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Emergence of a novel cluster of influenza A(H5N1) virus clade 2.2.1.2 with putative human health impact in Egypt, 2014/15

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A distinct cluster of highly pathogenic avian influenza viruses of subtype A(H5N1) has been found to emerge within clade 2.2.1.2 in poultry in Egypt since summer 2014 and appears to have quickly become predominant. Viruses of this cluster may be associated with increased incidence of human influenza A(H5N1) infections in Egypt over the last months.

In Egypt, highly pathogenic avian influenza (HPAI) influenza A(H5N1) viruses of clade 2.2.1 and their descendants have been circulating in poultry populations since 2006, causing sporadic human infections [1]. Human influenza A(H5N1) infections in Egypt have been reported since the introduction of the virus in 2006 with 204 cases occurring until end of 2014 and a fatality rate of 35,8% in laboratory-confirmed cases reported to the World Health Organization (WHO). However, since January 2015, the incidence of human H5N1 cases in Egypt has increased dramatically: as of 21 March 2015, 116 human cases including 36 deaths have been reported to WHO [2]. This study was initiated to analyse molecular properties of H5N1 viruses that have caused outbreaks in poultry in Egypt since summer 2014 and to compare them with published sequences from H5N1 viruses obtained from recent human cases.

Sample origin

Between October 2014 and February 2015, a new wave of 435 outbreaks of H5N1 infections in poultry in Egypt was reported to the National Laboratory for Quality Control on Poultry Production (NLQP) by Egyptian veterinary authorities (Figure 1). Affected poultry species included chickens, ducks, turkeys and quails on commercial farms as well as in backyard holdings. In this study, 29 H5N1-positive samples, mostly obtained by passive surveillance and submitted to NLQP for routine analysis, were selected so as to represent different poultry species, sectors of poultry holdings (commercial farms, backyards and live bird markets) and locations (Table 1).

Phylogenetic analyses

Nucleotide sequence data for the haemagglutinin (HA) gene of all 29 viruses and of the neuraminidase (NA) gene of 15 viruses were generated by Sanger sequencing; whole genome sequencing was carried out for four virus isolates selected to represent different locations, moments in time and sectors of poultry holdings.

Phylogenetic analysis of the HA and NA gene sequences was done with the maximum likelihood methodology using the IQTree software [3,4]. The authors gratefully acknowledge the originating and submitting laboratories who contributed sequences used in the phylogenetic analysis to the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database, and recognise in particular Alice Fusaro and colleagues (Istituto Zooprofilattico Sperimentale Delle Venezie, Padova, Italy) as well as Mee Poh (Centers for Disease Control and Prevention, WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza, Influenza Division, Atlanta, United States). Phylogenetic analysis placed the H5N1 viruses sequenced in this study into a separate cluster within the previously defined clade 2.2.1.2 (Figure 2A and 2B). While the H5N1 isolate A/duck/Egypt/14VIR784–4-133AD/2013 (KP035030) harboured the closest related ancestral sequences of this cluster, two further poultry viruses sampled in June and July 2014 were placed directly at the root of this cluster: A/quail/Egypt/BSU5514-AR2219/2014 (EPI557138) was at the basis of the HA phylogeny while A/duck/

Table 1

Influenza A(H5N1) viruses included in this study and collected from poultry, Egypt, June 2014–January 2015 (n=29)

No	Sample ID	Sequenced	Collection date	Governorate	Source	Accession number (EpiFlu)
1	A/turkey/Egypt/14139FAOS/2014	Whole genome	18 Jun 2014	Assiut	LBM	EPI574374–81
2	A/duck/Egypt/14154FAOS/2014	HA, NA	25 Jun 2014	Monofiya	LBM	EPI573331–2
3	A/chicken/Egypt/1427AF/2014	HA	3 Sep 2014	Fayoum	farm	EPI573333
4	A/chicken/Egypt/1476CA/2014	HA	14 Oct 2014	Al-Gharbiya	Household	EPI573330
5	A/turkey/Egypt/ AR235-S240NLQP/2014	HA, NA	31 Oct 2014	Cairo	Household	EPI573251–2
6	A/turkey/Egypt/14240FAOS/2014	Whole genome	31 Oct 2014	Cairo	LBM	EPI574382–89
7	A/chicken/Egypt/141/2014	HA	3 Nov 2014	Aswan	Household	EPI573334
8	A/duck/Egypt/14227FAOS/2014	HA	4 Nov 2014	Sohag	Household	EPI573335
9	A/chicken/Egypt/ AR234-FAOF8NLQP/2014	HA, NA	12 Nov 2014	Menia	Farm	EPI573249–50
10	A/chicken/Egypt/ AR231-CA113NLQP/2014	HA, NA	19 Nov 2014	Suez	Household	EPI573243–4
11	A/chicken/Egypt/1478CAL/2014	HA	23 Nov 2014	Qena	Household	EPI573318
12	A/duck/Egypt/144/2014	HA	4 Dec 2014	Giza	Household	EPI573323
13	A/chicken/Egypt/14140CA/2014	HA	5 Dec 2014	Assiut	Household	EPI573329
14	A/turkey/Egypt/ AR238-SD177NLQP/2014	Whole genome	6 Dec 2014	El-Beheira	Household	EPI573261–8
15	A/chicken/Egypt/14148CA/2014	HA	9 Dec 2014	Menia	Household	EPI573314
16	A/duck/Egypt-BS/146RS-f6/2014	HA	14 Dec 2014	Beni Suef	Farm	EPI573315
17	A/duck/Egypt/1427SL/2014	HA	14 Dec 2014	Sohag	Household	EPI573316
18	A/chicken/ Egypt/AR233-S283NLQP/2014	HA, NA	15 Dec 2014	Qena	Household	EPI573247–8
19	A/chicken/Egypt/14168CA/2014	HA, NA	16 Dec 2014	Menia	Household	EPI573327–8
20	A/duck/Egypt/ AR232-A13NLQP/2014	HA, NA	22 Dec 2014	Giza	Household	EPI573245–6
21	A/chicken/Egypt/152RS/2015	HA	29 Dec 2014	Monofiya	Household	EPI573322
22	A/chicken/Egypt/152/2015	HA	1 Jan 2015	South Sinai	Household	EPI573319
23	A/chicken/Egypt/153AF/2015	HA, NA	4 Jan 2015	Fayoum	Household	EPI573320–1
24	A/chicken/Egypt/1540S/2015	HA, NA	11 Jan 2015	Assiut	Household	EPI573336–7
25	A/chicken/Egypt/152AI/2015	HA, NA	11 Jan 2015	Ismailia	Household	EPI573325–6
26	A/chicken/Egypt/1510CA/2015	HA, NA	14 Jan 2015	Cairo	Household	EPI573312–3
27	A/duck/Egypt/ AR236-A3NLQP/2015	Whole genome	15 Jan 2015	Giza	Household	EPI573253–60
28	A/duck/Egypt/1560S/2015	HA	18 Jan 2015	Dakahliya	Household	EPI573324
29	A/chicken/Egypt/1575S/2015	HA	21 Jan 2015	Assiut	Household	EPI573317

HA: haemagglutinin; LBM: live bird market; NA: neuraminidase.

Egypt/14154-FAOS/2014 (EPI573331) marked the basis of the NA tree (Figure 2A and 2B, green colour). The cluster has expanded since October 2014; the fact that no more sequences of the older range of 2.2.1.2 viruses were detected thereafter indicates that this cluster had become predominant over previously circulating phylotypes. GenBank sequences of two recent H5N1 HPAI viruses obtained from infected humans in Egypt in November 2014 (AJM70734 and AJM70746), fell into the same expanding cluster (Figure 2A and 2B, blue colour). Calculation of the time to the most recent common ancestor (TMRCA) of the emerging phylotype by BEAST analysis (Figure 2C) [5] suggested that ancestors of this phylotype emerged around February 2014. Similar phylogenetic relationships were observed for the internal gene segments of these viruses (data not shown, available from authors upon request). The phylogenetic information of the internal genes supports the results for the HA and NA gene sequences, indicating that the new viruses represent a distinct cluster that originated from previously circulating viruses of clade 2.2.1.2. So far, no reassortment events were found to be involved in generating this newly emerging phylotype.

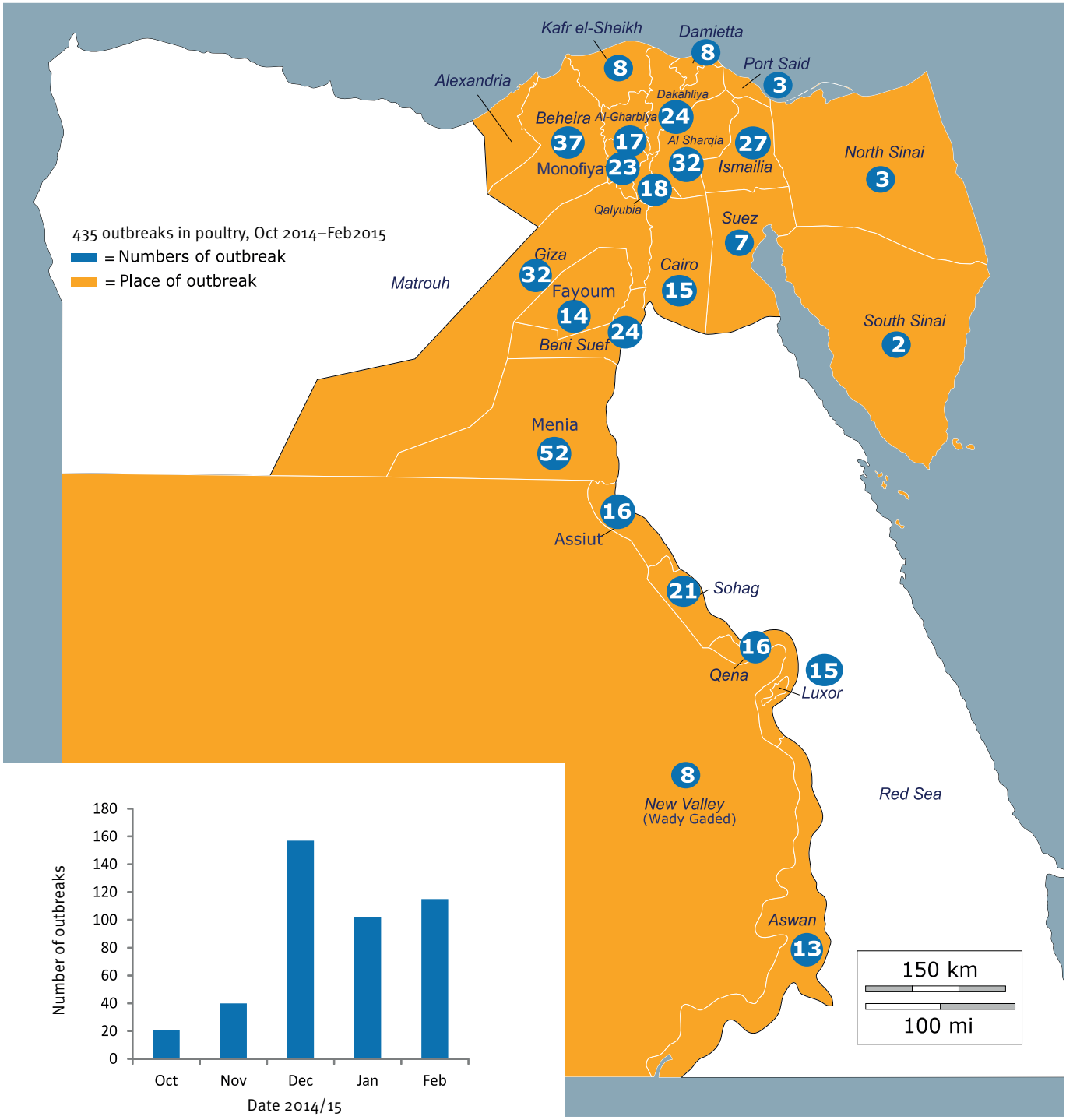
Genetic characterisation

Compared with viruses sampled before October 2014 in Egypt, the emerging cluster contained distinct fixed mutations in several genome segments (PB2, PB1-F2, HA, NA, M1).

In the HA gene, differences in the nucleotide composition of the coding sequence of up to 1.3% were found, which included up to 14 specific fixed nucleotide substitutions distinguishing these viruses from earlier

Figure 1

Temporal (graph) and geographic (map) distribution of outbreaks of highly pathogenic avian influenza A(H5N1) in poultry, Egypt, October 2014–February 2015 (n=435)



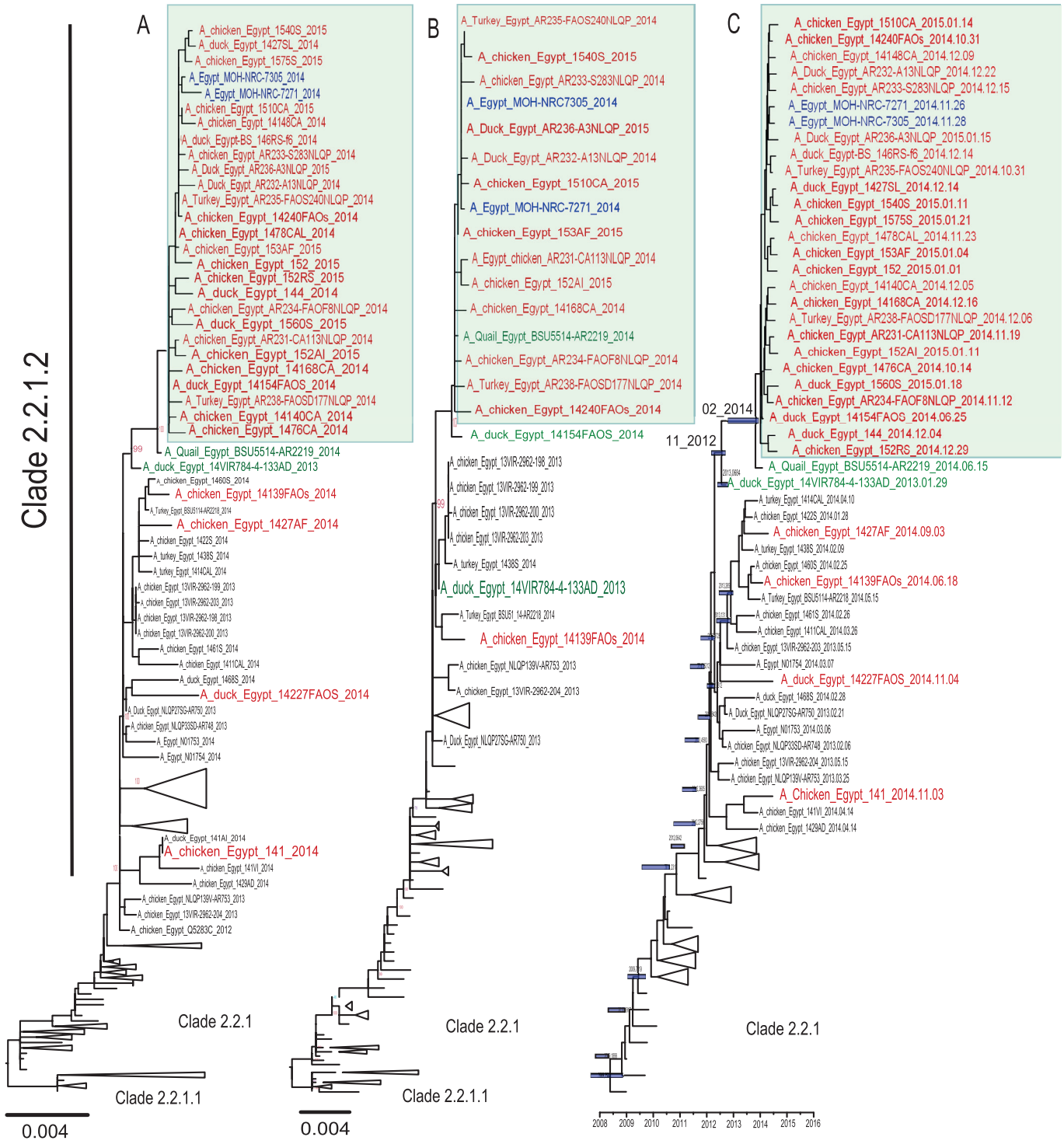
Source of the map: d-maps.com.

Egyptian isolates of 2014. A total of 12 of these mutations were synonymous (silent) and only two resulted in amino acid substitutions (K373R and F537S). Of these, only K373R, located in the stalk domain at the oligomerisation interface of the HA, is characteristic of the emerging cluster; this mutation has sporadically been reported in very few older isolates from Egypt [6].

The mutation F537S had already been detected in several older isolates. In addition, the HA protein of the viruses in the new cluster contained mutations D94N, T156A, K189R and P235S, which are associated with improved binding to SAα2,6-Gal, the human type of influenza virus receptors [7]. However, these mutations were present also in earlier clade 2.2.1 H5N1 viruses

Figure 2

Phylogenetic analysis of the HA (A) and NA (B) genes (coding regions) and maximum clade credibility tree (C) based on the HA open reading frame of selected highly pathogenic avian influenza A(H5N1) viruses, Egypt, June 2014–January 2015 (n=29)



HA: haemagglutinin; NA: neuraminidase.

The phylogenetic analysis was done by maximum likelihood method using the IQTree algorithm [3,4]. BEAST [5] analysis was carried out to determine time to the most recent common ancestor (TMRCA; indicated by horizontal blue bars overlaying nodes). TMRCA calculations were based on an uncorrelated log-normal relaxed clock model. The maximum clade credibility tree was scaled to time using the collection dates (day/month/year) of all samples. Red labels indicate poultry viruses sampled after August 2014 and sequenced in this study. Blue labels denote sequences of viruses retrieved from human influenza A(H5N1) infections (AJM70734; AJM70746). Green labels highlight viruses close to the basis of the emerging cluster of selected H5N1 HPAI viruses circulating in Egypt.

The authors gratefully acknowledge the originating and submitting laboratories who contributed sequences used in the phylogenetic analysis to the Global Initiative on Sharing All Influenza Data (GISAID) EpiFlu database.

Table 2

Antigenic characterisation of highly pathogenic avian influenza A(H5N1) viruses from Egypt and elsewhere, based on haemagglutination inhibition assays

Influenza antigens for immunisation	Immune sera	H5 LP		H5N1 HP				
		Am	EA	2.2.1	2.2.1N[a]	2.2.1.1	1	2.3.2.1
		A/H5N2 MX	A/H5N2 PTD	R740/09	AR238/15	R737/09	NIBRG-14	R1970/13
A/H5N2 LP, Mexico vaccine	S1	**9**	8	3	3	<1	7	<1
	S2	**9**	7	5	5	<1	5	1
	S3	**10**	8	6	5	<1	6	1
A/H5N2 LP, Potsdam (Germany)	S4	7	**8**	5	5	<1	7	3
	S5	8	**10**	8	8	<1	8	5
	S6	7	**8**	7	6	<1	5	<1
A/H5N1 R65	S13	7	7	**8**	7	3	7	6
A/H5N1 R737	S7	3	4	5	5	**9**	5	6
	S8	2	1	5	4	**10**	4	5
	S9	2	<1	3	3	**10**	3	4
Re-5	S10	6	6	3	3	<1	6	4
	S11	5	6	4	4	2	6	4
	S12	4	5	4	4	4	6	4
None	S-SPF	<1	<1	<1	<1	<1	<1	<1

Am: American; EA: Eurasian; HP: highly pathogenic; LP: low pathogenic; PTD: Potsdam.

