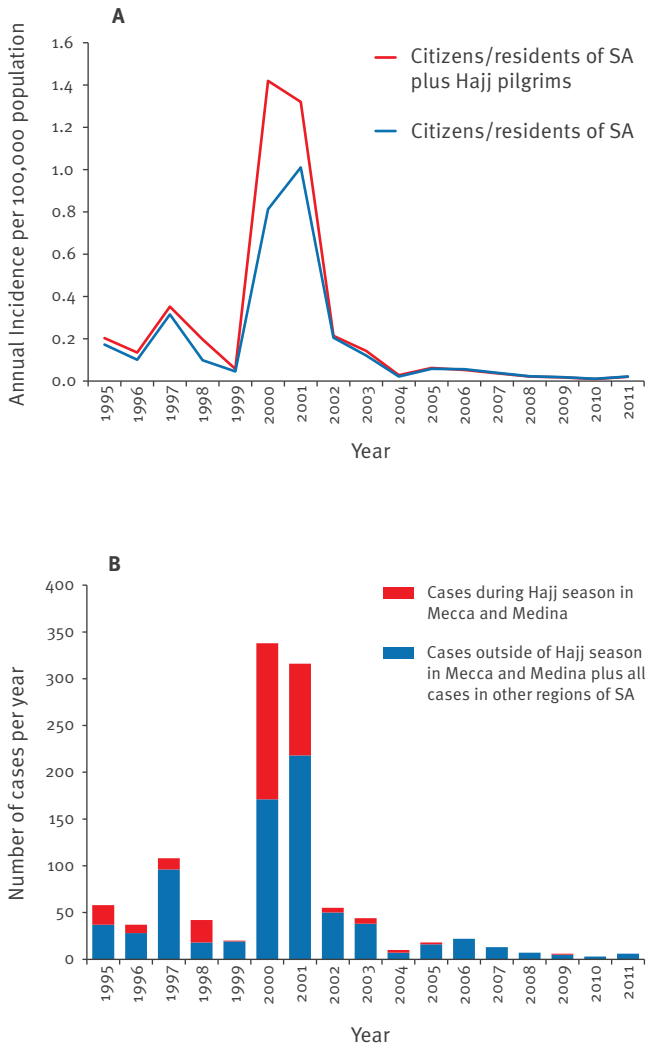
Age, sex and region specific incidence for Saudi Arabia citizens and residents

For SA citizens and residents, in the study period, the age group including less than four year-olds had the highest cumulative incidence, with both sexes equally affected (12.3 cases/100,000; Figure 2). Above four years of age, males had a higher cumulative incidence. Among those between 15 and 64 years of age, cumulative incidence among males (1.8 cases/100,000) was three times higher than among females (0.6 cases/100,000).

In the period between 1995 and 2011, citizens and residents in the main Hajj pilgrimage destinations had a high cumulative incidence (Mecca: 9.04 cases/100,000 n=175; Medina: 4.52 cases/100,000 n=84 and Jeddah: 2.28 cases/100,000 n=88), whereas urban regions, not visited by the Hajj pilgrims, such as the capital city Riyadh (1.85 cases/100,000 n=131) had lower cumulative incidences.

Figure 1

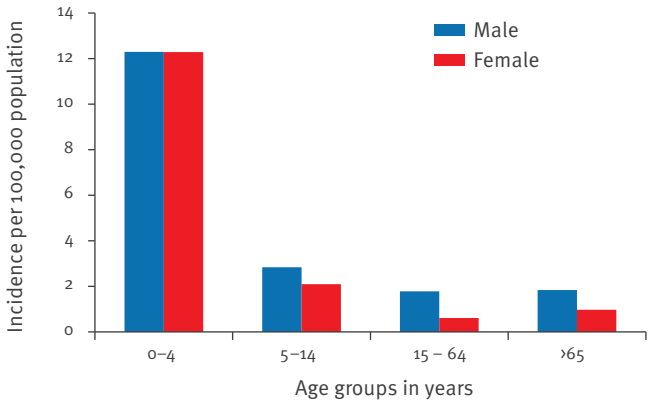
Annual incidence of invasive meningococcal disease (panel A) and distribution of invasive meningococcal disease cases linked to the Hajj (panel B), Saudi Arabia, 1995–2011



SA: Saudi Arabia.

Figure 2

Cumulative incidence of invasive meningococcal disease for citizens and residents of Saudi Arabia by age group and sex, 1995–2011 (n=645)



Hajj-related cases

In the 2000 and 2001 outbreak years, IMD cases from Mecca and Medina during the Hajj accounted for 49% and 31% of all notified annual IMD cases, respectively (Figure 1B). In contrast, between 2002 and 2011, only a mean annual 8.1% (standard deviation (SD): 10.1) of all IMD cases were reported from Mecca or Medina during the Hajj season. Since 2006, during Hajj seasons, Medina reported only one case of IMD. The mean numbers of Hajj-related cases was higher (13.4 cases/year; SD: 9.3) during the pre-epidemic than during the post-epidemic years (1.7 cases/year; SD: 2.3; p=0.02).

Distribution of mean age

In the period between 1995 and 2011, among SA citizens and residents, the age group comprising those younger than four years had the highest disease incidence. However, the mean age of all IMD cases, including pilgrims, between 1995 and 2011 was 25.8 (SD: 0.7) years. The mean age decreased from 31 years (SD: 1.3) in the pre-epidemic period to 18 years (SD: 1.4) in the post-epidemic period ($p<0.01$; b coefficient -1.27, Figure 3).

The mean age of IMD cases during the Hajj season (37.0 years; SD: 1.0) was higher ($p<0.01$) than among cases outside this season (17.4 years; SD: 0.8). Similarly, the mean age of IMD cases among foreign Hajj and Umrah pilgrims (48.9 (SD: 0.9); 51.4 (SD: 2.1), respectively) was higher ($p<0.01$) than among SA residents cases (14.4 years; SD: 1.0) or citizens (9.6 years; SD: 0.8). Finally, cases in Mecca (33.9 years; SD: 1.2) and Medina (31.4 years; SD: 1.8) had a higher mean age than in other health regions (16.7 years; SD: 0.8) ($p<0.01$).

Serogroup distribution

Serogroup results were available for 59% (656/1,103) of all cases reported between 1995 and 2011. In 33% (369/1,103) the serogroup could not be determined and in 7% (78/1,103) no information was submitted (Table 2). In one isolate, serogroups A and C and in nine isolates serogroups A, C, W135 and Y were not further subtyped. Of all serogrouped isolates, 89% (587/656) belonged to the vaccine preventable serogroups A, C, W135 and Y. From 1995 to 1999, the predominant serogroup was A, accounting for 49% (77/158) of all typed isolates, followed by B with 26% (41/158) and serogroup W135 with 20% (31/158) of isolates (Table 2). During the 2000 and 2001 outbreak years, the emerging serogroup W135 predominated, accounting for 78% (298/383) of typed isolates, while during the post-epidemic period between 2002 and 2011, serogroups A and W135 were almost equally distributed (36% (41/115) and 40% (46/115), respectively), while serogroup B accounted for 17% (19/115) of typed isolates.

The age group below one year is dominated by isolates of serogroup B (19% (14/74)) and serogroup W135 (70% (52/74)). In those aged one through four years, serogroup W135 is by far the most common serogroup (78%

Table 2

Distribution of serogroups for all typed isolates (n=656) among invasive meningococcal disease cases (n=1,103) and stratification by time periods, age groups, and outcome, Saudi Arabia

Confirmed IMD cases	Serogroups							
	A	B	C	W135	X	Y	Z	Total
Time period								
1995–1999[a] n(%)	77 (49)	41 (26)	6 (4)	31 (20)	0 (0)	3 (2)	0 (0)	158
2000–2001[b] n(%)	75 (20)	6 (2)	4 (1)	298 (78)	0 (0)	0 (0)	0 (0)	383
2002–2012[c] n%)	41 (36)	19 (17)	0 (0)	46 (40)	2 (2)	6 (5)	1 (1)	115
Age groups								
<1 year n(%)	4 (5)	14 (19)	1 (1)	52 (70)	1 (1)	2 (3)	0 (0)	74
1–4 years n(%)	15 (10)	12 (8)	3 (2)	112 (78)	0 (0)	1 (1)	0 (0)	143
5–14 years n(%)	25 (29)	4 (5)	1 (1)	51 (59)	0 (0)	4 (5)	1 (1)	86
15–45 years n(%)	82 (43)	21 (11)	2 (1)	81 (43)	1 (0)	2 (1)	0 (0)	189
>45 years n(%)	67 (41)	15 (9)	3 (2)	79 (48)	0 (0)	0 (0)	0 (0)	164
Deaths	42	14	2	74	0	3	0	135
Total number of confirmed IMD cases	**193**	**66**	**10**	**375**	**2**	**9**	**1**	**656**
CFR[d] (%)	21.8	21.2	20	19.7	0.0	33.3	0.0	20.6

CFR: case fatality ratio; IMD: invasive meningococcal disease.

Of 1,103 isolates from cases of invasive meningococcal disease 369 isolates were not typable and for 78 isolates serogroups were not reported. The CFR for cases with untypable isolates was 9.8% (36 deaths).

[a] Pre-epidemic period.

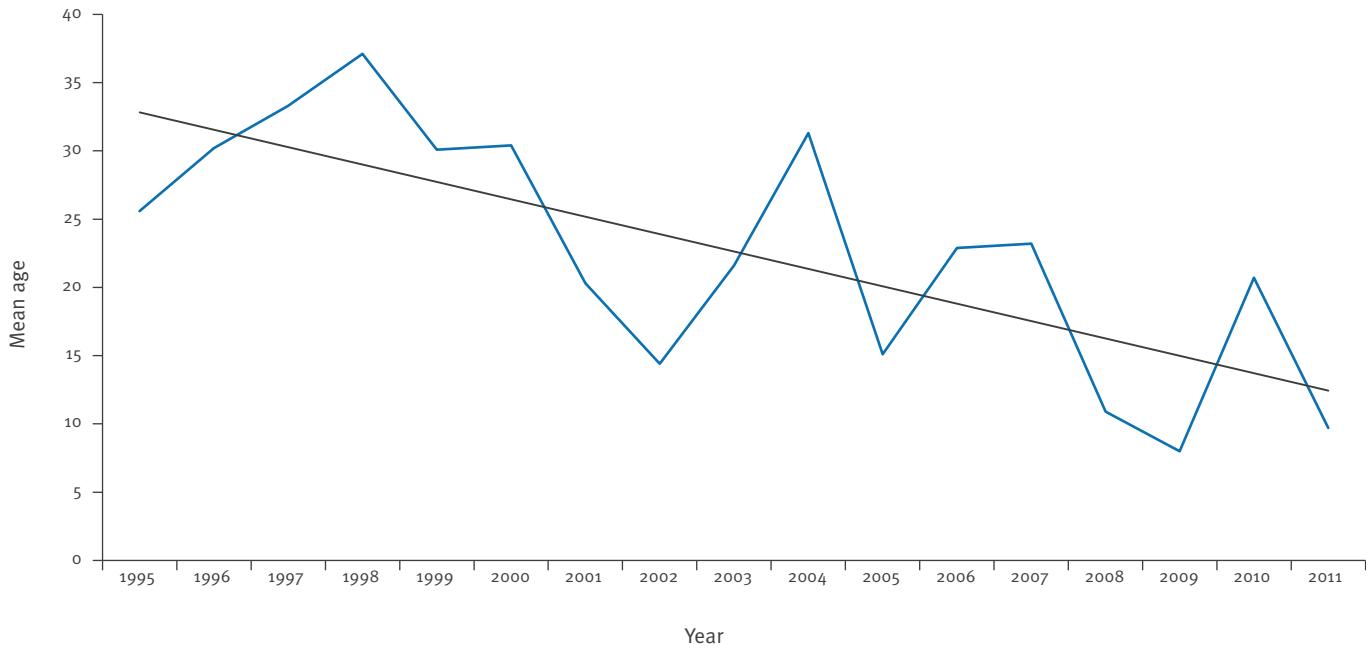
[b] Epidemic period.

