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Possible pandemic threat from new reassortment of influenza A(H7N9) virus in China

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Avian influenza A(H7N9) virus re-emerged in China in December 2013, after a decrease in the number of new cases during the preceding six months. Reassortment between influenza A(H7N9) and local H9N2 strains has spread from China's south-east coast to other regions. Three new reassortments of A(H7N9) virus were identified by phylogenetic analysis: between A(H7N9) and Zhejiang-derived strains, Guangdong/Hong Kong-derived strains or Hunan-derived A(H9N2) strains. Our findings suggest there is a possible risk that a pandemic could develop.

Recent re-emerged influenza A(H7N9) virus infections in China – especially the rapid outbreak in Zhejiang province in December 2013, involving 60 cases [1] – have raised concerns. Although several reports described the genetic characteristics of the virus [2-4], little is known about its further evolution after the initial outbreak in March 2013 [2] and the current re-emergence. As of 31 January 2014, there were a total of 260 cases: 127 of these have occurred in 2014 [5,6]. Cases have been reported from Zhejiang, Guangdong and Jiangsu provinces, Shanghai metropolitan area and Hong Kong in 2014 [6].

It is important to know whether new variants or lineages of influenza A(H7N9) virus are responsible for this re-emergence of the virus. In this study, four lineages and three new reassortments of A(H7N9) virus were identified by phylogenetic analysis and DNA mutation analysis of the PB1 gene.

Sequences analysis of PB1 genes from influenza A(H7N9) virus isolates

We retrieved 72 PB1 gene sequences of influenza A(H7N9) viruses, isolated from 11 Chinese provinces and cities, from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) deposited from March 2013 to January 2014 (Tables 1 and 2). In particular, the most recent A(H7N9) virus isolates from Hong Kong were also retrieved, through GISAID (A/Hong Kong/5942/2013 in November 2013 and A/Hong Kong/734/2014 in January 2014). We carried out a Basic Local Alignment Search Tool (BLAST) search to acquire related reference sequences in the National Center for Biotechnology Information (NCBI) Influenza Virus Resource [7]. Multiple alignments of sequences of eight genes of A(H7N9) virus isolates (PB2, PB1, PA, HA, NP, NA, MP, NS) were made using Bio-Edit7.0 software. We then carried out a phylogenetic analysis using MEGA6.1, as previously described [8,9].

In order to generate a neighbor-joining tree, the statistical robustness of the tree and the reliability of the branching patterns were confirmed by bootstrapping (1,000 replicates) and the effective transmission linkage was supported by a bootstrap value over 80% at the tree node. In accordance with previous studies reporting the virus as a triple reassortant A(H7N9) [2-4], we also observed that all A(H7N9) virus strains analysed, including the latest strains from Hong Kong (Hong Kong strains 5942 and 734), were part of one large cluster in an HA and NA gene-derived neighbor-joining tree (data not shown). However, analysis of six internal genes originating from influenza A(H9N2) virus identified multiple effective A(H7N9) clusters in PB2, PB1, NP, MP gene-derived neighbor-joining trees. As previously described, there is frequent PB2-PB1-PA-NP co-segregation during avian influenza virus reassortment [10]. Clusters of A(H7N9) consistent with this were observed in PB2, PB1 and NP gene-derived neighbor-joining trees (data not shown). Therefore, we then performed further phylogenetic analysis of A(H7N9) and A(H9N2) PB1 gene sequences.

At least four distinct clusters of A(H7N9) virus isolates were identified in a PB1 gene-derived neighbor-joining tree by high bootstrap value (›80%) (Figure 1). Cluster 1 containing poultry- or human-derived A(H7N9) virus isolates represents the earliest infections (shown by the collection date in Tables 1 and 2) and covers the majority of A(H7N9) virus infections in 2013, while the

Table 1A

Information on influenza A(H7N9) viruses in four distinct clusters, China, March 2013–January 2014

Cluster	Isolate name	Location	Host	Collection date
	A/Shanghai/3/2013	Shanghai		2013-Feb-27
	A/Shanghai/4664T/2013	Shanghai		2013-Mar-5
	A/Anhui/1/2013	Anhui		2013-Mar-20
	A/Changsha/1/2013	Hunan		2013-Mar-22
	A/Hangzhou/1/2013	Zhejiang		2013-Mar-24
	A/Hangzhou/2/2013	Zhejiang		2013-Mar-25
	A/chicken/Anhui-Chuzhou/01/2013	Anhui		2013-Mar-29
	A/environment/Nanjing/2913/2013	Jiangsu	Envir	2013-Mar-29
	A/Jiangsu/01/2013	Jiangsu		2013-Mar-30
	A/Wuxi/1/2013	Jiangsu		2013-Mar-31
	A/Wuxi/2/2013	Jiangsu		2013-Mar-31
	A/chicken/Shanghai/017/2013	Shanghai	Chicken	2013-Apr
	A/chicken/Zhejiang/DTID-ZJU01/2013	Zhejiang	Chicken	2013-Apr
	A/environment/Wuxi/1/2013	Jiangsu	Envir	2013-Apr-2
	A/Environment/Shanghai/S1088/2013	Shanghai	Envir	2013-Apr-3
	A/Environment/Shanghai/S1438/2013	Shanghai	Envir	2013-Apr-3
	A/Environment/Shanghai/S1439/2013	Shanghai	Envir	2013-Apr-3
	A/pigeon/Shanghai/S1421/2013	Shanghai	Pigeon	2013-Apr-3
	A/pigeon/Shanghai/S1423/2013	Shanghai	Pigeon	2013-Apr-3
	A/Zhejiang/02/2013	Zhejiang		2013-Apr-3
	A/Zhejiang/DTID-ZJU01/2013	Zhejiang		2013-Apr-3
	A/environment/Hangzhou/34-1/2013	Zhejiang	Envir	2013-Apr-4
	A/Jiangsu/04/2013	Jiangsu		2013-Apr-5
1	A/Shanghai/9/2013	Shanghai		2013-Apr-8
	A/Jiangsu/09/2013	Jiangsu		2013-Apr-9
	A/Shanghai/10/2013	Shanghai		2013-Apr-9
	A/Jiangsu/06/2013	Jiangsu		2013-Apr-10
	A/chicken/Zhejiang/SD033/2013	Zhejiang	Chicken	2013-Apr-11
	A/Beijing/01-A/2013	Beijing		2013-Apr-12
	A/Anhui/02/2013	Anhui		2013-Apr-14
	A/chicken/Jiangsu/S002/2013	Jiangsu	Chicken	2013-Apr-16
	A/chicken/Jiangsu/SC035/2013	Jiangsu	Chicken	2013-Apr-16
	A/chicken/Jiangsu/SC537/2013	Jiangsu	Chicken	2013-Apr-16
	A/Duck/Anhui/SC702/2013	Anhui	Duck	2013-Apr-16
	A/wildpigeon/Jiangsu/SD001/2013	Jiangsu	Pigeon	2013-Apr-17
	A/homingpigeon/Jiangsu/SD184/2013	Jiangsu	Pigeon	2013-Apr-20
	A/Anhui/03/2013	Anhui		2013-Apr-21
	A/Taiwan/S02076/2013	Taiwan		2013-Apr-22
	A/Taiwan/T02081/2013	Taiwan		2013-Apr-22
	A/Fujian/01/2013	Fujian		2013-Apr-23
	A/Fujian/1/2013	Fujian		2013-Apr-24
	A/Taiwan/1/2013	Taiwan		2013-Apr-24
	A/Environment/Guangdong/C13281025	Guangdong	Envir	2013-Apr-26
	A/Environment/Guangdong/C13281030	Guangdong	Envir	2013-Apr-26
	A/environment/Fujian/SC337/2013	Fujian	Envir	2013-Apr-30
	A/Zhejiang/DTID-ZJU10/2013	Zhejiang		2013-Oct-14
	A/shanghai/05/2013	Shanghai		2013-Apr-2

Envir: environment.

Table 1B

Information on influenza A(H7N9) viruses in four distinct clusters, China, March 2013–January 2014

Cluster	Isolate name	Location	Host	Collection date
2	A/chicken/Zhejiang/SD007/2013	Zhejiang	Chicken	2013-Apr-22
	A/environment/Hangzhou/37/2013	Zhejiang	Envir	2013-Apr-4
	A/chicken/Hangzhou/48-1/2013	Zhejiang	Chicken	2013-Apr-10
	A/environment/Hangzhou/109-1/2013	Zhejiang	Envir	2013-Apr-12
	A/Hangzhou/3/2013	Zhejiang		2013-Apr-2
3	A/Guangdong/1/2013	Guangdong		2013-Aug-10
	A/Duck/Zhejiang/SC410/2013	Zhejiang	Duck	2013-Apr-16
	A/chicken/Shanghai/S1080/2013	Shanghai	Chicken	2013-Apr-3
	A/HongKong/5942/2013	Hong Kong		2013-Nov-30
	A/HongKong/734/2014	Hong Kong		2014-Jan-7
4	A/chicken/Shanghai/019/2013	Shanghai	Chicken	2013-Apr-4
	A/Pigeon/Shanghai/S1069/2013	Shanghai	Pigeon	2013-Apr-2
	A/chicken/Shanghai/S1076/2013	Shanghai	Chicken	2013-Apr-3
	A/Shanghai/13/2013	Shanghai		2013-Apr-10
	A/environment/Henan/SC232/2013	Henan	Envir	2013-Apr-24
	A/Environment/Henan/SD429/2013	Henan	Envir	2013-Apr-24
	A/Jiangxi/01/2013	Jiangxi		2013-Apr-24
	A/Nanchang/1/2013	Jiangxi		2013-Apr-24
	A/Hunan/02/2013	Hunan		2013-Apr-25
	A/Environment/Shandong/1/2013	Shandong	Envir	2013-Apr-27
	A/chicken/Jiangxi/SD001/2013	Jiangxi	Chicken	2013-May-3
	A/Environment/Shandong/SD038/2013	Shandong	Envir	2013-May-3
	A/Shandong/01/2013	Shandong		2013-Apr-21
	A/Environment/Shandong/SD049/2013	Shandong	Envir	2013-May-3
	A/Hunan/01/2013	Hunan		2013-Apr-24

Envir: environment.

other three clusters indicate close phylogenetic links between A(H7N9) and A(H9N2) strains.

Generally, identification of distinct transmission clusters should meet the following criteria: a phylogenetic clade supported by both high bootstrap values (>80%) in a neighbor-joining tree and a posterior probability value of 1 at the Bayesian tree node [11,12]. For this purpose, a Bayesian phylogenetic inference was subsequently performed to confirm the distinct clusters of A(H7N9) isolates using MrBayes 3.1 as previously described [8,9]. As expected, the same four clusters (100% probability; posterior probability=1) were also seen in the Bayesian tree (Figure 2), as well as in the neighbor-joining tree (Figure 1), which further identified the effective transmission linkages inside these clusters (Figure 2).

Characterisation of transmission clusters

To further characterise the four transmission clusters of influenza A(H7N9) virus isolates, the mutation sites of the viral PB1 gene sequences were highlighted using Nucleotide Sequences v2.2.3 (Figure3), revealing a distinct DNA mutation pattern of the four transmission clusters. Cluster1 shared the most common mutation sites with Shanghai-derived A(H7N9) strains, while all A(H7N9) strains from the other three clusters carried the most common mutation sites of their local A(H9N2) strains. The A(H7N9) strains of Cluster 2 carried the most common mutation sites of a Zhejiang-derived A(H9N2) strain, whereas the A(H7N9) strains in Clusters 3 and 4 had the most common mutation sites of Guangdong/Hong Kong-derived A(H9N2) and Hunan-derived A(H9N2) strains, respectively. These distinct DNA mutation patterns further identified new reassortments between A(H7N9) isolates and local A(H9N2) strains.

Phylogeographical trees of the influenza A virus PB1 gene sequences were constructed to further confirm the phylogenetic linkage of A(H7N9)and A(H9N2) virus strains using the BEAST V1.6.2 package as described previously [13,14]. The most recent common ancestor of the four clusters was estimated to be from

Table 2a

Origin of the influenza A(H7N9) viruses used for the analyses

Segment ID	Segment	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI447643	PB1	China	2013-Apr-14	A/Anhui/02/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI447636	PB1	China	2013-Apr-21	A/Anhui/03/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI439508	PB1	China	2013-Mar-20	A/Anhui/1/2013		WHO Chinese National Influenza Center	
EPI447836	PB1	China	2013-Apr-12	A/Beijing/01-A/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI477319	PB1	China	2013-Mar-22	A/Changsha/1/2013		Other Database Import	Zhang RS et al.
EPI447889	PB1	China	2013-Mar-29	A/chicken/Anhui-Chuzhou/01/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI443657	PB1	China	2013-Apr-10	A/chicken/Hangzhou/48-1/2013(H7N9)	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Li J et al.
EPI457875	PB1	China	2013-Apr-16	A/chicken/Jiangsu/S002/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457867	PB1	China	2013-Apr-16	A/chicken/Jiangsu/SC035/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457851	PB1	China	2013-Apr-16	A/chicken/Jiangsu/SC537/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457843	PB1	China	2013-May-03	A/chicken/Jiangxi/SD001/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI471846	PB1	China	2013-Apr-01	A/chicken/Shanghai/017/2013		Other Database Import	Yang D-Q et al.
EPI471847	PB1	China	2013-Apr-01	A/chicken/Shanghai/019/2013		Other Database Import	Yang D-Q et al
EPI457827	PB1	China	2013-Apr-03	A/chicken/Shanghai/S1076/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457795	PB1	China	2013-Apr-03	A/chicken/Shanghai/S1080/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI442719	PB1	China	2013-Apr-01	A/chicken/Zhejiang/DTID-ZJU01/2013		Other Database Import	Wu H et al.
EPI457763	PB1	China	2013-Apr-22	A/chicken/Zhejiang/SD007/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457747	PB1	China	2013-Apr-11	A/chicken/Zhejiang/SD033/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457739	PB1	China	2013-Apr-16	A/duck/Anhui/SC702/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457731	PB1	China	2013-Apr-16	A/duck/Zhejiang/SC410/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457723	PB1	China	2013-Apr-30	A/environment/Fujian/SC337/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI490969	PB1	China	2013-Apr-26	A/Environment/Guangdong/C13281025/2013	Guangdong Provincial Center for Disease Control and Prevention	Guandong Centers for Disease Control	
EPI490977	PB1	China	2013-Apr-26	A/Environment/Guangdong/C13281030/2013	Guangdong Provincial Center for Disease Control and Prevention	Guandong Centers for Disease Control	
EPI443673	PB1	China	2013-Apr-12	A/environment/Hangzhou/109-1/2013(H7N9)	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Li J et al.
EPI443570	PB1	China	2013-Apr-04	A/environment/Hangzhou/34-1/2013(H7N9)	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Jing-Cao P

Table 2b

Origin of the influenza A(H7N9) viruses used for the analyses

Segment ID	Segment	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI443649	PB1	China	2013-Apr-04	A/environment/Hangzhou/37/2013	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Li J et al.
EPI457715	PB1	China	2013-Apr-24	A/environment/Henan/SC232/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457707	PB1	China	2013-Apr-24	A/environment/Henan/SD429/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI453621	PB1	China	2013-Mar-29	A/environment/Nanjing/2913/2013		Other Database Import	Bao C et al.
EPI447650	PB1	China	2013-Apr-27	A/Environment/Shandong/1/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI457699	PB1	China	2013-May-03	A/environment/Shandong/SD038/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457683	PB1	China	2013-May-03	A/environment/Shandong/SD049/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI440691	PB1	China	2013-Apr-03	A/Environment/Shanghai/S1088/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	
EPI457659	PB1	China	2013-Apr-03	A/environment/Shanghai/S1438/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457651	PB1	China	2013-Apr-03	A/environment/Shanghai/S1439/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI467334	PB1	China	2013-Apr-02	A/environment/Wuxi/1/2013		Other Database Import	Qi X et al.
EPI447713	PB1	China	2013-Apr-23	A/Fujian/01/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI453828	PB1	China	2013-Apr-24	A/Fujian/1/2013		Other Database Import	Weng Y et al.
EPI476699	PB1	China	2013-Aug-10	A/Guangdong/1/2013		Other Database Import	Guan W et al.
EPI441600	PB1	China	2013-Mar-24	A/Hangzhou/1/2013	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Li J et al.
EPI446450	PB1	China	2013-Mar-25	A/Hangzhou/2/2013	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Jing-Cao P
EPI446456	PB1	China	2013-Apr-02	A/Hangzhou/3/2013	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Jing-Cao P
EPI457643	PB1	China	2013-Apr-20	A/homing pigeon/Jiangsu/SD184/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI490880	PB1	Hong Kong (SAR)	2013-Nov-30	A/Hong Kong/5942/2013	Public Health Laboratory Services Branch, Centre for Health Protection	Public Health Laboratory Services Branch, Centre for Health Protection	Mak GC et al.
EPI498798	PB1	Hong Kong (SAR)	2014-Jan-07	A/Hong Kong/734/2014	Public Health Laboratory Services Branch, Centre for Health Protection	Public Health Laboratory Services Branch, Centre for Health Protection	Mak G et al.
EPI447699	PB1	China	2013-Apr-24	A/Hunan/01/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI447692	PB1	China	2013-Apr-25	A/Hunan/02/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI447920	PB1	China	2013-Mar-30	A/Jiangsu/01/2013		WHO Chinese National Influenza Center	Wang D et al.

Table 2c

Origin of the influenza A(H7N9) viruses used for the analyses

Segment ID	Segment	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	Authors
EPI447850	PB1	China	2013-Apr-05	A/Jiangsu/04/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI447678	PB1	China	2013-Apr-10	A/Jiangsu/06/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI447657	PB1	China	2013-Apr-09	A/Jiangsu/09/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI447706	PB1	China	2013-Apr-24	A/Jiangxi/01/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI467775	PB1	China	2013-Apr-24	A/Nanchang/1/2013		Other Database Import	Zhou X et al.
EPI440699	PB1	China	2013-Apr-02	A/Pigeon/Shanghai/S1069/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	
EPI457635	PB1	China	2013-Apr-03	A/pigeon/Shanghai/S1421/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI457627	PB1	China	2013-Apr-03	A/pigeon/Shanghai/S1423/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI447720	PB1	China	2013-Apr-21	A/Shandong/01/2013		WHO Chinese National Influenza Center	Wang Dayan et al.
EPI447910	PB1	China	2013-Apr-02	A/shanghai/05/2013		WHO Chinese National Influenza Center	Wang Dayan et al.
EPI447809	PB1	China	2013-Apr-09	A/Shanghai/10/2013		WHO Chinese National Influenza Center	Wang Dayan et al.
EPI447784	PB1	China	2013-Apr-10	A/Shanghai/13/2013		WHO Chinese National Influenza Center	Wang Dayan et al.
EPI447960	PB1	China	2013-Feb-27	A/Shanghai/3/2013		WHO Chinese National Influenza Center	Wang Dayan et al.
EPI446964	PB1	China	2013-Mar-05	A/Shanghai/4664T/2013		Other Database Import	Hu Y
EPI447755	PB1	China	2013-Apr-08	A/Shanghai/9/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI445910	PB1	Taiwan	2013-Apr-24	A/Taiwan/1/2013	National Influenza Center, Centers for Disease Control	Taiwan CDC	Ji-Rong Y et al.
EPI452258	PB1	Taiwan	2013-Apr-22	A/Taiwan/S02076/2013		Other Database Import	Chang SC et al.
EPI452266	PB1	Taiwan	2013-Apr-22	A/Taiwan/T02081/2013		Other Database Import	Chang SC et al.
EPI457619	PB1	China	2013-Apr-17	A/wild pigeon/Jiangsu/SD001/2013	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Zhang Q et al.
EPI467303	PB1	China	2013-Mar-31	A/Wuxi/1/2013		Other Database Import	Qi X et al.
EPI467311	PB1	China	2013-Mar-31	A/Wuxi/2/2013		Other Database Import	Qi X et al.
EPI447748	PB1	China	2013-Apr-03	A/Zhejiang/02/2013		WHO Chinese National Influenza Center	Wang D et al.
EPI441800	PB1	China	2013-Apr-03	A/Zhejiang/DTID-ZJU01/2013		Other Database Import	Chen H-L et al.
EPI477447	PB1	China	2013-Oct-14	A/Zhejiang/DTID-ZJU10/2013	The First Affiliated Hospital, College of Medicine, Zhejiang University	Shanghai Zhijiang Biotechnology Co., Ltd	Chen Y et al.

