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Early 2016/17 vaccine effectiveness estimates against influenza A(H3N2): I-MOVE multicentre case control studies at primary care and hospital levels in Europe

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We measured early 2016/17 season influenza vaccine effectiveness (IVE) against influenza A(H3N2) in Europe using multicentre case control studies at primary care and hospital levels. IVE at primary care level was 44.1%, 46.9% and 23.4% among 0–14, 15–64 and ≥ 65 year-olds, and 25.7% in the influenza vaccination target group. At hospital level, IVE was 2.5%, 7.9% and 2.4% among ≥ 65, 65–79 and ≥ 80 year-olds. As in previous seasons, we observed suboptimal IVE against influenza A(H3N2).

The 2016/17 influenza season in Europe is marked by the predominant circulation of influenza A(H3N2) viruses [1], with significant pressure on hospitals, mostly due to patients aged 65 years and older developing severe disease [1]. Many European countries have reported excess all-cause mortality [2]. Initial estimates based on Swedish and Finnish electronic databases suggest low influenza vaccine effectiveness (IVE) among older adults [3,4]. We measured early IVE at primary care and hospital levels against laboratory-confirmed influenza A(H3N2) in Europe.

Primary care and hospital-based multicentre case control studies in Europe to measure influenza vaccine effectiveness

We conducted separate multicentre primary care and hospital-based case–control studies and analyses using the test-negative design (TND). We have described the methods in detail previously [5-7].

In the primary care study, comprising 893 practitioners (including general practitioners and paediatricians) in 12 countries, we included a systematic sample of all community-dwelling patients presenting to their practitioner with influenza-like illness (ILI), as defined by the European Union ILI case definition (*sudden onset* of symptoms and at least one of the following systemic symptoms: fever or feverishness, malaise, headache, myalgia, and at least one of the following respiratory symptoms: cough, sore throat, shortness of breath). In the hospital study, comprising 27 hospitals from 11 countries, we included community-dwelling patients aged 65 years and older admitted to hospital for influenza-related clinical conditions with symptoms compatible with severe acute respiratory infection (SARI). Each study site adapted a generic protocol to their local setting [8,9].

At each study site, the study period commenced more than 14 days after the start of the vaccination campaign and lasted from the week of the first influenza case to the date of sending data for the interim analysis at the end of January 2017.

A case of confirmed influenza was an ILI (primary care) or SARI (hospital) patient who was swabbed and tested positive for influenza A(H3N2) virus using real-time RT-PCR. Controls were ILI (primary care) or SARI (hospital) patients who tested negative for any influenza virus using RT-PCR.

We excluded patients with contraindications for influenza vaccination, SARI patients discharged from a previous hospital stay within 48 hours of symptom onset (hospital), those with a previous laboratory-confirmed influenza in the season, those refusing to participate or unable to consent, those who had received antiviral drugs before swabbing (primary care), those swabbed more than 7 days after symptom onset, patients with missing laboratory results and any patients positive to any influenza virus other than influenza A(H3N2).

Practitioners and hospital teams collected clinical and epidemiological information including date of symptom onset and date of swabbing, 2016/17 seasonal

Table 1a

Influenza A(H3N2) cases and controls included in the 2016/17 season influenza vaccine effectiveness analysis, I-MOVE/I-MOVE+ multicentre case control studies (primary care (n = 5,023) and hospital (n = 635) levels) Europe, influenza season 2016/17

Variables	Primary care level						Hospital level					
	Number of A(H3N2) n = 2,250			Number of controls n = 2,773			Number of A(H3N2) n = 267			Number of controls n = 368		
	n	Total	%	n	Total	%	n	Total	%	n	Total	%
Median age (years)	29			28			79			80		
Age groups (years)												
0–4	276	2,242	12.3	723	2,766	26.1	NA			NA		
5–14	508	2,242	22.7	336	2,766	12.1	NA			NA		
15–64	1,177	2,242	52.5	1,438	2,766	52.0	NA			NA		
65–79	234	2,242	10.4	214	2,766	7.7	138	267	51.7	185	368	50.3
≥ 80	47	2,242	2.1	55	2,766	2.0	129	267	48.3	183	368	49.7
Missing	8			7			0			0		
Sex												
Female	1,126	2,237	50.3	1,407	2,758	51.0	141	267	52.8	190	368	51.6
Missing	13			15			0			0		
Chronic conditions												
At least one chronic condition	451	2,237	20.2	542	2,743	19.8	237	255	92.9	321	344	93.3
Missing	13			30			12			24		
At least one hospitalisation in the previous 12 months for chronic conditions	26	2,196	1.2	57	2,686	2.1	66	247	26.7	146	334	43.7
Missing	54			87			20			34		
Target group for vaccination												
Belongs to a target group for vaccination	616	2,241	27.5	706	2,755	25.6	267	267	100.0	368	368	100.0
Missing	9			18			0			0		
Swab delay												
Swabbed within 3 days of symptom onset	2,024	2,250	90.0	2,291	2,773	82.6	154	267	57.7	212	368	57.6
Vaccination status												
Seasonal flu vaccination 16–17	231	2,250	10.3	301	2,773	10.9	108	267	40.4	191	368	51.9
Seasonal flu vaccination 15–16	223	2,196	10.2	316	2,665	11.9	117	252	46.4	199	362	55.0
Missing	54			108			15			6		
Previous and current season influenza vaccination												
Not vaccinated in any season	1,929	2,196	87.8	2,284	2,665	85.7	128	252	50.8	147	362	40.6
Current season vaccination only	44	2,196	2.0	65	2,665	2.4	7	252	2.8	16	362	4.4
Previous season vaccination only	43	2,196	2.0	95	2,665	3.6	20	252	7.9	28	362	7.7
Current and previous season vaccination	180	2,196	8.2	221	2,665	8.3	97	252	38.5	171	362	47.2
Missing	54			108			15			6		
Type of vaccine												
Not vaccinated	2019	2,215	91.2	2,472	2,725	90.7	159	261	60.9	177	359	49.3
Inactivated subunit egg	97	2,215	4.4	108	2,725	4.0	65	261	24.9	101	359	28.1
Inactivated split virion egg	71	2,215	3.2	118	2,725	4.3	32	261	12.3	74	359	20.6
Adjuvanted	18	2,215	0.8	21	2,725	0.8	5	261	1.9	7	359	1.9
Quadrivalent vaccine	10	2,215	0.5	6	2,725	0.2	0	261	0.0	0	359	0.0
Missing vaccine type	35			48			6			9		
Month of onset												
October 2016	4	2,250	0.2	84	2,773	3.0	0	267	0.0	0	368	0.0
November 2016	154	2,250	6.8	759	2,773	27.4	3	267	1.1	6	368	1.6
December 2016	1,199	2,250	53.3	1,194	2,773	43.1	174	267	65.2	236	368	64.1
January 2017	893	2,250	39.7	736	2,773	26.5	90	267	33.7	126	368	34.2

NA: Not applicable.

Table 1B

Influenza A(H3N2) cases and controls included in the 2016/17 season influenza vaccine effectiveness analysis, I-MOVE/I-MOVE+ multicentre case control studies (primary care (n = 5,023) and hospital (n = 635) levels) Europe, influenza season 2016/17

Variables	Primary care level						Hospital level					
	Number of A(H3N2) n = 2,250			Number of controls n = 2,773			Number of A(H3N2) n = 267			Number of controls n = 368		
	n	Total	%	n	Total	%	n	Total	%	n	Total	%
Study sites												
Croatia	13	2,250	0.6	13	2,773	0.5	NA			NA		
Finland	NA			NA			14	267	5.2	17	368	4.6
France	584	2,250	26.0	609	2,773	22.0	35	267	13.1	116	368	31.5
Germany	28	2,250	12.8	873	2,773	31.5	NA			NA		
Hungary	39	2,250	1.7	84	2,773	3.0	NA			NA		
Ireland	135	2,250	6.0	113	2,773	4.1	NA			NA		
Italy	411	2,250	18.3	367	2,773	13.2	37	267	13.9	58)	368	15.8
Lithuania	NA			NA			30	267	11.2	18	368	4.9
Navarra	NA			NA			20	267	7.5	34	368	9.2
The Netherlands	47	2,250	2.1	142	2,773	5.1	6	267	2.2	19	368	5.2
Poland	9	2,250	0.4	33	2,773	1.2	NA			NA		
Portugal	156	2,250	6.9	80	2,773	2.9	36	267	13.5	14	368	3.8
Romania	27	2,250	1.2	9	2,773	0.3	60	267	22.5	37	368	10.1
Spain	474	2,250	21.1	303	2,773	10.9	29	267	10.9	55	368	14.9
Sweden	66	2,250	2.9	147	2,773	5.3	NA			NA		

NA: Not applicable.

vaccination status, date of vaccination and vaccine product administered, 2015/16 seasonal vaccination status, sex, age, presence of chronic conditions, whether the patient belonged to a target group for influenza vaccination (primary care) and number of hospitalisations for chronic conditions in the past 12 months.

We defined individuals as vaccinated if they had received at least one dose of the 2016/17 influenza vaccine at least 15 days before ILI/SARI symptom onset. We excluded individuals vaccinated less than 15 days before symptom onset and individuals with unknown vaccination date.

At primary care level, nine study sites (France, Germany, Hungary, Ireland, the Netherlands, Portugal, Romania, Spain and Sweden) participated in a substudy using an in-depth laboratory protocol, and randomly selected positive influenza A(H3N2) specimens for genetic sequencing.

We pooled individual patient data in each study and computed the pooled IVE as ((1–OR of vaccination between cases and controls) × 100) using logistic regression with study site as a fixed effect. We conducted a complete case analysis excluding patients with missing values for any of the variables in the model. All IVE estimates were adjusted for study site, calendar time of onset and age (where sample size allowed). Further potential confounding factors included sex, underlying chronic conditions and hospitalisations in the past year.

We stratified IVE by age group. We measured IVE among the target groups for influenza vaccination at primary care level, defined as older adults (aged over 54, 59 or 64 years depending on study site), individuals with chronic conditions and other groups for whom the vaccine was recommended in a given country (e.g. pregnant women, healthcare workers and other professional groups, depending on the study site).

Influenza vaccine effectiveness in primary care

In the primary care analysis, we included 2,250 cases of influenza A(H3N2) and 2,773 negative controls.

The 2016/17 seasonal influenza vaccine coverage was 10.3% among influenza A(H3N2) cases and 10.9% among controls. Compared with cases, a greater proportion of controls belonged to the age group of 0–4-year-olds (26.1% vs 12.3%) and a lower proportion belonged to the age group of 5–14-year-olds (12.1% vs 22.7%) (Table 1).

Nine study sites sequenced 204 randomly selected specimens out of 1,817 (11.2%) (Table 2). Of these, 156 (76.5%) belonged to the 3C.2a1 clade A/Bolzano/7/2016, 46 (22.5%) to A/Hong Kong/4801/2014 (3C.2a) and two (1.0%) to A/Switzerland/9715293/2013 (3C.3a).

Table 2

Influenza A(H3N2) viruses characterised by clade, amino acid substitutions and study site, at nine participating laboratories, I-MOVE/I-MOVE+ primary care multicentre case control study, Europe, influenza season 2016/17 (n = 1,817)

Characterised viruses (clade)	Germany n = 289		France n = 584		Hungary n = 39		Ireland n = 135		The Netherlands n = 47		Portugal n = 156		Romania n = 27		Spain n = 474		Sweden n = 66		Total n = 1,817	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
A/HongKong/4801/2014 (3C.2a)	10		6		3		0		8		8		4		3		4		46	
N121K + S144K	3	30	6	100	3	100	0		1	12	8	100	4	100	3	100	3	75	31	67
A/Bolzano/7/2016 (3C.2a1)	33		19		3		5		20		23		8		36		9		156	
N171K + N121K + I140M	10	30	0		0		0		7	35	2	9	4	50	8	22	3	33	34	22
N171K + N121K + T135K	2	6	0		2	67	0		3	15	0		0		1	3	3	33	11	7
N171K + N121K + K92R + H311Q	8	24	0		1	33	1	20	4	20	4	17	0		10	28	0		28	18
N171K + R142G	7	21	3	16	0		3	60	3	15	17	74	0		1	3	1	11	35	22
A/Switzerland/9715293/2013 (3C.3a)	0		0		0		2		0		0		0		0		0		2	
Total sequenced/total A(H3N2)	**43**	**15**	**25**	**4**	**6**	**15**	**7**	**5**	**28**	**60**	**31**	**20**	**12**	**44**	**39**	**8**	**13**	**20**	**204**	**11**

Table 3

Pooled adjusted seasonal vaccine effectiveness against laboratory-confirmed influenza A(H3N2) by age group and target group for vaccination, I-MOVE/I-MOVE+ multicentre case control studies (primary care (n = 4,937) and hospital (n = 635)), influenza season 2016/17

Analyses	Adjustment / stratification	Cases			Controls			Adjusted VE	95% CI
		All	Vaccinated	%	All	Vaccinated	%		
Primary care									
All ages	Adjusted by study site only	2,216	229	10	2,721	297	11	10.9	−8.3 to 26.6
	Adjusted by calendar time and study site	2,216	229	10	2,721	297	11	27.9	11.9 to 41.1
	Adjusted by calendar time, age and study site	2,216	229	10	2,721	297	11	38.4	22.2 to 51.3
	Fully adjusted: calendar time, age, study site, presence of chronic conditions, sex	2,216	229	10	2,721	297	11	38.0	21.3 to 51.2
By age group (years)[a]	0–14	773	20	3	1,043	27	3	44.1	−12.3 to 72.2
	15–64	1,164	69	6	1,410	126	9	46.9	25.2 to 62.3
	≥ 65	278	140	50	268	144	54	23.4	−15.4 to 49.1
Target group for vaccination[a]	All ages	606	201	33	698	235	34	25.7	1.5 to 43.9
Hospital									
≥ 65 years	Adjusted by study site only	267	108	40	368	191	52	−0.7	−46.8 to 30.9
	Adjusted by calendar time and study site	267	108	40	368	191	52	3	−42.2 to 33.8
	Adjusted by calendar time, age and study site	267	108	40	368	191	52	2.5	−43.6 to 33.8
	Fully adjusted: time, age, study site, sex, chronic condition (lung, heart, renal disease, diabetes, cancer, obesity) and hospitalisation in the past year	240	95	40	316	162	51	2.0	−51.7 to 36.8
By age group (years)[b]	65–79	130	38	29	165	70	42	7.9	−67.3 to 49.3
	≥80	115	59	51	167	102	61	2.4	−81.3 to 47.5

CI: confidence interval; VE: vaccine effectiveness at hospital level.

[a] Adjusted by study site, age, calendar time, presence of chronic conditions and sex.

[b] Adjusted by calendar time, age and study site.

Among the 156 viruses of the 3C.2a1 clade, further genetic groups have emerged in 108 (69.2%) (Table 2). These include 34 viruses in group 1 (22%), harbouring the I140M substitution located in the antigenic site A of the haemagglutinin, in addition to changes in amino acid positions 171 and 121, both located in the antigenic site D. Eleven viruses belonged to group 2 (7%), carrying the T135K mutation located in the antigenic site A and resulting in the loss of a glycosylation site, in addition to the already mentioned changes in positions 171 and 121. Twenty-eight viruses belonged to genetic group 3 (18%), carrying the K92R and H311Q substitutions located in the antigenic sites E and C, respectively, in addition to changes in positions 171 and 121. Finally, 35 viruses belonged to group 4 (22%), carrying the R142G mutation located in the antigenic site A and the N171K substitution. Thirty-one viruses (67%) belonging to the 3C.2a clade (A/HongKong/4801/2014) carried the substitutions N121K and S144K, the latter located in the antigenic site position A.