[a] 2.2.1N denotes a virus representative of the currently emerging cluster of highly pathogenic avian influenza A(H5N1) viruses in Egypt.

Immune sera were raised in chickens against low pathogenic (LP) A/chicken/Mexico/232/1994 (A/H5N2 LP, used as a vaccine virus and representing an American LP H5 strain), against A/duck/Potsdam/1402–6/1986 (A/H5N2 LP PTD, representing an Eurasian LP H5 strain) and against highly pathogenic (HP) A/whooper swan/Germany/R65/2006 (A/H5N1 R65, clade 2.2), A/chicken Egypt/0879-NLQP/2008 (A/H5N1 R737, clade 2.2.1.1) and reverse genetically-modified (rg) A/duck/Anhui/0.5006 (Re-5, clade 2.3.4). A specific pathogen-free chicken serum served as negative control. All viruses used for immunisation, except Re-5, also served as antigens in HI assays. Additional antigens were derived from A/chicken/Egypt/083-NLQP/2008 (R740/09, clade 2.2.1), A/turkey/Egypt/FAO-SD177/2015 (AR238/15, clade 2.2.1, emerging cluster), rg A/Vietnam/1194/2004 (NIBRG-14; clade 1) and A/Hill myna/Austria/R1970/2013 (R1970/13, clade 2.3.2.1). Homologous (same clade) serum–antigen reactions are shown in bold.

in Egypt. Since no substituting mutations were found in HA epitopes, we do not expect marked differences in the antigenic properties of the emerging phylotype compared with the previously circulating clade. This was partially confirmed by haemagglutination inhibition assays using sera against different clades of H5 viruses (Table 2).

The NA gene of the emerging cluster differed by seven nucleotide substitutions from recent H5N1 HPAI viruses of clade 2.2.1.2. Four of the seven mutations encoded amino acid substitutions not previously reported in 2.2.1.2 viruses: V34I, I74V, V244I and V284I (Table 3). Mutation V34I has been reported in H5N1 strains from Cambodia from 2013 where an increase in human H5N1 infections was observed [8]. No biological function has been associated with the V34I substitution, while positions 74, 244 and 284 are located in B- or T-cell antigenic regions of the NA protein [9].

For four viruses, whole genome sequences were generated and further signature mutations of the emerging viruses were found in the internal gene segments as well: Non-silent cluster-specific mutations were confined to the PB2 (M66I, T106A), PB1-F2 (G22E), and M1 (I15V) proteins (Table 3).

Discussion

Influenza pandemics remain one of the major threats posed by communicable diseases to the human population. The avian reservoir of influenza viruses contributed by reassortment to the emergence of most previous pandemic human influenza viruses [10]. Since their emergence in Asia in 2003, HPAI viruses of subtype H5N1 and their recent descendants continue to cause significant economic losses to commercial poultry not only in Asia, but also in Egypt where high mortality in poultry has continuously been observed since 2006 [11, 12]. They also exhibit strain-specific zoonotic potential resulting in sporadic avian-to-human spillover transmissions which lead to human infections associated with a high case fatality rate [13]. However, apart from sporadic cases (e.g., family clusters) sustained human-to-human transmission of any of these viruses has not ensued so far.

Our data confirm the emergence of an additional virus cluster within the Egyptian 2.2.1.2 clade of H5N1 HPAI viruses. Since November 2014, viruses of this new cluster appear to have become dominant over the previously described clade 2.2.1.2 phylotypes circulating

Table 3

Amino acid residues distinguishing recently emerging highly pathogenic avian influenza A(H5N1) viruses from virus lineages circulating before November 2014, Egypt, March 2015

Gene segment	Position	New cluster	clade 2.2.1.2	clade 2.2.1.1
PB2	66[a]	I	M	M
	106[a]	A	T	T
PB1-F2	22	E	G	G
HA	389[a]	R	K	K
NA	34	I	V	V
	74	V	I	I
	244	I	V	V
	284	I	V	V
M1	15	V	I	I

HA: haemagglutinin; NA: neuraminidase.

Data on the new cluster are based on sequences established in this study: HA (29 sequences), NA (15 sequences), internal gene segments (4 sequences). Data for older clades were retrieved from public databases.

[a] Some of the listed mutations have been infrequently observed among single isolates from previous years.

in various poultry species. The only two publicly available sequences of viruses isolated from recent human H5N1 cases in Egypt show similar mutation patterns and fall into the same phylogenetic group. The molecular determinants that may improve the evolutionary fitness of these viruses need to be further clarified. The emergence of new clusters of H5N1 HPAI viruses in Egypt is not without precedence: In late 2007, a subclade of antigenic drift variants, later designated 2.2.1.1, emerged and expanded (clade 2.2.1.1a) in commercial poultry in Egypt but disappeared until end of 2010 [14] and, contrary to the current situation, did not replace 2.2.1 viruses. Viruses of clade 2.2.1.1 that emerged in 2007 hardly caused any human cases: according to the OpenFlu database [15]: only one of 100 H5N1 isolates from humans in Egypt belonged to clade 2.2.1.1; all others belonged to clade 2.2.1 and 2.2.1.2. In contrast, the emerging cluster identified in this study seems to be predominant across all poultry production sectors and has already caused a third of all human infections reported in Egypt since 2006 in only three months of 2015.

Given the endemic status of influenza H5N1 in poultry and the limitations of the reporting system of H5N1 HPAI virus outbreaks in poultry in Egypt, it is difficult to assess whether the altered epidemiological pattern of the emerging phylotype is due to altered biological properties in poultry or whether the increased incidence of infections in poultry merely reflects an increased viral burden across all poultry sectors in Egypt. In any case, the observed recent rise in outbreaks in poultry probably resulted in increased exposure risks for humans in contact with poultry, which may have caused an increased incidence in human cases. However, it can at this point not be excluded with certainty that the emerging phylotype of viruses may have increased zoonotic potential and may be transmitted more efficiently to humans, although this assumption cannot be drawn from the molecular evidence described here. Further studies of the pathogenicity and transmissibility of these viruses in humans, e.g. in the ferret model, are required. Concerted efforts of both veterinary and public health authorities are urgently needed to interrupt virus circulation in poultry in Egypt efficiently. This will help decrease the risk of human exposure to the virus.

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Conflict of interest

None declared.

Authors' contributions

Abdel-Satar A. Arafa, Mahmoud M. Naguib and Timm Harder conceived the study. Walid H. Kilany, Ahmed Samy and Ahmed Abdelhalim were involved in the collection, identification and isolation of viruses from field samples. Christine Luttermann, Naglaa Hagag and Abdullah A. Selim conducted the Sanger sequencing. Christian Grund provided data from haemagglutination inhibition assays. Mahmoud M. Naguib, Abdel-Satar A. Arafa, E. M. Abdelwhab and Timm Harder produced, analysed and interpreted genetic and phylogenetic data. Gwenaelle Dauphin, Yilma Makonnen and Mohamed K. Hassan provided and analysed epidemiological data. Timm Harder and Mahmoud M. Naguib drafted the manuscript, Thomas C. Mettenleiter, Martin Beer, Juan Lubroth and all co-authors critically analysed and revised the manuscript and provided final approval.

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Emergence of tick-borne encephalitis in new endemic areas in Austria: 42 years of surveillance

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Human infections with tick-borne encephalitis (TBE) virus are a public health concern in certain regions of Europe, central and eastern Asia. Expansions of endemic areas and increased incidences have been associated with different factors including ecological changes supporting tick reproduction, socioeconomic changes increasing human outdoor activities and climatic changes favouring virus circulation in natural foci. Austria is among the most strongly affected countries in Central Europe, but the annual number of cases has strongly declined due to vaccination. Here, we have analysed changes of the incidence of TBE in the unvaccinated population of all federal states of Austria over a period of 42 years. The overall incidence in Austria has remained constant, but new strongly affected endemic regions have emerged in alpine valleys in the west of Austria. In parallel, the incidence in low-land regions in the north-east of the country is decreasing. There is no evidence for a shift to higher altitudes of infection sites in the traditional TBE zones, but the average altitudes of some newly established endemic areas in the west are significantly higher. Our analyses underscore the focal nature of TBE endemic areas and the potential of TBE virus to emerge in previously unaffected regions.

Introduction

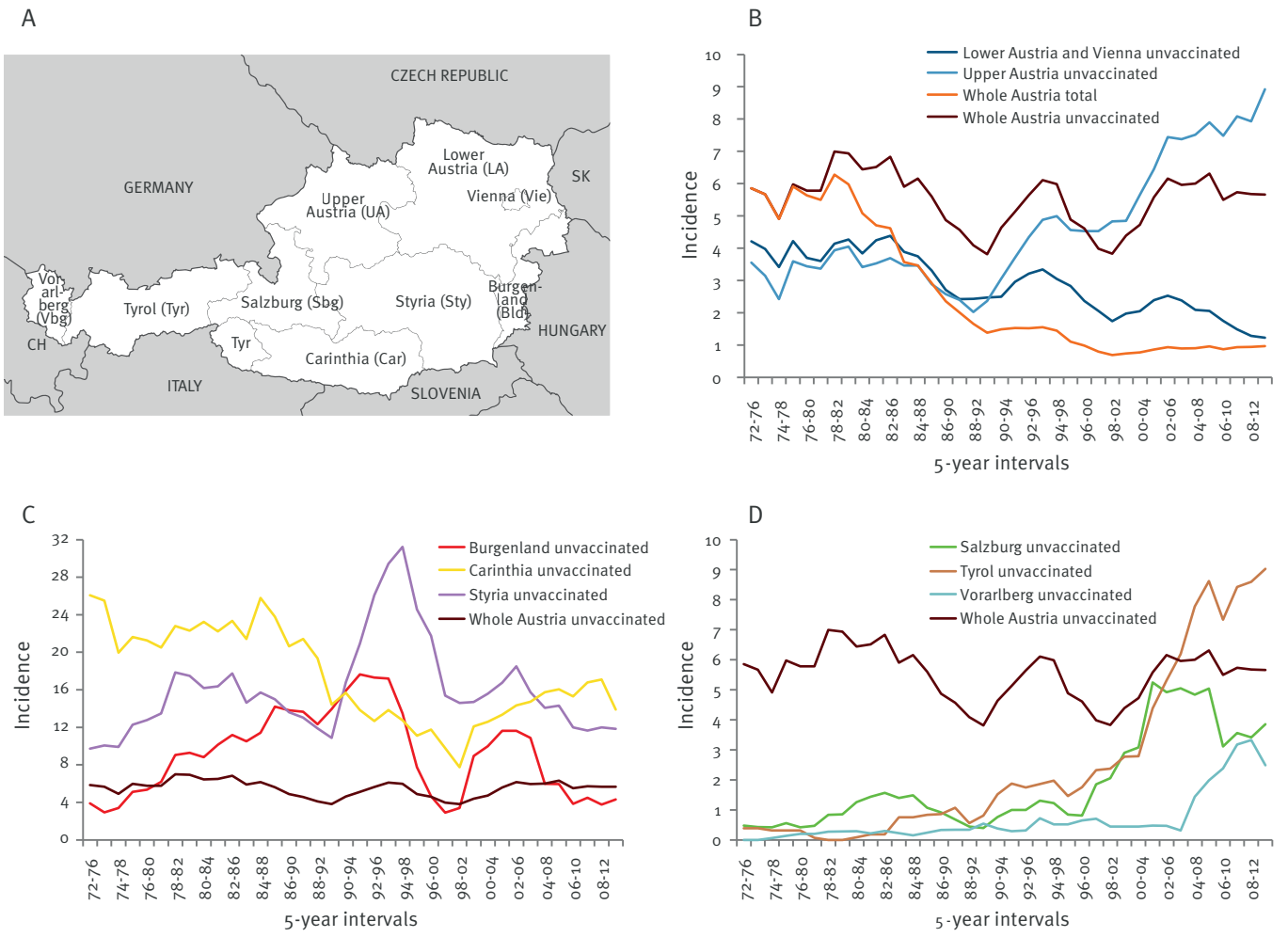
Tick-borne encephalitis (TBE) virus is endemic in many parts of Europe as well as central and eastern Asia and responsible for more than 10,000 hospitalised TBE patients every year [1]. Together with the mosquito-transmitted yellow fever, dengue, West Nile and Japanese encephalitis viruses it is a member of the genus *Flavivirus* in the family *Flaviviridae* and consists of at least three antigenically closely related subtypes [2]. The European subtype is primarily transmitted by *Ixodes ricinus*, whereas *Ixodes persulcatus* is the main vector of the Siberian and Far Eastern subtypes [3]. In the Baltic countries and Finland, an overlap of vectors and subtypes has been described [4,5]. The human disease can be effectively prevented by vaccination with formalin-inactivated vaccines, as demonstrated by epidemiological studies in countries and regions with high vaccination coverage, such as Austria or the Sverdlovsk district in Russia [6,7].

The ecological conditions for maintaining TBE virus replication and persistence in nature are very complex and fragile. They require not only the availability of specific animal hosts (rodents and larger mammals such as roe deer) for feeding the different stages of tick development (larvae, nymphs and adults) and increasing their density, but also favourable conditions for the transmission of the virus between different developmental stages. There is strong evidence that conditions allowing non-viraemic transmission from infected larvae to non-infected nymphs by co-feeding on the same rodent are a major prerequisite for the maintenance of virus circulation in nature [8]. Such a mechanism of tick infection requires specific climatic conditions (especially a swift rise of temperature in spring) that lead to a temporal coincidence of larval and nymph development and possibly contribute to the restriction of TBE virus to selected endemic regions. This is in contrast to other tick-transmitted pathogens such as *Borrelia burgdorferi* that are found in all areas populated with ticks [9-12].

Strong annual variations as well as long fluctuations over time are a characteristic feature of the TBE incidence in affected countries [6,13,14] and an overall upsurge has been reported in certain parts of Europe (reviewed in [15]). These changes have been related to climatic, ecological, environmental and socioeconomic factors that can lead to an increased risk of human exposure to infected ticks [8,16-19]. In addition, however, the establishment of new natural foci of TBE virus circulation has been described in areas previously considered free of TBE, as for instance in certain parts of Norway [20], Sweden [21,22], Finland [23], Denmark

Figure 1

Incidence rates of tick-borne encephalitis in Austria (total and unvaccinated population) and its federal states (unvaccinated population), 1972–2013



CH: Switzerland; SK: Slovakia.

Incidence: Number of cases/100,000 population. Assignment to federal states is based on the site of hospitalisation.

[24] and Switzerland [13,25] as well as areas of higher altitude in the Czech Republic [19,26] and Slovakia [27]. The mechanisms underlying the discontinuous spread of the virus and its long-distance transport to previously unaffected areas are largely unknown but may be related to (i) changes in climatic conditions that allow virus maintenance in natural hosts and (ii) birds [28-30] or larger mammals (including humans) serving as transport vehicles of infected ticks [21,31].

In Austria, the high vaccination coverage (more than 80% of the total population has received at least one TBE vaccination) has led to a substantial decline in the number of annual cases [6]. The incidence in the unvaccinated population, however, is virtually identical with that in the prevaccination era (on average ca 6 per 100,000 in the whole country), indicating that the overall risk of human exposure to TBE virus has remained constant [6]. In this work, we have addressed the question of possible regional changes of TBE endemicity by analysing the incidences of TBE in the unvaccinated population over a period of 42 years (1972 to 2013) in all federal states of Austria. Since the documentation of cases based on administrative districts blurs the situation of real risk areas, we also mapped the cases over the whole time period based on questionnaires indicating the most likely site of infection.

Methods

Documentation of tick-borne encephalitis cases

Data on TBE cases were documented by the Department of Virology, Medical University of Vienna, acting as the National Reference Laboratory for TBE virus and other flavivirus infections, on the basis of confirmed laboratory diagnosis of each case and on the basis of questionnaires. Only hospitalised patients with a serologically confirmed recent infection with TBE virus were counted as cases. Confirmation was based on TBE virus IgM and IgG ELISA results, which have replaced

the haemagglutination inhibition and/or complement fixation assays used until the early 1980s in Austria.

Each confirmed case received a questionnaire to provide information on tick bites and possible sites of infection. Since only ca 50% of the patients had a known history of a tick bite, the time elapsed between infection and hospitalisation is usually three to four weeks and the origin of the infecting tick can be uncertain, the geographical mapping of infection sites was based on a subset of patients (ca 36% of the total cases) who could provide data precise enough to make the site of infection 'most likely'.

Calculation of incidence rates

Overall incidence rates and incidence rates in unvaccinated persons were calculated for the Austrian federal states individually (because of its location, the capital state of Vienna was included in the counts of Lower Austria; Figure 1A) and for the whole of Austria, using population data from Statistics Austria [37] and data on vaccination status in each federal state collected by GfK Austria HealthCare (Vienna, Austria) through postal surveys [6]. Data on possible regional differences in vaccination coverages within federal states were not available. As described previously [6], the incidence among unvaccinated persons was estimated by assuming a vaccine efficacy of 97% for the years before 2000 [6]; since 2000, the actual number of cases that occurred among unvaccinated persons has been used for calculation. The assignment of cases to individual federal states was based on the site of hospitalisation.

Mapping of infection sites by ArcGIS

Infection sites were geocoded and further processed for spatial mapping by ArcGIS (Environmental Systems Research Institute; ESRI Inc.). Spatially close sites were aggregated to a single point using a 1 km buffer around the individual points, assembling the union of all buffers, and subsequently calculating the centroid of the area. For the display of aggregated infection sites in the maps, these centroids formed the centre of circles with diameters proportional to the number of infection sites found within this area.

For building the base map of Austria (Figure 2), we used data of Statistics Austria (http://www.statistik.at/web_de/services/geodaten/) for borders of Austria and its federal states, Natural Earth Data (http://www.naturalearthdata.com/downloads/10m-physical-vectors/) for rivers, lakes and cities, and Aster data of the United States Geological Survey (USGS; http://earthexplorer.usgs.gov/) for topography.

Altitudes of infection sites were derived from the digital elevation model (DEM) of USGS Aster data (spatial resolution of 30 m), using the original coordinates for each single documented 'most likely' infection site.

