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Original Article

Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002–2003

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Objectives Avian-like H1N1 and human-like H3N2 swine influenza viruses (SIV) have been considered widespread among pigs in Western Europe since the 1980s, and a novel H1N2 reassortant with a human-like H1 emerged in the mid 1990s. This study, which was part of the EC-funded 'European Surveillance Network for Influenza in Pigs 1', aimed to determine the seroprevalence of the H1N2 virus in different European regions and to compare the relative prevalences of each SIV between regions.

Design Laboratories from Belgium, the Czech Republic, Germany, Italy, Ireland, Poland and Spain participated in an international serosurvey. A total of 4190 sow sera from 651 farms were collected in 2002–2003 and examined in haemagglutination inhibition tests against H1N1, H3N2 and H1N2.

Results In Belgium, Germany, Italy and Spain seroprevalence rates to each of the three SIV subtypes were high (≥30% of the sows seropositive) to very high (≥50%), except for a lower H1N2 seroprevalence rate in Italy (13·8%). Most sows in these countries with high pig populations had antibodies to two or three subtypes. In Ireland, the Czech Republic and Poland, where swine farming is less intensive, H1N1 was the dominant subtype (8·0–11·7% seropositives) and H1N2 and H3N2 antibodies were rare (0–4·2% seropositives).

Conclusions Thus, SIV of H1N1, H3N2 and H1N2 subtype are enzootic in swine producing regions of Western Europe. In Central Europe, SIV activity is low and the circulation of H3N2 and H1N2 remains to be confirmed. The evolution and epidemiology of SIV throughout Europe is being further monitored through a second 'European Surveillance Network for Influenza in Pigs'.

Keywords ESNIP, Europe, pigs, seroprevalence, surveillance, swine influenza.

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Introduction

The epidemiology of swine influenza (SI) has become increasingly complex over the last decade. Three influenza A virus subtypes – H1N1, H3N2 and H1N2 – are currently circulating in swine worldwide, but the origins and the antigenic and genetic characteristics of these swine influenza virus (SIV) subtypes differ in different continents or regions of the world.[1] The first significant outbreaks of SI on the European mainland occurred in the late 1970s after the transmission of an H1N1 virus from wild ducks to pigs.[2] This 'avian-like' H1N1 virus has become established in the European pig population and ultimately became the dominant H1N1 SIV strain.[3] Viruses of human origin, A/Hong Kong/68-like H3N2 formed a stable lineage in European pigs since the early 1970s, but reassortant H3N2 viruses with human haemagglutinin (HA) and neuraminidase (NA) genes and avian-like swine H1N1 internal protein genes have become dominant since the mid 1980s.[4,5] Finally, 'triple reassortant' H1N2 viruses have been isolated frequently from pigs throughout Europe since the mid 1990s.[6–10] These viruses contain an HA of human influenza virus origin, a NA of swine H3N2 virus origin and internal protein genes of avian-like swine H1N1 virus origin.[11] The HA of these H1N2 viruses shows low antigenic and genetic homology (70·4% amino acid identity in the HA1 region) with avian-like H1N1 viruses and there is no cross-reaction between H1N1 and H1N2 viruses in the HI test.[12] Though most SIV infections are clinically mild or subclinical, all three subtypes have been associated frequently with typical outbreaks of 'swine flu' and SIV may be responsible for up to 50% of acute respiratory disease outbreaks in pigs.[1]

Viruses of H1N1 and H3N2 SIV subtypes are considered widespread and endemic in pig populations in Austria, Belgium, Denmark, France, Germany, Great Britain, Italy, The Netherlands and Spain.[13] Occasional serological investigations of pigs sampled at slaughter have been performed in most of these countries in the 1980s and early 1990s and revealed seropositivity to H1N1 and H3N2 in >50% of the tested population. However, over the last few years H3N2 activity is believed to be low or absent in France (Brittany) and Great Britain (Brown I., Veterinary Laboratories Agency, Weybridge, UK, unpublished observations; Kuntz-Simon G., Agence Française de Sécurité Sanitaire des Aliments, Ploufragan, France, personal communication). Either or both subtypes have also been reported in Bulgaria, the former Czechoslovakia, Greece, Hungary, Ireland, Macedonia, Poland and Sweden.[13] As for the H1N2 virus, a limited serosurvey in sows in Belgium in 1999 has shown seropositivity in 69% of the 443 sera examined.[7] However, there is limited H1N2 seroprevalence data for other European countries and there is little information on the evolution of H1N1 or H3N2 seroprevalence rates in Europe.

The 'European Surveillance Network for Influenza in Pigs 1' (ESNIP1) was a concerted action in the 5th Framework Research Programme of the European Commission (QLK2-CT-2000–01636, 01-01-2001 until 31-12-2003) that involved 14 partners from 10 different European countries. This action was initiated through the need for standardisation of diagnostic techniques for SI and for a more organised surveillance. The present paper reports the results of a first international serosurvey undertaken by ESNIP1 partners from Belgium, the Czech Republic, France, Italy, Ireland and Poland and by voluntary participants from Germany and Spain. The study aimed to determine the seroprevalence of the novel H1N2 virus in different geographic regions of Europe, and to compare the relative prevalences of each SIV between countries and regions where possible.

