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Comparing introduction to Europe of highly pathogenic avian influenza viruses A(H5N8) in 2014 and A(H5N1) in 2005

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Since the beginning of November 2014, nine outbreaks of highly pathogenic avian influenza virus (HPAIV) A(H5N8) in poultry have been detected in four European countries. In this report, similarities and differences between the modes of introduction of HPAIV A(H5N1) and A(H5N8) into Europe are described. Experiences from outbreaks of A(H5N1) in Europe demonstrated that early detection to control HPAIV in poultry has proven pivotal to minimise the risk of zoonotic transmission and prevention of human cases.

Highly pathogenic avian influenza virus A(H5N1) and A(H5N8) outbreaks in domestic poultry and wild birds in Europe

Outbreaks of highly pathogenic avian influenza virus (HPAIV) A(H5N8) in domestic poultry and wild birds in Europe in 2014 have some similarities to the previous introduction of HPAIV A(H5N1). Since beginning of November 2014, nine outbreaks have been detected in four countries in Europe. Comparisons between these incursions may help to better understand potential risks to public health.

In autumn 2005, Romania and Croatia were the first European countries reporting influenza A(H5N1) virus infections in wild birds and domestic poultry; hereafter, it spread rapidly across central Europe starting in February 2006 [1]. When HPAIV A(H5N1) finally disappeared in 2010, it had been detected in wild birds and poultry in 21 European countries (Table) [2]. Prior to the introduction into Europe, HPAIV A(H5N1) was extensively circulating in poultry, with recurrent spill-overs to wild birds, in Asia [3].

Influenza A(H5N8) virus is a complex reassortant virus carrying genes from A(H5N1) as one of its parental viruses. An ancestral strain was first reported in China in 2010 [4]. In 2014, following further virus evolution via reassortment, several outbreaks with HPAIV A(H5N8) viruses occurred in aquatic migratory birds, chickens, geese and ducks in China, Japan, and Republic of Korea [2,5-10]. Between 5 November and 16 December 2014, Germany, Italy, the Netherlands, and the United Kingdom (UK) reported HPAIV A(H5N8) outbreaks in nine holdings in total, with turkeys, chickens or ducks [11] (Figure, Table).

In November and December 2014, HPAIV A(H5N8) was detected in a healthy common teal (*Anas crecca*) in Germany and in two faecal samples from Eurasian wigeons (*Anas penelope*) in the Netherlands. The region in Germany currently affected by HPAIV A(H5N8) largely matches the area that was first affected by HPAIV A(H5N1), whereas in the UK, cases of A(H5N1) were initially located in eastern and southern regions and in Italy in central and southern regions, but not in the areas affected in November 2014. The Netherlands had not been affected by HPAIV A(H5N1) at all [12].

Influenza A(H5N8) virus infection does not appear to be associated with severe illness or excessive mortality in wild mallard ducks [10]. In contrast, in galliform poultry (turkey, chickens), HPAIV A(H5N8) causes mass mortality as seen in affected holdings [2]. The low to moderate virulence of HPAIV A(H5N8) in domestic ducks seen in the UK resembles the situation in wild mallards [2]. Many strains of HPAIV A(H5N1), in contrast, cause high mortality not only in galliformes but also in anseriform poultry and in wild birds [13,14].

Routes of introduction into Europe

The spread of HPAIV A(H5N1) from east Asia to Europe via central Asia may have occurred by a complex mixture of activities associated with poultry production,

Table

European countries with reported outbreaks of influenza A(H5N1) and influenza A(H5N8) virus infection in domestic poultry and wild birds, 2003 to 2014[a]

Country	Detections of influenza A(H5N1) virus infection in domestic poultry/wildlife 2003–2010	Number of outbreaks of influenza A(H5N1) virus infection in domestic poultry 2003–2010	Number of outbreaks of influenza A(H5N8) virus infection in domestic poultry 2014	Detections of influenza A(H5N8) virus infection in wildlife 2014
Albania	Yes	3	0	0
Austria	Yes	0	0	0
Bosnia and Herzegovina	Yes	0	0	0
Bulgaria	Yes	0	0	0
Croatia	Yes	0	0	0
Czech Republic	Yes	4	0	0
Denmark	Yes	1	0	0
France	Yes	1	0	0
Germany	Yes	8	2	1
Greece	Yes	0	0	0
Hungary	Yes	9	0	0
Italy	Yes	0	1	0
Netherlands	No	0	5	2
Poland	Yes	10	0	0
Romania	Yes	164	0	0
Serbia	Yes	1	0	0
Slovakia	Yes	0	0	0
Slovenia	Yes	0	0	0
Spain	Yes	0	0	0
Sweden	Yes	1	0	0
Switzerland	Yes	0	0	0
United Kingdom	Yes	2	1	0

[a] Data available from [2].

illegal poultry transports, spill-over infections to wild birds, and migratory bird dispersal, but no consistent route of infection into poultry holdings within Europe has been identified [15]. The sudden occurrence of HPAIV A(H5N1) in central Europe in 2006 was probably associated with cold spells in western Russia that drove out substantial numbers of putatively infected wild birds further west [16].

Mutual transmission of HPAIV between migratory birds and domestic poultry might occur through direct or indirect contacts [7]. Genetic analyses of the recent European influenza A(H5N8) viruses revealed very high similarity to Japanese wild bird isolates suggesting a common source of infection [17].

Import of live poultry and live captive birds as well as poultry commodities from affected Asian regions to the European Union (EU) is illegal but cannot be formally excluded as route of introduction for HPAIV A(H5N8); however, this seems unlikely considering the simultaneous nature and geographic spread of the outbreaks. No epidemiological links have been identified between the initially affected holdings in Germany, the Netherlands and the UK. Findings of HPAIV A(H5N8) in wild birds in Europe also open a possibility for an importation from Asia to Europe via migratory wild birds although this remains hypothetical until the virus would be found in further wild bird populations in central and western Asia.

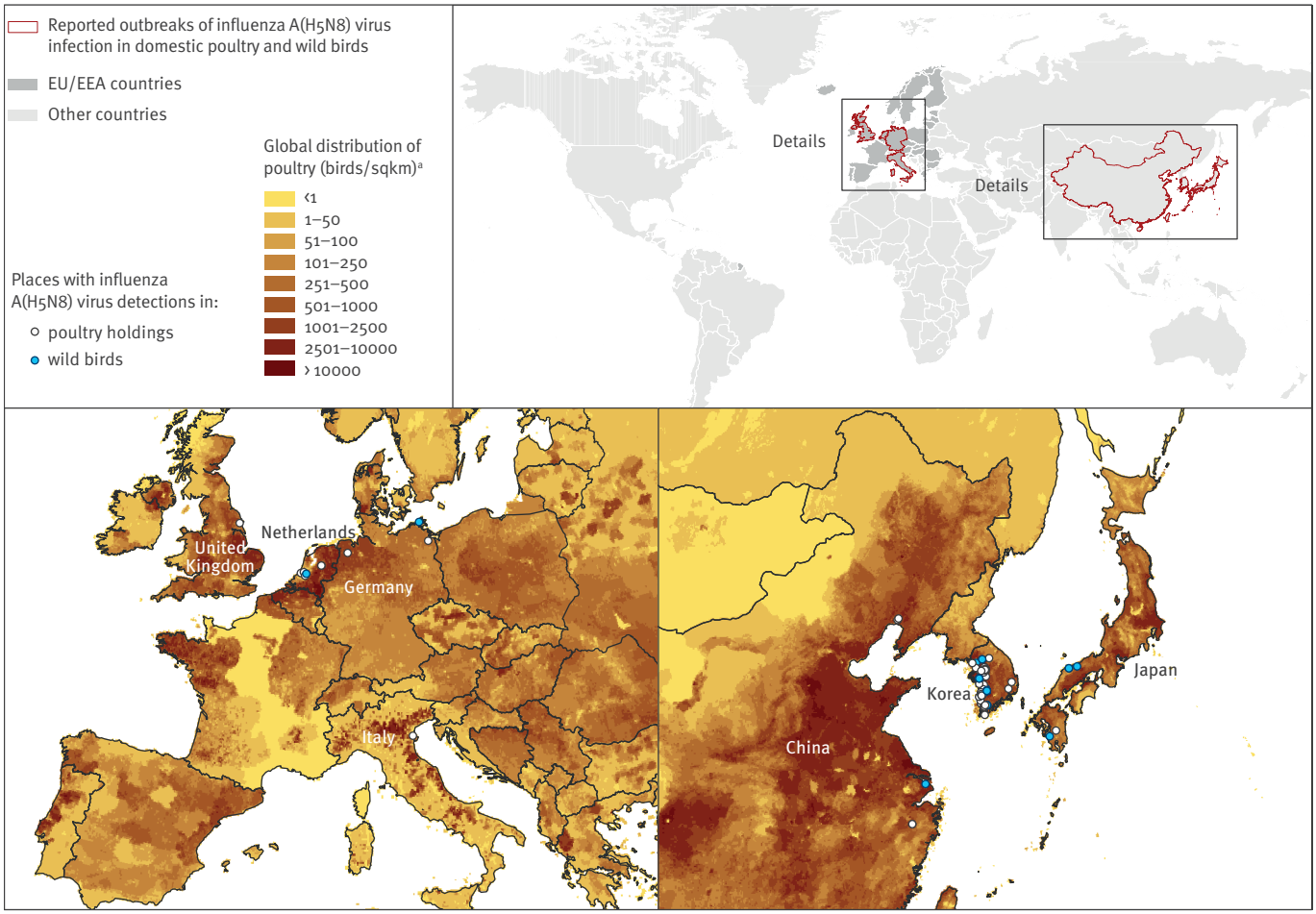
Affected holdings in Europe so far were indoor facilities with nominally good biosecurity, yet indirect contact between wild birds and poultry (via any material contaminated by infected wild birds, e.g. faeces) might be possible. The fact that in several of the holdings, stable units closest to the entrance of the compound were the first affected in the outbreak, might suggest the introduction of A(H5N8) from a focal source close to the compound

Surveillance of highly pathogenic avian influenza viruses in the European Union

The experience of large HPAIV outbreaks of in the Netherlands, Belgium and Germany, e.g. A(H7N7) in 2003, prompted the EU to better prepare for large scale incursions of HPAIV [18]. The establishment and implementation of surveillance systems, diagnostic tools for

Figure

Countries reporting outbreaks of influenza A(H5N8) virus infection in domestic poultry and wild birds, 2014



EU/EEA: European Union/European Economic Area.

[a] Recently published Gridded Livestock of the World (GLW) 2.0, May 2014.

early detection of HPAIV and harmonised restriction and control measures was laid down in the EU legislation [19]. This included introducing programmes of passive surveillance of wild bird populations as an early warning system, which proved to be a useful tool in detecting presence of HPAIV A(H5N1). This was complemented by active monitoring of poultry. In the current epizootic, enhanced wild bird monitoring might also help to further elucidate the role of wild birds transmitting A(H5N8), although the apparently asymptomatic presentation in wild bird species in Europe indicates potentially scaling up elements of targeted active rather than passive surveillance may be appropriate to better understand the distribution, prevalence and introduction pathways into Europe.

Risk for human transmission and resulting public health measures

Although to date, HPAIV A(H5N1) has caused 676 human cases worldwide, including 398 deaths [20], no human cases have ever been reported in Europe and these viruses lack the capability for sustained human-to-human transmission. Cats and mustelids were the only mammals found to be infected in Europe [13]. No human cases due to A(H5N8) have so far been reported anywhere. Mammals such as mice, ferrets, dogs and cats proved to be susceptible to experimental A(H5N8) infection, yet, only mild clinical disease, if any, ensued and so their role as amplification or reservoir hosts appears less likely [21].

Binding of avian influenza viruses to mammalian α2–6 sialic acid receptors is a prerequisite for transmission to humans. HPAIV A(H5N8) binds strongly to avian α2–3 receptors and, to a markedly lesser degree, to α2–6 receptors indicating limited zoonotic potential [21]. Antiviral resistance has not been described in the recent isolates [17]. Candidate vaccine viruses for human use have been proposed for pandemic preparedness [22].

People at risk of spill-over infections are those directly exposed to infected birds and their carcasses, e.g. during culling measures in affected holdings. Personal

protective equipment and other protective measures are a prerequisite for preventing zoonotic transmission. Passive or active monitoring of exposed persons enables early identification of transmission events. There is no evidence of human infection through consumption of contaminated food [23]. Recommended diagnostic tests for animal and human samples showed reliable detection of HPAIV A(H5N8).

Conclusions

The current simultaneous occurrence of A(H5N8) in different European countries is comparable to the introduction of A(H5N1) around a decade ago. These similarities may point to common routes of introduction into Europe, although these are not fully understood and the exact sources of infection of the affected indoor poultry holdings have not been identified yet. More outbreaks seem possible. Although HPAIV A(H5N8) might have the potential to transmit to humans, no human cases have been detected so far. Based on the experience from HPAIV A(H5N1) in Europe, efficient biosecurity, early detection, and stringent control measures are able to minimize risks of spill-over transmission to humans. The rapid geographic dispersion of yet another reassortant highly pathogenic avian influenza A virus underlines the need for enhanced preparedness.

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Authors' contributions

CA: study initiation, compilation of data, drafting the manuscript

CG: critical review and drafting of the manuscript

GK & RB: data provision from outbreaks in the Netherlands, critical review and approval of the manuscript

IB: providing data from the UK and EUR Lab, critical review and approval of the manuscript

FV: providing EFSA data, critical review and approval of the manuscript

PP: critical review and approval of the manuscript

TH: study concept and providing German data, manuscript draft, critical review and approval

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ESwab challenges influenza virus propagation in cell cultures

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Although the ESwab kit (Copan, Brescia, Italy) is intended for sampling bacteria for culture, this kit is increasingly also used for virus sampling. The effect of ESwab medium on influenza virus detection by real-time reverse transcription-polymerase chain reaction (RT-PCR) or virus propagation in Madin-Darby canine kidney (MDCK) cell culture was investigated. The ESwab medium was suitable for viral RNA detection but not for viral propagation due to cytotoxicity. Sampling influenza viruses with ESwab challenges influenza surveillance by strongly limiting the possibility of antigenic characterisation.

Background

Viral culture is a prerequisite for the surveillance of antigenic drift of influenza viruses as well as phenotypic antiviral resistance [1,2]. Antigenic drift of influenza virus can result in severe epidemics and vaccine failure, with consequences for especially the risk groups who are recommended the seasonal influenza vaccine [3]. Monitoring changes in the phenotypic traits of influenza virus is therefore an important component of the international and national surveillance systems.

At the National Influenza Center (NIC) in Denmark, we have experienced challenges during the recent 2012/13 and 2013/14 seasons with culture of influenza virus in clinical samples submitted from regional hospitals. Atypical cytopathogenic effect (CPE)/cytotoxicity in cell cultures appear for clinical materials which have been sampled using the ESwab sampling kit (ESwab, Copan, Brescia, Italy). The medium included in the sampling kit is modified Amies medium intended for bacterial culture and is not recommended for virus isolation by the manufacturer [4-6].