Statistical analyses of altitudes of infection sites

Sea level data of infection sites were log-transformed due to the skewed distribution. Normal distribution of residuals of the transformed data was tested by Kolmogorov-Smirnov test with Lilliefors' corrected p values. A two-factor analysis of variance of transformed sea level data with period (1972–1983, 1984–2013) and region (six affected federal states in 1972–1983 and eight in 1984–2013) as factors was performed. Comparison of periods within regions was done by linear contrasts. Differences in the scatter of altitudes were tested by Levene's test for homogeneity. Due to the fact that no cases occurred in Tyrol and Vorarlberg during the first period, differences between regions were tested separately for the two periods with one-way analyses of variance. Pairwise differences between regions were tested by Tukey's honest significant difference (HSD) test for unequal sample size.

Results

Changes in the incidence of tick-borne encephalitis in Austria

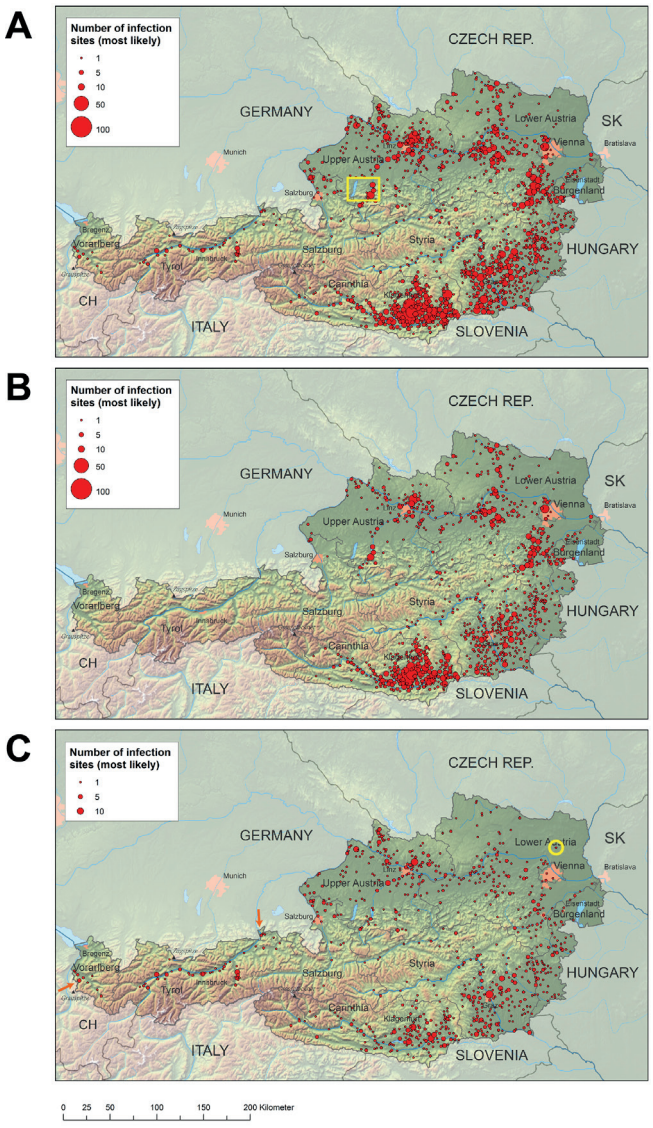
In the past two to three decades, the most remarkable change in TBE incidence in Austria was caused by vaccination, resulting in an 84% reduction of the annual number of cases, while the incidence in the unvaccinated population remained constant at ca 6 per 100,000 population [6]. On the background of this unaltered overall risk of TBE virus infection in Austria, we wanted to assess possible regional shifts and dynamics of endemic areas. For this purpose, we determined the incidence rate in the unvaccinated population for the whole of Austria and each of the Austrian federal states (Figure 1A) and assuming a vaccine protection rate of 97% [6]. To reveal long-range developments and to avoid confusion due to the strong annual fluctuations typical of TBE [6,13,14], we used a sliding window representation of the mean incidences in five year intervals. The overall situation in Austria (including the incidence in the unvaccinated population) is displayed in Figure 1B.

Disparate developments occurred in the northern states of Upper and Lower Austria (including the capital state of Vienna) which border Germany, the Czech Republic and Slovakia (Figure 1B). Both of these regions started with an incidence of ca 4 (Lower Austria/Vienna: 4.2; Upper Austria: 3.6) per 100,000 at the beginning of the observation period, but after 1990, the incidence in Upper Austria began to rise and reached 8.9 per 100,000 in the period 2009 - 2013. In contrast, in Lower Austria/Vienna, the incidence continuously declined to 1.2 per 100,000 (Figure 1B).

Figure 1C shows the development in the most strongly affected areas in the south of Austria, including the federal states of Carinthia, Styria and Burgenland at the borders to Italy/Slovenia, Slovenia and Hungary, respectively (Figure 1A). All three states had substantially higher mean incidences than the whole of Austria

Figure 2

Sites of tick-borne encephalitis virus infection, Austria, 1972–2013



A. Cumulative data for the whole period 1972 to 2013

B. Data from 1972 to 1983

C. Data from 1984 to 2013

CH: Switzerland; SK: Slovakia.

The size of the red circles is proportional to the number of documented infection sites within this area (see insert). (A) Yellow rectangle indicates the lake district east of Salzburg. (B) Yellow circle indicates last 'most likely' infection site in north-east Lower Austria in 1984. Orange arrows indicate possible sites of virus seeding into new endemic areas through valleys.

during the entire observation period (17.9/100,000 in Carinthia, 15.4/100,000 in Styria, and 8.6/100,000 in Burgenland) and displayed strong annual fluctuations of minimum and maximum incidences, ranging from 1.2 to 42.8 per 100,000 in Carinthia, 5.5 to 42.6 per 100,000 in Styria, and 0 to 32.6 per 100,000 in Burgenland. Importantly, the fluctuations were not synchronised in the three states, suggesting strong region-specific differences in the activity of natural foci and other factors potentially influencing the risk of human exposure to TBE virus.

The most striking changes were observed in the alpine regions in the west of Austria, including the states of Salzburg, Tyrol, and Vorarlberg (Figure 1D). Both Salzburg and Tyrol experienced strong increases in incidence around 2000 which later levelled off in Salzburg but continued to rise in Tyrol and reached more than 8 per 100,000, i.e. well above the Austrian average. In 2013, the incidence in Tyrol was almost as high as in the most strongly affected state Carinthia (13.9 vs 15.1 per 100,000) and higher than in Styria (11.3 per 100,000). In the western-most state Vorarlberg, a similar steep rise began ca 10 years later and appears to continue.

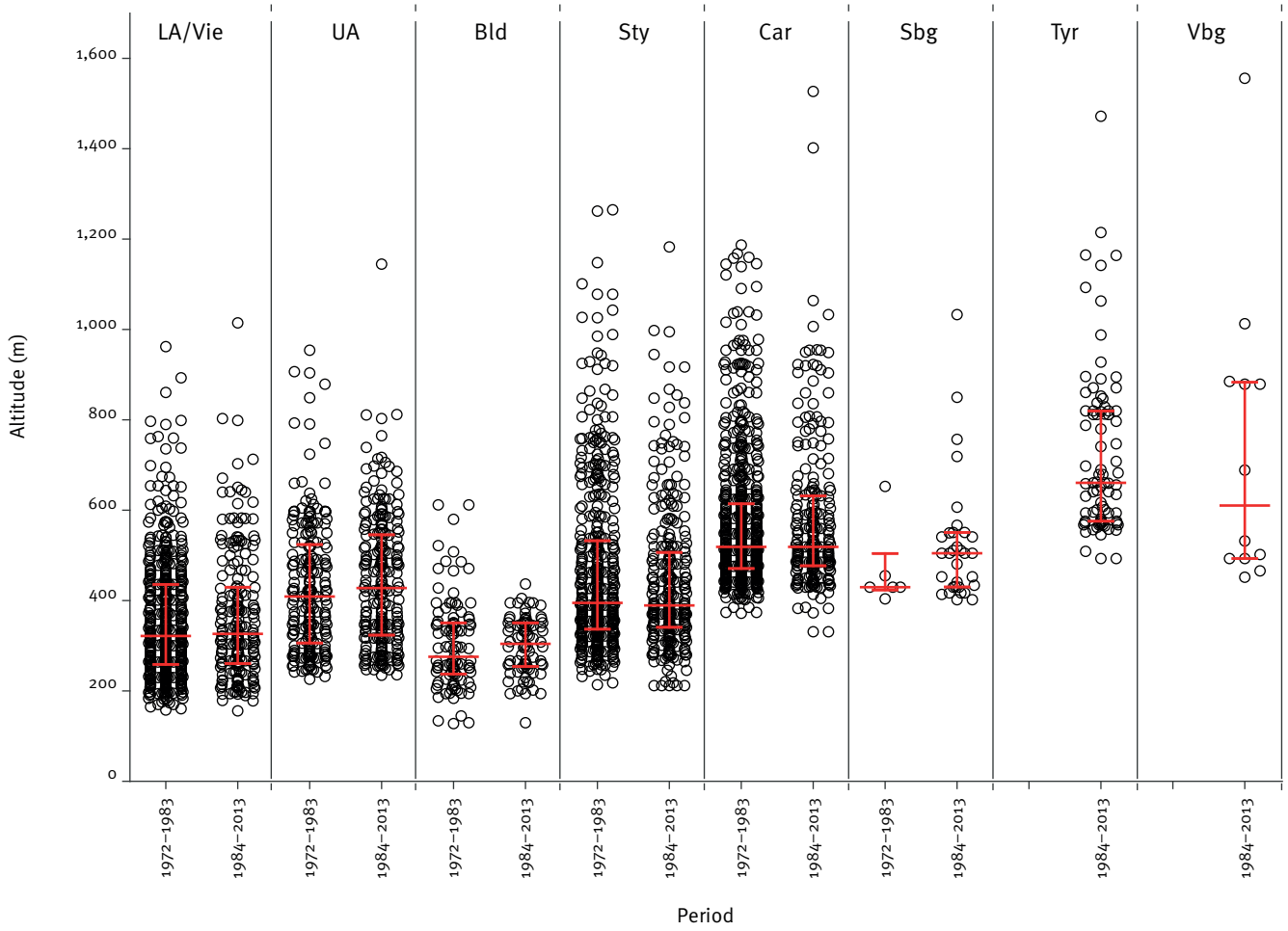
Changes in infection sites in Austria

Although the documentation of TBE cases according to hospitalisation in administrative regions such as the federal states provides good information about trends of shifting incidences of disease in different areas of the country, it is imprecise with respect to the true location of infection sites. To obtain a more detailed picture of risk areas, we evaluated the information provided through questionnaires received by the national reference laboratory for TBE in Austria. Considering the time elapsed between infection and hospitalisation and the inherent uncertainty of the origin of the tick leading to infection, we used very stringent criteria for the mapping of new infection sites. Figure 2 therefore contains only data from cases for whom other possible sites of infection could be excluded with high probability. One also needs to keep in mind that the total number of cases in Austria, and concomitantly the sampling of infection sites, has strongly declined over time due to vaccination (Figure 1B). Quantitative inferences can thus not be made from Figure 2 with respect to the prevalence of TBE virus and the total number of bites by infected ticks. The data are displayed for the complete observation period (1972–2013; Figure 2A) and for two time windows separately: 1972 to 1983 (the period before the first documentation of an autochthonous case in Tyrol in 1984; Figure 2B) and 1984 to 2013 (Figure 2C). A total of 5,148 and 3,495 cases were recorded from 1972 to 1983 and from 1984 to 2013, respectively. Over the whole time period, a 'most likely' site of infection could be identified for ca 36% of these cases.

The most striking changes in infection sites occurred in the alpine regions in the west of Austria, affecting especially Tyrol and Vorarlberg where no TBE was reported before 1984 and 2000, respectively. Since then, TBE has become highly endemic in the valleys of the rivers Inn and Ziller (Tyrol) and that of the river Ill (Walgau, Vorarlberg) (Figure 2C). First cases of TBE have also been documented since 2000 in another alpine valley (Salzach River) south of the city of Salzburg (Figure 2C). Further comparison of the maps

Figure 3

Altitudes of tick-borne encephalitis infection sites, Austria, 1972–1983 compared with 1984–2013



Bld: Burgenland; Car: Carinthia; LA/Vie: Lower Austria and Vienna; Sbg: Salzburg; Sty: Styria; Tyr: Tyrol; UA: Upper Austria; Vbg: Vorarlberg. Each circle represents a documented 'most likely' infection site. Error bars represent the medians and interquartile ranges.

also suggests the establishment of new endemic foci or at least increasing TBE activity in a region North of Salzburg (Figure 2C). The opposite effect, i.e. decreasing activity or complete disappearance, however, can be inferred from the comparison of the situation in the north-east of Lower Austria (Figure 2C) where the last 'most likely' infection site was recorded in 1984 (Figure 2C; yellow circle).

Altitudes of infection sites

Since the geographically most remarkable areas of TBE emergence were located in alpine regions of western Austria and data from other countries such as the Czech Republic [19,26] and Slovakia [27] suggest a rise of TBE foci at higher altitudes, we analysed the sea levels of all mapped sites of TBE infections in Austria and determined their distribution in the federal states in the time periods 1972 to 1983 and 1984 to 2013 (Figure 3). In accordance with the topography of Austria, the average altitudes of infection sites differed between individual federal states and range from 300 m in Burgenland to 730 m in Tyrol. For the traditional endemic areas, possible shifts to higher altitudes were evaluated by comparing the two time windows (1972–1983 and 1984–2013) using analysis of variance with linear contrasts for each region. No statistically significant difference was observed at the level of federal states, neither with respect to the average altitudes (p values between 0.154 and 0.705) nor the scattering of altitudes in individual states (p value: 0.272). Nevertheless, individual infection sites at altitudes around 1,500 m were only found in the later time window of 1984 to 2013 (two in Carinthia, one in Tyrol, one in Vorarlberg) (Figure 3). The new infection sites in Tyrol, however, proved to be significantly higher than those in the rest of the country (Table). Because of the relatively low number of cases and the strong scattering of altitudes, statistical significance was not reached in the comparisons of Vorarlberg with Tyrol, Salzburg and Carinthia (Table).

Table

Pairwise comparisons of sea levels of tick-borne encephalitis virus infection sites between federal states of Austria, 1972–2013

	LA/Vie	UA	Bld	Sty	Car	Sbg	Tyr	Vbg
LA/Vie		<0.001	0.036	<0.001	<0.001	0.473	no cases	no cases
UA	<0.001		<0.001	0.275	<0.001	0.981		
Bld	0.006	<0.001		<0.001	<0.001	0.088		
Sty	<0.001	1.000	<0.001		<0.001	0.125		
Car	<0.001	<0.001	<0.001	<0.001		0.900		
Sbg	<0.001	0.154	<0.001	0.999	0.939			
Tyr	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Vbg	<0.001	0.002	<0.001	0.002	0.751	0.283	1.000	

Bld: Burgenland; Car: Carinthia; LA/Vie: Lower Austria and Vienna; Sbg: Salzburg; Sty: Styria; Tyr: Tyrol; UA: Upper Austria; Vbg: Vorarlberg.
p values form Tukey's HSD test. Upper triangular matrix: period 1972–1983; lower triangular matrix: period 1983–2013.

Discussion

We have evaluated epidemiological changes of TBE in Austria over an observation period of more than 40 years at a regional level. A number of factors have been described that can affect the documented incidence of TBE. These include climatic or anthropogenic environmental changes influencing the ecosystems required for virus circulation in nature [8], socioeconomic changes increasing the risk of human exposure to tick bites [16], changes in the awareness, laboratory diagnosis and notification of the disease, as well as vaccination [6]. In Austria, a marked decline of TBE incidence was concomitant with a steadily increasing vaccination coverage, which started in the late 1970s and reached more than 80% of the total population around 2000 [6]. The incidence in the unvaccinated population, however, remained constant at ca 6 per 100,000 (Figure 1B), suggesting no major changes in the countrywide overall risk of human exposure to TBE virus-infected ticks. It is, however, apparent from our analysis of incidences in the unvaccinated population in the different federal states as well as the mapping of sites of infection, that this figure is an aggregate of opposing trends in different parts of the country, with increasing incidences in alpine regions in the west, decreasing incidences in the north-east and relative constancy in the high endemic areas in the south.

The development of new endemic regions in Tyrol (first documented case in 1984) and Vorarlberg (first documented case in 2000) deserves special attention. Since there is no evidence for socioeconomic or behavioural changes on such a small regional and temporal scale that might have increased the risk of exposure, the changes can best be explained by the establishment of truly novel natural foci and their further spread to cover large parts of the valleys of the rivers Inn and Ziller in Tyrol and the river Ill in Vorarlberg. A very similar expansion of TBE virus into a mountain valley also occurred recently in the Canton Valais in Switzerland [25,32], suggesting that similar mechanisms operate in alpine regions of Central Europe that allow the dispersion of this virus. It remains an unresolved issue, however, how the virus could have reached these locations. They are separated from other endemic regions by high mountain barriers, raising the possibility that the seeding with the virus occurred through the courses of rivers and valleys, e.g. from southern Germany to Tyrol through the Inn valley and from the Rhine valley in Liechtenstein/eastern Switzerland to Vorarlberg (Figure 2C). In this context, it is important to note that in the Austrian examples, both sides of the valleys became affected, i.e. the river did not represent a barrier in the establishment and spread of the virus. An extension of endemic areas along rivers would be consistent with recent phylogenetic analyses of virus isolates from Germany, the Czech Republic and Slovakia [33], which unveiled a predilection of long-distance migratory routes of TBE virus along river valleys in the past 300 years. Similar studies have not yet been conducted for Austria. Comparative analyses of the sequences of virus isolates from different parts of Austria and other European countries could shed light on the viral origins of the new endemic areas. Roe deer have been implicated in long-distance transport of infected ticks [33] but introduction by birds is also a possibility [28,29]. It cannot be excluded that the potential ways of virus dispersal in Europe have changed over time, but it appears more likely that the emergence of new endemic regions is caused by the establishment of previously non-existing suitable climatic and ecological conditions that are required for the maintenance of TBE virus circulation in nature [34].

Similar to the decreased incidence of TBE in Hungary (ca 2.8/100,000 until 1997 and 0.4 to 0.8 thereafter [35], although vaccination coverage was very low [36], the north-east of Austria (including the capital city of Vienna and some of its most popular recreational areas) also encountered a decline from an incidence of ca 4 to only ca1.3 per 100,000 (Figure 1C). In the absence of evidence for human behavioural changes at such a

small regional level and other factors that could affect TBE virus endemicity, these data suggest decreasing virus activity and even the loss of natural foci in these regions. Most of these areas have an altitude below 400 m, in contrast to those with increasing incidences in western Austria, with median altitudes of more than 600 m (Figure 3). A similar trend, i.e. reduction of cases in lowlands and increases in submountaineous areas has also been described in Slovakia [27] and may be associated with climatic changes that are unfavourable for the maintenance of virus circulation in its natural hosts in the lowland areas of these countries. With respect to the average overall altitudes of infection sites, however, no significant changes were observed over the whole period of observation in the traditional endemic areas in Austria (Figure 3), similar to the situation in the Czech republic [19].