Adjusted IVE against influenza A(H3N2) across all age groups was 38.0% (95% CI: 21.3 to 51.2). It was 44.1% (95% CI: −12.3 to 72.2), 46.9% (95% CI: 25.2 to 62.3) and 23.4% (95% CI: −15.4 to 49.1) in 0–14, 15–64 and ≥ 65 year-olds, respectively. The IVE in the target group for vaccination was 25.7% (95% CI: 1.5 to 43.9) (Table 3).

Influenza vaccine effectiveness at hospital level

In the hospital study, we included 267 cases of influenza A(H3N2) and 368 negative controls.

The 2016/17 seasonal influenza vaccine coverage was 40.4% among influenza A(H3N2) cases and 51.9% among controls. A higher proportion of controls were vaccinated with inactivated split-virion vaccine group (20.6% vs 12.3%). A higher proportion of controls had been hospitalised for chronic conditions in the past twelve months (43.7% vs 26.7%) (Table 1).

Adjusted IVE against influenza A(H3N2) among those aged 65 years and older was 2.5% (95% CI: −43.6 to 33.8), it was 7.9% (95% CI: −67.3 to 49.3) among those aged 65 to 79 years and 2.4% (95% CI: −81.3 to 47.5) among those aged 80 years and older (Table 3).

Discussion

In primary care, early estimates suggest moderate IVE against influenza A(H3N2) among 0–64-year-olds and low IVE in the target group for influenza vaccination. Among those aged 65 years and older, IVE was low at both primary care and hospital level, however precision was low.

Viruses of the 3C.2a1 clade (A/Bolzano/7/2016) predominated in the study sites participating in the laboratory protocol. Compared to the vaccine virus A/HongKong/4801/2014, they had the N171K substitution and in addition, most of them had the N121K substitution. This clade appears to be antigenically similar to the A(H3N2) vaccine component. However, our sequencing results suggest that this cluster is continuing to evolve: 70% of sequenced viruses had further mutations, forming clusters defined by new HA1 amino acid substitutions in antigenic sites, including antigenic site A. We did not measure IVE against A/Bolzano/7/2016 viruses, as estimates were not robust because of the small sample size.

The 2016/17 early primary care IVE estimate among all ages was 38% (95% CI: 21.3 to 51.2), similar to the early estimates from the Canadian Sentinel Practitioner Surveillance [10] and comparable to early estimates against influenza A(H3N2) in previous seasons: 43% (95% CI: -0.4 to 67.7) in 2011/12 and 41.9% (95% CI: −67.1 to 79.8) in 2012/13 [11,12]. This season, we reached better precision thanks to a larger sample size. The IVE estimates among those aged 65 years and older and target groups for vaccination were low and, despite low precision, reinforce the risk assessment from the European Centre for Disease Prevention and Control (ECDC), which suggests to consider administering antiviral drugs to populations vulnerable to severe influenza irrespective of vaccination status, in line with national and international recommendations [1].

These early results are included in the Global Influenza Vaccine Effectiveness (GIVE) report to contribute to the World Health Organization consultation and information meeting on the composition of influenza virus vaccines for use in the 2017/18 northern hemisphere influenza season [13].

Conclusion

The early season estimates presented here corroborate the suboptimal performance of inactivated influenza vaccine against influenza A(H3N2) that the I-MOVE team and others have reported in the previous post-2009 pandemic seasons [14,15].

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Conflict of interest

None declared.

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Combined effectiveness of prior and current season influenza vaccination in northern Spain: 2016/17 mid-season analysis

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The 2016/17 mid-season vaccine effectiveness estimate against influenza A(H3N2) was 15% (95% confidence interval: −11 to 35) in Navarre. Comparing to individuals unvaccinated in the current and four prior seasons, effectiveness was 24% for current and 3–4 prior doses, 61% for current and 1–2 prior doses, 42% for only current vaccination, and 58% for 3–4 prior doses. This suggests moderate effectiveness for different combinations of vaccination in the current and prior seasons.

During the early 2016/17 influenza season, influenza A(H3N2) was the main circulating virus in Europe [1]. Although most of the A(H3N2) viruses characterised genetically matched the vaccine component, a high incidence of severe cases was detected [1,2]. We present the 2016/17 mid-season vaccine effectiveness (VE) estimates in preventing laboratory-confirmed influenza A(H3N2), relative to different combinations of current and prior seasonal influenza vaccinations.

Setting and information sources

In the 2016/17 season the A(H3N2) component recommended for the influenza vaccine in the northern hemisphere was A/HongKong/4801/2014-like virus (group 3C.2a) [3], in the 2015/16 season A/Switzerland/9715293/2013-like (group 3C.3a) [4], and in seasons 2012/13 to 2014/15 it was A/Texas/50/2012-like or A/Victoria/361/2011-like (group 3C.1) [5].

The Influenza Surveillance System in Spain reported that as of 9 February 2017, 99% of the sentinel detections of influenza virus were A(H3N2), and sequence analysis of the HA1 fragment of the haemagglutinin gene found 74% of strains as A/Bolzano/7/2016 (group 3C.2a1) and 21% as A/HongKong/4801/2014, both of which matched the vaccine component [2].

A test-negative case–control study was conducted, based on epidemiological and virological surveillance of influenza in primary healthcare and hospitals in Navarre, northern Spain. The influenza vaccination campaign took place in October and November 2016. The trivalent inactivated non-adjuvanted vaccine was offered free of charge to a target group for vaccination, including people aged 60 years or over and those with major chronic conditions (body mass index ≥40 kg/m2, cancer, liver cirrhosis, dementia, diabetes mellitus, immunodeficiency, heart disease, renal disease, respiratory disease, rheumatic disease, and stroke).

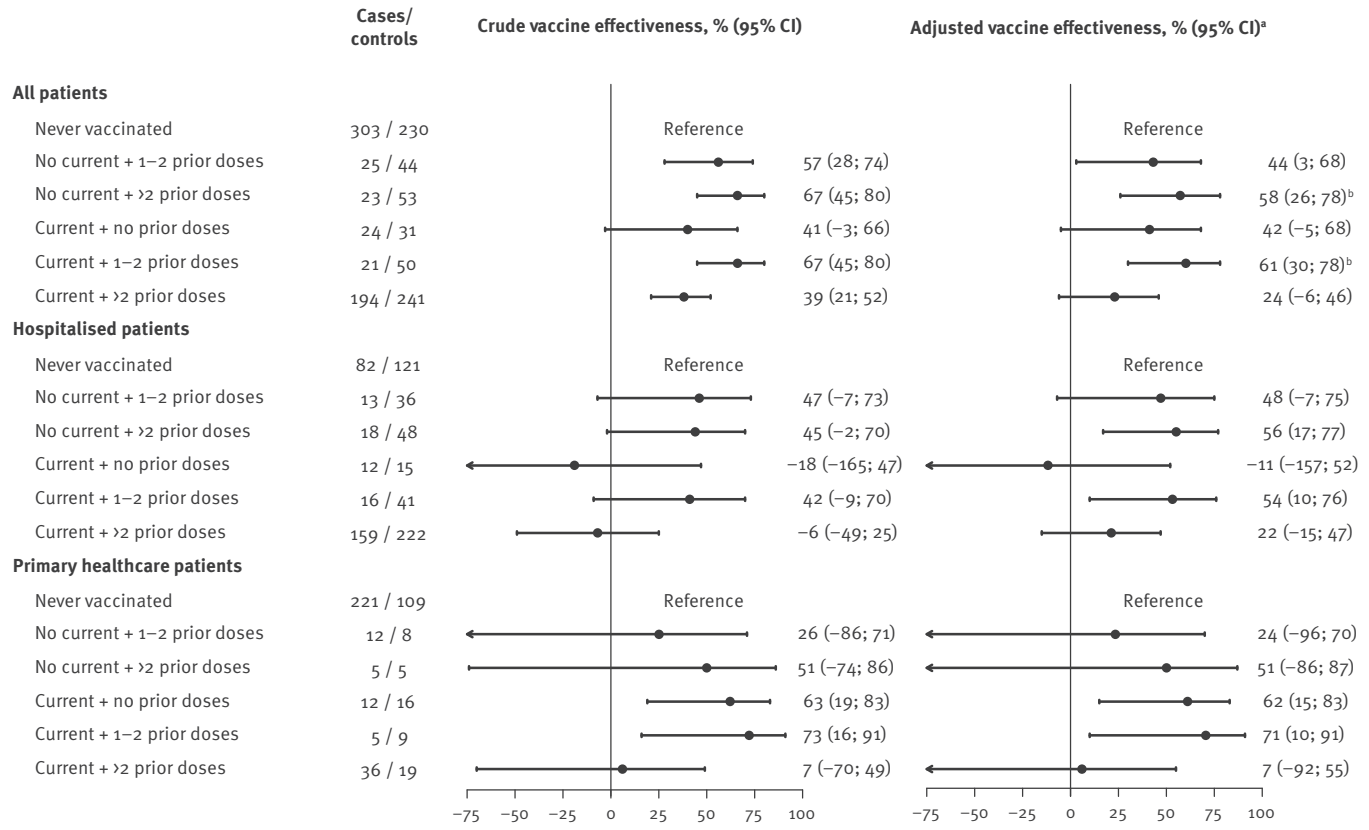
Influenza vaccine status in seasons 2012/13 to 2016/17 was obtained from the online regional vaccination register [6]. These five seasons were considered because for all of them the A(H3N2) component included in the vaccine belonged to clade 3C [3-5].

Patients were considered to be protected from influenza 14 days after vaccine administration in the current season.

Influenza surveillance was based on automatic reporting of cases of influenza-like illness (ILI) from all primary healthcare physicians and hospitals [7]. A sentinel network of primary healthcare physicians was requested to take nasopharyngeal and pharyngeal swabs from their patients diagnosed with ILI, whose symptoms had begun less than five days previously. In hospitals, the protocol specified early detection and swabbing of all hospitalised patients with ILI. Samples

Figure

Effectiveness of current season influenza vaccination and of vaccination in the four prior seasons in preventing laboratory confirmed influenza A(H3N2) cases among people ≥ 9 years-old in Navarre, Spain, 1 December 2016–31 January 2017 (n = 1,240 patients)



CI: confidence interval.

[a] Vaccine effectiveness adjusted by age groups (9–24, 25–44, 45–64, 65–84 and ≥ 85 years), sex, major chronic conditions(body mass index ≥40 kg/m2, cancer, liver cirrhosis, dementia, diabetes mellitus, immunodeficiency, heart disease, renal disease, respiratory disease, rheumatic disease and stroke), healthcare setting (primary healthcare and hospital), and month of swabbing.

[b] $p < 0.05$ for comparisons with the category 'Current season vaccination + >2 prior doses'.

were processed by reverse transcription-polymerase chain reaction assay.

Statistical analysis

The study population included persons covered by the Navarre Health Service since 2012 (96% of the population). All patients who were swabbed between 1 December 2016 (beginning of continued detection of influenza virus) and 31 January 2017 were initially considered. Healthcare workers, persons living in nursing homes, children less than 9 years-old and patients hospitalised before ILI symptom onset were excluded. We compared seasonal vaccination status in patients for whom A(H3N2) influenza virus was detected (cases) and in those who were negative for influenza (controls). Crude and adjusted odds ratios (OR) with their 95% confidence intervals (CI) were calculated using logistic regression models. Adjusted models included sex, age group (9–24, 25–44, 45–64, 65–84 and ≥ 85 years), major chronic conditions, month of swabbing, and healthcare setting (primary healthcare and hospital). Six categories combining current vaccination status with vaccination in the four prior seasons and thus distinguishing between frequent and infrequent vaccinees were considered [8,9]: current-season vaccination and > 2 prior doses, current-season vaccination and 1–2 prior doses, current-season vaccination and no prior doses, no current-season vaccination and > 2 prior doses, no current-season vaccination and 1–2 prior doses, and no current-season vaccination and no prior doses (reference group). To compare VE among categories, the model was repeated using the category with current season vaccination and > 2 prior doses as the reference. VE was estimated as a percentage: (1–OR) × 100.

Early estimation of influenza vaccine effectiveness

Of 1,243 ILI initial patients, one case of non-subtyped influenza A and two influenza B cases were not further

Table 1

Characteristics, according to the healthcare setting and test result, of patients with medically-attended influenza-like illness included in the test-negative case–control analysis, Navarre, Spain, 1 December 2016–31 January 2017 (n = 1,240 patients)

Characteristics	All patients				Hospitalised patients				Primary healthcare patients			
	Controls		Cases		Controls		Cases		Controls		Cases	
	N	%	N	%	N	%	N	%	N	%	N	%
Age groups in years												
9–24	37	6	56	9	14	3	1	0	23	14	55	19
25–44	76	12	99	17	22	5	7	2	54	33	92	32
45–64	135	21	121	20	80	17	33	11	55	33	88	30
65–84	269	41	197	33	236	49	143	48	33	20	54	19
≥ 85	132	20	118	20	131	27	116	39	1	1	2	1
Sex												
Male	331	51	274	46	269	56	151	50	62	37	123	42
Female	318	49	317	54	214	44	149	50	104	63	168	58
Residence												
Rural	237	37	213	36	213	44	154	51	24	15	59	20
Urban	412	63	378	64	270	56	146	49	142	86	232	80
Major chronic conditions												
No	189	29	242	41	78	16	43	14	111	67	199	68
Yes	460	71	349	59	405	84	257	86	55	33	92	32
Month of swabbing												
December	159	24	139	24	106	22	58	19	53	32	81	28
January	490	76	452	76	377	78	242	81	113	68	210	72
Target group for vaccination[a]												
No	124	19	182	31	36	7	11	4	88	53	171	59
Yes	525	81	409	69	447	93	289	96	78	47	120	41
2016/17 season vaccine												
No	327	50	351	59	205	42	113	38	122	73	238	82
Yes	322	50	240	41	278	58	187	62	44	27	53	18
Total	**649**	**100**	**591**	**100**	**483**	**100**	**300**	**100**	**166**	**100**	**291**	**100**

[a] Target group for vaccination includes people ≥ 60 years old and people with major chronic conditions (body mass index ≥ 40 kg/m², cancer, liver cirrhosis, dementia, diabetes mellitus, immunodeficiency, heart disease, renal disease, respiratory disease, rheumatic disease and stroke).

considered. The remaining 1,240 ILI patients were included in the study and consisted of 783 (63%) hospitalised patients and 457 primary healthcare patients. A total of 591 (48%) were confirmed cases for influenza A(H3N2) and were compared with 649 controls negative for any influenza virus.

Compared with test-negative controls, A(H3N2) influenza cases had a lower proportion of persons over 65 years-old (53% (315/591) in cases vs 62% (401/649) in controls; p = 0.003), with major chronic conditions (59% vs 71%; p < 0.001; Table 1) and who were treated in hospital (51% (300/591) vs 74% (483/649; p < 0.001)). Among the cases, 41% had received the 2016/17 seasonal vaccine, vs 50% of the controls (p = 0.001) (Table 1).