According to the feedback that the Danish NIC has received from the clinical microbiology laboratories, the ESwab kit is increasingly popular for multipurpose sampling due to the ease of use and low cost. The sampling kit is useful for analysing both bacterial and virus samples for diagnostic purposes using polymerase chain reaction (PCR) techniques [7,8]. Studies of cell propagation of influenza virus stored in the ESwab sampling kit have only been reported in one previous study by Indevuyst et al. [9], who describe that atypical CPE can be attributed to the flocked swab included in the kit. However, the swab alone cannot explain the widespread atypical CPE we have observed, and to explore this further we have studied the effect of ESwab's modified Amies medium directly onto Madin-Darby canine kidney (MDCK) cell culture as well as the effect on virus propagation. The suitability of ESwab medium for influenza virus detection by real-time reverse transcription-polymerase chain reaction (RT-PCR) was also investigated.

Methods

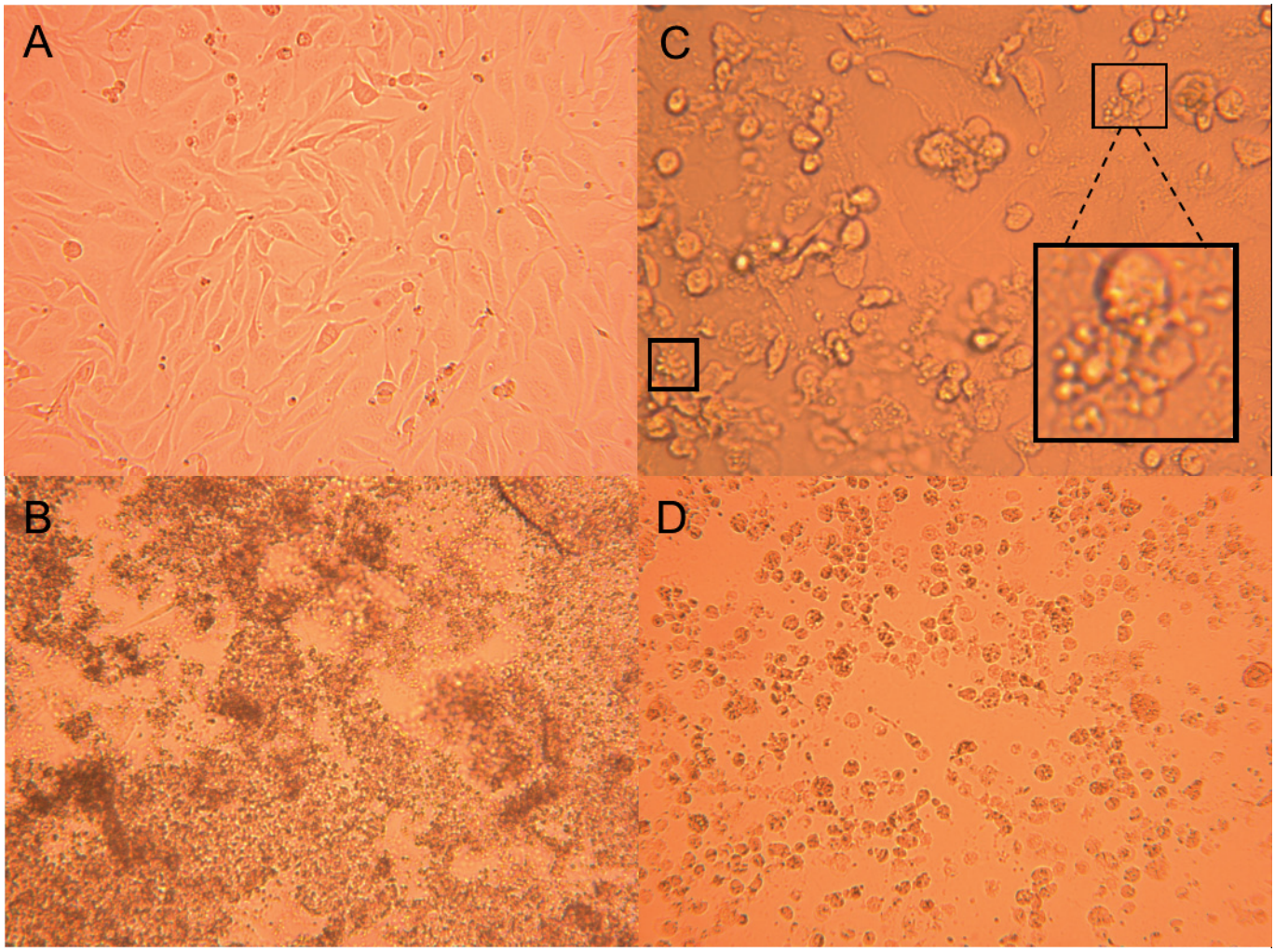
Testing ESwab medium suitability for viral culture

Twenty ESwab medium solutions were each used as a starting point to generate twofold dilution series in replicate of ESwab medium (without the swab) in Eagles minimum essential medium (MEM). The dilution ranged from undiluted to 1:128. Three different batches of the medium were used in the experiments to exclude lot variation. Pure Eagles MEM was used as negative control for each ESwab dilution series. Once established all dilutions and controls were stored at 4 °C for 24 hours before being tested for their effect on MDCK cells.

To test the effect of pure ESwab medium and the diluted ESwab medium solutions described above on MDCK cells, these solutions as well as negative controls were respectively inoculated on the cells. The degree of cytotoxicity was evaluated by scoring from 0 to 5, where 0 was no cytotoxicity, 1 was low and 5 was extensive cytotoxicity.

Figure 1

Cytotoxic effect observed in Madin-Darby canine kidney cell cultures inoculated with solutions consisting of varying proportions of ESwab and Eagles minimum essential media



MDCK: Madin-Darby canine kidney cells.

A: Negative MDCK cell control inoculated with pure Eagles minimum essential medium after 24 hours of incubation, magnification x20, B: MDCK cells inoculated with pure ESwab medium after 24 hours of incubation, magnification x20, C: MDCK cells inoculated with ESwab diluted 1:2 in Eagles minimum essential medium after 24 hours of incubation. Black squares indicate apoptotic cells surrounded by apoptotic bodies. One of the squares is magnified (x60) to enhance visibility of apoptotic bodies surrounding a degenerated cell, magnification x20 D: MDCK cells inoculated with influenza virus (H1N1pdm09), rounded cells typical for influenza virus cytopathogenic effect are visible, magnification x10.

To evaluate the direct effect of ESwab medium and diluted ESwab medium solutions on virus propagation, reference virus isolates as well as virus isolates from clinical samples were respectively diluted 10-fold in these solutions. The reference virus isolates were A/California/07/2009 (H1N1pdm09) (passage history: two times in primary swine kidney cells (sw)/four times in MDCK (2sw/4MDCK)), A/Denmark/42/2014 (H1N1) (2MDCK), A/Brisbane/10/2007(H3N2) (3MDCK), and A/Denmark/43/2014 (H3N2) (2MDCK-SIAT/1MDCK). Identical virus dilutions were also made in Eagles MEM as controls. Negative controls of pure Eagles MEM were also included.

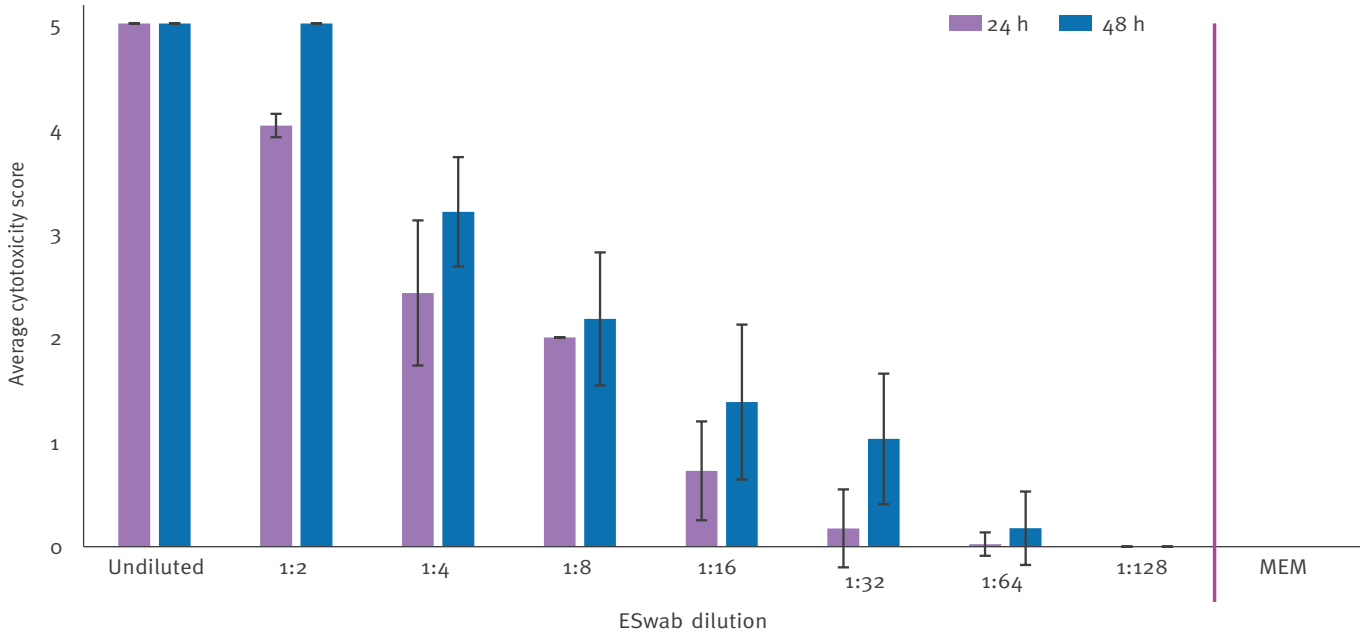
Dilutions were stored at 4 °C for 24 hours before inoculation following normal procedures in confluent monolayers of MDCK cells. The cells were observed daily and CPE was scored from 0 to 4, where 0 was no CPE, 1 was up to 25% CPE, 2: 25–50%, 3: 50–75% and 4: 75–100% CPE. Cell cultures were fixed and immunostained for influenza virus after 48 hours. Cell cultures were considered influenza positive if characteristic influenza virus CPE appeared and if the cells were positive for influenza virus by immunostaining.

Testing ESwab medium for real-time reverse transcription-polymerase chain reaction

In order to confirm the suitability of virus samples submitted in ESwab medium for diagnostics using PCR technology, H1N1pdm09 virus was subjected to a tenfold dilution series either in ESwab medium or in phosphate-buffered saline (PBS). The viral solutions obtained were tested by two in-house real-time reverse transcription (RT)-PCR assays. One assay used primers

Figure 2

Average scores[a] of cytotoxicity[b] in Madin-Darby canine kidney cells inoculated with solutions consisting of varying proportions of ESwab and Eagles minimum essential media



MEM: Eagles minimum essential medium.

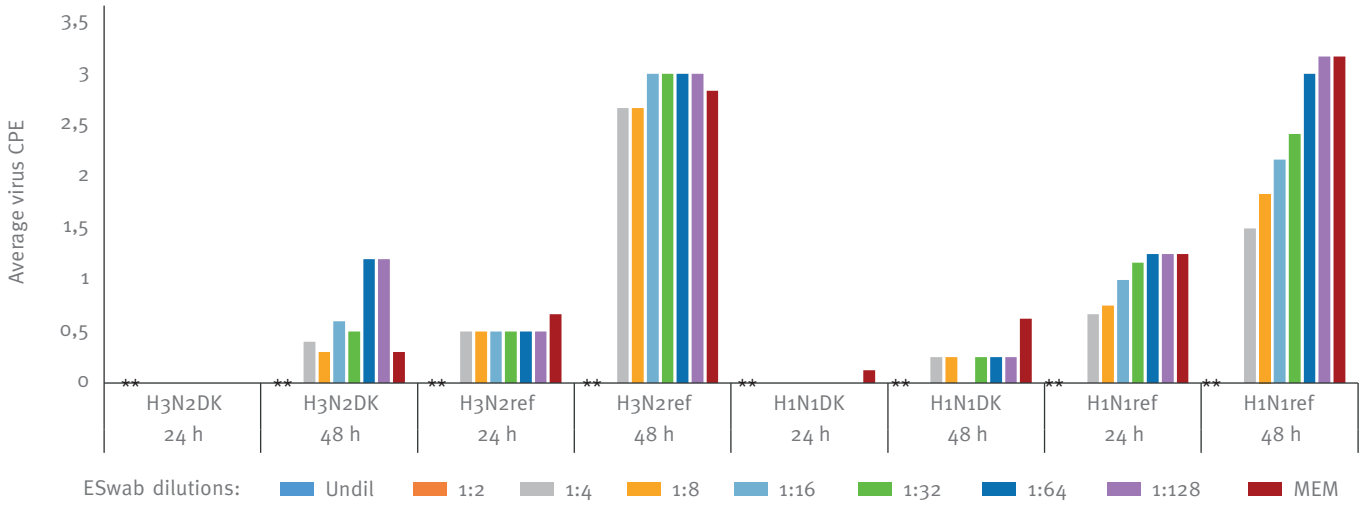
Twenty ESwab medium solutions were used to generate the eight dilutions shown on the X axis. To evaluate cytotoxicity, Madin-Darby canine kidney cells were inoculated with the 20 replicates for each dilution.

[a] All dilutions were scored for cytotoxicity after 24 and 48 hours (h) incubation with the Madin-Darby canine kidney cells respectively.

[b] Cytotoxicity was evaluated by scoring from 0 to 5, where 0 was no toxicity and 5 extensive toxicity. The mean cytotoxicity obtained with replicate experiments is shown on the Figure. Standard deviations are indicated with black bars.

Figure 3

Average scores of cytopathogenic effect[a] caused by propagation in Madin-Darby canine kidney cells of influenza virus isolates[b] in solutions with varying proportions of ESwab and Eagles minimum essential media



CPE: cytopathogenic effect; MEM: Eagles minimum essential medium; undil: undiluted Eswab medium.

Due to the highly toxic effect of the undiluted and 1:2 dilution of the ESwab medium it was not possible to evaluate influenza virus CPE in these dilutions. This is indicated by asterisks (*) in the Figure.

[a] CPE was scored from 0 to 4, where 0 was no CPE, 1 was up to 25% CPE, 2: 25–50%, 3: 50–75% and 4: 75–100% CPE. Scoring was performed after 24 and 48 hours incubation of the inoculated cells. Virus CPE was confirmed by immunostaining of influenza virus infected cells.

[b] Dilutions of the following influenza virus isolates were used: A/California/07/2009 (H1N1pdm09) (H1N1ref (n = 6)), A/Denmark/42/2014 (H1N1pdm09) (H1N1DK (n = 4)), A/Brisbane/10/2007(H3N2) (H3N2ref (n = 3)), and A/Denmark/43/2014 (H3N2) (H3N2DK (n = 5)). The solutions used to dilute the virus isolates consisted of varying proportions of ESwab medium and Eagles MEM. Control dilutions of the virus isolates were in pure Eagles MEM.