Overall, our data document strong region-specific differences in the changing epidemiology of TBE and conform to a tendency of establishment of new natural foci of TBE in Europe. This has been observed on a wide geographical scale and includes countries with different climates and topographies such as Norway, Sweden, Finland, Denmark, and Switzerland [13,20-25]. The mechanisms of establishment as well as spread and the factors controlling these processes, however, are still far from clear. This may be exemplified by the situation in the lake district east of Salzburg. Here, the virus is highly endemic in certain locations but several lakes and intervening hill sides are completely spared (Figure 2A) and not a single case has been documented in the 42 years of observation, although all known parameters potentially affecting TBE virus circulation and human infection (climate, landscape and geography, human outdoor activities, animal reservoirs etc.) are seemingly identical in the whole region. The elucidation of the interplay of processes driving or restricting the spread of TBE virus and, consequently, a detailed understanding of the focal nature of TBE endemic areas remain a formidable challenge for the future.

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Conflict of interest

None declared.

Authors' contributions

Study design, data compilation and analysis: FXH, KS, HH, CK Statistical analyses: MK Geographical data analysis: MW, WK Data collection on vaccination coverage: AE Data collection of infection sites in Styria (until 1983): WS Writing of manuscript: FXH, KS Revision and approval of manuscript: all.

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Survey of surveillance systems and select prevention activities for hepatitis B and C, European Union/ European Economic Area, 2009

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Hepatitis B and C viral infections are leading causes of hepatic cirrhosis and cancer. The incidence and prevalence of both hepatitis B and C varies across European countries. European wide surveillance data help to understand the dynamic epidemiology of hepatitis B and C, which is important for the implementation and effectiveness of prevention and control activities. Comparison of surveillance data between countries in Europe is hampered by the differences in national healthcare and reporting systems. This report presents the results of a survey in 2009 which was undertaken to collect baseline information on surveillance systems and core prevention programmes for hepatitis B and C in individual European Union/ European Economic Area countries. The results provide key information to aid the interpretation of surveillance data, and while indicating heterogeneity in national surveillance systems and programmes, they highlight the potential of these systems. This resource has supported the implementation of a standardised European enhanced surveillance programme.

Introduction

Infections with hepatitis B (HBV) and C (HCV) viruses can result in acute and chronic hepatitis and are leading causes of hepatic cirrhosis and cancer. Both infections are globally prevalent. According to the World Health Organization (WHO), one third of the world's population has been infected with HBV and around 240 million people have chronic infection [1,2]. The WHO additionally estimates that 2.8% of the global population have been infected with HCV, resulting in 185 million people with antibodies to HCV [3,4]. Across Europe, HBV and HCV are prevalent, but the incidence and prevalence vary between countries [5], with some specific subpopulations within countries particularly affected, such as people who inject drugs (PWIDs) and men who have sex with men (MSM) [6-8].

The modes of transmission of HBV and HCV differ and vary considerably around the world [6-10]. In areas where the prevalence of HBV is high (defined as prevalence of hepatitis B specific antigen (HBsAg) ≥ 2%), transmission is mostly perinatal or during childhood through horizontal transmission to close household contacts. In areas of lower prevalence, HBV transmission usually occurs later in life mostly through injecting drug use (IDU) and sexual exposure [11]. HCV is most commonly transmitted through percutaneous exposure. In countries that have introduced blood screening and have good systems of infection control, most infections appear to have occurred through IDU. Some infections however, occur among renal dialysis patients, patients who have undergone surgical procedures and individuals exposed through body piercing or tattooing [12]. Sexual and perinatal transmission of HCV is uncommon [13].

Because transmission of HBV and HCV varies between countries, the most effective prevention strategy depends on the underlying epidemiology or its drivers. Prevention strategies tackling HBV in all European countries include either a universal or targeted vaccination programme. The prevention of HCV infection, however, is more problematic, as there is currently no effective vaccine. Reducing the HCV disease burden is achieved through early diagnosis and effective prevention strategies to reduce or eliminate the risk for transmission from nosocomial exposures (e.g. blood transfusion, unsafe injection practices) and high-risk practices (e.g. IDU) [9,14].

Harmonisation of the surveillance of viral hepatitis in the European Union (EU) was identified by the European Parliament in 2006 as one of the priorities for the European Centre for Disease Prevention and Control (ECDC) [15,16]. Robust surveillance information is important for effective public health action. Comparison of surveillance data between countries is nevertheless hampered by differences in healthcare, screening practices and surveillance systems [15]. Detailed information on national surveillance and

prevention programmes is important for a clear interpretation of epidemiological data at the international level. There is known to be variation in the case definitions used and no clear distinction in the reporting between acute and chronic hepatitis B and C cases in many countries [15].

In order to provide a foundation for the development of enhanced surveillance of hepatitis B and C across Europe, ECDC undertook a survey in 2009 to describe existing national surveillance systems and core prevention programmes among EU/European Economic Area (EEA) countries. The survey aimed to build upon the findings of Rantala and van de Laar in 2008 whereby a preliminary review of programmes in a select number of European countries was undertaken [15].

Box

European Union 2002 and 2008 case definitions for hepatitis B and C[a]

EU 2002/253/EC Hepatitis B (acute) case definition:

Clinical criteria – In symptomatic cases, clinical picture compatible with hepatitis e.g. discrete onset of symptoms and jaundice or elevated serum aminotransferase levels.

Laboratory criteria – IgM antibody to hepatitis B core antigen (anti-HBc) positive. Detection of hepatitis B virus (HBV) nucleic acid in serum.

EU 2002/253/EC Hepatitis C case definition:

Clinical criteria – in symptomatic cases, clinical picture compatible with hepatitis, e.g. discrete onset of symptoms and jaundice or elevated serum aminotransferase levels.

Laboratory criteria – Detection of hepatitis C virus (HCV) specific antibodies. Detection of HCV nucleic acid from clinical samples.

EU 2008/426/EC Hepatitis B (acute) case definition:

Clinical criteria - Any person with a discrete onset of symptoms (e.g. fatigue, abdominal pain, loss of appetite, intermittent nausea and vomiting) AND at least one of the following three: fever; jaundice; and elevated serum aminotransferase levels.

Laboratory criteria – Hepatitis B virus core IgM antigen specific antibody response. Laboratory results need to be interpreted according to the vaccination status.

Epidemiological criteria -An epidemiological link by human to human transmission (e.g. sexual contact, vertical transmission or blood transmission).

EU 2008/426/EC Hepatitis C (acute) case definition:

Clinical criteria – not relevant for surveillance purposes.

Laboratory criteria – at least one of the following two: Detection of HCV specific antibodies; detection of HCV nucleic acid in serum OR HCV specific antibody response confirmed by a different antibody test.

[a] Replaced in 2012 by new case definitions, European Commission 2012/506/EU, which capture data on acute and chronic cases of hepatitis B and C.

Methods

All 27 EU Member States as well as Iceland, Liechtenstein and Norway were invited to participate in a web-based survey on surveillance and prevention of HBV and HCV. Nominations of technical experts for hepatitis B and C surveillance were requested from the formal ECDC contact point at each of the national organisations for surveillance. The link to the questionnaire, along with a cover letter, was sent to these nominated contacts or to the general ECDC contact person if no nomination was received in September 2008. All non-responder countries were followed up with a reminder email and countries were able to upload their data until October 2009.

The survey was divided into separate parts for hepatitis B and C and further sub-divided into four sections covering: (i) general aspects of hepatitis surveillance (including case definitions and objectives); (ii) key sources and the type of data collected; (iii) other questions related to surveillance e.g. linkage to other data sources; and (iv) local provision of screening and vaccination services.

Survey data were analysed in Excel version 2007 (Microsoft, Redmond/Washington, United States). The results were collated into a report and the participants were asked to validate their country-specific information to check that it had been correctly analysed and interpreted.

Data collected on vaccination programmes were validated and completed with data from the Vaccine European New Integrated Collaboration Effort (VENICE) project (http://venice.cineca.org/) and data from the European surveillance network for selected vaccine-preventable diseases (EUVAC.NET) (http://www.euvac.net/).

Results

All 30 countries participated in the survey, the Czech Republic only completed the hepatitis C section and Liechtenstein completed only the hepatitis B section. The overall response rate was high at 29 of 30 for each disease.

Surveillance systems

A detailed summary of the information on national surveillance systems for hepatitis B and C is shown in Table 1. All countries reported having a system in place for the surveillance of hepatitis B and C and this system is mandatory for most of these countries for both hepatitis B (27/29) and hepatitis C (26/29). The survey asked if the surveillance system could be defined as active, which meant that the surveillance system was based on the initiative of public health officials to actively contact physicians, laboratory, hospital staff or other relevant sources to report data. Only four countries reported having an 'active' surveillance system for hepatitis B and five countries for hepatitis C. Around half the countries (15/29 for hepatitis B and 14/29 for

Table 1

Summary, by disease, of information obtained on national surveillance systems for hepatitis B and C, European Union/ European Economic Area countries, 2009 (n=30)[a]

Properties	Number of countries	
	Hepatitis B	Hepatitis C
Type of surveillance		
Mandatory	27	26
Voluntary	2	3
Passive[b]	25	24
Active[c]	4	5
Case-based data (individual anonymised patient data)	26	26
Aggregate data (data aggregated at regional or national level)	8	9
Type of surveillance system		
A hepatitis specific surveillance system	15	14
Several different hepatitis specific surveillance systems, one of which is the most comprehensive	3	3
Several different hepatitis specific surveillance systems, none is the most comprehensive	1	2
Syndromic surveillance[d] of viral hepatitis	5	5
Other	5	5
Objectives		
Monitor trends	29	29
Detect outbreaks	26	25
Monitor changes in disease distribution	28	27
Evaluate and plan control measures	28	28
Improve knowledge of epidemiology	27	28
Other	5	2
Case definitions		
EU 2002/253/EC[e]	3	4
EU 2008/426/EC[e]	8	11
Possibly European Union (lack of information)	5	5
Extended European Union	5	4
No case definition	3	2
Other	5	3
Case classification[f]		
Possible	1	1
Probable	15	6
Confirmed	28	28
Acute	29	27
Chronic	17	18
Asymptomatic	9	12
Suspected	1	1
Duplicates		
Including duplicates	4	9
Under-reporting		
No	3	2
Exists	26	27

[a] Of the 30 countries, all but Liechtenstein completed the survey for hepatitis B, and all but the Czech Republic completed the survey for hepatitis C.

[b] A surveillance system based on healthcare providers reporting notification data on their own initiative without being reminded.

[c] A surveillance system based on a public health officials initiative to actively contact physicians, laboratory or hospital staff or other relevant sources to report data.

[d] A surveillance system where public health officials monitor disease indicators in real-time or near real-time to detect outbreaks of disease earlier than would otherwise be possible with traditional public health methods.

[e] See text box.

[f] As defined by country.

Table 2

Set of variables in national surveillance systems for hepatitis B and C, European Union/European Economic Area countries, 2009 (n=30)[a]

Characteristics		Number of countries	
		Hepatitis B	Hepatitis C
Basic data	Patient ID	24	22
	Date of birth or age	29	29
	Sex	29	29
	Country of birth	16	16
	Place of residence	28	27
	Date of onset of the disease	26	23
	Date of diagnosis	21	21
	Date of reporting/notification	27	28
	Date used for statistics	19	18
	Country where infection has most likely been acquired	19	19
	Immunisation status	24	11
	Outcome	18	15
Clinical and case classification information	Clinical symptoms	16	13
	Laboratory results	23	24
	Epidemiological information	21	22
Transmission route/risk factors	Homosexual contact	16	14
	Heterosexual contact	16	13
	Injecting drug use	21	21
	Mother HBsAg/HCV-positive	19	15
	Close family member HBsAg/HCV- positive	20	17
	Sex partner HBsAg-positive	17	17
	Blood or blood product transfusion	21	21
	Invasive healthcare procedure/dental treatment	18	20
	Organ transplantation	16	17
	Haemodialysis	18	19
	Needle injury or other occupational exposure	18	19
	Tattooing/body piercing	18	19
	Other	8	8
Other factors	Hospitalisation	19	17
	Length of hospitalisation	8	8
	Genotype information	1	3

HCV: hepatitis C virus; HBsAg: hepatitis B specific antigen; ID: identity.

[a] Of the 30 countries, all but Liechtenstein completed the survey for hepatitis B, and all but the Czech Republic completed the survey for hepatitis C.

hepatitis C) reported that they had a specific hepatitis surveillance system in place and several countries (4 for hepatitis B and 5 for hepatitis C) reported more than one surveillance system for hepatitis B or C. In five countries (Hungary, Italy, Latvia, Romania and Slovakia), the reporting systems for hepatitis B and C are part of a syndromic surveillance system.

Most countries accorded with the specific objectives for surveillance listed in the questionnaire with only a few countries identifying 'other' objectives (Table 1). These 'other' objectives included 'the resource allocation and healthcare planning' identified by Ireland and the 'monthly publication of statistics required by law' noted by Luxembourg.

There were differences in the case definitions between countries (Table 1). A total of 11 of 29 countries confirmed that they used one of the standardised EU case definitions (textbox) for hepatitis B and 15 of 29 reported they did so for hepatitis C. Three countries reported there being no case definition in use for hepatitis B and two countries reported no case definition for hepatitis C.

Of the 29 countries participating in the hepatitis B questionnaire, 28 reported that confirmed cases were included in surveillance and 15 reported that probable cases were also included. All 29 countries included acute hepatitis B cases and 17 countries included chronic cases. Thirteen of the 17 countries that include

both acute and chronic cases reported that they could differentiate between acute and chronic infection. In relation to hepatitis C surveillance, 28 countries included confirmed cases in their national systems and five countries included probable cases. Twenty-seven of the 29 countries included acute hepatitis C cases. Eighteen countries included both acute and chronic cases and half of these countries reported that they were able to differentiate between these cases.

Data sources were very similar for both diseases with physicians being cited as the main source. In addition, nine countries reported sexually transmitted infection (STI) clinics as a source of data for hepatitis B and six countries reported these clinics as a source for hepatitis C. Seven countries also collect data for these infections through laboratory networks, four countries collect the data through sentinel surveillance and five countries collect it through serosurveys in the general population.

Electronic data collection was the most common route reported (23/29 for hepatitis B; 25/29 for hepatitis C). Four countries (Bulgaria, Norway, Poland and Romania) collect hepatitis C data using a paper-based system and three countries use this system for hepatitis B (Poland, France and Liechtenstein). Some countries reported using both paper and electronic data collection.

Twenty-six of the countries had case-based data available for both hepatitis B and C while three countries (Bulgaria, Poland and Romania) reported the availability of only aggregated data at the time of the survey. Several countries reported that duplicates may be included in the national surveillance system (4/29 for hepatitis B; 9/29 for hepatitis C). Twenty-six countries reported that under-reporting exists for hepatitis B and 27 countries reported this for hepatitis C.

Countries collected data on a number of different variables (Table 2). Over two-thirds of the countries collected a broad set of data covering demographic and clinical data as well as information on transmission routes. Other countries, such as Belgium, Luxembourg and Spain, collected a much more select dataset focused on basic demographic data. Few countries collected data on genotype information or length of hospitalisation.

Ten of the countries reported that they can link their hepatitis data to local registers on liver transplants, liver cancer, mortality and/or hospital admissions. Five countries (Denmark, Finland, Iceland, Slovakia and the United Kingdom (UK)) reported links to all these registers.

Prevention programmes

Screening

All countries (except Luxembourg) reported at least one national screening programme in place for HBV or HCV (Table 3). The most commonly reported screening programmes included antenatal screening of pregnant women for HBV (24/29) and the screening of blood and organ donors for HBV (26/29) and HCV (28/29). Many of the countries had hepatitis screening programmes in place for specific risk groups including PWIDs (15/29 for HBV; 15/29 for HCV), prisoners (11/29 for HBV; 11/29 for HCV) and attendees of STI clinics (10/29 for HBV; 8/29 for HCV). Very few countries reported national screening programmes for military recruits, people with multiple sexual partners or residents of long-term health facilities.

Table 3

Screening programmes for hepatitis B and C, European Union/European Economic Area countries, 2009 (n=30)[a]

Screening programme	Number of countries	
	Hepatitis B	Hepatitis C
Pregnant women	24	3
Military recruits	3	1
People who inject drugs	15	15
STI clinic patients	10	8
Multiple sex partners	2	1
Prisoners	11	11
Haemodialysis patients	21	22
Long-term healthcare facilities	2	0
Healthcare workers	7	7
Workers who are occupationally exposed to the virus	11	10
Blood and organ donors	26	28
Other groups	4	4

STI: sexually transmitted infection.

[a] Of the 30 countries, all but Liechtenstein completed the survey for hepatitis B, and all but the Czech Republic completed the survey for hepatitis C.

Four of 30 countries reported 'other' types of national screening programmes for HBV and HCV. Where specified, these included all people with human immunodeficiency virus (HIV) infection or HIV-infected MSM.

Immunisation

Twenty-two countries reported that they have a universal hepatitis B vaccination programme in place. The other seven countries (Denmark, Finland, Iceland, the Netherlands, Norway, Sweden and the UK) reported that they have opted for selective vaccination programmes targeting specific risk groups at the time of the survey. In addition to the routine childhood vaccination programmes in place, 11 countries reported catch-up programmes for older children and adolescents. Four countries reported 'other' universal vaccination programmes, which included a programme targeting children before entry to primary school in Slovenia.

Over half of the countries with a universal vaccination programme provided information on coverage and ten countries reported coverage rates in infants younger than two years of over 95%. The reported coverage did vary between countries and age groups ranging from between 30% and 99% for coverage among infants to between 31% and 98% for coverage among adolescents.

In addition to universal programmes, most countries have implemented selective vaccination programmes for key groups. The main risk groups targeted for vaccination included 'individuals at risk for HBV due to occupation' (27/29 countries), household contacts of HBsAg positive patients (23/29 countries), haemodialysis patients (23/29 countries), and neonates born to HBsAg positive mothers (22/29 countries). Two countries with universal vaccination programmes in place (Austria and Liechtenstein) reported no targeted vaccination programme for specific risk groups. Each of the seven countries with no universal vaccination programme in place reported at least five targeted vaccination programmes for risk groups. Many countries reported vaccinating a range of 'other' risk groups such as HIV and chronic liver disease patients, MSM, prisoners, PWIDs sex workers, and travellers to countries with a high prevalence of HBV.