The overall adjusted estimate of influenza VE was 15% (95%CI: −11 to 35). The estimates were similar in the analysis of the target group for vaccination (16%), and were somewhat better in persons younger than 65 years (24%) than in the older age group (≥ 65 years; 11%). The point estimates suggested higher VE in outpatients (48%; 95%CI: −1 to 65) than in inpatients (0%; 95%CI: −38 to 27) (Table 2).

In the pooled analysis of all patients, as compared with individuals unvaccinated in the current and four prior seasons, the preventive effect was 61% (95%CI: 30 to 78) in those vaccinated in the current season who had also received 1–2 doses of vaccine in the prior seasons, 24% (95% CI: −6 to 46) in those vaccinated in the current season after 3–4 doses, 42% (95% CI: −5 to 68) in those vaccinated only in the current season, 58% (95%CI: 26 to 78) in individuals without current vaccination but with > 2 prior doses, and 44% (95% CI: 3 to 68) in those unvaccinated in the current season but with 1–2 prior doses. Current and 1–2 dose prior season vaccination, or current season non-vaccination in people with > 2 prior doses showed statistically significant higher protection than current and > 2 prior season vaccinations (Figure).

Table 2

Influenza vaccine effectiveness in preventing laboratory-confirmed influenza A(H3N2) among individuals ≥ 9 years-old in Navarre, Spain, 1 December 2016–31 January 2017 (n = 1,240 patients)

Characteristics	Controls Vaccinated/unvaccinated	Cases Vaccinated/unvaccinated	Crude VE % (95% CI)	Adjusted VE % (95% CI)[a]
Both healthcare settings				
All swabbed patients	322/327	240/351	31 (13 to 45)	15 (–11 to 35)
Target group for vaccination[b]	307/218	225/184	13 (–13 to 33)	16 (–12 to 37)
Age group in years				
9–64	56/192	37/239	47 (16 to 66)	24 (–26 to 55)
≥65	266/135	203/112	8 (–25 to 33)	11 (–23 to 35)
Hospitalised patients				
All swabbed patients	278/205	187/113	–22 (–64 to 9)	0 (–38 to 27)
Target group for vaccination[b]	272/175	185/104	–14 (–55 to 16)	2 (–36 to 29)
Age group in years				
9–64	33/83	14/27	–30 (–179 to 39)	–27 (–188 to 44)
≥65	245/122	173/86	0 (–40 to 29)	5 (–34 to 33)
Primary healthcare patients				
All swabbed patients	44/122	53/238	38 (3 to 61)	48 (–1 to 65)
Target group for vaccination[b]	35/43	40/80	39 (–10 to 66)	54 (10 to 77)
Age group in years				
9–64	23/109	23/212	49 (4 to 72)	43 (–8 to 70)
≥65	21/13	30/26	29 (–70 to 70)	44 (–41 to 78)

CI: confidence interval; VE: vaccine effectiveness.

[a] Logistic regression model adjusted for sex, age group (9–24, 25–44, 45–64, 65–85 and ≥85 years), major chronic conditions, month of swabbing and healthcare setting (primary healthcare and hospital).

[b] Target group for vaccination includes people ≥60 years old and people with major chronic conditions (body mass index ≥40 kg/m², cancer, liver cirrhosis, dementia, diabetes mellitus, immunodeficiency, heart disease, renal disease, respiratory disease, rheumatic disease and stroke).

In separated analyses of outpatients and inpatients, vaccination only in the current season was protective for primary healthcare consultations but not for hospitalisations. In hospitalised patients however, a history of vaccination in the prior seasons appeared to confer enhanced protection, whether the inpatients were vaccinated or not in the current season (Figure).

Discussion and conclusion

Estimates of VE during the influenza season help guide health interventions aimed at reducing the impact of influenza in the population [10] and may help in the selection of strains to be contained in the next season's vaccine. For the 2016/17 season in Navarre, when vaccination status in the prior influenza seasons was not considered, we found low VE (15%) in the whole pool of patients, null VE for hospitalised patients and better protection (48%) for outpatients; the higher protection level in outpatients is consistent with the early estimates reported from the Canadian Sentinel Practitioner Surveillance Network [11].

In the analysis considering vaccination history; however, better levels of protection were observed for many of the combinations of current and prior season vaccination, especially for hospitalised patients. The results of the overall analysis suggest that the protective effect of the influenza vaccination against A(H3N2) virus in Navarre in the early 2016/17 season ranged from 24% to 61%, depending on the vaccination status in the current and prior seasons.

The VE estimates were strongly related to the vaccination history. One or two vaccine doses over the four prior seasons maintained or increased the protection of the current season vaccination, but three or more prior doses had a negative interference with the current season vaccine effect. A similar interference was described in previous seasons by other authors [8,12], and inverse exposure-response association has been reported between repeated influenza vaccination and haemagglutinin antibodies titres for A(H3N2) virus [13].

Our results obtained from two independently recruited groups, inpatients and outpatients, were broadly consistent. The main difference was that vaccination only in the current season was protective for influenza cases attended in primary healthcare but not against influenza hospitalisations, which may be due to the poorer immune response among patients that need hospitalisation. Especially remarkable is the preventive effect observed for the vaccine doses received in prior seasons in individuals without current season vaccination.

This study has some limitations. Natural immunity due to exposure to influenza virus was not considered; however, in a previous study we demonstrated that it was not a relevant confounding factor or effect modifier of influenza VE [7]. Since these results are preliminary and have limited statistical power for some analyses, the final results for the season may be different. The study compared laboratory-confirmed cases with controls recruited in the same settings before either patient or physician knew the laboratory result, an approach that reduced selection bias [14]. We included patients recruited in primary care and hospitals, thus achieving representation of the whole spectrum of patients with influenza. The healthcare setting could have acted as a confounding factor, therefore the analyses were adjusted for this variable. This study evaluates a particular situation of circulating virus and composition of the vaccines; caution should be taken in generalising its outcome.

In conclusion, the results suggest that, overall, the different combinations of vaccination in the current and prior seasons were moderately effective against influenza A(H3N2) in the early 2016/17 season in northern Spain. In spite of the possible interferences between the effects of the current season vaccine and frequent prior vaccination, these findings highlight the net benefit of immunisation against influenza.

Primary Health Care Sentinel Network of Navarre

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Conflict of interest

None declared.

Authors' contributions

J Castilla, I Casado and I Martínez-Baz designed the study and coordinated the activities. I Martínez-Baz, I Casado and J Castilla undertook the statistical analysis. A Navascués, A Aguinaga, A Pérez-García, C Ezpeleta and F Pozo were responsible of the virological analysis and the interpretation of laboratory results. J Díaz-González, L Fernandino and I Casado participated in the data collection. J Castilla, C Ezpeleta, F Pozo and I Martínez-Baz wrote the draft manuscript, and all authors revised and approved the final version.

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Multinational outbreak of travel-related *Salmonella* Chester infections in Europe, summers 2014 and 2015

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Between 2014 and 2015, the European Centre for Disease Prevention and Control was informed of an increase in numbers of *Salmonellaenterica* serotype Chester cases with travel to Morocco occurring in six European countries. Epidemiological and microbiological investigations were conducted. In addition to gathering information on the characteristics of cases from the different countries in 2014, the epidemiological investigation comprised a matched case–case study involving French patients with salmonellosis who travelled to Morocco that year. A univariate conditional logistic regression was performed to quantify associations. The microbiological study included a whole genome sequencing (WGS) analysis of clinical and non-human isolates of *S.* Chester of varied place and year of isolation. A total of 162 cases, mostly from France, followed by Belgium, the Netherlands, Spain, Denmark and Sweden were reported, including 86 (53%) women. The median age per country ranged from 3 to 38 years. Cases of *S.* Chester were more likely to have eaten in a restaurant and visited the coast of Morocco. The results of WGS showed five multilocus sequence types (ST), with 96 of 153 isolates analysed clustering into a tight group that corresponded to a novel ST, ST1954. Of these 96 isolates, 46 (48%) were derived from food or patients returning from Morocco and carried two types of plasmids containing either *qnrS1* or *qnrB19* genes. This European-wide outbreak associated with travel to Morocco was likely a multi-source outbreak with several food vehicles contaminated by multidrug-resistant *S.* Chester strains.

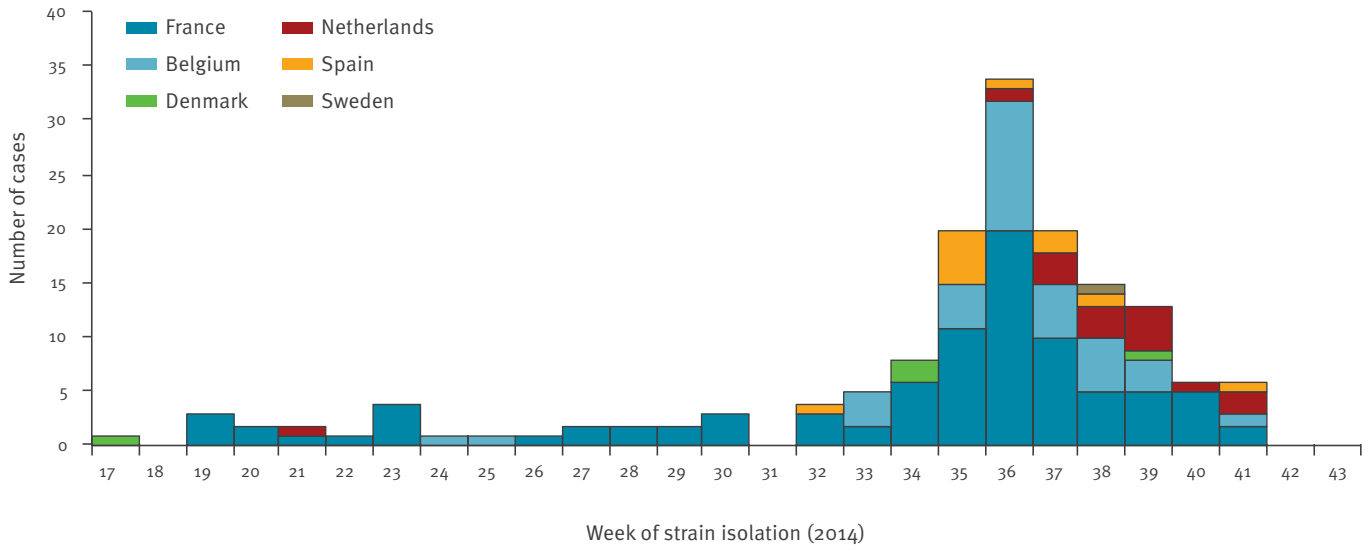
Introduction

Non-typhoidal *Salmonella* infections are the most common cause of reported food-borne outbreaks in the European Union (EU) [1,2]. These infections mostly cause mild disease (gastroenteritis), however life-threatening infections (e.g. bacteraemia) may occur, particularly in cases involving patients at the extremes of age or who are immunocompromised. Due to the large animal reservoir, including farm animals, pets and wild animals, *Salmonella* is mainly transmitted by consumption of contaminated food and to a lesser extent by contaminated environments, contact with animals, or person-to-person [3]. The mean incubation period is between 1 and 3 days. More than 2,500 serotypes of the genus *Salmonella* have been described so far [4].

Of these, serotype Chester is not commonly identified through human surveillance. Between 2009 and 2013, EU and European Economic Area (EU/EEA) countries

Figure 1

Distribution of *Salmonella* Chester cases by respective isolate week and country of residence, European Union, 2014 (n = 162)



reported through The European Surveillance System (TESSy) a mean of 91 *S.* Chester cases per year, which accounts for only 0.1% of all annual salmonellosis cases notified in the EU/EEA [5]. Outbreaks associated with *S.* Chester have been reported: in Australia, associated with sea turtle meat in 1998 and with tap water in 2005; in the United States, associated with cantaloupe in 1990 and with frozen meals (cheesy chicken and rice) in 2010; in Japan associated with cuttlefish chips in 1999 and in Canada, associated with headcheese in 2010 [6-11]. *S.* Chester was also the second most common serotype in poultry, in 2010, in Burkina Faso [12]. From 2005 to 2015, according to the Rapid Alert System for Food and Feed (RASFF, http://ec.europa.eu/food/safety/rasff/index_en.htm) database, a EU tool to share information when cross-border risks to public health are detected in the food chain, *S.* Chester was found in kangaroo meat (twice in 2007 and 2011 respectively), peppermint (once in 2005), dog chew (once in 2005) and fishmeal (six times in 2014) [13].

In France, the human *Salmonella* surveillance system is based on a voluntary network of laboratories that send or report their *Salmonella* isolates to the French National Reference Center for *Escherichia coli*, *Shigella* and *Salmonella* (NRC) [14,15]. Travel information is collected from laboratory surveillance forms (completed for ca 30% of the patients in 2014). In addition, foodborne outbreaks of salmonellosis (at least two cases clustered in time and place) are subject to mandatory notification to the French Institute for Public Health Surveillance (Santé publique France; SpF).

In September 2014, the French NRC notified SpF of an increase in numbers of *S.* Chester isolates, with 31 isolates received between August and September 2014, slightly more than twice the number observed for the same period in 2013 (n = 14). Most cases had travelled to Morocco within two weeks prior to their symptom onset. During the same period, Belgium had initiated a similar notification to the European Epidemic Intelligence Information System (EPIS) of the European Centre for Disease Prevention and Control (ECDC) with 18 *S.* Chester cases. The Netherlands, Spain and Denmark reported clusters of, respectively eight, six, and four cases, and Sweden reported one case [1]. In September 2014, a European investigation was launched in order to identify the vehicle(s)/source(s) of infection and implement control measures. France, the country with the highest number of cases, coordinated this investigation with the support of the ECDC.

In this article, we describe the epidemiological and microbiological investigations of the outbreak and report and discuss their outcome.

Methods

We carried out both epidemiological and microbiological investigations. The epidemiological investigation only included cases with symptom onset in 2014 while the microbiological investigation considered cases with onset occurring over a larger time frame as further described.