Table

Results of real-time reverse transcription-polymerase chain reaction depending on the dilution level of H1N1pdm09 reference virus template in ESwab medium or phosphate-buffered saline, with effect of different storage conditions

Real-time RT-PCR target	Storage time and temperature	Medium	Ct values obtained with A/California/07/2009 (H1N1pdm09)[a] dilutions							
			Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Matrix gene	24 h and 4 °C	ESwab	19.75	22.70	26.15	29.72	32.68	35.68	41.48	-
		PBS	19.48	24.21	27.67	29.56	33.29	36.18	43.67	-
	7 d and 4 °C	ESwab	19.53	23.12	26.66	29.88	32.32	36.74	38.84	-
		PBS	20.12	25.20	26.71	31.03	33.66	37.60	39.96	-
	24 h and 20 °C	ESwab	19.95	22.26	25.97	29.31	33.36	36.12	39.98	-
		PBS	19.40	25.29	26.13	29.87	35.19	36.11	41.67	-
	7 d and 20 °C	ESwab	20.33	23.67	27.47	31.43	32.03	37.97	38.16	-
		PBS	20.15	24.90	26.09	30.16	34.30	37.70	-	-
N1pdm09 gene	24 h and 4 °C	ESwab	18.95	21.68	25.83	29.16	32.36	36.50	39.52	-
		PBS	19.08	23.23	26.51	29.59	33.35	36.00	39.82	-
	7 d and 4 °C	ESwab	18.81	22.12	26.37	29.48	32.37	36.40	39.76	-
		PBS	19.56	25.53	27.82	32.24	35.22	-	-	-
	24 h and 20 °C	ESwab	18.80	21.14	25.66	29.03	32.47	35.76	37.84	-
		PBS	18.93	23.97	26.00	29.60	34.02	35.40	39.90	-
	7 d and 20 °C	ESwab	18.95	22.88	27.21	30.49	33.42	35.90	39.93	-
		PBS	19.08	25.21	27.14	31.40	35.96	38.39	-	-

Ct: cycle threshold; d: days, h: hours; MDCK: Madin-Darby canine kidney cells; PBS: phosphate-buffered saline; RT-PCR: reverse-transcription polymerase chain reaction; sw: primary swine kidney cells; TCID: tissue culture infective dose; -: negative.

[a] A/California/07/2009 (H1N1pdm09) (passage history: two times in sw/four times in MDCK) with titre of 2 x 10^6 $TCID_{50}$/mL.

and a probe for the matrix gene of influenza A virus, the other, primers and a probe for the neuraminidase (NA) gene of H1N1pdm09. The dilution series were stored at 4 °C for 24 hours or seven days before testing by PCR, this to mimic a realistic time span for samples submitted to the laboratory vs optimal conditions.

Results

ESwab medium and viral culture

MDCK cells inoculated with undiluted ESwab medium, were completely lysed after 24 hours of incubation and only granulated cell debris was observed (Figure 1 and 2). Cytotoxic effects were observed from dilution 1:2 until 1:32 but were clearly decreasing by each dilution step of the ESwab medium (Figure 1 and 2). The cytotoxic effects were recognised as granulated cell debris from lysed cells, apoptotic cells displaying apoptotic bodies, and irregular cells loosing attachment. After 48 hours the cytotoxic effects were more pronounced and were observed until at a dilution of 1:64, however, with only minor cytotoxicity (mean score: 0.176) in this dilution step (Figure 2).

Due to the highly cytotoxic effect of the undiluted and 1:2 dilution of the ESwab medium it was not possible to evaluate influenza virus CPE at these dilutions. However, the success of influenza virus infection, scored by CPE and confirmed by immunostaining, was increasing by each dilution step of the ESwab medium from 1:4 until 1:128 (Figure 3). This finding is most notable for the H1N1pdm09 virus isolates.

ESwab medium and real-time reverse transcription-polymerase chain reaction

The suitability of the ESwab medium for diagnostics using PCR techniques was confirmed, as the H1N1pdm09 virus diluted in both ESwab and PBS, respectively, was detected equally well by two different real time RT-PCR assays (Table). In addition to this, the PCR results were not affected by storage of the dilutions series for seven days compared with 24 hours at 4 °C before PCR testing (Table).

Discussion

In this study we document that the ESwab medium severely affects the MDCK cells. Cell lysis and apoptosis in such cells suggest that the medium creates hyperosmolarity. The ESwab declaration lists a range of salts included in the medium and a milky appearance suggests high concentrations of salts. The MDCK cells are seemingly unaffected when the ESwab medium is diluted 1:64 to 1:128 in Eagles minimum essential medium, before inoculation in cells.

In agreement with this, we find that virus propagation is most successful when the medium is diluted substantially. Dilution to this degree is however not an

option for most clinical samples, as the virus concentration varies greatly between samples, and low virus concentrations are common. The virus isolates, which are used in the experiments described in this report, have been propagated in cell culture beforehand and viral cell adaptation must be expected [10]. Influenza viruses in clinical samples are by nature not adapted to the MDCK cells used for viral propagation, which means that we should expect an even lower success rate.

From our results, we can confirm that samples in ESwab medium are appropriate for diagnostics using real-time RT-PCR, as viral RNA appears unaffected by the otherwise cell toxic ingredients of this medium.

The increased use of ESwab as a multipurpose swab (for bacteria and virus sampling) at the hospitals in Denmark, despite it being intended for bacterial sampling, challenges the national influenza surveillance programme, as lack of successful virus culture prevents antigenic characterisation using haemagglutination inhibition test, as well as functional testing for resistance to antiviral drugs. The phenotypic assays for antigenic characterisation and resistance testing require virus isolates [1,2,11] and important information regarding phenotypic drift, not necessarily recognised by genome sequencing, will be lost. If the increasing trend using the ESwab sampling kit continues, we face a new risk of losing important data on vaccine match and on determination of antiviral treatment failure as well as information on new virus candidates for vaccine formulation.

To exemplify the problem, in the 2013/14 season NIC Denmark received a sample harbouring influenza A(H3N2) virus. Sequencing revealed, in collaboration with the World Health Organization Collaborating Center for Influenza in London, that the sample was probably antigenically distinct from the otherwise circulating dominant H3N2 viruses in Denmark and Europe. At that time only one identical H3N2 virus had so far been described in the Global Initiative on Sharing Avian Influenza Data (GISAID). This virus had been identified in an American patient by the World Health Organization Collaborating Center at the United States Centers for Disease Control and Prevention in Atlanta. The sample had been submitted to NIC Denmark in ESwab, preventing the preparation of a virus isolate for phenotypic antigenic characterisation. The opportunity was therefore lost to further explore this strain of concern.

The ESwab medium is highly unfit for viral propagation in cells and to avoid cytoxicity substantial dilution of the medium is required. This in practice is feasible only with the few samples containing high viral loads. Feedback from the hospitals in Denmark upon request from the Danish NIC to change the medium to a virus-friendly medium, is that the ESwab sampling kit is convenient because it is useful for PCR [7], and the staff only need to relate to one sampling material. Another argument is that the ESwab is also cheaper than the available universal/viral transport media (UTM/VTM). Therefore, most regional clinical microbiology departments so far have not changed medium. As a consequence, the Danish NIC has kindly requested twin-samples to be collected from the critically ill patients highly suspected for influenza disease during the coming 2014/15 influenza season. Feedback when informally addressing the challenges experienced in Denmark at international influenza meetings and virus symposia during 2014 has led us to believe that the increased use of ESwab may also be occurring in other European countries and we therefore expect our findings to have implications for the influenza surveillance of such countries.

Conclusion

The results from this study expand our understanding further of the cytotoxic effect of the ESwab sampling kit on cells used to propagate viruses, and indicate that whereas the ESwab sampling kit can be used for virus diagnostic purposes using RT-PCR, it is highly unfit for viral propagation in cell cultures and the continuing widespread use of the ESwab may pose difficulties for influenza surveillance.

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Conflict of interest

None declared.

Authors' contributions

RT: Conceived and designed the experiments in collaboration with BA and TKF, analysed the data, and wrote the paper. BA and JR: Performed the experiments, and analysed the data. JM: Contributed to discussion and information regarding the ESwab sampling kit as well as antigenic analysis perspectives. TKF: Contributed further with discussion of data and perspectives, as well as reviewing of the paper. All authors have seen, commented, and approved the paper.

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A large community outbreak of Legionnaires' disease in Vila Franca de Xira, Portugal, October to November 2014

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An outbreak of Legionnaires' disease with 334 confirmed cases was identified on 7 November 2014 in Vila Franca de Xira, Portugal and declared controlled by 21 November. Epidemiological, environmental and microbiological analysis identified industrial wet cooling systems to be the probable source of infection. Preliminary results from sequence-based typing of clinical specimens and environmental isolates confirmed this link. A series of meteorological phenomena are likely to have contributed to the scale of this outbreak.

On Friday 7 November, the Portuguese Directorate General of Health (DGS) was informed of 18 cases of Legionnaires' disease admitted to two hospitals within 24 hours in the municipality of Vila Franca de Xira, Lisbon. No cases reported foreign travel and all had positive urinary antigen tests for *Legionella pneumophila* serogroup 1. Between 2007 and 2012, the annual number of cases reported in Portugal had ranged from 82 to 140 [1,2]. Within hours, the Ministries of Health and Environment convened a multidisciplinary task force to investigate and control the outbreak.

Description of the investigation

Epidemiological investigation

Cases of Legionnaires' disease and/or laboratory diagnoses of *Legionella* are mandatorily notifiable in Portugal. All notifications since 1 October 2014 were reviewed. The DGS and the national reference laboratory (NRL) actively searched for cases through hospital and laboratory networks.

A confirmed case was defined as a person with pneumonia, laboratory confirmation of *L. pneumophila*, symptom onset after 1 October and a history of living in, working in, or visiting Vila Franca de Xira from 15 September onwards. All reported cases with clinical symptoms of pneumonia and an epidemiological connection to the affected area were treated as suspected cases and investigated further.

Staff from regional health authorities interviewed patients applying a standard questionnaire for symptoms, risk factors, place of residence and work, recent travel and daily habits during the incubation period. The outbreak was described in terms of date of symptom onset, age and sex distributions. Street addresses were geocoded using Google Earth to determine spatial distribution.

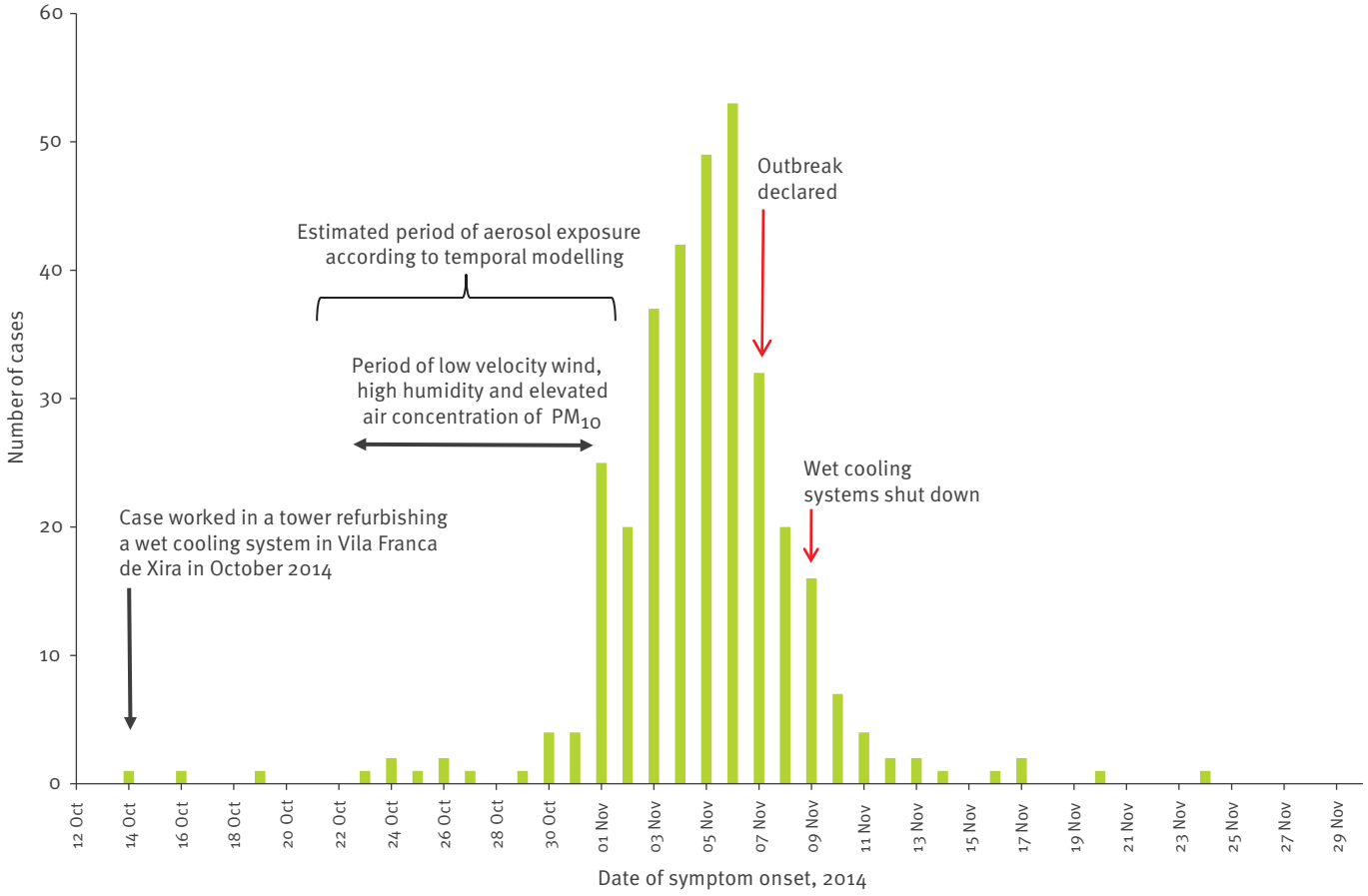
Back-calculation models developed by Egan et al. [3] were applied using epidemiological data available on 12 November. The models allowed determination of the period of aerosol release (period of exposure) and estimation of the number of expected cases. At the early phase of the outbreak, we had limited information on exposure. It was not clear which case represented the index case, therefore two scenarios were considered based on the available date of symptom onset: 14 October and 21 October.

Environmental investigation

As the cooling towers of wet cooling systems are often implicated in large outbreaks of Legionnaires' disease, the municipality identified all premises with wet cooling systems and other aerosol-generating sources in Vila Franca de Xira. Water samples were taken from public, domestic and industrial sources. The locations of wet cooling systems were georeferenced using visual pinpointing in Google Earth. Meteorological and

Figure 1

Confirmed cases of Legionnaires' disease by date of symptom onset, Vila Franca de Xira, Portugal, 14 October – 24 November 2014 (n = 334)



environmental agencies analysed weather data from 1 October until 7 November.

Microbiological investigation

The NRL tested clinical specimens and environmental samples for *Legionella* species. They performed sequence-based typing (SBT) following the protocol of the European Working Group for *Legionella* infections [4] with the aim of matching environmental isolates with isolates from clinical specimens.

Measures taken

On 7 November, hospitals in the region of Lisbon and the Tagus Valley activated the contingency plan to respond to the increased demand for ventilator systems. As the investigations were ongoing, the following actions were taken in the municipality: on 8 November, the public water authority raised chlorine levels and public pools, spas and fountains were shut down and disinfected after sampling; on 9 November, the Environmental Inspectorate shut down and ordered the disinfection of all wet cooling systems operating in the municipality.