Discussion

The results of the survey conducted in this study provide an overview of national surveillance systems and key aspects of prevention programmes for hepatitis B and C across Europe. The survey aimed to pull together detailed information particularly on the existing surveillance systems at country level to better understand the European landscape before embarking upon the implementation of an EU/EEA wide surveillance system. There are some key limitations to this survey. Firstly, although the overall response rate from countries was high there were gaps in the completeness of data provided with countries not completing all questions, especially those on vaccine coverage. Secondly, although additional explanatory information was provided to countries to help clarify terms used in the survey, respondents may have understood and interpreted terms differently. For example, there were differences in the interpretation of the ECDC definition of an active surveillance system that was provided in the questionnaire (Table 1) with some countries describing active surveillance as a system which stipulates that physicians or laboratories report cases directly to public health authorities. Thirdly, the questionnaire was quite broad in its scope, covering both hepatitis B and C prevention and surveillance activities and it is possible that this contributed to the incompleteness of the data collected as the questionnaire was quite lengthy. Also in terms of prevention, the questionnaire collected basic information on screening programmes and HBV vaccination so only provides a limited overview. Programmes have continued to evolve since the survey was undertaken and Romania, for example, started to implement case-based data collection. A further and more comprehensive survey would therefore provide a more accurate picture of current prevention activities across EU/EEA countries.

All countries participating in the survey undertook surveillance of both hepatitis B and C. However, there is considerable variation in surveillance systems across Europe and earlier surveys have also found differences between countries in terms of surveillance system structures, reporting practices, data collection methods and case definitions [15]. In this survey, the main objectives for hepatitis surveillance were found to be similar across countries and included the monitoring of trends, detecting outbreaks, and the evaluation and planning of control measures. The consensus across countries around these core objectives was obviously important during the development and implementation of the enhanced surveillance programme.

Most countries included acute cases of HBV and HCV in their systems which may reflect the fact that most national systems historically focused on newly acquired infections in patients with clinical symptoms of hepatitis [16] Some countries reported that they were unable to differentiate between acute and chronic cases of HBV and HCV. This inability to differentiate data and the lack of chronic hepatitis data from some countries has obvious implications for the comparison of data between countries and for a clear interpretation of the data.

Case definitions varied between countries, although most countries used an EU-related case definition. It should be noted that this survey took place during 2008 and 2009 in a period of transition as the EU 2008 case definitions (2008/426/EC) replaced previous case definitions (2002/253/EC) for hepatitis and this may explain some of the variation. Indeed, subsequent to the initial survey, there was a validation process for countries and a number of countries changed their information on case definitions at this time. Since the survey was undertaken, the EU case definitions were further revised in 2012 to incorporate acute and chronic cases of both infections based on laboratory criteria only [17]. Countries demonstrated flexibility in being able to adapt their data, as in the first data collections most countries were able to provide data defined by these new case definitions, although the differentiation of hepatitis C cases as acute or chronic was problematic [18].

In the survey, deviation from the EU case definitions was observed. Some countries who reportedly used the EU 2008 case definition for hepatitis B included chronic and asymptomatic cases, even though these are not covered by this case definition. There was similar variation for hepatitis C. The heterogeneity around case definitions, data collected and the possibility of duplicate records and under-reporting have all been

hurdles for the harmonisation of surveillance activities at EU level. These potential difficulties were also previously identified and highlighted by the former EUROHEPNET team [16]. This team, established in 2002 by the European Commission to develop a European network on suveillance and prevention of vaccine preventable hepatitis, undertook a similar scoping survey and identified similar issues. The information collected in this current survey however, undoubtedly helped in addressing these differences during the implementation phase of the enhanced surveillance programme at the EU level. While this programme aims to harmonise surveillance, differences in surveillance systems inevitably exist between countries and a clear understanding of local systems therefore aids the interpretation of data and of any differences in these data between countries.

The survey highlighted that most countries have case-based hepatitis B and C data available at the national level which is mostly in an electronic format. The existence of these national surveillance systems across Europe provided an essential platform for building the EU-wide enhanced surveillance system. The collection of a vast body of data from countries and the possibility in some countries to link these data to other registers of morbidity and mortality offer exciting prospects for taking forward surveillance data of hepatitis B and C at EU level.

In relation to prevention activities, the survey was restricted to screening and HBV immunisation programmes and these programmes may have changed since the survey was undertaken. For example, subsequent to the undertaking of survey, universal hepatitis B vaccination began to be implemented in the Netherlands. Nevertheless, the information collected provides a valuable resource to facilitate the interpretation of data. Comparison of vaccination coverage figures between countries is somewhat challenged by the differences in the denominators and dates for the data provided. The results however highlight that while some countries reported low coverage, many countries reported high coverage, particularly among infants, confirming the findings of the VENICE project [19]. This project, conducted in 2009, included 27 EU Member States and two EEA countries (Norway and Iceland) and found routine vaccination programmes in 74% of countries, with reported coverage ranging from 29% to 99%. Further evaluation of vaccination strategies across Europe is important and would benefit from greater harmonisation around coverage and surveillance data. The targeted screening of risk groups also showed considerable variation. Further cost-effectiveness studies of screening different risk groups would help countries target their resources more efficiently.

Following this survey, a working group was established consisting of national experts from a number of EU/EEA countries to assist the ECDC in preparing the protocol for European-wide hepatitis surveillance. By evaluating the common denominators in national surveillance systems and by establishing the core values and objectives of European surveillance, a protocol was developed and discussed at the first European network meeting on hepatitis in 2011. It was recognised that not all countries would be able to comply with the new EU case definitions and not all countries would be able to collect data on the defined set of variables (of which only a few were compulsory such as age, data source, date of diagnosis, date used for statistics, classification, record type, reporting country, sex, stage of hepatitis, transmission and subject). However, it was agreed that implementation should start in 2011 with a retrospective data collection of five years. Indeed, the first data collections were challenging in terms of data comparability and completeness, as described in the report published by ECDC in 2013 [18]. Future analysis will aim to improve the interpretation of the surveillance results by directly linking the surveillance data with current screening and vaccination programmes.

In conclusion, the epidemics of HBV and HCV infections in Europe have emerged over recent decades to pose major challenges to public health and both epidemics continue to evolve [7,20-23]. The strengthening and standardisation of national surveillance systems is widely recognised as important to assess the burden of diseases, evaluate prevention and control strategies and identify epidemiological trends [16,24]. While standardisation across countries is considered a huge challenge [25], the results of this survey provided a foundation which assisted in the development of a common enhanced European surveillance system. Although harmonising systems across Europe will take time, as demonstrated by the experience with HIV, there is great potential to improve surveillance of hepatitis B and C at the European level with interesting possibilities for data linkage which may maximise the utility of this information.

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Conflict of interest

None declared.

Authors' contributions

Marita van de Laar commissioned the original survey and contributed to the production of the survey report. Erika Duffell wrote the paper for publication and undertook revisions through close collaboration with Marita van de Laar.

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Lower seroreactivity to European than to North American H3N2 swine influenza viruses in humans, Luxembourg, 2010

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Seroreactivity to H3N2 swine influenza viruses (SIVs) was evaluated in serum samples collected from 843 people aged 0 to 100 years in 2010 in Luxembourg. Sera were analysed by haemagglutination inhibition (HI) and virus neutralisation (VN) assays targeting a European H3N2 SIV, a North American H3N2 variant of swine origin (H3N2v) and human seasonal H3N2 viruses isolated in 1975, 1995 and 2005. HI antibodies (titre ≥ 10) against European H3N2 SIV were almost exclusively detected in those born before 1990, of whom 70% were seropositive. HI antibodies against H3N2v were predominantly found in those born before 2000, with 86% seropositive. Titres against the North American H3N2v were higher than against the European H3N2 SIV. VN patterns were similar, but with higher rates and titres. We also demonstrated lower seroreactivity to European H3N2 SIV than to North American H3N2v virus. Finally, we found a strong correlation between HI titres against the European H3N2 SIV and H3N2v and their respective human ancestors, A/Victoria/3/75 and A/Nanchang/933/95. This finding and the minimal contacts between humans and pigs in Luxembourg suggest that anti-SIV antibodies in human serum samples reflect serological cross-reactivity with historical human H3N2 viruses. Our findings help assess the pandemic risk of H3N2 SIV.

Introduction

Three swine influenza virus (SIV) subtypes, H1N1, H1N2 and H3N2, are enzootic throughout the world in regions with a high density of pigs. The haemagglutinin (HA) and neuraminidase (NA) genes of most, if not all, H3N2 SIVs have been derived from human seasonal influenza A(H3N2) viruses. In Europe, H3N2 SIVs are derived from descendants of the A/Hong Kong/1/68 pandemic influenza A(H3N2) virus, but they have evolved further through genetic reassortment with the endemic avian-like H1N1 SIVs present in western Europe since the late 1970s. This has resulted in H3N2 SIVs with human-like HA and NA genes and avian-like internal genes [1,2]. In North America, H3N2 viruses have become established in swine since 1998. They are known as 'triple-reassortant' viruses because their HA, NA and polymerase B1 genes stem from human seasonal H3N2 viruses and the remaining internal genes from avian influenza virus and classical H1N1 SIV [3]. Since 2009, novel reassortant H3N2 viruses with variable numbers of internal genes derived from the 2009 pandemic influenza A(H1N1)pdm09 virus have been reported frequently and this has further complicated the epidemiology of swine influenza in the United States (US) [4]. From 2009 to 2012, these novel influenza A(H1N1)pdm09 reassortants accounted for 54% of H3N2 SIVs isolated [4]. Reassortant viruses with seven genes from the triple-reassortant H3N2 SIVs and only the matrix (M) gene from the A(H1N1)pdm09 virus have become the dominant genotype. These viruses, called H3N2 variant or H3N2v when isolated from humans, have caused many zoonotic infections since 2011 [5].

Antigenic drift in the HA is generally slower in SIVs than in human influenza viruses, and pigs can therefore serve as reservoirs of older human HAs [6-8]. Swine-adapted viruses with an HA of human origin could initiate a pandemic once immunity within the human population has waned sufficiently to allow widespread infection, provided that the viruses also have the ability to spread efficiently from person to person. This was observed for the A(H1N1)pdm09 virus that contains the classical swine H1. Evolutionarily, the 1918 H1N1 pandemic influenza virus was the common ancestor of human seasonal and classical swine H1N1 influenza viruses; it has undergone significant antigenic drift in humans but remained largely in antigenic stasis in swine [9,10]. Consequently, only people born before the 1940s had been previously exposed to human seasonal H1N1 viruses with an H1 related to that of A(H1N1)pdm09, and a pandemic was possible because younger people lacked cross-reactive anti-H1 antibodies [11-14]. A similar situation could occur with human-adapted H3N2 SIVs in the future if they carry the HA of seasonal H3N2 viruses that have not circulated in decades.

Before 2011, only sporadic dead-end zoonotic infections with H3N2 SIVs had been reported, in humans in close contact with pigs. Recently, the H3N2v virus has caused 343 human infections in the US from August 2011 through October 2014 [15]. These infections occurred primarily in young children visiting agricultural fairs, and the H3N2v virus did not spread widely through the human population [16]. These zoonotic infections prompted serological investigations for cross-reactive antibodies against H3N2v in people of various ages in the US, Canada, Norway and England. These studies found that more than half of the adolescents and young adults tested had haemagglutination inhibition (HI) antibody titres ≥ 40, which is considered as seroprotective [17]. In contrast, younger children and older adults typically exhibited lower or negative antibody titres [18-22].

Antibodies against the antigenically distinct European H3N2 SIV have been reported in ca 50% of humans in studies in Italy and Germany between 2008 and 2010 [23,24]. However, these studies sought to compare antibody prevalences in swine workers and non-swine workers with a mean age of 45 years, rather than using an age-stratified design to assess seropositivity in the general population. In this study, we primarily sought to compare the seroreactivity to a European H3N2 SIV with that to a North American H3N2v virus in people in various age groups in Luxembourg who were very unlikely to have been exposed to pigs. Importantly, we also examined the association between antibody titres against swine-origin and those against human seasonal H3N2 influenza viruses.

Methods

Serum samples

A total of 843 anonymised human serum samples were randomly selected from the Serum Bank of the Laboratoire National de Santé, Luxembourg. The sera were collected from patients admitted to hospital for various reasons in April or May 2010. The sera were from people born between 1910 and 2010 and were divided into 10 groups by birth decade. As an example, 1910s refers to people born between 1910 and 1919. Ca 10 sera per year of birth and 100 sera per birth decade were tested. Only the youngest (n = 40 samples) and oldest age group (n = 9 samples) had fewer samples. The sex ratio in each age group was ca 50:50, except for the participants born in the 1910s (two male vs seven females). No further personal data were collected due to ethical constraints.

H3N2 influenza viruses

We measured serum antibody titres against human seasonal H3N2 influenza viruses A/Victoria/3/75, A/Nanchang/933/95 and A/Wisconsin/67/05. These viruses were circulating worldwide during 1976–78, 1996–98 and 2006–08, respectively, and were recommended as the influenza vaccine strains by the World Health Organization during their time of circulation. We also measured antibody titres against sw/Gent/172/08, a virus representative of H3N2 SIVs that are currently circulating in western Europe, and against A/Indiana/08/11, which represents swine-origin H3N2v viruses isolated from humans in the US since 2011.

The HA1 amino acid sequences of these five viruses, a selection of human seasonal influenza A(H3N2) viruses (1968–2012), and European and North American H3N2 SIVs (1984–2012) were downloaded from GenBank. The sequences were compared using the MegAlign programme with DNASTAR 5.01 software (DNASTAR, Inc., Madison, WI, US). A neighbour-joining phylogenetic tree was constructed to compare amino acid sequences with MEGA 5.05 software (http://www.megasoftware.net/). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are expressed as the number of amino acid substitutions per site.

Antigenic characterisation of the five viruses used for serology was performed by HI and virus neutralisation (VN) assays with hyperimmune swine serum (for sw/Gent/172/08) or post-infection ferret sera (for all other viruses). We used ferret serum against A/Wuhan/359/95 instead of A/Nanchang/933/95 because a ferret serum against the latter virus was not available. The two viruses have only two amino acid differences in the HA1, outside the antigenic region. Viruses used in HI assays were propagated in 10 day-old embryonated chicken eggs (≤ 4 passages); viruses used in VN assays underwent an additional passage in Madin-Darby canine kidney (MDCK) cells.

Serological assays

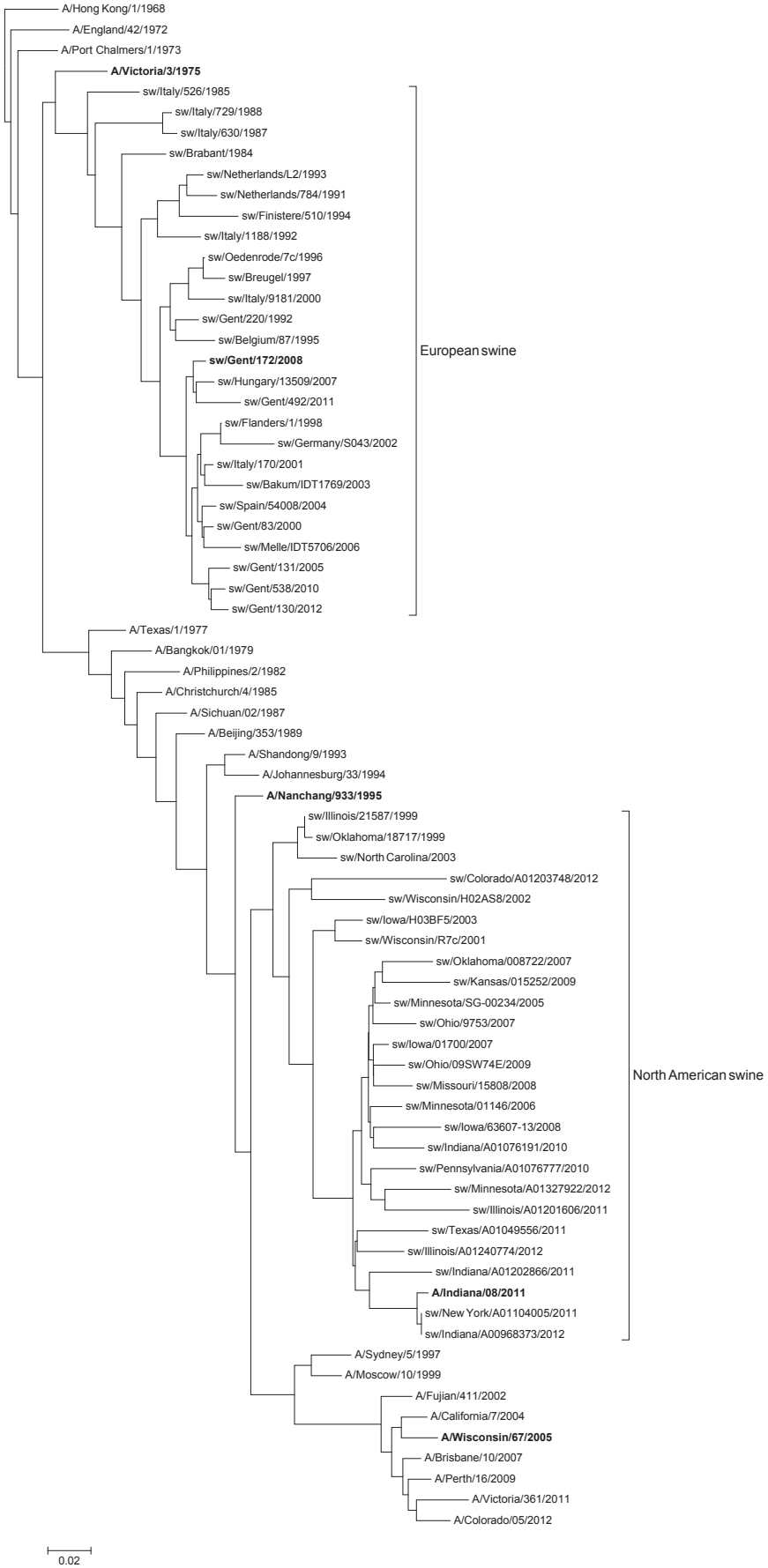
All sera were examined in HI assays against the three human seasonal H3N2 viruses and the two swine-origin H3N2 viruses. Since the VN assay is more sensitive than the HI assay and highly relevant for protection, sera from people born after 1940 were also tested in VN assays against the swine-origin viruses. HI and VN assays were performed following standard procedures [25,26]. Antibody titres were expressed as the reciprocal of the highest serum dilution that showed complete inhibition of HA of 4 hemagglutinating units of virus (HI assay), or 50% neutralisation of 10^2 50% tissue culture infectious doses ($TCID_{50}$) of virus in MDCK cells (VN assay). The starting serum dilution was 1:10 for both assays. Sera with titres ≥ 10 were considered as seropositive. HI titres ≥ 40 were considered as seroprotective.