[c] Post-epidemic period.

[d] CFR=Deaths/total number of confirmed IMD cases.

Figure 3

Mean age of invasive meningococcal disease cases, Saudi Arabia, 1995–2011 (n=1,103)



The black line can be expressed by the equation y =34.108-1.2747x ; (R^2 = 0.54).

The mean age of invasive meningococcal disease cases was 31 years (standard deviation (SD): 1.3) in the pre-epidemic period (1995–1999) and 18 years (SD: 1.4) in the post-epidemic period (2002–2011).

Table 3

Mortality, case fatality ratio and relative risk of dying from invasive meningococcal disease by residency status, Hajj season, age group and sex, Saudi Arabia, 1995–2011 (n=1,103)

Characteristics	Number of deaths	CFR (%)[a]	Relative Risk[b]	95% CI	p-value
Saudi citizen/resident					
Yes	67	10.39	1.00 (reference)	NA	NA
No	131	28.85	2.78	2.12–3.63	‹0.01
Hajj season					
Yes	135	28.60	2.86	2.18–3.77	‹0.01
No	63	9.98	1.00 (reference)	NA	NA
Age group					
‹1 year	8	6.84	1.00 (reference)	NA	NA
1–4 years	20	9.35	1.37	0.62–3.01	0.43
5–14 years	13	9.29	1.36	0.58–3.16	0.48
15–45 years	72	19.41	2.84	1.41–5.72	0.01
›45 years	85	32.57	4.76	2.39–9.51	‹0.01
Sex					
Male	117	17.23	0.90	0.70–1.17	0.43
Female	81	19.10	1.00 (reference)	NA	NA

CFR: case fatality ratio; CI: confidence interval; NA: not applicable.

[a] CFR=(Number of deaths /Number of confirmed IMD cases) in each category.

[b] The relative risk of dying from invasive meningococcal disease is calculated by dividing the CFR of the relevant row by the CFR of the reference row.

(112/143)) among typed isolates. Above four years of age, serogroups A and W135 comprise 87% (385/439) of all typed isolates in those age groups. Serogroups other than A, B and W135 contribute only marginally to the IMD burden in SA (Table 2).

Case fatality

Between 1995 and 2011, the overall reported CFR was 18.0% (198/1,103), with a decrease from an annual mean of 19.3% (SD: 1.8) in the pre-epidemic years to 11.4% (SD: 7.0) in the post-epidemic years (p=0.04). The CFR increased with age, from 6.8% in the ‹1 year-olds to 32.6% in the ›45 years age group (Table 3). When stratified by age groups, no significant changes in the CFR for the ‹5 years group between pre-epidemic (13.6%; 95% CI: 0.0–39.6), epidemic (6.3%; 95% CI: 0.0–40.6) and post-epidemic years (12.4%; 95% CI: 3.2–21.6) were observed (Table 4). For cases ›5 years of age, the CFR decreased significantly between the pre-epidemic (20.72%; 95% CI: 18.2–23.2) and post-epidemic period (8.72%; 95% CI: 2.6–14.8). During the epidemic the CFR for cases above five years-old was significantly elevated (25.6; 95% CI: 24.9–26.2). The CFR was similar for the most common serogroups (A: 21.8%; B: 21.2%; C: 20.0%; W135: 19.7%). Among Hajj or Umrah pilgrims from foreign countries the CFR was 2.8 times higher than Saudi citizens/residents (Table 3). In addition, the CFR during the Hajj season (28.6%) was 2.9 times higher than outside the season (10.0%).

Discussion

According to the present study and confirming previous reports, the Hajj season constitutes a special opportunity that is very favourable to IMD epidemics [7,9,10]. The high morbidity and fatality for pilgrims during the Hajj season might be largely explained by epidemiologically unfavourable conditions (e.g. crowding, delayed clinical and laboratory diagnosis). Our study indicates that the incidence of IMD decreased in SA following the introduction of the ACWY vaccine in 2002. More specifically, Hajj-related IMD cases declined after the introduction of the vaccine compared to pre-epidemic years. The results suggest that the compulsory use of the ACWY vaccine for pilgrims and residents of Mecca and Medina may have played a role in reducing not only Hajj-related IMD cases, but also the overall disease incidence in SA.

Children under five, predominantly affected by serogroup B and W135 infections, suffered the highest age-specific incidence of IMD throughout the study period. This is consistent with experience from the United Kingdom (UK), the US and Germany, where B is the most prevalent serogroup in children below five years [23-25]. The mean age of cases decreased during the study period. Two factors may explain this evolution. First, there could have been a decrease in IMD in adolescent or adult Hajj pilgrims since 2002 because of the vaccine introduction for all Hajj pilgrims. Second,

Table 4

Annual mean case fatality ratio (CFR) by age groups, Saudi Arabia, 1995–2011

Age group	Mean of the annual CFR[a] (95% CI)		
	1995–1999	2000–2001	2002–2011
<5 years-old	13.64 (0.00–39.56)	6.30 (0.00–40.61)	12.4 (3.21–21.59)
≥5 years-old	20.72 (18.24–23.20)	25.55 (24.91–26.19)	8.72 (2.62–14.82)

95% CI: 95% confidence interval.

[a] CFR = (Number of deaths /Number of confirmed IMD cases) in each category.

no meningococcal vaccines are yet given to children below three years-old according to the Hajj vaccination policy from 2002.

The CFR of IMD was highest among older age groups, as reported elsewhere [23,26]. Our data indicate that one third of deaths occurred among persons >45 years of age. Following introduction of ACWY meningococcal vaccine, both the IMD morbidity and the CFR for above four year-olds decreased among the SA population including Hajj pilgrims. A number of factors could be responsible for this finding. First, the reduced number of cases among pilgrims during the Hajj season in recent years contribute to a lower CFR, as clinical and laboratory diagnosis might be delayed during the Hajj and pilgrims seek medical care too late while being on the pilgrimage. Second, the reduction in the mean age of cases might contribute to the decreased CFR and third, there might have been increased awareness after the two outbreak years 2000 and 2001 leading to a more rapid diagnosis and therefore improved healthcare measures. The reported CFR outside of the Hajj season (10%, and 11% for the whole post-vaccination period) were comparable to reports from non-endemic European countries. Austria, France, Germany, UK reported CFRs ranging from 8.2 to 12.5% [24,25,27-29].

The reported mean annual incidence of IMD for citizen, residents and Hajj pilgrims in SA during the post-epidemic period was low. The US reported 0.35 IMD cases/100,000 in 2007, New Zealand 2.6 cases/100,000 in 2007 and Taiwan 0.2 cases/100,000 in 2001 [10]. The European Invasive Bacterial Infection Surveillance (EU-IBIS) project reported 1.01 cases/100,000 in 2006 from 27 European countries [10]. However, those incidence rates cannot be compared to those in SA, as the serogroup distribution in those countries differs, which changes the disease impact completely. Differences in case definitions, which include different laboratory methods as well as clinical and epidemiological criteria, impede international comparisons, as examples from European and Australian guidelines illustrate [30,31]. In the future, introduction of new sensitive polymerase chain reaction (PCR) methods for the detection of *N. meningitidis* in SA, could enable laboratories to confirm a higher number of suspected IMD cases [32,33].

In the current IMD surveillance database, it proved difficult to distinguish between Hajj related cases and non-Hajj cases. We used the time of disease onset and the region to infer a potential connection to the pilgrimage. As per our criteria, cases outside of Mecca and Medina with contact to Hajj pilgrims, or Hajj pilgrims moving outside both cities, would not be identified as Hajj-related. As this information could be used to implement public health control measures, reporting health personnel should verify the Hajj-related status of every case.

The majority of outbreak-related cases were caused by *N. meningitidis* serogroup W135. The responsible clone was later found to belong to the ST-11/ET-37 complex [34]. Genetic analysis of the clone suggested that a capsular switch from serogroup C to serogroup W135 may have occurred years before the onset of the outbreak [34]. The same hypervirulent clone caused serogroup C disease in Belgium, Iceland, Ireland, the Netherlands, Portugal and the UK in the late 1990s and early 2000s [10]. Concerns had been raised that mass immunisation with vaccines that do not protect against all serogroups could lead to an increase in incidence of meningococcal disease due to strains not included in the vaccine [35,36]. Following the introduction of ACWY vaccines, this phenomenon of serotype replacement, or a major emergence of non-vaccine serotypes, has not been reported in Mecca and Medina, or elsewhere.

Vaccine coverage data are needed to determine whether the vaccine contributed to reduced morbidity. While no coverage data are available for foreign Hajj pilgrims, the entry requirement that is enforced through border checks suggest that coverage should be high. In comparison, compliance among Saudi pilgrims may be poorer, since Saudi citizens do not have to provide proof of vaccination at border controls or check points. In 2006, a study of 134 British and 109 Saudi Hajj pilgrims reported that vaccine coverage was lower among local residents (64%) than among British pilgrims (100%) [37]. According to this study, 50% of local pilgrims residing in Mecca and Jeddah had been

vaccinated, compared with 71% from the rest of the country. Overall, a larger vaccine coverage study would obviously help interpret results of IMD surveillance data.

Conclusion

Saudi-Arabian IMD surveillance data highlight the shift from Hajj-related cases towards non-Hajj related ones, following the introduction of the ACYW vaccine in 2002. The number of cases and the CFR also declined, suggesting a potential positive effect of the current Hajj vaccination policy, among other factors. On the basis of our investigations, we can formulate a number of recommendations. First, regular monitoring of vaccination coverage would help interpret trends of IMD. Second, the surveillance system could be improved through notification of clinically suspected and epidemiologically-linked cases as well as including more sensitive molecular biology based diagnostic methods. Third, inclusion of information on possible links to the Hajj could be considered for the surveillance form. Continued surveillance with annual data analysis remains necessary to drive adapted public health measures and avoid future IMD epidemics during the Hajj seasons of the coming years.

