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Schistosoma haematobium infections acquired in Corsica, France, August 2013

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A 12 year-old boy in Germany developed urinary schistosomiasis in January 2014. He had bathed in rivers in south-eastern Corsica five months earlier. Before this case, human schistomiasis had not been reported on the island, although its vector, the snail *Bulinus truncatus*, locally transmitted the zoonotic Schistosoma bovis. The boy's father excreted *S. haematobium* ova that were not viable; the boy's three siblings had a positive serology against schistosomes.

Schistosomiasis cases reported in Germany in 2014

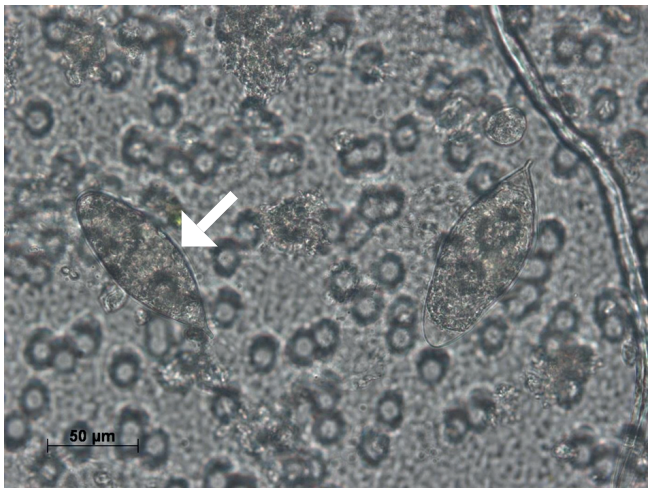
In January 2014, a 12 year-old German boy noticed painless excretion of bloody urine. He was referred by his paediatrician to a nearby urological hospital where ultrasonography showed focal thickening of the bladder wall. Since no bacterial infectious agent was detected, cystoscopy was performed, as a result of which severe cystitis was detected. Histological examination of a biopsy taken from the bladder wall showed granulomatous inflammation and schistosomiasis was suspected. As the boy had had no exposure in known schistosomiasis-endemic regions, his family took him to the Tropical Diseases Service in Düsseldorf, Germany.

Microscopy of a 24-hour urine sample collected from on the first day he presented until the following day and filtered through a nuclepore microfilter revealed viable ova of *Schistosoma haematobium* (Figure).

The boy was treated with a single standard dose of praziquantel (40mg/kg body weight) and re-treated three weeks later with the same dose, to be sure to achieve complete cure of the infection. During 24 days after therapy, he had various attacks of haematuria, sometimes with temporary large blood clots inside the bladder. Upper urinary tract infection was not observed. Bacterial coinfections including tuberculosis were ruled out by urine cultures, PCR and interferon-gamma release assay.

Figure

Ova of *Schistosoma haematobium* seen by microscopy of a microfiltrate of urine from a boy with urinary schistosomiasis, Germany, March 2014



Ova are indicated by arrows.

Investigations of the boy's five family members revealed non-viable *S. haematobium* ova in the urine of his father. Repeated examinations of enriched stool samples were negative for all family members. On the other hand, schistosomiasis serology against cercarial and adult antigens (by enzyme-linked immunosorbent assay (ELISA), immunofluorescence test (IFT)) was highly positive in all four children (including the boy) and the father, but not the mother.

Detailed history again confirmed that the boy and his family had never travelled outside Europe. His most

southerly travel was to Spain, where he had not bathed in surface fresh water, and southern France including south-eastern Corsica, where the boy had spent his holidays together with his family in August 2013 and bathed frequently in various rivers. The only place where the mother did not bathe together with her family was Cavu River.

Background

Schistosomiasis is endemic in in 78 countries of the tropical and subtropical world [1]. The World Health Organization in 2012 estimated that 249 million people required preventive antiparasitic therapy for schistosomiasis [1]. One of the six *Schistosoma* species, i.e. *S. haematobium*, causes urinary schistosomiasis and is endemic in Africa and the Arabian Peninsula [2].

The life cycle of this helminth involves man and freshwater snails [2]. Humans constitute the definitive host, the freshwater snail of the genus *Bulinus* spp. the intermediate host. The habitat of the snail is shallow waters of rivers or lakes, where the snail usually lives attached to water plants. The snail releases mobile worm larvae called cercariae, which actively reach the definitive host and penetrate their skin. The larvae reach the bloodstream and end up in the portal system, where the worm larvae grow and mature to female and male adult worms. The adult worms mate and descend to the plexus vesicalis, where the female worms start to lay their ova into the urinary bladder mucosa. The ova are excreted into the urine of the definitive host. When the ova reach fresh water, the intraovular larvae called miracidia hatch and swim towards the snails, where the cycle is completed. The bladder mucosa is damaged by the inflammatory response to the intraovular antigens resulting in ulcerations, papillomata and polyps of the bladder wall. Complications include bladder carcinoma, upper urinary tract infection and involvement of the reproductive tract [2].

Discussion

To the best of our knowledge, this is the first report in the scientific literature of autochthonous human schistosomiasis acquired in Europe since its elimination in the 1960s [2]. Similar cases among French tourists visiting the same sites in Corsica have been reported recently [3,4].

As the family had never travelled to known schistosomiasis-endemic areas, the most likely location for acquiring schistosomiasis was south-eastern Corsica, as supported also by the French tourists who were presumably infected in the same area [3,4]. Schistosomiasis has not been described as endemic in Corsica so far; however, *Bulinus truncatus*, the vector (snail) of *S. haematobium* is known to be endemic in some areas of south Europe, including Corsica, where it transmitted zoonotic *S. bovis*, which in humans may cause skin irritation (cercarial dermatitis) due to the unsuccessful skin penetration of zoonotic cercariae [5-7]. Generally, *Bulinus* spp. snails are very tolerant to temperature change as they can survive temperatures between 2 °C and 40 °C [8,9]. Malacological investigations have shown that *S. haematobium* can develop in *Bulinus* spp. at temperatures around 20 °C [9]; however, the optimum temperature for infection of *Bulinus* spp. with *S. haematobium* miracidia is between 20 °C and 30 °C [9,10]. Cercarial shedding is also temperature dependent, with more cercariae shed at higher temperatures than at lower temperatures [11]; however, cercarial shedding can occur between 10 °C and 30 °C [12].

Physicians, especially urologists, should be alerted to the potential risk of schistosomiasis in individuals with haematuria or unclear genital symptoms who have never visited known schistosomiasis-endemic areas. Besides Corsica, schistosomiasis might be reintroduced to other endemic or formerly endemic areas for *Bulinus* snails, including southern Portugal, Sardinia, Sicily, Cyprus and Greece [11,13-16]. Since schistosomiasis is a chronic disease that may start with mild non-specific pruriginous cutaneous, urinary or genital tract symptoms [2,17,18], people who experience such symptoms and were exposed to surface fresh water in southern Corsica during past summers should be investigated [4].

Further studies are needed to identify the location of transmission sites, the focus and seasonality of transmission of *S. haematobium* in southern Corsica and to determine the origins of its introduction. Results of molecular genetic studies to identify the origin of the parasite and malacological studies to study the biology of the vector snails as well as the parasite's presence in the vector snails are pending.

Acknowledgments

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Conflict of interest
None declared.

Authors' contributions

JR and IMS took clinical care of the patients. MH, HM and GM performed the parasitological investigations. JR, MH, HM and GM looked for infected snails in Cavu River. All authors participated in writing the manuscript.

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Uptake and impact of a new live attenuated influenza vaccine programme in England: early results of a pilot in primary school-age children, 2013/14 influenza season

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As part of the introduction and roll-out of a universal childhood live-attenuated influenza vaccination programme, 4–11 year-olds were vaccinated in seven pilot areas in England in the 2013/14 influenza season. This paper presents the uptake and impact of the programme for a range of disease indicators. End-of-season uptake was defined as the number of children in the target population who received at least one dose of influenza vaccine. Between week 40 2013 and week 15 2014, cumulative disease incidence per 100,000 population (general practitioner consultations for influenza-like illness and laboratory-confirmed influenza hospitalisations), cumulative influenza swab positivity in primary and secondary care and cumulative proportion of emergency department respiratory attendances were calculated. Indicators were compared overall and by age group between pilot and non-pilot areas. Direct impact was defined as reduction in cumulative incidence based on residence in pilot relative to non-pilot areas in 4–11 year-olds. Indirect impact was reduction between pilot and non-pilot areas in <4 year-olds and >11 year-olds. Overall vaccine uptake of 52.5% (104,792/199,475) was achieved. Although influenza activity was low, a consistent, though not statistically significant, decrease in cumulative disease incidence and influenza positivity across different indicators was seen in pilot relative to non-pilot areas in both targeted and non-targeted age groups, except in older age groups, where no difference was observed for secondary care indicators.

Background

The United Kingdom (UK) has had a long-standing selective influenza vaccination programme that aims to directly protect populations at higher risk of severe disease due to influenza. This approach, as in many other countries in Europe, has been targeted at all those over 64 years of age and those less than 65 years in clinical risk groups, including pregnant women [1].