Statistical analysis

Geometric mean titres (GMTs) of antibody with 95% confidence intervals (CI) were calculated for each age group against each of the five influenza viruses. A numeric value of 5 was assigned to samples with antibody titres < 10. Antibody titres between age groups

Figure 1

Neighbour-joining phylogenetic tree of HA1 amino acid sequences from human seasonal influenza A(H3N2) viruses and European and North American swine influenza A(H3N2) viruses



The viruses used for the present serological studies are indicated in bold.

were compared using the nonparametric Wilcoxon signed-rank test, and differences in the proportion of sera with HI titres ≥ 40 were analysed using Fisher's exact test. A p value < 0.05 was considered statistically significant. Pearson correlation tests were used to compare HI antibody titres against human and swine-origin viruses. All analyses were performed with Graphpad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, US).

Results

Relationships between human seasonal and swine-origin influenza A(H3N2) viruses

Phylogenetic relationships between the HA1 amino acid sequences of human seasonal H3N2 viruses and H3N2 SIVs from Europe and North America are shown in Figure 1. European and North American H3N2 SIVs formed separate clusters, and branched off from human viruses at different apparent time points. The HA1 of the human A/Victoria/3/75 virus was most closely related to the European H3N2 SIVs (87.0% identity to sw/Gent/172/08). The HA1 of the human A/Nanchang/933/95 virus was most closely related to North American H3N2 SIVs (89.7% identity to A/Indiana/08/11).

Antigenic relationships between human seasonal and swine-origin H3N2 viruses are shown in Table 1. All viruses reacted with their homologous antisera at HI and VN titres ≥ 160. There was minimal cross-reactivity between the H3N2v virus A/Indiana/08/11 and the European H3N2 SIV sw/Gent/172/08 using hyper-immune swine serum against sw/Gent/172/08, and ferret serum against A/Indiana/08/11 failed to cross-react with sw/Gent/172/08. Sw/Gent/172/08 showed cross-reaction with antiserum against the human A/Victoria/3/75 virus in both HI and VN assays (titre = 80). A/Indiana/08/11, on the other hand, reacted with antiserum against the human A/Wuhan/359/95 virus, which is similar to A/Nanchang/933/95, in the VN assay (titre = 240), but not in the HI assay. Both swine-origin viruses had negligible cross-reactivity with A/Wisconsin/67/05.

Serological status to human seasonal influenza A(H3N2) viruses

HI antibody titres ≥ 10 against the A/Victoria/3/75 virus were detected in 78% of individuals born before 1990 (Figure 2). In contrast, only 10% of those born in the 1990s and none of the children born after 2000 had detectable antibodies. HI titres ≥ 40 were most common in persons born in the 1950s, 1960s and 1970s. A larger proportion of people had detectable HI antibodies and titres ≥ 40 against the A/Nanchang/933/95 virus. Detectable HI antibodies against the latter virus were observed in 85% of individuals born before 2000, but only in 20% of the children born after 2000. More than half of those born in the 1970s, 1980s and 1990s had HI titres ≥ 40. Seroprevalence rates against the A/Wisconsin/67/05 strain had the least variation across different age groups. More than half of the people in each age category had detectable antibodies, except for those born in the 1960s (42%). HI titres ≥ 40 were most common in those born after 1990.

GMTs of HI antibodies against the three human seasonal H3N2 viruses showed similar age-dependent trends as the seroprevalence rates (Table 2). Antibody titres against A/Victoria/3/75 were highest in people born in the 1950s, 1960s and 1970s. Antibody titres against A/Nanchang/933/95 and A/Wisconsin/67/05, on the other hand, were highest in people born in the 1980s and 1990s and those born in the 1990s, respectively. Peak antibody titres against A/Nanchang/933/95 were higher than those against either of the other viruses.

Table 1

Cross-reactivity between human seasonal A(H3N2) viruses (A/Victoria/3/75, A/Nanchang/933/95 and A/Wisconsin/67/05) and swine-origin A(H3N2) viruses (sw/Gent/172/08 and A/Indiana/08/11) in haemagglutination inhibition and virus neutralisation assays, Luxembourg, 2010 (n = 843)

Virus strain	Antibody titres with serum against									
	A/Victoria/3/75		A/Wuhan/359/95[a]		A/Wisconsin/67/05		sw/Gent/172/08[b]		A/Indiana/08/11	
	HI	VN	HI	VN	HI	VN	HI	VN	HI	VN
A/Victoria/3/75	160	960	<10	<10	<10	15	20	120	10	<10
A/Nanchang/933/95	<10	20	320	1,280	<10	15	<10	10	<10	80
A/Wisconsin/67/05	<10	10	<10	20	160	160	<10	10	<10	<10
sw/Gent/172/08	80	80	<10	<10	<10	15	2,560	5,120	<10	<10
A/Indiana/08/11	20	30	<10	240	<10	15	10	40	320	2,560

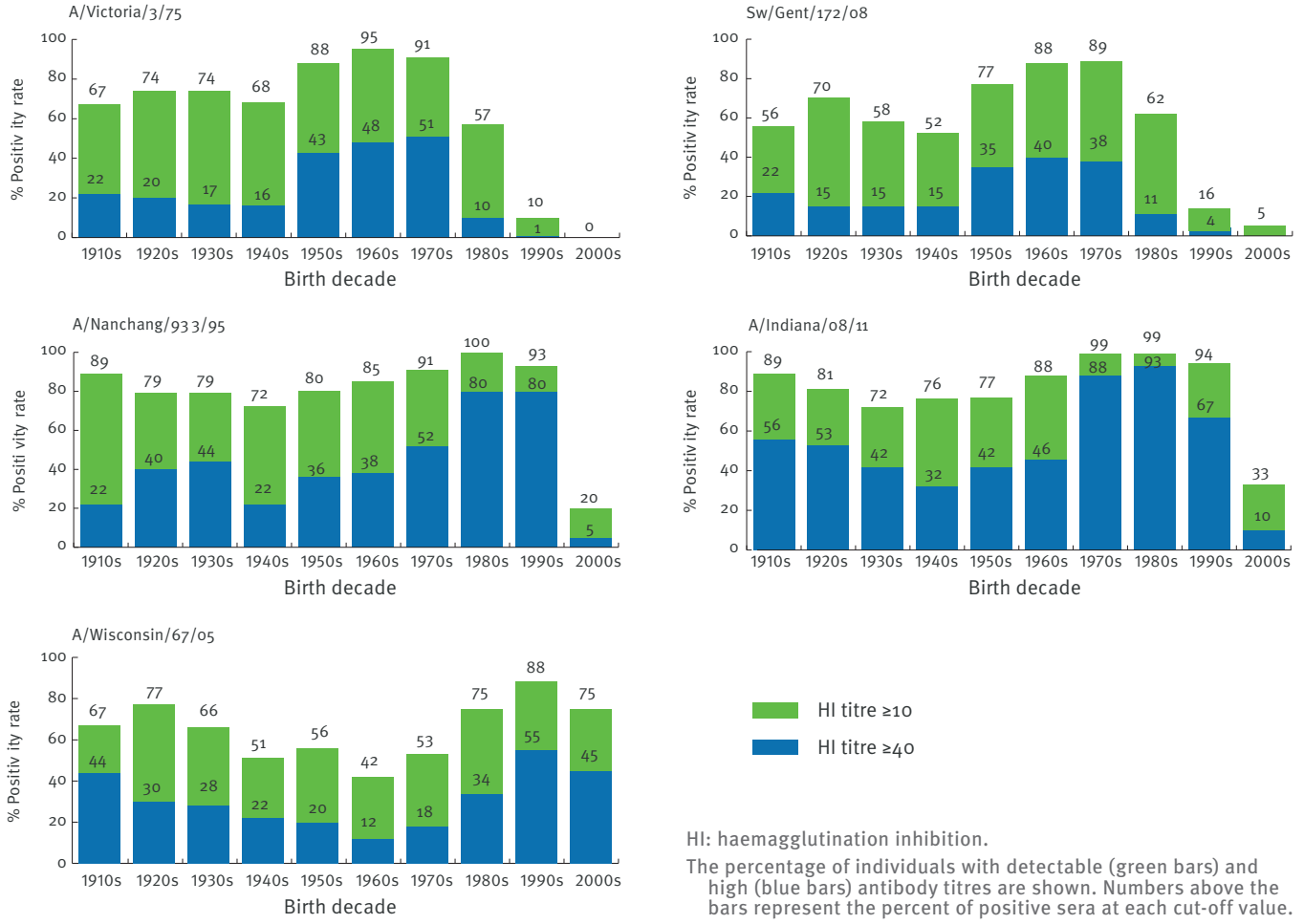
HI: haemagglutination inhibition; VN: virus neutralisation.

[a] Ferret serum against A/Wuhan/359/95 was used because ferret serum against A/Nanchang/933/95 was not available. The two viruses are antigenically identical.

[b] Serum against sw/Gent/172/08 was obtained by hyper-immunisation of swine; the other sera were post-infection ferret sera.

Figure 2

Prevalence of haemagglutination inhibition antibodies against three human seasonal influenza A(H3N2) viruses, an endemic European H3N2 swine influenza virus and a North American H3N2v virus, by decade of birth, Luxembourg, 2010 (n = 843)



HI: haemagglutination inhibition.

The percentage of individuals with detectable (green bars) and high (blue bars) antibody titres are shown. Numbers above the bars represent the percent of positive sera at each cut-off value.

Serological status to European and North American swine-origin influenza A(H3N2) viruses

Rates of seroprevalence against the European H3N2 SIV sw/Gent/172/08 and North American H3N2v virus A/Indiana/08/11 were similar to those against the human seasonal viruses A/Victoria/3/75 and A/Nanchang/933/95, respectively (Figure 2). HI antibodies (titres ≥ 10) against sw/Gent/172/08 were almost exclusively found in those born before 1990, of whom 70% were seropositive. HI antibodies against A/Indiana/08/11 were mainly detected in people born before 2000, of whom 86% were seropositive. In contrast, fewer than 20% of those born after 1990 had antibodies against sw/Gent/172/08, and only 33% of children born after 2000 showed antibodies against A/Indiana/08/11. Seroprevalences and HI antibody GMTs followed similar age-specific patterns for both viruses (Figure 2, Table 2). The highest proportions of HI titres ≥ 40 and the highest GMTs were observed in individuals born in the 1950s to 1970s for sw/Gent/172/08, and in those born in the 1970s to 1990s for A/Indiana/08/11. Nonetheless, the prevalence of titres ≥ 40 and the GMTs were higher for A/Indiana/08/11 than for sw/Gent/172/08 in all age categories ($p < 0.05$), except for those born in 1950s and 1960s ($p > 0.05$).

Sera from individuals born after 1940 were also examined for antibodies against the two swine-origin H3N2 viruses with the VN assay (Table 3). For both viruses, VN titres were higher than HI titres ($p < 0.05$), except for antibodies against sw/Gent/172/08 in children born after 2000 ($p > 0.05$). Higher VN antibody titres were detected against A/Indiana/08/11 than against sw/Gent/172/08 ($p < 0.05$).

Correlations between antibody titres against human seasonal and swine-origin influenza A(H3N2) viruses

There was a strong correlation between HI antibody titres against sw/Gent/172/08 and A/Victoria/3/75 viruses ($r = 0.71$), and between those against A/Indiana/08/11 and A/Nanchang/933/95 viruses ($r = 0.69$) (Table 4). Correlations were low between HI

Table 2

Haemagglutination inhibition antibody titres against human and swine-origin influenza A(H3N2) viruses, by age group, Luxembourg, 2010 (n = 843)

Birth decade	n	A/Victoria/3/75	A/Nanchang/933/95	A/Wisconsin/67/05	sw/Gent/172/08	A/Indiana/08/11
1910s	9	13.6 (6.4–29.1)	20.0 (7.9–50.3)	25.2 (7.0–90.4)	12.6 (4.3–36.6)	29.4 (10.1–85.6)
1920s	91	13.8 (11.5–16.5)	21.4 (17.1–26.8)	18.0 (14.5–22.3)	12.1 (10.2–14.4)	28.2 (21.8–36.5)
1930s	100	12.7 (10.9–14.8)	22.4 (18.3–27.3)	16.1 (13.0–20.0)	11.2 (9.5–13.2)	22.1 (17.4–28.0)
1940s	100	12.4 (10.5–14.7)	14.9 (12.4–17.8)	12.0 (9.7–14.9)	11.1 (9.2–13.4)	17.6 (14.3–21.6)
1950s	100	25.0 (20.2–30.9)	19.2 (15.6–23.5)	12.8 (10.3–16.0)	16.8 (13.7–20.7)	22.0 (17.3–28.1)
1960s	100	30.1 (24.9–36.5)	24.0 (19.2–29.9)	9.7 (7.9–11.8)	24.1 (19.3–30.1)	28.7 (22.8–36.1)
1970s	100	28.7 (23.5–35.0)	29.1 (23.8–35.6)	10.8 (8.9–13.1)	23.1 (19.1–28.0)	69.6 (58.0–83.7)
1980s	104	10.0 (8.7–11.5)	71.0 (59.1–85.3)	18.2 (14.8–22.4)	10.9 (9.4–12.7)	117.8 (97.5–142.2)
1990s	99	5.5 (5.2–5.8)	77.8 (59.5–101.6)	34.3 (26.9–43.7)	6.2 (5.5–7.0)	58.8 (44.6–77.5)
2000s	40	5.0 (5.0–5.0)	6.5 (5.4–7.8)	20.4 (14.6–28.3)	5.2 (4.9–5.4)	7.7 (6.1–9.7)

CI: confidence interval; GMT: geometric mean titre

titres against A/Wisconsin/67/05 and sw/Gent/172/08 (r=0.25), as well as between those against A/Wisconsin/67/05 and A/Indiana/08/11 (r=0.42) (all p<0.01).

Discussion

The present study was designed to investigate the extent to which prior exposure, through infection or vaccination, to earlier antigenic variants of seasonal influenza A(H3N2) viruses was associated with the presence of antibodies against swine-origin H3N2 viruses from Europe and North America in people in Luxembourg born between 1910 and 2010. Our results demonstrate that as many as 70% of people in the study born before 1990 had detectable HI antibodies against the European H3N2 SIV, whereas such antibodies were generally lacking in those born after 1990. The prevalence of antibodies against the antigenically distinct H3N2v swine-origin virus was also age-dependent: antibodies were predominantly found in people born before 2000, of whom 86% were seropositive. Our data are consistent with previous studies [18-23,27], but we have for the first time demonstrated a lower level of seroreactivity to the European H3N2 SIV than to the H3N2v virus. Although HI titres of ≥40 are generally considered as seroprotective, people with lower antibody titres may also have some protection against H3N2 viruses from swine. Indeed, HI assays do not measure mucosal antibodies, antibodies against NA and cell-mediated immunity, which will also contribute to protection [28]. The VN assay yielded higher seroprevalence rates and antibody titres against

Table 3

Virus-neutralising antibody titres against swine-origin influenza A(H3N2) viruses sw/Gent/172/08 and A/Indiana/08/11 in people born after 1940, Luxembourg, 2010 (n = 643)

Birth decade	n	sw/Gent/172/08			A/Indiana/08/11		
		% sera with titre		GMT (95% CI)	% sera with titre		GMT (95% CI)
		≥10	≥40		≥10	≥40	
1940s	100	77	36	24.0 (18.6–31.0) [a]	98	71	80.6 (62.6–103.6) [a]
1950s	100	94	68	76.4 (56.8–102.7) [a]	96	69	90.1 (67.3–120.6) [a]
1960s	100	93	62	62.6 (46.6–84.0) [a]	100	82	112.4 (87.7–144.2) [a]
1970s	100	99	79	90.4 (70.9–115.1) [a]	100	94	247.2 (199.7–306.0) [a]
1980s	104	79	30	22.7 (18.4–28.1) [a]	100	99	488.1 (410.4–580.5) [a]
1990s	99	30	9	8.0 (6.7–9.7) [a]	97	89	235.4 (172.3–321.7) [a]
2000s	40	10	0	5.4 (5.0–5.7)	58	15	13.3 (9.2–19.2) [a]

CI: confidence interval; GMT: geometric mean titre.

[a] GMTs significantly higher (p<0.05, by Wilcoxon signed-rank test) in the virus neutralisation than in the haemagglutination inhibition assay.

Table 4

Pearson correlation coefficients between haemagglutination inhibition antibody titres against human and swine-origin influenza A(H3N2) viruses, Luxembourg, 2010 (n = 843)

	A/Victoria/3/75	A/Nanchang/933/95	A/Wisconsin/67/05	sw/Gent/172/08	A/Indiana/08/11
A/Victoria/3/75	1	0.17	0.24	0.71	0.09
A/Nanchang/933/95		1	0.48	0.26	0.69
A/Wisconsin/67/05			1	0.25	0.42
sw/Gent/172/08				1	0.31
A/Indiana/08/11					1

All $p < 0.01$.

both swine-origin influenza viruses than the HI assay. This is not surprising, because the VN assay detects a broader range of antibodies than the HI assay [29].

We have several reasons to believe that antibodies against current swine-origin H3N2 viruses result from exposure to historical human H3N2 strains rather than from infection with SIVs. Firstly, Luxembourg has a low pig density compared with other European regions and only 0.07% of the population are employed in the swine industry. Furthermore, only 0.99% of the Luxembourg swine population tested seropositive against H3N2 SIV in 2013 [30]. Secondly, H3N2 SIVs from North America have never been detected in swine in Europe. Finally, we found a strong correlation between antibody titres against swine-origin viruses sw/Gent/172/08 and A/Indiana/08/11 and their respective human ancestor A/Victoria/3/75 and A/Nanchang/933/95. Similar correlations have previously been reported between antibody titres against the European H3N2 SIV and A/Port Chalmers/1/73 [27], and between the H3N2v virus and A/Wuhan/359/95 and A/Sydney/5/97 viruses [18,20]. In contrast, younger people who had not been exposed to these human H3N2 strains in past decades, were generally seronegative against the swine-origin viruses. In addition, our phylogenetic and antigenic analyses, in agreement with previous studies [31,32], confirm the close relationship between these swine-origin viruses and their human H3N2 ancestors.

Despite lower seroreactivity to the European H3N2 SIV than to the North American H3N2v virus, only three human infections with the European H3N2 SIV have been reported between 1993 and 2014 [33,34]. The H3N2v viruses, in contrast, have caused 343 human infections in the US between 2011 and 2014. It is possible that the H3N2v virus is more infectious for humans than other H3N2 lineages from swine or that more people in the US may have opportunities for exposure to pigs. The H3N2v is the only North American H3N2 SIV genotype that has caused widespread infections in humans, and although some believe this is due to the presence of the pandemic M gene segment, this has not been firmly proven [35]. Most H3N2v cases reported exposure to pigs at agricultural fairs [16,36]. Thousands of fairs are held in summer and autumn in North America, and these fairs provide unique settings where pigs from numerous sources can come into contact with millions of persons, which may facilitate interspecies transmission of influenza viruses. Such large-scale pig shows are rare in Europe. Furthermore, human cases of animal influenza have been notifiable in the US since 2007, which is not the case in Europe, and there is much more extensive surveillance for influenza in humans and swine in the US than in Europe.