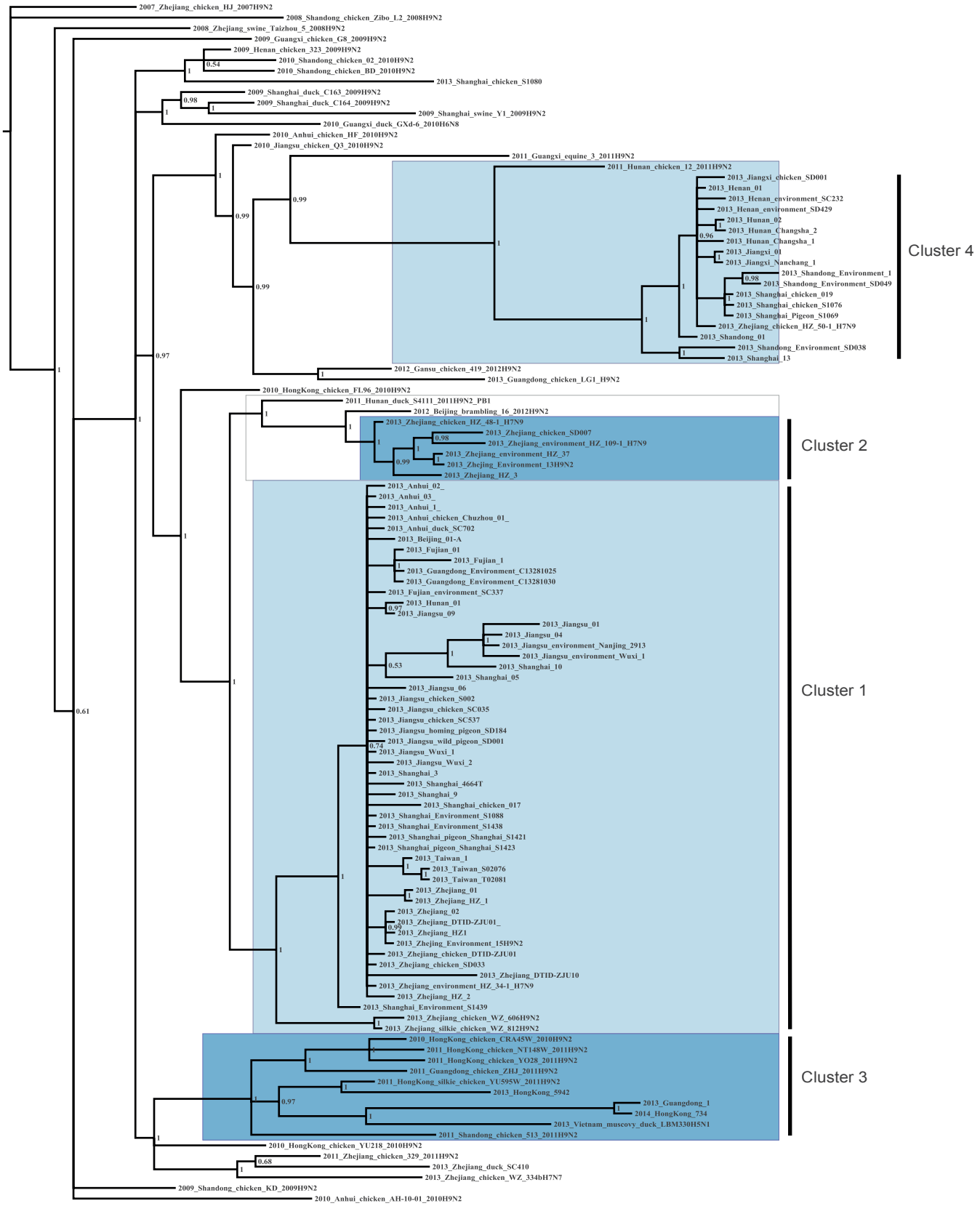
Figure 1

Neighbor-joining tree of PB1 gene sequences of influenza A(H7N9) and A(H9N2) viruses, China, March 2013–January 2014

The tree was constructed using MEGA6.1. Four distinct clusters supported by over 80% bootstrap probability were identified (subtrees with a thick black line). The A(H7N9) virus sequences of 2013 clustered with those of A(H9N2) strains with 100% bootstrap probability in three subtrees shown as Clusters 2–4. Notably, Guangdong- and Hong Kong-derived A(H7N9) sequences (empty triangles) showed a close transmission linkage with local A(H9N2) strains.

Figure 2

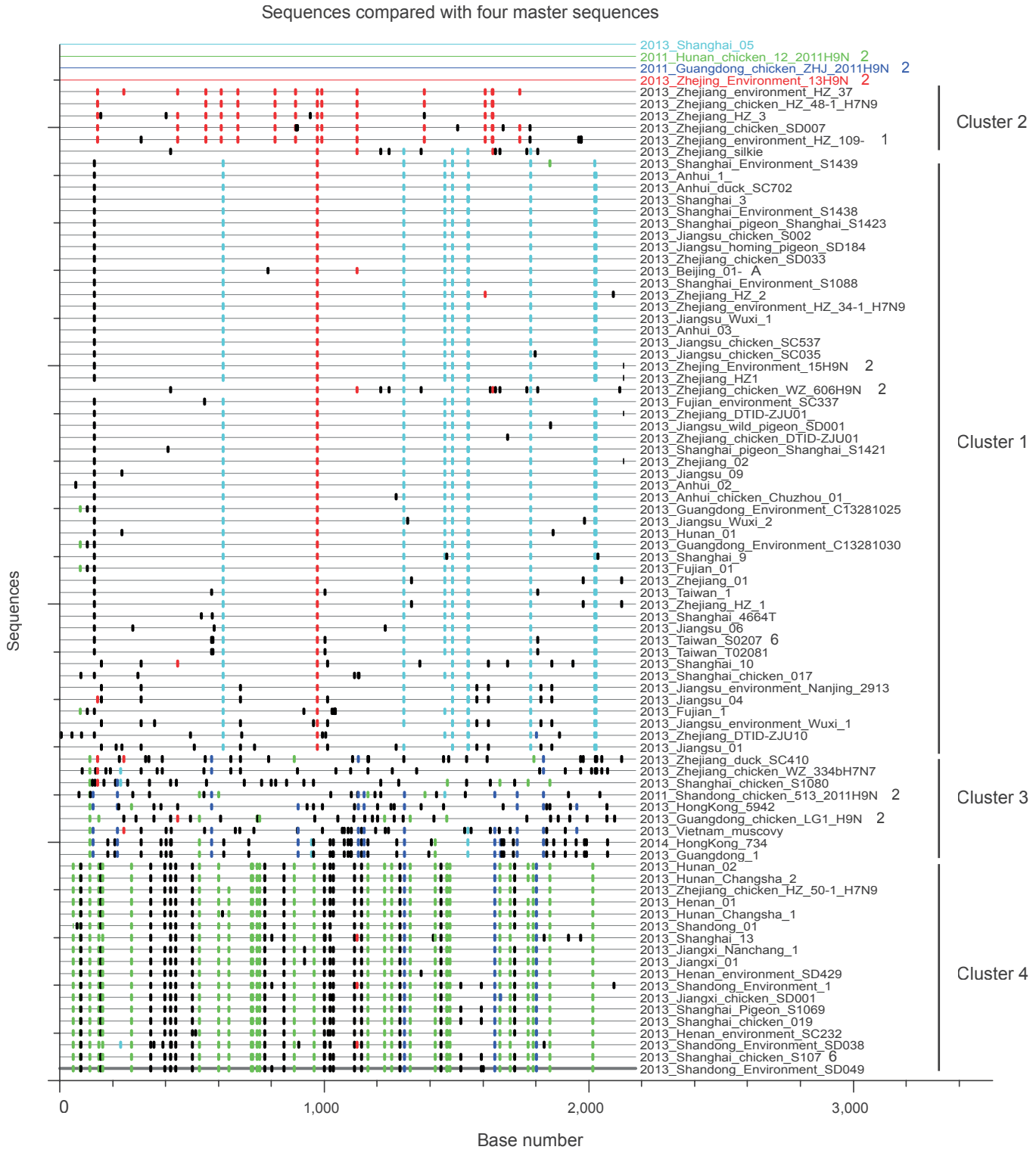
Bayesian tree of PB1 gene sequences of influenza A(H7N9) and A(H9N2) viruses, China, March 2013–January 2014



The tree was constructed using MrBayes 3.1. Significant linkages in Bayesian phylogenetic inference analysis were considered as those having posterior probabilities of 100%. Four transmission clusters (Clusters 1–4) with 100% posterior probability are indicated. Influenza A(H7N9) strains shows close transmission linkage with A(H9N2) strains in Clusters 2–4.

Figure 3

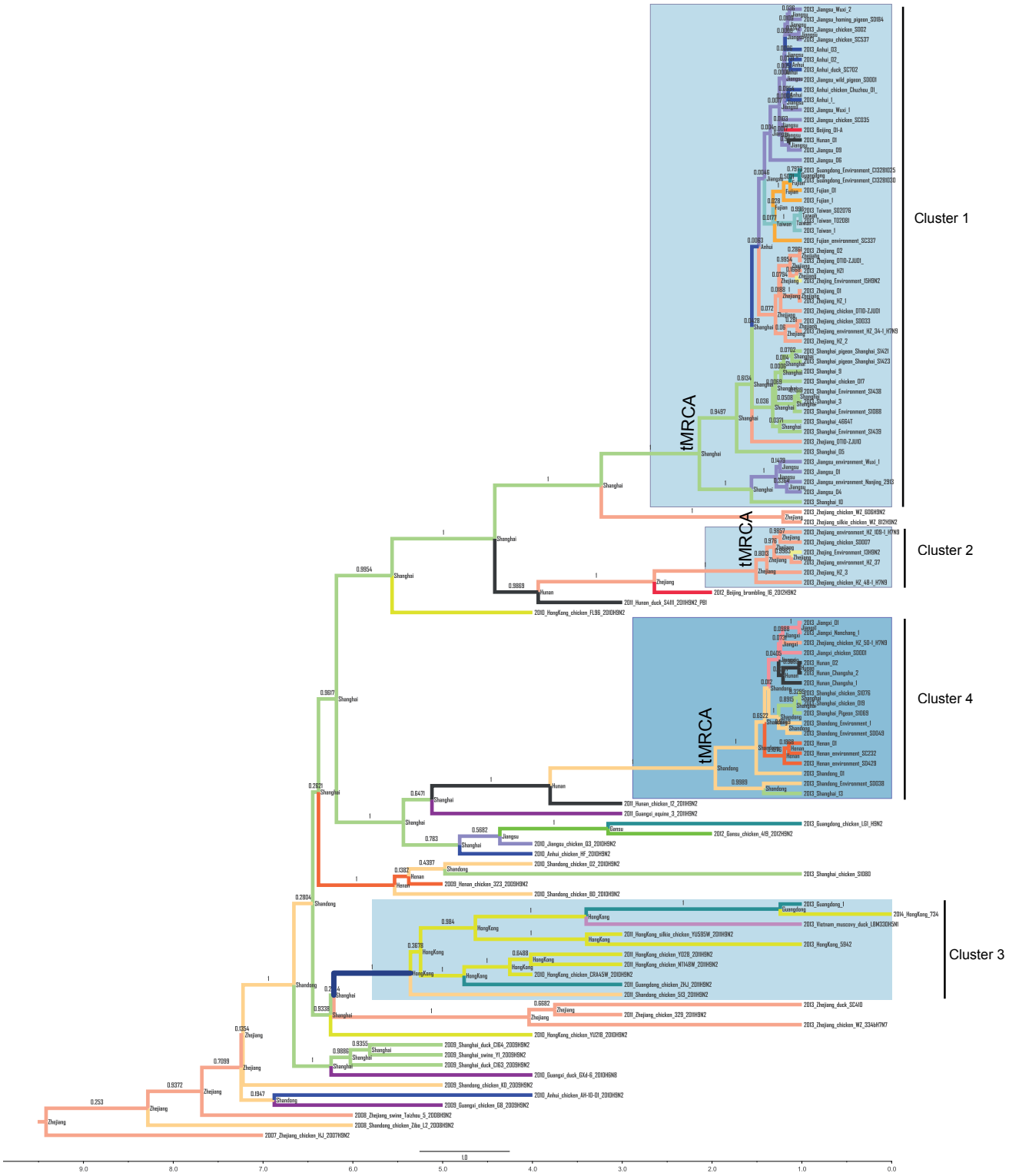
Highlighter analysis of mutation sites of influenzaA(H7N9) virus PB1 gene sequences, China, March 2013–January 2014



The analysis was carried out using Nucleotide Sequences v2.2.3. Four isolates (one from each cluster) were considered as master sequences based on the phylogenetic analysis: 2013_Shanghai_05 (cyan), 2011_Hunan_Chicken_12_H9N2 (green), 2011_ Guangdong_Chicken_ZHJ_ H9N2 (blue), 2013_Zhejiang_Environment_13H9N2 (red).

Figure 4

Maximum clade credibility trees of PB1 gene sequences of influenza A viruses, China, March 2013–January 2014

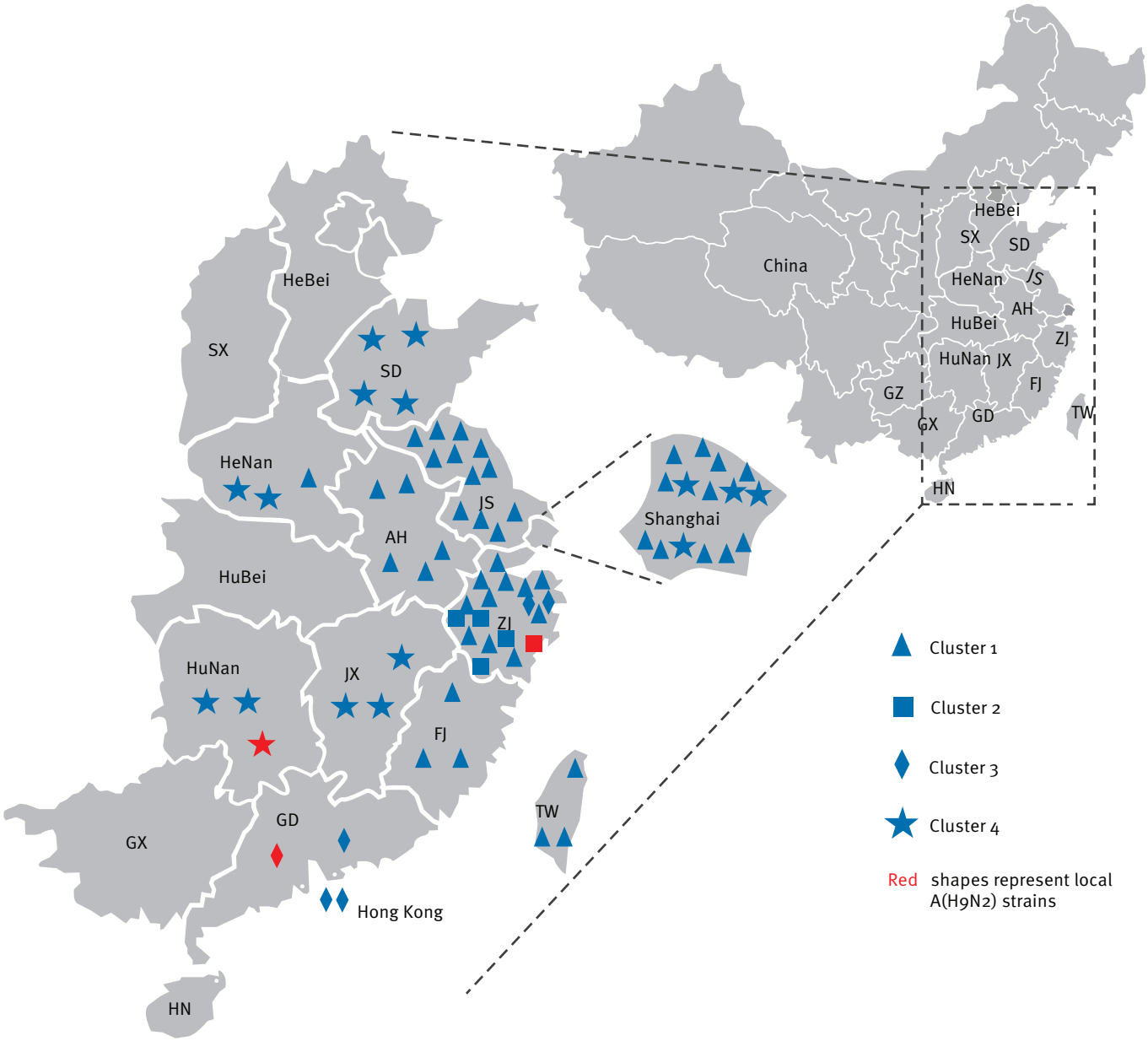


tMRCA: time to the most recent common ancestor.

Phylogeographical trees were constructed using BEAST V1.6.2 package. The tree branches are coloured according to their respective geographical regions. The percentage possibility of the most recent common ancestor of each cluster is labelled at the tree nodes. The four clusters shown are consistent with the four transmission clusters identified in the neighbor-joining tree (Figure 1) and Bayesian tree (Figure 2).

Figure 5

Geographical distribution of influenza A(H7N9) virus strains from four transmission clusters, China, March 2013–January 2014

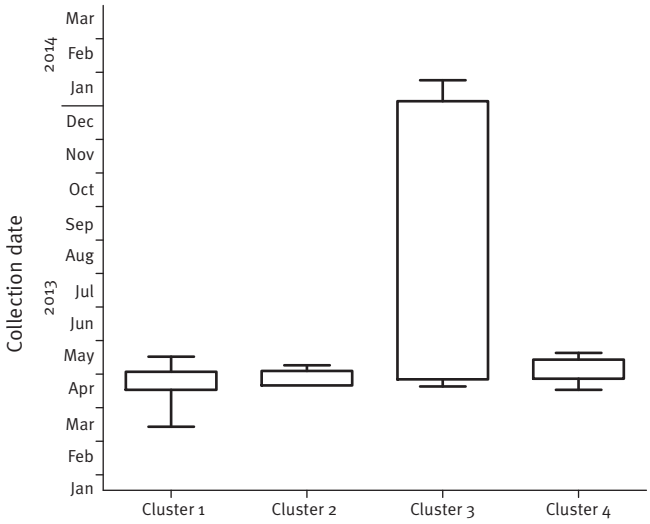


AH: Anhui; FJ: Fujian; GD: Guangdong; GX: Guangxi; HN: Hainan; JS: Jiangsu; JX: Jiangxi; SD: Shandong; SX: Shanxi; TW: Taiwan; ZJ: Zhejiang.

Shanghai-derived strains (Figure 4). In addition, the collection dates of Shanghai A(H7N9) strains were earlier than those of other strains in Clusters 1 and 4 (Tables 1 and 2), suggesting a critical role of Shanghai strains in dissemination of the virus (Figure 5). A new reassortment involving Zhejiang-derived A(H7N9) and Guangdong local A(H9N2) strains formed one independent cluster (Cluster 3), representing the latest and furthest A(H7N9) strains (from the earliest infecting strains in Shanghai) [2] (Figure 4). Moreover, as indicated by Cluster 4 in Figures 4 and 5, the new reassortment of Shanghai A(H7N9)- and Hunan A(H9N2)-derived strains may facilitate further dissemination of A(H7N9) virus from the Yangtze River Delta Economic Zone (including Shanghai, Jiangsu, Zhejiang, Anhui) into neighbouring provinces (such as Shandong, Hunan, Henan and Jiangxi). Meanwhile, Shanghai and Zhejiang A(H7N9) strains were involved in Clusters 3 and 4, indicating that the new reassortment may occur in poultry. Moreover, both the time of the most recent common ancestor and collection date of strains in Clusters 2 and 4 (Figures 4 and 6) showed an obvious delay in comparison with those in Cluster 1, suggesting that reassortment probably occurred during the initial outbreaks. Unlike Clusters 3 and 4, Cluster 2 represents

Figure 6

Collection dates of influenza A(H7N9) virus isolates from four transmission clusters, China, March 2013–January 2014



The bars represent the standard deviation of the collection dates of the A(H7N9) strains in each cluster.

the reassortment between local A(H7N9) and A(H9N2) strains (both from Zhejiang).