Outbreak description

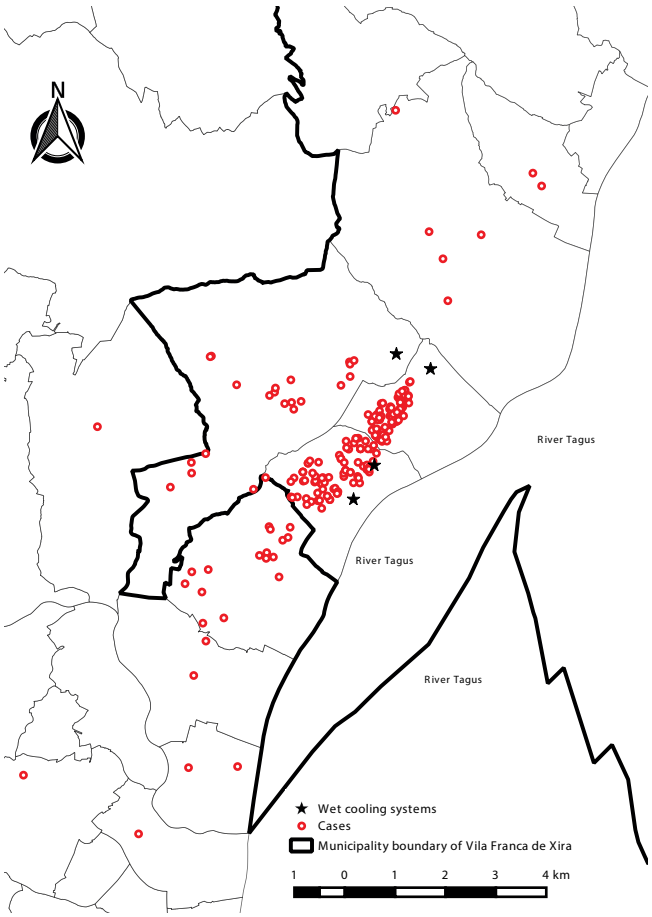
By 11 December 2014, the DGS had received reports of 417 suspected cases of Legionnaires' disease of which 334 were confirmed. All 334 had positive urinary antigen tests for *L. pneumophila* serogroup 1. Of the confirmed cases, 221 (67%) were male, the median age was 58 years (range: 25–92 years). Ten deaths were reported in confirmed cases and a further two deaths were awaiting post mortem investigations.

The outbreak started on 14 October, with a sharp increase in cases from 1 November, and peaked on 6 November (Figure1). The last reported case developed symptoms on 24 November. No confirmed cases with symptom onset after 24 November have been notified. The outbreak was considered controlled on 21 November. By 11 December, two incubation periods had passed since the peak of the outbreak and the implementation of control measures, and the number of notified cases from the area had returned to levels observed before the outbreak.

Place of residence was mapped for 250 of the 294 cases notified by 14 November, and the geospatial distribution was analysed: 90% (n=226) lived within 3 km of a wet cooling system. The task force hypothesised

Figure 2

Mapping of cases of Legionnaires' disease by place of residence, Vila Franca de Xira, Portugal, notified by 14 November 2014 (n = 250)



that the most likely source of infection was aerosolised release from one or more of these systems (Figure 2).

The back-calculation models using 21 October as the date of first symptom onset, suggested a logistic growth of the environmental exposure, with greatest intensity most probably between 23 October and 2 November. From this analysis, we estimated a total outbreak size of 325 cases (95% CI: 287–422). Since cases had been identified with symptom onset as early as 14 October, detailed interviews were conducted with them to ascertain if they were indeed linked to this outbreak. The patient who developed symptoms on 14 October was found to have been involved in maintenance works in the towers of local industrial wet cooling systems in the two weeks before onset of symptoms.

Meteorological data from the weather station in Alverca, 8 km from Vila Franca de Xira, indicated north-easterly wind with a velocity of 2–3 m/s between 18 October and 1 November. This wind coincided with high humidity (80%) from 26 October onwards, particularly at night. In addition, between 19 October and 1 November, a cloud of sand and dust lifted by a storm in the Sahara desert, covered Portugal and increased airborne concentrations of small particulate matter (PM_{10}).

By 20 November, 129 respiratory secretion specimens and 101 environmental samples were under analysis. Preliminary results from 12 clinical specimens identified the causative organism as *L. pneumophila* serogroup 1, sequence type 11, 14, 16, 10, 15, 13, 2ST 1905. Preliminary results of an environmental sample from once cooling system also showed sequence type 11, 14, 16, 10, 15, 13, 2 ST 1905.

Discussion

This was the largest outbreak of Legionnaires' disease in Portugal to date and among the largest reported in Europe. In the largest outbreak with 449 confirmed cases in Murcia, Spain, in 2001, a microbiological link between cases and a hospital cooling tower was demonstrated in the months after the outbreak [5]. Timely collection of respiratory secretion specimens by hospitals and widespread environmental sampling in the early phase of the outbreak allowed identification of a microbiological link between the environment samples and patient isolates [6].

To the best of our knowledge, this is the first time the models of Egan et al. [3] have been used in the early stage of an outbreak investigation. At the time of applying the models, we strongly suspected cooling towers as a source but had not ruled out other sources. Using the models we were able to identify cases whose pattern of illness did not fit with an aerosolised exposure. The occupational exposure of these outliers was identified within one week of the declaration of the outbreak.

Microbiological results suggested a link between the isolate from a wet cooling system and the isolates from the clinical specimens of patients, consistent with previously described large community outbreaks in Spain and the United Kingdom [5,7].

The concordance between the independent analysis of meteorological conditions and temporal modelling supports the theory that the prevailing weather conditions created a unique setting for *Legionella* multiplication and may explain the large scale of the outbreak. Unseasonable weather [8] and periods of humidity [9] have previously been described as facilitating the circulation of aerosolised *Legionella*.

Conclusion

Although the outbreak is considered controlled, detailed epidemiological investigations and microbiological investigations continue and may result in the reclassification of cases and an increase in confirmed cases. In addition, determination of the monoclonal subtype and molecular sequence typing to provide further evidence about the link between the environment and patients are underway. An evaluation of the outbreak with the view to improving early detection will be developed. .

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Conflict of interest

None declared.

Authors' contributions

Tara Shivaji wrote the manuscript. Lucilia Carvalho modelled the outbreak and provided insight into interpretation of results. Jorge Machado provided details on microbiological investigation and results. Teresa Marques provided technical details and insight on microbiological investigation and interpretation of results. Paulo Jorge Nogueira: modelled the outbreak. Baltazar Nunes modelled the outbreak and interpreted results for the manuscript. Catia Sousa Pinto provided technical details on epidemiological investigations and results Ana San-Bento was involved in epidemiological analysis for the manuscript. Luis Antonio Oliveira Serra georeferenced data and produced the map and technical commentary. João Valente was involved in epidemiological analysis and provided technical commentary on the manuscript. Paula Vasconcelos provided technical information and edited the manuscript.

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Foodborne hepatitis A outbreak associated with bakery products in northern Germany, 2012

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In October 2012, a hepatitis A (HA) outbreak with 83 laboratory-confirmed cases occurred in Lower Saxony. We defined primary outbreak cases as people with laboratory-confirmed HA and symptom onset between 8 October and 12 November 2012, residing in or visiting the affected districts. Secondary outbreak cases were persons with symptom onset after 12 November 2012 and close contact with primary cases. We identified 77 primary and six secondary cases. We enrolled 50 primary cases and 52 controls matched for age and sex, and found that 82% of cases and 60% of controls had consumed products from a particular bakery (OR=3.09; 95% CI: 1.15–8.68). Cases were more likely to have eaten sweet pastries (OR=5.74; 95% CI: 1.46–22.42). Viral isolates from five selected cases and three positively tested surfaces in the bakery had identical nucleotide sequences. One additional identical isolate derived from a salesperson of the bakery suffering from a chronic disease that required immunosuppressive treatment. Epidemiological and laboratory findings suggested that the salesperson contaminated products while packing and selling. Future risk assessment should determine whether food handlers with chronic diseases under immunosuppressive treatment could be more at risk of contaminating food and might benefit from HAV immunisation.

Introduction

Background

On 31 October 2012, the local health authorities of the neighbouring districts of A and B in Lower Saxony, northern Germany, detected an increase of hepatitis A (HA) cases. Thirteen serologically-confirmed HA cases had been notified within three days (incidence: 7.76 per 100,000 inhabitants) whereas during October to December 2011, only one case (incidence: 0.37 per 100,000 inhabitants) was notified in the same area. Notification of laboratory-confirmed infection with hepatitis A virus (HAV) and notification of clinical cases of viral hepatitis are mandatory in Germany. From 2001 to 2011 in Lower Saxony, the incidence of notified HA cases declined substantially. Fitting a linear trend to the incidence rate per 100,000 population reveals an average reduction by 0.13 per year, from 2.13 in 2001 down to 0.78 in 2011. During this time, the proportion of cases that could be assigned to known outbreaks was 28% on annual average with a slightly increasing trend (unpublished data).

In general, the true number of infections is presumed to be higher than the number of notified cases due to underreporting and the fact that many individuals, especially children, may experience HAV infection without symptoms [1].

HAV is transmitted by the faecal-oral route, either by a direct contact with a contagious person infected with HAV or by ingestion of HAV-contaminated food or water or from contaminated surfaces [2]. The average incubation period of HA is 28 days (range: 15–50 days) [3]. The period of highest infectiousness is assumed to range from two weeks before until one week after onset of jaundice. Viral shedding with highest HAV concentration in stool occurs before jaundice [4].

Food items that have been described or assumed to have caused HA outbreaks include mussels, oysters,

vegetables and salad fertilised with faeces [5,6], fruits including raw blueberries [7], frozen raspberries [8], frozen strawberries [9], green onions [10] and shellfish [11]. Contamination of food by infected food handlers in restaurants has also been described [12]. Several HA outbreaks have been associated with bakeries [13]. In an outbreak observed in 1968 in Michigan, United States (US), the vehicle of infection was pastries covered with glaze or icing [14]. In an outbreak in New York, US, HA was associated with sugar-glazed baked goods [15]. An HA outbreak took place in Germany in 2004 where a bakery employee most probably contaminated the products that caused the outbreak [13].

The tenacity of HAV is higher than of other non-enveloped viruses belonging to the family of Picornaviridae. The ability of HAV to survive on hands and on inanimate surfaces plays an important role for HAV transfer from hands of infectious persons to food items and from contaminated surfaces to fingertips [16,17]. Disinfection with alcohol and hydrogen peroxide (3% and 6% concentration) has been found ineffective against HAV on surfaces [18]. According to Mbithi, unmedicated liquid soap was ineffective for reducing virus titre on hands [18], indicating that hand washing with ordinary soap is not sufficient to prevent HAV transmission. HAV is acid-stable [19] and seems to remain viable while passing through the stomach [1]. The minimum infectious dose is estimated to be very low with 10–100 virus particles. The amount of HAV shed by infected humans ranges from 106 to 108 virus particles per 1 gram of stool [20,21].

In cooperation with local and regional public health and food safety authorities, we conducted epidemiological, laboratory and molecular investigations to identify the vehicle of the outbreak to prevent further cases. The local health authorities were aware that both of the most-affected villages had a shop of a bakery company (bakery X); this bakery runs four shops in total. Additionally, during the standardised interviews, cases reported, without being asked, having observed signs of jaundice in a bakery X employee. Taking into consideration the literature on bakery-associated HA outbreaks [13–15], a causal link to bakery X or its products was a major hypothesis for the outbreak investigation.

Methods

Case definition

Based on the regional and temporal distribution of cases, we defined primary outbreak cases as persons:

- residing in the affected districts (A or B) or having visited the outbreak area during 15 September to 03 October 2012 and
- reported with serologically-confirmed HAV infection and
- with disease onset between 8 October and 12 November 2012.

Serologically confirmed HAV infection was defined as IgM and/or IgG positive results, depending on the time lag between apparent symptoms and taking of blood sample. If sequencing data were available and did not match the outbreak strain, the person was not regarded as case. We excluded cases with travel history to HA-endemic areas during the probable period of infection.

Secondary outbreak cases were defined as persons:
- reported with serologically-confirmed HAV infection and
- suffering from HA with onset after 12 November 2012 and
- having close contact with primary outbreak cases.

In the course of the outbreak investigation, two HAV-infected persons were detected who did not meet the primary and secondary outbreak case definition but appeared to be important to clarify the outbreak. We refer to them as early cases and they were defined as persons:

- with serologically-confirmed HAV infection (IgG and IgM) and
- with symptom onset before 8 October 2012 and
- residing in the outbreak area.

Cases are described in terms of time, place and demographic information.

Case–control study

In a case–control study, we investigated the hypotheses that the occurrence of disease was associated with bakery X in general, with particular food items from bakery X, or with other exposures like festivals and markets in the outbreak area during time of infection. We also considered well-known risk factors for HAV infection such as travelling abroad or consuming seafood.

We aimed to recruit primary outbreak cases according to the case definition with the following additional restrictions:

- at least 18 years of age and
- physical and mental state and language skills sufficient to allow successful participation in the interview and
- no household contact with the suspected early cases (because such persons might have had other routes of transmission than the majority of cases).

We recruited control persons by random-digit dialling, aiming for a case–control ratio of 1:1. We used an approach adapted to the German landline system [22] since landlines are still common in the outbreak region. The landline telephone numbers of residential cases were modified by incrementing the last two digits. This was to ensure a regional matching of controls with the cases. In addition, the controls were

frequency-matched to the cases by sex and five-year age bands. Persons were not selected as controls if they reported symptoms of HAV infection, a history of HAV infection, or an HAV vaccination (other than recent post-exposure vaccination). For the interviews, we used standardised questionnaires for cases and controls. Both cases and controls were interviewed within two weeks after notification of the cases by trained interviewers. The exposure data were dichotomised by classifying 'don't know' and 'no reply' into one group. The associations were estimated by univariable and multivariable logistic regression analyses. All exposures were investigated and assessed by odds ratios (OR) with 95% confident intervals (CI) and two-tailed p-values of chi-squared test. Exposure variables with an OR>1 and a p-value<0.2 in univariable analysis were included in a multivariable logistic regression analysis. A stepwise backward elimination was used until all remaining variables were significant at the 0.05 level, keeping age and sex in the model. Statistical analyses were performed with Stata version 12.1.

Laboratory diagnostics of human samples

During their initial diagnostic workup, specimens from the cases were usually analysed in the contract laboratories used by the cases' various general practitioners. Thus, the investigating authorities did not have direct access to these samples and we performed additional laboratory investigations.

In order to compare the HAV sequences, we investigated specimens from one of the two suspected early cases, a bakery X employee, together with five of the suspected primary outbreak cases, particularly primary outbreak cases who were not resident in the two affected districts, to identify whether they all belonged to the same outbreak. Blood and faecal specimens of bakery X staff were screened with the aim to clarify their infection status, to detect potentially HAV positive persons and to determine whether contamination by a virus shedding employee could have been the source of the outbreak.