Acknowledgements

We wish to acknowledge Yvan Hutin for his contribution to critical review of the manuscript.
Novartis vaccine and diagnostic provided financial support (including travel, transportation and accommodation, meals expenses) for the mission of EUPHEM fellow Daniel Eibach to the Kingdom of Saudi Arabia. The salary for the EUPHEM fellow is supported by a grant from ECDC and he did not receive any salary or honorariums from other sources. Novartis has not been involved in the study design, analysis, or writing of the paper nor has it influenced the analyses or results of the study.

Conflict of interest

None declared.

Authors' contribution

All authors contributed to the drafting, writing, and reviewing the manuscript.

References

1. Visintin C, Mugglestone MA, Fields EJ, Jacklin P, Murphy MS, Pollard AJ et al. Management of bacterial meningitis and meningococcal septicaemia in children and young people: summary of NICE guidance. BMJ. 2010;340:c3209. http://dx.doi.org/10.1136/bmj.c3209. PMid:20584794.
2. Pollard AJ, Nadel S, Ninis N, Faust SN, Levin M. Emergency management of meningococcal disease: eight years on. Arch Dis Child. 2007;92(4):283-6. http://dx.doi.org/10.1136/adc.2006.102384. PMid:17376933. PMCid:PMC2083684.
3. Chang Q, Tzeng YL, Stephens DS. Meningococcal disease: changes in epidemiology and prevention. Clin Epidemiol. 2012;4:237-45. PMid:23071402. PMCid:PMC3470458.
4. Wilder-Smith A. Meningococcal vaccine in travelers. Curr Opin Infect Dis. 2007;20(5):454-60. http://dx.doi.org/10.1097/QCO.0b013e3282a64700. PMid:17762777.
5. Vipond C, Care R, Feavers IM. History of meningococcal vaccines and their serological correlates of protection. Vaccine. 2012;30 Suppl 2:B10-7. http://dx.doi.org/10.1016/j.vaccine.2011.12.060. PMid:22607894.
6. World Health Organization. Global Advisory Committee on Vaccine Safety, 12-13 December 2007. Wkly Epidemiol. Rec. 2008;83(4):37–44. PMid:18219807.
7. Ahmed QA, Arabi YM, Memish ZA. Health risks at the Hajj. Lancet. 2006;367(9515):1008-15. http://dx.doi.org/10.1016/S0140-6736(06)68429-8
8. Aguilera JF, Perrocheau A, Meffre C, Hahné S; W135 Working Group. Outbreak of serogroup W135 meningococcal disease after the Hajj pilgrimage, Europe, 2000. Emerg Infect Dis. 2002;8(8):761-7. http://dx.doi.org/10.3201/eid0808.010422. PMCid:PMC2732506.
9. Memish ZA. Meningococcal disease and travel. Clin Infect Dis. 2002;34(1): 84-90. http://dx.doi.org/10.1086/323403. PMid:11731951.
10. Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. Vaccine. 2009; 27 Suppl 2:B51-63. http://dx.doi.org/10.1016/j.vaccine.2009.04.063. PMid:19477562.
11. al-Gahtani YM, el Bushra HE, al-Qarawi SM, al-Zubaidi AA, Fontaine RE. Epidemiological investigation of an outbreak of meningococcal meningitis in Makkah (Mecca), Saudi Arabia, 1992. Epidemiol Infect. 1995;115(3):399-409. http://dx.doi.org/10.1017/S0950268800058556. PMid:8557071. PMCid:PMC2271597.
12. Trotter CL, Greenwood BM. Meningococcal carriage in the African meningitis belt. Lancet Infect Dis. 2007;7(12):797-803. http://dx.doi.org/10.1016/S1473-3099(07)70288-8
13. Caugant DA, Høiby EA, Magnus P, Scheel O, Hoel T, Bjune G, et al. Asymptomatic carriage of Neisseria meningitidis in a randomly sampled population. J Clin Microbiol. 1994;32(2):323-30. PMid:8150942. PMCid:PMC263032.
14. Centers for Disease Control and Prevention (CDC). VPD Surveillance Manual, 5th Edition, Atlanta; CDC; 2011. Chapter 8, Meningococcal Disease. [Accessed 09 Aug 2013]. Available from: http://www.cdc.gov/vaccines/pubs/surv-manual/chpto8-mening.pdf
15. Memish ZA, Venkatesh S, Ahmed QA. Travel epidemiology: the Saudi perspective. Int J Antimicrob Agents. 2003;21(2): 96-101. http://dx.doi.org/10.1016/S0924-8579(02)00364-3
16. Wilder-Smith A, Memish Z. Meningococcal disease and travel. Int J Antimicrob Agents. 2003;21(2):102-6. http://dx.doi.org/10.1016/S0924-8579(02)00284-4
17. Al-Mazrou YY, Al-Jeffri MH, Abdalla MN, Elgizouli SA, Mishskas AA. Changes in epidemiological pattern of Meningococcal disease in Saudi Arabia. Does it constitute a new challenge for prevention and control? Saudi Med J. 2004;25(10):1410-3. PMid:15494812.
18. World Health Organization. Health conditions for travellers to Saudi Arabia pilgrimage to Mecca (Hajj). Wkly Epidemiol Rec. 2005;80(49-50):431-2. PMid:16372666.
19. Olcén P, Fredlund H. Isolation and characterization of Neisseria meningitidis in the vaccine era. Who needs what and when? Scand J Infect Dis. 2010;42(1):4-11. http://dx.doi.org/10.3109/00365540903311177. PMid:19883161.
20. World Health Organization (WHO). Control of epidemic meningococcal disease. WHO practical guidelines. 2nd edition. Geneva: WHO; 1998.
21. United Nations Development Programme (UNDP). International Human Development Indicators. New York: UNPD. [Accessed 29 Jan 2013]. Available from: http://hdr.undp.org/en/statistics/
22. Ministry of Hajj, Kingdom of Saudi Arabia. [Accessed 10 Sep 2013]. Available from: http://www.hajinformation.com

23. Harrison LH, Pass MA, Mendelsohn AB, Egri M, Rosenstein NE, Bustamante A, et al. Invasive meningococcal disease in adolescents and young adults. JAMA. 2001;286(6):694-9. http://dx.doi.org/10.1001/jama.286.6.694. PMid:11495619.

24. Shigematsu M, Davison KL, Charlett A, Crowcroft NS. National enhanced surveillance of meningococcal disease in England, Wales and Northern Ireland, January 1999-June 2001. Epidemiol Infect. 2002;129(3):459-70. http://dx.doi.org/10.1017/S0950268802007549. PMid:12558328. PMCid:PMC2869907.

25. Robert Koch Institut (RKI). Invasive Meningokokken-Erkrankungen im Jahr 2007. Epidemiologisches Bulletin. 2008;32:265-72. German.

26. Ladhani SN, Flood JS, Ramsay ME, Campbell H, Gray SJ, Kaczmarski EB, et al. Invasive meningococcal disease in England and Wales: implications for the introduction of new vaccines. Vaccine. 2012;30(24):3710-6. http://dx.doi.org/10.1016/j.vaccine.2012.03.011. PMid:22429756.

27. Olivares R, Bouyer J, Hubert B. Risk factors for death in meningococcal disease. Pathol Biol (Paris). 1993;41(2):164-8.

28. Steindl G, Liu YL, Schmid D, Orendi U, Kormann-Klement A, Heuberger S. Epidemiology of invasive meningococcal disease in Austria 2010. Wien Klin Wochenschr. 2011;123 Suppl 1:10-4. http://dx.doi.org/10.1007/s00508-011-0058-0. PMid:21935643.

29. Connolly M, Noah N. Is group C meningococcal disease increasing in Europe? A report of surveillance of meningococcal infection in Europe 1993-6. European Meningitis Surveillance Group. Epidemiol Infect. 1999;122(1):41-9. http://dx.doi.org/10.1017/S0950268898001848. PMid:10098784. PMCid:PMC2809586.

30. European Centre for Disease Prevention and Control (ECDC). Case definitions. 2008 EU Case Definition (2008/426/EC) for meningococcal disease, invasive. Stockholm: ECDC. [Accessed 29 Jun 2013]. Available from: http://www.ecdc.europa.eu/en/activities/surveillance/EU_IBD/Pages/Case_definition.aspx

31. Australian Government, Department of Health and Ageing. Australian national notifiable diseases case definitions. Meningococcal disease (invasive) surveillance case definition - V1.4. Canberra: Australian Government, Department of Health and Ageing. [Accessed 29 Jun 2013]. Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-cd_mening.htm

32. Ni H, Knight AI, Cartwright K, Palmer WH, McFadden J. Polymerase chain reaction for diagnosis of meningococcal meningitis. Lancet. 1992;340(8833):1432-4. http://dx.doi.org/10.1016/0140-6736(92)92622-M

33. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. Simultaneous Detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in Suspected Cases of Meningitis and Septicemia Using Real-Time PCR. J Clin Microbiol. 2001;39(4):1553-8. http://dx.doi.org/10.1128/JCM.39.4.1553-1558.2001. PMid:11283086. PMCid:PMC87969.

34. Wilder-Smith A, Goh KT, Barkham T, Paton NI. Hajj-associated outbreak strain of Neisseria meningitidis serogroup W135: estimates of the attack rate in a defined population and the risk of invasive disease developing in carriers. Clin Infect Dis. 2003;36(6):679-83. http://dx.doi.org/10.1086/367858. PMid:12627350.

35. Swartley JS, Marfin AA, Edupuganti S, Liu LJ, Cieslak P, Perkins B, et al. Capsule switching of Neisseria meningitidis. Proc Natl Acad Sci USA. 1997;94(1):271-6. http://dx.doi.org/10.1073/pnas.94.1.271. PMid:8990198. PMCid:PMC19312.

36. Vogel U, Claus H, Frosch M. Rapid serogroup switching in Neisseria meningitidis. N Engl J Med. 2000;342(3):219-20. http://dx.doi.org/10.1056/NEJM200001203420319. PMid:10651563.