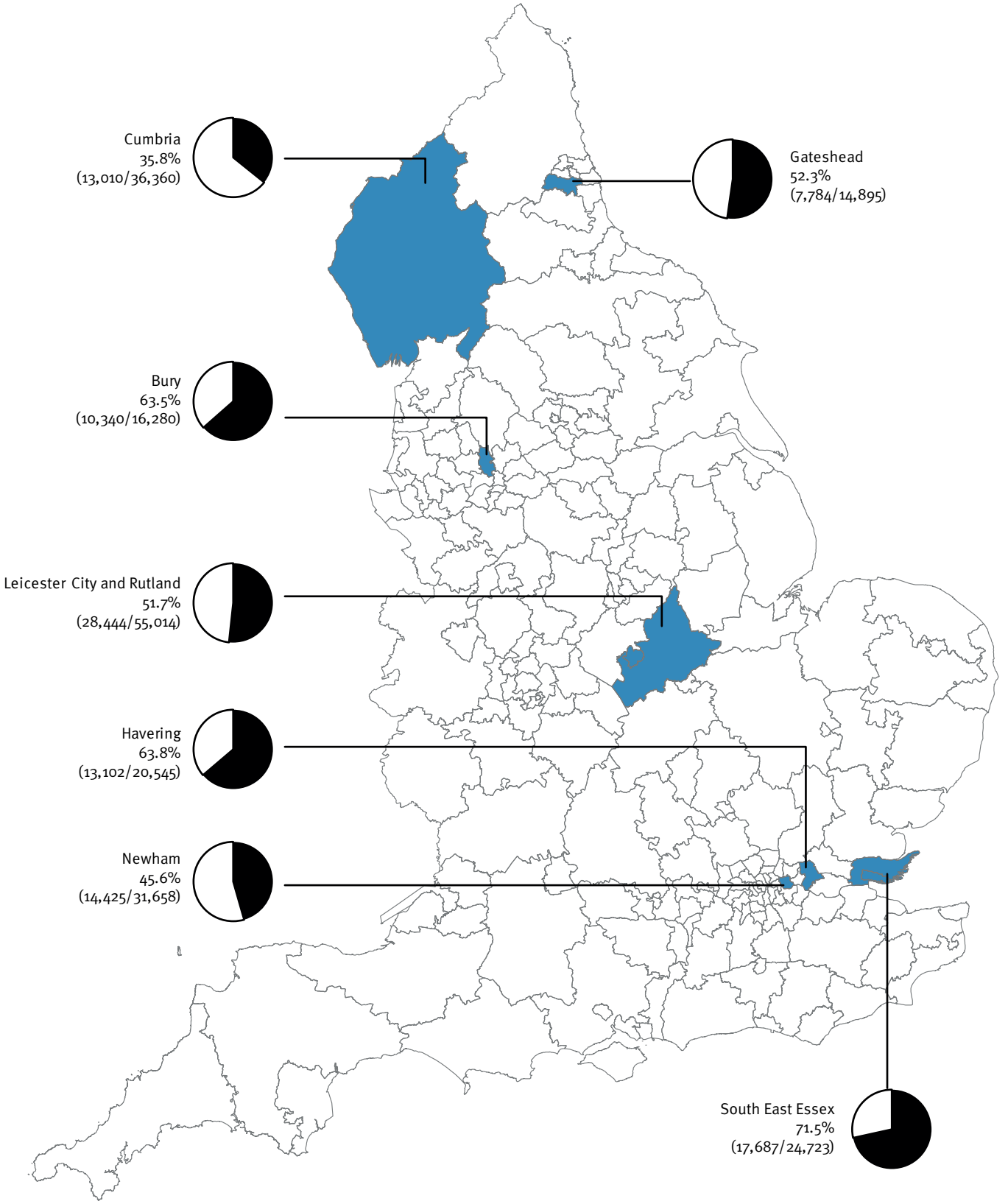
Although published work has demonstrated that the UK selective programme is cost-effective [2], it is apparent that there still remains a considerable burden of disease due to influenza in the population [3,4]. Children are recognised to play a key role in the transmission of influenza virus [5], with mathematical modelling predicting that targeting this group with influenza vaccine would not only reduce infection in immunised children themselves (direct programme impact) but also reduce influenza-related disease in other age groups, including elderly people, and individuals in high-risk groups (indirect programme impact) [6,7].

On the basis of this evidence and recommendations from the Joint Committee of Vaccination and Immunisation [8], the UK initiated a universal childhood immunisation programme with a newly licensed intranasally administered trivalent live attenuated influenza vaccine (LAIV) in the 2013/14 influenza season [9]. This programme is being rolled out over several seasons, with the ultimate intention of offering a single dose of LAIV to all healthy children aged 2–16 years annually. This is based upon published evidence that a second dose of LAIV provides only modest additional protection against laboratory-confirmed influenza infection (e.g. 60% versus 77% vaccine effectiveness for one and two doses, respectively) [11]. Influenza vaccine-naive children aged six months to less than nine years in clinical risk groups are offered two doses of vaccine, either LAIV or inactivated influenza vaccine for those in whom LAIV is contraindicated [10].

In this first season, the UK influenza vaccine programme targeted all children aged two and three years, reaching

Figure 1

Cumulative uptake of live attenuated influenza vaccine in primary school-age children[a] in pilot areas, England, 2013/14 influenza season



The pilot areas are shown on the map. The shaded area of each pie chart indicates the percentage of target children vaccinated.

[a] Aged 4–11 years.

a provisional uptake of 42.6% (308,925/724,747) and 39.6% (285,616/722,048) respectively in England [12]. In addition, a series of geographically discrete pilots of LAIV vaccination for primary school-aged children (4–11 years) were organised in England. Local NHS England teams interested in running pilot immunisation programmes submitted business cases which were evaluated and sites selected by the national team. Different models of delivery (in particular, school- versus community-based) were evaluated in these pilots.

Despite recommendations for universal childhood influenza immunisation in several countries, only limited observational data have been published on the impact of such programmes [13-15]. The implementation of the new UK childhood influenza vaccine programme provides an opportunity to add to this evidence base. This paper presents early results from the primary school-age pilots of the direct and indirect impact of such a programme for a range of disease indicators during the 2013/14 influenza season (over and above vaccination of preschool age children). As seen elsewhere in Europe, the 2013/14 season was dominated by the circulation of influenza A(H1N1)pdm09 virus, with evidence of community transmission at low intensity from weeks 5 to 15 (27 January to 13 April) 2014 [12].

Methods

Uptake of live attenuated influenza vaccine

Seven geographically discrete pilot areas were selected in England. The target population was defined as children of primary school age (4 to 11 years-old) resident in seven pilot areas: Bury, Cumbria, Gateshead, Leicester City and Rutland, Havering and Newham boroughs (in London) and South East Essex (Figure 1), covering about 5% of the population of this age in England. End-of-season programme uptake was calculated based on number of children in the target population who received at least one dose of influenza vaccine during the campaign period (September 2013 to January 2014). Uptake data were reported weekly by each NHS England pilot area team during the season to PHE using a bespoke web-based portal.

Disease indicators

LAIV programme impact was measured for a range of clinical and virological respiratory end points in primary and secondary care from week 40 2013 to week 15 (30 September 2013 to 13 April 2014), the end of notable community transmission of influenza [12]. To ensure appropriate surveillance coverage for each sentinel surveillance scheme (in primary care, hospital emergency departments and general hospital admissions), additional participating sites were recruited in each pilot area where required.

Surveillance in primary care was undertaken through monitoring the weekly influenza-like illness (ILI) consultation rates through the Royal College of General Practitioners (RCGP) Research and Surveillance Centre (RSC) sentinel general practitioner (GP) network, with nine practices participating in pilot areas and 78 in non-pilot areas. Sentinel practices, in conjunction with practices from the Sentinel Microbiology Network (SMN) scheme, undertook respiratory swabbing and testing with influenza virus polymerase chain reaction (PCR) assays for a proportion of patients presenting with ILI, including all patients under 17 years of age. Influenza swab positivity rates and GP consultation rates in pilot and non-pilot areas were compared by age group.

The Emergency Department Sentinel Surveillance System (EDSSS) monitors routine syndromic surveillance data, in real-time, using anonymised emergency department attendances, across a sentinel network of emergency departments [16]. Attendances monitored included those for acute respiratory illness (two emergency departments in pilot and 30 in non-pilot areas). The proportion of all EDSSS admissions coded as 'respiratory' in pilot and non-pilot areas was compared by age group.