Notably, the distinct time to the most recent common ancestor of Clusters 1, 2 and 4 (Figure 4) is consistent with the time course of collection date of the strains in the three clusters (Figure 6, Tables 1 and 2), suggesting distinct phases for transmission and reassortment of A(H7N9) virus in China. Cluster 1, with the earliest most recent common ancestor (Figure 4), may represent the first wave and main body of the A(H7N9) outbreak during first half of 2013, which facilitated the subsequent reassortment between A(H7N9) and local A(H9N2) strains, as Clusters 2 and 4 indicate. Additionally, although no time to the most recent common ancestor is indicated for Cluster 3 (Figure 4), all A(H7N9) strains in this cluster have been isolated very recently (Tables 1, and 2, Figure 6), which may represent the latest reassortment of A(H7N9) and A(H9N2) strains. The association between the expanding transmission and appearance of reassortments suggests a tendency for A(H7N9) evolution towards more and more geographical localisation. In addition, Shanghai or Zhejiang poultry-derived A(H7N9) strains may also play active roles in the process of reassortment and localisation (Tables 1 and 2).

Discussion

Our analysis revealed dynamic reassortments between influenza A(H7N9) and A(H9N2) viruses since the outbreak of A(H7N9) virus infection in March 2013.To some extent, the continuous transmission of H7N9 in Chinese poultry has led to increasing diversity and new reassortment of A(H7N9) with local A(H9N2) strains. Our findings suggest that the re-emerged H7N9 infections may be triggered by new reassortment strains, such as those in the Guangdong/Hong Kong transmission of Cluster 3. In this regard, these infections may have implications for the traditional strategies of drug and vaccine development targeted against HA and NA genes [15].In particular, the new reassortments generated by A(H7N9) and local A(H9N2) strains may produce avian influenza virus strains that are more adaptive and have a higher pathogenicity in humans [16], emphasising the importance of continuously monitoring the A(H7N9) epidemic.

To date, 127 cases of A(H7N9) virus infections have been reported in January 2014, almost the same number as reported in the spring of 2013 (n=133) [5,6]. Notably, Zhejiang and Guangdong provinces and the Shanghai metropolitan area, where new reassortment of A(H7N9) strains is being identified, have been the worst affected regions in China in 2014 [1,17,18]. Although the case-fatality rate in January 2014 (24%, 31/127) is not higher than that seen in the spring of 2013 (29%, 39/133) [5,6], the rapidly increasing number of cases of A(H7N9) virus infection in these three regions may raise concerns as to whether there is an association between circulation of the new A(H7N9) reassortment strains identified and accelerated transmission of A(H7N9) virus in humans. Therefore, it is of the utmost importance to monitor the risk of a potential pandemic initiated by various influenza virus strains.

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Conflict of interest

None declared.

Authors' contributions

Z.M. and J.X. conceived and designed the experiments. Z.M. performed the experiments and analysed the data. Z.Y., Y.H. and X.Z. contributed reagents/materials/analysis tools. Z.M. and R.H. wrote the paper.

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Vaccine effectiveness in preventing laboratory-confirmed influenza in Navarre, Spain: 2013/14 mid-season analysis

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We estimate mid-2013/14 season vaccine effectiveness (VE) of the influenza trivalent vaccine in Navarre, Spain. Influenza-like illness cases attended in hospital (n=431) and primary healthcare (n=344) were included. The overall adjusted VE in preventing laboratory-confirmed influenza was 24% (95% CI: –14 to 50). The VE was 40% (95% CI: –12 to 68) against influenza A(H1)pdm09 and 13% (95% CI: –36 to 45) against influenza A(H3). These results suggest a moderate preventive effect against influenza A(H1)pdm09 and low protection against influenza A(H3).

2013/14 influenza season: early assessment of vaccine effectiveness

Spain was one of the European countries affected earliest by influenza in the 2013/14 season. During the early part of the season (October 2013 to January 2014), influenza A(H1N1)pdm09 and A(H3N2) viruses co-circulated in Spain and elsewhere in Europe: most characterised isolates were A/StPetersburg/27/2011(H1N1)pdm09-like and A/Texas/50/2012(H3N2)-like [1-3]. The composition of the influenza vaccine in the northern hemisphere for 2013/14 comprises an A/California/7/2009(H1N1)pdm09-like virus, an A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011 and a B/Massachusetts/2/2011-like virus [4]. We provide early indicators of the effectiveness of the 2013/14 seasonal vaccine in preventing laboratory-confirmed influenza in Navarre, Spain, by assessing patients in three settings: primary healthcare, hospitalised patients and nursing homes.

Setting and information sources

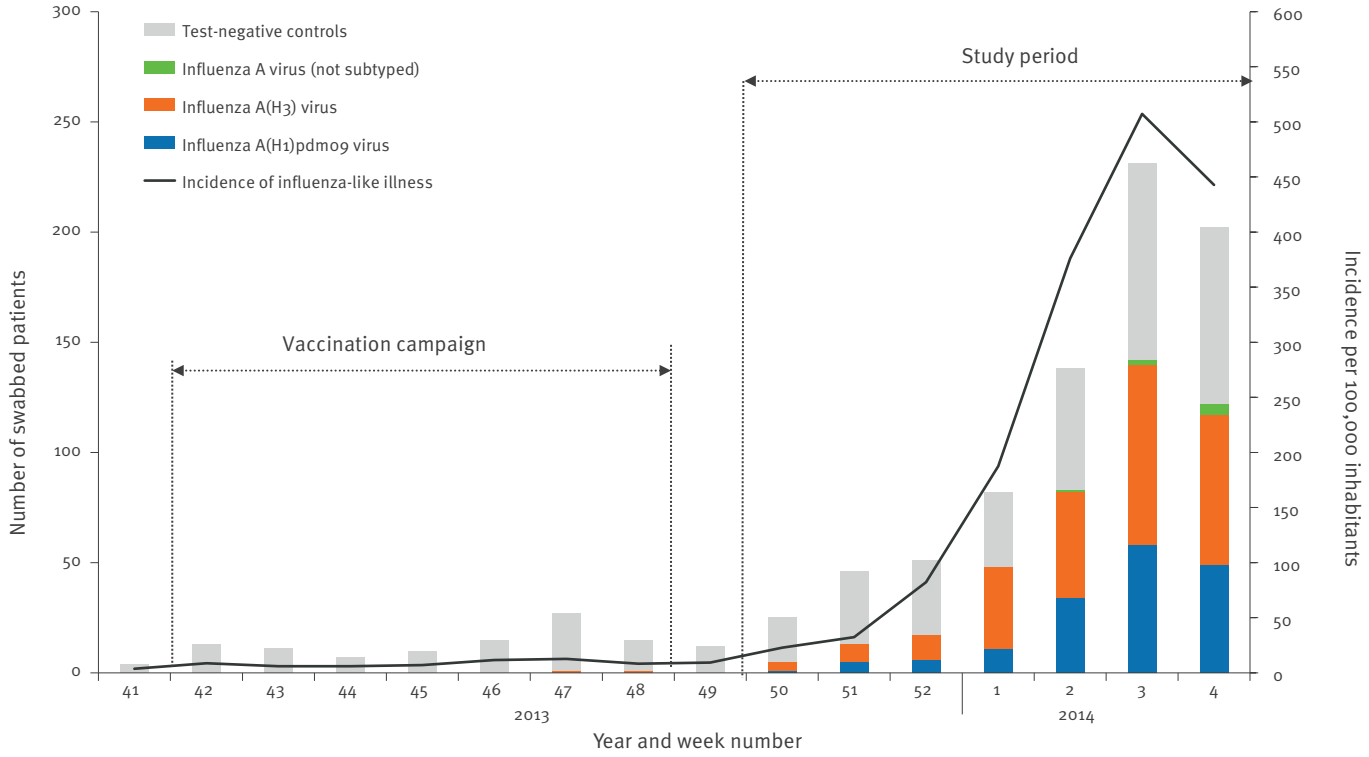
Estimates of vaccine effectiveness (VE) during the influenza season help guide health interventions aimed at reducing the impact of influenza in the population [5,6]. As part of a multicentre European study Influenza Monitoring Vaccine Effectiveness (I-MOVE) [5], Navarre, an autonomous community in northern Spain, has since 2009 provided regular mid-season estimates of influenza VE, which have been supported by estimates at the end of the season [6]. This evaluation of VE is based on electronic clinical records and on epidemiological and virological surveillance of influenza in primary healthcare, hospitals and nursing homes.

In Navarre, the seasonal influenza vaccination campaign took place from 14 October to 30 November 2013. The trivalent inactivated non-adjuvanted vaccine (Vaxigrip, Sanofi Pasteur MSD) was offered free of charge to people aged 60 years or over, to those with major chronic conditions (outlined below) and to people living in institutions. Other people could also be vaccinated if they paid for the vaccine. Precise instructions for registering each dose of vaccine were communicated to all vaccination sites [7]. Influenza vaccine status was obtained from the online regional vaccination register [8] and people were considered to be protected 14 days after vaccine administration. Those for whom the period between vaccination and symptom onset was less than 14 days were excluded, as their immune status is unknown.

Influenza surveillance was based on automatic reporting of cases of influenza-like illness (ILI) from all primary

Figure

Weekly incidence of medically attended influenza-like illness patients and number of swabbed patients by test result, Navarre, Spain, 7 October 2013–26 January 2014



healthcare physicians and searching of ILI cases by public health nurses among admitted patients in hospitals. All of them followed the European Union ILI case definition [9]. A sentinel network composed of a representative sample of 80 primary healthcare physicians, covering 16% of the population, was requested to take nasopharyngeal and pharyngeal swabs, after obtaining verbal informed consent from all their patients diagnosed with ILI, whose symptoms had begun less than five days previously. In hospitals, an agreed protocol was applied, which specified early detection and nasopharyngeal and pharyngeal swabbing of all hospitalised patients with ILI, but only swabs taken within 10 days of symptom onset were considered in our analysis. Swabs were processed by RT-PCR assay and samples positive for influenza A(H1)pdm09, A(H3) and B viruses were identified.

From the electronic primary healthcare records, we obtained the following baseline variables: sex, age, migrant status, district of residence and major chronic conditions (heart disease, lung disease, renal disease, cancer, diabetes mellitus, cirrhosis, dementia, stroke, immunodeficiency, rheumatic disease and body mass index ≥40 kg/m2).

Interim estimation of influenza VE in non-institutionalised inpatients and outpatients

This analysis included persons covered by the Regional Health Service, except healthcare workers, persons living in nursing homes and children under six months of age (96% of the population of the region). All primary healthcare patients and hospitalised patients who were swabbed between 9 December 2013 (the first week with continuous influenza virus detections) and 26 January 2014 were included in an interim test-negative case–control analysis. We compared the seasonal vaccination status of patients in whom any influenza virus was detected (cases) and those who tested negative for influenza (controls). Crude and adjusted estimators of the effect of vaccination were quantified by odds ratios (ORs) with their 95% CIs, calculated using logistic regression models. The adjusted models included sex, age group (<5, 5–14, 15–44, 45–64 and ≥65 years), major chronic conditions, month of sample collection and healthcare setting (primary healthcare and hospital). Separate analyses were carried out by type/subtype of influenza, age group, healthcare setting, and for patients for whom influenza vaccination was indicated because they were 60 years of age or older or had a major chronic condition.

Percentages were compared by chi-squared test. VE was estimated as a percentage: (1–rate ratio)×100 or (1–OR)×100.

During mid-2013/14 season in Navarre, the incidence of ILI cases, number of swabbed patients and number of influenza-positive cases followed a similar trend,

peaking in week 3 (which began on 13 January) of 2014 (Figure).

During the study period, a total of 1,112 ILI patients were swabbed: 775 were included in the VE analysis, of whom 431 were hospitalised patients and 344 were primary healthcare patients recruited by sentinel practitioners. The distribution of these patients by age group (<15, 15–64 and ≥65 years) was, respectively, 21%, 29% and 50% in hospitalised patients, and 13%, 80% and 7% in primary healthcare patients (p<0.001). Influenza virus was laboratory confirmed in 430 (56%) cases: all were infected with influenza A virus. Influenza A(H3) virus was detected in 258 cases, influenza A(H1)pdm09 in 164, and eight remained non-subtyped.

Compared with confirmed cases of influenza, the group of test-negative controls had a higher proportion of persons under the age of five years or 65 years and older, persons with major chronic conditions and persons treated in hospital. As compared with influenza A(H1)pdm09 detections, influenza A(H3) was more frequently detected in persons aged 65 years or older, persons with major chronic conditions and individuals who were hospitalised. The percentage of hospitalised patients was similar in cases infected with influenza A(H1)pdm09 virus (43%) and those with A(H3) virus (46%, p=0.623) (Table 1).

Among the 430 laboratory-confirmed influenza cases, 98 (23%) had received the 2013/14 seasonal vaccine, versus 113 (33%) of the 345 influenza-negative controls (p=0.002) (Table 1).

In the logistic regression analysis, the overall adjusted estimate of the influenza VE was 24% (95% CI: −14 to 50). The VE estimates were similar in the analysis restricted to primary healthcare patients (23%; 95% CI: −87 to 68), to hospitalised patients (22%; 95% CI: −25 to 52), or to the target population for vaccination (23%; 95% CI: −20 to 51). However, the estimated VE in persons aged 65 years or over (11%; 95% CI: −53 to 48) was lower than the estimate in persons younger than 65 years (39%; 95% CI: −15 to 68) (Table 2).

The VE against influenza A(H1)pdm09 virus was 40% (95% CI: −12 to 68) and against influenza A(H3) was 13% (95% CI: −36 to 45). The estimates restricted to primary healthcare patients, to hospitalised patients and to the target population for vaccination were quite similar (Table 2). However, relevant differences were found in the VE against influenza A(H1)pdm09 virus between persons younger than 65 years (59%; 95% CI: 4 to 83) and those aged 65 or more (4%; 95% CI: −162 to 65) (Table 2).

Influenza outbreaks in nursing homes

Influenza surveillance in Navarre includes the detection and study of influenza outbreaks in nursing homes for elderly people or people with physical or mental disabilities. Outbreaks are passively reported by physicians, actively detected by sentinel general practitioners who cover six nursing homes or actively searched when a nursing home resident is confirmed with influenza in a hospital. Influenza vaccine coverage is usually near 90% or higher in all these institutions (unpublished data from the Vaccination Register of Navarre). From 2009 to 2013, outbreaks of laboratory-confirmed influenza in nursing homes were only detected in the 2011/12 season [10], a season with predominance of influenza A(H3) and low VE in the general population [11]. In the other seasons, the estimated VE

Table 1

Characteristics of patients with medically attended influenza-like illness included in test-negative case–control analysis, by test result, Navarre, Spain, 9 December 2013–26 January 2014 (n=775)

Characteristic	Test-negative controls n (%)	Influenza cases[a] n (%)	Influenza virus	
			A(H1) pdm09 n (%)	A(H3) n (%)
Age groups in years				
<5	69 (20)	22 (5)	7 (4)	14 (5)
5–14	25 (7)	21 (5)	10 (6)	11 (4)
15–44	70 (20)	141 (33)	63 (38)	75 (29)
45–64	61 (18)	127 (30)	64 (39)	61 (24)
≥65	120 (35)	119 (28)	20 (12)	97 (38)
Sex				
Male	170 (49)	212 (49)	76 (46)	132 (51)
Female	175 (51)	218 (51)	88 (54)	126 (49)
Month				
December	98 (28)	49 (11)	15 (9)	34 (13)
January	247 (72)	381 (89)	149 (91)	224 (87)
Residence				
Rural	90 (26)	138 (32)	52 (32)	84 (33)
Urban	255 (74)	292 (68)	112 (68)	174 (67)
Migrant status				
No	325 (94)	398 (93)	143 (87)	248 (96)
Yes	20 (6)	32 (7)	21 (13)	10 (4)
Major chronic conditions				
No	159 (46)	233 (54)	103 (63)	126 (49)
Yes	186 (54)	197 (46)	61 (37)	132 (51)
Healthcare setting				
Primary healthcare	107 (31)	237 (55)	93 (57)	140 (54)
Hospital	238 (69)	193 (45)	71 (43)	118 (46)
Seasonal influenza vaccine 2013/14				
No	232 (67)	332 (77)	142 (87)	183 (71)
Yes	113 (33)	98 (23)	22 (13)	75 (29)
Total	**345 (100)**	**430 (100)[a]**	**164 (100)**	**258 (100)**

[a] Includes eight cases of infection with influenza A virus that was not subtyped.

Table 2

Influenza vaccine effectiveness in preventing laboratory-confirmed influenza in Navarre, Spain, 9 December 2013–26 January 2014

Category of patients	Controls	All influenza viruses		Influenza A(H1)pdm09 virus		Influenza A(H3) virus	
	Number vaccinated/ total	Cases Number vaccinated/ total	VE % (95% CI)[a]	Cases Number vaccinated/ total	VE % (95% CI)[a]	Cases Number vaccinated/ total	VE % (95% CI)[a]
All swabbed patients	113/345	98/430	–	22/164	–	75/258	–
Crude	–	–	39 (17 to 56)	–	68 (47 to 81)	–	16 (–19 to 41)
Adjusted	–	–	24 (–14 to 50)	–	40 (–12 to 68)	–	13 (–36 to 45)
Target population for vaccination[b]	105/204	91/232	–	20/73	–	70/154	–
Crude	–	–	39 (11 to 58)	–	64 (36 to 80)	–	21 (–19 to 48)
Adjusted	–	–	23 (–20 to 51)	–	36 (–28 to 68)	–	15 (–37 to 47)
Age <65 years	35/225	25/311	–	9/144	–	16/161	–
Crude	–	–	53 (18 to 73)	–	64 (22 to 83)	–	40 (–12 to 68)
Adjusted	–	–	39 (–15 to 68)	–	59 (4 to 83)	–	15 (–77 to 59)
Age ≥65 years	78/120	73/119	–	13/20	–	59/97	–
Crude	–	–	15 (–45 to 50)	–	0 (–170 to 63)	–	16 (–45 to 52)
Adjusted	–	–	11 (–53 to 48)	–	4 (–162 to 65)	–	10 (–59 to 49)
Primary healthcare patients	12/107	22/237	–	5/93	–	17/140	–
Crude	–	–	19 (–70 to 62)	–	55 (–33 to 85)	–	–9 (–140 to 50)
Adjusted	–	–	23 (–87 to 68)	–	42 (–97 to 83)	–	11 (–123 to 41)
Hospitalised patients	101/238	76/193	–	17/71	–	58/118	–
Crude	–	–	12 (–30 to 40)	–	57 (22 to 77)	–	–31 (–104 to 16)
Adjusted	–	–	22 (–25 to 52)	–	34 (–41 to 69)	–	14 (–45 to 49)

CI: confidence interval; VE: vaccine effectiveness.

[a] Logistic regression model adjusted for sex, age group (<5, 5–14, 15–44, 45–64 and ≥65 years), month, major chronic conditions and healthcare setting (primary healthcare and hospital).

[b] Target population for vaccination includes people ≥60 years-old and people with major chronic conditions.

was higher and only sporadic cases were detected in nursing homes [12-14].

In mid-2013/14 season, influenza outbreaks in five nursing homes in Navarre were detected (Table 3). All five had carried out an influenza vaccination campaign in October and November 2013, reaching coverages of 89% to 100%. The influenza outbreaks occurred in January 2014, coinciding with the epidemic wave in the region. In each institution, influenza was laboratory confirmed for three or more ILI patients. Influenza virus A(H3) was identified in 18 patients in four outbreaks in homes for elderly people. Another outbreak occurred in a home for persons with physical disabilities aged 18 to 64 years old, where influenza A(H1)pdm09 virus was detected in the three swabbed patients. In total, 20 of the 22 laboratory-confirmed cases had received the trivalent 2013/14 seasonal vaccine.

Virus characterisation

Although, to date, antigenic tests are pending, we found some genetic differences between circulating and vaccine viruses. Sequence analysis of the product of amplification (HA1 fragment of the haemagglutinin gene) showed that all four influenza A(H1N1)pdm09 viruses studied clustered into the group 6B [15], represented by A/Norway/2417/2013 and defined by D97N, K163Q, S203T, S185T, A256T and K283E amino acid mutations compared with the vaccine virus A/California/07/2009. Nevertheless, all six mutations had already been detected in previous seasons and did not have an important influence on the VE.