37. El Bashir H, Rashid H, Memish ZA, Shafi S; Health at Hajj and Umra Research group. Meningococcal vaccine coverage in Hajj pilgrims. Lancet. 2007;369(9570):1343. http://dx.doi.org/10.1016/S0140-6736(07)60625-4

Investigating the link between the presence of enteroaggregative *Escherichia coli* and infectious intestinal disease in the United Kingdom, 1993 to 1996 and 2008 to 2009

M A Chattaway[1], R Harris[2], C Jenkins[1], C Tam[3], J E Coia[4], J Gray[5], M Iturriza-Gomara[6], J Wain (J.Wain@uea.ac.uk)[1,5]
1. Gastrointestinal Bacteria Reference Unit, Public Health England, Colindale, London, United Kingdom
2. Statistics, Modelling and Economics Department, Public Health England, Colindale, United Kingdom
3. Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, United Kingdom
4. Scottish Salmonella, Shigella and Clostridium difficile Reference Laboratory, Glasgow, United Kingdom
5. Microbiology, Norwich and Norfolk University hospital and the Norwich Medical School, University of East Anglia, Norfolk, United Kingdom
6. Clinical Infection, Microbiology and Immunology, University of Liverpool, United Kingdom

Citation style for this article:
Chattaway MA, Harris R, Jenkins C, Tam C, Coia JE, Gray J, Iturriza-Gomara M, Wain J. Investigating the link between the presence of enteroaggregative Escherichia coli and infectious intestinal disease in the United Kingdom, 1993 to 1996 and 2008 to 2009. Euro Surveill. 2013;18(37):pii=20582. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20582

Article submitted on 24 January 2013 / published on 12 September 2013

There are an estimated 17 million human diarrhoea cases annually in the United Kingdom. In 2008 and 2009, enteroaggregative *E. coli* (EAEC) were identified in 1.9% of stools. However, it remains unclear whether there is a causal link between presence of EAEC and disease. This study used bacterial load, the presence of co-infections and demographic data to assess if EAEC was independently associated with intestinal infectious disease. Quantitative real-time PCR data (Ct values) generated directly from stool specimens for several pathogen targets were analysed to identify multiple pathogens, including EAEC, in the stools of cases and healthy controls. Sensitivity and specificity using Ct value (60% and 60%) was not useful for identifying cases or controls, but an independent association between disease and EAEC presence was demonstrated: multivariate logistic regression for EAEC presence (odds ratio: 2.41; 95% confidence interval: 1.78–3.26; p<0.001). The population-attributable fraction was 3.3%. The group of bacteria known as EAEC are associated with gastrointestinal disease in at least half of the cases with EAEC positive stools. We conclude that the current definition of EAEC, by plasmid gene detection, includes true pathogens as well as non-pathogenic variants.

Introduction

Measuring the burden of infectious disease is essential for the rational design of public health intervention strategies and for the allocation of resources. For intestinal infectious diseases (IID) there is a massive global burden; the World Health Organization (WHO) estimates around 2 billion cases every year [1]. Detailed surveillance studies have shown that there are up to 17 million sporadic community cases of IID and one million general practitioner (GP) consultations annually in the United Kingdom (UK) [2]. Routine investigations of IID in the UK include salmonellosis, shigellosis, campylobacteriosis, cholera, infection with verotoxin-producing *Escherichia coli* O157 (VTEC), rotavirus, norovirus and parasitic infections and yet no cause is identified for over half of the laboratory-investigated diarrhoeal episodes [3]. One, often undiagnosed, potential pathogen is enteroaggregative *E. coli* (EAEC). In England, this pathotype of *E. coli*, defined by the ability to aggregate to HEp-2 cells [4], has been associated with cases of gastrointestinal infection [2,5,6] at a level comparable to *Salmonella* [6,7]. EAEC gained notoriety during a recent outbreak in Germany and France caused by an *E. coli* strain that was both a verotoxin-producing and enteroaggregative [8]. This outbreak was unusual due to the scale of morbidity and mortality, high even for VTEC infection, and the acquisition of the EAEC plasmid which may have played an important role in adherence to the human gut; the *E. coli* strain that caused the outbreak lacked the attachment and effacement (*eae*) gene for intimate adherence to human gut epithelium normally associated with severe disease caused by VTEC [9]. The emergence of this hybrid pathogen has been described before in 1996, when an O111:H2 strain had caused an outbreak of haemolytic uraemic syndrome (HUS) in France [10], in 1999, when an O86:H strain associated with HUS was isolated in Japan [11], and most recently in 2011, when an O111:H21 strain was associated with a family outbreak in Ireland [12]. All of these cases were associated with severe disease. It is likely that there are more cases of IID caused by EAEC and VTEC hybrids, but the EAEC pathotype is not routinely looked for.

Although EAEC itself has been associated with disease globally [13-19] including outbreaks (most notably a large outbreak in Japan involving 2,697 children [20]), a considerable proportion of healthy controls in case–control studies (16–31%) also harbour this pathotype [21-23]. Furthermore, research data describing the association of genetic factors with virulence are contradictory [21,24,25]. The reliability of virulence factors to identify EAEC for diagnostic purposes is therefore unclear [16]. The situation is further complicated by the presence of co-infections in IID [7]. When multiple pathogens are present in a diarrhoeic stool, defining which are causing the symptoms can be problematic, and as diagnostic tools improve, mixed infections in the gut are being recognised more frequently [26]. This is especially true in studies looking at EAEC infection; in Peru, for instance, multiple pathogens are found in 40% of infants with diarrhoea and with EAEC in their stool [27].

The successful completion of two IID burden studies in the UK [2,6] using quantitative PCR, presented the opportunity to investigate the causal link between gastrointestinal disease and the presence of EAEC in the stool. We estimated bacterial load for EAEC and the presence of co-infection in a well-defined population in the UK and tested the independent association between EAEC presence and disease.

Methods

Datasets

Data from two IID studies were used in this analysis: the IID1 case–control study (August 1993–January 1996) [6,7,28] and the IID2 case-only study (April 2008–March 2009) [29]. The data had been generated by testing stool samples by real-time PCR for the presence of a range of pathogens and recording the number of PCR cycles (Ct) needed before detection of product, to give a semi-quantitative estimate of bacterial load. The EAEC probe was the anti-aggregation protein transporter gene CVD432/*aatD* [30].

Cases of IID were defined in the same way in both studies as having had more than one loose stool, or clinically significant vomiting, over a two week period with no underlying non-infectious cause, followed by a symptom-free period of three weeks [2]. Healthy controls (IID-free) were only recruited in IID1 and were selected from the study cohort, matched for age and sex, and asked to submit a stool specimen.

The dataset for the IID1 case–control study contained 4,664 stool specimens (2,443 cases, 2,221 controls); EAEC was detected, by PCR, in 113 cases and in 38 controls but real-time Ct values (for the EAEC probe) were only available for 102 cases and 31 controls; in this study, all 151 positive cases were used for descriptive comparisons, and the 133 with Ct values for quantitative analysis.

The dataset for the IID2 case-only study [29] contained PCR Ct values from 3,966 stools (all of which were from individuals with diarrhoea); EAEC was detected in 83 of them. These data were used for burden estimations and comparisons of demographic data for cases; there had been no controls recruited in IID2 case-only study, and so IID1 data only were used for comparison of cases with controls.

Statistical methods

One aim of this study was to assess the methods for estimating burden of EAEC in England from the current IID2 study results. However, no controls were recruited to the IID2 study and so a receiver-operating characteristic (ROC) analysis was constructed from the case–control data (IID1), and used to look for a cut-off between case and control in the Ct values. We compared the distribution of Ct values from EAEC-positive cases and controls using Student's t-test.

It is clear that the relationship between presence of EAEC and disease is not absolute and so several methods were used to investigate the association of EAEC with disease:

Carriage rates of EAEC in healthy controls, compared to other pathogens

For each infection, the chi-squared test was used to test if the distribution of the pathogen between cases and controls was as expected by chance.

Association of disease with individual pathogens in persons with multiple pathogens in their stool

For all EAEC-positive individuals with multiple pathogens (both cases and controls), we tested whether individual pathogens were equally distributed between cases and controls using chi-squared tests for independence. Because norovirus was the most common pathogen, we also compared by chi-squared test co-infection in all individuals positive for EAEC and all individuals positive for norovirus to see if the presence of other individual pathogens was dependent on infection with EAEC or norovirus.

Independent association of EAEC presence with disease

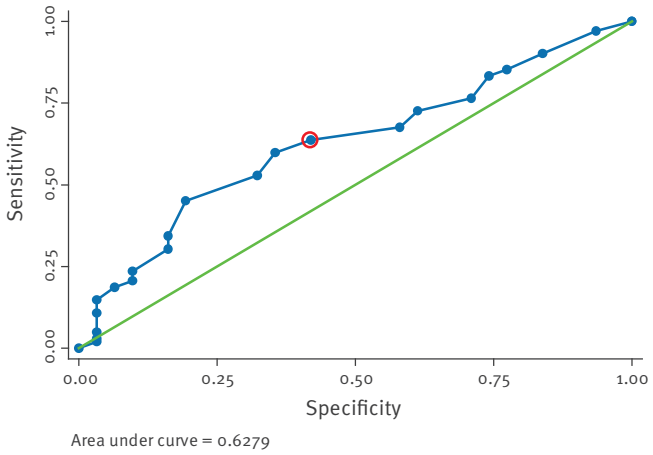
A logistic regression of univariate and multivariate analysis was carried out using case or control as outcome, and infecting agent and age as independent variables. In this way we assessed the independent association between EAEC and disease, while controlling for other pathogens. Model results were then used to calculate the population attributable fraction (PAF):

$$PAF = P_e (RR_e - 1) / RR_e,$$

where P_e is the proportion of cases with the exposure (EAEC) and RR_e the relative risk of disease. This form allows for confounding of the exposure if an adjusted RR is used, as recommended in Rockhill et al. [31]. In that case, adjusted odds ratios (OR) are substituted

Figure 1

Receiver-operating characteristic analysis of Ct values for enteroaggregative *Escherichia coli* from gastrointestinal disease cases (n=102) and controls (n=31) , United Kingdom, August 1993–January 1996



The red circle at Ct value 31 indicates the cut-off value which was chosen at the point where sensitivity and specificity were equivalent.

into this equation to give an approximate, adjusted PAF.

Results

Defining diagnostic cut-off values for Ct values in EAEC infection

In order to investigate the link between Ct value and disease, the sensitivity and specificity of the Ct value was assessed in EAEC-positive specimens from the case–control study (dataset IID1); Ct values were obtained and included 102 cases and 31 controls. Figure 1 shows the resulting ROC curve, and Figure 2 the distribution of Ct values in cases and controls. The cut-off was chosen to balance sensitivity and specificity and was set at a Ct value of 31 (Figure 1). The ratio of false positives versus false negatives with this cut-off point was 1.09 (95% confidence interval (CI): 0.79–1.53) (Figure 2), so the total number of test-positives, although not a good diagnostic for the individual, was a reasonable estimate of the total number of cases. Importantly however, in the population studied, there was a significant association between bacterial load and disease state (p=0.039), and further investigations were carried out using the point of ‹40 to indicate presence of EAEC.

Descriptive statistics

To test if the analysis of data from the IID1 case–control study remained relevant in 2009, we compared the demographic data from the two periods. There was no significant difference between the rate of EAEC in the IID1 case–control study (1993–96) and IID2 case-only study (2008–09), with 1.4% and 1.9%, respectively; individuals with EAEC present in their stool were distributed evenly across all age groups in both IID1 and IID2 (chi-squared p value for non-independence: 0.253). For EAEC-positive individuals, there was no significant difference in age between cases and controls (p=0.237). We therefore believe that the epidemiology did not change significantly for EAEC infection between the two periods. Cases tended to be slightly older than controls in IID1 (mean age of cases: 30.1 years, standard deviation (SD): 24.7 years; mean age of controls 28.7 years, SD: 23.9 years; p value for difference: 0.051).