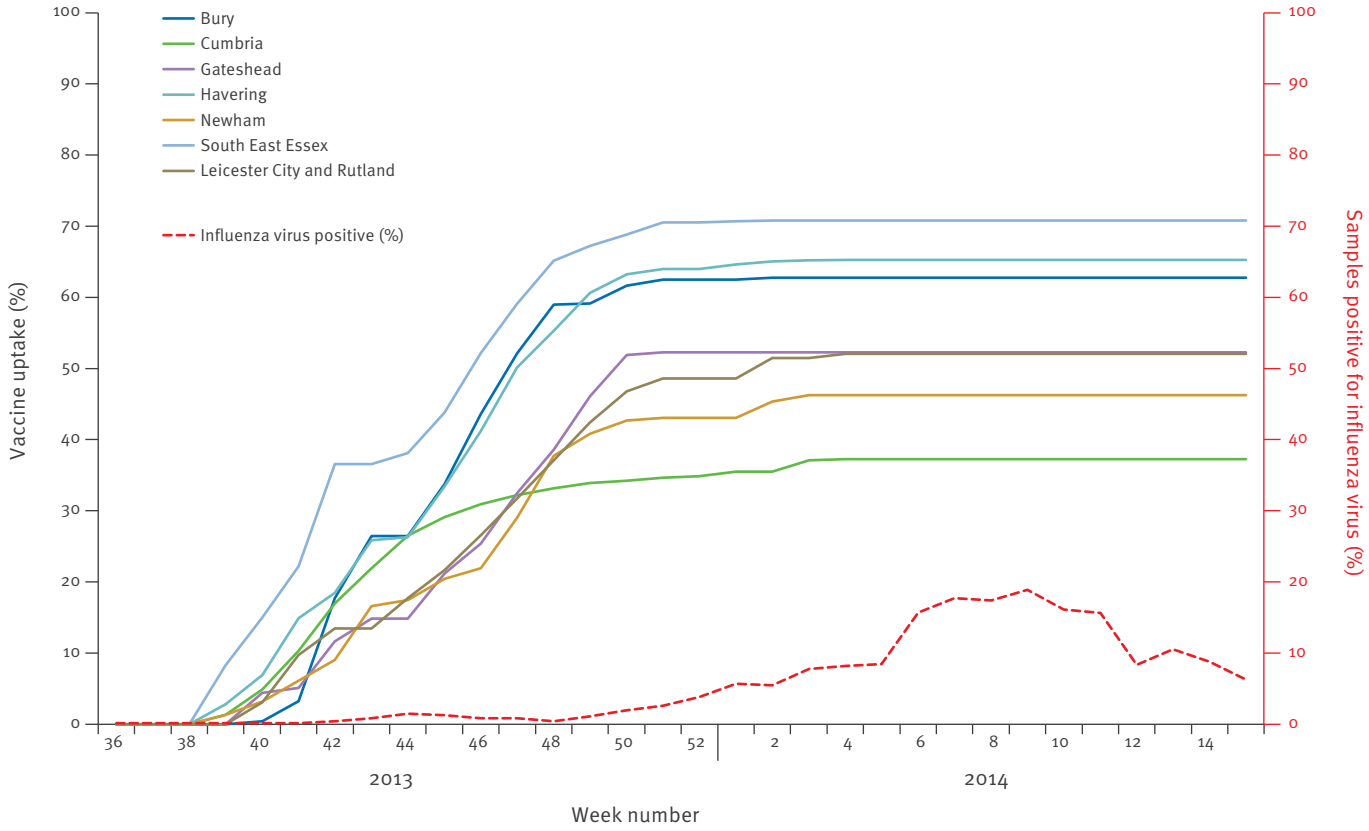
The UK Severe Influenza Sentinel Surveillance System (USISS) [17] consists of a network of 35 National Health Service (NHS) hospital trusts (nine in pilot areas and 26 in non-pilot areas) that report the number of laboratory-confirmed hospital and intensive-care unit (ICU) weekly admissions due to influenza. As routine USISS data were not in the LAIV target age groupings, influenza hospitalisation rates by age group for primary school-age children and non-targeted age groups were calculated for pilot and non-pilot areas using estimated hospital catchment populations [18]. As the age groups of hospital catchment populations did not match our targeted and non-targeted age groups, population estimates were adjusted in line with Office for National Statistics age-specific population proportions from mid-2012 population estimates [19].

The Respiratory DataMart scheme (RDMS) [20] reports all influenza virus PCR respiratory swab results from a network of PHE and NHS laboratories, with the majority of samples (>68%) [20] taken from patients in secondary care. Postcode of patients' residence was used to allocate patients to pilot and non-pilot areas. Influenza swab positivity rates in pilot and non-pilot areas were compared by age group.

Weekly excess mortality due to all causes and to respiratory illness was estimated in pilot and non-pilot areas based upon place of residence. The EuroMOMO (European monitoring of excess mortality for public health action) standard algorithm was used to calculate number of deaths expected for a given week in the year [21]. The number of observed deaths (corrected for reporting delay) was compared with the modelled number expected each week to determine if statistically significant excess mortality was seen in pilot and non-pilot areas.

Figure 2

Estimated weekly proportion of uptake of live attenuated influenza vaccine in primary school-age children[a] by pilot area and weekly proportion of samples positive for influenza virus[b], England, 2013/14 influenza season[c]



[a] Aged 4–11 years.

[b] Through the Respiratory DataMart scheme (RDMS).

[c] Week 36 2013 to week 15 2014 (2 September 2013 to 13 April 2014).

Measuring impact of the live attenuated influenza vaccine programme

Cumulative disease incidence rates per 100,000 population were calculated by summing the number of disease episodes each week from week 40 2013 to week 15 2014 relative to the population at risk. Cumulative influenza swab positivity was calculated by summing the number of positive samples and the number of samples tested each week from week 40 2013 to week 15 2014, with a similar calculation done for EDSSS respiratory attendances.

As a sample of primary and secondary care centres were recruited, sampling based statistical methods were used. Cumulative indicators were statistically compared overall and by age group between pilot and non-pilot areas for different indicators. Direct impact was defined as reduction in cumulative disease incidence based on residence in pilot and non-pilot areas in the target age group (4–11 year-olds). Indirect impact was defined as reduction in cumulative disease incidence over the same period between pilot and non-pilot areas in non-target age-groups (<4 years of age and >11 years of age). Cumulative incidence rates were compared between pilot and non-pilot areas by calculating risk ratios with 95% confidence intervals. Negative binomial regression was used to account for extra-Poisson variability between GPs or NHS hospital trusts within pilot and non-pilot areas. The cumulative proportion of samples positive for influenza virus and EDSSS admissions coded as respiratory were compared between the areas using logistic regression (giving odds ratios) with adjustment for overdispersion.

Results

Vaccine uptake

The total target population for the pilot study was estimated to be 199,475 children aged 4–11 years of age. Six of the seven pilot areas chose to deliver the programme through a school-based approach, while Cumbria delivered through community pharmacies and primary care. A total of 104,792 primary school-age children received at least one dose of LAIV or inactivated vaccine during the study period, an uptake of 52.5%. This ranged from 35.8% (Cumbria) to 71.5% (South

East Essex) at pilot level (Figure 1), with final uptake in all pilot areas reached when there was evidence of community influenza transmission from week 5 2014 onwards (Figure 2). Uptake by school year decreased from 56.1% (16,727/29,826) in reception class children (aged 4–5 years) to 49.7% (12,859/ 25,864) in children in year 6 (aged 10–11 years), with a steady decline in uptake with increasing age (chi squared test for trend p<0.0001).

Programme impact

The cumulative all-age ILI GP consultation rate was higher in non-pilot (64.5/100,000 population) than in pilot areas (17.7/100,000), with a similar pattern for all three age groups (Figure 3). The overall risk difference of pilot relative to non-pilot cumulative incidence was –46.8/100,000. Using data from RCGP, the risk ratio was 0.34 (an estimated impact of vaccination of 66%), though this was not statistically significant (Table).

The overall cumulative influenza swab positivity rate in primary care in pilot areas was 8.5% (15/176) compared with 16.2% (265/1,634) in non-pilot areas, with a consistent pattern for all three age groups (Figure 3). Derived from RCGP/SMN influenza virus positivity data, the odds ratios for pilot relative to non-pilot areas for children aged ≥12 years and all ages, with values of 0.54 and 0.53 respectively, were not statistically significant (Table).

Through EDSSS, the overall cumulative proportion of emergency department attendances coded as respiratory was 5.5% (2,804/51,413) in pilot compared to 8.7% (83,224/954,225) in non-pilot areas (Table), with a consistently lower cumulative proportion in children <4 years and aged 4 to <12 years, but no apparent difference in people older than 12 years. The overall odds ratio was 0.60 (estimated impact of 40%), which was not statistically significant, as was the case for age-specific estimates.

The cumulative all-age incidence of laboratory-confirmed influenza hospitalisations reported through the USISS sentinel scheme was 5.5 per 100,000 population in pilot compared with 7.0 per 100,000 in non-pilot areas (Table). The cumulative incidence of hospitalisations in <4 year old and 4–11 year old was higher in non-pilot compared with pilot areas; however, it was very similar for people aged 12 years or more (Figure 3). The overall risk difference of pilot vs non-pilot areas was –1.5/100,000 and risk ratio was 0.76 (an estimated impact of 24%), which was not statistically significant.