All 17 influenza A(H3N2) viruses studied clustered into the group 3C [15], which includes the A/Texas/50/2012 vaccine virus strain, but harbouring some amino acid changes that make it possible to find some genetic differences. All 17 A(H3N2) viruses clustered within the

Table 3

Interim description of influenza outbreaks in five nursing homes, Navarre, Spain, January 2014

Characteristic	Nursing home				
	1	2	3	4	5
Number of residents	40	82	523	55	78
2013/14 influenza vaccine coverage	100%	91%	89%	100%	96%
Number of influenza-like illness cases	8	19	10	6	26
Swabbing criteria	Cases referred to hospital	Hospitalised cases	All cases	All cases[a]	Hospitalised cases
Number of patients with nasopharyngeal swab	3	4	10	5	4
Number of patients confirmed with influenza virus infection	3	3	8	4	4
Number vaccinated/unvaccinated	3/0	3/0	6/2	4/0	4/0
Age range in years	28–44	85–90	69–92	82–90	85–93
Virus type/subtype	A(H1)pdm09	A(H3)	A(H1)pdm09 A(H3)[b]	A(H3)	A(H3)
Influenza-related hospitalisations	2	3	1	2	3

[a] The first case could not be swabbed.

[b] The first laboratory-confirmed case was infected with influenza A(H1)pdm09 virus and the other seven cases with A(H3) virus.

subgroup 3C.3, represented by A/Samara/73/2013 and defined by N128A and R142G amino acid substitutions. Interestingly, we could differentiate 16 viruses within the 3C.3 subgroup with an additional double L157S and N122D mutation. Another virus harbouring the K160R amino acid substitution could be identified within the 3C.3 subgroup. Changes in influenza A(H3N2) viruses are referred to the A/Texas/50/2012 vaccine virus strain.

Discussion and conclusion

In mid-2013/14 influenza season, our analysis suggests low effectiveness of the trivalent influenza vaccine in preventing laboratory-confirmed influenza in Navarre. Similar estimates were obtained for hospitalised patients and primary healthcare patients. In both groups, estimates suggest a moderate VE against influenza A(H1)pdm09 virus and a low VE against influenza A(H3) virus.

We also detected an unusually high number of outbreaks of laboratory-confirmed influenza A(H3) in nursing homes in Navarre with high vaccination coverage, which also suggests low VE. Information on influenza virus infection and vaccine coverage in nursing home workers could not be systematically collected, although it can be related to the occurrence of outbreaks. The outbreaks and lower VE in older people could be due to immunosenescence; however, the VE against influenza A(H3) virus was also low in people under 65 years. Some pre-existing immunity and the higher VE that we found against A(H1N1)pdm09 virus can explain the absence of outbreaks caused by this virus subtype in older people.

In the 2013/14 influenza season to date, influenza activity has peaked in Spain, but it is still increasing in many other European countries. Influenza A(H3) and A(H1)pdm09 viruses are co-circulating in Europe, with different proportions in different countries [1-3]. Both virus components were the same in the 2013/14 and 2012/13 seasonal vaccines. In the 2012/13 season, low VE against influenza A(H3N2) virus was also observed among elderly people in Denmark [16]. Although antigenic tests of influenza A(H3) strains from Navarre are pending, we have found some genetic differences between circulating and vaccine viruses.

In a recent report from Canada, the interim estimate of 2013/14 VE was 74% against A(H1N1)pdm09 viruses. Relative to vaccine, these viruses were antigenically similar and genetically well conserved [17]. Our results suggest a lower VE against this serotype in Navarre, but as yet, we do not have final antigenic results.

The estimates of the VE in Navarre are not representative of Europe, and studies in other countries or regions are necessary to draw conclusions about the influenza VE in Europe in the 2013/14 season.

The results presented here are preliminary, and have limited statistical power and wide CIs for some analyses. Therefore the final results for the season may be different. The case–control analysis included only laboratory-confirmed influenza cases and compared them with controls recruited in the same healthcare settings before either patient or physician knew the laboratory result, a feature that reduces selection bias [18].

The differences between crude and adjusted VE estimates were, in general, greater in the analysis of the influenza A(H1)pdm09 cases. This can be explained because the controls and the influenza A(H3) cases were more similar in their characteristics. In any event, the differences in these characteristics were controlled for in the adjusted analysis.

In our analysis, we included patients recruited in primary healthcare and in hospitals in Navarre, thus achieving representation of the whole spectrum of influenza patients seeking medical care. As the healthcare setting could have acted as a confounding factor, the analyses were stratified or adjusted for this variable. The possibility that the healthcare setting might have modified the effect or biased the results can be ruled out given the similarity of the estimates obtained in these two patient groups separately and in the joint analysis.

These results support a moderate protective effect of the trivalent seasonal vaccine against influenza A(H1) pdm09 virus and a low effect against A(H3) virus in Navarre mid-2013/14 season. These results should serve as a stimulus to design better influenza vaccines [19], to improve the selection of strains contained in the vaccine and to highlight the importance of other preventive measures that complement vaccination in high-risk populations, such as promotion of basic hygiene measures, use of face masks and avoidance of contact with influenza cases [20]. Early treatment with antiviral drugs should be considered in persons diagnosed with influenza who have a high risk of complications, regardless of vaccination status [21].

Even in seasons in which the effectiveness of influenza vaccine is low, vaccination may appreciably reduce the number of cases and hospitalisations in high-risk persons. In the 2013/14 season in Navarre, vaccination resulted in avoiding almost a quarter of the possible influenza cases in the vaccinated at-risk population; while not entirely satisfactory, this result is important in terms of individual and public health.

Network members

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Conflict of interest

None declared.

Authors' contributions

J Castilla, I Martínez-Baz and M Guevara designed the study, coordinated the activities and undertook the statistical analysis. E Albeniz coordinated the activities in primary healthcare. A Navascués, M Fernández-Alonso, G Reina and C Ezpeleta were responsible for the virological analysis and interpretation of laboratory results. J Chamorro and MT Ortega coordinated the activities in hospitals. E Albeniz coordinated the activities in primary healthcare. F Pozo was responsible for the virus characterisation. J Castilla, I Martínez-Baz and M Guevara wrote the draft manuscript, and all authors revised and approved the final version.

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Influenza vaccine effectiveness estimates in Europe in a season with three influenza type/subtypes circulating: the I-MOVE multicentre case–control study, influenza season 2012/13

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In the fifth season of Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE), we undertook a multicentre case–control study (MCCS) in seven European Union (EU) Member States to measure 2012/13 influenza vaccine effectiveness against medically attended influenza-like illness (ILI) laboratory confirmed as influenza. The season was characterised by substantial co-circulation of influenza B, A(H1N1)pdm09 and A(H3N2) viruses. Practitioners systematically selected ILI patients to swab ≤7 days of symptom onset. We compared influenza-positive by type/subtype to influenza-negative patients among those who met the EU ILI case definition. We conducted a complete case analysis using logistic regression with study as fixed effect and calculated adjusted vaccine effectiveness (AVE), controlling for potential confounders (age, sex, symptom onset week and presence of chronic conditions). We calculated AVE by type/subtype. Study sites sent 7,954 ILI/acute respiratory infection records for analysis. After applying exclusion criteria, we included 4,627 ILI patients in the analysis of VE against influenza B (1,937 cases), 3,516 for A(H1N1)pdm09 (1,068 cases) and 3,340 for influenza A(H3N2) (730 cases). AVE was 49.3% (95% confidence interval (CI): 32.4 to 62.0) against influenza B, 50.4% (95% CI: 28.4 to 65.6) against A(H1N1)pdm09 and 42.2% (95% CI: 14.9 to 60.7) against A(H3N2). Our results suggest an overall low to moderate AVE against influenza B, A(H1N1)pdm09 and A(H3N2), between 42 and 50%. In this season with many co-circulating viruses, the high sample size enabled stratified AVE by type/subtype. The low estimates indicate seasonal influenza vaccines should be improved to achieve acceptable protection levels.

Introduction

The 2012/13 influenza season in Europe was characterised by an extended season where the three influenza viruses A(H1N1)pdm09, A(H3N2), B/Yamagata lineage all contributed substantially to morbidity although marked geographical differences were noted [1]. Currently, the best preventive method against influenza is receipt of the influenza vaccine. The composition of the 2012/13 northern hemisphere influenza vaccine included A/California/7/2009 (H1N1)pdm09, A/Victoria/361/2011 (H3N2) and B/Wisconsin/1/2010-like (Yamagata lineage) viruses. The A(H3N2) and influenza B components were changed from those of the 2011/12 influenza season [2].

Influenza vaccine effectiveness (VE) studies are essential to monitor how the vaccine performs in the target populations. If VE estimates are available early in the season they can lead to additional preventive measures if they are low, such as stronger recommendations for antiviral treatment for those at risk of severe disease.

Since 2008/09, using a European multicentre case–control study, a component of the Influenza Monitoring Vaccine Effectiveness (I-MOVE) network, we have estimated the effectiveness of the seasonal and pandemic influenza vaccine to prevent medically attended influenza-like illness (ILI) laboratory confirmed as

influenza [3-6]. In February 2013, early influenza VE estimates from the multicentre case–control study for the 2012/13 influenza season by type/subtype were included among the VE estimates provided to the World Health Organization (WHO) for the vaccine strain selection meeting for the 2013/14 influenza vaccine: 78.2% (95% confidence interval (CI): 18.0 to 94.2) against influenza B, 62.1% (95% CI: -22.9 to 88.3) against A(H1) pdm09, 41.9 (95% CI: –67.1 to 79.8) against A(H3N2) [7]. Estimates were also sent to other major bodies supporting public health decision-making: The European Centre for Disease Prevention and Control and the European Medicines Agency. In this article we present the 2012/13 end of season pooled VE estimates, from study sites in seven European Union (EU) Member States. The objective of the fifth I-MOVE multicentre case–control study was to provide pooled adjusted influenza VE estimates by influenza type/subtype and age group for the overall population and for the target group for vaccination.

Methods

The seven study sites undertaking case–control studies included in the 2012/13 analysis were based in France, Germany, Ireland, Poland, Portugal, Romania and Spain. All study sites used the test-negative design; detailed methods on the I-MOVE multicentre case–control study are described elsewhere [4-6]. In summary, participating practitioners interviewed and carried out nasopharyngeal swabbing of all or of a systematic sample of patients presenting with influenza-like illness (ILI) or acute respiratory infection (ARI); in France practitioners sampled ARI patients exclusively and in Germany in case of no patients consulting for ILI, practitioners sampled those consulting for ARI.

Practitioners from all study sites collected information on date of symptom onset and date of swabbing, 2012/13 seasonal influenza vaccination status, date and brand, 2011/12 seasonal influenza vaccination status, ILI symptoms, sex, presence of a chronic condition (including obesity, except Germany and Poland), number of hospitalisations for chronic conditions in the previous 12 months and pregnancy. Six study sites collected information on number of practitioner visits in the previous 12 months (not collected in Germany), five collected information on receipt of antivirals (not collected in Spain and France) and five on smoking status (not collected in Germany and France). To identify individuals belonging to the target group for vaccination, four study sites included a specific question and three used variables such as age, chronic conditions, and pregnancy to enable their identification.

We included patients in the study if they met the EU ILI case definition (sudden onset of symptoms and at least one of the following four systemic symptoms: fever or feverishness, malaise, headache, myalgia; and at least one of the following three respiratory symptoms: cough, sore throat, shortness of breath), if they were swabbed ≤7 days of symptom onset, had no contraindications to influenza vaccination and did not receive antivirals prior to swabbing [8].

We defined the study period as at least 15 days after the beginning of the 2012/13 country-specific seasonal influenza vaccination campaigns and excluded controls that had symptom onset before the week of onset of the first influenza type/subtype case depending on the outcome used. We also dropped ILI patients presenting after the week of onset of the last influenza type/subtype case depending on outcome used, after which there were at least two consecutive weeks with no further influenza positive cases of this type/subtype.

Swabs were tested for influenza at the respective country's National Influenza Reference Laboratory. In France and Spain, tests were also conducted in other laboratories participating in the National Influenza Sentinel Surveillance System. All the laboratories contributing to the National Influenza Sentinel Surveillance Systems are certified. At all study sites a subset of isolates were genetically and/or antigenically characterised. Details of laboratory viral detection, typing, subtyping and variant analysis performed in each country are described elsewhere [9].

Among study participants fulfilling the inclusion criteria, we defined a case as a patient who tested positive for influenza virus by reverse-transcription polymerase chain reaction (RT-PCR) or culture. We classified patients with swabs testing negative for influenza virus as controls.

We classified cases and controls as vaccinated if they had received at least one dose of 2012/13 seasonal influenza vaccine at least 15 days before ILI symptom onset. All other patients were classified as unvaccinated.

Study sites sent their anonymised data to EpiConcept, where we pooled them and carried out a complete case analysis (where records with missing values were dropped). Using a one-stage method with study site as fixed effect in the model, we estimated the pooled influenza VE as 1 minus the odds ratio (OR) of being vaccinated in cases versus controls multiplied by 100.

To test for heterogeneity by influenza type/subtype between study sites, we used Cochran's Q-test and the I^2 index [10]. In study sites with sample sizes large enough we used adjusted ORs and their standard error, otherwise we used the crude ORs. In study sites with no vaccinated cases, we reclassified one unvaccinated case as vaccinated chosen at random, in order to calculate the OR and standard error.

We used a logistic regression model to calculate VE including potential confounding factors: age (modelled as a restricted cubic spline with 4 knots [11]), sex, presence of at least one chronic condition (including

Table 1

Study details for the I-MOVE multicentre case–control study to measure 2012/13 influenza vaccine effectiveness, study sites in seven European Union Member States, ISO week 43 in 2012–ISO week 18 in 2013

Study site	Week/year of start of influenza season[a]	Week/ year of peak of influenza season[a]	Number of practitioners recruiting at least one ILI patient[b]	Number of ILI patients[b] included in study	Inclusion period for the final analysis (ISO weeks/year)[c]	Number of included ILI patients positive for influenza and with known vaccination status[d]		Number of included ILI patients negative for any influenza and with known vaccination status[d]	
						Total	Vaccinated	Total	Vaccinated
France	51/2012	5/2013	318	1,613	51/2012–15/2013	950	33	619	33
Germany	50/2012	8/2013	137	2,875	43/2012–18/2013	1,407	95	1,305	123
Ireland	50/2012	1/2013	21	264	48/2012–16/2013	167	10	96	10
Poland	47/2012	3/2013	4	54	49/2012–4/2013	24	0	30	0
Portugal	3/2013	10/2013	44	335	51/2012–15/2013	152	8	183	37
Romania	3/2013	8/2013	69	196	2/2013–17/2013	130	1	66	7
Spain	3/2013	8/2013	194	1,297	50/2012–16/2013	823	40	473	45
Total	–	–	787	6,634	43/2012–18/2013	3,653	187	2,772	255

ILI: Influenza-like illness; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; ISO: International Organization for Standardization.

The study sites for the 2012/13 influenza vaccine effectiveness analysis were respectively located in the seven following European Union Member States: France, Germany, Ireland, Poland, Portugal, Romania and Spain.

[a] According to the thresholds used to define the start of the influenza season in each of the countries.

[b] ILI patients meeting the European Union case definition, swabbed ≤7days after onset of symptoms within the study period.

[c] From 15 days after the start of the vaccination campaign to week 18, 2013. We excluded controls with onset of symptoms in the weeks before the week of the first influenza case and after the week of the influenza case after which there were two or more consecutive weeks with no further cases in the study site.

[d] ILI patients included in the study, after excluding those with missing information on vaccination status or date of vaccination.

pregnancy and obesity where available) and week of symptom onset.

We calculated VE against influenza type/subtype and carried out stratified analyses by age group (0–14 years, 15–59 years and ≥60 years). We categorised vaccines according to vaccine group: inactivated subunit egg-based, inactivated subunit cell-based, inactivated split virion egg-based and inactivated adjuvanted (squalene MF59) and calculated VE by influenza vaccine group, by age group and for the target group for vaccination.

In a sensitivity analysis we restricted to those swabbed within three days of symptom onset also and we also calculated VE adjusted by current smoking status and number of general practitioner (GP) visits in the previous 12 months (0–1 visits, 2–4 visits and ≥5 visits) among the five study sites collecting this information. We calculated VE with those vaccinated within 14 days of symptom onset excluded, instead of coded as unvaccinated. We also carried out a multiple imputation using chained equations to assess if there was any bias in dropping records with missing data. We used missing at random assumptions and independently analysed 20 copies of the data using 30 cycles of regression. The variables included in the model to create the imputation database were the respective outcomes and the predictors: vaccination status for the 2012/13 season, age group, sex, presence of chronic conditions and associated hospitalisations in the previous 12 months, 2011/12 seasonal vaccination status, belonging to a target group for vaccination, delay between onset and swabbing, onset month and study site.

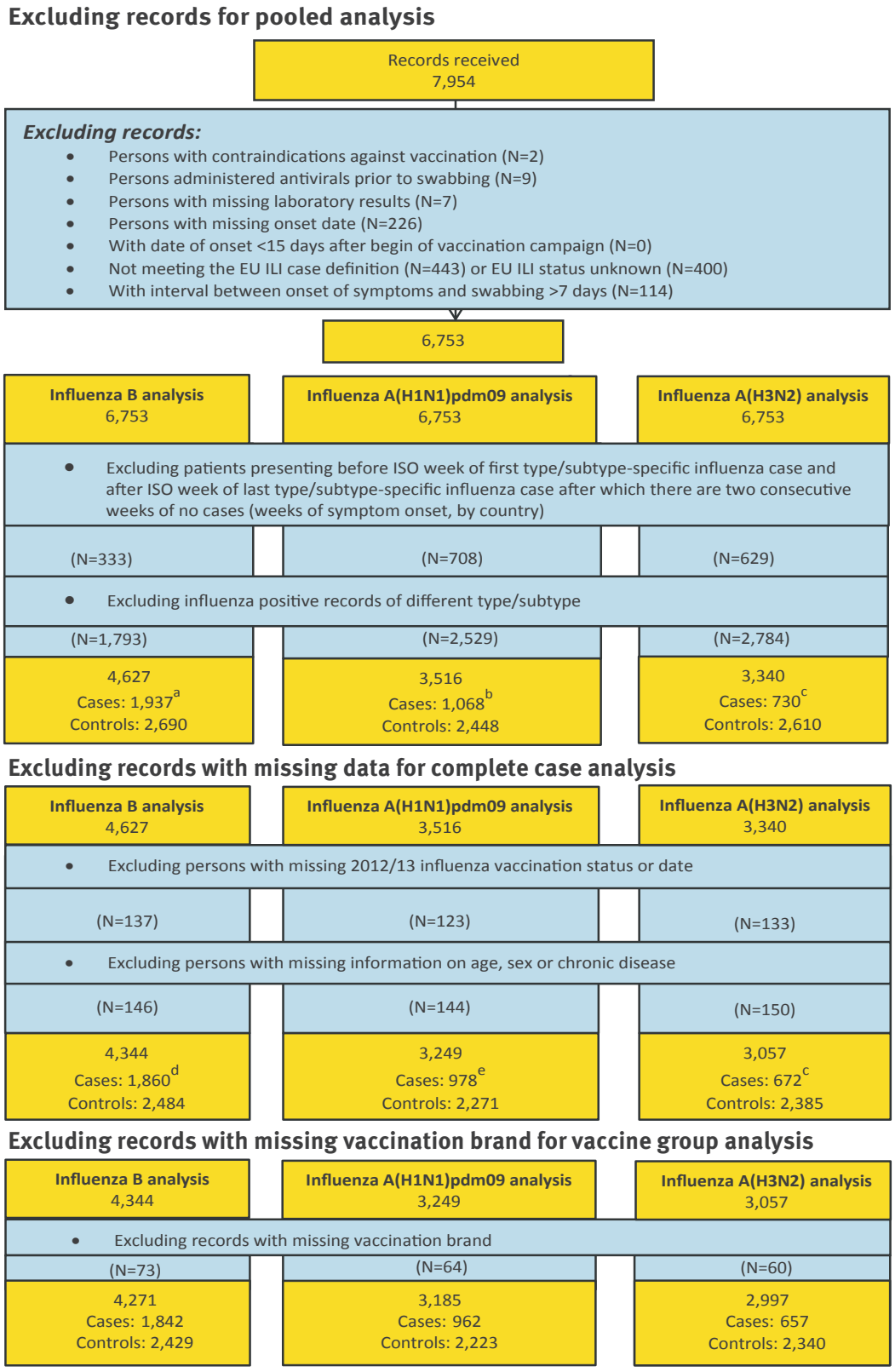
Results

The influenza season in the seven countries where the seven respective study sites were located in started and peaked at different times, as defined by national thresholds (Table 1). The season started earliest in Poland (week 47, 2012) and latest in Portugal, Romania and Spain (week 3, 2013). The peak of the influenza season varied from week 1, 2013 in Ireland to week 10, 2013 in Portugal.