Investigation of the association of EAEC presence with disease

Carriage rates of EAEC, compared to other pathogens, in healthy controls

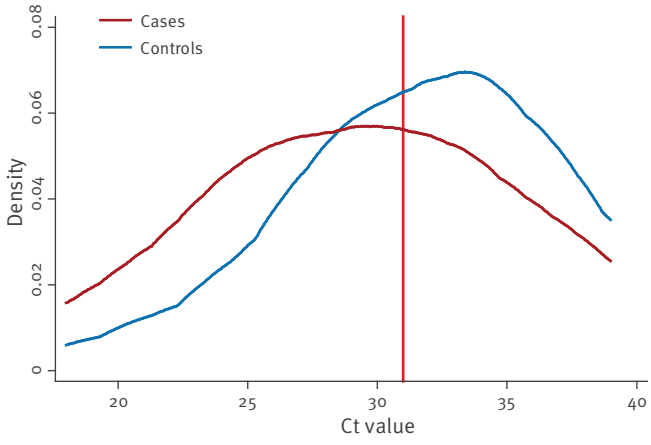
Submitting a stool specimen that was positive for EAEC was positively associated with having disease (Figure 3). However, one quarter of all EAEC positive individuals were asymptomatic (38/151).

Association of disease with individual pathogens in persons with multiple pathogens in their stool

The presence of co-infection was almost three times higher in EAEC-positive cases (74/113, 66%) than in EAEC-positive asymptomatic controls (9/38, 24%)_ (Figure 4). Cases had more multiple co-infections

Figure 2

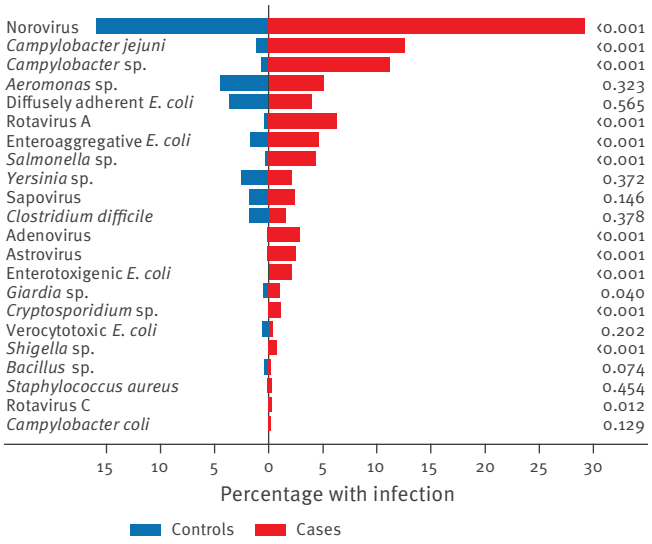
Distribution of Ct values for curve analysis of enteroaggregative *Escherichia coli* in gastrointestinal disease cases (n=102) and controls (n=31), United Kingdom, August 1993–January 1996



Fitted Curve distribution of Ct values. The red line indicates the cut-off point where the ratio of false positives versus false negatives with this cut-off point was closest to equivalent 1.09; 95% confidence interval: 0.79–1.53.

Figure 3

Organisms present in stool samples from gastrointestinal disease cases (n=2,221) and controls (n=2,243) in the IID1 study, United Kingdom, August 1993–January 1996



Submitting a stool specimen that was positive for enteroaggregative *Escherichia coli* (EAEC) was positively associated with having disease. EAEC was found in <2% of controls, indicating that EAEC is not a ubiquitous commensal organism.

The p values are indicated on the right.

(38/113, 34%) than controls (1/38, 3%) (chi-square test, p<0.001).

Investigation of the independent association of EAEC presence with disease

The logistic regression of EAEC status (but not Ct value) in univariate analysis gave an OR of 2.55 (95% CI: 1.91–3.39, p≤0.001); in multivaritate analysis, the OR was 2.41 (95% CI: 1.78–3.26, p≤0.001). This means that among IID cases, the odds of EAEC infection were 2.5 times higher compared with asymptomatic controls. The resulting adjusted PAF was 0.033% (95% CI: 0.024–0.039), suggesting that around 3.3% of cases of IDD in the UK were attributable to EAEC. This confirmed that EAEC was an independent cause of IID.

A comparison of co-infections with the most common cause of IID, norovirus, is presented in Figure 5.

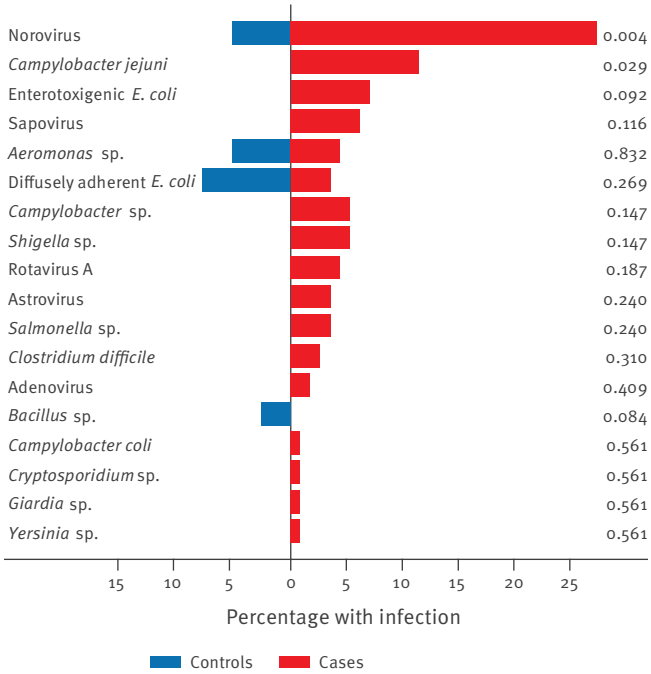
Discussion

Although described as a pathogenic group of *E. coli*, it is well documented that EAEC may be associated with asymptomatic infection [21-23]. In this study we asked the question how much disease EAEC is responsible for. In an attempt to remove healthy carriers from the case definition (a lower bacterial load might be expected in carriers than in cases), we analysed data from a PCR-based case–control study (IID1). Using the Ct value as an indicator of bacterial load, we were only able to define a cut-off with 60% sensitivity and specificity. These values suggest that estimation of bacterial load by the Ct value of a quantitative PCR for virulence factors is not a useful diagnostic test for EAEC infection.

However, there was a strong association between higher load (low Ct) and being a case, so we tried to define more accurately in which positive individuals EAEC was the causal agent of diarrhoea. The bacterial load data revealed the presence of two overlapping normally distributed data sets for EAEC: one representing the load in health (controls) and one in disease (cases) (see Figure 2). We further addressed any possible confounding effects of age (i.e. acquired immunity) and co-infection using logistic regression confirmed by univariate analysis; the results showed that an individual was 2.5 times more likely to be a case than a control if they had EAEC. Therefore we concluded that EAEC was

Figure 4

Co-infection with enteroaggregative *Escherichia coli* in gastrointestinal disease cases (n=113) and controls (n=38) in the IID1 study, United Kingdom, August 1993–January 1996



EAEC: enteroaggregative *E.coli*.

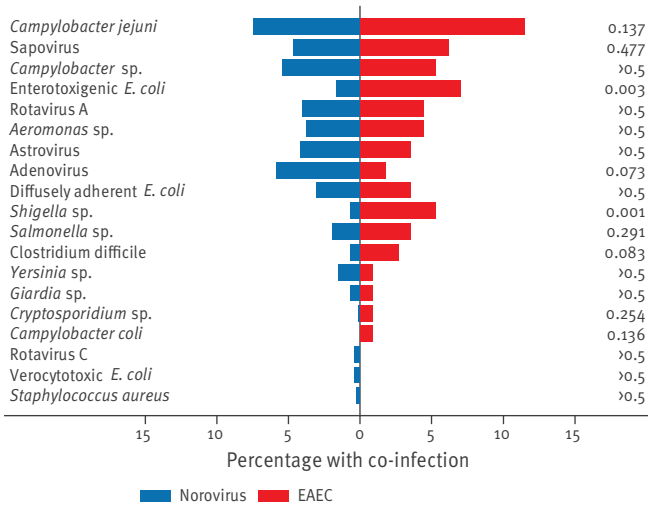
There were a higher variety of co-infection types, a higher percentage of co-infections and more multiple co-infection in EAEC-positive cases than in EAEC-positive controls.

Note: organisms designated sp. include all species of that genus (except *Campylobacter* sp. which list *C. jejuni* and *C. coli* separately), *Staphylococcus aureus* refers to all *S. aureus* >10^6/g.

The p values are indicated on the right.

Figure 5

Comparison of co-infections with enteroaggregative *Escherichia coli* (n=113) or norovirus (n=715), United Kingdom, August 1993–January 1996



EAEC: enteroaggregative *E.coli*.

Co-infection with EAEC was more common than with norovirus (66% versus 43%).

The p values for individual agents are indicated on the right.

independently associated with disease and we investigated the factors influencing this association.

Our results suggest that EAEC is common in the absence of disease. This situation is similar for gastrointestinal viral infection where post-infection levels of virus particles, although reduced, persist up to 56 days after symptoms have cleared [32,33]. Another possibility is pre-existing immunity to the infection at the time of exposure, which could result in reduced viral replication and a failure to develop symptoms. If pre-existing immunity was the cause of symptomless EAEC carriage we would expect to find an age distribution where adults are less frequently infected (older individuals have a higher chance of exposure and therefore a higher chance of immunity). The age distribution was even across the age groups and, as seen in the ROC analysis, the association between bacterial load and symptoms was not strong. Therefore we investigated an alternative explanation, the presence of a co-infecting pathogen.

The presence of increased co-infection in cases raises the possibility that the co-infecting pathogen rather than the EAEC, or a combination of both, is causing disease. To test this hypothesis we took norovirus, an infectious agent known to be present in both symptomatic and asymptomatic infection, as a comparator. As norovirus was a very common infection, we removed cases infected simultaneously with both norovirus and EAEC from the calculation: there were slightly more co-infections in EAEC-positive cases than in norovirus-positive cases (66% versus 43%). For EAEC co-infection, 12.6% were explained by enterotoxigenic *E. coli* (ETEC) and *Shigella* co-infections (Figure 5). This suggests that a proportion of EAEC cases can be explained by other pathogens (ETEC and *Shigella* are associated almost exclusively with symptomatic infection), but by no means all cases.