Through RDMS, overall cumulative influenza swab positivity was similar in pilot and non-pilot areas (Table). Similar age-specific cumulative positivity was seen for each age group (Figure 3), although time to cumulative peak positivity was shorter in non-pilot compared with pilot areas for 4–11 year-olds. The overall odds ratio (0.99) showed that there was little difference in pilot relative to non-pilot areas.

No significant excess all-cause or all-respiratory mortality was observed in pilot or non-pilot areas in children aged <4 years, 4–11 years or people aged ≥12 years.

Discussion

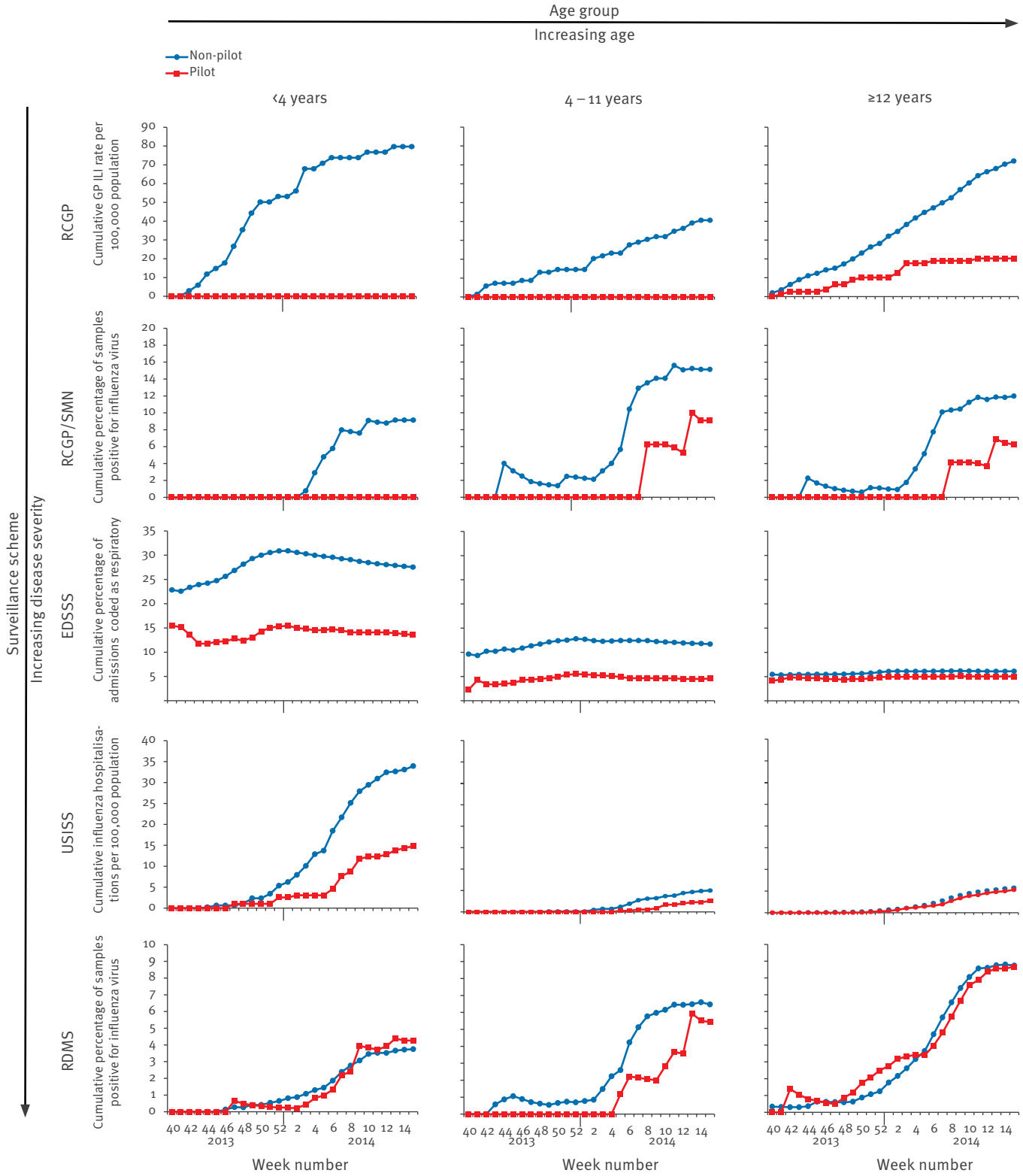
This pilot universal paediatric influenza vaccination programme achieved an overall uptake of 53% (ranging from 36 to 72% in individual pilot areas) in primary school-age children in the first year of implementation in England. Although the results were not statistically significant, the cumulative disease incidence was lower in pilot relative to non-pilot areas in both targeted and non-targeted age groups for a range of influenza indicators – both laboratory-confirmed and syndromic. These observed differences were smaller for more severe disease end-points.

The LAIV programme delivered in primary school settings (in six of the seven pilot areas) achieved a relatively good uptake in the target population, although there was variation in coverage by pilot area. The lowest uptake was observed in the one pilot area where delivery was through a community pharmacy/primary care setting. There was also significant variation in uptake by year group, with coverage levels highest among the youngest, with a steady decline with increasing age. These levels compare favourably with those achieved in the United States, where LAIV has been recommended for all children for several years. Implementation has been varied in the United States [14], with uptake of 41% reported in children 5–12 years of age from one study in 2011/12 [22]. The modelling work of Baguelin et al. suggests that reaching levels of 30% vaccine coverage in children would already start to produce substantial benefits [6]. Thus, the overall high uptake achieved in our target population in the first year, particularly with a school-based delivery model, augers well for the future. Further evaluation into factors that might explain local variation in uptake is under way and will inform future programme implementation.

These early results suggest a direct programme impact, with reductions in incidence seen for a wide range of influenza indicators including primary care consultations, swab positivity, hospitalisation of laboratory-confirmed cases and percentage of respiratory-coded emergency department attendances in pilot vs non-pilot areas for 4–11 year-olds. A direct impact among the immunised group of at least 25–30% would be expected, based on the observed uptake of 53% with a moderately effective vaccine (with a vaccine effectiveness of 50–60%). No evidence of a reduction, however, in swab positivity from RDMS data, which relate mainly to samples taken in secondary care settings, was seen in pilot compared with non-pilot areas for the same age group. Direct impact of such school-based programmes has previously been demonstrated in North America for end points such as emergency department

Figure 3

Cumulative disease indicators in pilot vs non-pilot areas by age group across surveillance schemes, England, 2013/14 influenza season[a]



EDSSS: Emergency Department Sentinel Surveillance System; GP: general practitioner: ILI: influenza-like illness; RCGP: Royal College of General Practitioners; SMN: Sentinel Microbiology Network; USISS: UK Severe Influenza Sentinel Surveillance System; UK: United Kingdom.

[a] Week 40 2013 to week 15 2014 (30 September 2013 to 13 April 2014).

Table

Cumulative primary care consultations, hospitalisations, influenza positivity and emergency department attendances in children (<4, 4–11 years) and ≥12 year-olds in pilot and non-pilot areas, England, 2013/14 influenza season[a]

Surveillance scheme	Disease indicator	Age group (years)	Pilot areas	Non-pilot areas	Ratio[b] (95% CI)	p value
RCGP	**Number of sentinel GPs**		**9**	**78**		
	Cumulative GP ILI consultation rate per 100,000 population[c]	<4	0.0	73.6	0 (0–1.47)	0.170
			(0/3,641)	(27/36,672)		
		4–11	0.0	37.9	0 (0–1.33)	0.110
			(0/7,809)	(28/73,957)		
		≥12	20.3	66.8	0.38 (0.08–1.86)	0.232
			(16/78,953)	(483/723,075)		
		Total	17.7	64.5	0.34 (0.07–1.72)	0.194
			(16/90,403)	(538/833,704)		
RCGP/SMN	**Number of swabbing GPs**		**10**	**76**		
	Cumulative proportion (%) of swabs positive for influenza (n/N)[d]	<4	NA	9.1	1 (0–4.58)	1.000
			(0/9)	(17/186)		
		4–11	9.1	15.1	0.49 (0.07–3.26)	0.462
			(2/22)	(23/152)		
		≥12	9.1	17.3	0.54 (0.28–1.04)	0.067
			(13/143)	(221/1,276)		
		Total	8.5	16.2	0.53 (0.28–1.01)	0.055
			(15/176)	(265/1,634)		
EDSSS	**Number of sentinel emergency departments**		**2**	**30**		
	Cumulative percentage of emergency department admissions coded as respiratory (n/N)[d]	<4	13.6	27.5	0.42 (0.16–1.09)	0.075
			(361/2,658)	(26,645/96,747)		
		4–11	4.6	11.7	0.36 (0.10–1.33)	0.127
			(141/3,080)	(8,950/76,471)		
		≥12	5.0	6.1	0.81 (0.38–1.72)	0.583
			(2,302/45,675)	(47,629/781,007)		
		Total	5.5	8.7	0.60 (0.30–1.19)	0.146
			(2,804/51,413)	(83,224/954,225)		
USISS	**Number of sentinel NHS hospital trusts**		**9**	**26**		
	Cumulative incidence of laboratory-confirmed influenza hospitalisations per 100,000 population[c]	<4	14.8	31.4	0.37 (0.11–1.25)	0.111
			(29/195,379)	(146/465,442)		
		4–11	2.6	5.0	0.28 (0.13–1.56)	0.203
			(9/352,911)	(42/840,722)		
		≥12	5.3	5.8	0.93 (0.43–2.04)	0.858
			(174/3,293,487)	(452/7,845,918)		
		Total	5.5	7.0	0.76 (0.33–1.75)	0.516
			(212/3,841,777)	(640/9,152,082)		
RDMS	Cumulative percentage of swabs positive for influenza virus (n/N)[d]	<4	4.3	3.7	1.13 (0.30–4.26)	0.858
			(31/727)	(262/6,991)		
		4–11	5.4	6.5	0.79 (0.21–3.05)	0.735
			(7/129)	(98/1,514)		
		≥12	8.7	8.7	1.02 (0.39–2.66)	0.966
			(91/1,050)	(1,110/12,704)		
		Total	6.8	6.9	0.99 (0.35–2.80)	0.988
			(129/1,885)	(1,432/20,820)		