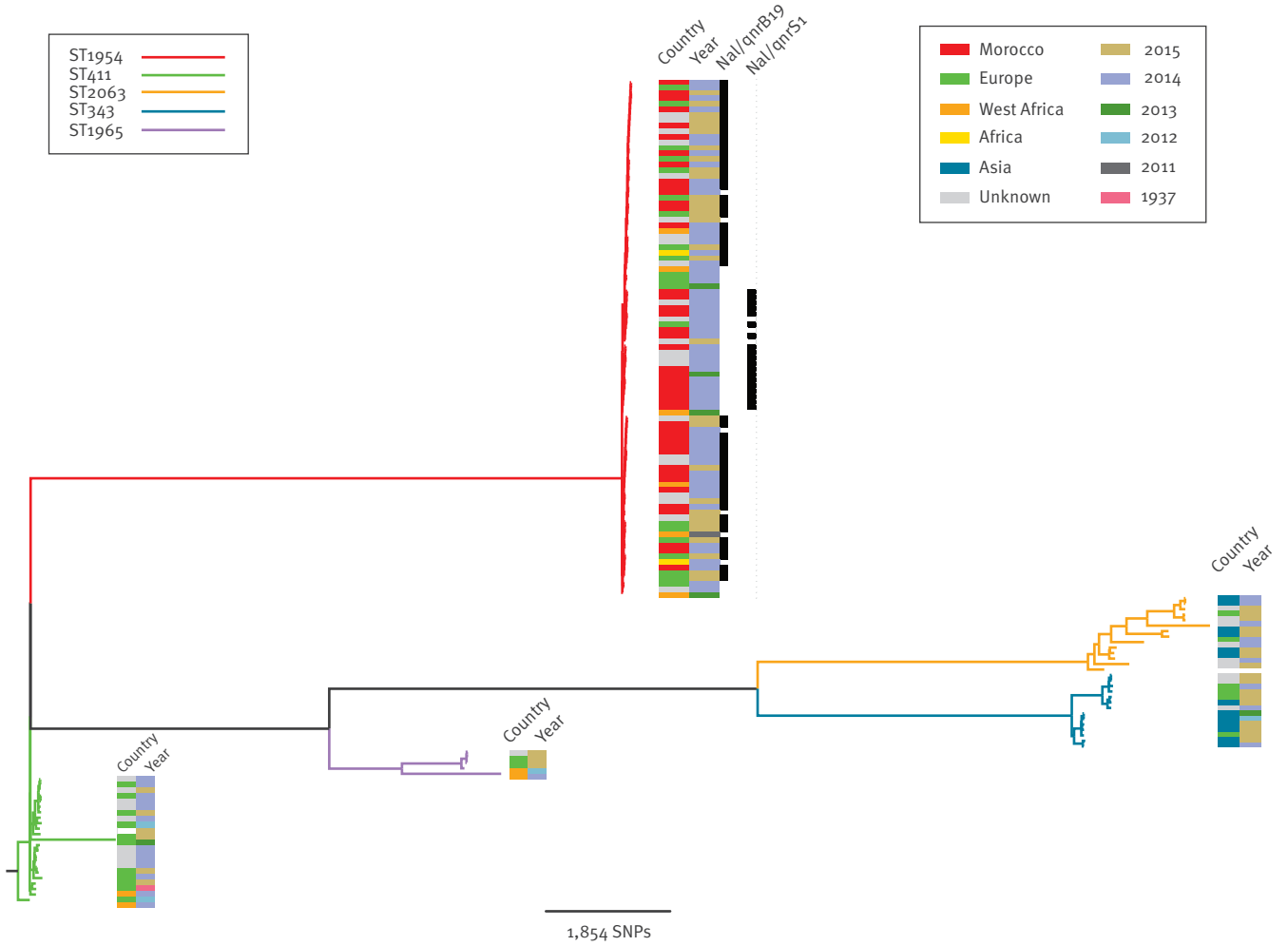
Epidemiological investigation

We defined a case as a symptomatic resident of the EU/EEA with laboratory-confirmed *S.* Chester infection and with symptom onset (or date of strain isolation in case of unavailable onset date) between week 17 (April) and week 41 (October) of 2014.

We described cases in terms of age, sex and travel history to Morocco.

Figure 2

Phylogenetic tree of *Salmonella enterica* serotype Chester, European Union, 1937–2015 (n=153 isolates)



Nal: nalidixic acid; SNP: single nucleotide polymorphism; ST: sequence type.

Maximum-likelihood phylogenetic tree based on the analysis of 11,879 chromosomal SNPs from the 153 short read sequences of Salmonella Chester using harvest v1.0.1 f parsnp function against the 17K reference strain and rooted on the 201009678 genome. Branches distributing in a cluster corresponding to a given ST are assigned a specific colour and different colours are respectively used for ST1954, ST411, ST2063, ST343 and ST1965. The geographical area of acquisition/collection of the strain and the year of isolation are shown on the right of each branch together with information as to whether the strain has a Col/qnrB19 plasmid or an IncN/qnrS1 plasmid (whereby presence of either plasmid is indicated by a black box). The remaining quinolone-susceptible isolates are indicated by the absence of a black box (in particular for all the non-ST1954 isolates).

The SNP difference among the 96 isolates of the ST1954 cluster ranged between 0 and 214 SNPs; the ST1954 cluster itself was at a distance of 8, 453 SNPs from the reference strain.

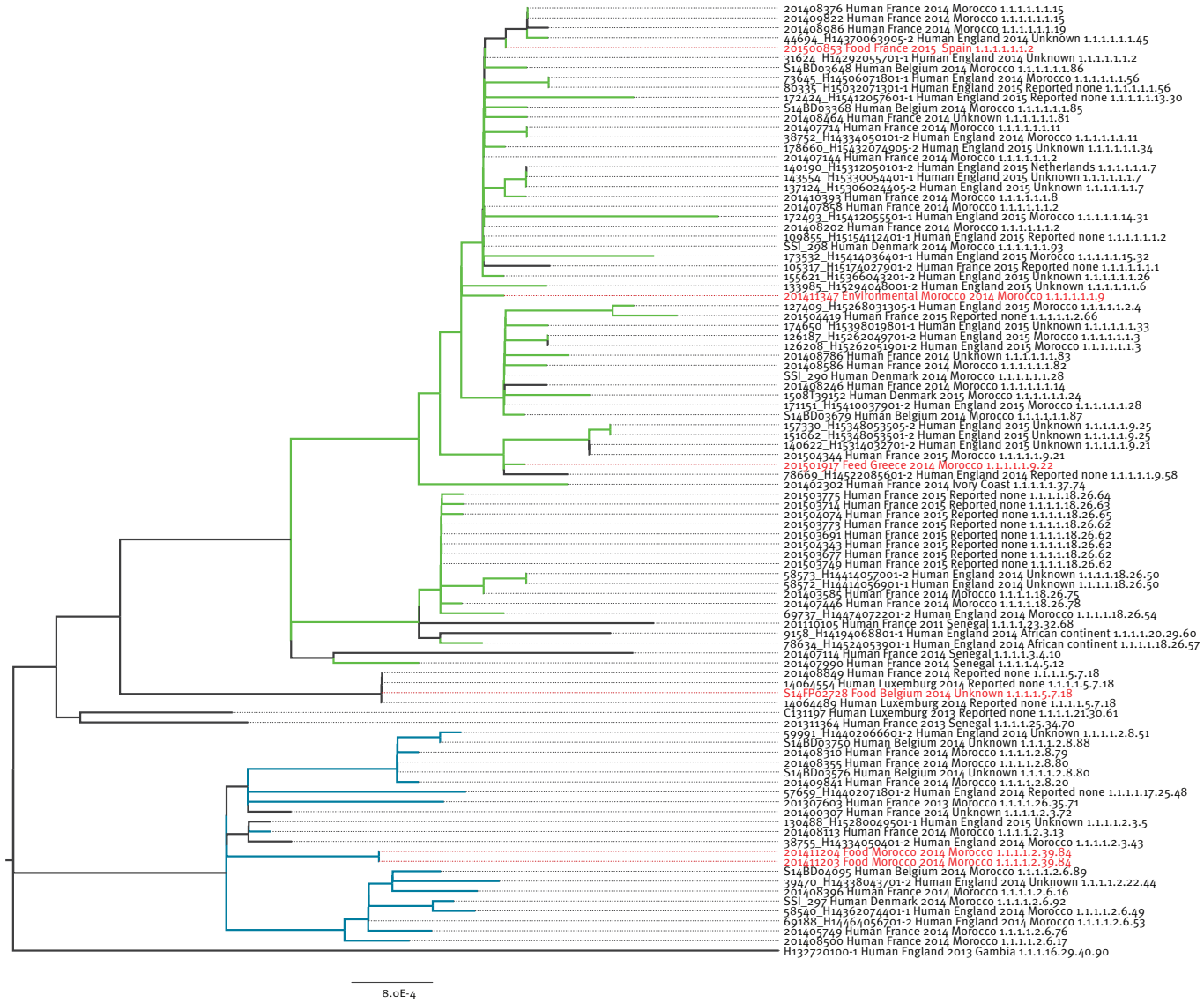
In France, we interviewed the most recently infected cases (with isolates obtained from week 33 of 2014) using a trawling questionnaire. This gathered information on demographics, clinical details, travel within the seven previous days with a focus on Morocco, contact with symptomatic persons, attended events, visited places and a detailed food history for the week before symptom onset. If the case was a child aged less than 15 years, we interviewed one of his/her parents.

The Dutch and Belgian Institutes used the same questionnaire to interview their cases. The Danish and Spanish cases were interviewed using a different questionnaire focusing on travel destination and whereabouts during the travel. The Swedish case was not interviewed on exposures.

In France, SpF carried out a matched case–case study to test the hypotheses raised by the exploratory investigation. We interviewed cases, with symptom onset between week 31 and week 40, who were not included in the exploratory investigation. We selected controls, among non-typhoidal *Salmonella* cases, who were diagnosed with an infection by other serotypes than

Figure 3

Maximum Likelihood phylogeny of the *Salmonella* Chester ST1954 strains, European Union (n=96 strains)



ST1954 strains were mapped to the Spades v2.5.1 de novo assembly of isolate 60056_H14424061601–2 using BWA-MEM. The taxa are labelled with the strain number, isolate source, country of origin, year of isolation, travel information, and single nucleotide polymorphism (SNP) address. Hierarchical single linkage clustering was performed on the pairwise SNP difference between all isolates at various distance thresholds. The SNP address is a seven number indicating the range of SNPs as follow: Δ250, Δ100, Δ50, Δ25, Δ10, Δ5, Δ0. In green, the QnrB19-producing isolates; in blue, the QnrS1-producing isolates; in black the susceptible-quinolone isolates and in red, the non-human isolates.

Chester, who reported travel history to Morocco in the week before symptoms and whose symptoms started between week 27 and week 40 of 2014. We selected two controls for each case. After excluding cases who did not travel to Morocco before being symptomatic, we performed a crude analysis and three matched analyses. We separately matched cases with controls according to their age group (‹1, 1–5, 6–15, 16–40,›40 years of age), according to the week of their symptom onset (plus or minus two weeks) and according to both their age group and week of symptom onset.

We performed a univariate conditional logistic regression to quantify associations. We calculated matched odds ratios (mORs) and their 95% confidence interval (CI). We used Stata v12.1 (Stata Corporation, Texas, US) for analysis.

Microbiological investigations conducted at the European Union level

A microbiological investigation was conducted at the EU level, whereby countries were also asked whether they could participate in a whole genome sequencing (WGS) study. Five EU countries consisting of England and Wales (which conduct WGS routinely), France,

Table 1

Characteristics of *Salmonella* Chester cases, European Union, 2014 (n=162)

Characteristics	France (n = 90)	Belgium (n = 35)	Netherlands (n = 15)	Spain (n = 11)	Denmark (n = 10)	Sweden (n = 1)	All (n = 162)
Proportion of women n (%)	51 (57)	15 (43)	10 (67)	6 (55)	4 (40)	0	86 (53)
Median age (years)	3	14.5	5	6	38	NA	NA
Number of cases with known travel information	20	8	12	5	9	1	55
Number of cases that travelled to Morocco	17	8	10	5	4	1	45

NA: not applicable.

Denmark, Belgium and Luxemburg, took part, making, overall, a total of 153 *S.* Chester isolates available for the investigation. One hundred and forty seven human isolates were selected so as to reflect a significant diversity in terms of year of isolation, geographical area of acquisition and potential link with the present multinational outbreak. Six non-human isolates from 2014 and 2015 were also added. Of the 147 human isolates, 82 were isolated in England and Wales between 2012 and 2015, 45 in France between 2011 and 2015 (including 26 isolates obtained during the epidemiological investigation), nine in Denmark in 2014 and 2015 (including 6 from the epidemiological investigation), six in Belgium in 2014 (all were from the epidemiological investigation) and three in Luxemburg between 2013 and 2014. The two remaining isolates were the reference strains (17K and ATCC 11997). 17K represents the historical reference strain first isolated from humans during a food poisoning outbreak in the hospital of Chester, United Kingdom (UK) in 1937 [16] and the ATCC 11997 is a reference strain from the Centers for Disease Control and Prevention of the United States (US CDC) [17]. Among these 145 patients, 71 (49%) reported international travel two weeks before illness onset (mainly in Morocco, n = 42 and West African countries, n = 10), 34 reported no travel and for the 40 remaining patients, this information was unknown. Of the six non-human isolates, three were collected from food (two from chicken sausages in Casablanca, Morocco and one from poultry in Belgium), one from decanted water in a treatment plant in Agadir, Morocco, one from turkey meat isolated in France but imported from Spain and the remaining isolate was isolated from fishmeal. Contrary to the other isolates obtained from random controls, this latter strain was sent upon request by the Greek authorities to the NRC in 2015 after a notification through RASFF about border rejection of fishmeal from Morocco in October 2014.

For all isolates sent to the French NRC (including the six human isolates from Belgium, the 45 human isolates from France and the six non-human isolates), the serotype was confirmed by agglutination tests with antisera (Bio-Rad, Marnes-la-Coquette, France) according to the White-Kauffmann-Le Minor scheme [4]. For the 82 English and Welsh, the nine Danish and the three Luxemburgish isolates, the serotype was determined from genome sequences, which were shared.

A total of 105 *S.* Chester isolates were selected for antimicrobial susceptibility testing (AST). These consisted of 35 of the 82 isolates from England and Wales, and all human isolates from France (n=45), Denmark (n=9), Belgium (n=6), Luxemburg (n=3) as well as the six hon-human isolates and the 17K reference strain. AST was carried out by the disk diffusion method, with a panel of 32 antimicrobial drugs (Bio-Rad) as previously described [18]. The minimal inhibitory concentration (MIC) of nalidixic acid, ciprofloxacin, azithromycin and colistin were determined by using Etests (BioMérieux, Marcy l'Etoile, France) and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical guidelines [19].

PulseNet standard pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested chromosomal DNA was performed on a subset of 36 isolates. PFGE profiles were compared using Bionumerics software, v6.6 (Applied Maths, Sint Martens Latem, Belgium) and by the molecular typing clusters detection tools of ECDC.

For English and Welsh, French, Danish, Belgian and Luxemburgish *S.* Chester strains, genomic DNA was extracted and purified using different kits (Wizard of Promega or QiaAmp of Qiagen) and DNA samples were processed according to Illumina systems (MiSeq, NextSeq or HiSeq) generating 150 bp paired-end reads. Sequences were transferred to NRC for compiled analysis. Reads were trimmed and filtered using AlienTrimmer [20] with a quality Phred score threshold of 28 on a minimum length of 30 nt. De novo assembly was performed with SPAdes assembler version v2.5.1 [21]. Assembled sequences were analysed using web-tools available from the Center for Genomic Epidemiology (CGE) website (http://www.genomicepidemiology.org/) to obtain the multilocus sequence typing (MLST) type, to detect resistance genes (ResFinder) and to detect and type plasmids (PlasmidFinder and pMLST). New MLST types were confirmed by Sanger

Table 2

Assessing associations between exposures and cases of *Salmonella* Chester infection by univariate conditional logistic regression, France, 2014 (n=14 cases)

Exposure	Cases (N=14)		Control-cases (N=26)		Matched OR[b]	95% CI
	n	%[a]	n	%[a]		
Meat						
Beef	12	92	20	80	2.7	0.26–28
Lamb/sheep meat	5	42	14	54	0.5	0.12–2.2
Chicken	11	79	22	85	0.6	0.12–3.2
Chicken sausage	3	21	4	16	1.3	0.22–8.0
Turkey ham	1	7	8	31	0.2	0.02–1.7
Cachir	2	14	6	23	0.6	0.10–3.5
Poultry meat sandwich	2	25	5	24	1	0.05–19
Milk and eggs products						
Pasteurised milk	6	55	16	76	0.2	0.02–1.7
Yogurt	8	57	19	76	0.4	0.06–2.2
Spreadable cheese	7	54	16	64	0.7	0.08–5.3
Scrambled eggs	6	49	6	29	3	0.54–16
Vegetables and fruits						
Tomato	11	78	18	69	1.4	0.33–6.0
Cucumber	9	69	16	64	1.2	0.28–5.3
Grapes	10	71	18	72	1	0.27–0.7
Melon	9	64	20	77	0.6	0.12–2.8
Water melon	10	71	21	81	0.5	0.08–2.7
Olives	7	50	15	60	0.7	0.16–3.1
See food and fish						
Sardine	6	43	8	31	1.5	0.44–5.0
Shrimp	7	50	4	15	5.6	**1.1–28**
Squid	6	43	4	15	3.3	0.81–14
Sweets						
Ice cream	10	71	15	60	1.6	0.36–7.2
Popcorn	4	31	8	32	0.9	0.21–0.9
Eating place						
Fast food X attendance	4	29	5	19	1.9	0.39–8.9
Restaurant attendance	14	100	18	69	6.2	1.1–295[c]
Shrimp consumption in restaurant	6	43	1	4	11.1	1.3–92.5[c]
Place of residence						
Residing on the coast	11	92	9	41	9.3	**1.1–78**

CI: confidence interval; OR: odds ratio.