Study sites sent a total of 7,954 records of ILI/ARI patients for analysis (Figure 1). After applying exclusion criteria, we included 4,627 ILI patients in the analysis of VE against influenza B (1,937 cases), 3,516 in the analysis of VE against influenza A(H1N1)pdm09 (1,068 cases) and 3,340 in the analysis of VE against influenza A(H3N2) (730 cases). The maximum weekly

Figure 1

Flowchart of data exclusion, I-MOVE multicentre case–control study to measure 2012/13 influenza vaccine effectiveness, study sites in seven European Union Member States, influenza season 2012/13



EU: European Union; ILI: influenza-like illness; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; ISO: International Organization for Standardization.

[a] Includes 6 influenza B+A(H1N1)pdm09 and 3 influenza B+A(H3N2) co-infections.
[b] Includes 6 influenza B+A(H1N1)pdm09 and 7 A(H1N1)pdm09+A(H3N2) co-infections.
[c] Includes 3 influenza B+A(H3N2)pdm09 and 7 A(H1N1)pdm09+A(H3N2) co-infections.
[d] Includes 5 influenza B+A(H1N1)pdm09 and 3 influenza B+A(H3N2) co-infections.
[e] Includes 5 influenza B+A(H1N1)pdm09 and 7 A(H1N1)pdm09+A(H3N2) co-infections.

number of cases recruited by type/subtype occurred at different times in the study (Figure 2).

After excluding patients with missing information on 2012/13 seasonal vaccination status or date, age, sex or presence of chronic condition, we included 4,344, 3,249 and 3,057 individuals for the complete case analysis of VE against influenza B, influenza A(H1N1)pdm09 and A(H3N2) respectively.

The median age was 15 years for influenza B cases (interquartile range (IQR): 7–43 years), 31 years for A(H1N1)pdm09 cases (IQR: 10–46 years) and 20 years for A(H3N2) cases (IQR: 5–46 years). Controls had a median age of 22 years (IQR: 4–45 years).

Among controls, 18% had at least one chronic condition, compared to 15%, 15% and 13% of influenza B, A(H1N1)pdm09 and A(H3N2) cases respectively. The proportion vaccinated with the 2012/13 influenza vaccine was 9% among controls, 5% among influenza B cases, 5% among A(H1N1)pdm09 cases and 7% among A(H3N2) cases (Table 2).

Among controls, 22% belonged to the target group for vaccination compared to 19%, 20%, and 20% of influenza A(H1N1)pdm09, influenza A(H3N2) and influenza B cases respectively (Table 2).

Overall 90% of controls were swabbed within three days of symptom onset compared to 91%, 90% and 86% of influenza A(H1N1)pdm09, influenza A(H3N2) and influenza B, cases respectively.

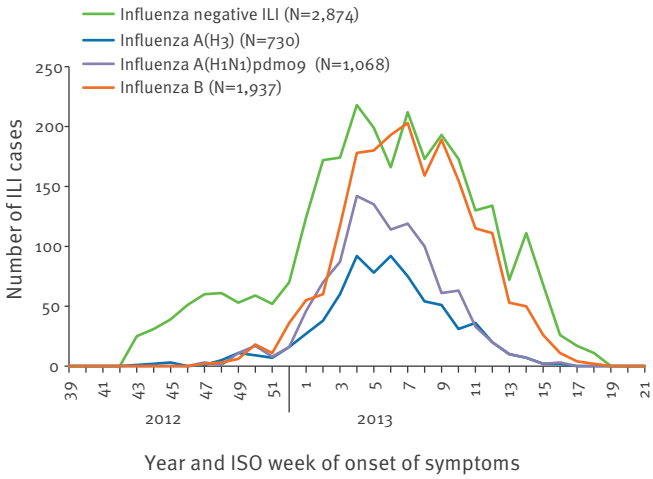
Among 331 vaccinated participants with known type of vaccine, 64% (213/331) had received split virion vaccine (note: one participant was co-infected with influenza A(H3N2) and influenza B). The proportion receiving split virion vaccine by country was: 0% (0/8) in Romania, 56% (87/156) in Germany, 71% (30/42) in France, 73% (62/85) in Spain, 76% (19/25) in Portugal and 100% (15/15) in Ireland. Ireland, however, was omitted from the vaccine type analysis as only one vaccine type was available. Four study participants in Spain and two in Germany had received an adjuvanted vaccine and one participant in Germany received a cell-mediated subunit vaccine. All others, including all eight vaccinated participants from Romania received an egg-derived subunit vaccine.

The Q test and I^2 index testing for heterogeneity of VE between study sites suggested no heterogeneity for influenza A(H1N1)pdm09 (p=0.849, I^2=0%), low heterogeneity for influenza B, (p=0.249, I^2=24.7%) and low to moderate heterogeneity for influenza A(H3N2) (p=0.168, I^2=37.9%).

Influenza VE adjusted by onset week, presence of chronic conditions age and sex was 49.3% (95% CI: 32.4 to 62.0) against influenza B, 50.4% (95% CI: 28.4 to 65.6) against influenza A(H1N1)pdm09 and 42.2% (95% CI: 14.9 to 60.7) against influenza A(H3N2) (Table 3).

Figure 2

Number of ILI cases included in the pooled analysis by influenza type/subtype and week of symptom onset, I-MOVE multicentre case–control study to measure 2012/13 influenza vaccine effectiveness, influenza season 2012/13 (n=6,609)



ILI: influenza-like illness; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; ISO: International Organization for Standardization.

The seven European Union Member State study sites were respectively located in France, Germany, Ireland, Poland, Portugal, Romania and Spain.

Among those aged 0 to 14 years, the adjusted VE was 22.3% (95% CI: -37.0 to 55.9) against influenza B, 36.5% (95% CI: -44.1 to 72.0) against influenza A(H1N1)pdm09 and 36.1% (95% CI: -41.1 to 71.0) against influenza A(H3N2). Among those aged 15 to 59 years, the adjusted VE was 63.6% (95% CI: 42.1 to 77.1) against influenza B, 55.6% (95% CI: 28.3 to 72.5) against influenza A(H1N1)pdm09 and 43.6% (95% CI: -3.8 to 69.4) against influenza A(H3N2).The sample size did not enable measuring adjusted VE among those aged 60 years and above. The crude VE in this age group was 44.0% (95% CI: 8.9 to 65.5) against influenza B, 59.1% (95% CI: 14.3 to 80.5) against influenza A(H1N1)pdm09 and 37.3% (95% CI: -13.0 to 65.3) against influenza A(H3N2).

Due to small numbers, VE for the inactivated subunit cell-based vaccine and the adjuvanted vaccine were not estimated. The adjusted VE for the inactivated subunit vaccine group was 47.8% (95% CI: 12.0 to 69.0) against influenza B, 68.8% (95% CI: 32.3 to 85.6) against influenza A(H1N1)pdm09 and 63.1% (95% CI: 20.1 to 82.9) against influenza A(H3N2). The adjusted VE for the inactivated split virion vaccine group was 52.5% (95% CI: 29.5 to 68.0) against influenza B, 48.9% (95% CI:

Table 2

Details for influenza B (n=1,937), A(H3N2) (n=730), A(H1N1)pdm09 (n=1,068) cases and controls (n=2,874) included in the 2012/13 season trivalent influenza vaccine effectiveness analysis, I-MOVE multicentre case–control study in seven European Union study sites, ISO week 43 in 2012–ISO week 18 in 2013, influenza season 2012/13

Variables	Number of test-negative controls[a]/ total (%)	Number of influenza B cases[b,c]/total (%)	Number of influenza A(H1N1)pdm09 cases[b,d]/total (%)	Number of influenza A(H3N2) cases[c,d]/total (%)
Age groups in years				
0–4	739/2,871 (26)	290/1,937 (15)	162/1,068 (15)	169/729 (23)
5–14	474/2,871 (17)	650/1,937 (34)	160/1,068 (15)	154/729 (21)
15–64	1,396/2,871 (49)	860/1,937 (44)	693/1,068 (65)	328/729 (45)
≥60	262/2,871 (9)	137/1,937 (7)	53/1,068 (5)	78/729 (11)
Sex–Female	1,458/2,854 (51)	965/1,933 (50)	562/1,059 (53)	355/727 (49)
Days between onset of symptoms and swabbing				
0	220/2,874 (8)	73/1,937 (4)	61/1,068 (6)	58/730 (8)
1	1,214/2,874 (42)	652/1,937 (34)	448/1,068 (42)	306/730 (42)
2	714/2,874 (25)	626/1,937 (32)	308/1,068 (29)	201/730 (28)
3	429/2,874 (15)	324/1,937 (17)	155/1,068 (15)	93/730 (13)
4–7	297/2,874 (10)	262/1,937 (14)	96/1,068 (9)	72/730 (10)
Seasonal vaccination, 2012/13[e]	255/2,772 (9)	94/1,898 (5)	48/1,031 (5)	47/699 (7)
2012/13 influenza vaccine group				
Inactivated subunit egg-based	68/2,713 (3)	25/1,879 (1)	9/1,013 (1)	9/683 (1)
Inactivated split virion egg-based	123/2,713 (5)	48/1,879 (3)	21/1,013 (2)	22/683 (3)
Inactivated subunit cell-based	1/2,713 (0)	0/1,879 (0)	0/1,013 (0)	0/683 (0)
Inactivated adjuvanted (squalene MF59)	4/2,713 (0)	2/1,879 (0)	0/1,013 (0)	0/683 (0)
At least one chronic condition	495/2,733 (18)	278/1,896 (15)	154/1,014 (15)	91/690 (13)
At least one hospitalisation in the previous 12 months for chronic condition	29/2,519 (1)	18/1,816 (1)	9/947 (1)	5/632 (1)
Belongs to target group for vaccination	624/2,801 (22)	376/1,916 (20)	199/1,045 (19)	140/707 (20)
Study sites				
France	643/2,874 (22)	534/1,937 (28)	234/1,068 (22)	179/730 (25)
Germany	1,383/2,874 (48)	519/1,937 (27)	508/1,068 (48)	471/730 (65)
Ireland	96/2,874 (3)	119/1,937 (6)	17/1,068 (2)	29/730 (4)
Poland	30/2,874 (1)	1/1,937 (0)	22/1,068 (2)	0/730 (0)
Portugal	183/2,874 (6)	66/1,937 (3)	80/1,068 (7)	6/730 (1)
Romania	66/2,874 (2)	74/1,937 (4)	52/1,068 (5)	2/730 (0)
Spain	473/2,874 (16)	624/1,937 (32)	155/1,068 (15)	43/730 (6)

I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; ISO: International Organization for Standardization.

Denominators of the fractions vary in each category of variables when the information in question is not known for all cases and/or controls. 36 influenza A cases that were not subtypable are not included in the Table.

[a] Controls from 'any influenza' analysis used.

[b] Six influenza cases positive for both influenza B and for influenza A(H1N1)pdm09 were included in the analysis.

[c] Three influenza cases positive for both influenza A(H3N2) and for influenza B were included in the analysis.

[d] Seven influenza cases positive for both influenza A(H3N2) and for influenza A(H1N1)pdm09 were included in the analysis.

[e] Vaccination more than 14 days before onset of influenza-like illness symptoms.

Table 3a

Pooled crude and adjusted seasonal vaccine effectiveness against laboratory-confirmed influenza by influenza type/subtype, overall and among target groups for vaccination, I-MOVE multicentre case–control study in seven European Union study sites to measure 2012/13 influenza vaccine effectiveness, ISO week 43 in 2012–ISO week 18 in 2013, influenza season 2012/13

Analysis scenarios, population included	Influenza B VE (95%CI)	Influenza A(H1N1)pdm09 VE (95%CI)	Influenza A(H3N2) VE (95%CI)
Primary analysis			
All age groups[a]			
N (cases/vaccinated; controls/vaccinated)	4,344 (1,860/92; 2,484/236)	3,196 (978/44; 2,218/214)	3,012 (672/46; 2,340/212)
Crude (study site as fixed effect)	46.5 (30.9 to 58.6)	56.1 (38.6 to 68.7)	22.5 (-8.6 to 44.7)
Adj. for onset week	50.2 (35.4 to 61.6)	57.5 (40.2 to 69.8)	29.1 (-0.5 to 50.0)
Adj. for sex	46.6 (31.0 to 58.7)	56.2 (38.7 to 68.7)	22.4 (-8.7 to 44.6)
Adj. for chronic condition	43.2 (25.9 to 56.5)	54.0 (34.9 to 67.5)	17.4 (-17.2 to 41.8)
Adj. for age	45.7 (28.3 to 59.0)	50.3 (28.9 to 65.2)	38.6 (11.1 to 57.5)
Adj. for onset week, age	50.1 (33.8 to 62.5)	51.9 (30.9 to 66.6)	45.7 (20.5 to 63.0)
Adj. for onset week, sex	50.3 (35.5 to 61.7)	57.6 (40.4 to 69.9)	29.0 (-0.6 to 49.9)
Adj. for onset week, chronic condition, age, sex	49.3 (32.4 to 62.0)	50.4 (28.4 to 65.6)	42.2 (14.9 to 60.7)
0–14 year-olds[b]			
N (cases/vaccinated; controls/vaccinated)	1,969 (905/26; 1,064/40)	1,210 (292/8; 918/35)	1,252 (296/9; 956/34)
Crude	8.0 (-54.4 to 45.2)	30.9 (-52.0 to 68.6)	28.0 (-53.2 to 66.2)
Adj. for onset week, chronic condition, age, sex	22.3 (-37.0 to 55.9)	36.5 (-44.1 to 72.0)	36.1 (-41.1 to 71.0)
15–59 year-olds[c]			
N (cases/vaccinated; controls/vaccinated)	1,994 (824/28; 1,170/95)	1,709 (636/25; 1,073/85)	1357 (303/15; 1,054/85)
Crude	55.6 (30.8 to 71.6)	52.9 (25.5 to 70.3)	41.0 (-4.6 to 66.8)
Adj. for onset week, chronic condition, age, sex	63.6 (42.1 to 77.1)	55.6 (28.3 to 72.5)	43.6 (-3.8 to 69.4)
≥60 year-olds			
N (cases/vaccinated; controls/vaccinated)	362 (131/38; 231/100)	266 (50/11; 216/94)	277 (73/22; 204/89)
Crude	44.0 (8.9 to 65.5)	59.1 (14.3 to 80.5)	37.3 (-13.0 to 65.3)
Adj. for onset week, chronic condition, age, sex	Too few cases	Too few cases	Too few cases
Analysis by vaccine group[d]			
N (cases/vaccinated subunit/vaccinated split virion; controls/vaccinated subunit/vaccinated split virion)	4,058 (1,724/24/44; 2,334/61/106)	3,038 (945/8/20; 2,093/55/99)	2,830 (630/9/20; 2,200/54/99)
Crude subunit Crude split virion	41.0 (3.7 to 63.9) 48.6 (25.7 to 64.4)	72.0 (40.7 to 86.8) 55.0 (26.6 to 72.4)	47.0 (-8.9 to 74.2) 19.0 (-33.5 to 50.9)
Fully adjusted[e] subunit Fully adjusted[e] split virion	47.8 (12.0 to 69.0) 52.5 (29.5 to 68.0)	68.8 (32.3 to 85.6) 48.9 (13.7 to 69.8)	63.1 (20.1 to 82.9) 41.7 (-1.3 to 66.5)

Adj: adjusted; CI: confidence interval; GP: general practitioner; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; ISO: International Organization for Standardization; obs: observations; VE: vaccine effectiveness.

[a] Week 46 dropped (45 obs) for influenza A(H3N2); week 48 dropped (53 obs) for influenza A(H1N1).

[b] Weeks 48 and 13 dropped (27 obs) for influenza A(H1N1); weeks 46, 47 and weeks 14–16 and Romania dropped (119 obs) for influenza A(H3N2).

[c] Week 47 dropped (19 obs) for influenza B; weeks 48 and 15 dropped (37 obs) for influenza A(H1N1); weeks 43–46 dropped (52 obs) for influenza A(H3N2).

[d] Ireland excluded as only one vaccine group available (split virion). Records with adjuvanted or inactivated subunit cell-based vaccine group excluded due to very low sample sizes (8 and 1 respectively). Unknown or missing vaccine group excluded from analysis.

[e] Adjusted for onset week, chronic condition, age and sex.

[f] Weeks 18 and 47 dropped for influenza B (14 obs). Weeks 47,48 and 50 dropped for A(H1N1) (36 obs). October dropped for A(H3N2) – adjusted by onset month, not week, due to small sample size (3 obs dropped).

[g] Week 48 dropped for influenza A(H1N1) (47 obs). Week 46 dropped for influenza A(H3N2) (40 obs).

[h] Week 48 dropped for influenza A(H1N1) (53 obs). Week 46 dropped for A(H3N2) (45 obs).

[i] Numbers of vaccinated and unvaccinated are approximate, due to the nature of the imputed database. Week 46 dropped for influenza A(H3N2) (52 obs). Adjusted for age group (10 year age bands), sex, presence of chronic condition and week of symptom onset.

Table 3b

Pooled crude and adjusted seasonal vaccine effectiveness against laboratory-confirmed influenza by influenza type/subtype, overall and among target groups for vaccination, I-MOVE multicentre case–control study in seven European Union study sites to measure 2012/13 influenza vaccine effectiveness, ISO week 43 2012–ISO week 18 2013, influenza season 2012/13

Analysis scenarios, population included	Influenza B VE (95%CI)	Influenza A(H1N1)pdm09 VE (95%CI)	Influenza A(H3N2) VE (95%CI)
Sensitivity analysis			
Restricted to target group for vaccination[f]			
N (cases/vaccinated; controls/vaccinated)	875 (356/69; 519/155)	648 (184/29; 464/142)	593 (126/29; 467/139)
Crude	40.7 (17.2 to 57.6)	55.8 (30.4 to 71.9)	23.2 (-22.9 to 52.0)
Adj. for onset week, chronic condition, age, sex	43.4 (17.9 to 61.0)	35.7 (-6.0 to 61.0)	39.0 (-3.4 to 63.9)
Restricted to those with delay onset and swabbing <4 days[g]			
N (cases/vaccinated; controls/vaccinated)	3,855 (1,609/79; 2,246/196)	2,912 (894/40; 2,018/178)	2,721 (609/39; 2,112/174)
Crude	40.3 (21.1 to 54.8)	51.9 (31.3 to 66.3)	20.1 (-15.3 to 44.6)
Adj. for onset week, chronic condition, age, sex	45.1 (24.9 to 59.8)	47.8 (23.2 to 64.6)	40.3 (9.1 to 60.8)
Those vaccinated <15 days excluded[h]			
N (cases/vaccinated; controls/vaccinated)	4,336 (1,858/92; 2,478/236)	3,190 (978/44; 2,212/214)	3,002 (670/46; 2,332/212)
Crude	46.6 (31.0 to 58.7)	56.3 (38.8 to 68.8)	22.6 (-8.5 to 44.8)
Adj. for onset week, chronic condition, age, sex	49.6 (32.8 to 62.2)	50.7 (28.9 to 65.9)	42.3 (15.0 to 60.8)
Adjusting by GP visits and smoking (Ireland, Poland, Portugal, Romania and Spain)			
N (cases/vaccinated; controls/vaccinated)	1,678 (875/39; 803/97)	1,105 (319/15; 786/93)	684 (79/5; 605/73)
Crude	62.5 (44.3 to 74.7)	63.5 (35.5 to 79.4)	47.3 (-37.4 to 79.8)
Adj. for onset week, chronic condition, age, sex	62.4 (41.0 to 76.1)	48.7 (3.3 to 72.8)	Too few cases
Adj. for onset week, chronic condition, age, sex, smoking	62.3 (40.7 to 76.0)	48.7 (3.2 to 72.8)	Too few cases
Adj. for onset week, chronic condition, age, sex, GP visits	60.0 (37.0 to 74.6)	46.0 (-2.0 to 71.4)	Too few cases
Adj. for onset week, chronic condition, age, sex, smoking, GP visits	59.9 (36.8 to 74.6)	45.9 (-2.1 to 71.4)	Too few cases
Imputed analysis[i]			
N (cases/vaccinated; controls/vaccinated)	4,993 (2,016/101; 2,977/282)	3,842 (1,138/54; 2,704/258)	3,652 (793/55; 2,859/262)
Crude	46.2 (31.4 to 57.8)	53.4 (36.7 to 65.7)	24.7 (-4.9 to 45.9)
Fully adjusted[e]	48.9 (33.0 to 61.1)	48.1 (27.3 to 63.0)	48.2 (23.8 to 64.8)

Adj: adjusted; CI: confidence interval; GP: general practitioner; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; ISO: International Organization for Standardization; obs: observations; VE: vaccine effectiveness.