CI: confidence interval; EDSSS: Emergency Department Sentinel Surveillance System; GP: general practitioner: ILI: influenza-like illness; n/N: number positive/number tested; RCGP: Royal College of General Practitioners; SMN: Sentinel Microbiology Network; USISS: UK Severe Influenza Sentinel Surveillance System; UK: United Kingdom.

[a] Week 40 2013 to week 15 2014 (30 September 2013 to 13 April 2014).

[b] When the numerator was zero in the pilot area, ratio confidence intervals were calculated using Fisher's exact test.

[c] Risk ratio calculated with negative binomial regression.

[d] Odds ratio calculated with logistic regression, correcting for overdispersion.

consultations and school absenteeism [13,14], but, as in this study not for other more severe disease end points [15].

There was also a suggestion of an indirect impact of the programme, which was an important contributor to the estimated cost-effectiveness of the new universal childhood influenza vaccine programme in the earlier modelling work [6]. Reductions, albeit non-significant, in GP ILI consultation rate and proportion of respiratory swabs positive for influenza in primary care for non-targeted age groups, particularly in children under 4 years and also to some extent in people older than 11 years were seen. Such indirect effects have been seen previously for less severe end points in the United States [15]. Little evidence of indirect impact, however, was seen in our study for influenza hospitalisations, swab influenza positivity rate (from RDMS), emergency department admissions coded as respiratory and excess mortality in older people. Some potential explanations for this are outlined below. Further work is required to understand these differences between schemes and disease severity.

There are several potential limitations to this study. Firstly, the 2013/14 influenza season in the UK was characterised by influenza A(H1N1)pdm09 virus circulation, the novel pandemic strain that first emerged in 2009: across surveillance schemes, only moderate influenza activity was seen predominately in the hospital-based surveillance systems and mainly in younger adults. There was little signal of influenza activity either in primary care or from syndromic surveillance, nor was there evidence of excess mortality in elderly people. Along with the small geographical coverage of the pilot areas, this will have limited the ability of the school-age pilot programme to detect evidence of direct and indirect impact. Secondly, older people, who are typically susceptible to severe disease following influenza virus infection, are recognised to have background immunity to influenza A(H1N1)pdm09 [23], hence the lack of impact in relation to excess mortality among elderly people and why so few lives are likely to have been saved by the LAIV programme in the 2013/14 influenza season. Thirdly, the potential indirect effects of the programme (through reduction in transmission) would be diluted through opportunities for populations (e.g. adult unvaccinated groups) to move back and forth into pilot areas, thus reducing the potential herd effects of vaccinated paediatric groups. This may also explain why the time to peak positivity was shorter for non-pilot compared with pilot areas for some indicators. Fourthly, we were very aware of the possibility of cluster effects, with the data being at the GP or hospital trust level. For this reason, we carefully examined each outcome indicator for evidence of over-dispersion and as a consequence employed the more conservative negative binomial regression (rather than Poisson regression). Fifthly, a sample of GP practices and hospitals were newly recruited to surveillance schemes in pilot areas raising the possibility of differential reporting.

These early, first season findings, which are consistent across a range of surveillance indicators, highlight the apparent value of vaccinating primary school children. The encouraging uptake levels achieved in most pilot areas demonstrate the feasibility of delivering such a programme in this population. While the estimates of programme impact were not statistically significant, it is encouraging that both direct and indirect impact (higher estimates in non-pilot relative to pilot areas) was seen across a range of surveillance schemes in primary care. The results were more nuanced for severe end points, where an impact was observed in children aged under 11 years (both targeted and non-targeted), but not in older age groups, which is an important contributor to the cost-effectiveness of the programme. These findings highlight the importance of further evaluation of data from the 2013/14 season. In 2014/15, pilot areas will continue to administer LAIV in primary school, with additional pilots in secondary school-age children (age 11–13 years) [24]. It will be important to continue the surveillance started in 2013/14, to determine if the observations presented here are repeatable and further quantify them to inform optimal roll-out.

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Conflict of interest

None declared.

Authors' contributions

RP led the design of the study and study group; all co-authors were members of the study group and involved in data collection, management and analyses; HG, NA and RP led the data analysis; HG undertook the summary analyses; HZ was responsible for the RDMS system, data management and analysis; NB was responsible for the USISS system, data management and analysis; ZB, AE and GES were responsible for the EDSSS data system management and analysis; HD and SL were responsible for the RCGP data system management and analysis; JE, MD and MZ were responsible for virological testing schemes; NS, AS and LL were responsible for monitoring of vaccine uptake in the pilot sites; RP drafted

the initial manuscript with HG; all co-authors reviewed and commented including approval of the final version.

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Emergence and molecular characterisation of non-toxigenic *tox* gene-bearing *Corynebacterium diphtheriae* biovar mitis in the United Kingdom, 2003–2012

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Non-toxigenic *Corynebacterium diphtheriae* have become increasingly recognised as emerging pathogens across Europe causing severe invasive disease. A subset of non-*tox*igenic *C. diphtheriae* are 'non-toxigenic *tox* gene-bearing' (NTTB) strains; these strains are genotypically *tox*positive, but do not express the protein. The circulation of NTTB strains was first observed during the 1990s upsurge of diphtheria in Eastern Europe but has not been reported in other European countries. Circulation of NTTB strains could be considered an increased risk for diphtheria and other related diseases, given their possible role as a *tox* gene reservoir with the theoretical risk of re-emerging toxin expression. Here we report the characterisation of 108 non-toxigenic *C. diphtheriae* biovar mitis isolates submitted to the World Health Organization (WHO) Global Reference Centre for Diphtheria at Public Health England, London, between 2003 and 2012, in order to determine the presence of NTTB strains. Using molecular methods, five NTTB isolates were identified; four human isolates (MLST type 212) and one isolate from a companion cat (MLST type 40). The emergence of these strains could indicate continuation of the circulation of potentially toxigenic strains and appropriate laboratory diagnostic methods should be used for detection. Given the complacency that currently exists in Europe awareness with regards to diphtheria diagnostics must be enhanced.

Introduction

Infections caused by toxigenic strains of *Corynebacterium diphtheriae* have become uncommon in Europe as a result of widespread immunisation, implemented during the first half of the 20th Century. In the European Union (EU)/European Economic Association (EEA) countries, the number of reported (toxigenic) diphtheria cases has declined over the past years to 46, 15 and 16 confirmed cases in 2008, 2009 and 2010, respectively [1]. However, diphtheria is still present in all six World Health Orgainization (WHO) regions and new epidemics are regularly reported [2]. These and the Eastern European epidemic in the 1990s with more than 157,000 cases and 5,000 deaths between 1990 and 1998, clearly demonstrate the unbroken threat of the disease in the post-vaccine era [3].

The toxigenicity of *C. diphtheriae* strains is solely attributed to the expression of a very potent exotoxin (DTX) which inhibits protein synthesis in mammalian cells [4]. The structural gene (*tox*), consisting of subunits A and B, is carried by a corynebacteriophage and regulated by the chromosomally encoded regulator DtxR (diphtheria toxin repressor). Integration of *tox*-carrying bacteriophages into the bacterial genome can convert non-toxigenic strains into toxigenic and virulent strains. This transformation has been described for example in patients but is generally believed to occur rarely in nature [4,5].