[a] Percentages are based on the number of cases or control-cases who answered the questionnaire about a given exposure. These numbers can be less than the totals provided in respective column headers.

[b] OR matched on age categories and week of symptom onset.

[c] Crude odds ratio.

sequencing according to the MLST database website (http://mlst.warwick.ac.uk/mlst/).

As there is no complete *S.* Chester reference genome in public databases, core-genome multi-alignment of assembled genomes was done using harvest v1.0.1 f parsnp function [22] against the 17K reference strain or the ATCC 11997 *S.* Chester assembly [16,17]. The software uses FastTree2 to infer an approximately maximum-likelihood phylogenetic tree based on the analysis of 11,879 chromosomal single-nt polymorphisms (SNPs) from the 153 short read sequences of *S.* Chester [23]. The final tree was visualised in FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

A sequence type (ST)1954 specific *S.* Chester phylogeny was constructed. ST1954 short-read sequences were mapped to the SPAdes v2.5.1 [21] de novo assembly of isolate 60056_H14424061601–2 using BWA-MEM [24]. SNPs were identified using GATK2 [25] in unified genotyper mode. Genome positions that had a high quality SNP (›90% consensus, minimum depth 10x, GQ≥30) in

at least one isolate were extracted. Pseudosequences of polymorphic positions were used to create maximum likelihood trees using RAxML [26] and pairwise SNP distances between each pseudosequence calculated. Hierarchical single linkage clustering was performed on the pairwise SNP difference between all isolates at various distance thresholds (Δ250, Δ100, Δ50, Δ25, Δ10, Δ5, Δ0). The result of the clustering is a SNP address that can be used to describe the population structure based on clonal groups [27].

FASTQ sequences were deposited in the National Center for Biotechnology Information (NCBI) Short Read Archive under the BioProject PRJNA248792.

Results

Epidemiological investigations

Between week 17 and 41 of 2014, six EU countries (France, Belgium, the Netherlands, Spain, Denmark and Sweden) reported 162 cases through EPIS. The number of reported cases peaked on the first week of September (week 36, 2014) (Figure 1).

Of the 162 EU cases, about half of the cases (86; 53%) were women and the median age ranged from 3 to 38 years according to the country of notification. We obtained the travel history for 55 cases and 45 (82%) had recently travelled to Morocco (Table 1).

In France, 16 cases were interviewed (8 females, 8 males) with a median age of 2 years (range: 1–32 years). Four cases aged between 1 and 3 years had been hospitalised (median length of hospitalisation: 5.5 days). Fifteen of the 16 cases had travelled to Morocco before symptom onset, staying there between two and six weeks. In the following analysis, we describe these 15 cases. The majority (10 of 15) arrived in Morocco by car and boat through the ports of Tanger (n = 7), Nador (n = 1) or Ceuta (n = 2), five cases travelled by plane landing in different airports in Morocco. The period between the date of arrival in Morocco and the symptom onset was always longer than seven days (median: 20; range: 8–48). We did not identify any common place (city, hotel, restaurant, supermarket) or activity shared by all cases. The food exposures most frequently mentioned were ice cream (14 of 15 cases; 14/15), grapes (10/11), chicken (13/15), pasteurised milk (13/15) and spreadable cheese (13/15). Shrimps were mentioned by five of the 15 cases. Eleven of the 15 the cases mentioned eating in a restaurant located in different cities.

In the Netherlands, 10 cases were interviewed, in Belgium, seven, in Spain, eight and in Denmark, nine. The food exposures most frequently mentioned were chicken (22 cases of 25 for which this information was available), grapes (17/20) and pasteurised milk (21/25).

Eighteen cases and 26 control-cases were interviewed, four of the 18 cases were excluded because they did not travel to Morocco (n = 2) or because they were considered to be secondary cases (n = 2). Two of the cases were matched with only one control respectively. Cases were more likely than controls to have eaten shrimps (mOR: 5.6; 95% CI: 1.1–28), to have resided on the Moroccan coast (mOR: 9.3; 95% CI: 1.1–78) and to have eaten shrimp in a restaurant (mOR: 11.1; 95% CI: 1.3–92.5). Cases were also more likely to have eaten in a restaurant before symptom onset (crude OR: 6.2; 95% CI: 1.1–295; we could not estimate the mOR because of the small number of cases). Consumption of squid was more frequent among cases (43%) compared with controls (15%), but the association was not significant (Table 2).

Microbiology

The AST showed that 63 *S.* Chester isolates (of 105 isolates tested, i.e. 60%) were resistant to at least nalidixic acid with a MIC range of 24–64 mg/L (Table 3 and data not shown). Among the 39 tested strains, which were acquired in Morocco (human and non-human, 2013–2015), 35 were resistant to at least nalidixic acid. Quinolone-resistant *S.* Chester isolates were also found from travellers returning from the African continent (Côte d'Ivoire, n = 1; Senegal, n = 1 or unspecified, n = 1) in 2014, from 13 French and English cases from 2014 and 2015 with no reported travel and from turkey meat imported from Spain in 2015. In silico MLST indicated that 61 of 63 quinolone-resistant isolates (including the 35 Moroccan ones) belonged to a new type, ST1954. This ST was also found in 15 quinolone-susceptible isolates (Table 3). The 61 quinolone-resistant *S.* Chester ST1954 isolates contained plasmid-mediated quinolone resistance (PMQR) genes. Seventeen isolates (28%) harboured a *qnrS1* gene associated with an IncN-pST7 plasmid (including the two Moroccan chicken sausage isolates) and 44 isolates (72%) contained a *qnrB19* gene associated with a Col plasmid (including the turkey meat, fishmeal and the sewage water isolates) (Table 3). Resistance to quinolones was only supported by these *qnr* genes, as no mutation was found in quinolone-resistant determining regions (*gyrA*, *gyrB*, *parC* and *parE* genes). Furthermore, a transposon belonging to the Tn*3*-like family was also identified and carried *strA*, *strB, sul2, tet(A*) and/or *floR* genes conferring resistance to streptomycin, sulfonamides, tetracycline, and/or chloramphenicol, respectively. The *floR* gene was only associated with the IncN-qnrS1 plasmid (Table 3).

Two main pulsed-field gel electrophoresis (PFGE) patterns, XCHE_1440 and XCHE_2010, were observed among the outbreak isolates by using the ECDC cluster detection tools (data not shown). A few other patterns were also observed among the ST1954 outbreak strains.

The WGS results showed that 153 human and non-human *S.* Chester isolates clustered phylogenetically into five tight groups. The grouping was concordant with MLST distribution, ST1954 (n = 96), ST411 (n = 23),

Table 3

Microbiological characteristics of *Salmonella enterica* serotype Chester, European Union, 1937–2015 (n=153 isolates)

MLST	Number of isolates	Source (n)	Country of acquisition (n)[a]	Year (n)	AST profile[b] (n)	PFGE type	Plasmid type_pMLST	Resistance genes patterns (n)
343	14	Human (14)	Reported none (4) Unknown (4) Cambodia (1) India (1) Indonesia (1) Maldives (1) Sri Lanka (1) Thailand (1)	2012 (1) 2013 (1) 2014 (3) 2015 (9)	Susceptible (4) Not tested (10)	XCHE_1887 (1) Not tested (13)	Absence (8) IncFII (6)	Absence (14)
411	23	Human (23)	Reported none (8) Unknown (8) Reported yes (3) Greece (2) Burkina Faso (1) Togo (1)	1937 (1) 2012 (2) 2013 (1) 2014 (13) 2015 (6)	Susceptible (15) ACroCazKGSuTmpTeNal (1) Not tested (7)	XCHE_1 (1) XCHE_3 (1) XCHE_4 (1) XCHE_1949 (1) Not tested (19)	Absence (15) Col (2) incFII (6)	Absence (22) *strA, strB, sul1, dfrA18, tet(D),qnrB4,bla*$_{DHA-1}$ (1)
1954	96	Human (90) Non human (6)	Reported none (18) Unknown (22) African continent (2) Côte d'Ivoire (1) The Gambia (1) Morocco (46) Netherlands (1) Senegal (4) Spain (1)	2011 (1) 2013 (4) 2014 (59) 2015 (32)	Susceptible (13) AKNTGNal (1) ASSpSuTmpCTeNal (1) ASSuTmpCTeNal (2) Nal (24) SSuTmpCTeNal (13) SuTmpCTeNal (1) SuTmpNal (1) SuTmpTe (2) SuTmpTeNal (18) Not tested (20)	Lane4 (1) XCHE_1440 (16) XCHE_2 (1) XCHE_2010 (4) XCHE_2011 (1) XCHE_5 (1) XCHE_X1 (2) Not tested (70)	Absence (11) Col (64) IncN_ST7 (19) IncI1 (6) IncX1 (1)	Absence (15) *qnrB19* (26) *strA, strB, sul2, dfrA14, floR, tet(A), qnrS1* (16) *strA, strB, sul2, dfrA14, tet(A), qnrB19* (33) *strA, strB, sul2, dfrA14, tet(A)* (2) *qnrB19, bla*$_{TEM-1}$ (1) *strA, strB, sul2, dfrA14, floR, tet(A), qnrS1, bla*$_{TEM}$ (3)
1965	5	Human (5)	Reported none (2) Unknown (1) Ghana (1) Senegal (1)	2012 (1) 2014 (1) 2015 (3)	Susceptible (2) Not tested (3)	Not tested (5)	Absence (1) IncFII (4)	Absence (5)
2063	15	Human (1) ATCC_11997	Reported none (2) Unknown (7) India (2) Sri Lanka (2) Thailand (1) Vietnam (1)	Unknown (1) 2014 (6) 2015 (8)	Susceptible (5) SuTmp (1) ASuTmpNal (1) Not tested (8)	XCHE_1951 (1)	Absence (11) IncI1 (2) Col (2)	Absence (13) *strA, strB, sul2, dfrA14 (1)* *strA, strB, sul2, dfrA14, qnrS1, bla*$_{TEM}$ (1)

AST: antimicrobial susceptibility testing; MLST: multilocus sequence type; PFGE: pulsed-field gel electrophoresis.

[a] For the country of acquisition 'reported none' indicates that the patients specified that they did not travel prior to the two weeks before their onset of symptoms, while 'unknown' indicates that no information was available as to the country of acquisition of the strain.

[b] A: ampicillin; C: chloramphenicol; G: gentamicin; K: kanamycin; N: netilmicin; Nal: nalidixic acid; S: streptomycin; Sp: spectinomycin; Su: sulfonamide; T: tobramycin; Te: tetracycline; Tmp: trimethoprim.

ST2063 (n = 15), ST343 (n = 14) and ST1965 (n = 5) (Figure 2).

Within the ST1954 outbreak-cluster the SNP distance between strains was between 0 and 214 SNPs, the cluster itself being 8,453 SNPs distant from the reference 17K genome. The epidemic ST1954 clone encompassed all *S.* Chester QnrS1- and QnrB19 producers of the outbreak period as well as the 15 ST1954 quinolone-susceptible strains that had been isolated since 2011. Furthermore, all the six non-human *S.* Chester isolates were distributed throughout this cluster and some of them had < 5 SNP of difference with human cases (Figure 3).

Discussion

This multinational outbreak of *S.* Chester cases associated with travel to Morocco has affected at least six EU countries since 2014. The true extent of the outbreak has probably been larger than observed, with unreported cases both in visitors to and residents of the affected area. Morocco is a popular holiday destination welcoming more than 10 million international travellers in 2014. The most common countries of origin of visitors registered at the Moroccan border were France (n = 3,494,112 visitors) and Spain (n = 2,134,610) [28]. In relation to the populations of the country of residence, the highest proportions of travellers to Morocco

in 2014 came from France (5,405/100,000 inhabitants), Belgium (5,320/100,000 inhabitants) and Spain (4,567/100,000 inhabitants). This could explain the predominance of French residents among outbreak cases. The very young age of the cases we report could be an observational bias because we caught cases who had consulted a medical doctor after their return to Europe. These cases were more likely the very young and more severely affected by salmonellosis.

In September 2014 and during the investigation, Moroccan health authorities were kept informed by ECDC and SpF and attended telephone meetings held to discuss the event. The French ministry of health informed in October 2014, through the International Health Regulation mechanism, the Moroccan ministry of health of the increase in *S.* Chester cases among French travellers returning from this country. Outbreaks of food-borne infection, only, are reportable in Morocco. No *S.* Chester outbreak was reported by the Moroccan authorities before and during the investigation.

The epidemiological investigations suggest that the source of the outbreak was in Morocco. We found significant associations between *S.* Chester infection and shrimp consumption, visiting the coast and restaurant attendance before symptoms. The OR associated with squid consumption was high although it did not reach statistical significance. These results suggest that seafood, shrimp in particular, could be one of the sources of this outbreak. Multiple other sources of human contamination are suggested by the molecular and WGS analysis of the non-human strains: the chicken sausage could explain the human cases with isolates carrying the incN-*qnrS1* plasmid that appeared in 2014 and the turkey meat, some of human cases of 2015 with the Col-*qnrB19* positive isolates. The fishmeal and the decanted water samples, also contaminated by Col-*qnrB19* strains, may indicate the possible contamination of the environment by Moroccan poultries. Interestingly, fishmeal has been a major component of industrialised poultry feed [29]. Thus, to explain this contamination of different food chains, further environmental studies in Moroccan flocks are needed to highlight the potential cross-contamination/transmission mechanisms. The Col-*qnrB19* and incN-*qnrS1* types of plasmids have been widely described in *E. coli* and *Salmonella* from animals, the environment and humans worldwide [30] but IncN-pST7 plasmid has never been reported to date.