[a] Week 46 dropped (45 obs) for influenza A(H3N2); week 48 dropped (53 obs) for influenza A(H1N1).

[b] Weeks 48 and 13 dropped (27 obs) for influenza A(H1N1); weeks46, 47 and weeks 14–16 and Romania dropped (119 obs) for influenza A(H3N2).

[c] Week 47 dropped (19 obs) for influenza B; weeks 48 and 15 dropped (37 obs) for influenza A(H1N1); weeks 43–46 dropped (52 obs) for influenza A(H3N2).

[d] Ireland excluded as only one vaccine group available (split virion). Records with adjuvanted or inactivated subunit cell-based vaccine group excluded due to very low sample sizes (8 and 1 respectively). Unknown or missing vaccine group excluded from analysis.

[e] Adjusted for onset week, chronic condition, age and sex.

[f] Weeks 18 and 47 dropped for influenza B (14 obs). Weeks 47,48 and 50 dropped for A(H1N1) (36 obs). October dropped for A(H3N2) – adjusted by onset month, not week, due to small sample size (3 obs dropped).

[g] Week 48 dropped for influenza A(H1N1) (47 obs). Week 46 dropped for influenza A(H3N2) (40 obs).

[h] Week 48 dropped for influenza A(H1N1) (53 obs). Week 46 dropped for A(H3N2) (45 obs).

[i] Numbers of vaccinated and unvaccinated are approximate, due to the nature of the imputed database. Week 46 dropped for influenza A(H3N2) (52 obs). Adjusted for age group (10 year age bands), sex, presence of chronic condition and week of symptom onset.

13.7 to 69.8) against influenza A(H1N1)pdm09 and 41.7% (95% CI: -1.3 to 66.5) against influenza A(H3N2).

The adjusted VE for the inactivated subunit vaccine group was 28.9% (95% CI: -60.6 to 68.5), 68.5% (95% CI: 32.6 to 85.3) and 64.6% (95% CI: 21.6 to 84.0) for the 0 to 14 year-olds, 15 to 59 year-olds and those aged 60 and older respectively (Figure 3). The adjusted VE for the inactivated split virion vaccine group was 12.2% (95% CI: -85.6 to 58.5), 63.7% (95% CI: 39.7 to 78.2) and 54.1% (95% CI: 16.8 to 74.7) for the 0 to 14 year-olds, 15 to 59 year-olds and those aged 60 and older respectively.

Discussion

In the 2012/13 influenza season, all the I-MOVE multicentre case–control VE adjusted point estimates were below 70%. The design of the case–control study, which involved a multicentre approach, enabled to obtain a sample size large enough to calculate adjusted VE with reasonable precision by age group and type/subtype in this season with significant proportions of influenza B and both influenza A subtypes circulating in Europe.

Adjusted VE estimates against influenza type/subtype for all ages were lowest for influenza A(H3N2) and highest for A(H1N1)pdm09.

The obtained VE estimates by age group suggested differences in terms of age. Indeed, VE estimates were slightly higher among 15 to 59 year-olds while VE point estimates among children aged 0 to 14 years were low, with the lowest VE against influenza B. Two doses of vaccine are often recommended for children up to a certain age, usually before nine years of age, however information on number of doses received was not collected, so the results need to be interpreted with caution. Among the elderly, there were too few cases included in the study to estimate adjusted VE, despite GPs from five study sites including all elderly consulting for ILI in the study. The crude estimates however, suggest low VE against influenza B and influenza A(H3N2). Low VE among the elderly was also seen this season in Denmark (in mainly a hospital setting) and against A(H3N2) in the United States in the early adjusted VE estimates [12,13]. Influenza A(H1N1)pdm09 was less common than A(H3N2) and influenza B among the elderly in this study.

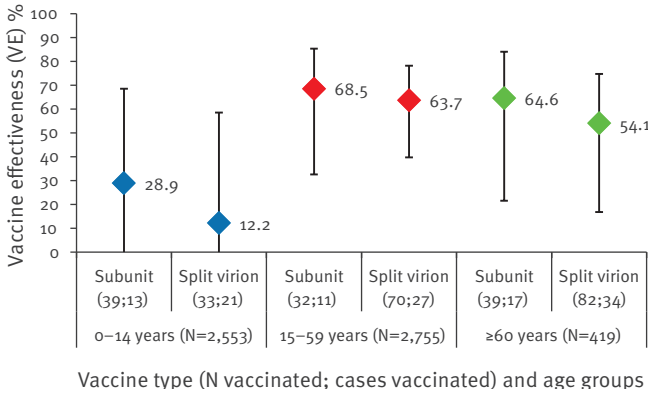
In four study sites, sampling schemes were different for elderly and other age groups. Therefore in analyses where it is not possible to stratify type/subtype-specific estimates further by age group, incorporating, in the future, a sampling fraction by age group and site may correct for any oversampling of a given age group by study site. This could render the comparison of results between countries and pooled estimates more accurate.

The analysis of VE by vaccine group suggested some differences between inactivated subunit and split virion vaccine types for VE against influenza A(H1N1)pdm09 and influenza A(H3N2), although no differences were statistically significant. Some of the vaccines targeted specific age groups, e.g. only the elderly, or only children. So the observed differences in VE may be due to differences in ages of persons using these vaccines. Again, an analysis by influenza type/subtype, age group and vaccine group was not possible due to low sample sizes. However an age-specific analysis of VE by vaccine group against any influenza showed smaller differences in adjusted VE of subunit and split virion vaccines among the different age groups.

Data completeness was good, with only between six and 10% of observations dropped for the type/subtype-specific complete case analyses. The multiple imputation sensitivity analysis showed very similar VE estimates to the complete case analysis for influenza B and influenza A(H1N1)pdm09), suggesting no bias was introduced by missing values for these viruses. The imputed analysis suggested a slightly higher VE for influenza A(H3N2), indicating there may be a small bias in the missing data for A(H3N2). There is a higher proportion of children aged under five years among influenza A(H3N2) cases in this study and there is a higher proportion of missing values in the age group 0 to 4 years. However practitioners are blinded to the outcome in this study design, so there should be no reason for differential incompleteness of data for cases and controls.

Figure 3

Pooled adjusted seasonal vaccine effectiveness by vaccine group against any laboratory-confirmed influenza by age group, I-MOVE multicentre case–control study in seven European Union study sites, ISO week 43 in 2012–ISO week 18 in 2013, influenza season 2012/13



I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; ISO: International Organization for Standardization.

Below the X axis, the two numbers in parentheses for each vaccine type indicate the following: the first number represents all vaccinated with the vaccine group, while the second number represents the cases vaccinated: (N vaccinated; cases vaccinated).

The seven European Union study sites were respectively located in France, Germany, Ireland, Poland, Portugal, Romania and Spain.

Study sites collected potentially important information on positive and negative confounders [14]. Overall the magnitude of confounding was small for VE estimates against influenza B and A(H1N1)pdm09 (‹3% and ‹6% absolute difference between crude and adjusted VE estimates for all ages respectively). Confounding was much greater for VE against influenza A(H3N2), with around 20% absolute difference, mainly due to the age adjustment. Information on smoking and practitioner visits in the previous 12 months was not collected by two study sites, however the sensitivity analysis restricted to the five study sites collecting these variables, suggests no major change in VE following inclusion (‹3% absolute difference).

Influenza type/subtype VE estimates varied by study site (data not shown). An analysis of heterogeneity using the I^2 and Cochrane's Q test showed no, low or moderate heterogeneity between study sites depending on type/subtype, none of which was statistically significant. A possible reason for heterogeneity may be different age distributions by study site among circulating influenza type/subtypes. However an analysis of heterogeneity by age group and type/subtype together was not possible due to the small sample size. Ideally we need a large sample size by study site to carry out detailed study site-specific analyses; this will also enable a two-stage pooled analysis [15]. Other reasons for heterogeneity may include use of different vaccine brands and different healthcare seeking behaviour by study site.

Adjusted VE estimates among the target groups for vaccination were similar compared to the overall population for influenza B and A(H3N2), however lower for influenza A(H1N1)pdm09, although confidence intervals overlap. Influenza A(H1N1)pdm09 cases belonging to the target group for vaccination had a higher proportion of younger people with chronic conditions than other influenza type/subtypes. This suggests that the vaccine may not protect as well against influenza A(H1N1)pdm09 among those who are vulnerable to complications.

The inclusion weeks for the 2012/13 study ranged from week 43 2012 to week 18 2013, making this a very extended study period compared to previous I-MOVE multicentre case–control studies [3-6]. The ratio of controls to cases varied along the season, with a higher ratio of controls to cases at the very beginning of the study. An analysis restricted to peak weeks (weeks 1–12, 2013) showed very similar type/subtype-specific VE, suggesting that this season, there was no bias introduced by including periods with a high ratio of controls to cases (data not shown).

In the 2011/12 influenza season, the I-MOVE study looked at waning of vaccine-induced protection later in the season. In 2012/13, no vaccine-related waning of protection against A(H1N1) was evident, although sample sizes were low. Sample sizes for A(H3N2) were also too low to draw conclusions. For influenza B, there may have been some suggestion of waning of protection with more days between vaccination and onset of symptoms, however sample sizes are also too low to draw conclusions (data not shown).

While the test-negative design is commonly used in vaccine effectiveness studies, it remains a study design that needs to be validated [16-19]. Assumptions behind this study design and other biases associated with the test negative study design (e.g. representativeness of the test negative controls with regards the vaccine coverage of the source population of cases, role of other ARI virus infections among the control group, etc.) have been described elsewhere [20-24]. Larger sample sizes are needed to perform robust validation analyses to determine if controls properly reflect the vaccine coverage of the source population for cases in general and over time.

The predominant influenza B lineage circulating in the 2012/13 influenza season in Europe was the B/Yamagata lineage, which was also the vaccine virus lineage [1]. Among the 1,860 influenza B cases included in the study, 694 (37.3%) specimens were ascribed to a lineage; 630 of these (90.7%) were influenza B/Yamagata lineage (data not shown). Because of limited information on influenza B lineage in the study, we were unable to estimate VE by lineage, which would be important in the context of the introduction of the quadrivalent vaccines. Throughout the 2012/13 season in Europe, B/Yamagata-lineage viruses from an antigenically distinguishable genetic clade from the vaccine virus clade have increasingly been detected [1]. These are from clade 2 represented by B/Massachusetts/2/2012, which is a recommended vaccine component for the 2013/14 influenza vaccine [25]. While this may explain some of the lower VE estimates for influenza B in our study, we do not have virological data on an individual level to test this hypothesis.

The I-MOVE multicentre case–control study provided adjusted type/subtype-specific VE estimate early in this season, as did other studies [7,12,13,26-29]. The information was shared with WHO in time for the 2013/14 influenza vaccine composition meeting in February 2013. Early adjusted VE estimates for influenza B were higher than the final season adjusted estimates (78.2% vs. 49.3%), as well as for influenza A(H1N1)pdm09 (62.1% vs. 50.4%). Estimates for influenza A(H3N2) remained similar (41.9% vs. 42.2%). Differences between early and overall estimates may be due to different proportions of cases occurring by age group over time; however this cannot be verified as sample sizes were too small to provide early type/subtype estimates stratified by age. This strongly suggests that effort should be made worldwide to increase the sample size for precise and stratified early and overall estimates each season.

In conclusion, our estimates suggest that the 2012/13 influenza vaccine has low to moderate effectiveness in preventing medically attended laboratory-confirmed influenza, with varying effectiveness in different age groups. In a season with the co-circulation three influenza types and subtypes, the large sample size achieved by the multicentre case–control study was necessary to provide the important age-specific estimates. However an even greater sample size is needed to provide robust results among the elderly and to provide age group-specific estimates by type/subtype and vaccine type. While the vaccine remains the best method of protection against influenza, the low estimates emphasise the need for an improved influenza vaccine. Influenza VE studies worldwide need to continue with sample sizes large enough to enable precise stratified estimates in order to better understand the mechanisms for varying VE by season and age groups.

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Conflict of interest

EpiConcept analyses pooled data of a hospital-network multicentre case-control (HNMCC) study measuring influenza VE. The HNMCC analysis was co-funded in 2012/13 by Sanofi Pasteur, SPMSD, GlaxoSmithKline, EpiConcept and study sites.

Authors' contributions

EpiConcept: Marta Valenciano coordinated the I-MOVE multicentre case control study network. Marta Valenciano and Esther Kissling led the writing of the research article. Esther Kissling undertook the statistical analysis on which the research article is based. All authors provided contribution to the research article and approved the final version. Alain Moren, Marta Valenciano, Esther Kissling were involved in the original methodological design. In general: Alain Moren, Marta Valenciano, Esther Kissling, Amparo Larrauri, Silvia Jiménez- Jorge, Joan O'Donnell, Emilia Lupulescu, Daniela Pitigoi, George Necula, Baltazar Nunes, Raquel Giuomar, Annicka Reuss, Udo Buchholz, Jean Marie Cohen and Isabelle Daviaud have all had a role in modification of this design over the years. All authors read, contributed and approved the manuscript final version. Germany: Annicka Reuss and Udo Buchholz were responsible for validation of data and interpretation of results. Spain: Amparo Larrauri and Silvia Jiménez-Jorge were responsible for the study design and coordination of the Spanish study and the national database. France: Jean Marie Cohen and Isabelle Daviaud were responsible for the study design in French study site, participated in the coordination of the French study and management of the French database. Portugal: Baltazar Nunes and Raquel Guiomar were responsible for the study design in Portugal study site. Ireland: Justyna Rogalska was involved in the collection and collation of the data. Joan O'Donnell was involved in the original methodology and final review of the paper. She was also coordinating the project in Ireland. Romania: Daniela Pitigoi and George Necula coordinated the Romanian study. Daniela Pitigoi was responsible for the study design in Romania study site. Daniela Pitigoi and George Necula collected data. Daniela Pitigoi enrolled patients. Poland: Iwona Paradowska-Stankiewicz and Malgorzata Głuchowska were responsible for the study design and coordination in the Polish study site.

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Climatic suitability of *Aedes albopictus* in Europe referring to climate change projections: comparison of mechanistic and correlative niche modelling approaches

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The Asian tiger mosquito, *Aedes albopictus*, is capable of transmitting a broad range of viruses to humans. Since its introduction at the end of the 20th century, it has become well established in large parts of southern Europe. As future expansion as a result of climate change can be expected, determining the current and projected future climatic suitability of this invasive mosquito in Europe is of interest. Several studies have tried to detect the potential habitats for this species, but differing data sources and modelling approaches must be considered when interpreting the findings. Here, various modelling methodologies are compared with special emphasis on model set-up and study design. Basic approaches and model algorithms for the projection of spatio-temporal trends within the 21st century differ substantially. Applied methods range from mechanistic models (e.g. overlay of climatic constraints based on geographic information systems or rather process-based approaches) to correlative niche models. We conclude that spatial characteristics such as introduction gateways and dispersal pathways need to be considered. Laboratory experiments addressing the climatic constraints of the mosquito are required for improved modelling results. However, the main source of uncertainty remains the insufficient knowledge about the species' ability to adapt to novel environments.

Background

In recent years, European awareness concerning the introduction and establishment of invasive mosquitoes has increased, most notably due to the incursion of the Asian tiger mosquito, *Aedes albopictus* – the most invasive disease vector globally [1,2]. This mosquito has spread from its original distribution area in southeast Asia [3] to all continents via shipping of goods [4]. After its initial introduction to Europe at the end of the 20th century, *A. albopictus* became well established in southern Europe [2]. Recent observations hint towards a spread of this vector to the continental interior of Europe [5]. This mosquito is capable of transmitting several viruses that are pathogenic to humans [1,6]. Most strikingly, *A. albopictus* was the vector that caused the first autochthonous transmission of chikungunya virus [7,8] and dengue virus [9-11] in the Mediterranean area. More recently, a dengue outbreak occurred in the autonomous region of Madeira, Portugal. In this case, *A. aegypti*, the yellow fever mosquito, acted as the vector [12].

Several studies have aimed to determine the climatic suitability of *A. albopictus* at the end of 20th and the beginning 21st century [13-15], as well as the expected future tendencies in Europe [16-19]. Most recently, Caminade et al. [19] implemented three established modelling approaches [14,16,20], making use of new observations, climate data and a report on model quality. However, a comparative methodological evaluation of the different approaches was still missing. Here, we provide a comprehensive comparison of studies assessing the climatic suitability of European regions for *A. albopictus* as a result of a rapidly changing climate during the 21st century. General information as well as limitations in study design and data quality is highlighted. Uncertainties related to climate change and insect vectors are identified. In so doing, we aim to provide guidance for future research.

Review of distribution models for *Aedes albopictus*

In order to assess knowledge about the responses of *A. albopictus* to climate change in Europe, we conducted a literature search, using the Thomson Reuters Web of Knowledge research portal (which includes the databases Web of Science, BIOSIS, Current Contents Connect, MEDLINE and Journal Citation Reports) as well as Google Scholar. Search terms were built from all possible combinations of the keywords '*Aedes albopictus*', 'Stegomyia albopicta' or 'Asian tiger mosquito' in combination with 'climat* change', 'climat* warming'

or 'global warming'. We considered only those research studies with a detailed analysis of methodological tasks and comparison of results, in which distribution modelling approaches were applied to European regions.

Our search identified six studies (up to November 2012) that aimed to determine the distribution of *A. albopictus* in Europe [14-19]. Methodological details and study design are described (Tables 1 and 2). Four of them analysed changing spatial patterns of *A. albopictus* in Europe by using climate change scenarios [16-19]. The studies with projections were used to derive general trends concerning the future development arising from a comparison of the resulting projections. In this review, we bring these specific studies for Europe into a wider context, as we account for the (methodological) development in the creation of risk maps of *A. albopictus* in order to understand the philosophy behind the work more intuitively.

Generally, two methodological approaches seem to be appropriate for the projection of climatic suitability of European habitats for *A. albopictus*: mechanistic models and correlative niche models. Mechanistic models do not require geographical occurrence data for species. They are either based on the construction of overlay functions for climatic constraints in geographic information system (GIS) environments or process-based models with mechanistic principles. The aim of such models is to simulate and project the response of an individual organism or a population by explicitly incorporating biological processes calibrated with observations on individuals in natural populations and controlled field or laboratory studies [21]. Thus, mechanistic models rely on the implicit assumption that the model structure and process formulations are correct [22].

A second, rather statistical approach is the use of correlative environmental niche models. Here, species presence and, in some approaches, also absence locations are related to environmental or climatic variables with the aim of determining the species-specific niche (synonymously used: 'envelope') that is defined by the parameter values – including the multivariate combinations – from the known occurrences. This niche can be interpolated or extrapolated to infer species' geographical distribution. Advanced modelling techniques offer novel opportunities for the determination of species changing spatial distribution patterns as a response to environmental and climatic changes [23]. The main issue with correlative models is their dependence on the amount, quality and relevance of the data used [22]. Commonly, niche-modelling algorithms require presence as well as absence records. However, some models make use of pseudo-absence data or even presence-only data, as in many cases, absence data are not available. The lack of absence data may also suggest that areas where the species is missing might be suitable, but the insect may simply not be present yet. Consequently, presence-only models are appropriate to handle most of the data for mobile and invasive insects in the course of climate impact research.

Distribution models devoid of climate change projections

Several studies identified the past or current climatic suitability for *A. albopictus*, based either on mechanistic [13,14,20] or correlative [15,16,24,25] distribution modelling approaches for specific regions or globally. Here, we highlight studies with relevance for Europe.

Mechanistic approaches

Kobayashi et al. identified a close connection with the annual and January mean temperature for the distribution of *A. albopictus* in northern Japan [20]. In addition, a period with daily temperature continuing above at least 11 °C during summer months (more than 186 days per year) was observed and interpreted as a requirement for larval development.