The logistic regression of co-infection univariate and multivaritate was statistically significant and again confirmed that EAEC was independently associated with disease; the odds of disease were 2.4 times higher if EAEC was present than if not and were still highly significant after controlling for co-infections. The PAF adjustments indicated that EAEC would be responsible for disease in 3.3% of cases, a significant proportion in gastrointestinal disease, higher than for *Salmonella* [2]. Although age was an independent predictor for disease overall, controlling for age did not change the association of disease with EAEC, and there was no interaction between EAEC and age.

This study did not directly address causality over association, but we believe that bacterial variation best explains the observed association of EAEC with disease for the following reasons. There are two common arguments for EAEC being found in high levels in healthy individuals: (i) Low levels of EAEC are present in a symptomless commensal relationship in the human gut and only increase to detectable levels after infection with a true pathogen because adherence of EAEC to the gut epithelium is stronger than for other commensals; an independent association of EAEC with disease argues against this for at least half of the infections in this study. (ii) Post-infection immunity leads to carriage in apparently healthy individuals; lack of any detectable trends in age distribution and no clear association between pathogen load and disease, as seen in norovirus infection [34], suggest that acquired immunity against EAEC does not protect against infection and is therefore unlikely to lead to symptomless carriage. Transient passage, as with plant viruses, is also unlikely, as there is no known reservoir for exposure to EAEC from outside the human gut.

It seems therefore clear that some, but not all, EAEC cause disease. The explanation for this may be that EAEC are defined by in vitro phenotype rather than by the ability to cause disease: non-pathogenic EAEC, able to agglutinate cells in the laboratory but unable to cause disease in the human host, are found in controls and in co-infections with true pathogens, but pathogenic variants are found as the sole pathogen detected in diarrhoeic stools. Attempts to define genetic markers for EAEC using alternative probes still do not define those EAEC capable of causing disease: the presence of the *aat* (anti-aggregative transporter) [35] or *agg*R (a transcriptional activator) [13,18,35] does not correlate

precisely with disease, but rather with the ability to agglutinate cells in the laboratory.

It may be that the genetic factors used for EAEC diagnostics are not true virulence factors and that they rather encode the ability to adhere to human intestinal cells and allow colonisation (especially during infection with a true pathogen). It is likely that a combination of the EAEC-associated adherence factors and a true virulence factor allows EAEC to cause primary infection. This was seen in the German ST678 (O104:H4) outbreak [36], where the EAEC adherence genes were present in the same bacterial host as the Shiga-like toxin gene (*stx*). We suggest that an appropriate diagnostic test for pathogenic EAEC should look for the EAEC plasmid genes and other virulence factors. More work is still needed to define those other virulence factors in diarrhoeagenic EAEC.

The main limitation of this study is the lack of controls in the IID2 study. Although there were 20 years between the IID1 and IID2 studies, the demographic data for cases suggest that the epidemiology has not changed during that period. Although there may have been some change in co-infection rates, we believe the data to be relevant in 2013. Another limitation, but also a strength, of the study is the range of infectious agents identified. Small numbers in some groups of cases with co-infections (six cases or less for EAEC co-infections with *C. difficile, Yersinia, Giardia, Cryptosporidium*, rotavirus C, VTEC and *Staphylococcus*) mean that the ability to detect statistical differences between cases and controls was limited. However, the study allowed us, for the first time, to explore the association between EAEC and all potential co-infecting agents as well as the more common pathogens norovirus (n=29) and *Campylobacter* (n=12).

Conclusion

This study highlights the importance of EAEC as a pathogenic group of bacteria which caused disease in more than 1% of all IID cases in the UK in 2008–09. The EAEC group is most likely to be a mixture of pathotypes which needs to be split into rational subgroups before tests for detection and typing can be implemented. Detailed studies of the genetic content of EAECs from case–control studies are warranted.

Acknowledgements

We would like to thank the study teams for allowing us access to the data generated from the two IID studies and in particular Dr Corrine Amar from the Laboratory of Gastrointestinal Pathogens (LGP) Unit, Health Protection Agency (HPA), London and Sarah O'Brien, University of Liverpool, Liverpool. We would also like to thank Prof Iruka Okeke from the Molecular Microbiology Department, Haverford College, Pennsylvania, USA and Kathie Grant from LGP, HPA, London for valuable advice with this manuscript.

Conflict of interest

None declared.

Author's contributions

Conception, design of study, interpretation of data, drafting and revising manuscript: MA Chattaway and J Wain; Acquisition and analysis of statistical data: R Harris; Drafting manuscript and interpretation of data: T Clarence, M Iturriza-Gomara, Claire Jenkins and John Coia.

References

1. World Health Organization (WHO). Diarrhoeal disease. Fact Sheet no 330. Geneva: WHO; Apr 2013. Available from: http://www.who.int/mediacentre/factsheets/fs330/en/
2. Tam CC, Rodrigues LC, Viviani L, Dodds JP, Evans MR, Hunter PR, et al. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut. 2012;61(1):69-77. http://dx.doi.org/10.1136/gut.2011.238386. PMid:21708822. PMCid:PMC3230829.
3. Tam CC, O'Brien SJ, Tompkins DS, Bolton FJ, Berry L, Dodds J, et al. Changes in causes of acute gastroenteritis in the United Kingdom over 15 years: microbiologic findings from 2 prospective, population-based studies of infectious intestinal disease. Clin Infect Dis. 2012;54(9):1275-86. http://dx.doi.org/10.1093/cid/cis028. PMid:22412058.
4. Nataro JP, Kaper JB, Robins-Browne R, Prado V, Vial P, Levine MM. Patterns of adherence of diarrheagenic Escherichia coli to HEp-2 cells. Pediatr Infect Dis J. 1987;6(9):829-31. http://dx.doi.org/10.1097/00006454-198709000-00008. PMid:3313248.
5. Nataro JP, Mai V, Johnson J, Blackwelder WC, Heimer R, Tirrell S, et al. Diarrheagenic Escherichia coli infection in Baltimore, Maryland, and New Haven, Connecticut. Clin Infect Dis. 2006;43(4):402-7. http://dx.doi.org/10.1086/505867. PMid:16838226.
6. Tompkins DS, Hudson MJ, Smith HR, Eglin RP, Wheeler JG, Brett MM, et al. A study of infectious intestinal disease in England: microbiological findings in cases and controls. Commun Dis Public Health. 1999;2(2):108-13. PMid:10402744.
7. Amar CF, East CL, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case-control Infectious Intestinal Disease Study (1993-1996). Eur J Clin Microbiol Infect Dis. 2007;26(5):311-23. http://dx.doi.org/10.1007/s10096-007-0290-8. PMid:17447091.
8. Rubino S, Cappuccinelli P, Kelvin DJ. Escherichia coli (STEC) serotype O104 outbreak causing haemolytic syndrome (HUS) in Germany and France. J Infect Dev Ctries. 2011;5(6):437-40. http://dx.doi.org/10.3855/jidc.2172. PMid:21727641.
9. Jenkins C, Willshaw GA, Evans J, Cheasty T, Chart H, Shaw DJ, et al. Subtyping of virulence genes in verocytotoxin-producing Escherichia coli (VTEC) other than serogroup O157 associated with disease in the United Kingdom. J Med Microbiol. 2003;52(Pt 11):941-7. http://dx.doi.org/10.1099/jmm.0.05160-0. PMid:14532337.
10. Mellmann A, Harmsen D, Cummings CA, Zentz EB, Leopold SR, Prior K, et al. Prospective genomic characterization of the German enterohemorrhagic Escherichia coli O104:H4 outbreak by rapid next generation sequencing technology. PLoS One. 2011;6(7):e22751. http://dx.doi.org/10.1371/journal.pone.0022751. PMid:21799941. PMCid:PMC3140518.
11. Iyoda S, Tamura K, Itoh K, Izumiya H, Ueno N, Nagata K, et al. Inducible stx2 phages are lysogenized in the enteroaggregative and other phenotypic Escherichia coli O86:HNM isolated from patients. FEMS Microbiol Lett. 2000;191(1):7-10. http://dx.doi.org/10.1111/j.1574-6968.2000.tb09311.x. PMid:11004392.
12. Dallman T, Smith GP, O'Brien B, Chattaway M, Finlay D, Grant KA, et al. Characterization of a verocytotoxin-producing enteroaggregative Escherichia coli serogroup O111:H21 strain associated with a household outbreak in Northern Ireland. J Clin Microbiol. 2012;50(12):4116-9. http://dx.doi.org/10.1128/JCM.02047-12. PMid:23035193. PMCid:PMC3502983.
13. Antikainen J, Tarkka E, Haukka K, Siitonen A, Vaara M, Kirveskari J. New 16-plex PCR method for rapid detection of diarrheagenic Escherichia coli directly from stool samples. Eur J Clin Microbiol Infect Dis. 2009;28(8):899-908. http://dx.doi.org/10.1007/s10096-009-0720-x. PMid:19238467.