We compared the exposures of controls to the exposures of cases divided into two different groups according to the two plasmids' distribution in their *S.* Chester strains. However, this analysis did not reinforce existing associations or highlight any new association between an exposure and the *S.* Chester infection (data not shown). Only 50% of cases could be explained by self-reported consumption of shrimp. This low proportion might be due to the recall period bias or to the fact that shrimp is a stealth food vehicle used in many common dishes (salads, pizza, sauce). No association between poultry (chicken and turkey) consumption and *S.* Chester infection could be identified, probably because chicken is widely consumed by the population (79% of the cases and 85% of the controls in our study) and due to the low power of our study. Poultry and seafood are very commonly implicated in *Salmonella*-related food-borne outbreaks [31,32]. Furthermore, seafood and chicken meat have been identified, along with beef, as products most involved in the spreading of *Salmonella* in Morocco [33]. *Salmonella* contamination of the Moroccan coast, between 2002 and 2005, has already been shown in previous studies [34,35]. Moreover, according to the RASFF database, various serotypes of *Salmonella* were found 27 times in fishmeal from Morocco during the period ranging from January 2010 to June 2015 (at least six *S.* Chester) [13].

The number of reported *S.* Chester cases in affected EU countries decreased after week 37 (mid-September) 2014 probably because most travellers came back from Morocco before the beginning of the school year. Indeed, we observed a new increase in number of *S.* Chester cases in September 2015 with 55 cases (at least 16 with travel history to Morocco) in France, 36 cases in Belgium, seven cases in Spain and four cases in Denmark. In 2016 in France we observed an increase again with 70 cases on the same period (between April and October), 16 had travel history to Morocco. Retrospectively, we also observed in France a slight increase in numbers of *S.* Chester cases during the summer 2013 with 14 cases during the period August–September 2013 compared with four in 2012 for the same period. The hypothesis that there are persistent sources of contamination in Morocco but also, to a lesser extent, in other West African countries is raised. In case of persistent sources and if no control measure is taken in Morocco a new increase could be observed every summers.

There were several limitations to our investigation. First, we observed that symptom onset of most of cases occurred at the end of their stay in Morocco or after they were back in France. The investigation probably missed cases who were sick during their stay and whose symptoms were already resolved before returning to France. Moreover, cases did not accurately represent all French tourists visiting Morocco, as only those seeking healthcare in France and that were tested for *Salmonella* were identified. Nevertheless, this should not affect the ORs, as the same limitations pertain to controls. We selected controls among other non-typhoidal *Salmonella* cases with other serotypes and matched them by age, travel to Morocco and exposure period. This could result in 'overmatching' and as a consequence lead to underestimating our ORs. One possible drawback of this design is that the aetiological exposures were different between serotypes, which could lead to false associations. Among our control group, nine different serotypes were included which

would reduce this risk. The advantage of this design, in comparison with healthy people as controls, was the likely reduction of the recall bias, as ill people tend to recall different food exposures more accurately [36]. The case–case study design was already successfully used in several studies [37,38]. Unfortunately, we could not perform a multivariate analysis due to the small number of interviewed cases and controls. Finally, to our knowledge no environment and food investigations were conducted in Morocco.

The source(s) of this outbreak was located in Morocco, making it more difficult to investigate than an outbreak with a source in a EU country. However, the multinational collaboration was very helpful to share information for both the epidemiological and microbiological investigations. In this context, EPIS was very a useful tool. This kind of collaboration should be promoted and reinforced in case of outbreaks affecting several countries and occurring at a holiday destination. Specific recommendations for this outbreak were not taken because the risk posed by *Salmonella* in Morocco was already known and prevention and information messages already broadcasted.

In conclusion, this outbreak is probably a multi-source outbreak with several contaminated foods and likely also food chains. Chicken and shrimp in Morocco could be one of the sources of this outbreak. We recommend continuing collaboration and communication at EU level, in particular to report cases or new outbreaks through EPIS, and also to reinforce collaboration with Moroccan health authorities. Local epidemiologists could be involved in investigating such events in the field.

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Conflict of interest

None declared.

Authors' contributions

LFo, NJD designed, conducted and analysed the epidemiological investigations. LM, IF, CVM, CMG and ES participated to the epidemiological study. SLH, LFa and FXW designed, conducted and analysed the microbiological investigations. PA, JM, MT, KG, LF and TD participated to the WGS and exchanged sequences. MT, WM, SB, SHL performed PFGE and exchanged strains. BB, AB and EV participated to the food traceback linked to Morocco and exchanged strains. LFo, NJD and SLH wrote the MS. All authors commented and agreed upon the final manuscript.

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Research article

Inference and forecast of H7N9 influenza in China, 2013 to 2015

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The recent emergence of A(H7N9) avian influenza poses a significant challenge to public health in China and around the world; however, understanding of the transmission dynamics and progression of influenza A(H7N9) infection in domestic poultry, as well as spillover transmission to humans, remains limited. Here, we develop a mathematical model–Bayesian inference system which combines a simple epidemic model and data assimilation method, and use it in conjunction with data on observed human influenza A(H7N9) cases from 19 February 2013 to 19 September 2015 to estimate key epidemiological parameters and to forecast infection in both poultry and humans. Our findings indicate a high outbreak attack rate of 33% among poultry but a low rate of chicken-to-human spillover transmission. In addition, we generated accurate forecasts of the peak timing and magnitude of human influenza A(H7N9) cases. This work demonstrates that transmission dynamics within an avian reservoir can be estimated and that real-time forecast of spillover avian influenza in humans is possible.

Introduction

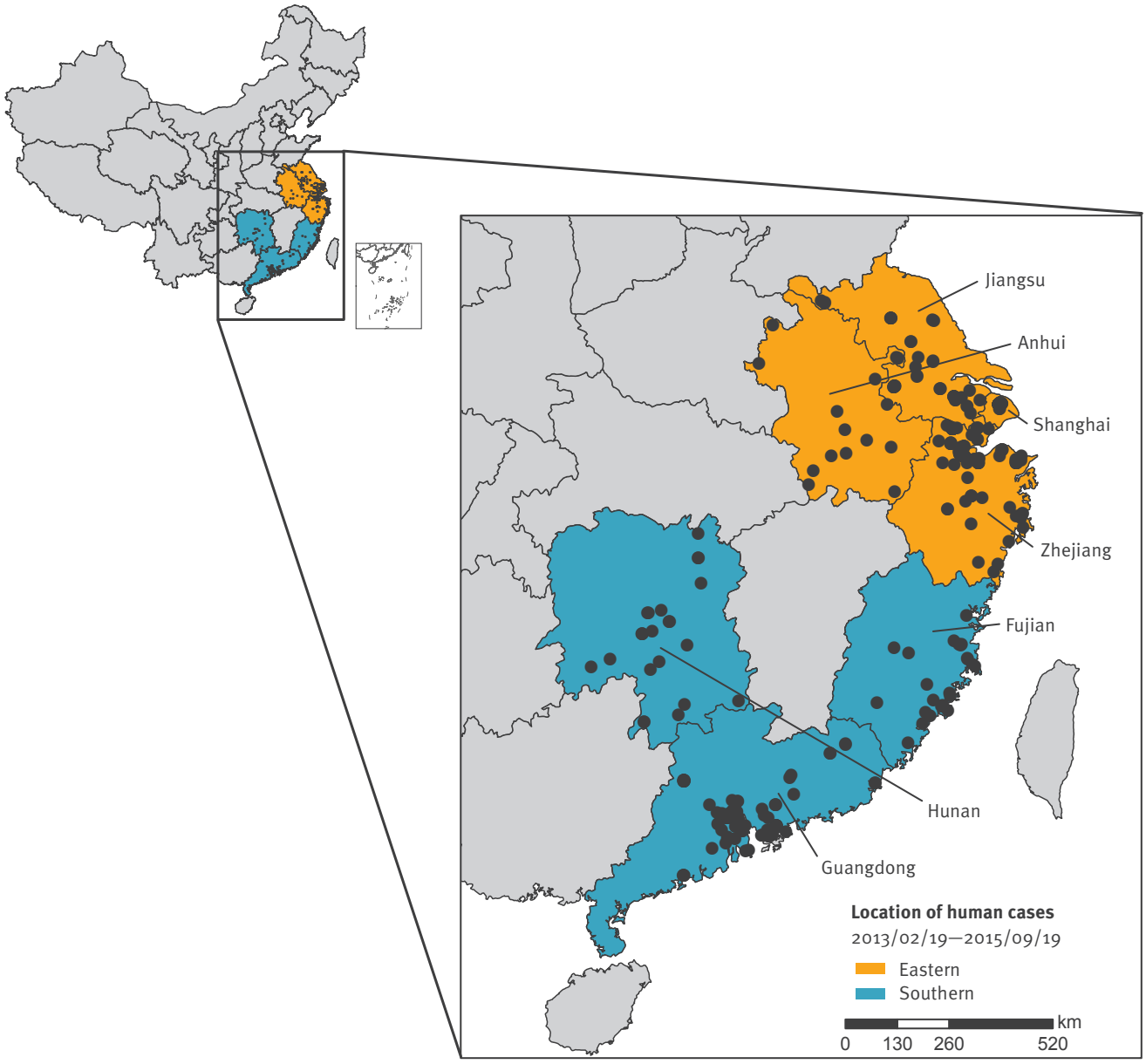
Wild birds, particularly *Anseriformes* and *Charadriformes*, are thought to be the principal natural reservoir of low pathogenic avian influenza (LPAI) viruses [1,2], as well as the source of influenza A viruses infecting all other animals [3]. Indeed, LPAI includes nearly all influenza subtypes, and wild bird migration can bring viruses to new areas and species [1,4]. The LPAI A(H7N9) virus was first identified in humans in China in early 2013 [5]. As at 15 October 2015, 678 confirmed human infections have been documented, with a case fatality rate of ca 40% [6]. The virus most probably originated in wild bird populations [7,8], was introduced into domestic ducks and chickens and has since become well established in poultry populations in south-eastern China [6]. Transmission to humans occurs primarily at live bird markets (LBMs), where direct contact between humans and infected poultry leads to spillover transmission [9].

Human influenza A(H7N9) infections have been well documented by the Chinese government and public health authorities. Outbreaks of human influenza A(H7N9) cases peak in winter months [10] and geographical diffusion from the eastern to the southern region of China has been observed [11]. As is true for most LPAI viruses, influenza A(H7N9) does not produce significant illness in domestic poultry, implying that poultry can be infected asymptomatically [6]. Consequently, poultry infections are likely to be under-reported even though LBMs are being closely and actively monitored [12,13]. This limited, partial observation of influenza A(H7N9) infection in poultry poses a challenge to the study and quantification of the transmission potential of H7N9 viruses in poultry populations, as well as spillover transmission from poultry to humans. However, owing to the transmission link between influenza A(H7N9) infection in poultry and human infection through LBMs [9], and because human influenza A(H7N9) cases have been well documented, these human cases serve as a sentinel proxy for infection rates among domestic poultry.

Mathematical approaches can be used to infer critical epidemiological processes and parameters. Traditional methods of epidemic curve fitting regard the increase in cumulative cases as an exponential with set doubling times [14]. This approach uses surveillance data during the early exponential growth period of an outbreak to provide retrospective estimates of R_0 [15,16]. However, these estimates rely on specific assumptions, such as the initial susceptibility of the population and the infectious period. In contrast, a Bayesian approach [17] can provide continuous estimation of all system parameters without specific assumptions and is therefore more suitable for nonlinear epidemic modelling. In

Figure 1

Spatial distribution of human influenza A(H7N9) cases and classification of study regions, China, 19 February 2013–19 September 2015 (n = 526)



The human influenza A(H7N9) cases (black dots indicate case locations) in the study area are grouped into the eastern region (Jiangsu, Zhejiang, Shanghai and Anhui provinces: pink) and the southern region (Guangdong, Fujian and Hunan provinces: blue). These cases are shown in the whole map of China and the enlarged map of the study area. Also shown is the South Sea but with no human cases.

previous work, we used Bayesian inference methods to infer disease transmission dynamics, estimate critical epidemiological parameters, and generate forecasts of seasonal and pandemic human influenza (i.e. H1N1, H3N2, B) in both temperate [18-20] and subtropical regions [21].

Here we used human case data and a combined framework of mathematical model and Bayesian inference to simulate influenza A(H7N9) virus transmission among poultry and generate retrospective forecasts of influenza A(H7N9) incidence for both poultry and humans in the eastern and southern regions of China (Figure 1).

Specifically, human influenza A(H7N9) case data in the period from 2013 to 2015 were used in conjunction with a model-inference framework that combines a susceptible-infected-recovered (SIR) compartmental model of influenza A(H7N9) virus transmission among poultry and the ensemble adjustment Kalman filter (EAKF) to simulate influenza A(H7N9) virus transmission among poultry, estimate critical epidemiological parameters,

and generate forecasts of influenza A(H7N9) infections for both humans and poultry.

Methods

Data

From 19 February 2013 until 19 September 2015, a total of 526 human influenza A(H7N9) cases were extracted from official reports of the National Health and Family Planning Commission (NHFPC) in China. Associated record attributes included location, observation and reporting date, and descriptive information including age, sex and contact history.

These records were processed into biweekly counts during the 2012/13, 2013/14 and 2014/15 seasons and aggregated into two spatial regions, the southern region (Guangdong, Fujian and Hunan provinces) and the eastern region (Jiangsu, Zhejiang, Shanghai and Anhui provinces). This spatial grouping was based on the geographical location, common sources for poultry and virus spatial transmission patterns among the provinces. Specifically, provinces in the same region are geographically conjoined, and influenza A(H7N9) virus appeared to diffuse from the eastern region, where chicken farming and consumption occur locally, to the southern region where chickens are imported from northern China (e.g. Hebei and Shandong provinces).

Description of the epidemical model

The epidemical model used for this study simulates the transmission of influenza A(H7N9) among poultry as well as spillover transmission from poultry to humans. The model is described by the following equations:

(1)

$$\frac{dS}{dt} = -\frac{\beta I_c S}{N}$$

(2)

$$\frac{dI_c}{dt} = -\frac{\beta I_c S}{N} - \frac{I_c}{D}$$

(3)

$$I_h = \frac{I_c}{\gamma}$$

where *S* is the number of susceptible poultry, *Ic* and *Ih* are the number of infectious poultry and humans, respectively, *N* is chicken population size, β is the contact rate among poultry, *D* is the mean infectious period, and γ is the scaling factor linking the number of infected poultry with human infections. The basic reproductive rate, *Ro*, is calculated from the infection rate and mean infectious period as $Ro = \beta D$, while the effective reproductive rate is also determined from susceptibility as $Re = Ro\ S / N$.

This modelling framework was implemented with the assumption of homogenous mixing among chicken and human populations, indicating that spillover transmission from poultry to human was constant through time and that no transmission among humans occurred. In essence, we used human influenza A(H7N9) case data as a proxy for infection among poultry. We took this approach because infections among poultry are likely to be greatly under-reported and human influenza A(H7N9) incidence data are much more reliable.