All serum specimens were serologically tested by enzyme immuno assay (EIA) for IgM and IgG anti-HAV using an automated system (Architect i2000SR, Abbott Diagnostics). The serum and faecal specimens were analysed for the presence of viral HAV RNA using a reverse transcription PCR.

Environmental investigation

We analysed surface swabs which were taken indoors in all four bakery X shops (hereafter referred to as A, B, C, D, Figure 1). HAV RNA was isolated by using a QIAmp Viral RNA Mini Kit (Qiagen). Subsequently, a real-time reverse transcription PCR (RT-PCR) was used for detecting HAV RNA in surface specimens following the methods of Costafreda et al. [23].

Genotyping/sequencing

At the German Consultant Laboratory for Hepatitis A and Hepatitis E, HAV RNA was amplified from IgM positive serum and stool specimens as well as from positive surface specimens.

Nucleic acid isolation was performed using the RNeasy Mini Kit (Qiagen) with 100 µl elution volume (RNase-free water). A 10 µl aliquot of the eluate was used for reverse transcription in a total reaction volume of 20 µl using Moloney murine leukaemia virus (M-MuLV) reverse transcriptase (Applied Biosystems) and random hexamers according to the manufacturer's instructions (42 degrees Celsius, 30 minutes). Two replicates were analysed in 30 µl PCRs each containing 10 µl of the

Table 1

Primer and probe sequence, hepatitis A outbreak, northern Germany, 2012

Assay and gene target	Primer or probe	Amplicon size (bp)	Primer or probe sequence (5'–3')[a]	Nucleotide positions or accession number[b]
RT-qPCR				NC_001489
Polymerase	SH-Poly-A[c]	107	GARTTTACTCAGTGTTCAATGAATGT	5964–5989
Polymerase	SH-Poly-1[c]		GGCATAGCTGCAGGAAAATT	6051–6070
Polymerase	SH-Poly-Q[c]		FAM-TCTCCAAAACGCTTTTTAGAAAGAGTCC-BHQ-1	5992–6019
Sequencing				
VP1/P2A	HAV6[d]	542	TGTCTGGAGCACTGGATGG	2839–2857
VP1/P2A	HAV7[d]		CATTTCAAGAGTCCACACACTTCT	3357–3380
VP1/P2A	HAV8[d]	397	TGGTTTCTATTCAGATTGCAAATTA	2890–2914
VP1/P2A	HAV9[d]		TTCATTATTTCATGCTCCTCAGT	3264–3286

NT: nucleotide.

[a] Hydrolysis probes were labelled with 6-carboxyfluorescein (FAM) at the 5' end and a nonfluorescent quencher (BHQ-1) at the 3' end.

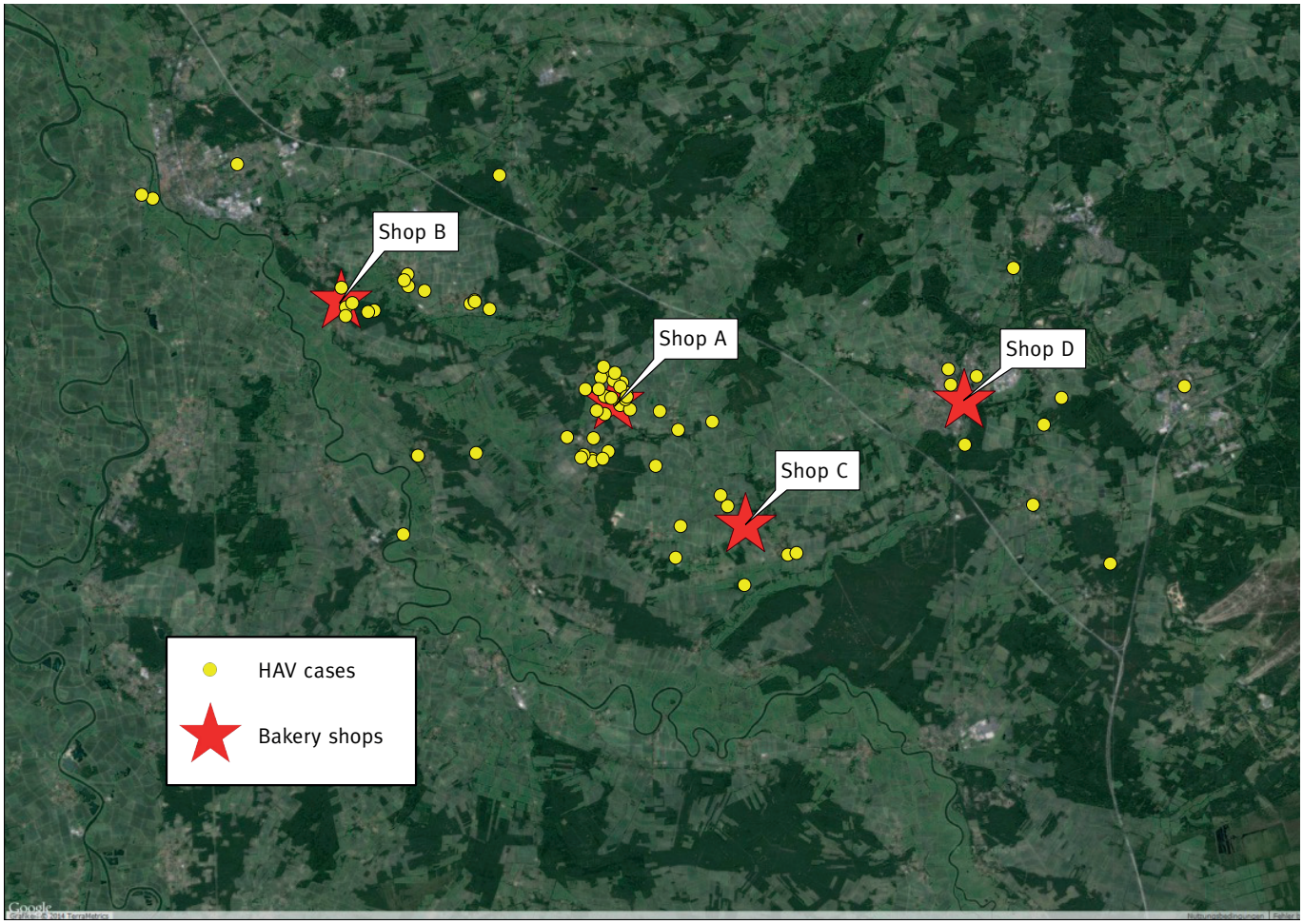
[b] Reference sequence accession numbers are for the databases of GenBank, the European Nucleotide Archive (ENA), and the DNA Data Bank of Japan (DDBJ).

[c] Primer reported by Houde et al. 2007 [25].

[d] Primer reported by Grinde et al. 1997 [26].

Figure 1

Primary cases of hepatitis A, by residence, and bakery X shops selling bakery X goods, hepatitis A outbreak, northern Germany, 2012



For data protection purposes, cases' home addresses were randomly distributed an average of 250m east/west and 250m north/south for locations within villages, and 1 km east/west and 1 km north/south for locations outside villages.

RT product (corresponding to 5 µl eluate), ROX buffer, 5.0 mmol/l $MgCl_2$, 1.25 U AmpliTaq Gold DNA polymerase (all Applied Biosystems), dNTPs, specific primers (300 nmol/l each) and TaqMan hydrolysis probe (200 nmol/l). Table 1 shows the nucleotide sequences of the primers and probe used for the HAV reverse-transcription quantitative real-time PCR (RT-qPCR) assay in the viral polymerase region: SH-Poly-A, SH-Poly-1 and SH-Poly-Q [24]. Thermal cycling was performed on a StepOnePlus instrument (Applied Biosystems) and comprised a 10-minute initial enzyme activation step at 95 degrees Celsius, and 45 cycles of 95 degrees Celsius for 15 seconds and 60 degrees Celsius for 1 minute.

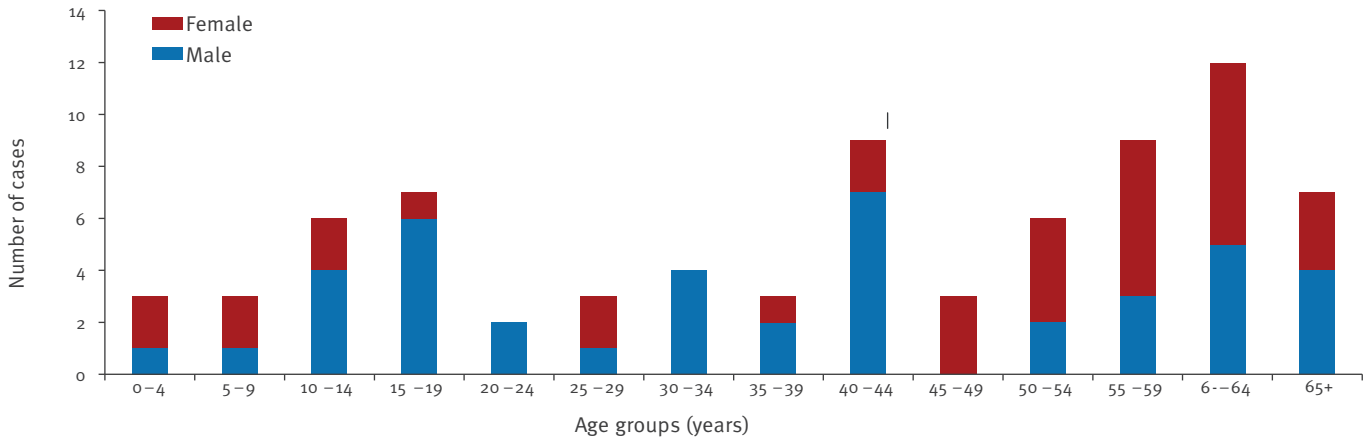
RT-qPCR positive isolates were further characterised by amplicon sequencing. The initial amplification was performed by using specific primers [25] for the HAV VP1/P2A genomic region (HAV6, HAV7, 542 bp). A 2.5 µl aliquot from the first round of PCR was then used as a template in the second round of PCR with primers HAV8 and HAV9 (397 bp). The PCR products were purified by using QIAquick columns (Qiagen) and sequenced in both directions with the second-round amplification primers.

Nucleotide sequences of amplicons were determined by using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and separated on a model 3730xl genetic analyser (Applied Biosystems). Nucleotide sequences of PCR products were analysed by using CodonCode Aligner 4.1.1 software (CodonCode Corporation, Centerville, MA, USA).

GenBank was searched for sequences with high similarity using the BLAST and FASTA algorithms as implemented by the HUSAR Biocomputing Service at DKFZ, Heidelberg, Germany. A rooted maximum likelihood phylogenetic consensus tree for VP1/P2A nucleotide sequences was inferred using PHYLIP 3.69 software.

Figure 2

Age and sex distribution of primary hepatitis A outbreak cases, northern Germany, 2012 (n=77)



Results

Descriptive epidemiology

A total of 83 outbreak cases with disease onset between 8 October and 29 December 2012 were identified (77 primary and six secondary outbreak cases).

The median age of the primary cases was 44 years (range: 4–77 years) and 78% of the cases (n=61) were 18 years and older. A total of 45% of the cases were female (Figure 2).

We found that 77 cases (91%) were residents of the districts of A (n=52) and B (n=25) in Lower Saxony. The primary cases' places of residence (not including the visitors) were clustered mainly around two bakery X shops (shop A and B, see Figure 1).

Seven primary cases were visitors to A or B from different German districts during the possible period of infection (15 September–3 October 2012). Three of these seven stayed in the area for the weekend of 22–23 September 2012, two more spent a holiday from 15 to 29 September 2012, and two worked regularly in this area but lived in other districts.

Disease onset for the first primary outbreak case was 8 October 2012 (calendar week (CW) 41). The outbreak peaked in CW 43 and 44 and ended in CW 52 (Figure 3). Besides the notified cases, the outbreak investigation revealed two persons having experienced HAV infection with an earlier disease onset than the primary outbreak cases. Thus we categorised them as 'early cases'. One was a bakery X employee. This person was not involved in preparing products but sold products in shop A and B and packed products for shop C and D. Henceforward this particular employee will be referred to as 'the salesperson' (although the bakery X shops had several other salespersons), and we will use the pronoun 'they'. Onset of jaundice was early October 2012 (CW 39). The other early case was a close contact with disease onset early September 2012 (CW 36, Figure 3). These two infections only became known to the health authorities in the course of the outbreak investigation. They had not previously been notified to the surveillance system. A second employee of bakery X was notified as a case, but their disease onset occurred at the peak of the outbreak (CW 44).

Case-control study

Of the total 77 primary outbreak cases, we enrolled 50 cases (including six who were not resident in district A or B) in the case–control study together with 52 frequency-matched controls. The median age of enrolled cases was 51 years (range: 18–77 years) and of controls 52 years (range: 27–80 years). The sex distribution was almost equal. A total of 25 of the 50 cases were female, as were 30 (58%) of 52 controls.

Figure 3

Hepatitis A outbreak cases by reported onset of illness per calendar week, northern Germany, 2012 (n=85)

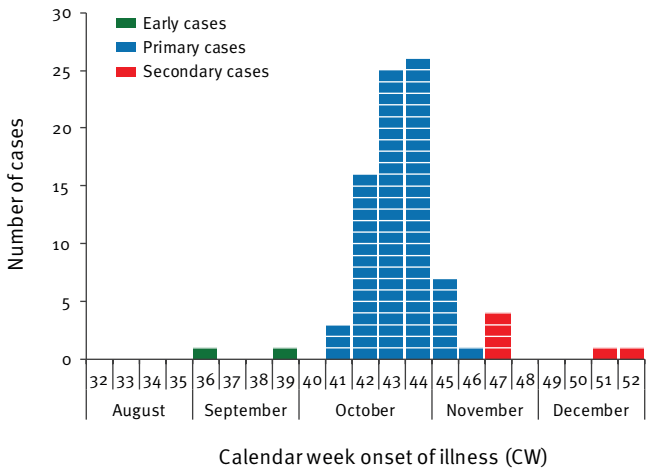


Table 2

Results of univariable analysis of the case–control study among cases and controls, hepatitis A outbreak, northern Germany, 2012 (n=102)

Exposure	Cases (n=50)	Controls (n=52)	OR	95% CI	p-value
Dried tomatoes	5	3	1.80	0.39–9.63	0.458
Mussels	2	4	0.50	0.06–2.97	0.428
Event/fair	23	20	0.93	0.41–2.15	0.879
Frozen berries	8	9	0.91	0.31–2.65	0.865
Bakery X customer	41	31	3.09	1.15–8.86	0.013

OR: odds ratio; CI: confidence intervals.

The univariable analysis revealed a significant association between HAV infection and being a customer of bakery X (Table 2).

A total of 41 of the 50 cases reported eating bakery X products, as did 31 of 52 controls (OR: 3.09; 95% CI: 1.15–8.68; p=0.013). None of the other exposures, which were unrelated to bakery X, was significantly associated with the disease. To find out more about the specific products of bakery X, we performed a nested analysis (Table 3) of cases and controls who were customers of the bakery. Sweet pastries (OR: 5.97; 95% CI: 1.43–34.91; p=0.005), apple cake (OR: 3.86; 95% CI: 0.89–23.18; p=0.043), and Bienenstich (a German cake typically made with a custard, buttercream or cream filling) (OR: 4.68; 95% CI:0.87–46.58; p=0.043) were significantly associated with HAV infection in univariable models. In a multivariable modelling approach adjusted for age and sex and other bakery products, only consumption of sweet pastries remained significant (OR_{adj}: 5.74; 95% CI: 1.46–22.42; p=0.012). Five of six non-residents were among the group of bakery X customers who reported having eaten sweet pastries or cake.