Typical diphtheria is caused by infection with toxigenic strains of *C. diphtheriae*, leading to respiratory or cutaneous symptoms. The characteristic of severe respiratory diphtheria is the presence of a strongly adherent greyish-white pseudomembrane, typically progressing from the tonsils into the larynx and trachea and suffocation following aspiration of the membrane is a common cause of death in untreated cases [6]. Cutaneous diphtheria is more common in tropical regions with a usually mild non-systemic clinical presentation in the form of infected skin lesions and shallow ulcers often occurring in combination with poor hygienic conditions [6]. The most effective treatment against diphtheria is the diphtheria antitoxin (DAT) which binds and neutralises circulating toxin which has not yet bound to tissue. DAT is therefore only recommended for treatment of acute disease. The only way to induce long lasting immunity and to prevent the disease is vaccination; the diphtheria vaccine is one of the oldest vaccines and available as bi- (in combination with tetanus toxoid (DT/dT)) or trivalent vaccine (tetanus, diphtheria,

pertussis (DTacP)) or as combination vaccine with *Haemophilus influenzae* type b (Hib) vaccine) or inactivated polio vaccine (IPV), depending on national vaccination schedules [7].

Non-toxigenic strains have become increasingly recognised as causes of severe invasive disease causing e.g. endocarditis and bacteraemia [8-10]. Infections caused by non-toxigenic *C. diphtheriae* are not preventable by vaccination and pathogenic mechanisms are generally not well understood.

Non-toxigenic *C. diphtheriae* usually completely lack the *tox* gene; exceptionally some non-toxigenic strains also bear the *tox* gene. These strains are genotypically tox-positive, but do not express the protein. They are called 'non-toxigenic *tox* gene-bearing strains' (NTTB).

During and after the 1990s diphtheria epidemic in countries in the Eastern part of the WHO European region, circulation of NTTB strains was widely observed. In 2004, Melnikov et al. published a study on 828 *C. diphtheriae* non-toxigenic strains isolated in different regions of Russia between 1994 and 2002, and found approximately 10% to be NTTB strains [11,12]. All NTTB strains were found to belong to *C. diphtheriae* biovar mitis; some of the isolates were from patients with severe respiratory illnesses. Molecular analyses of the NTTB isolates suggested two mechanisms contributing to the blockage of *tox* gene expression: a single base deletion (52-55 bp), resulting in a frame shift, or the presence of an insertion element (38-46 bp), both localised in the A-subunit of the *tox* gene. Similar studies have, however, not been conducted in other parts of the European region.

At present, the prevalence and epidemiological significance of *C. diphtheriae* NTTB strains across Europe is unknown. The circulation of NTTB strains could be considered an increased risk for diphtheria, given the possible role of these strains as a *tox* gene reservoir in the population and the theoretical possibility of re-emerging toxin expression through spontaneous reversion into toxigenic strains or through homologous recombination between different corynebacteriophages [5].

A recent and unique European screening study comprising 10 European countries and coordinated by the Diphtheria Surveillance Network (DIPNET) (13), showed that toxigenic, non-toxigenic and NTTB strains of *C. diphtheriae* are circulating in Europe, despite a high vaccination coverage among children in many European countries and a presumed absence of clinical disease [14]. Several European countries reported an increase in non-toxigenic *C. diphtheriae* infections in recent years based both on surveillance data and also related to outbreaks [10,15].

The gold standard for laboratory diagnosis of diphtheria is the phenotypic Elek test for toxigenicity characterisation; strains that phenotypically express the toxin always carry the gene. However, if Elek is the sole test used, the presence or absence of the *tox* gene cannot be determined in strains not expressing toxin. The aim of this study was therefore, to determine the presence, estimate the prevalence and characterise, using molecular methods, NTTB strains submitted to the WHO Global Reference Centre for Diphtheria at Public Health England, London, United Kingdom (UK) between 2003 and 2012 in order to support public health management of diphtheria.

Methods

One-hundred and twenty-two *C. diphtheriae* biovar mitis isolates were received during the period from 15 November 2003 to 16 July 2012, all were human isolates from the UK, except for one, which was originally isolated in Belgium from a cat's nasal swab (this was referred to the reference unit for confirmation of toxigenicity). In case of multiple specimens from one individual only the first isolate was considered. All specimens received in the laboratory were cultivated on blood agar and tellurite agar for initial screening. Typing and biochemical characterisation of all isolates was performed using the API® Coryne test (API bioMérieux, Marcy l'Etoile, France) in addition to the pyrazimidase, the cystinase and the toxigenicity tests (Elek test, polymerase chain reaction (PCR)) if applicable [16]. Five isolates had been previously identified as toxigenic (tox +) by Elek test [17,18] and were excluded from the analysis together with nine specimens that could not be recultivated. PCR analysis was performed for 108 specimens. Two strains, NTTB strain 99/CD/196 (ribotype St. Petersburg, isolated in 1999) and NCTC 13129 (GenBank: NC_002935.2) were included in the molecular analysis as reference strains.

For extraction of bacterial DNA, overnight cultures of *C. diphtheriae* strains cultivated on blood agar were incubated at 37°C (16). A loopful of bacterial culture was added to 500 µl sterile water and heat-treated for 30 minutes at 100°C. Cell debris were separated by centrifugation (12,000 rpm, 1 min) and the supernatant containing DNA was transferred to a sterile tube. For PCR reactions, 1-5 µl of supernatant was used.

Polymerase chain reaction and sequencing of the *tox* gene

Amplification of the *tox* gene (subunit A) was performed as described previously [19,20] using the Novocastra *C. diphtheriae* Primer Set (Leica Microsystems) according to manufacturer's instructions; each reaction included an internal positive control as amplification control. For direct DNA sequencing, the *tox* gene was amplified using the HotStarTaq Master Mix (Qiagen) and selected primers as previously described [21] or primers specifically designed for this study (Table 1 and Figure 1). Sequencing was performed with a BigDye Terminator v1.1 or v3.1 Cycle Sequencing kit (Applied Biosystems) and sequencing reactions were set up using the PCR primers. Sequence data were analysed using the

Table 1

Primers for polymerase chain reaction analysis and sequencing of non-toxigenic *tox* gene-bearing *Corynebacterium diphtheria*, United Kingdom 2012

Primer	Position[a]	Nucleotide sequence (5' -3')	length (bp)
Dipht 1F [21]	(-130) - (-110)	TTGCTAGTGAAGCTTAGCTAG	**NA**
Dipht 4R [21]	817-837	TGCCGTTTGATGAAATTCTTC	**973**
Dipht 4F [21]	535-555	GAACAGGCGAAAGCGTTAAGCG	**NA**
Dipht 8R [21]	1731-1751	TCTACCTGTGCATACTATAGC	**1217**
NTTB 10F[b]	(-25)-(-4)	ATGAGTCCTGGTAAGGGGATACGTTGT	**581[c]**
NTTB 11F[b]	(-694)-(-669)	AGCTGCATGAGTGTTGTAGCTGCTT	**840[d]**
NTTB 12F[b]	(-399)- (- 372)	GGCCTGATGATATTGATCTAGATGAGA	**545[d]**
NTTB 12R[b]	117 - 146	TTAGTCCCGTGGTACGAAGAAAAGTTTTC	NA

NA: not applicable

[a] relative to the first nucleotide of the start codon (+1)
[b] this study
[c] with Dipht 4R
[d] with Diph 12R

Nucleic acid position indicated according to *Corynebacterium diphtheriae* NCTC 13129 (GenBank Reference Sequence: NC_002935.2)

softwares BioNumerics (Applied Maths) and BioEdit (http://www.mbio.ncsu.edu/BioEdit/page2.html).

Multilocus sequence typing (MLST) based on a sequencing scheme comprising seven housekeeping genes was performed as described previously [22].

All isolates were subjected to both the conventional and the modified Elek tests as described previously [16-18].

Antimicrobial sensitivity testing

Antimicrobial sensitivity testing of NTTB isolates was performed according to British Society for Antimicrobial Chemotherapy (BSAC) [23]/The European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2013 guidelines for coryneform organisms [24]. Using Etest strips (bioMérieux, Marcy l'Etoile, France) minimum inhibitory concentration (MIC) breakpoints in mg/L of ›0.12 (resistant (R)) and ‹0.12 (sensible (S)) were considered for Penicillin [25].

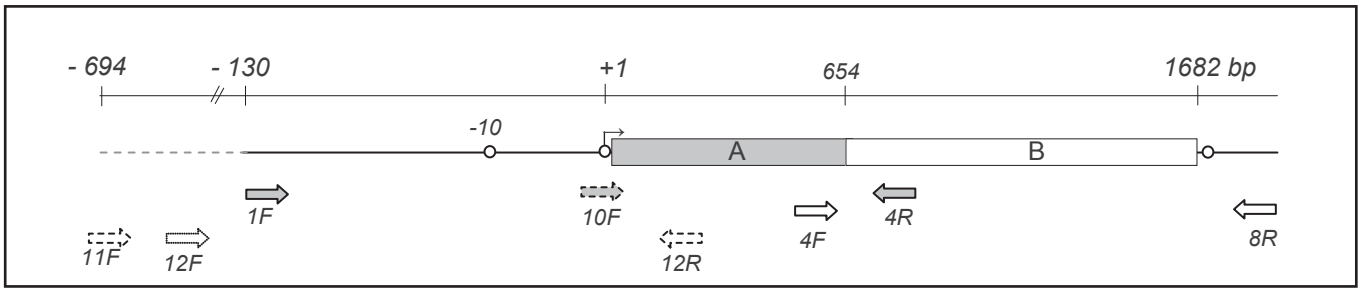
Results

Pure cultures for the 108 *C. diphtheriae* biovar mitis strains were mainly referred from throat swabs (n=87; 80.6%), wound swabs (n=10; 9.3%) and furthermore from blood culture (n=1), nasal swab (n=2) swab/other (n=1), pus (n=1), skin (n=2), ulcer (n=1), for three specimens, no information was provided; 62 specimens were from men (57.4%) and one from an animal (cat). The average age of the human patients was 27.3 years (range 1-72).