14. Cennimo D, Abbas A, Huang DB, Chiang T. The prevalence and virulence characteristics of enteroaggregative Escherichia coli at an urgent-care clinic in the USA: a case-control study. J Med Microbiol. 2009;58(Pt 4):403-7. http://dx.doi.org/10.1099/jmm.0.005793-0. PMid:19273633.
15. Jenkins C, Tembo M, Chart H, Cheasty T, Willshaw GA, Phillips AD, et al. Detection of enteroaggregative Escherichia coli in faecal samples from patients in the community with diarrhoea. J Med Microbiol. 2006;55(11):1493-7. http://dx.doi.org/10.1099/jmm.0.46683-0. PMid:17030907.
16. Okeke IN, Nataro JP. Enteroaggregative Escherichia coli. Lancet Infect Dis. 2001;1(5):304-13. http://dx.doi.org/10.1016/S1473-3099(01)00144-X
17. Okhuysen PC, Dupont HL. Enteroaggregative Escherichia coli (EAEC): a cause of acute and persistent diarrhea of worldwide importance. J Infect Dis. 2010;202(4):503-5. http://dx.doi.org/10.1086/654895. PMid:20594108.
18. Toma C, Lu Y, Higa N, Naksone N, Chinen I, Baschkier A, et al. Multiplex PCR assay for identification of human diarrheagenic Escherichia coli. J Clin Microbiol. 2003;41(6):2669-71. http://dx.doi.org/10.1128/JCM.41.6.2669-2671.2003. PMid:12791900. PMCid:PMC156568.
19. Usein CR, Tatu-Chitoiu D, Ciontea S, Condei M, Damian M. Escherichia coli pathotypes associated with diarrhea in Romanian children younger than 5 years of age. Jpn J Infect Dis. 2009;62(4):289-93. PMid:19628907.
20. Itoh Y, Nagano I, Kunishima M, Ezaki T. Laboratory investigation of enteroaggregative Escherichia coli O untypeable:H10 associated with a massive outbreak of gastrointestinal illness. J Clin Microbiol. 1997;35(10):2546-50. PMid:9316905. PMCid:PMC230008.
21. Huang DB, Mohamed JA, Nataro JP, DuPont HL, Jiang ZD, Okhuysen PC. Virulence characteristics and the molecular epidemiology of enteroaggregative Escherichia coli isolates from travellers to developing countries. J Med Microbiol. 2007;56(10):1386-92. http://dx.doi.org/10.1099/jmm.0.47161-0. PMid:17893178.
22. Okeke IN, Lamikanra A, Czeczulin J, Dubovsky F, Kaper JB, Nataro JP. Heterogeneous virulence of enteroaggregative Escherichia coli strains isolated from children in Southwest Nigeria. J Infect Dis. 2000;181(1):252-60. http://dx.doi.org/10.1086/315204. PMid:10608774.
23. Regua-Mangia AH, Gomes TA, Vieira MA, Irino K, Teixeira LM. Molecular typing and virulence of enteroaggregative Escherichia coli strains isolated from children with and without diarrhoea in Rio de Janeiro city, Brazil. J Med Microbiol. 2009;58(4):414-22. http://dx.doi.org/10.1099/jmm.0.006502-0. PMid:19273635.
24. Boisen N, Scheutz F, Rasko DA, Redman JC, Persson S, Simon J, et al. Genomic characterization of enteroaggregative Escherichia coli from children in Mali. J Infect Dis. 2012;205(3):431-44. http://dx.doi.org/10.1093/infdis/jir757. PMid:22184729. PMCid:PMC3256949.
25. Jenkins C, van Ijperen C, Dudley EG, Chart H, Willshaw GA, Cheasty T, et al. Use of a microarray to assess the distribution of plasmid and chromosomal virulence genes in strains of enteroaggregative Escherichia coli. FEMS Microbiol Lett. 2005;253(1):119-24. http://dx.doi.org/10.1016/j.femsle.2005.09.040. PMid:16243450.
26. Lindsay B, Ramamurthy T, Sen Gupta S, Takeda Y, Rajendran K, Nair GB, et al. Diarrheagenic pathogens in polymicrobial infections. Emerg Infect Dis. 2011;17(4):606-11. PMid:21470448. PMCid:PMC3377398.
27. Ochoa TJ, Ecker L, Barletta F, Mispireta ML, Gil AI, Contreras C, et al. Age-related susceptibility to infection with diarrheagenic Escherichia coli among infants from Periurban areas in Lima, Peru. Clin Infect Dis. 2009;49(11):1694-702. http://dx.doi.org/10.1086/648069. PMid:19857163. PMCid:PMC2779581.
28. Sethi D, Wheeler JG, Cowden JM, Rodrigues LC, Sockett PN, Roberts JA, et al. A study of infectious intestinal disease in England: plan and methods of data collection. Commun Dis Public Health. 1999;2(2):101-7. PMid:10402743.
29. O'Brien SJ, Rait G, Hunter PR, Gray JJ, Bolton FJ, Tompkins DS, et al. Methods for determining disease burden and calibrating national surveillance data in the United Kingdom: the second study of infectious intestinal disease in the community (IID2 study). BMC Med Res Methodol. 2010;10:39. http://dx.doi.org/10.1186/1471-2288-10-39. PMid:20444246. PMCid:PMC2886083.
30. Baudry B, Savarino SJ, Vial P, Kaper JB, Levine MM. A sensitive and specific DNA probe to identify enteroaggregative Escherichia coli, a recently discovered diarrheal pathogen. J Infect Dis. 1990;161(6):1249-51. http://dx.doi.org/10.1093/infdis/161.6.1249. PMid:2189007.
31. Rockhill B, Newman B, Weinberg C. Use and misuse of population attributable fractions. Am J Public Health. 1998;88(1):15-9. http://dx.doi.org/10.2105/AJPH.88.1.15. PMid:9584027. PMCid:PMC1508384.
32. Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, et al. Norwalk virus shedding after experimental human infection. Emerg Infect Dis. 2008;14(10):1553-7. http://dx.doi.org/10.3201/eid1410.080117. PMid:18826818. PMCid:PMC2609865.
33. Partridge DG, Evans CM, Raza M, Kudesia G, Parsons HK. Lessons from a large norovirus outbreak: impact of viral load, patient age and ward design on duration of symptoms and shedding and likelihood of transmission. J Hosp Infect. 2012;81(1):25-30. http://dx.doi.org/10.1016/j.jhin.2012.02.002. PMid:22445090.
34. Phillips G, Lopman B, Tam CC, Iturriza-Gomara M, Brown D, Gray J. Diagnosing norovirus-associated infectious intestinal disease using viral load. BMC Infect Dis. 2009;9:63. http://dx.doi.org/10.1186/1471-2334-9-63. PMid:19442278. PMCid:PMC2698835.
35. Denno DM, Shaikh N, Stapp JR, Qin X, Hutter CM, Hoffman V, et al. Diarrhea Etiology in a Pediatric Emergency Department: A Case Control Study. Clin Infect Dis. 2012:55(7):897-904. http://dx.doi.org/10.1093/cid/cis553. PMid:22700832.
36. Chattaway MA, Dallman T, Okeke IN, Wain J. Enteroaggregative E. coli O104 from an outbreak of HUS in Germany 2011, could it happen again? J Infect Dev Ctries. 2011;5(6):425-36. http://dx.doi.org/10.3855/jidc.2166. PMid:21727640.

First joint meeting of three European tuberculosis networks

M J van der Werf (Marieke.vanderWerf@ecdc.europa.eu)[1], C Erkens[2], A Gebhard[2], F Voitzwinkler[3], M Dara[4]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. KNCV Tuberculosis Foundation, The Hague, the Netherlands
3. Global Health Advocates, Brussels, Belgium
4. World Health Organization Regional Office for Europe, Copenhagen, Denmark

Citation style for this article:
van der Werf MJ, Erkens C, Gebhard A, Voitzwinkler F, Dara M. First joint meeting of three European tuberculosis networks. Euro Surveill. 2013;18(37):pii=20583. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20583

Article submitted on 12 August 2013 / published on 12 September 2013

On 29 May 2013 three European tuberculosis (TB) networks met for the first time to discuss TB prevention, control and care in the World Health Organization (WHO) European Region including the European Union (EU). This meeting, which took place in The Hague, the Netherlands, provided a unique opportunity to discuss progress with the implementation of the Berlin Declaration on TB [1], the European Centre for Disease Prevention and Control (ECDC) Framework Action Plan to fight tuberculosis in the EU [2,3], and the Consolidated Action Plan to prevent and combat multidrug- and extensively drug-resistant tuberculosis (M/XDR-TB) in the WHO European Region [4]. Surveillance focal points, laboratory experts, and National TB Programme Managers (NTPs) exchanged lessons learned and discussed next steps to reach the targets defined in the plans.

To coordinate and improve TB prevention, control and care in the WHO European Region including the EU, professional networks have been established: (i) the Wolfheze movement aims to strengthen TB control in the WHO European Region [5]; the workshops of the Wolfheze movement provide a platform for NTPs, health authorities, laboratory experts, national TB surveillance correspondents, civil society organisations and other partners to discuss achievements, challenges and way forward; (ii) the European Tuberculosis Surveillance Network aims to improve the contribution of surveillance to TB control, and to promote standardised methods; (iii) the European Reference Laboratory Network for Tuberculosis (ERLN-TB) was established to strengthen TB diagnosis in the EU [6].

Implementation of European TB prevention and control plans

Masoud Dara, WHO Regional Office Europe, Copenhagen, Denmark, and Marieke van der Werf, ECDC, Stockholm, Sweden, presented progress made with the implementation of the WHO European Region and EU TB prevention and control plans, respectively. They also presented the TB and MDR-TB epidemiological situation [7], the status of the indicators of the Berlin Declaration Monitoring and Evaluation Framework [7] and, finally, the epidemiological and core operational indicators of the Follow-up of the Framework Action Plan to fight tuberculosis in the EU [3] were assessed.

Since 2011, treatment of MDR-TB patients has been substantially scaled up with more than 97% of patients reported to be on treatment. However, treatment success rate of MDR-TB is far below the target of 75% envisaged for 2015. Furthermore there are frequent stockouts of second-line drugs for TB. The following key steps were presented to support further implementation of the Consolidated Action Plan:

- continued technical support to Member States;
- identify and address the social determinants of TB and M/XDR-TB;
- prepare a compendium of best practices;
- scale up best practices and patient-centred ambulatory care;
- strengthen country capacity in surveillance for producing reliable estimates of MDR-TB;
- introduce rational use of new TB medicines;
- develop interventions to move towards TB elimination in low TB incidence countries;
- define the role of surgery in TB and M/XDR-TB.

In the EU, only one of four epidemiological targets was met (i.e. overall decline of the five-year trend in TB case notification rates). Furthermore only one of five measured core targets was met (i.e. 100% of the national TB reference laboratories achieved a performance level of >80% for smear microscopy, culture and DST for first- and second-line drugs). It was therefore concluded that continued implementation of the Framework Action Plan to Fight Tuberculosis in the EU is needed.

After the general session the meeting focussed on four specific areas: (i) diagnosis; (ii) treatment and care; (iii) infection control, and; (iv) advocacy, partnerships and political commitment. First, selected low incidence and

high TB priority countries presented their key achievements and challenges. This was followed by four working groups on the aforementioned themes.

Diagnosis

Gulnoz Uzakova, Global Fund to Fight AIDS, Tuberculosis and Malaria TB grant manager, Tashkent, Uzbekistan, considered the improved diagnostic infrastructure and implementation of an external quality assurance scheme (EQA) in Uzbekistan as key achievements. Also, rapid drug susceptibility tests are implemented both at central and peripheral level. Good political commitment, as well as partnership and collaboration between the national and international organisations have been established. However, the targets for culture confirmation, coverage of first-line drug sensitivity testing (DST), and coverage of second-line DST are not met. The logistics, such as transportation of sputum samples, need to be improved, as well as data interpretation and information flows.

In Italy, key achievements presented by Daniela Cirillo, San Raffaele Scientific Institute, Milan, include a well-functioning infrastructure of laboratories on three levels and implementation of EQA for both first and second line DST. There is also a good system for regular training of laboratory staff. The key challenges are the need to strengthen the surveillance system and data reporting within Italy and its regions and from the regional to the national level. There is also a lack of funds for the implementation and support of the regional and national laboratory networks.

The working group on diagnosis addressed several questions, among which the role of national TB reference laboratories, access to rapid TB diagnosis and existing problems in implementation of novel technologies and drug susceptibility testing to second line drugs. The main conclusions were that in principle the national reference laboratory is responsible for the quality of the TB laboratory network at country level. However, in practice not all national reference laboratories are able to perform this function because they are not authorised or because of funding. To ensure a high quality of laboratory test results, TB laboratories should be accredited by designated national bodies and/or have a quality management system. A challenge is that national legislations and regulations differ in the requirements and levels of laboratory accreditation. Universal access to rapid diagnosis of TB is currently hampered by the costs of the tests, administrative obstacles, lack of training and managerial capacity, and inappropriate requests of the tests by clinicians. The working group emphasised the need for a prioritisation of DST for fluoroquinolones (FQ) and injectable second line drugs to detect early XDR cases. Major challenges include the unavailability of laboratory supplies, the lack of EQA, high costs, a lack of standardisation and need to improve the clinical interpretation of test results.