Laboratory diagnostics

Six persons fulfilling the spatial and temporal requirements of the primary outbreak case definition provided specimens for additional testing. In addition, specimens were collected from both the early cases. They all tested positive for recent HAV infection (IgG and IgM positive). HAV RNA in stool specimens was detected for one early case, the salesperson. No HAV RNA was available for the salesperson's contact. The salesperson suffered from an underlying condition that may prolong shedding of the virus. Follow up investigations revealed that the salesperson was shedding a large amount of HAV ($4.5x10_4$ copies/ml) for about six months.

There were 43 other bakery staff members, of whom 14 did not provide specimens (including the HAV positive employee in CW 44). A total of 24 employees tested HAV-antibody negative. Of the remaining five, four had been vaccinated or had a previous HAV infection, results showing them to be IgG positive and IgM negative; the salesperson had an acute HAV infection, as indicated above.

Environmental investigation

Surfaces in all four bakery X shops were examined. Overall, 129 swabs were taken from surfaces in the kitchen, bathrooms and sales spaces. HAV RNA was detected by PCR in four swabs from two bakery shops (A and B, Figure 1) at the following locations:

Table 3

Results of univariable and multivariable analysis of the case–control study among bakery X customers, hepatitis A outbreak, northern Germany, 2012 (n=72)

Univariable analysis					
Exposure (products of bakery X)	Cases exposed to bakery X (n=41)	Controls exposed to bakery X (n=31)	OR	95% CI	p-value
Bread and rolls	35	26	1.12	0.24 - 4.95	0.861
Tarts	6	1	5.14	0.56–244.26	0.106
Apple cake	12	3	3.86	0.89–23.18	0.043
Bienenstich	12	2	4.68	0.87–46.58	0.043
Sweet pastries	16	3	5.97	1.43–34.91	0.005
Multivariable analysis adjusted for age and sex					
Sweet pastries	16	3	5.74	1.46–22.42	0.012

OR: odds ratio; CI: confidence intervals.

- cash desk and refrigerator handle (shop A)
- PC keyboard and door handle of staff toilet (shop B)

Three of the four surface specimens contained enough HAV RNA for molecular sequencing.

Analyses of food items were not conducted because no leftover samples were available.

Molecular biology

The PCR analysis for HAV demonstrated that seven human and three surface specimens contained sufficient HAV RNA to be molecularly sequenced. These 10 specimens were reanalysed using a nested PCR protocol targeting the variable VP1/P2A genomic region (Table 1).

Sequence analysis of the 349 bp amplicons (PCR primer sequences not included) revealed nine isolates (three surface and six human specimens) with identical nucleotide sequences (Figure 4).

For the outbreak isolate (accession number HF677201), the closest matches in the EMBL viral sequence database (accessed April 2013) were strain TunS4–01, isolated in 2001 in Tunisia (acc. AY875664) and strain number 148, isolated in 2007 in Germany (acc. EU416238), both with 98.9% identity. Phylogenetic analysis allowed the two novel isolates to be classified as HAV genotype I, subgenotype IA (Figure 4). Genotype and subgenotype nomenclatures were applied according to a previously proposed classification scheme [26].

One of the seven human isolates (HSCK100034 (acc. HF677202)), differs significantly from the sequence with 21 mismatches, translating into 94% similarity (Figure 5).

Consequently, this person was not regarded as a case belonging to this outbreak. They were a sporadic visitor to the B district, had not visited to the narrower outbreak region, and had not consumed products from bakery X.

Outbreak management

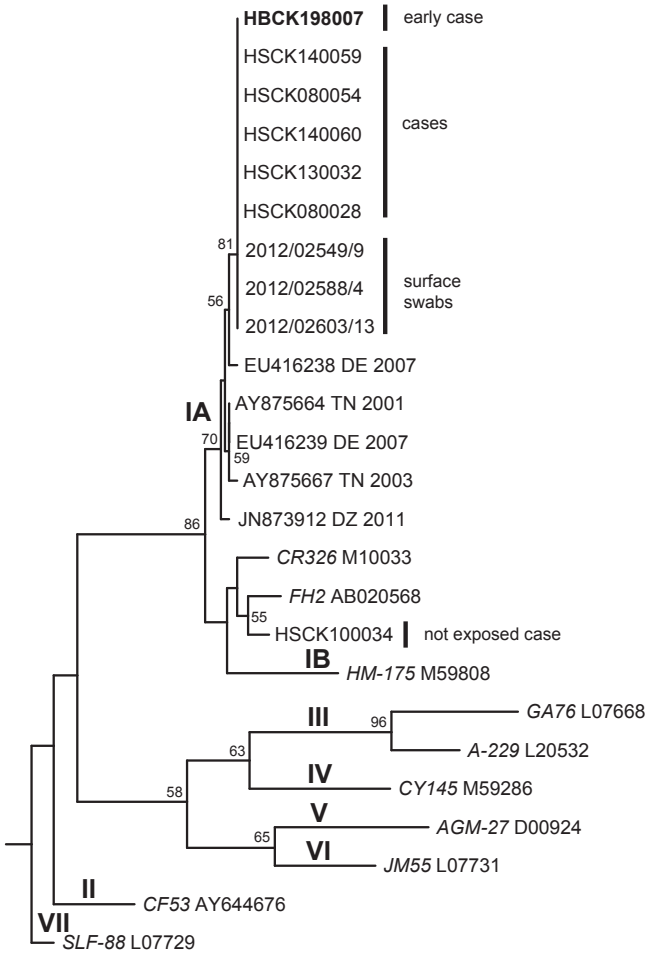
The local health authorities began management of the outbreak immediately after the first notifications. The most important measures to control the outbreak were the vaccination of all susceptible bakery X employees and cases' household contacts, and the cancellation of a fair that was to be held in the most affected village. In addition, the above-mentioned laboratory testing of bakery X staff and surface sampling in bakery X were part of the control strategy, as they facilitate the exclusion of infectious staff members from work and detect contaminated surfaces in food processing facilities.

Discussion and conclusions

We described a point-source HA outbreak in northern Germany with 83 laboratory-confirmed cases who developed symptoms between 8 October and 27 December 2012. This is the largest recorded autochthonous HA outbreak since the start of standardised surveillance in Germany in 2001. By means of the case–control study, we found a significant association between HAV infections and being a customer of bakery X. In particular, consuming sweet pastries was identified as a significant risk factor. This epidemiological evidence is supported by the strong laboratory evidence of the molecular identity of HAV sequences of

Figure 4

Rooted maximum likelihood phylogenetic consensus tree for VP1/P2A nucleotide sequences of selected hepatitis A virus isolates, hepatitis A outbreak, northern Germany, 2012



DE: Germany; TN: Tunisia; DZ: Algeria.

The sequences of the outbreak related strain cluster in Hepatitis A virus (HAV) subgenotype IA. The early case is shown in bold. The selected sequences represent the nearest homologs in GenBank and typical members of genotype I-VII (27).Genotype VII was used as an outgroup. Numbers at the nodes indicate bootstrap values of greater than 50%. Sequences are denoted by GenBank ID, isolate name (reference strains in italic), International Organization for Standardization (ISO) country code and year of isolation. Sequence data from this paper have been deposited with the European Nucleotide Archive under Accession Nos. HF677201–HF677202.

specimens from cases, the bakery X salesperson and surfaces inside bakery X shops. In addition, sequencing of non-residential cases confirmed their relation to the outbreak.

Onset of cases' symptoms and travel data from non-residential cases indicate that the period of infection may have lasted from 15 September to 3 October 2012, but definitely covered the weekend of 22–23 September 2012. In the telephone interviews, all five visitors with sequence-identical isolates reported having eaten sweet pastries or cake (apple cake or Bienenstich) from bakery X. Thus, epidemiological and laboratory results suggest that contaminated bakery X products were vehicles of infection.

It was not possible to test bakery X products for containing HAV. There were no leftover samples from the period of infection due to the short shelf life of bakery products and the long incubation period of HA.

Considering the time of the salesperson's disease onset and their work schedule, they might have worked while being infectious and contaminated baked goods and surfaces. A potential reason for sweet pastries being the vehicle of transmission could be the fact that those often are coated with fatty substances that help the virus to persist on the surface and maybe as well help to weather the acidic milieu of the stomach. Furthermore, inactivation of HAV requires heating foods to›85 degrees Celsius for at least one minute and sweet pastries are usually not fully baked or have unheated fillings or toppings.

Since the salesperson was suffering from a chronic disease and was treated with cortisone, the secretion of virus in their stool might have started at very high titres one to two weeks prior to their onset of illness, and continued for several months at lower titres after their jaundice occurred. One month after onset of jaundice, the titre was still at a high level ($4.5x10_4$ copies/ml).

Figure 5

Alignment of hepatitis A virus VP1/P2A nucleotide sequences, hepatitis A outbreak, northern Germany, 2012

```
HBCK198007     1 CAATCATTCT GATGAATATT TGTCCTTTAG CTGTTACTTG TCTGTTACAG 50
HSCK100034     1 .......... .......... .......... T.....T... .....C.... 50

HBCK198007    51 AACAATCAGA GTTTTATTTT CCTAGAGCTC CATTGAATTC AAATGCTATG 100
HSCK100034    51 ....G..... ...C...... .......... ....A..... .......... 100

HBCK198007   101 TTGTCCACTG AGTCTATGAT GAGTAGAATT GCAGCTGGAG ACTTGGAGTC 150
HSCK100034   101 .......... .......... .......... .......... .T........ 150

HBCK198007   151 ATCAGTGGAT GATCCTAGAT CAGAGGAGGA CAGGAGATTT GAGAGTCATA 200
HSCK100034   151 ...G...... .......... .......... ...A...... .......... 200

HBCK198007   201 TAGAATGTAG AAAACCATAC AAGGAATTGA GATTGGAGGT TGGGAAACAA 250
HSCK100034   201 .......... G......... ..A....... ....A..... ...C...... 250

HBCK198007   251 AGACTTAAAT ATGCTCAGGA AGAGTTGTCA AATGAAGTGC TTCCACCTCC 300
HSCK100034   251 ..G..C.... .......... .......... .......... .......... 300

HBCK198007   301 TAGGAAAATG AAAGGGGTTT TTTCCCAGGC TAAAATTTCT CTTTTTTAT 349
HSCK100034   301 .......... ..G...C.A. ....A..A.. ...G...... ......... 349
```

The upper sequence was derived from the presumptive index case (HBCK198007, salesperson) and represents further eight identical sequences from five cases (serum specimen) and three surface specimens collected in the bakery X. The sequence from one notified non-residential case differs significantly (HSCK100034, 21 mismatches, 94% similarity).

It remains unclear where the infected salesperson and their close contact acquired their infections. However, we learned that the salesperson had packed and sold bakery products one to two weeks prior to the onset of jaundice, which correlates with the time period the visitors reported eating bakery X products. The epidemiological results may raise questions about how nine of the cases might have been exposed as they were not costumers of bakery X. In fact, further investigation of these cases revealed that it was very likely that they all had consumed bakery X products, for example when visiting the homes of other bakery X customers. However, as we did not perform these extensive additional investigations for controls in the same way, we did not include this information into the case-control study's analysis.

The laboratory investigation showed that HAV-positive human and surface specimens had identical RNA sequences, while one case differed. Therefore, we excluded this case from the case–control study and the outbreak. This shows the importance of accomplishing outbreak investigation by sequence-based molecular methods which can verify or disprove an assumed epidemiological link.

As a consequence of prompt outbreak management, the cooperation of all involved parties and follow-up with the cases by the regional health authority, only six secondary cases occurred and the outbreak was interrupted.

There were several limitations. Based on the long incubation period of HAV, a recall bias regarding the answers of consumed bakery items may have occurred. And, considering the age distribution and knowing that children have more often asymptomatic HAV infection [27], we assume that we did not identify every case.

Recommendations

Nationwide, two previously-reported HA outbreaks associated with baked goods were documented [13, 28]. In both, contamination of filled or glazed pastries by a baker was the probable source of HAV infection. Both of these conclusions were based on epidemiological evidence alone. In our study, we were able to sequence the HAV strains of the salesperson, primary cases and environmental specimens. It may be possible to avoid similar outbreaks in the future by addressing the potential spread of HAV through food handlers and educating food handlers as well as their employer to recognise hepatitis A symptoms and to be aware that HAV has a long incubation period.

Considering the high tenacity of HAV, we strongly recommend the supervised use of disinfectants with virucidal activity against HAV and appropriate application of virucidal disinfection in outbreak situations.

For all food handlers, we recommend wearing plastic gloves while handling baked and unbaked products provided that correct handling is assured (e.g. changing gloves at the recommended intervals).

In view of the availability of an effective HAV vaccine [29] and several HA outbreaks with food handlers being the probable source, the evidence for the role of food handlers in HAV transmission and disease burden in Germany should be reviewed to inform a decision for a vaccination recommendation targeting food handlers.

Future risk assessment should determine whether food handlers with chronic diseases requiring immunosuppressive treatment could be more at risk of contaminating food and might therefore benefit from HA immunisation.

Acknowledgements

We sincerely thank colleagues from the local public health departments for collecting case data and sending in blood and stool specimens. Our thanks also go to all patients and controls for participating in the interviews. Finally, a warm thank you to Sandra Heidrich, Ina Holle and Nicola Jahn for conducting interviews, developing the database and entering the data.

Conflicts of interest

None

Authors' contributions

Author contribution for Foodborne Hepatitis A outbreak associated with bakery products in Northern Germany, 2012.
The work presented here was carried out in collaboration between all authors. Elke Mertens and Manuela Harries provided the surveillance and human exposure data. Johannes Dreesman, Elke Mertens and Manuela Harries designed methods and analysed the epidemiological data. Masyar Monazahian carried out the human laboratory analyses. Martina Weber investigated the environmental (surface) specimens. Joachim Ehlers was part of the response team in the veterinary section and provided the reinforcement of food control administration data. Jürgen J. Wenzel and Wolfgang Jilg carried out the molecular genetic studies of human and surface specimens including the sequence alignment. Elke Mertens and Manuela Harries interpreted the results and wrote the manuscript. All authors read and critically revised the first as well as the subsequent and final drafts of this manuscript.

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Innovative applications of immunisation registration information systems: example of improved measles control in Taiwan

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Immunisation registry systems have been shown to be important for finding pockets of under-immunised individuals and for increasing vaccination coverage. The National Immunisation Information System (NIIS) was established in 2003 in Taiwan. In this perspective, we present the construction of the NIIS and two innovative applications, which were implemented in 2009, which link the NIIS with other databases for better control of measles. Firstly, by linking the NIIS with hospital administrative records, we are able to follow up contacts of measles cases in a timely manner to provide the necessary prophylaxis, such as immunoglobulin or vaccines. Since 2009, there have been no measles outbreaks in hospitals in Taiwan. Secondly, by linking the NIIS with an immigration database, we are able to ensure that young citizens under the age of five years entering Taiwan from abroad become fully vaccinated. Since 2009, the measles-mumps-rubella vaccine coverage rate at two years of age has increased from 96% to 98%. We consider these applications of the NIIS to be effective mechanisms for improving the performance of infectious disease control in Taiwan. The experience gained could provide a valuable example for other countries.