Our data further support the notion that pigs serve as reservoirs for older human H3 HAs against which immunity in the human population will gradually decrease over time. Experimental infection studies in pigs and in ferrets have shown that prior infection with recent seasonal H3N2 viruses offers limited or no protection against challenge with the European H3N2 SIV or H3N2v [31,37]. As such, H3N2 SIVs, and the European strains in particular, could potentially contribute to pandemic viruses in the future as seroreactivity to the respective HAs wanes over time in the human population. Yet, it is highly likely that the current substantial immunity in people born before 1990 would prevent extensive spread of H3N2 SIVs. As for the swine-origin A(H1N1)pdm09 virus, pre-pandemic antibodies against A(H1N1)pdm09 were present in most individuals born before 1944, but were low or absent in younger people [11-14]. It may take nearly 50 years before a substantial proportion of the human population would be fully susceptible to swine-origin H3N2 viruses. To assure the best preparation for swine-origin influenza virus pandemics, surveillance of influenza in pigs should be expanded and integrated with human public health surveillance efforts, and additional studies on the cross-reactivity between human and swine influenza viruses are warranted.

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Conflict of interest

None declared.

Authors' contributions

Yu Qiu and Kristien Van Reeth conceived and designed the experiments. Yu Qiu performed the experiments and analysed the data. Yu Qiu and Kristien Van Reeth wrote the paper. Claude P Muller provided sera, read and revised manuscript.

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Streptococcus pneumoniae nasopharyngeal colonisation in children aged under six years with acute respiratory tract infection in Lithuania, February 2012 to March 2013

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Data on distribution of *Streptococcus pneumoniae* (SPn) serotypes among children in Lithuania are limited. A prospective study was carried out from February 2012 to March 2013 to evaluate the circulation of SPn serotypes among young children in five cities of Lithuania before the introduction of universal vaccination with pneumococcal conjugate vaccine (PCV). A total of 900 children under six years of age who presented to primary care centres or a hospital emergency department with acute respiratory tract infection (RTI) were enrolled in the study. The SPn colonisation rate was 40.8% (367/900), with a peak at two and three years-old (48.8% and 45.4%, respectively). Of the 367 SPn isolates, the most common serotypes were 6B (15.8%, n = 58), 19F (13.9%, n = 51), 23F (13.9%, n = 51), 15 (10.1%, n = 37), 14 (9.5%, n = 35), 6A (9.3%, n= 34), 11 (4.6%, n = 17), 3 (3.0%, n = 11) and 18C (3.0%, n = 11); less frequent were 23 (non-23F) (2.7%, n = 10), 19A (2.2%, n = 8) and 9V (1.6%, n = 6). Serotypes 6A and 11 were more common in children under two years-old; 18C was found only in children aged two to five years. The serotypes found might be an important predictor of the likely effectiveness of the PCVs currently available in Lithuania

Introduction

Streptococcus pneumoniae (SPn) is one of the major bacterial pathogens colonising the nasopharynx, which can cause a wide spectrum of illnesses from upper respiratory tract infection (RTI) to invasive pneumococcal disease (IPD), or the infection can be asymptomatic [1]. SPn infection is also associated with high mortality – an estimated 1.6 million people, including 0.7–1 million children under five years of age, die of pneumococcal diseases each year worldwide [2].

Pneumococcal disease is preceded by asymptomatic colonisation: the colonisation rate is especially high in children under six years of age [1,3]. In addition, nasopharyngeal carriage is a major factor in the horizontal transmission of SPn strains and nasopharyngeal isolates reflect currently circulating strains in the community [4]. Young children are thought to be the most important source in horizontal dissemination of pneumococcal strains due to the high frequency of pneumococcal colonisation and high crowding in day-care centres and families [1].

There is a wide variation in SPn capsule polysaccharides: currently 94 serotypes have been identified [5]. Different SPn serotypes have different propensities to cause disease [6]. The distribution of SPn serotypes varies by country, age or ethnic group, and study design [4,7-13]. Data on distribution of SPn serotypes among children in Lithuania are limited. The National Public Health Surveillance Laboratory in Vilnius collects all invasive pneumococcus strains nationwide. A total of 45 SPn strains, which caused IPD in children under five years of age, were serotyped during 2006 to 2011. Some 14 different serotypes were identified, with 23F, 14, 6B, 1, 18C being the most prevalent serotypes [14]. As the amount of data was low, however, the findings do not necessarily reflect the actual SPn serotype prevalence among IPD paediatric patients in Lithuania.

The presence of SPn in the nasopharynx of children with acute RTI might be considered as an additional risk factor for development of mucosal or invasive pneumococcal disease [15]; however, data on the carriage rate and SPn serotype distribution among such patients worldwide are limited. The data in our study will be analysed further to evaluate whether the presence of SPn in the nasopharynx had an influence on the outcome of acute RTI among our patients.

At the time of the study, routine PCV vaccination was not yet part of the national vaccination schedule in Lithuania and PCVs (10-valent (PCV10) and 13-valent

Table

Demographic data of enrolled children under six years of age with acute respiratory tract infection in the study sites, Lithuania, February 2012 to March 2013 (n=900)

Characteristic	Number (%)[a]							
	Vilnius			Kaunas	Klaipeda	Panevezys	Alytus	Total
	PCC	ED	Total	PCC	PCC		PCC	
Children enrolled	173 (19.2)	264 (29.3)	437 (48.6)	159 (17.7)	63 (7.0)	223 (24.8)	18 (2.0)	900 (100)
Sex								
Female	84 (48.6)	110 (41.7)	194 (44.4)	78 (49.1)	28 (44.4)	104 (46.6)	4 (22.2)	408 (45.3)
Male	89 (51.4)	154 (58.3)	243 (55.6)	81 (50.9)	35 (55.6)	119 (53.4)	14 (77.8)	492 (54.7)
Age in months								
Range	4–69	1–71	1–71	1–71	5–71	1–71	23–71	1–71
Mean (SD)	32.3 (16.3)	33.5 (16.4)	33.1 (16.4)	33.6 (18.9)	38.8 (18.5)	37.9 (17.6)	51.6 (16.1)	35.1 (17.6)
Age groups in months								
0–24	57 (32.9)	87 (33.0)	144 (33.0)	58 (36.5)	14 (22.2)	55 (24.7)	1 (5.6)	272 (30.2)
25–48	86 (49.7)	128 (48.5)	214 (49.0)	67 (42.1)	29 (46.0)	102 (45.7)	7 (38.9)	419 (46.6)
49–71	30 (17.3)	49 (18.6)	79 (18.1)	34 (21.4)	20 (31.7)	66 (29.6)	10 (55.6)	209 (23.2)

ED: emergency department of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos ; PCC: primary care centre; SD: standard deviation.

[a] Unless otherwise indicated.

(PCV13)) were available only on private market. As vaccination costs were not reimbursed, vaccination coverage was unknown but was probably rather low due to the relatively high price of the vaccines.

The nasopharynx is the reservoir for SPn and the carriage of SPn in children with acute RTI has not been studied widely. This study was undertaken to evaluate the circulation of SPn serotypes among children with acute RTI under six years of age in Lithuania before the introduction of universal pneumococcal vaccination in the country in October 2014 [16]. It was expected that the data collected will be helpful in decision-making regarding universal PCV vaccination in Lithuania. They may also be the basis for further investigations into the impact of PCV vaccination on the distribution of SPn serotypes in Lithuania, including the widely discussed phenomenon of replacement, i.e. changes in circulating SPn serotypes due to vaccination [17].

Methods

This prospective study was carried out from February 2012 to March 2013. Eight primary care centres in Lithuania's five biggest cities (Vilnius (n = 2), Kaunas (n = 2), Klaipeda (n = 2), Panevezys (n = 1), Alytus (n = 1)), from all main regions of the country, and the emergency department (ED) of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos in Vilnius were involved in examining children for SPn nasopharyngeal carriage.

Children under six years old, who visited a primary care physician because of acute RTI and who met all inclusion criteria were enrolled into the study. The main symptoms of acute RTI were acute onset, fever, runny nose, cough, throat redness and otalgia. Children were excluded if they had been vaccinated with any pneumococcal vaccine, had taken antibiotics during the previous month or another cause of fever was identified (e.g. it was confirmed not due to respiratory infection).

Local ethics committee approval was obtained and the parents were asked to sign an informed consent form before the child was enrolled in the study.

Nasopharyngeal swabs were taken at the time of enrolment in the study using Culturette with Amies transport medium (Deltalab, Spain) and transported to the bacteriology laboratory of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos in Vilnius within 48 hours from collection. Classic cultural methods (cultivation in 5% CO_2, colony morphology, optochin sensitivity) were used to isolate SPn from the swabs [18]. Serotypes were determined by means of latex agglutination reaction using the Pneumotest-Latex kit (Statens Serum Institut, Copenhagen, Denmark).

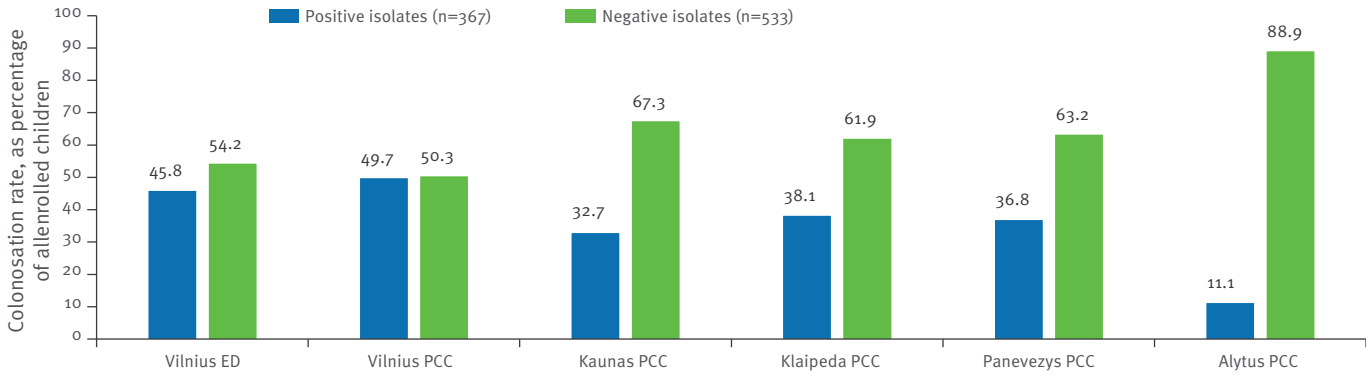
Statistical analysis

The data were analysed using SPSS software 16. Chi-squared test was used to test statistical significance for differences between two groups. Fisher's exact test was used when cell values in the SPSS table had an expected frequency of five or less. Statistical significance was defined by $p < 0.05$.

The expected theoretical protection of PCVs was calculated by comparing the isolated SPn serotypes with the serotypes included in currently available PCV vaccines.

Figure 1

Streptococcus pneumoniae nasopharyngeal colonisation among enrolled children aged under six years with acute respiratory tract infection in the study sites, Lithuania, February 2012–March 2013 (n=900)



ED: emergency department of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos ; PCC: primary care centre.

Multivariable Poisson regression with robust variance estimation was used to assess the association of site, season, age and sex with SPn colonisation. SPn colonisation prevalence ratios (PRs) with 95% confidence intervals (CIs) associated with the site (PCC of Vilnius, Kaunas, Klaipeda, Alytus and Panevezys vs Vilnius ED), season (spring vs winter, summer vs winter, and autumn vs winter), sex (female vs male), and age (25–48 vs 1–24 months and 49–71 vs 1–24 months) were calculated.

Results

During the one-year study period, 908 children were examined for SPn nasopharyngeal carriage. Due to the exclusion criteria, eight were excluded from the analysis. The data collected from the 900 study participants were analysed: 636 patients at the PCCs and 264 at the hospital ED.

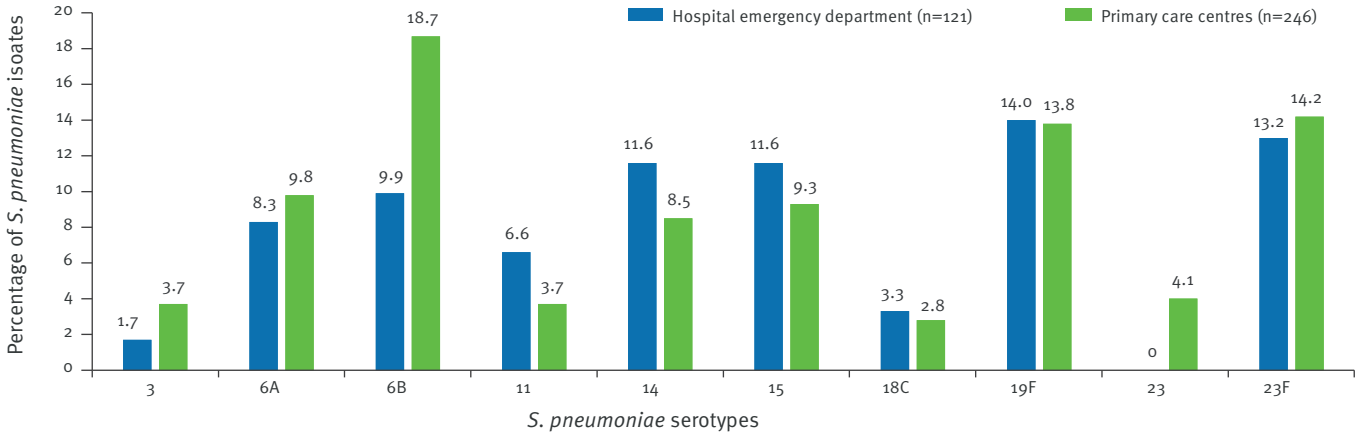
The enrolled children comprised 408 girls and 492 boys under six years of age with acute RTI. The participants were enrolled throughout the study period. Two thirds were enrolled during spring and autumn (35.0% (n = 315) and 32.9% (n = 296), respectively), the others during winter (22.0% (n = 198) and summer (10.1% (n = 91)). The distribution of enrolled children by sex and age was similar in all the cities, with the exception of Alytus (Table), which may be due to a very small number of study participants in this city (n = 18).

Streptococcus pneumoniae colonisation rate

A total of 367 SPn strains (one per patient) were isolated from the 900 samples collected, giving a colonisation rate of 40.8%. The rate was higher among patients admitted to the ED of the Children's Hospital in Vilnius than among patients at the PCCs (45.8% (121/264) vs 38.7% (246/636); p = 0.047). The colonisation rate was

Figure 2

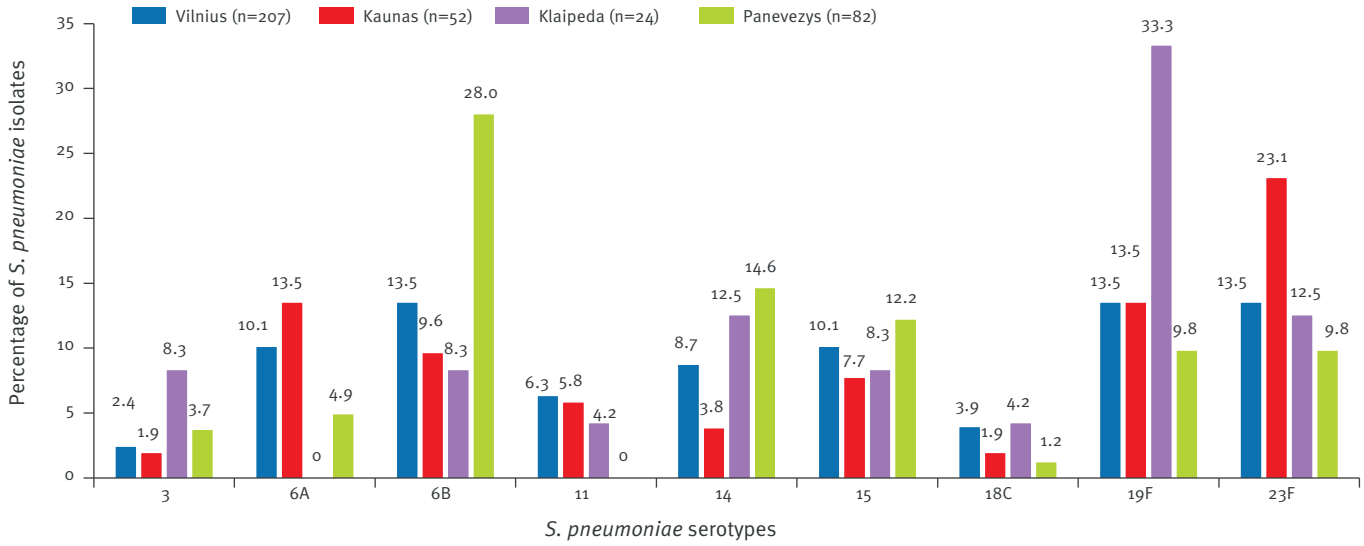
Most common Streptococcus pneumoniae serotypes isolated from enrolled children aged under six years with acute respiratory tract infection the study sites[a], Lithuania, February 2012–March 2013 (n=367)



[a] Study sites were primary care centres of Vilnius, Kaunas, Klaipeda, Panevezys and Alytus and the emergency department of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos in Vilnius.

Figure 3

Distribution of the most common Streptococcus pneumoniae serotypes isolated from enrolled children aged under six years with acute respiratory tract infection in the study sites[a], Lithuania, February 2012–March 2013 (n=365)



[a] Study sites were primary care centres of Vilnius, Kaunas, Klaipeda, Panevezys and Alytus and the emergency department of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos in Vilnius. Note that Alytus was excluded from this comparison because of the small number of *S. pneumoniae* isolates (n=2).

higher in Vilnius PCC and ED (47.4%, 207/437) than in Kaunas (32.7%, 52/159; p=0.001), Panevezys (36.8%, 82/223; p=0.009) and Alytus (2/18; p=0.002).

There was also a higher colonisation rate in Vilnius (both PCC and ED) than in Klaipeda (38.1%, 24/63), but the difference was not statistically significant (p=0.168). The distribution of pneumococcal colonisation rates in the enrolled children in the five cities in Lithuania is shown in Figure 1.

Seasonality differences were detected in SPn colonisation rates: they were higher in spring (43.2%, 136/315) and autumn (44.6%, 132/296) than in summer (35.2% (32/91), p›0.05 for both comparisons) and winter (33.8% (67/198), p=0.035 and p=0.017, respectively). The sex of the patients had no influence on the pneumococcal colonisation rates: 40.4% (165/408) of the girls and 41.1% (202/492) of the boys carried SPn in their nasopharynx.

The youngest child with a positive pneumococcal sample was two months-old. The colonisation rate of SPn in infants aged up to one year was 28.0% (26/93). A peak level was reached at two to three years of age (48.8% (101/207) and 45.4% (98/216), respectively). A slight decrease in the colonisation rate was found among children aged four to five years (38.1% (45/118) at the age of four years and 30.7% (31/101) at the age of five years).