Description of the ensemble adjustment Kalman filter

The EAKF is a sequential Monte Carlo, or data assimilation, method that is used to iteratively update the model state variables and parameters with each new observation [22]. This update follows Bayes' rule:

(4)

$$p(Z_t|y_t, y_{t-1}, \ldots) \alpha\, p(y_t|Z_t) p(Z_t|y_{t-1}, \ldots)$$

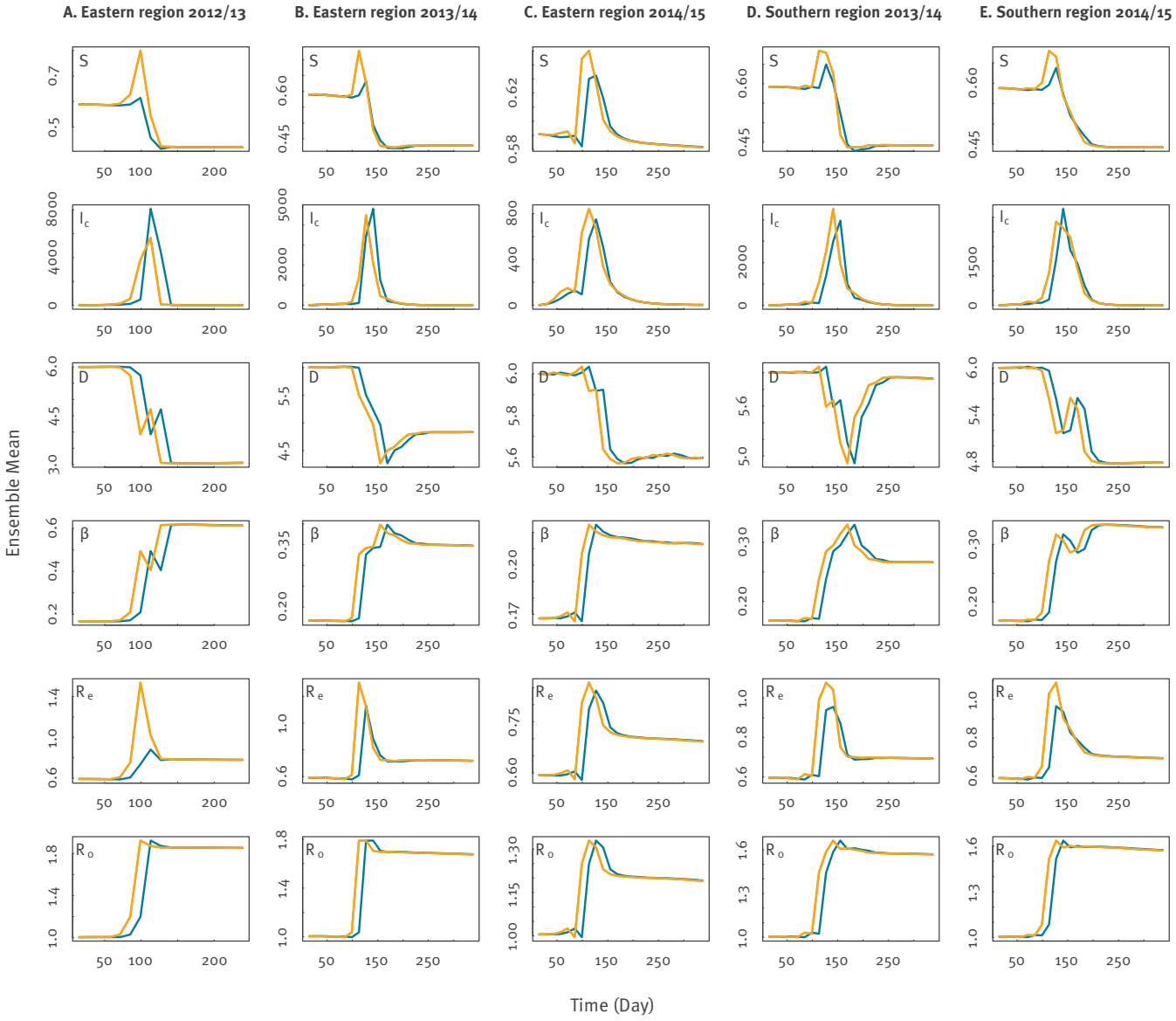
where *Zt* is the system state, including model variables and parameters *S*, *Ic*, *Ih*, *D*, *Ro*, and *yt* is the observation at time *t*. Formula 4 shows that the updated (i.e. posterior) probability distribution is proportional to the product of the likelihood of the occurrence of new observations given the current system state and the prior probability distribution of the system state. The EAKF uses an assumption of normality for the likelihood and prior distribution. In doing so, only the first two statistical moments are needed to characterise the distributions on the right hand side of Formula 4.

The EAKF was selected for iterative Bayesian inference in this work because it was already being used for state space estimation in the geosciences (e.g. climate and weather simulation and prediction) and also in conjunction with influenza state space models to generate seasonal influenza forecasts [18,19,21].

All simulations of influenza A(H7N9) transmission and incidence among poultry and spillover transmission to humans with the model-inference system (i.e. the SIR dynamic model and EAKF inference) were run using a 300-member ensemble of simulations. These simulations were run simultaneously and linked through the EAKF. Before integration with the model equations, each simulation (i.e. ensemble member) was randomly assigned an initial combination of state variables and parameters from specified uniform distributions (see below). These comprised the initial conditions, or 'initial prior', for each simulation before integration. Each initialised ensemble member was then integrated through time using the equations of the model; as each simulation has a different initial array of state variables and parameters, the trajectory of each simulation differs.

Figure 2

Parameter dynamics of H7N9 influenza across seasons for the eastern and southern region, China, 2012–2015



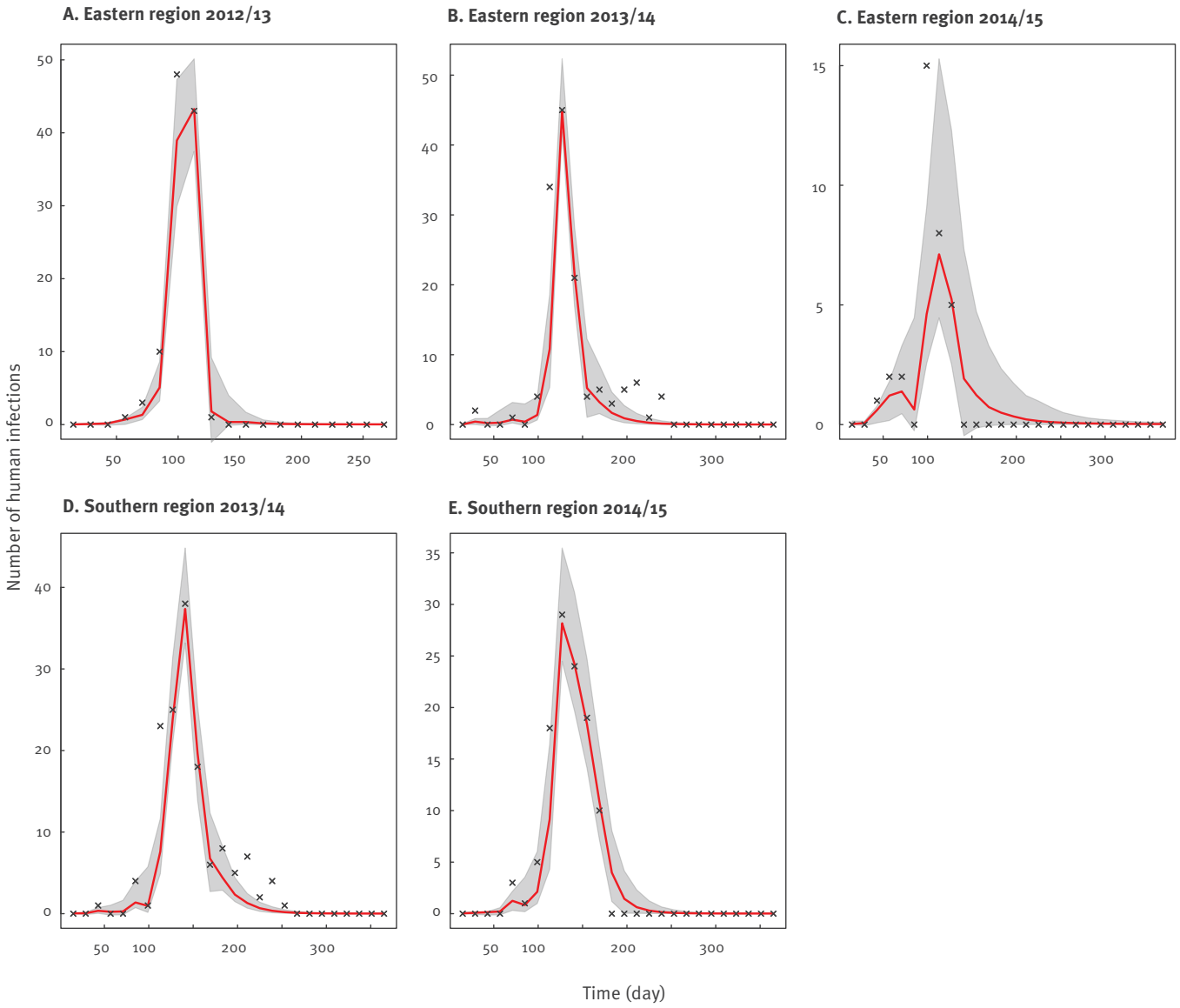
Time series of 300-member ensemble mean posterior (red) and prior (blue) for key epidemiological variables (susceptibility (S) and the number of infections in chicken (Ic)), and for parameters (the infectious period (D), chicken-to-chicken contact rate (β) and the effective and basic reproductive rate (Re and Ro)), during different seasons and regions. In each season, variables and parameters were adjusted from the beginning of the season to the two-week period with the last recorded case. Both the prior and posterior mean estimates are shown; EAKF adjustment at a given time is the difference of the mean posterior minus the mean prior.

The ensemble was integrated until the time point of the first observation at which the run was halted and the EAKF algorithm and observation were used to update the ensemble mean and variance of the observed state variable (here incidence) according to Formula 4, as well as all the unobserved variables and parameters [19,22]. The conditions upon halting before the EAKF update are termed the 'prior'; the conditions after the EAKF update are termed the 'posterior'. The mean prior and posterior are averages across the ensemble; for example, the mean prior and posterior of susceptibility (S) is simply the ensemble average value of S before and after EAKF updating at a particular point in time.

The use of an ensemble of simulations provided an easy means of estimating credible intervals and uncertainties, both for parameter estimates and forecasts. Indeed, for the EAKF, the prior and posterior moments (i.e. mean and variance) can be calculated directly from the average prior and posterior estimates of all the 300 ensemble members.

Figure 3

SIR-EAKF simulations of human H7N9 influenza across seasons and regions, China, 2012–2015



300-member ensembles are simulated and repeated 10 times for each season. Initial conditions were varied with each repetition. The ensemble mean prior (blue) and posterior (red) estimates and the corresponding 5th and 95th percentiles of the ensemble posterior estimates of human infections were averaged over all 10 runs and compared with observed case numbers (black). The area between the 5th and 95th percentiles of prior and posterior estimates is shaded blue and grey, respectively.

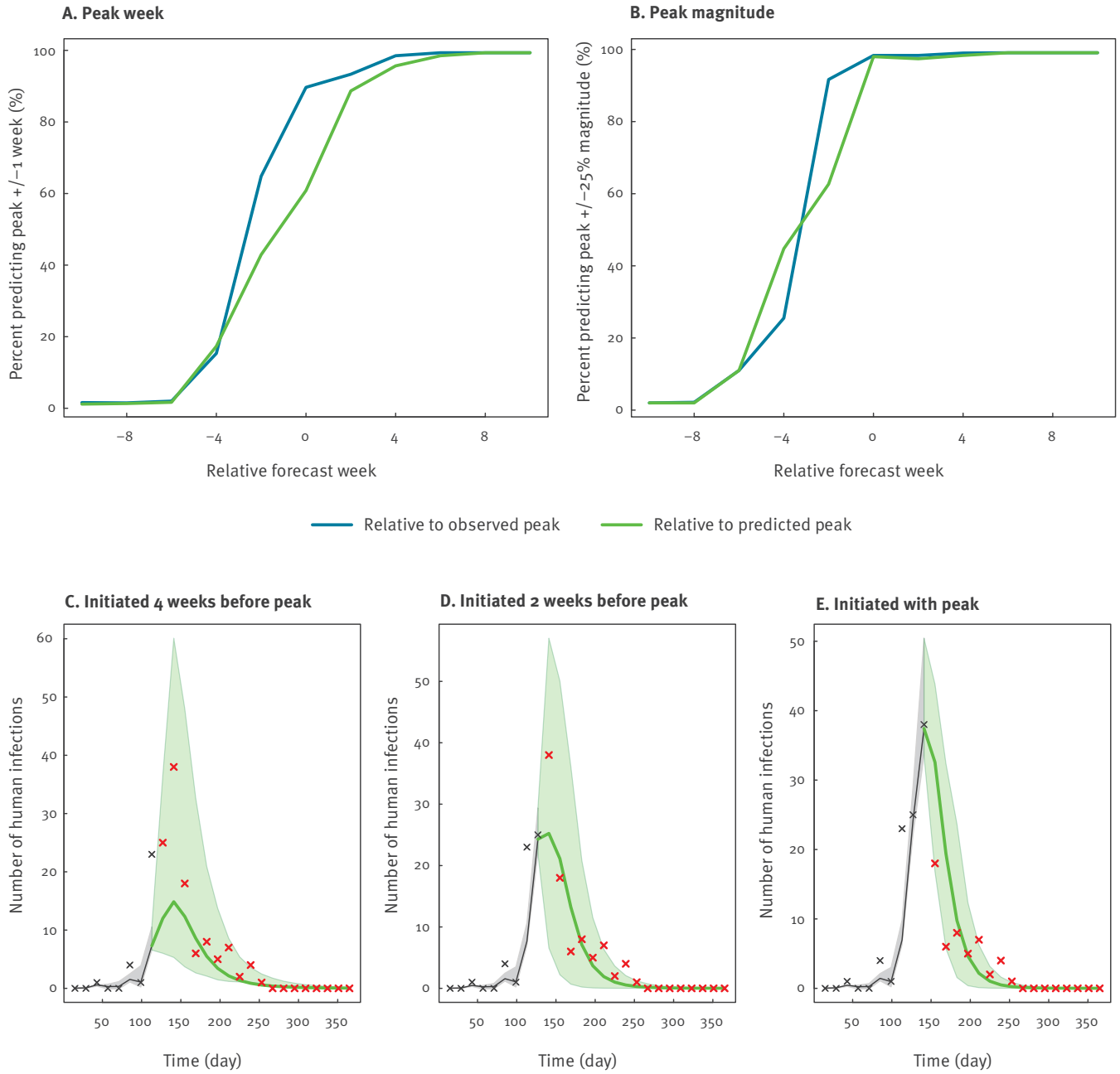
The described cycle of integration and adjustment was repeated for each successive observation, i.e. after updating, the posterior was integrated through time until the next observation, at which point it became the prior. Then the EAKF and observation were used to generate a new posterior. Through this iterative updating process, the estimates of the state variables and parameters converge to a combination capable of simulating the outbreak as observed up to that point. The intention was that by optimising the model to simulate conditions as observed from the past to present, a better forecast of the future can be generated using that optimised ensemble of simulation.