Introduction

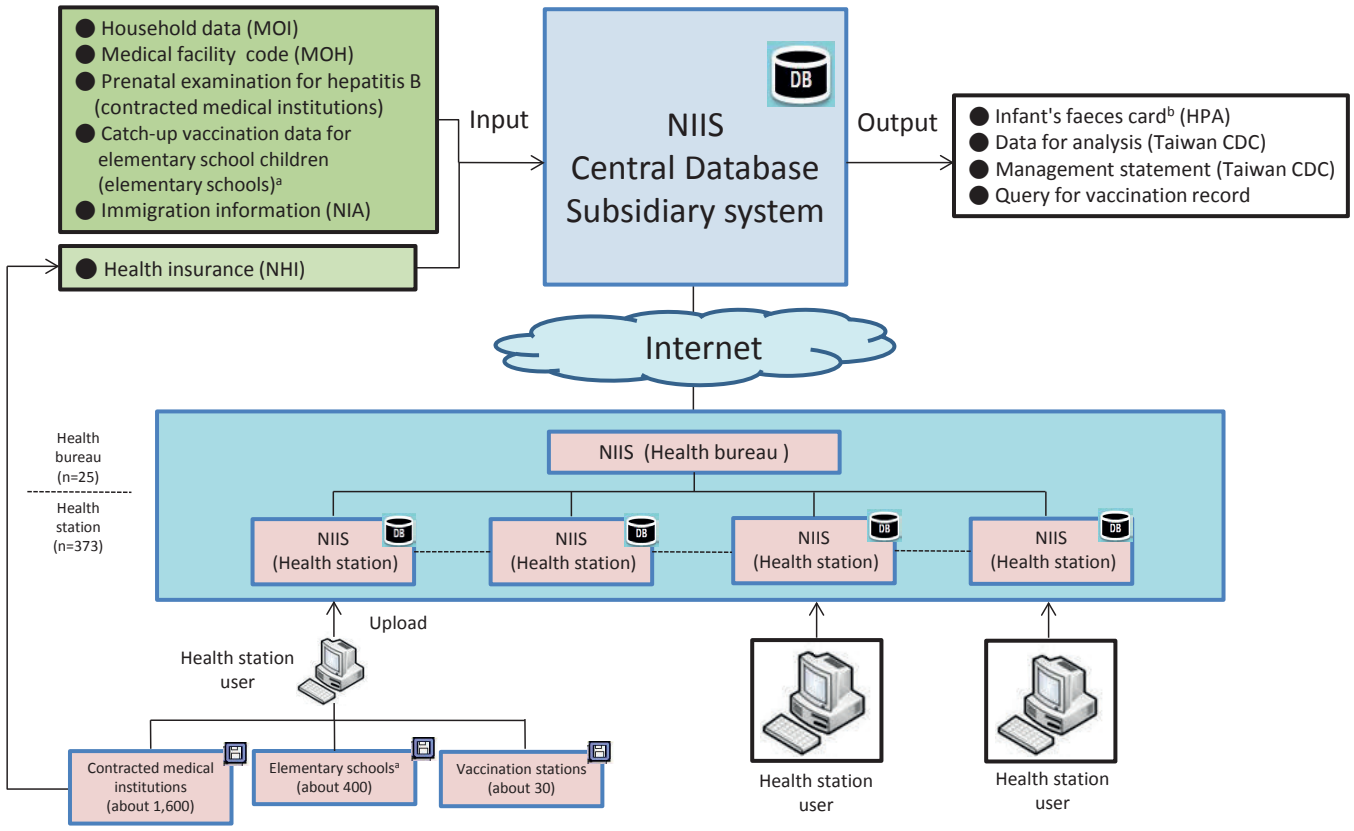
Immunisation is the most effective and efficient way to prevent vaccine-preventable diseases. However, the appropriate coverage rate is still critical for a comprehensive immunisation programme, especially for highly contagious diseases such as measles. The World Health Organization (WHO) recommends that countries should achieve coverage greater than 95% for a two-dose measles-containing vaccine in every district to eliminate measles [1]. The WHO European Region has set 2015 as the target for interrupting transmission of measles and rubella [2]; however, according to the *2014 Assessment report of the global vaccine action plans* [3], among the three WHO regions aiming for measles elimination by the end of 2015, the Eastern Mediterranean Region and the European Region 'are markedly off track'. The WHO Western Pacific Region has made the most progress, but outbreaks in China, the Philippines and Vietnam in 2013 proved a setback. National coverage is above 95% in many countries in the three WHO regions, but 'just one weak district is enough for this highly contagious virus to continue circulating' [3].

In many countries, comprehensive immunisation registry information systems are well established and have proved helpful in identifying pockets of under-immunised individuals and improving vaccination coverage [4]. This perspective aims to present innovative applications of the system used for measles prevention and control in Taiwan and to share the preliminary experiences. New concepts of immunisation database linkage could help to address the weakness in measles control in mobile populations and within medical settings, even in countries with comprehensive vaccination polices.

Taiwan is well known for its information technology industry, and technology advances have been readily adopted in health management for over 20 years. The Primary Health Information System (PHIS) was first established for public health stations in every township in the 1990s. The vaccination registry system was developed independently and was extended to a web-based system to fulfil the demand to keep up with the mobile population of Taiwan. The planning and construction of a new registry system, the National Immunisation Information System (NIIS), started in 2001. Since the end of 2003, the system has been operating online, running at all public health bureaus in 25 counties/cities and public health stations in all 373 townships. It took a year to train the users and to extend its use from government public health services to all of the 1,600 contract clinics and hospitals, covering all service points nationwide. Since 2005, all of the local health agencies have been participating in the

Figure 1

Structure and information flow of the National Immunisation Information System in Taiwan



CDC: Centers for Disease Control; HPA: Health Protection Administration; MOH: Ministry of Health and Welfare; MOI: Ministry of the Interior; NHI: National Health Insurance Administration; NIA: National Immigration Agency; NIIS: National Immunisation Information System.

[a] Elementary schools: for children 6 to 11 years of age.

[b] Infant's faeces card: NIIS has a function reminding healthcare workers to check and record stool colour of infants for early diagnosis of biliary atresia.

NIIS and upload complete information to a central database. As several imported measles cases and clusters occurred from the end of 2008 to spring 2009 [5], NIIS data linkage was considered in 2009 to help to control the disease.

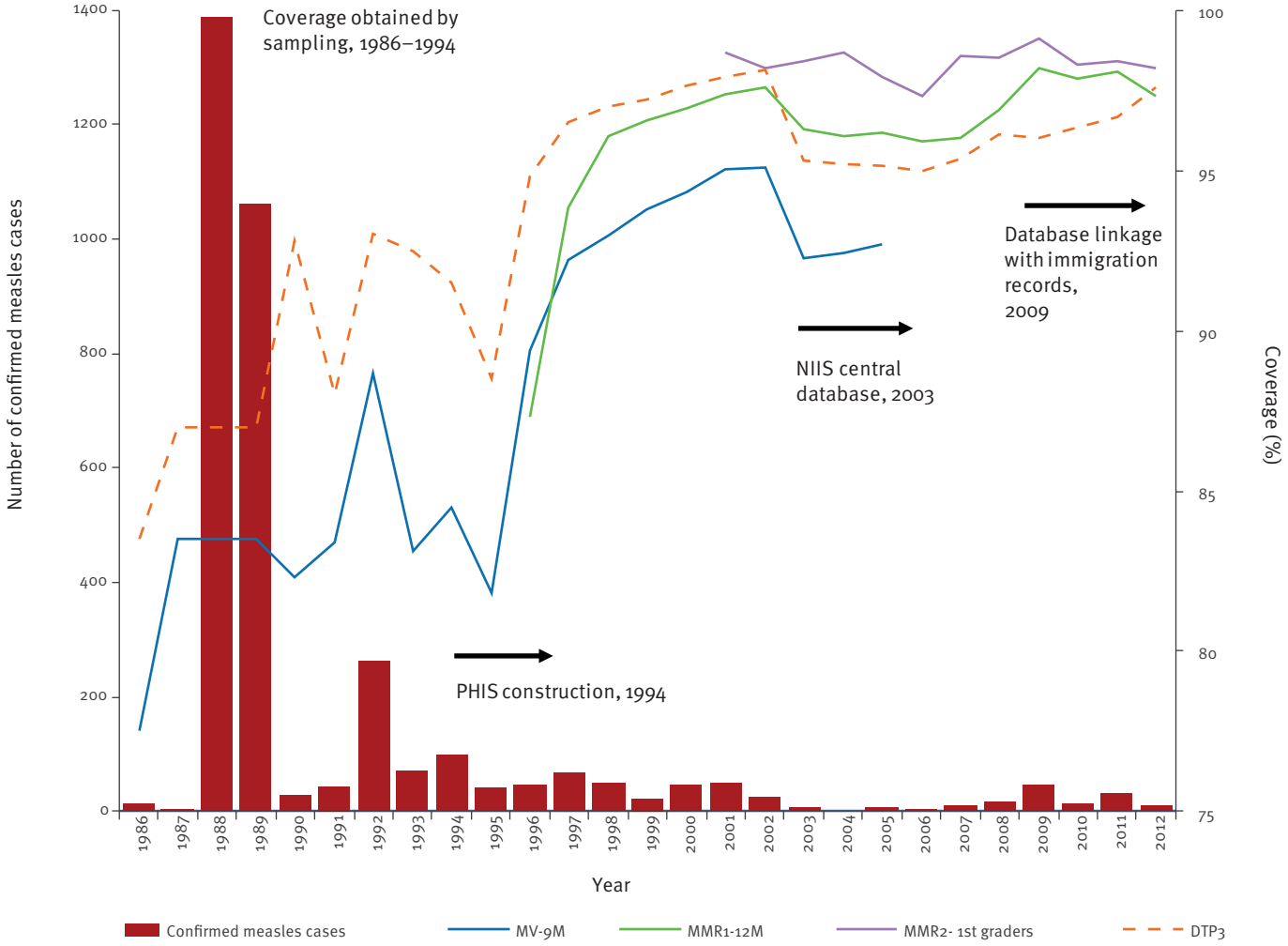
The National Immunisation Information System

The NIIS contains most of the information for immunisation affairs, including an immunisation registry, vaccine management, lists of unvaccinated children, reports of coverage rates and related statistics. For example, once a baby is born in Taiwan, hepatitis B vaccine is administered within 24 hours and the baby is registered in an interim NIIS account by the clinic or hospital. Hepatitis B carriage status at the prenatal examination is also recorded in the system. By matching the baby with the mother's ID, hepatitis B immunoglobulin can be provided in a timely manner to high-risk newborns. After three to four weeks, when the baby gets its ID number, a permanent account is generated to replace the interim one in the NIIS, and the parent or guardian's name and address are updated by transferring data from the Ministry of the Interior's National Household Information System every 24 hours. Through this mechanism, the NIIS database has been able to cover the whole population born after 1995 in Taiwan. More detailed contact information is provided by the parents or guardians voluntarily and is recorded in the NIIS, including mobile telephone numbers in over 50% of cases. All of the subsequent vaccination doses for the baby are recorded in the ID account and compiled through the data exchange between the NIIS local and central databases. This mechanism allows children to be vaccinated anywhere in the country and helps to ensures that all the immunisations will not be missed or repeated. In addition, related information such as vaccination dose, place, time and lot number is also required on the data entry sheet: NIIS records are also critical for vaccine safety investigation. The structure of the NIIS is demonstrated in Figure 1.

Before computerisation, it was impossible to calculate immunisation coverage rates precisely using paper records. From 1986 to 1994, the Taiwan national vaccination coverage rates were estimated through surveys for

Figure 2

Measles-containing vaccine coverage, compared with coverage of the third dose of diphtheria-tetanus-pertussis vaccine, and number of measles cases (n=3,467), Taiwan, 1986–2012



DTP3: third dose of diphtheria-tetanus-pertussis vaccine at six months of age (since 1955); MMR: measles-mumps-rubella; MMR1-12M: first dose of MMR vaccine at 12 months of age (since 1992; national coverage data available since 1996); MMR2-first graders: second dose of MMR vaccine for children aged six to seven years old (since 2001); MV-9M: measles-containing vaccine scheduled to be vaccinated at nine months of age (from 1978 to 2005; national coverage data available since 1986); NIIS: National Immunisation Information System; PHIS: Primary Health Information System.

sampled counties every one to three years. As the PHIS was being constructed, the coverage for each administration area became available. After the construction of the NIIS, local records could be integrated and updated for every individual; all of the PHIS records since 1995 were compiled into the NIIS central database. Since then, public health workers have been able to follow up on unvaccinated individuals easily, and the coverage rates for measles-containing vaccines and the third dose of diphtheria-tetanus-pertussis vaccine (DTP3) have been increasing and maintained at over 95% since 2001 (Figure 2). NIIS data show that the coverage rates for other childhood vaccinations have all reached and been maintained at≥95% for the primary doses, so higher standards for timeliness and completeness have also been set by the Taiwan Centers for Disease Control (CDC). For example, in 2012 in Taiwan, measles-mumps-rubella (MMR) vaccine coverage by 15 months of age (scheduled to be vaccinated at 12 months-old) was 88.8%. Coverage of MMR vaccination at 12 months is usually calculated when the child is aged 18 or 24 months. Checking vaccination status at 15 months of age will protect children earlier and decrease the number of post-vaccination seizures, but a lower vaccination rate at this time point is also expected. As the time available to trace unvaccinated children and complete their vaccination is shorter (as checked at 15 months of age), coverage of 88.8% is considered good. Full immunisation in children at two years of age (i.e. they have received one dose of Bacillus Calmette–Guérin (BCG) vaccine, varicella vaccine and MMR vaccine, two doses of Japanese encephalitis vaccine, three doses of hepatitis B vaccine, four doses of DTP-*Haemophilus influenzae* type b-inactivated polio vaccine) was 94.1%. The index 'full immunisation coverage' is much more stringent than coverage for an individual vaccine, as a child

Table

Follow-up activities for contacts (aged six years or less) of measles cases who presented at hospital, Taiwan, 2009–2012 (n=640)

Hospital	Month, year	Contacts traced[a]							
		Under one year of age				1–6 years of age			
		Number	Followed up	IMIG administration[b]	Infected[c]	Number who had not received MCV	Followed up	Vaccinated	Infected[c]
A	Feb 2009	13	13	5	1	10	10	9	1
B	Mar 2009	264	264	43	0	85	85	64	0
C	Mar 2009	88	88	2	2	24	23	22	0
D	May 2009	42	42	22	1	14	14	13	0
E	Jul 2010	12	12	6	0	37	37	36	0
F	Jun 2011	49	49	36	0	2	2	2	0
Total	–	**468**	**468**	**114**	**4**	**172**	**171**	**146**	**1**

IMIG: intramuscular immunoglobulin; MCV: measles-containing vaccine.