C. diphtheriae biovar mitis isolates analysed in this study were submitted between July 2003 and November 2012 with the highest number of isolates submitted (per calendar year) in 2005 (n=14) and 2004 (n=10) and

Figure 1

Scheme of the *tox* gene and location of sequencing primers used in this study



Primers were selected for sequencing of subunit A (grey) and subunit B (white) of the *tox* gene.Primers indicated in dotted lines were designed for sequencing and determination of the promoter and upstream region of the four human non-toxigenic *tox* gene-bearing *Corynebacterium diphtheria* isolates.

Position numbers correspond to *Corynebacterium diphtheriae* NCTC 13129 (GenBank Reference Sequence: NC_002935.2)

Table 2

Identified non-toxigenic *tox* gene-bearing and toxigenic *Corynebacterium diphtheriae* biovar mitis strains among isolates submitted to the Diphtheria Reference Unit, United Kingdom, 2003–2012 (n=10)

Isolate	Sex	Age[a]	Specimen	Characteristics
2011/1	M	27	Throat swab	NTTB (PCR+, tox–)
2011/2	M	24	Throat swab	NTTB (PCR+, tox–)
2011/3	M	24	Throat swab	NTTB (PCR+, tox–)
2012/1	M	27	Throat swab	NTTB (PCR+, tox–)
2012/cat	Cat		Nasal swab	NTTB (PCR+, tox–)
2012/1	M	67	Wound swab	Toxigenic (PCR+, tox+)
2010/1	F	57	Skin swab	Toxigenic (PCR+, tox+)
2009/1	M	72	Wound swab	Toxigenic (PCR+, tox+)
2008/1	M	17	Throat swab	Toxigenic (PCR+, tox+)
2008/2	F	7	Bronchoalveolar lavage	Toxigenic (PCR+, tox+)

F: female; M: male; NTTB: non-toxigenic *tox* gene-bearing; PCR: polymerase chain reaction; PCR+: positive; tox: result from conventional and modified tox+/tox–: tox-positive/tox-negative in conventional and modified Elek test.

[a] Age refers to age at time point when specimen was taken.

Toxigenicity was determined for all strains using the conventional and modified Elek test and molecular methods (PCR). Full antibiotic sensitivity to penicillin was determined for all non-toxigenic *tox* gene-bearing isolates.

a consistent number of isolates submitted between 2006 and 2011 (n= 5 to 8 isolates per year).

Overall, five *C. diphtheriae* biovar mitis NTTB strains (4.6 %) were identified through PCR analysis and the phenotypic non-toxigenicity for all five NTTB strains was subsequently reconfirmed, using the conventional and modified Elek tests. All five *C. diphtheriae* biovar mitis NTTB strains were referred between 2011 and 2012. One of the NTTB strains originated from a nasal swab of a cat. All four human strains were isolated from throat swabs taken from men (Table 2).

Molecular characterisation of *tox* gene-bearing- and toxigenic *Corynebacterium diphtheriae* biovar mitis strains

The five identified *C. diphtheriae* biovar mitis NTTB isolates were subjected to further molecular analysis which included whole *tox* gene sequencing (Figure 2) and molecular typing using MLST [22]. For control and reference purposes, three of the toxigenic strains (*C. diphtheriae* biovar mitis, 2010/1, 2008/1 and 2008/2 (Table 1)) and one previously described NTTB strain from the 1990s Eastern European diphtheria epidemic (St. Petersburg 1999/196) were also included.

Analysis of the sequencing data revealed the presence of the whole *tox* gene, including subunits A and B for all five NTTB isolates. In contrast, the NTTB strain St. Petersburg 1999/196 contained only subunit A of the *tox* gene. Subsequent sequence analysis revealed identical sequences for all isolates subjected to sequencing for the *tox* subunit B (data not shown) and sequence variations for toxsubunit A and within the respective promoter regions (Figure 2).

All four human NTTB strains showed identical sequences for the *tox* subunit A which included a one base pair (bp) deletion at position 25 (Figure 2). The region upstream from the start codon could not be sequenced in the four human NTTB isolates, which included the promoter region, approximately 690 bp, from position -25 to -694, strains 2011/1-3, 2012/1 (Figure 2).

Interestingly, *tox* subunit A in the animal NTTB isolate (2012/cat) was identical to the Russian NTTB strain (St. Petersburg 1999/196) and included an identical promoter region, a one base pair deletion (bp 55) and a one base pair substitution (bp 60) in comparison to the toxigenic strains (Figure 2).

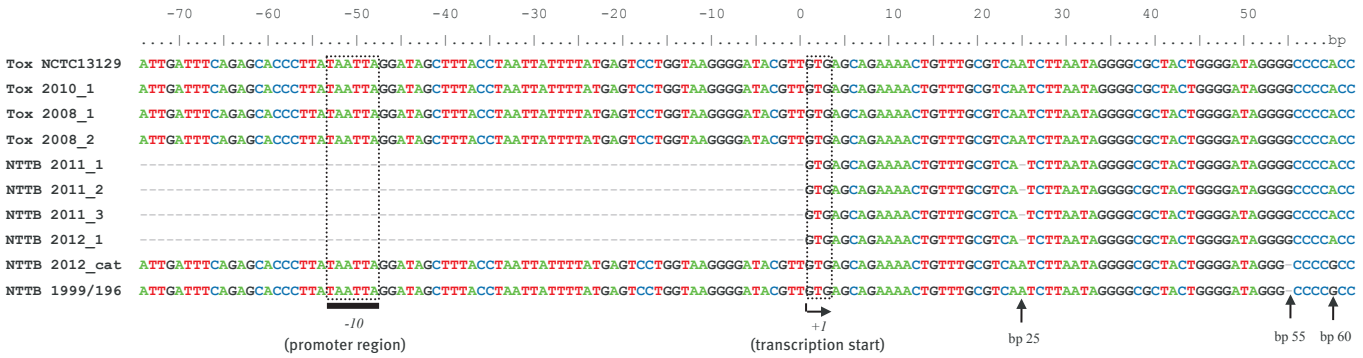
Molecular typing, using MLST, revealed similar MLST sequence types (ST) for the four human NTTB strains (ST 212) and different MLST sequence types for the NTTB isolate from the cat (ST 40) and the toxigenic isolates (STs 500 and 67).

Basic epidemiological features

Only basic epidemiological information for the NTTB isolates could be extracted from the laboratory referral forms. All four human NTTB isolates were throat swabs and collected from young men (Table 1) temporally and/or spatially dispersed. Specimens 2011/1 and 2011/2 were both collected during the same week in April 2011 but in different cities in the UK (distance › 300 km). Isolate 2011/3 was collected November 2011 and in the same city as isolate 2011/2. Subsequently, isolate 2012/1 was collected in January 2012 in the same city as isolate 2011/1. No clinical or further epidemiological information was provided on the referral

Figure 2

tox gene sequence alignment (-75-+62 bp) of non-toxigenic *tox* gene-bearing *Corynebacterium diphtheriae* biovar mitis strains identified and selected reference strains, United Kingdom 2012



NTTB: non-*tox*igenic *tox* gene-bearing; Tox: toxigenic strains.

Sequencing analysis revealed identical sequences for the four human isolates (NTTB 2011/1-3, 2012/1) including a one base pair deletion (bp 25) and a C>T replacement (bp 244, data not shown). Sequence similarity was revealed for the animal NTTB isolate (NTTB 2012/cat) and the reference Russian isolates (NTTB 199/196) including a one base pair deletion (bp 55) and base pair replacements (bp 60 (A>G) and 431 (G>A, not shown)). All deletions and replacements were located within subunit-A of the *tox* gene.

forms. The animal isolate was from a nasal swab taken from a cat whose owner was diagnosed with a *C. ulcerans* leg ulcer infection.

Discussion

Non-toxigenic *C. diphtheriae* are increasingly recognised as pathogens across Europe (26). However, in contrast to the toxigenic *C. diphtheriae* strains, disease and epidemiology is less well understood and monitored and currently, non-toxigenic *C. diphtheriae* are not included in the case-definitions for surveillance of diphtheria in Europe [27] and no public health action beyond antibiotic treatment is implemented [28].