Materials and methods

Serum samples

European Surveillance Network for Influenza in Pigs 1 partners from Belgium, the Czech Republic, Italy, Ireland and Poland together with diagnostic laboratories from Germany (Impfstoffwerk Dessau-Tornau, Rodleben) and Spain (Laboratorios HIPRA, Amer) participated in the serosurvey. Serum samples were collected from first parity sows (i.e. sows younger than a year old) only (Italy, Poland) or from sows of various ages (other countries) during 2002 and 2003. None of the animals had been vaccinated against SIV and they originated from herds with a minimum of 100 sows. A total of 4190 sera from 651 farms were collected. Details on sample origin and sample size in the different countries are given in Table 1. In Belgium, Germany, Italy and Spain, sera were collected from regions containing the highest density of pigs. These regions included 10 of the 19 main swine production regions in the 15 countries comprising the EU until 2004. In the Czech Republic, Ireland and Poland, sera from larger areas or from throughout the country were examined.

Table 1. Overview of countries participating in the serosurvey, regions involved and sample sizes

Country	Specific region(s) sampled	Sample sizes: Number of herds	Total number of sera	N sera/herd
Belgium	Flanders	100	600	6
Czech Rep.	All 13 regions of the country	84	555	6–10
Germany	Lower Saxony, North Rhineland-Westphalia	93	811	5–10
Ireland	20 of 26 counties of the Republic of Ireland	98	527	5–7
Italy	Lombardia, Emilia Romagna, Veneto	76	456	6
Poland	8 of the 16 provinces of Poland, located in the North and West	100	641	5–10
Spain	Cataluña, Aragon, Andalucia, Castilla and Leon	100	600	6

Haemagglutination-inhibition test

All sera were tested in haemagglutination-inhibition (HI) tests against one H1N1, one H3N2 and two H1N2 SIV. Haemagglutination-inhibition tests were performed in each participant's laboratory according to a standardized protocol established during the ESNIP1 concerted action, which is based on the original protocol by Palmer *et al.* (1975).[14] All sera were treated with receptor-destroying enzyme from *Vibrio cholerae* and adsorbed with chicken red blood cells to remove non-specific inhibitors of agglutination and natural serum agglutinins respectively. Twofold serum dilutions were tested starting at a dilution 1:20. Haemagglutination-inhibition tests were performed with four haemagglutinating units (HAU) of virus and 0·5% chicken red blood cells. Titres were expressed as the reciprocal of the highest dilution inhibiting four HAU.

The H1N1 and H3N2 strains used in HI tests were representative of avian-like swine and human-avian reassortants respectively and they differed between countries. All countries except the Czech Republic and Poland used local H1N1 and H3N2 strains isolated during the last 15 years, which had been shown to be appropriate for the serological detection of SIV isolated in the respective countries during the study period. The H1N1 strains were as follows: sw/Belgium/1/98 (Belgium and Poland), sw/Italy/1513-1/98 (Italy), sw/Eire/0756/92 (Ireland), sw/Finistère/2899/82 (Czech Republic), sw/Bakum/3543/98 (Germany) and sw/Spain/45304/03 (Spain). The H3N2 strains included sw/Flanders/1/98 (Belgium and Poland), sw/Italy/1523/98 (Italy), sw/Eire/933/93 (Ireland), sw/Gent/1/84 (Czech Republic), sw/Bakum/909/93 (Germany) and sw/Spain/46356/03 (Spain). Two H1N2 isolates with some antigenic differences in their HA, sw/Scotland/94 and sw/England/96,[11] were used by all participants. Both H1N2 isolates have a human-like HA and they do not cross-react with avian-like H1N1 viruses in cross-HI tests with post-infection swine sera[12] or sera prepared in ferrets or chickens.[7,8,11]

Pigs with an antibody titre ≥20 to a given subtype were considered seropositive for that subtype. A farm with one or more seropositive pigs for a given subtype was considered positive for that subtype.

Results

Seroprevalence of H1N1, H3N2 and H1N2 SIV in the sow population

Table 2 shows the number and percentage of seropositive sows to tested SIV and the geometric mean antibody titre of the positive animals in each of the seven countries. The incidence of antibodies to H1N2 was higher in HI tests with Scotland/94 than in tests with Engand/96. A total of 1061 sera tested positive to H1N2: of these 45·6% were positive to both H1N2 strains, 49·3% were positive to Scotland/94 alone and 5·1% were positive to England/96 alone. Only 10 of the total 651 H1N2-positive farms were positive to England/96, usually at low antibody titres, and not to Scotland/94. This indicates that Scotland/94 is most representative for the H1N2 viruses circulating in European pigs and therefore only serological responses to the Scotland strain are further discussed.