[a] Once the exposure has been reported, the contacts should be identified and followed up with within 48 hours. Follow-up activities include checking for symptoms and signs, evaluation for providing IMIG or vaccination, and health education.

[b] IMIG administered was within six days after the exposure in contacts under one year of age for whom it was determined to be necessary.

[c] None of the five contacts infected received post-exposure prophylaxis.

has to receive all the recommended doses; coverage of over 90% for children aged two years is not easy to obtain.

Two innovative applications for measles prevention and control that link the NIIS with other data sources are described below.

Application 1: linking the National Immunisation Information System with hospital administrative records to identify and prevent potential measles transmission within hospitals

Medical facilities have been considered high-risk settings for measles transmission [6,7]. In Taiwan, there were three measles outbreaks resulting in 22 cases from five hospitals across Taiwan from November 2008 to February 2009 [5]. To address within-hospital measles transmission, the Taiwan CDC requires hospitals to provide them with a list with names of possible contacts when a measles case is identified. A possible measles contact is defined as any person present in the same room or ward 30 minutes before or two hours after an infectious measles patient (four days before or after rash onset [8]) stayed there. With a universal health insurance scheme, relatively low co-payment for visiting a doctor and completely free choice of healthcare providers, however, 'doctor shopping' is frequent in Taiwan [9]; consequently, a person with measles may have visited several clinics or hospitals before being diagnosed. Public health workers thus face difficulties to identify large numbers of contacts in healthcare facilities and to follow them up in time.

Since February 2009, all contacts of measles cases who presented to a hospital have been identified via the hospital's computerised health information system. The electronic files of the names of the contacts, with their ID numbers are put into the NIIS, to check the contacts' vaccination history. Children under six years-old have been considered a high-risk group: the first priority is those under one year of age, who are evaluated for administration of intramuscular immunoglobulin (IMIG). Parents or guardians of children from one to six years-old without complete vaccinations are contacted immediately and the children are vaccinated as soon as possible. Other contacts (or their parents or guardians, if the contact is a child older than six years) will receive a letter informing them of the exposure and giving them recommended self-health management advice.

As it takes only five minutes to check the vaccination records of hundreds of contacts using an NIIS batch-screening function, public health workers can identify high-risk contacts and take the necessary actions in a timely manner. From February 2009 to 2012, there were six occurrences of large-scale measles exposure in Taiwanese hospitals, with between 556 and 1,266 contacts identified per case. After screening the contacts' vaccination history through the NIIS, almost all (639/640) of those who were susceptible to measles completed follow-up (Table). Through linkage with the NIIS database and with a rapid response, 146 individuals were vaccinated within three days after exposure. Among those contacts under one year of age (n=468), 114 were considered to have required post-exposure prophylaxis and IMIG was provided within six days after exposure. As measles cases are infectious four days before rash onset [8], five contacts were exposed to three cases during the infectious period and were identified too late after exposure to benefit from prophylaxis and subsequently developed measles. Nevertheless, all the contacts who received post-exposure prophylaxis remained free of symptoms and there

has been no measles outbreak in hospitals since the intervention in 2009.

To maintain the achievement of measles elimination in the WHO Region of the Americas, high standards of case investigation and containment measures have cost vast resources in the United States [7,10]. The experience in Taiwan presented here shows that by batch-screening vaccination histories, public health workers were able to identify high-risk hospital contacts quickly and take the necessary actions accordingly, saving time and manpower in checking vaccination status, and enabling them to trace contacts at low risk.

Application 2: linking the National Immunisation Information System with immigration records to monitor children entering Taiwan without measles vaccination

During 2007 to 2009, eight of 18 measles cases imported to Taiwan were incompletely vaccinated children returning from travel abroad with their parents (unpublished data). Such travel may lead to missed vaccination opportunities according to the Taiwanese vaccination schedule. For example, from 1998 to 2012, 10% of the babies in Taiwan were born to mothers from other countries [11]: these mothers may have travelled with their babies to visit their homeland within one year after the birth. In addition, measles case investigation reports and feedback from public health workers involved in tracing unvaccinated children showed that increasing numbers of families travelled back and forth between mainland China and Taiwan for work during 2007 and 2009.

Given the above, the Taiwan CDC called for assistance from the National Immigration Agency. Since February 2009, immigration records have been provided to the Taiwan CDC on a daily basis and have been linked with the NIIS database. Any child in the NIIS under five years of age with no record of MMR vaccination in Taiwan is identified, and public health workers attempt to contact the parents or family members as soon as possible. The records are completed if the child has already been vaccinated abroad, or MMR vaccine is offered immediately.

From February 2009 to May 2010, a total of 206,367 children entered Taiwan [12]; among them 9,961 were without measles vaccination and were identified via the data-linking process. Follow-up was successfully carried out for 5,185 (52%) of them. Among these children, 3,114 were vaccinated according to Taiwan's immunisation schedule as soon as possible; the others (n=2,071) presented certificates of vaccination from abroad and their NIIS records were updated. In 2012, a total of 4,975 children entering Taiwan without MMR vaccination were identified, and 2,627 (53%) were successfully followed up. The reasons some children could not be followed up included travelling abroad again, no longer living at the known address, no one answered when called by telephone or visited, or the telephone number or address were unavailable. Because of the implementation of this project, the coverage rate of the first dose of MMR vaccine for children aged two years since 2009 has increased from 96% to roughly 98%. As second-dose vaccination (recommended to be administered at six years of age) is a school-based programme and elementary school (for 6–11 year-olds) attendance rate in Taiwan is high – for example, 99.52% for the 2013 school year [13] – coverage of the second dose of MMR vaccine is over 97%; thus, the NIIS has only a limited effect on it. Since 2012, when other vaccines were also included for follow-up using this NIIS data linkage mechanism, the coverage of DTP3 increased to 98% (from 95% in 2006) (Figure 2).

Although there are very effective and safe vaccines to control measles, the coverage of measles-containing vaccines is still a key issue, even in industrialised countries [14]. In addition, industrialised countries are at increasing risk because of migration, air travel and working personnel exchange due to global economic mobility. Inconsistencies between vaccination rates and measles incidence have been found in some instances [15,16] and supplementary immunisation activity is not always as effective as is expected [17]; all of these issues could be a result of unreliable coverage calculation, repeated vaccinations or recordings, and, ultimately, failure to reach vulnerable populations, for example, mobile populations and abused children. A comprehensive registry information system could help to find any weaknesses regarding vaccination and thus help in trying to solve them. Optimal methods for estimating vaccination coverage and additional strategies for achieving high coverage in migrant, nomadic and displaced populations have been identified as research priorities for global measles and rubella control and eradication [18].

Experiences and challenges

The development of a new information system under the public health administration was not an easy task. In the beginning, the construction of Taiwan's NIIS experienced resistance from users in local health agencies because they had been accustomed to working only with paper documents for many years. To understand users' opinions and the system's effectiveness, the Taiwan CDC conducted two comprehensive surveys, at the end of 2003 and 2004. The surveys indicated that users complained about the change at first but that satisfaction rates improved greatly a year after the project was implemented [19]. With the recognition that children could move around and get vaccinated in any part of Taiwan and that a centralised database was needed, all of the local health agencies finally agreed to participate in the NIIS in 2005.

Despite the support for the information system, immunisation programs still rely on the performance of public health workers and clinicians. Unfortunately, compared with medical care services (24/56 practicing doctors/nurses per 10,000 population in Taiwan

in 2010 [20]; 32/88 respectively per 10,000 population for member countries of the Organisation for Economic Co-operation and Development in 2011 [21]), the resources allocated for public health in Taiwan are relatively insufficient. In 2013, there were only 3,429 first-line public health workers at local health bureaus and stations, i.e. 1.5 public health workers per 10,000 population (unpublished data). The NIIS has provided an efficient way to reduce interface costs and provide greater consistency in data exchange. We also noticed that in both of the examples presented in this article, intersectoral cooperation was essential for both the public (immigration or household registry agencies) and private (medical facilities participating in the NIIS) sectors. In addition to comprehensive legislation that gave the authority for disease control policies to be enforced, awareness of the benefits of immunisation and commitment to better disease control play key roles in the success of these extended applications of the NIIS.

The power of linking databases with immunisation information systems has been shown [22-24], mostly involving links with health records. Linking a hospital's information concerning measles contacts to the NIIS can help to prevent secondary transmission at a hospital. To the best of our knowledge, linking an immunisation information system with immigration records is a new concept. For infectious diseases, particularly in the context of rapid and extensive world travel, there is a need for systematic, concerted cooperation among government agencies. Moreover, unvaccinated children are usually at high risk and may be difficult to reach. Linking the NIIS with the immigration database facilitated successful follow-up of over 50% of the target population. The other 50% were mobile, either people who had left Taiwan again or whose contact information was unavailable or incorrect. With better collaboration among health, social and educational departments, an even higher percentage of follow-up may be attainable. By using innovative applications of immunisation information systems, it is possible to achieve over 95% vaccine coverage and timely case investigation and intervention.

One issue concerning the application of immunisation information systems is the protection of personal information; however, regional or national immunisation information systems do exist and, as of 2012, were compliant with national data protection laws in 14 countries in the European Union [25]. Examples of using immunisation information systems and other databases with personal details to promote public health are present in Australia, Canada and the United States [22-24]. In Taiwan, the Personal Information Protection Act states that the government agency should use personal information in accordance with the scope of its job functions provided by laws or regulations, and in compliance with the specific purpose of collection [26]. However, the information may be used outside this scope when it is used to prevent harm to life, body, freedom or property and where it is used to prevent harm to the rights and interests of other people. This legislative process was not developed specifically for NIIS linkage and parents do not have to give explicit permission for data linkage. With a rigorous policy review and compliance with national data protection laws in Taiwan, public trust in data collection and linking of databases with the NIIS has been maintained and public health protected and promoted. With the necessary protection of personal information, the experience of Taiwan's immunisation information system and its application could be valuable for other countries now that international travel and workforce exchanges have become commonplace.

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Conflict of interest

None declared.

Authors' contributions

Ding-Ping Liu, En-Tzu Wang and Yi-Hsin Pan were involved in collection of data and information. Ding-Ping Liu drafted the manuscript, which was reviewed by Shou-Hsia Cheng.

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Letter to the editor: Management of patients with Ebola virus disease in Europe: high-level isolation units should have a key role

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To the editor:

We read with interest the article by de Jong and colleagues, who provide an initial insight into European hospital preparedness level for the admission of a patient with Ebola virus disease (EVD) [1].

In the past, the rare imported cases of Ebola and Marburg in western European countries and the United States were managed in high-level isolation units (HLIUs) [2]. Subsequently, reported experiences indicate that strict contact-droplet isolation is enough for preventing transmission. From this hypothesis, the idea may derive that HLIUs are not strictly necessary for the management of EVD patients, who may be safely managed in non-specialised hospitals, as suggested by some international recommendations elaborated during the current Ebola outbreak in West Africa [3,4]. Even if we concur that strict contact-droplet isolation is enough to prevent transmission during routine care, we believe that HLIUs should have a key role in EVD containment in countries where such facilities are available. An HLIU is a healthcare facility specifically designed to provide safe, secure, high-quality, and appropriate care, with optimal infection containment and infection prevention and control procedures, for a single patient or a small number of patients who have, or who may have, a highly infectious disease [5].

In hospitals, breaches in infection control may occur; many healthcare associated infections could be prevented by standard precautions and contact isolation measures, but despite this, they continue to hit thousands of patients and to increase health-related costs [6]; measures for preventing needlestick and sharp injuries are well-known, but many of these accidents occur every day; hand hygiene alone may prevent many infections, but this simple procedure is often poorly applied [6]. We believe that such breaches are not acceptable when managing a disease with 50% of case fatality rate such as EVD. Data from de Jong and colleagues, reporting that practical exercises have been performed in 28.4% of responding hospitals only [1], as well as the secondary transmissions that occurred in Spain and the United States, reinforce this position.

Indeed, establishing precautions is not equal to their adherence. Well-trained staff, awareness about personal protective equipment and other infection control procedures, continuous practice, appropriate supervision, and adequate logistics are needed; in other words, an established 'infection control culture and practice'. Moreover, rooms with special technical air-handling features are necessary for aerosol-producing procedures [7].

We believe that this unique combination of technical and logistic equipment, well-trained and experienced staff, and long-term established and updated procedures, is available within HLIUs only, thereby representing the safest place to manage EVD.

In Europe, an assessment of isolation capabilities for the management of highly infectious diseases was performed in 2009–2010 within the European Network for Infectious Diseases/European Network for Highly Infectious Diseases (EUNID/EuroNHID) projects coordinated by the National Institute for Infectious Diseases 'Lazzaro Spallanzani' in Italy [8]. The EuroNHID Consortium currently includes 47 isolation facilities identified by the national health authorities as referral centres for highly infectious diseases (including EVD), in 20 countries (Austria, Bulgaria, Belgium, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Malta, Norway, Poland, Portugal, Spain, Slovenia, Sweden and the United Kingdom). The survey results are being updated in 2014: complete data are available from 12 countries; from the remaining eight countries, partial data are available. According to currently available data, among

the 47 isolation facilities 17 HLIUs are present in nine European countries, with at least 92 beds available, 57 of which with intensive care capacity. Additional capacity may be present in other countries not participating to EuroNHID Consortium. This bed capacity (not expected to change significantly after the collection of pending data) is surely enough to effectively manage Ebola patients in Europe, in the current epidemiological situation.

In conclusion, we strongly believe that HLIUs should play a crucial role in management of patients, and preparedness plans should include referral of EVD patients to these facilities as early as possible.

Other members of EuroNHID Consortium are:

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Conflict of interest

None declared.

Authors' contribution

All authors equally contributed to manuscript concept and writing. All authors gave their final approval to the manuscript contents.

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Authors' reply: Management of patients with Ebola virus disease in Europe: high-level isolation units should have a key role

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Ippolito and colleagues suggest a key role of high-level isolation units (HLIUs) for patient management and containment of Ebola virus disease (EVD) in Europe [1]. In principle, we do agree with this notion, particularly in relation to repatriated or evacuated patients with confirmed EVD. However, realities are (i) that patients with (suspected) EVD who are in need of care may present at any hospital anywhere in Europe, and (ii) that the number and geographic distribution of HLIUs are limited which pose difficulties particularly in the unlikely event of multiple introductions or spread of EVD (or other highly infectious diseases) in Europe. For these reasons, preparedness for admission of suspected patients or procedures for transfer of such patients to other hospitals are essential, and this is what we sought to assess in our survey [2].

As noted by Ippolito et al., practical exercises of preparedness are important and were performed in only 28% of hospitals overall at the time of the survey. However, somewhat reassuringly, it should be noted that this percentage was substantially higher in hospitals that would admit suspected patients (46%). Also, it should be noted that this survey was initiated less than three weeks after the World Health Organization's Public Health Emergency of International Concern (PHEIC) declaration [3], and that preparedness activities, including exercises, will likely have intensified since then.

In conclusion, efforts to identify and address gaps in preparedness of European hospitals are essential to assess and manage the risk of possible spread of EVD or the next emerging highly infectious disease in Europe. Notwithstanding their importance, reliance solely on HLIUs for containment of EVD or other highly infectious diseases may be unrealistic.

Conflict of interest

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Authors' contribution

MDdJ, MK and HG wrote the letter.

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