The first GIS-based risk maps were developed by Mitchell [13] for the Mediterranean Basin. Expert knowledge on temperature, rainfall and humidity as well as the photoperiod was applied in order to frame climatic constraints. For the United Kingdom, Medlock et al. [14] used temperature and daylight thresholds to simulate life cycle dynamics via overlay functions in GIS. Furthermore, they created different scenarios by altering the diurnal length of the photoperiod. This was done to assess the ability of eggs to survive in winter and predict the hatching in spring and the subsequent production of diapausing eggs in autumn. Consequently, the potential responses to these alterations in mosquito life cycle can be determined. It should be noted that the scenarios of Medlock et al. [14] do not refer to scenarios announced in the special report on emissions scenarios (SRES) [26] from the Intergovernmental Panel on Climate Change (IPCC). In the technical report of the European Centre for Disease Prevention and Control (ECDC), *Development of Aedes albopictus risk maps* [16], the approach from Medlock [14] is adapted, but was expanded to cover Europe. In this ECDC report [16], two further modelling approaches were used: one further mechanistic and one correlative approach, which are described below.

Correlative approaches

Presence/absence models

Many niche modelling algorithms require both documented presence, as well as absence localities in order to build statistical relationships. In its report, ECDC deployed random forest models (based on regression trees) in order to estimate the current climatic suitability for *A. albopictus* in Europe [16]. In short, random forest is an ensemble classifier that consists of combined decision trees and gives the class that is the mode of the classes by individual trees as an output. Centroids (geometric centres) of the European municipalities

Table 1

Studies addressing current and projected climatic suitability of *Aedes albopictus* in Europe

Study	Region	Model	Input data: climate/ environmental	Validation or data/ model predictive power	Climate projection or climate model	Scenario	Time step
Medlock et al. 2006 [14]	UK	GIS overlay (MA)	• Climate data: annual mean rainfall and monthly mean temperature from 1971 to 2000 provided by the UK Meteorological Office (1 km) • Weekly weather data: derived from monthly temperature data using a continuous piecewise quadratic functio	–	Own alteration	Own scenarios	–
ECDC 2009 [16]	Europe	Random forest (CA)	• World climatic zones • Temperature data archive at the University of Daytona, US: daily mean temperatures (1995–2007) • MODIS: day- and night-time LST (1 km) • CRU: monthly mean temperatures and rainfall variables averaged from 1961 to 1990 (5 km) • NDVI and EVI (1 km)	• n=1,525 (presences and absences, due to centroids of municipalities) • training sample (n=300), divided over both the presence (n=165) and absence (n=135) • AUC	No projection	–	–
	Europe	GIS overlay (CA) sensu Medlock et al. [14]	• Same climate data source as for the random forest (CA)	–	No projection	–	–
	Europe	MCDA (CA)	• Same climate data source as for the random forest (CA)	–	According to IPCC (no further details)	Minimal and maximum impact scenarios	2010, 2030
Fischer et al. 2011 [17]	Europe	MaxEnt (CA)	• Worldclim: 19 bioclimatic variables derived from monthly temperature, rainfall values and altitude (10 km)	• Presence point data worldwide (n=1,199) • Randomly selected test (30%) and training (70%) data; the split into training and test data was replicated 100 times • AUC	Regional climate model COSMO-CLM rescaled to 10 km	A1B[a] B1[a]	2011–2040, 2041–2070, 2071–2100
Roiz et al. 2011 [18]	Trentino (north-east Italy)	GLM (CA)	• Daily LST (MODIS Terra and Aqua satellites), reprojected to 200 m • Human population data from official population census (2001) and from Landscan Global Population Database	• Absence and presence point data at 145 sample stations • AIC	No specific climate model: increase in mean January temperature (1.5 °C) and mean annual temperature (1 °C) with respect to reference period 1961–1990	A2[a]	2040–2050
Caminade et al. 2012 [19]	Europe	GIS overlay (MA) sensu Kobayashi et al. [20] and Medlock et al. [14], MCDA sensu ECDC [16	• Gridded climate dataset based on station measurements at daily and monthly temporal resolution (25 km²)	• Absence and presence data at the regional administrative level of the European Union • AUC	10 selected regional climate models (ensembles), 0.25 ° step: C4IRCA3, CNRM-RM4.5, DMI-HIRAM5, ETHZ-CLM, ICTP-RegCM3, KNMI-RACMO2), METO-HC-HadRM3.0, MPI-M-REMO, OURANOSMRCC4.2.1, SMHIRCA	A1B[a]	2030–2050
ECDC 2012 [15]	Europe	Non-linear discriminant analysis (CA)	• Fourier transformation of MODIS temperature (Terra satellite) and elevation data • Worldclim data • Human population density from Global Rural-Urban Mapping Project	• Thousands of occurrence records via existing databases and own literature search (for *A. albopictus* and *A. aegypti*) • Generation of pseudo-absences via environmental (MD) and geographical distance measure	No projection	–	–

AIC: Akaike´s Information Criterion; AUC: area under the receiver operator characteristic curve; CA: correlative approach; CRU: Climate Research Unit; ECDC: European Centre for Disease Prevention and Control; EVI: enhanced vegetation index; GIS: geographic information system; IPCC: Intergovernmental Panel on Climate Change; LST: land surface temperature; MA: mechanistic approach; MCDA: multi criteria decision analyses; MD: Mahalanobis distance; MODIS: Moderate Resolution Imaging Spectroradiometer; NDVI: normalised difference vegetation index UK: United Kingdom; US: United States.

[a] Emissions scenarios are based on the IPCC special report on emissions scenarios (SRES), where different storylines describe the relationships between the driving forces of climate change. The A1B scenario describes a future world of very rapid economic growth, global population that peaks in mid-century and declines thereafter and the rapid introduction of new and more efficient technologies. The A2 scenario assumes a continuously increasing global population, the economic development is primarily regionally oriented and per capita, economic growth and technological changes are more fragmented and slower than in other storylines. The B1 scenario is based on the assumption that economic structures will change rapidly towards a service and information economy and resource-efficient technologies will be introduced [26].

were used as presence or absence localities [27]. It should be noted that these municipalities differ in their spatial extent. The average area calculated from the political boundaries of the municipalities in southern Europe (e.g. Italy or Spain) may be up to three times bigger than in those in central Europe (e.g. Germany), which limits the ability to account for landscape heterogeneity. The centroids indicating species presences or absences are correlated with 57 (standardised) climate data layers, from which four variables are chosen as predictors via a backward stepwise procedure. All selected predictors are related to temperature.

Another approach recently published in a later ECDC technical report, The climatic suitability for dengue transmission in continental Europe, is based on multivariate discriminant analyses [15]. Again, this approach concentrates on modelling the current climatic suitability for *A. albopictus*. Here, global occurrence of this species was used as a model input. Accounting for the global dimension offers the opportunity to include the entire environmental space occupied by the species. However, this neglects the role of adaptation in regional populations. As discriminant analyses require absence records, (global) pseudo-absences were generated by evaluating localities that were geographically and environmentally dissimilar to presences. The models aim to discriminate between these two categories using the predictor variables available. The final risk maps were produced by averaging over 100 bootstrap samples [15].

Presence-only models

Many insect databases rely on documented presence localities, especially if a species is globally distributed. As the generation of pseudo-absences is ambitious (see [15]), novel ways to cope with presence-only data have been developed. In presence-only models, relationships are based on comparison of a species presence with the environmental background. Within this environmental background, the species were not recorded, which could also mean that data collection was not attempted in the respective region. Thus, at those sites, no information on the suitability of the environment or climate exists.

Employing the correlative environmental niche model Genetic Algorithm for Rule-set Prediction (GARP), Benedict et al. determined the global risk of invasion by *A. albopictus* [24]. A model built with GARP is iteratively chosen from non-random correlations between environmental and occurrence data. The non-random correlations describe environmental thresholds, depending on the chosen type of mathematical rule. Apparently, *A. albopictus* occupies different environmental niches on the invaded continents, which is revealed by Medley by applying correlative niche models for isolated geographical occurrence localities from the native and invaded range [25]. For all comparisons, the niche for introduced distributions was not equivalent to the native niche. For this purpose, Medley [25] applied the Maximum Entropy approach (implemented in MaxEnt software) [28]. MaxEnt has replaced GARP as a preferred modelling algorithm for presence-only data during the past years, due to improved model performance [23]. The idea behind MaxEnt is to find the probability distribution of maximum entropy (most spread out) that is subject to constraints imposed by information available on the species presence and the environmental conditions across the study area [28,29].

Distribution models that consider climate change projections

Until November 2012, there were four studies that aimed to determine potential future climatic suitability of *A. albopictus* in Europe (summarised in Table 1 and 2) [16-19]. In two studies [16,19], climatic suitability was projected via mechanistic models, while the results of the two other studies [17,18] were based on correlative approaches. One study [18] was applied to a limited study region, while the other three [16,17,19] cover the entire European continent. In order to detect methodological qualities and constraints, these studies are compared in detail.

Information concerning input data is given including: climate variables, model validation and source and steps, e.g. of climate data for the respective emission scenario as well as addressed future time steps.

Mechanistic approaches

Within the technical report of the ECDC, a mechanistic multi criteria decision analysis (MCDA) was performed [16]. In contrast to the correlative approaches of this report, the results of the MCDA were projected to future conditions. An MCDA is a structured tool within a decision support framework. This enables evaluation of multiple decision constraints based on previously defined estimation criteria. The exploration of such decision alternatives for complex problem settings was recently developed within GIS frameworks in order to achieve accurate spatial risk assessment of vectors and vector-borne diseases [30]. In order to detect climatic suitability for *A. albopictus*, sigmoidal or symmetric sigmoidal membership functions were generated for the standardised variables and combined linearly with equal weight [16]. This was done based on expert advice. Generally, MCDA applications for spatial pattern analysis offer an opportunity to identify gaps and limits in knowledge; however, they are limited in determining causality [30]. Projections were applied for the MCDA approach and applied to the expected situation in 2010 and 2030, using SRES-scenarios with minimal or maximal impact [25]. Detailed information concerning the climate model and scenario characteristics was not given.

The mechanistic approaches used by Kobayashi et al. [20], Medlock [14] and the MCDA by ECDC [16] were adapted by Caminade et al. [19]. In contrast to previous approaches, Caminade et al. evaluated model performance via the area under the receiver operator

Table 2a

Variables and model set-up in studies addressing current and projected climatic suitability of *Aedes albopictus* in Europe

Study	Variables	Method
Medlock et al. 2006 [14]	Overwintering criteria - Mean January temperature >0 °C - Annual mean rainfall > 500 mm Spatio-temporal activity Scenario 1 Low risk - Spring mean temperature 10–10.5 °C - Spring photoperiod 11–11.25 h (daylight) - Temperature for cessation of egg/larval activity <9.5 °C - Critical photoperiod for autumn diapause 13–13.25 h Medium risk - Spring mean temperature 10.5–11 °C - Spring photoperiod 11.25–11.5 h - Temperature for cessation of egg/larval activity 9.5–10 °C - Critical photoperiod for autumn diapause 13.5–14 h High risk - Spring mean temperature >11 °C - Spring photoperiod >11.5 h - Temperature for cessation of egg/larval activity >10 °C - Critical photoperiod for autumn diapause >14 h Scenario 2 - Critical photoperiod for autumn diapause 11 h, 11.5 h and 12 h for high, medium and low risk, respectively. The other three parameters stay the same. Photoperiod is based on astronomical equations of sunrise and sunset.	GIS-based overlay Assessing the potential for survival and spatio-temporal activity dynamics (number of weeks between the first hatching of overwintered eggs in spring and the production of diapausing eggs)
ECDC 2009 [16] GIS overlay	Adapted by Medlock et al. [14] but no overwintering criteria - Critical photoperiod for autumn diapause 13.5 h - Spring photoperiod 11–11.5 h - Spring mean temperature 10–11 °C	GIS-based overlay sensu Medlock et al. [14]
ECDC 2009 [16] Random forest	Four predictor variables chosen from 57 data layers - Maximum night-time LST - Mean annual daytime LST - Minimum daytime LST - Second amplitude of daytime LST	Random forest - 200 aggregated classification trees for classification - Stepwise backward reduction of the number of variables until accuracy dropped below 90%.
ECDC 2009 [16] MCDA	Annual mean rainfall - No suitability <450 mm - Maximum suitability >800 mm Summer temperature (June–August) - No suitability <15 °C or >30 °C - Maximum suitability 20–25 °C Mean January temperature - No suitability <–1 °C - Maximum suitability >3 °C	MCDA - Sigmoidal transformation of mean annual rainfall and temperature in January - Symmetrical sigmoidal transformation of summer temperatures - Linear combination for suitability data layers, whereby each factor was assigned with equal weight
Fischer et al. 2011 [17] Expert knowledge-based model	Selection from 20 bioclimatic variables - Annual mean temperature - Mean temperature of the warmest quarter - Mean temperature of the coldest quarter - Annual precipitation - Altitude	MaxEnt - Selection of variables based on expert knowledge
Fischer et al. 2011 [17] Statistic based model	Selection from 20 bioclimatic variables - Annual mean temperature - Annual precipitation - Precipitation of the warmest quarter - Precipitation of the coldest quarter - Altitude	MaxEnt - Jackknife test to measure variables´ importance - Calculations of models´ training gains for variables´ in isolation and for remaining dataset if this variable is dropped
Roiz et al. 2011 [18]	Survival of overwintering eggs January mean temperature >0 °C Annual mean temperature >11 °C Highly suitable: January mean temperature ($JanT_{mean}$) >0 °C and Annual mean temperature ($AnnT_{mean}$) >11 °C Moderately suitable: $JanT_{mean}$ >0 °C and $AnnT_{mean}$ <11 °C or $JanT_{mean}$ <0 °C and $AnnT_{mean}$ >11 °C Unsuitable: $JanT_{mean}$ <0 °C and $AnnT_{mean}$ <11 °C Human population data - Human population density - Distance to human settlements	GLM with binomial distribution (multiple logistic regression) - Relating species' presences/ absences to variables

Table 2b

Variables and model set-up in studies addressing current and projected climatic suitability of *Aedes albopictus* in Europe

Study	Variables	Method
Caminade et al. 2012 [19] Model 1	Annual mean temperature - Totally suitable >12°C - High risk 11–12 °C - Moderate risk 10–11 °C - Low risk 9–10 °C Overwintering criterion Highly unsuitable - Mean January temperature <0 °C - Annual mean rainfall <500 mm Medium unsuitable - Mean January temperature 0–1 °C - Annual mean rainfall 500–600 mm Low unsuitable - Mean January temperature 1–2 °C - Annual mean rainfall 600–700 mm Suitable - Mean January temperature >2 °C - Annual mean rainfall >700 mm	GIS-based overlay sensu Kobayashi et al. [20]
Caminade et al. 2012 [19] Model 2	See ECDC [16] (MCDA)	MCDA sensu ECDC [16]
Caminade et al. 2012 [19] Model 3	Overwintering criterion (see model 1) Weeks of activity - Mean weekly temperatures - Mean weekly photoperiods Hatching onset (medium scenario) - Spring temperature >10.5 °C - Photoperiod >11.25 h Autumn diapause - Temperature >9.5 °C - Photoperiod >13.5 h	GIS-based seasonal activity model sensu Medlock et al. [14] - Overwintering criterion to mask the areas where the mosquito would not be able to survive - Photoperiod calculation as the period between sunrise and sunset - Computation of the start of spring hatching and autumn egg diapause is based on medium scenario
ECDC 2012 [15]	Clear documentation of pre-processing MODIS data; no further information about the chosen variables	Non-linear discriminant analysis - Preliminary k-means cluster analysis to analyse outliers in training set for exclusion in modelling process - 100 random bootstrap samples with equal number of presences and absences - Stepwise inclusion of 10 environmental variables - 100 results were averaged to produce the final risk maps

GIS: geographic information system; GLM: generalised linear model; LST: land surface temperature; MCDA: multi criteria decision analyses; MODIS: Moderate Resolution Imaging Spectroradiometer.

characteristic curve (AUC) [19]. AUC is based on signal detection theory and illustrates the performance of a binary classifier system when the discrimination threshold varies. Hence, it is typically used to determine performance of correlative niche models. Although it is a mechanistic approach, presence and absence localities based on centroids created from administrative level are generated [27]. These data were used as an evaluation of their results of the mechanistic classification in order to measure model performance. A novel feature was that Caminade et al. considered the role of climate change in Europe in past years (1960–1989, 1990–2009, 2005–2009) in the spread of the mosquito [19]. Furthermore, ensemble data of climate change projections were used, which were given by 10 regional climate models. Regional climate models are driven, at their boundaries, by global climate models. Employing ensemble data enables variations of future projections to be assessed and, consequently, reduces uncertainty [31]. Usually, projections based on ensemble data include a multitude of potential variations by averaging over all possible developments. In the study of Caminade et al., projections were solely based on the A1B emission scenario [19]. The A1 storyline describes a future world with very rapid economic growth and a rapid introduction of new and more efficient technologies. Thereby, the global population peaks mid-century and declines thereafter. In the A1B scenario, a balanced use across all energy resources is expected [26].

Correlative approaches

Previous findings hint towards niche shifts of *A. albopictus* during the global invasion process [25]. In order to account for this, Fischer et al. applied two models

built on presence-only data beyond the European distribution with MaxEnt [17]. Firstly, global occurrence was used for training. Secondly, the native (Asian) distribution served as a training region. Both models were tested for the current European climatic conditions. The database contains more than 6,000 occurrence records of which 1,200 were selected as model input. The initial database was reduced by using geographically weighted correction to minimise spatial bias and autocorrelation in data. Geographically explicit point localities were taken from the literature and completed with presences reported on county level from the United States for the generation of the global database. The problematic issue with political or administrative borders in datasets was mentioned before. While the native range models, containing the Asian distribution and environments, fail to predict the current distribution in Europe, the global-trained model predicts the current European distribution with highly satisfactory quality. This suggests the use of the entire 'climatic niche' for projections. Two sets of bioclimatic variables provided by WorldClim (global climate data) [32] were used as model input. The first set was based on expert knowledge on species' ecology. The second set was chosen via statistical tests to determine the highest explanatory power of the model. All models were validated with AUC values. As both the expert knowledge- and statistical-based models of the global range yield high AUC values, they were both projected to future climate conditions in Europe. The training region seemed to be more important than the chosen set of climatic variables. Projections were based on data given by the regional climate model COSMO-CLM, applying the two scenarios A1B and B1. The A1B scenario has been described above. The B1 storyline describes the same development of the global populations in a globalised world, as in the A1B scenario, but with a rapid change in economic structures towards a service- and information-oriented economy with environmental sustainability [26]. The B1 scenario is a rather moderate scenario and corresponds to the aim of the European Union of keeping anthropogenic warming below 2 Kelvin in comparison to the pre-industrial level [33]. Non-analogue climate is a problematic issue in species distribution modelling, as the observed distribution of a species provides no information about species response under novel climates, e.g. [22,34,35]. Hence, projections (in space and/or time) to regions with non-analogue climate are biased and require caution in interpretation. In the study of Fischer et al. [17], however, non-analogue climate in projections were excluded via multivariate environmental similarity surface analysis as state-of-the-art evaluation (see [36]).