Treatment and care

According to Armen Hayrapetyan National TB Programme, Yerevan, Armenia, the TB notification rate is steadily decreasing in Armenia. Since 2007, the treatment success of MDR-TB has remained low at about 53-55%. Another challenge is the increasing number of individuals co-infected with TB and human immunodeficiency virus (HIV). Key achievements in Armenia are the availability of a TB/MDR/XDR response plan, and national guidelines for TB care. Also drugs for treatment of M/XDR-TB patients are now available. A programme for TB home-based care has been introduced. The key challenges for Armenia are the modernisation of the TB services, and the training of physicians, nurses and TB specialists.

In Hungary, there has been a steady decline of TB and the level of MDR-TB is low (<3%) as reported by Kovács Gábor, Koranyi National Institute for Tuberculosis and Pulmonology, Budapest. There is political commitment and a new national TB control plan is under development. Hungary has protocols for TB treatment and diagnosis which are in line with international recommendations. The country lacks adequate human resources in the healthcare sector in general and for TB specifically. There is a need to improve the model for treatment support to improve the treatment outcomes.

The working group on TB treatment and care discussed progress in improving universal access to TB treatment and care. Considerable progress has been made, especially by involving non-governmental organisations (NGOs) which link with vulnerable and high-risk groups. A key recommendation is to further increase the collaboration with NGOs, especially those working in HIV care. The availability of new drugs, such as bedaquiline, widens the treatment arsenal. Participants agreed that stringent pharmacovigilance, careful planning, ethical clearance and quality controls must be in place to ensure rational and effective use of new medicines. Countries are optimising their TB models of care, shifting from hospital based towards more ambulatory care, while improving quality of care and rational use of inpatient facilities with infection control standards. These need to be accompanied with training and retraining health staff in TB services and primary health care facilities. Another key recommendation is that a shift towards more ambulatory TB models of care should be accompanied by a reform of TB financing, well planned and intensive training of the healthcare workers involved and adequate psycho-social patient support throughout treatment.

Infection control

In Georgia, key achievements within the area of infection control as presented by Nestan Tukvadze, National Center for Tuberculosis and Lung Diseases, Tbilisi include the finalisation of national TB infection control guidelines in line with WHO recommendations, the implementation of a basic infection control risk assessment for TB service points, and the renovation of TB

outpatient service points and microscopy laboratories. Currently Georgia is working on expanding the 'three I's' (intensified TB case-finding, initiate TB prevention with isoniazid preventive therapy and early antiretroviral therapy, and TB infection control [8]), training of healthcare workers and developing infection control standards for renovation and construction of healthcare facilities. Some of the key challenges that need to be addressed are monitoring and evaluation of implementation and impact of infection control measures, and adequate maintenance of engineering control measures.

Norway has comprehensive guidelines for TB control, care and prevention which are well disseminated and accepted by the healthcare providers according to Karin Rønning, Norwegian Institute of Public Health, Oslo. National infection control guidelines are available; however, they do not contain a separate TB infection control plan. The implementation of infection control measures is supported by comprehensive regulations and guidelines. The standards of healthcare facilities are generally very high, with adequate isolation capacity available. To stop TB transmission the challenge for Norway is to quickly identify cases and provide them with high quality treatment and care.

The working group discussed topics important for adequate TB infection control, such as national TB infection control guidelines and strategic plans, funding and other organisational mechanisms, human resources capacity building, and monitoring and evaluation of TB infection control activities.

The main conclusions were that progress has been made on TB infection control across the Region and that infection control should be an integral part of national strategic TB plans. Countries should focus on best possible (cost)-effective infection control measures.

Key recommendations included:

- ensure a proper funding mechanism for TB infection control measures;
- update regularly national TB infection control regulations;
- adapt TB infection control training modules based on country context;
- advocate for and develop plans for TB infection control operational research;
- develop country-adapted standardised specifications for ventilation systems, and for ultraviolet germicidal irradiation equipment.

Advocacy, partnerships and political commitment

Jonathan Stillo, TB Europe Coalition, Bucharest, reported that Romania has a significant TB burden with a high number of MDR-TB cases and a treatment success rate for MDR-TB cases below the target. Frequent changes of high level decision-makers have resulted in a lack of coherence and continuity in TB control. There are serious challenges related to financing of TB control. A step towards strengthening advocacy and a push for political commitment was the formation of the Romanian Stop TB Partnership including 19 NGOs. The presence of the international community is important to build partnerships and to ensure political commitment.

Simon Logan, All-Party Parliamentary Group on Global Tuberculosis, London, United Kingdom, informed about this group which is working across political parties to address TB by arranging meetings in the parliament, working through parliamentary procedures such as debates, oral and written questions and publishing reports. Partnerships among NGOs, academics, civil servants and public health professionals are key to building a strong and united approach.

The working group gathered participants from civil society, national TB programmes and international organisations and aimed to identify the main challenges to advocacy in the region, what action could be taken to remedy these challenges and the tools and resources needed.

The main conclusions are that there is insufficient involvement of all stakeholders in advocacy and a general lack of understanding of its added value. Hence, the inadequacy of funds for advocacy activities, and the lack of capacity and expertise for civil society and others to run such activities.

Key recommendations were to document evidence of the added value of advocacy efforts, build the capacity of TB stakeholders in advocacy, enhance coordination and partnership at country level, increase political awareness of TB and advocate for more funding for advocacy.

Conclusions

The joint day finished with a presentation of the EU Standards for Tuberculosis Care [9]. This document describes the standards for four areas, i.e. TB diagnosis, TB treatment, HIV infection and comorbid conditions, public health and TB prevention (including infection control). Three of these had been discussed during the day as well.

This first joint meeting helped to facilitate exchange of good practices between professionals working in different areas of TB control and prevention in Europe. Key areas that need strenghtening to reach the targets of the TB control and prevention plans were identified and key recommendations were agreed on.

Acknowledgements

We acknowledge the contributions of Gulnoz Uzakova (Uzbekistan), Daniela Cirillo (Italy), Armen Hayrapetyan (Armenia), Kovács Gábor (Hungary), Nestan Tukvadze (Georgia), Karin Rønning (Norway), Jonathan Stillo (Romania), Simon Logan (UK), Kristin Kremer (WHO Regional Office for Europe), Francis Drobniewski (UK), Vladyslav Nikolayevskyy (UK), Vlad Furman (Kazachstan), Patrick Bertrand Belgium), Ieva Leimane (Netherlands), and Nina Bjerglund Andersen (WHO Regional Office for Europe). We especially would like

to acknowledge Gerard de Vries (Netherlands), Barbara Hauer (Germany), Lena Fiebig (Germany), Andreas Sandgren (European Centre for Disease Prevention and Control), Martin van der Boom (WHO Regional Office for Europe), for their input for specific parts of the manuscript, and for reviewing the draft manuscript.

Conflict of interest

None declared.

Authors' contribution

Marieke J. van der Werf designed and drafted the manuscript and coordinated the input from the other authors. Connie Erkens provided input for specific parts of the manuscript, reviewed the draft manuscript, and gave final approval of the version to be published. Agnes Gebhard provided input for specific parts of the manuscript, reviewed the draft manuscript, and gave final approval of the version to be published. Fanny Voitzwinkler provided input for specific parts of the manuscript, reviewed the draft manuscript, and gave final approval of the version to be published. Masoud Dara provided input for specific parts of the manuscript, reviewed the draft manuscript, and gave final approval of the version to be published.

References

1. World Health Organization (WHO). The Berlin Declaration on Tuberculosis. Berlin: World Health Organization Regional Office for Europe and All Against Tuberculosis. Copenhagen: WHO; 2007. Available from: http://www.euro.who.int/__data/assets/pdf_file/0008/68795/E91369.pdf
2. European Centre for Disease Prevention and Control (ECDC). Framework action plan to fight tuberculosis in the European Union. Stockholm: ECDC; 2008. Available from: http://ecdc.europa.eu/en/publications/publications/0803_spr_tb_action_plan.pdf
3. European Centre for Disease Prevention and Control (ECDC). Progressing towards TB elimination. Stockholm: ECDC; 2010. Available from: http://ecdc.europa.eu/en/publications/publications/101111_spr_progressing_towards_tb_elimination.pdf
4. World Health Organization (WHO) Regional Office for Europe. Roadmap to prevent and combat drug-resistant tuberculosis. Copenhagen, WHO Regional Office for Europe; 2011. Available from: http://www.euro.who.int/__data/assets/pdf_file/0014/152015/e95786.pdf
5. Veen J, Migliori GB, Raviglione M, Rieder HL, Dara M, Falzon D, et al. Harmonisation of TB control in the WHO European region: the history of the Wolfheze Workshops. Eur Respir J. 2011 Apr;37(4):950-9. http://dx.doi.org/10.1183/09031936.00019410. PMid:20530031.
6. Drobniewski FA, Nikolayevskyy V, Hoffner S, Pogoryelova O, Manissero D, Ozin AJ. The added value of a European Union tuberculosis reference laboratory network--analysis of the national reference laboratory activities. Euro Surveill. 2008 Mar 18;13(12): pii=8076. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8076. PMid:18761994.
7. European Centre for Disease Prevention and Control (ECDC)/ World Health Organization (WHO) Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe, 2013. Stockholm/Copenhagen: ECDC / WHO Regional Office for Europe; 2013. Available from: http://www.ecdc.europa.eu/en/publications/Publications/Tuberculosis-surveillance-monitoring-2013.pdf
8. World Health Organization (WHO). WHO policy on collaborative TB/HIV activities: Guidelines for national programmes and other stakeholders. Geneva: WHO; 2012. Available from: http://whqlibdoc.who.int/publications/2012/9789241503006_eng.pdf
9. Migliori GB, Zellweger JP, Abubakar I, Ibraim E, Caminero JA, De Vries G, et al. European union standards for tuberculosis care. Eur Respir J. 2012 Apr;39(4):807-19. http://dx.doi.org/10.1183/09031936.00203811. PMid:22467723. PMCid:PMC3393116.

ESCAIDE 2013 - Call for 'late breaker' abstracts

Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Citation style for this article:
Eurosurveillance editorial team. ESCAIDE 2013 - Call for 'late breaker' abstracts. Euro Surveill. 2013;18(37):pii=20584.
Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20584

Article published on 12 September 2013

The 2013 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 again at ESCAIDE this year and the call to submit abstracts for this session is open from 9 to 24 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. As in previous years, it is anticipated that the conference will be accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide CME credits. For further information, contact: escaide.conference@ecdc.europa.eu

ESCAIDE 2013 will take place in Stockholm, Sweden, from 5 to 7 November.