Using multivariable Poisson regression analysis, the prevalence of SPn colonisation was higher in children aged 25–48 months (PR: 1.301), but not in children aged 49–71 months (PR: 0.986), compared with children aged up to 24 months. The PCCs of Kaunas, Alytus and Panevezys had significantly lower colonisation rates than the Vilnius ED (PR: 0.689 for Kaunas PCC vs ED, PR: 0.241 for Alytus PCC vs ED, and PR: 0.775 for Panevezys vs ED). The Vilnius PCC had similar colonisation rates as the Vilnius ED (PR: 1.046) and there were no significant differences between Klaipeda PCC and the Vilnius ED (PR: 0.768). The colonisation rate was significantly higher during autumn than winter (PR: 1.355), but not during other the seasons (PR: 1.262 for spring vs winter and PR: 0.950 for summer vs winter).

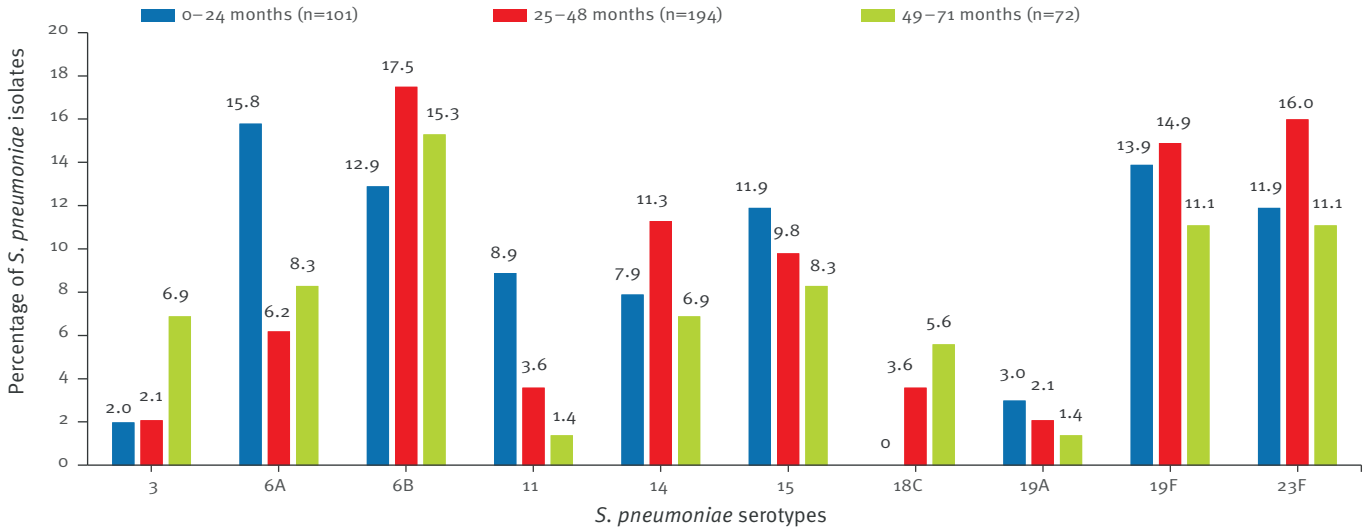
Streptococcus pneumoniae serotype distribution

Of the 367 SPn strains isolated, 22 different serotypes were detected. The most common serotypes were 6B (15.8%, n = 58), 19F (13.9%, n = 51), 23F (13.9%, n = 51), 15 (10.1%, n = 37), 14 (9.5%, n = 35), 6A (9.3%, n = 34), 11 (4.6%, n = 17), 3 (3.0%, n = 11) and 18C (3.0%, n = 11). Other SPn serotypes constituted 16.9% (n = 62) of all isolates and were as follows: serotypes 23 (non-23F) (2.7%, n = 10), 19A (2.2%, n = 8), 9V (1.6%, n = 6), 9 (1.1%, n = 4), 10 (1.1%, n = 4), 22 (0.8%, n = 3), 6C (0.5%, n = 2) and single isolates of 4, 7, 7F, 12, 17 and 19 serotypes; 5.2% (n = 19) were non-typable.

Differences in the distribution of SPn serotypes between those isolated from patients at the Vilnius ED and the PCCs were not statistically significant, except for serotypes 6B and 23, which were more common at the PCCs (p=0.03 and p=0.034, respectively) (Figure 2).

Figure 4

Distribution of Streptococcus pneumoniae serotypes by age group of enrolled children aged under six years with acute respiratory tract infection in the study sites[a], Lithuania, February 2012–March 2013 (n=367)



[a] Study sites were primary care centres of Vilnius, Kaunas, Klaipeda, Panevezys and Alytus and the emergency department of Children's Hospital, Affiliate of Vilnius University Hospital Santariskiu Klinikos in Vilnius.

A slightly different distribution of SPn serotypes was found in the study sites (Figure 3). Serotype 6B was more prevalent in Panevezys, compared with Vilnius (p = 0.004), Kaunas (p = 0.011) and Klaipeda (p = 0.045). Serotype 19F was more common in Klaipeda, compared with Vilnius (p = 0.018) and Panevezys (p = 0.009). Serotype 23F was more prevalent in Kaunas, compared with Panevezys (p = 0.035). Serotype 6A was not found in Klaipeda but was observed in Vilnius, Kaunas, Panevezys and Alytus; serotype 11 was not found in Panevezys but was identified in the other cities studied. Alytus was excluded from this comparison because of the small number of SPn isolates (n = 2).

The prevalence of serotypes 6B, 19F and 23F varied during the seasons. Serotype 6B was more prevalent during autumn (22.0% (29/132) and winter (19.4% (13/67) as compared with spring (11% (15/136), p = 0.018 and p=0.108, respectively) and summer (1/32, p = 0.014 and p = 0.03, respectively). Conversely, serotype 19F reached a peak (8/32) during summer; serotype 23F peaked during spring (21.3%, 29/136). The fluctuation rates of other SPn serotypes according to the season were not statistically significant. The numbers are small, however, thus limiting the ability to meaningfully compare between the seasons.

Of the 165 isolates from girls, serotypes 6B (20.0%, n = 33), 23F (12.7%, n = 21), 19F (10.9%, n = 18), 15 (10.3%, n = 17), 6A (9.7%,n = 16) and 14 (9.7%, n = 16) were the most common, whereas of the 202 isolates from boys, serotypes 19F (16.3%, n = 33), 23F (14.9%, n = 30), 6B (12.4%, n = 25), 15 (9.9%, n = 20), 14 (9.4%, n = 19) and 6A (8.9%, n = 18) were the most common. There were no sex-related differences, except for 6B, which was more common in girls (p = 0.046).

In addition, the study showed an age-related SPn serotype distribution, with serotypes 6A and 11 being more common in the youngest age group (0–2 years), while 18C was found only in the older age groups (Figure 4).

Among all serotypes isolated from our 367 patients, 58% (n = 214) were present in PCV10 (serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F) and 73% (n = 267) in PCV13 (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F).

Discussion

Data concerning the SPn nasopharyngeal carriage rates among healthy children in eastern Europe, including Lithuania, are limited. During the last decades, a number of studies on SPn nasopharyngeal carriage in healthy children were performed in western and southern Europe, with carriage rates ranging from 14.9% to 58.5% [8,10,11,13]. The reported rates of pneumococcal carriage among healthy children under eight years of age varied, with a wide range from 3.5% to 97% among different studies worldwide [19,20]. Surprisingly low carriage rates (3.5–14.9%) were observed in a few Italian studies performed in 1996 and 1999 [10,19], whereas much higher rates were seen in Romania (51% during 2008–09) [12], France (54% in 1999) [8], Russia (60% in 2007) [21] and Norway (77.7% in 2006) [22]. The highest SPn carriage rates among healthy children (70–97%) have been found in some Asian and African countries, e.g. in India (70.2% in 1998–99) [23], Gambia (97% in 2004–06) [20] and Malawi (84% in 1997) [24].

In Mozambique in 2003, SPn carriage was more common among children who had lower RTI symptoms at the time of enrolment (93%) than among healthy children (84%) [25].

Three studies of SPn nasopharyngeal carriage in healthy children have been performed in Lithuania (in 1999, 2001 and 2006) [26-28], in which a total of 1,625 children from the same 13 day-care centres were enrolled. On average, SPn was found in the nasopharynx of every second child (51% in 1999, 55% in 2001 and 43% in 2006) and the most prevalent serotypes were 19F, 23F, 6B, 6A, 3 and 18C. These studies were rather limited because all of them were performed during a short period of time (in February and March) and the data presented were from only one city of the country (Vilnius).

In our findings presented here, the SPn colonisation rate among young children with acute RTI in the study sites in Lithuania was 40.8%. It is important to note that most studies have focused on SPn carriage in healthy children while in our study, children with RTI were enrolled. The carriage rate seen was similar to those observed in healthy children in Estonia (44%), Czech Republic (38.1%) and Hungary (37.71%) [9,29,30]. It is also similar to those reported in healthy children in Vilnius in 2006 (43%) and slightly lower than that in 1999 and 2001 (51% and 55%, respectively) [26-28].

Different findings are reported concerning SPn nasopharyngeal colonisation among children with RTI. For example, Revai et al. (Texas, United States, in 2003–07) reported a relatively low SPn carriage rate (34%) in children with upper RTI [31]. Conversely, Syrjänen et al. (Finland in 1994–97) showed that nasopharyngeal SPn carriage rates increased during respiratory infections from 13–43% to 45–56%, depending on age [32]. It has also been shown that the SPn carriage rate during the first days of acute RTI is comparable to that in healthy children [33]. However, in RTI patients carrying SPn, a more severe course of RTI or activation of pneumococcal disease can occur and this is a subject for further analysis of our data.

Commonly, SPn nasopharyngeal colonisation begins in infancy, with peak incidence occurring at two to three years of age and decreases among children over the age of four to five years [12,13,20]. Pneumococcal colonisation occurs earlier in developing countries as compared with developed ones: high SPn carriage rates (70–98%) have been observed in infants in some Asian and African countries such as India (70.2% in 1998–99), Gambia (97% in 2004–06) and Kenya (98% in 2004) [20,23,34], in contrast to Finland, where only 9–22% of infants were colonised by SPn (1994–97) [32].

We found a seasonal fluctuation in the rates of SPn nasopharyngeal colonisation, with an increase during spring and autumn. Similarly, spring appeared to be a favourable season for SPn colonisation in comparison to winter in Poland [35] or winter in the United States [36]. In contrast, there was a reported increase of pneumococcal carriage in winter in Israel [37]. Other studies have found the seasonal effect to be negligible or absent [38,39]. Social factors such as being longer inside in day-care centres and higher crowding during the winter season or higher air pollution can be speculated as additional factors leading to higher colonisation rates.

In our study, the most predominant colonising serotypes were 6B, 19F, 23F, 15, 14 and 6A, which accounted 72.5% of the isolates. Previously reported data on SPn colonisation show that serogroups 6, 19 and 23 were the most common before widespread use of PCV routine vaccination in other European countries such as Estonia, Poland, Italy, Hungary, Romania, France and the Netherlands [4,7-12]. Serotypes 19F, 23F and 6B were also predominant among nasopharyngeal isolates in healthy Lithuanian children in 2006 [26,27].

SPn serotype fluctuation has been observed during different study years in Lithuania. The prevalence of serotype 3 decreased from 13% in 1999 to 3% in our study, while serotype 14 became three times more common than in 2006 according to our data (3.2% and 9.5%, respectively). Serotype 15 constituted 9% of all isolated pneumococci in 1999; it was 2.5% in 2006 [26,27] and 10% in our study. The distribution of other SPn serotypes remained stable, with small differences in their rates. It is important to note, however, that the study sites and the type of children studied differed, which limit the comparison.

Although the data on SPn serotypes in children with IPD in Lithuania are rather limited, we have tried to compare the serotype distribution among IPD patients and nasopharyngeal carriers. Serotypes 23F, 14, 6B, commonly found in the nasopharynx in our study, were the most prevalent among all SPn isolates from children with IPD identified during 2006 to 2011 in Lithuania [14]. Serotypes 1 and 18C each constituted 8.9% of the invasive isolates [14], 18C was three times less common (3.0%, 11/367) and serotype 1 was absent in nasopharyngeal carriage in our study. According to data reported by other authors, serotype 1 is among the most commonly isolated serotypes in IPD but it is rarely found among nasopharyngeal carriers [40].

Age-related differences of pneumococcal serotype distribution were found in our study, with serotypes 6A and 11 being more common in the youngest children, while 18C was found only in the older age groups. A study conducted by Bogaert et al. in 2002 showed an age-related serotype distribution in healthy children in the Netherlands, with a primary peak of serotypes included in the seven-valent conjugate vaccine (PCV-7) at the age of one year, followed by a secondary peak of non-vaccine serotypes at the age of four years [13]. However, few data are available concerning SPn

serotype distribution in the nasopharynx according to age.

The serotypes found in a population might be an important predictor of the likely effectiveness of a pneumococcal vaccine in that population. Our findings suggest a rather high theoretical coverage (58–73%) of nasopharyngeal pneumococcal isolates by the currently available PCVs (PCV-10 and PCV-13). This is similar to the theoretical coverage reported in Estonia (64% in 1999–2000, 2003), Hungary (55.6–69.6% in 2009–10) and Italy (56.5% in 1999) [9,10,30]. Lower theoretical coverage by PCVs has been reported in the Netherlands (41.6% in 2002), Norway (37% in 2006) and Russia (45%, published 2007) [21,22,41]. Conversely, PCV coverage rates were estimated to be higher in Romania (66–80% in 2008–09), France (76.5% in 1999) and Poland (73.7–80.1% in 2002–03) [4,8,12].

As our study was performed before the implementation of the universal programme of PCV vaccination in Lithuania, it provides a basis for future comparisons of SPn carriage and serotype distribution between the pre- and post-vaccination era in the country.

Acknowledgements

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Conflict of interest

None declared.

Authors' contributions

Vytautas Usonis had primary responsibility for the study design and development, data collection, outcome assessment and contributed in the writing of the manuscript. Sigita Petraitienė participated in the development of the protocol, nasopharyngeal sample and data collection, data analysis and writing of the manuscript. Daiva Vaičiūnienė contributed in nasopharyngeal sample and data collection and data analysis. Indrė Stacevičienė participated in nasopharyngeal sample and data collection, data analysis and writing of the manuscript. Tomas Alasevičius participated in nasopharyngeal sample and data collection, data analysis and writing of the manuscript. Jūratė Kirslienė was responsible for isolating *Streptococcus pneumoniae* and serotyping at the microbiology laboratory.

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Letter to the editor: Is a reduced duration of post-discharge surgical site infection surveillance really in our best interests?

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To the editor:

In a recent issue of *Eurosurveillance*, a well-considered study by Koek et al., among other objectives, investigated the impact of reducing the post-discharge surveillance duration on surgical site infection (SSI) incidence [1] The premise for this was the redefinition in the United States (US) of surveillance for surgeries involving implants, to 90 days, compared with the previously accepted one-year end point. The authors indicate that a similar change is expected in Europe and go on to support this redefinition in their conclusion, despite a potential 14% of SSIs being overlooked.

Our experience at a single neurosurgical centre would be similar. Of the 1,778 procedures including an implant between October 2011 and February 2014, 61 SSIs were identified after one-year follow-up. If follow-up were restricted to 90 days, this number would be reduced by 15%. Equally, the likelihood of developing an SSI significantly drops after this point. Other studies have identified comparable patterns [2].
So, clearly, redefining the end point will reduce the incidence of SSI. In the US, where financial penalties exist for the development of SSI, some will welcome a reduction in its 'reported' incidence. Koek et al. suggest that by shortening the duration of surveillance, there would be greater consistency among centres, allowing for more accurate and real-time inter-centre comparison. Such comparison has allowed individual centres to successfully recognise and respond to relatively high infection rates [3].

However, if our goal is to advance our knowledge and eliminate the problem entirely, is an artificial reduction in SSI incidence in our best interests? SSI is a relatively uncommon problem with a multifactorial aetiology. Research into combative strategies are challenged by the low event rate, demanding and often failing to attain the large sample numbers required to identify individual advances [4,5]. If the goal posts are magically changed to overlook a large number of SSI, are we not further handicapping our efforts to eliminate this significant problem?

Conflict of interest

None declared.

Authors' contributions

Both authors contributed to the writing of this letter.

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Authors' response: Is a reduced duration of post-discharge surgical site infection surveillance really in our best interests?

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To the editor:

We would like to thank Dr Davies and Dr Patel for their interest in our paper. We agree with them that it is important to advance our knowledge in order to try to eliminate the occurrence of surgical site infections (SSIs) [1]. In order to do so there are several instruments, and surveillance of SSIs is only one of them. Surveillance of SSIs is a standardised way to monitor and report SSIs, allowing comparison within and between hospitals and triggering improvement of internal processes.

For surveillance purposes, we consider it justified to shorten the duration of post-discharge surveillance (PDS), as not only the calculated SSI incidence but also the workload involved in PDS and the speed of feedback to the healthcare professional must be considered. The results of our paper clearly demonstrate that the majority of the SSIs occur within 90 days, and a shorter PDS will substantially facilitate prompt feedback of surveillance results to the healthcare professionals [2].

For clinical purposes however, it is still possible (and sometimes even advisable) to trace all SSIs, regardless of the time when they arise or of their cause. While the 90-day incidence of SSIs might be slightly lower than the one-year incidence, it might also be more accurate since it better represents truly surgery-related SSIs (instead of SSIs due to secondary causes, such as for instance bacteraemia). As such, the use of a 90-day PDS may improve the accuracy of studies investigating preventive or combative interventions.

Finally, we would like to emphasise that incidence by definition is a measure of frequency during a given time period [3]. Therefore, by reducing the duration of PDS, the incidence itself is not artificially reduced as Davies et al. conclude, but the incidence is measured during a shorter given time period. We would therefore not recommend to directly compare one-year incidences with 90-day incidences without taking into account the shorter duration of PDS.

Conflict of interest

None declared.

Authors' contributions

Mayke BG Koek wrote the letter on behalf of the authors of the original article.

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ESCAIDE 2015: registration and call for abstracts now open

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The call for abstracts for the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) is now open and abstracts can be submitted via the dedicated 'call for abstracts' portal on the **ESCAIDE website**.

The deadline for abstract submission is 11 May, at 24:00 (CET).

The conference welcomes abstracts on topics from field epidemiology to molecular biology, from vaccines and antimicrobial resistance to public health policy, and more.

Online registration for ESCAIDE 2015 will be open until 1 November and can be done via the dedicated **ESCAIDE registration page**.

Onsite registration during the conference will also be possible.

ESCAIDE 2015 will be held in Stockholm, Sweden between 11 and 13 November.

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