In the 1990s and during the upsurge of epidemic diphtheria in Eastern Europe, a subset of non-toxigenic *C. diphtheriae* were described which were *tox* gene-positive, but did not express the protein, the so called NTTB. In this study, we describe, to our knowledge, for the first time after the epidemic upsurge of diphtheria in the 1990s, systematic screening for NTTB strains, conducted among non-toxigenic *C. diphtheriae* biovar mitis isolates submitted from the UK (plus one animal isolate from Belgium) to the WHO Global Reference Centre at Public Health England, between 2003 and 2012. Overall, five NTTB isolates (4.6 %) were identified among 108 specimens, isolated from four human patients and one animal (cat) carrier. Unfortunately, no information could be obtained on the nature of symptoms or the severity of disease amongst the patients.

MLST of the four human NTTB isolates revealed sequence type (ST) 212 for all four isolates. This ST has until now only been documented twice in the published literature (29, 30). Interestingly, both reports described men; one symptomatically infected with a toxigenic *C. diphtheriae* biovar mitis (ST 212) strain and one asymptomatic carrier of *C. diphtheriae* biovar mitis (ST 212), retrospectively identified as an NTTB strain [29,30]. Furthermore, all six ST 212 *C. diphtheriae* biovar mitis isolates described thus far were collected between March 2011 and January 2012; however, they were widely dispersed across Europe (France, Germany, UK).

This study shows that *C. diphtheriae* biovar mitis NTTB strains are circulating in the UK. The temporal pattern, the limited information about patients and the basic molecular analysis of the four human isolates suggests a recent clonal introduction and spread rather than a wide circulation of NTTB strains in the UK. Sequence type 212 described in the German case report (30) was associated with sexual transmission and interestingly, all six ST 212 isolates described to date were isolated from men. Sexually transmitted or -associated diphtheria infections have been described rarely in the literature thus far. A prospective screening study from the 1990s performed at a Genitourinary Medicine (GUM) Clinic in the UK found six (1%) mildly symptomatic pharyngeal carriers of *C. diphtheriae* in a cohort of 578 homosexual men and one (0.05%) asymptomatic carrier in a cohort of 653 heterosexual men and no carriers among 1,043 women [31], but similar and more recent studies are lacking. However, a more detailed and discriminatory molecular analysis (e.g. ribotyping) in addition to detailed epidemiological information is required to allow definite conclusions about the route of transmission.

Molecular analysis of the four human NTTB isolates revealed a new genetic pattern, including a potential deletion of approximately 600 bp´s upstream of the start codon which could not be sequenced and a one

base pair deletion at position 25 within *tox* subunit A . Previously and during the 1990s diphtheria epidemic characterised NTTB strains were described with a single base pair deletion (bp 52-55) or the presence of an insertion element (bp 38-46) [11,12]. Despite various attempts using PCR-based methods, the nature of genetic rearrangements in the promoter region of the four human NTTB isolates could not be determined. Further studies including e.g. whole-genome sequencing are needed to analyse the genetic rearrangements and understand the phylogenetic evolution of these strains. Ideally this study could be aimed at a more comprehensive strain collection of NTTB strains circulating in different countries in Europe and beyond.

Interestingly, sequencing analysis of subunit A of the *tox* gene of the animal NTTB isolate (2012/cat) revealed identical sequences with the Russian NTTB isolate (1999/96), representing the 'old' NTTB strains circulating during the 1990 Eastern European epidemic. Thus far, only one similar case has been reported in the literature; in 2010 a non-toxigenic *C. diphtheriae*, biovar belfanti though, was isolated from a domestic cat and retrospectively identified as an NTTB strain [32].

In a recent study on 103 non-toxigenic *C. diphtheriae* isolates collected between 1977 and 2011 in France, the authors described an increasing resistance to selected antibiotics and the potential risk of non-toxigenic strains expressing the diphtheria toxin after being lysogenised by a corynephage harbouring the *tox* gene [33]. However, the role and function of NTTB strains within this context and from a public health point of view has yet to be determined. Firstly, the emergence of these potentially 'toxigenic' strains indicates a potential reservoir for *tox* sequences among circulating strains in the population, which, together with effects of waning immunity [34] and decreasing laboratory capacity for detection of diphtheria in many countries, poses an increased risk for disease and outbreaks. Secondly, the public health management of these patients is unclear; no general guidelines are available on the evaluation of cases and contacts including therapeutic measures such as antibiotic eradication therapy.

Here we report that *C. diphtheriae* NTTB strains are circulating in the UK, associated with a very distinct molecular pattern among the four human isolates, and interestingly, also associated with animal carriage (cat). The presence of *C. diphtheriae* NTTB strains has also been reported from other European countries but only associated with individual cases and systematic data are not available.

Our data together with data from other European countries provides evidence that non-toxigenic, toxigenic and NTTB strains of *C. diphtheriae* are still circulating in Europe. The occurrence of diphtheria has always been characterised by periodicity and epidemic waves and while disease caused by toxigenic strains of *C. diphtheriae* has become rare in the majority of countries in the WHO European region, non-toxigenic strains of *C. diphtheriae* have been increasingly recognised as emerging pathogens in several European countries. This together with a changing epidemiological pattern (e.g. shift in the age of patients) [35], the detection of multidrug-resistant (MDR) strains [36], reports of zoonotic transmission of C. ulcerans [37] and the global circulation of toxigenic strains [2,38], demonstrates the continuous threat posed by this ancient disease.

Complacency that currently exists in Europe with regards to diphtheria circulation and diagnostics should be addressed through regular snapshot or screening studies across European countries, together with both laboratory and epidemiological vigilance and the maintenance of high vaccination coverage. Our study also highlights the importance of close collaboration between clinicians, public health experts and microbiologists to ensure timely data exchange and information flow as our study was hampered in parts by the unavailability of supporting clinical data. Complex public health situations, such as diphtheria management require multidisciplinary approaches and rely on effective communication both on national and international levels.

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Conflict of interest

None declared.

Authors' contributions

Conceived the project: AE, SN, performed the experiments: KZ, analysed the data: KZ, SN, wrote the paper: KZ, contributed to paper writing/editing: AE, SN.

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Letter to the editor: *Salmonella* Stanley outbreaks – a prompt to reevaluate existing food regulations

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To the editor:

In a recent *Eurosurveillance* issue, Kinross et al. [1] describe a cross-border outbreak of *Salmonella* Stanley in the European Union, which could be traced back to a contamination in the turkey production chain. The aetiological clone is mono-resistant to nalidixic acid and characterised by a novel pulsed-field gel electrophoresis (PFGE) type. We agree with Kinross et al. that the exchange of molecular data has to be improved to speed up outbreak investigations. However, although control measures were adequate to contain the multistate outbreak, they were not sufficient to eradicate the new clone, seeing as two outbreaks that occurred in Germany 12 months and Austria 16 months later [2] were caused by kebab contaminated with the newly described *Salmonella* Stanley outbreak clone. Rather, there is a considerable risk that the clone will become endemic in the turkey or poultry production chain in Europe.

In an editorial on this outbreak report, Hugas and Beloeil from the European Food Safety Agency conclude: *If sufficient information becomes available to reliably identify particular strains of public health significance, the inclusion of such strains as part of the EU-wide targets should be considered* [3]. In Austria we are already observing rising infection rates with *Salmonella* Stanley, with nine documented human infections in 2010 versus 101 documented infections in 2013. Moreover, the problem of antibiotic resistance inherent to the *Salmonella* Stanley outbreak clone was not addressed in this editorial. During the recent outbreak in Austria, we isolated three strains from infected humans that had developed resistance even against third generation cephalosporins and gentamicin. All strains harboured a CTX-M-15 extended-spectrum beta-lactamase, rendering standard therapy regimens ineffective. To prevent further evolution and spread of *Salmonella* Stanley, countries must undertake every effort to eradicate this outbreak clone in the poultry production chain in Europe now.

Although European regulations have contributed substantially to reducing *Salmonella* infections, the recent *Salmonella* Stanley outbreaks should be seen as an opportunity to re-evaluate existing regulations in view of efficient risk management and consistency. According to Regulation (EC) No 178/2002 [4], food shall not be placed on the market if it is unsafe. Regulation (EC) No 2073/2005 [5] further specifies that *Salmonella* has to be absent in minced meat and meat preparations made from poultry meat. However, in 2011, Regulation 1086/2011 [6] set a food safety criterion for fresh poultry meat that unfortunately only covers *Salmonella* Enteritidis and *Salmonella* Typhimurium, leaving food inspectors in the difficult situation that safety criteria for meat preparations differ from those for raw meat. Further, in our opinion, Regulation 1086/2011 weakened the stricter standards originally intended by part E of Annex II to Regulation No 2160/2003 [7] specifying that fresh poultry meat may not be placed on the market for human consumption when contaminated with *Salmonella*.

Along with harmonisation and refinement of food safety criteria, inclusion of *Salmonella* Stanley in the community targets for the reduction of the prevalence of zoonoses and zoonotic agents should be implemented to efficiently support control measures.

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