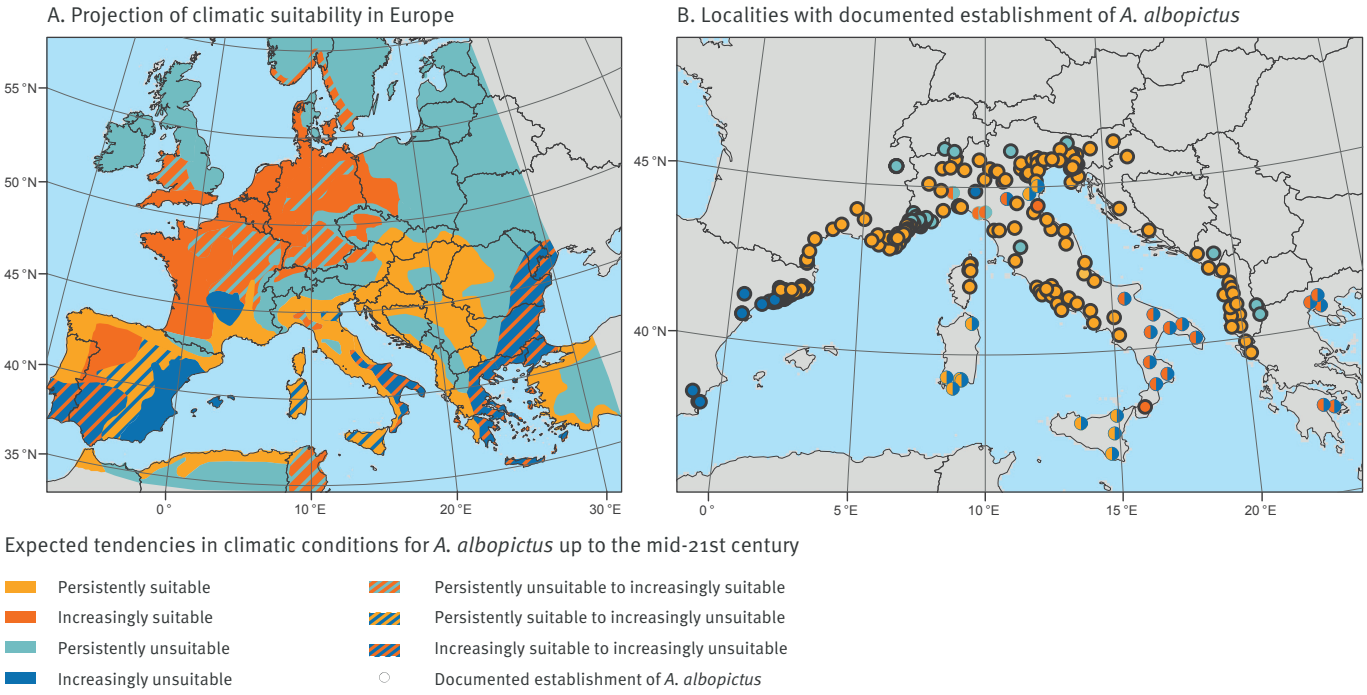
Roiz et al. focused on the potential spread of *A. albopictus* to higher altitudes in the Alps of northern Italy using binomial generalied linear model (GLM) as a logistic regression [18]. They related presences and absences of *A. albopictus* in ovitraps to land surface temperature (LST) data from satellite and human population data. Multiple years of daily LST data from the Moderate Resolution Imaging Spectroradiometer (MODIS) were reprocessed at increased spatial resolution of 200 m pixels. The geographically explicit presence/absence data offers the opportunity to correlate them with the background data at this high spatial resolution. A temperature-gradient-based model was used to fill no-data areas from more than 11,000 daily MODIS LST scenes from 2000 to 2009. On the basis of this, threshold conditions for the survival of eggs in the winter, alongside the survival of the adults, were determined. The best models were selected via Akaike's Information Criterion (AIC). AIC is grounded on the concept of information entropy and evaluates the information loss, when a given model should describe reality. It can be interpreted as a trade-off between model accuracy and complexity. In concurrence with previous results [20], Roiz et al. identified annual mean temperature (11 °C) and January mean temperature (o °C) as best predictors for identifying areas suitable for *A. albopictus* establishment [18]. Applying the A2 scenario, they considered an increase of the annual mean temperature of 1 and 1.5 Kelvin in winter in order to simulate the expected climatic conditions in 2050. Using data obtained directly from regional climate models would be inappropriate as these data are given in a resolution of 10–20 km. The A2 storyline describes a heterogeneous regionally oriented world and economy with a continuously increasing global population. Warming tendencies are more pronounced than in the previously described A1B and B1 scenarios [26].

Evaluation of climate change effects on the habitat suitability

Evidently, several distribution modelling efforts have been used to project the future climatic suitability of *A. albopictus* in Europe, which differ in model algorithm, climate data and scenarios. Here, we generated a simple GIS overlay (Figure 1A) to compare the risk map from the technical report of ECDC [16] with the results from Fischer et al. [17] and Caminade et al. [19]. However, an accurate comparison concerning the results of future projections cannot be presented, for several reasons. Firstly, there were clear differences regarding the chosen time-steps, emission scenarios and spatial resolution (Tables 1 and 2). Secondly, both, geographical and projected coordinate systems were used in the different studies. Hence, the comparison must be considered as a schematic and qualitative generalisation rather than a quantitative detailed compilation. Furthermore, we labelled localities with documented establishments of *A. albopictus* with the colour of the local climatic suitability (Figure 1B), to indicate how accurate the models reflect these occurrences. In general, the models under investigation were capable of predicting well the current localities of *A. albopictus* in Europe (Figure 1B). Only a few presences were observed in regions with rather unsuitable conditions.

Figure 1

Projections of climatic suitability of *Aedes albopictus* in Europe (A) and in European localities with documented establishment of *A. albopictus* (B)



A. Evaluation of projections of climatic suitability of *A. albopictus* within the first half of the 21st century in Europe in comparison with the situation at the end of the 20th century. Results of the mechanistic models based on multi criteria decision analyses of ECDC [16] and Caminade et al. [19] were compared with the statistical-based correlative niche model of Fischer et al. [17]. This is simply a schematic and qualitative generalisation, due to differences in time periods, scenarios and spatial resolution.

B. The records are coloured according to the evaluation of the changing climatic suitability of *A. albopictus* (ranging from the end of the 20th century up to the first half of the 21st century) presented in panel A.

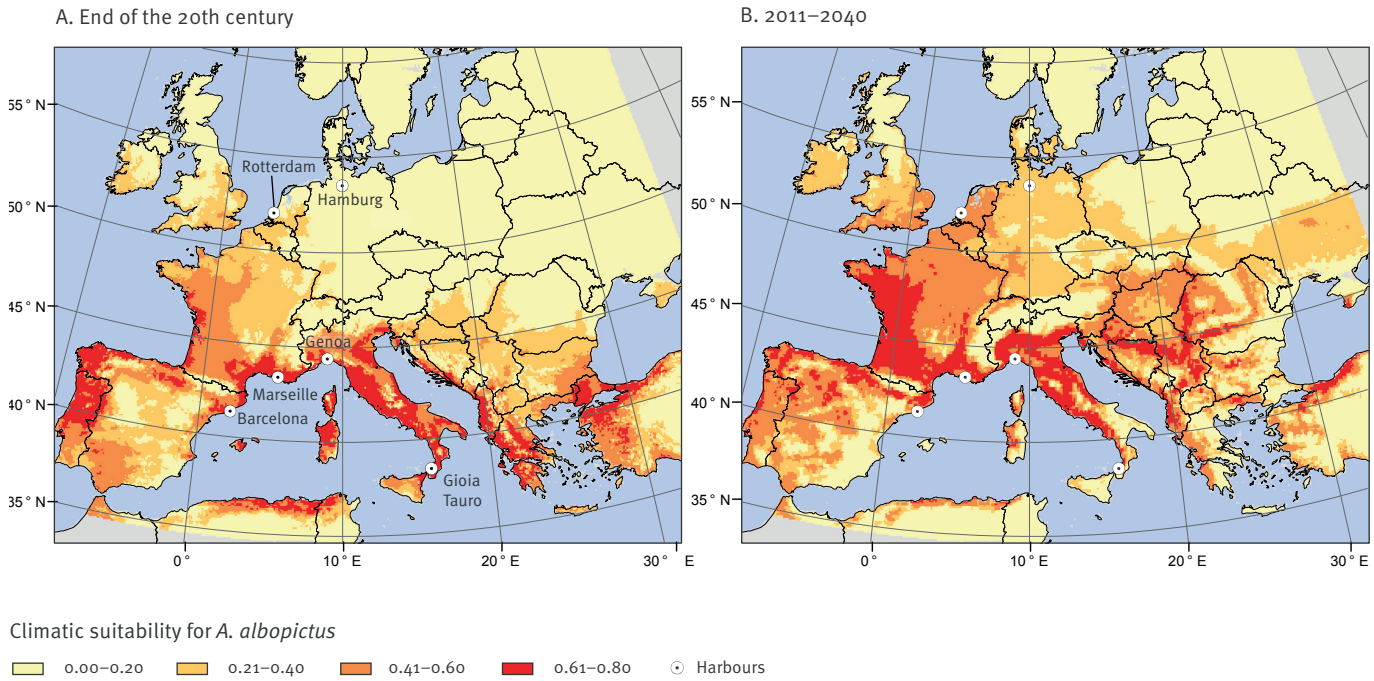
General trends arising from comparison of the studies

Regardless of the above-mentioned differences and obstacles for comparisons, some general tendencies concerning the evolving climatic suitability for *A. albopictus* in Europe within the first half of the 21st century can be derived. Projections indicate that climatic suitability will especially increase in many regions where the species is not yet established. Regions that are currently characterised by a rather low or moderate suitability have the potential for invasion by mid-century, due to increasing climatic suitability (Figure 1A). As a general tendency of all studies at the continental scale [16,17,19] it can be inferred that especially western Europe (Belgium, France, Luxembourg and the Netherlands) will provide favourable climatic conditions within the next decades. Furthermore, climatic suitability can be expected to increase in central Europe (e.g. parts of Germany) and the southernmost parts of the United Kingdom. Climatic conditions will continue to be suitable in southern France, as well as most parts of Italy and Mediterranean coastal regions in south-eastern Europe. Astonishingly, decreasing suitability for *A. albopictus* is projected for the western Mediterranean coast of Spain. This is very likely a consequence of an increased expectancy of drier conditions during the summer months.

However, some uncertainties in projections of the different studies are worth mentioning (see Figure 1A): differences between projections are evident in France, Germany, and western parts of the United Kingdom (Wales), where projections range from persistently unsuitable to increasingly suitable. In central parts of the Iberian Peninsula, Sardinia and Sicily, it is uncertain whether climatic conditions will continue to be suitable or will become less suitable in the future. Deviations between projections are most pronounced in the south-western parts of the Iberian Peninsula, south-eastern Italy and parts of eastern parts of Greece including also the west coast of the Black Sea. In these regions, uncertainties in model outputs vary strongly in projections: climatic suitability is expected to persist or increase in the projections of ECDC [16] and Caminade et al. [19], while Fischer et al. [17] identified decreasing climatic suitability. Generally, projections are more sensitive to uncertainties for precipitation

Figure 2

End of the 20th century (A) and projected (2011–2040) (B) climatic suitability of *Aedes albopictus* in Europe, with locations of important harbours



Data concerning the current and projected climatic suitability (A1B scenario) for *A. albopictus* refer to results of the statistical-based niche model of Fischer et al. [17]. Values for establishment theoretically range from 0 (completely unfavourable) to 1 (extremely favourable). Additionally, the changes in climatic suitability for 2011 to 2040 become obvious. Suitability will increase for the biggest European harbours of Rotterdam and Hamburg, which marks these as potential gateways for unintended mosquito introduction. In order to account for areas involved in cargo transport on a regional scale, we created buffer zones with different radii around the harbours of Rotterdam, the Netherlands, and Hamburg, Germany. This was done in order to detect examples of climatic suitability of the regions surrounding harbours with expected container transport. Climatic suitability was averaged for each buffer zone. Currently, climatic suitability is rather low for regions around Hamburg (radius (r) 50 km = 0.12 ± 0.01; r 100 km = 0.12 ± 0.02; r 200 km = 0.11 ± 0.03), while moderate suitability can be found for areas around Rotterdam (r 50 km = 0.21 ± 0.02; r 100 km = 0.23 ± 0.05; r 200 km = 0.23 ± 0.07). For 2011 to 2040, suitability of both regions of interest will increase remarkably. Regions around Hamburg will provide moderate suitability (r 50 km = 0.27 ± 0.03; r 100 km = 0.28 ± 0.05; r 200 km = 0.29 ± 0.06), while climatic suitability will even favour establishment of *A. Albopictus* in zones around Rotterdam (r 50 km = 0.50 ± 0.03; r 100 km = 0.51 ± 0.03; r 200km = 0.48 ± 0.05).

than for temperature, which is particularly evident in southern Europe. Compared with the studies of ECDC [16] and Caminade et al. [19], the influence of precipitation in climatic suitability is more pronounced within the statistical-based model of Fischer et al. [17] (see also Table 2).

Further trends to be expected

The general trend of increasing climatic suitability in regions that are currently rather unfavourable for *A. albopictus* establishment leads to the assumption of a northward spread in western but also central Europe up to the middle of the century. This is the time frame of results published by ECDC [16] and Caminade et al. [19]. From then on, trends can only be obtained by accounting solely for the study of Fischer et al. [17]. According to their projections, climatic suitability will further increase in central Europe and climate will become suitable for mosquito establishment in eastern Europe during the second half of the century [17].

Besides the continental dimension, potential range expansions on a local scale become crucial for the spread of *A. albopictus* in Europe as well. For instance, increasing temperatures may facilitate an upward spread in alpine regions, which has been demonstrated in northern Italy (Trentino) [18].

Future research avenues

In a warmer world, invasion processes of species may exhibit novel dynamics [37,38]. Thus, new challenges arise concerning the surveillance of invasive mosquitoes in Europe with high ability to colonise new territories as it is the case with *A. albopictus* [39]. Future research addressing invasive species that are of societal importance (e.g. regarding health issues) requires a comprehensive strategy for embedding climatic risk analyses in a broader scientific context. The main issues, such as transport mechanisms, alterations of habitats due to climatic extremes and biotic interactions, are highlighted below, as they are the most challenging tasks in modelling.

Continental dispersal pathways

None of the studies on potential future European occurrence of *A. albopictus* explicitly addresses processes such as the introduction and dispersal of the species. The introduction of this mosquito in Europe can be attributed to the global shipping of goods, especially by the world trade of used tyres or the import of tropical plants such as 'Lucky Bamboo' (*Dracaena braunii*) [1,2]. Undoubtedly, shipping is extremely effective in overcoming long-distance oceanic barriers [2,40,41]. Thus, the intercontinental range expansions of *A. albopictus* proved to be predictable using this combination of frequencies and traffic volumes of shipping lines in combination with climatic data at the target region around harbours [35]. The establishment of *A. albopictus* evidently took place around Mediterranean harbours, e.g. around the seaports of Genoa, La Spezia and Gioia Tauro in Italy as well as Barcelona, Spain – regions that are considered to be climatically suitable for the species today (Figure 2).

Intensified monitoring systems are installed in harbour regions at higher latitudes. After introduction, *A. albopictus* populations were found in glasshouses in the Netherlands used by Lucky Bamboo importers [42]. Such unintended import of the mosquito to the Netherlands seems to be a repeated phenomenon [43], although no evidence consists concerning the establishment of *A. albopictus* in Dutch landscapes. This is probably related to their low climatic suitability. This is also still true for other regions around the most important European harbours of Rotterdam, the Netherlands, and Hamburg, Germany,) that are characterised by the highest number of import containers, coming from endemic regions. Obviously, the harbours are not the final destination of the containers, as they are transported to the continental interior. We calculated the averaged climatic suitability within buffer zones of different radii (50–200 km) around the harbours of Rotterdam based on the results of Fischer et al. [17]. Increasing climatic suitability within these buffer zones around the introduction gateways may become crucial for future *A. albopictus* spread (Figure 2).

Once *A. albopictus* has been introduced and established, the question arises how to determine the risk of the mosquitoes spreading to further potentially climatically suitable habitats. Using the example of sandflies, it has been demonstrated that the dispersal of disease vectors on the continental scale can be evaluated by creating artificial cost surfaces that include several landscape features that are attributed with cost factors [44]. Consequently, the pathway with least costs for a species' dispersal can be considered as the most likely path of the species to move across landscapes. However, in contrast to sandflies, the dispersal of *A. albopictus* is mainly driven by unintended human transport through trade and traffic as opposed to natural dispersal. Hence, accounting anthropogenic factors in dispersal analyses is ambitious and acquires attribution of (rail-) roads and resting places in analyses. Consideration of these dispersal mechanisms, combined with current risk mapping and climate change assessments, suggests that further expansion across much of Europe is probable [2]. The necessity of dispersal analyses on the continental scale is highlighted by the recent incursion of *A. albopictus* in south-westernmost parts of Germany [45]. Thus, it has been concluded that *A. albopictus* crossed the Alps via transportation on motorways [46]. Another striking example is the recent importation of the mosquito to southernmost parts of the Czech Republic due to transit traffic [47]. Further spreading pathways need to be identified, as invasive mosquitoes may also be adaptable to new environments in a target region [2,36,48,49]. Without human transportation, the spreading potential of *A. albopictus* is limited to the local scale. In Italy, a flight range up to 300 m around their breeding containers has been observed [50]. This short-distance natural dispersal can be only assessed with high-resolution (250 m pixel resolution), gap-filled daily LST satellite data to predict areas that are potentially affected by infestation of *A. albopictus* [51,52].

Climatic constraints and novel scenarios

Integration of expert knowledge in modelling approaches demands detailed information on mosquitoes' ecology. In temperate regions, diapausing is a strategy to maintain species' typical life cycle traits, as diapausing eggs show remarkable desiccation resistance aside from increased cold tolerance [53]. In Italy, either favourable microclimates or cold acclimation may play a decisive role in the context of overwintering [54]. Likewise overwintering was identified as a constraint also in Switzerland [52]. Under laboratory conditions, the low-temperature thresholds for the survival of eggs of European populations of *A. albopictus* have been identified [55]. Such experiments help to detect potential regions, capable of overwintering populations. To date, information is mostly obtained by field observations; however, the thresholds for survival can be derived by simulating extremes that then can be transferred to climate change scenarios.

Currently, the development of the next generation of IPCC climate change scenarios is under way. Until now, a sequential approach has been used for scenario development [56]. These scenarios depict a linear chain of causes and consequences of anthropogenic climate change, handed from one research community to the next in a lengthy process, leading to inconsistencies. The new parallel process begins with the identification of radiative forcing characteristics that support modelling a wide range of possible future climates. In parallel, new socio-economic scenarios will be developed to explore important socio-economic uncertainties affecting both adaptation and mitigation. This is directly linked to, and integrated within, the new climate scenarios [5,57]. The extensive exchange between scientific disciplines acquired a more sophisticated design matching. Then, projections based on climatic extremes and their ecological consequences

will be improved. To date, projections concerning future climatic suitability of *A. albopictus* in Europe are based on long-term changes and do not consider the decisive role of rather short-term extremes. Modified climatic variability and associated sporadic extreme conditions are likely to create windows of opportunity for the establishment and reproduction of disease vectors such as *A. albopictus*, even if this is not reflected in trends of long-term average values [58].

Projections for the climatic suitability of *A. albopictus* can be combined, for instance, with the temperature-dependent extrinsic incubation period of an arbovirus, the time between pathogen infection of the insect vector and the vector's ability to infect the next vertebrate host. An accurate risk assessment of a climate-driven shift or spread of a vector-borne disease can then be obtained by combining risk maps of vector and transferred pathogen amplification in the light of a rapidly changing European climate for dengue [15,59,60] or chikungunya [61,62].

Further challenges for risk assessment

Aside from the above-mentioned novel opportunities, some challenges pertaining to future developments and their analyses need to be mentioned. A combination of phylogenetic analyses with distribution models was used to reconstruct the spatial occurrence of *A. albopictus* during the Pleistocene [63]. Such combined approaches seem to be a promising effort to support future projections. However, mutations and rapid adaptations of short-lived species to changing environment must be expected. Furthermore, outside of its native range *A. albopictus* acts as a strong competitor to local mosquitoes [49]. This not only affects the vectors' occurrence, but also the activity phase and population dynamics [64].

As *A. albopictus* prefers anthropogenic habitats, modified human behaviour is also a source of uncertainty. For instance, humans provide breeding sites for this container-breeder that enable survival in dry regions due to water storage [40]. Thus, changes in human behaviour or more general in human societies demand a comprehensive philosophy that must be implemented in risk assessments of climate change effects on emerging diseases. Estimating climatic suitability should be considered as a first step in risk assessment. Once future climatic suitability is detected for specific regions, societal and demographic aspects must be considered and regional specifics of healthcare systems can then be designed in a more specific and efficient way [65-67]. Such hierarchical and logical strategies may contribute to lowering the risks of vector spread and pathogen transmission. Recently, ECDC has launched the E3 Geoportal as a (spatial) data dissemination platform to facilitate data sharing and usability [68]. In order to guarantee accuracy for environmental risk mapping of *A. albopictus*, a proof of concept was given [69]. Furthermore, ECDC initiated research activities on assessing the related risk of chikungunya [62] and dengue virus transmission in Europe [70].

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