Initialisation and simulation with the SIR-EAKF framework

The state variable-parameter vector of the SIR-EAKF framework included optimisation of three variables (*S*, *Ic* and *Ih*) and two parameters (*D* and *Ro*). At the beginning of each outbreak, we initialised each simulation (i.e. each ensemble member) using a random selection from uniform ranges of the parameters and variables ($2 < D < 10$ days, $0.01 < Ro < 2.0$, $0.5 < So < 0.6$, $0 < Ico < 250$). These initial uniform ranges were based on prior modelling efforts simulating and forecasting human seasonal influenza [18,20]. In addition, as the transmission potential of influenza A(H7N9) virus among poultry is not well described, a broad initial prior

Figure 4

Forecast accuracy for all seasons and example forecasts of H7N9 influenza in the southern region, China, 2013/14 season



Top panels: Accuracy was measured as the percentage of ensembles predicting (A) the week with the most human influenza A(H7N9) cases within one week of the observed peak week and (B) the peak magnitude of human influenza A(H7N9) cases within 25% of the observed peak magnitude. It is presented as a function of the forecast week relative to observed (blue) and predicted (green) peak timing.

Bottom panels: Forecasts initiated (C) 4 weeks, (D) 2 weeks and (E) 0 weeks ahead of the observed peak week for the southern region during the 2013/14 season. The SIR model was recursively optimised up to the week of forecast initiation using observations (black x) and the EAKF; the red x are future observations which were not used in the model optimisation. The black and green lines are the mean trajectory of the ensemble and areas shaded grey and green are the 5th and 95th percentiles of the ensemble posterior for simulation and forecast periods, respectively.

range for *R*o was used; however, note that the EAKF in the presence of observations can adjust the model parameters and variables to values outside these initial ranges. A Latin hypercube sampling approach was used to generate a near-random initial prior sample across this multidimensional distribution of parameter and variable values.

Multiplicative inflation was used to increase the ensemble variance of all model variables and parameters by 2% before EAKF adjustment. Inflation is commonly applied to ensemble Kalman filters in order to avoid 'filter divergence', the situation in which the variance across the ensemble of simulations has contracted so much that the EAKF updating algorithm

Table 1

Estimates of key epidemiological parameters and variables for H7N9 influenza, China, 2012–2015

Region	Season	R_e maximum (IQR)	R_0 at maximal R_e (IQR)	D at maximal R_e (IQR)	β at maximal R_e (IQR)	S maximum % (IQR)
Eastern (Jiangsu, Zhejiang, Shanghai, Anhui)	2012/13	1.56 (1.53–1.59)	1.94 (1.92–1.96)	3.95 (3.76–4.13)	0.49 (0.47–0.51)	80.72 (79.34–82.47)
	2013/14	1.34 (1.30–1.38)	1.81 (1.79–1.83)	5.69 (5.37–6.05)	0.32 (0.30–0.33)	73.98 (72.41–75.51)
	2014/15	0.86 (0.84–0.87)	1.32 (1.31–1.33)	5.94 (5.90–6.02)	0.22 (0.21–0.23)	64.86 (64.19–65.55)
Southern (Guangdong, Fujian, Hunan)	2013/14	1.08 (1.04–1.09)	1.59 (1.56–1.63)	5.60 (5.41–5.69)	0.28 (0.27–0.29)	69.45 (68.80–70.37)
	2014/15	1.06 (1.05–1.07)	1.62 (1.61–1.64)	5.29 (5.18–5.43)	0.31 (0.30–0.32)	68.94 (67.01–70.54)

IQR: interquartile range.

The posterior means and IQR of the number of chicken infections (I_c), chicken-to-chicken contact rate (β), the infectious period (D) and the basic reproductive rate (R_0) were estimated at maximal epidemic forcing (maximal R_e). The level of initial susceptibility (S) was defined and estimated in the two-week period with maximal susceptibility.

effectively ignores the observations and model simulations diverge from the truth [22]. The 300-member ensemble simulations were repeated 10 times each season to account for stochastic effects due to the random selection of initial conditions. The average of the 10 repeated runs, each made up of a 300-member ensemble simulation, was used to derive mean posterior estimates of the model parameters.

Parameter estimation

Several epidemiological parameters are critical for characterising the transmission potential of infectious diseases. The basic reproductive number *R*o, defined as the number of secondary infections an infectious host would produce in a completely susceptible population, signals the potential of an infectious agent to start an outbreak as well as the transmissibility of a virus in the absence of intervention. The effective reproductive number *R*e quantifies the transmission force during the actual outbreak and can be used to monitor the impact of control strategies. An *R*e›1 indicates epidemic growth, while an *R*e‹1 indicates that sustained transmission cannot persist and that an outbreak will subside.

Epidemiological parameters, namely β, D, *Ro* and *Re*, were estimated for each of three seasons and two regions from the start of the season to the last two-week period with a recorded case. In a given season, the posterior mean and interquartile range of *Ic*, *Ih*, β, *D*, Ro and Re were estimated at the time of maximal epidemic forcing or the time point of highest transmission potential, i.e. the two-week period with the highest effective reproductive number. The level of initial susceptibility, however, was defined as and estimated for the two-week period with maximal susceptibility. We have previously presented parameter estimates at these key time points in studies of seasonal influenza [18,20]. The prior and posterior means during each outbreak for each variable and parameter were also recorded (Figure 2).

Parameter estimate changes during the entirety of an outbreak were used to inspect filter adjustment. Such parameter changes over time may reflect changes in the estimation or actual changes to the parameter values. For the former, the observations contain noise and the estimation of the parameters by the EAKF is neither perfect nor instantaneous; consequently, the parameter estimates move through time. For the latter, actual shifts in parameter value can occur, e.g. representing changing contact rates and control measures, as the pathogen moves through different subpopulations and/or geographical areas.

Sensitivity analysis

The parameter estimates were inferred using a scaling γ, representing a rate of spillover transmission from chicken to human, equal to 300. This value was selected following tests with γ ranging from 100 to 1,000 in increments of 100. For each value of γ, mean human case forecast error was used to calculate total outbreak root mean squared error (RMSE) and correlation, as well as attack rate error, peak weak error and peak magnitude error between observations and the predicted estimates. A ranking approach was used to identify the scaling with the lowest error. Specifically, for each metric (RMSE, correlation, attack rate error, peak weak error and peak magnitude error), the scaling levels were ranked. The scaling with the highest overall rank, i.e. $\gamma = 300$, was selected and used in all simulations and forecasts presented here.

Retrospective forecasts

Retrospective forecasts were run for the seasons 2012/13, 2013/14 and 2014/15 for the eastern region and for the last two seasons for the southern region. The model-inference system was again implemented

Table 2

Forecast accuracy for H7N9 influenza in all seasons, China, 2013–2015

	Relative forecast lead time (weeks)										
	−10	−8	−6	−4	−2	0	2	4	6	8	10
Proportion predicting peak ± 1 week (%)											
Relative to observed peak	1.59	1.50	2.00	15.33	64.84	89.67	93.33	98.50	99.34	99.34	99.34
Relative to predicted peak	1.17	1.33	1.67	17.33	42.92	60.83	88.67	95.67	98.50	99.34	99.34
Proportion predicting peak ± 25% magnitude (%)											
Relative to observed peak	2.00	2.17	11.00	25.50	91.67	98.33	98.33	99.00	99.08	99.08	99.08
Relative to predicted peak	2.00	2.00	11.00	44.75	62.67	98.00	97.42	98.33	99.08	99.08	99.08

Accuracy was measured as the percentage of ensembles predicting the week with the most human cases of influenza A(H7N9) within ± 1 week of the observed peak week and the peak magnitude of human H7N9 influenza cases within ± 25% of observed peak magnitude. The values are the same as those in Figure 4 and presented as a function of the forecast lead time from 10 weeks before to 10 weeks after the observed and predicted peak timing.

using 300-member ensembles and reinitialised with randomly selected variable and parameter combinations at the beginning of each season. All simulations and forecasts were repeated 10 times for each outbreak and were initialised with a random selection of parameter and variable values, as described above. Forecasts were generated beginning with the two-week period of the first recorded case and repeated every 2 weeks following the generation of a new posterior. Specifically, for the eastern region, separate ensemble forecasts were run from the 4th to 9th, 2nd to 17th and 3rd to 9th two-week period for the 2012/13, 2013/14 and 2014/15 seasons, respectively; for the southern region, forecasts were generated from the 3rd to 19th and 5th to 12th two-week period for the last two seasons.

To evaluate the accuracy of our SIR-EAKF system, we determined two measurements: the peak week and peak magnitude, or the percentage of ensemble mean trajectories predicting human influenza A(H7N9) case peak timing within ± 1 week of the observed peak week, and peak magnitude within ± 25% of the observed peak magnitude. These two indices were then plotted as a function of the relative forecast week, i.e. the week of forecast generation minus either the observed or predicted peak week, to show the relationship between predictive skill and lead time.

The combined SIR-EAKF system was coded in R. These codes are available from the corresponding author upon request.

Results

The mean posterior estimates of human influenza A(H7N9) incidence produced by the model-inference system matched the observed influenza A(H7N9) human case counts well (Figure 3).

These simulations captured the timing and magnitude of the epidemic. Mean posterior estimates of *Ro* ranged from 1.327 to 1.941 (Table 1) with the highest and lowest estimates occurring in seasons with the largest and smallest numbers of human cases, i.e. the 2012/13 and 2014/15 seasons in the eastern region, respectively. The mean infectious period *D* was estimated at 5 to 6 days for outbreaks during the seasons 2013/14 and 2014/15. For the first human influenza A(H7N9) outbreak in 2012/13 in the eastern region, the estimate for *D* was much lower (mean: 3.95 interquartile range (IQR): 3.76–4.13) and the estimate of β, the contact rate among poultry, was higher (mean: 0.49/day; IQR: 0.47–0.51/day).

The susceptibility of the chicken population was high in earlier outbreaks and dropped to around 65% in more recent outbreaks. For the effective transmission number *Re*, which quantifies the transmission force during the outbreak, the mean posterior estimates were greater than 1 during four of the five outbreaks analysed here, indicating a clear transmission potential among LBM poultry. The *Re* estimate was highest during the initial outbreak in 2012/13 when the two associated parameters, *Ro* and susceptibility, were also highest. The scaling factor γ, selected by the rank correlation approach (see Methods) mapped the observed human cases to simulated poultry infections and indicated that the daily poultry-to-human spillover transmission rate was low, around 3.3 × 10-3 per infected LBM chicken.

Estimates of all parameters remained stable during the seasons 2013/14 and 2014/15 in the southern region where outbreaks were of similar severity in both epidemic waves. However, there was an apparent decrease in *Ro*, *Re*, β and susceptibility from the first to the third outbreak in the eastern region, which was in accordance with the change of outbreak severity in this region.

The accuracy of the forecast for peak timing and magnitude increased as the week of forecast initiation got closer to the observed and predicted peak (Figure 4).

Specifically, the percentage of forecasts predicting the peak week within ± 1 week increased sharply from 6 weeks ahead of the observed peak week and reached 90% when a forecast was generated at the observed peak. For peak magnitude, the percentage of forecasts predicting the peak magnitude within ± 25% of the observed magnitude increased from 8 weeks before the observed peak, and almost all forecasts were accurate when predicting at the observed peak. However, as knowledge of the observed peak was unavailable for real-time forecasting, we also present overall accuracy as a function of predicted lead time. Here, the accuracy was 43% and 63% at 2 weeks lead time and 61% and 98% at 0 weeks lead time for peak timing and magnitude, respectively (Table 2). Example forecasts are also presented in Figure 4.

Discussion

Our findings indicate that data assimilation methods and a simple epidemic model can be combined to infer the transmission dynamics of H7N9 influenza in both chicken and human populations using only human infection data. Moreover, the model-inference system can produce accurate predictions of the peak timing and magnitude of human infections.

The estimated potential of chicken-to-human spillover transmission was low, even with the high transmission rate among poultry. Specifically, estimates of R_0 were greater than 1 and the mean contact rate among poultry was 0.326 across all seasons and regions, whereas the daily chicken-to-human infection rate reflected by the linkage parameter γ indicated that the mean number of human infections per infectious chicken was 3.3×10^{-3}. Our estimates of R_0, among poultry were similar to those of past pandemic influenza viruses in humans (e.g. 1.2–2.3 for influenza A(H1N1)pdm09) [23], which implies that influenza A(H7N9) has the potential to cause pandemics in chicken populations. This result is similar to earlier findings [24]; however, our estimates for three other parameters, the mean infectious period, the basic reproductive rate and the chicken-to-human infection rate, were smaller, which may be due to the finer spatial and longer temporal scales used in this study, as well as the difference in modelling approach. Specifically, our study used a dynamic model, Bayesian inference framework and regional bi-weekly counts of human infections, covering three epidemic waves. Our findings thus represent more detailed, localised and long-term patterns of transmission dynamic than earlier work using least-square methods in conjunction with daily human infection data at the beginning of the outbreak at a national scale [24].

The dynamic patterns of influenza A(H7N9) differed in the two regions studied here, although with the limited number of outbreaks available for validation, these differences must be interpreted with caution. The transmission potential among chicken flocks and initial susceptibility decreased across three seasons in the eastern region, but remained stable in the southern region. These differences were dynamically consistent with observed outbreak severity in both regions and may have been caused by a difference in control methods implemented by the government. In the eastern region, approaches such as closing of LBMs [25,26] and halting live poultry trade were implemented during the early stages of the outbreaks. This probably reduced chicken-to-human exposure and chicken-to-chicken mixing and consequently may have attenuated the severity of the outbreak. On the other hand, for southern provinces such as Guangdong (where LBM closure was implemented later, in the second half of February 2014), co-circulation of a diverse array of avian influenza subtypes as well as multiple strains of H7N9 and H9N2 influenza viruses has been documented. This abundance of viruses creates an environment primed for influenza reassortment, resulting in diversified and more adaptive genotypes and a higher risk of infections in both poultry and humans [27,28] and may therefore keep susceptibility high and stable across seasons.

The mean estimate of D, the mean infectious period, for the 2012/13 outbreak in the eastern provinces was lower (3.95) than for the later outbreaks, which ranged from 5.29 to 5.94. Given the limited number of total outbreaks investigated, the exact causes for this difference are difficult to pinpoint; however, factors could include actual changes to the virus between the first and later outbreaks, errors in the observed number of cases or errors in the estimation process. That the 2013/14 and 2014/15 outbreaks yielded consistent estimates, including similar values for D and β, and decreasing maximal S over time suggests that these findings are credible.

Our inference and forecasting framework was implemented with a simple SIR model and the assumption of homogeneous mixing among human and chicken populations, i.e. a constant chicken-to-human transmission rate. Our model only simulated chicken-to-chicken and chicken-to-human transmission (Formulas 1–3) and did not consider environmental transmission. Given the limited data on infection and transmission among poultry, inferred distinctions of alternate transmission modes, i.e. chicken-to-chicken vs environment-to-chicken, are likely to be poorly constrained. Further, prior attempts to simulate these different pathways suggest that the rates of chicken-to-environment shedding are low [29]. Loss of immunity was not modelled either, as birds are either slaughtered or, when infection is suspected, culled, as required by the Chinese government [30].

Despite these shortcomings, the combined model-inference system matched the observations well, and provided sensible estimates of key epidemiological parameters, including rates of chicken-to-human spillover infection. The analyses revealed the transmission potential of H7N9 influenza among poultry, the stability and changes of that transmission potential over time, and that real-time forecasting of influenza

A(H7N9) incidence in both human and poultry is possible. In the future, such methods could be applied in real time to newly emerged avian influenza subtypes.

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Conflict of interest

JS discloses partial ownership of SK Analytics.

Authors' contributions

RL and JS designed research; RL, BX, YB, JC and XZ compiled the data; RL, SK and AH performed the simulations; RL, JS, SK, and AH analysed data; RL and JS wrote the paper.

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