In Belgium, Germany, Italy and Spain, a minority of the sows were negative to all SIV tested. Seroprevalence rates for H1N1 and H3N2 were higher than 50% in Belgium (80·8% and 53·8% respectively) and in Germany (70·8% and 58·6% respectively) but slightly lower in Italy (46·4% and 41·7% respectively) and Spain (38·5% and 38·0% respectively). The seroprevalence rate for H1N2 was higher than 50% in Belgium and Spain (57·8 and 52·8% respectively), 32·1% in Germany but lower in Italy (13·8%).

Table 2. Prevalence of antibodies to H1N1, H3N2 and H1N2 swine influenza viruses (SIV) in sows in seven European countries

Country	No. sera tested	Sera with antibodies to												Sera without SIV antibodies	
		H1N1			H3N2			H1N2 Scotland/94			H1N2 England/96				
		No.	%	GMT*	No.	%	GMT	No.	%	GMT	No.	%	GMT	No.	%
Belgium	600	485	80·8	75·98	323	53·8	43·21	347	57·8	40·08	160	26·7	36·36	36	6·0
Germany	811	574	70·8	107·58	475	58·6	178·65	260	32·1	59·49	158	19·5	49·16	120	14·8
Italy	456	212	46·4	75·18	190	41·7	77·70	63	13·8	41·34	32	7·0	46·55	170	37·3
Spain	600	231	38·5	114·12	228	38·0	194·48	317	52·8	160·83	181	30·2	86·48	125	20·8
Czech Rep.	555	65	11·7	58·10	4	0·1	56·56	17	3·0	32·62	7	1·3	44·16	483	87·0
Ireland	527	94	17·8	64·59	22	4·2	62·17	3	0·6	63·49	0	0	0	415	78·8
Poland	641	51	8·0	91·60	0	0	0	0	0	0	0	0	0	590	92·0

*Geometric mean antibody titre of positive sera only.

In the Czech Republic, Ireland and Poland, most sows were negative for antibodies to all SIV tested. In these countries seroprevalence rates for H1N1 (11·7, 17·8 and 8·0% respectively) were clearly lower than in Belgium, Germany, Italy and Spain. Antibodies to H3N2 and H1N2 were found occasionally in the Czech Republic and in Ireland but were not detected in Poland.

Geometric mean antibody titres were largely comparable between the countries participating in ESNIP1. Spain reported higher mean antibody titres to each SIV subtype, and Germany reported relatively high titres to H1N1 and H3N2.

Seroprevalence of H1N1, H3N2 and H1N2 SIV on a farm basis

Table 3 shows SIV seroprevalence rates at the farm level in the seven European countries. The proportion (%) of seropositive farms in each country correlated with the proportion (%) of seropositive sows but was higher, because antibodies to a given SIV subtype were usually present in only part of the sow population from the same herd. In Belgium, Italy and Spain, for example, where exactly six animals per farm were sampled, the median number of H1N1 seropositive sows per farm was five, three and four respectively. Similar within herd seroprevalences were found for H3N2 and H1N2.

In Belgium, Germany and Spain SIV seronegative farms were rare (1·0–4·0%), while 11·8% of the Italian farms tested SIV seronegative. Furthermore, seroprevalence rates for all three subtypes were higher than 50% in these countries, with the exception of the H1N2 seroprevalence rate in Italy (35·5%).

Fewer SIV seropositive farms were detected in Ireland and the Central European countries. In the Czech Republic and Ireland, approximately 40% of the farms were positive for H1N1 and seropositivity rates for the other subtypes were considerably lower. The small number of sows with H3N2 antibodies in the Czech Republic (4) and with H1N2 antibodies in Ireland (3) originated from different farms. In Poland, where only H1N1 antibodies were detected, only 9% of the farms were seropositive.

Incidence of infections with multiple SIV subtypes in sows

The individual serological data of all countries were further analyzed to determine the proportion (%) of sows that had been infected with multiple SIV subtypes (Table 4). In the four countries with highest SIV seroprevalences, the proportion (%) of sows with simultaneous presence of antibodies to two or three different subtypes exceeded the proportion (%) of sows with antibodies to only a single subtype. In the remaining countries, most (Czech Republic, Ireland) or all sows (Poland) had antibodies to H1N1 only.

Discussion

This limited but targeted serological survey was undertaken to determine the prevalence of H1N2 SIV in different European countries and to compare it with that of H1N1 and H3N2. The approach used varied somewhat between countries: there were differences in sample numbers, the age of the sows sampled, the time of sample collection, and the H1N1 and H3N2 strains used as antigens in the HI test. In contrast the strength of the study, was that all participants used identical H1N2 strains and a standardized protocol for the HI test. To assure quality control and standardisation of HI test results, ESNIP1 partners participated in HI ring trials, i.e. parallel tests with well defined SIV and sera. Our data clearly show two distinct SIV seroprevalence patterns. In Belgium, Germany, Italy and Spain, H1N2 has become widespread and cocirculates with H1N1 and H3N2, which have been enzootic in these countries

Table 3. Number of farms with antibodies to H1N1, H3N2 and H1N2 swine influenza viruses (SIV) in seven European countries

Country	Number of farms tested	Farms with antibodies to								Farms without SIV antibodies	
		H1N1		H3N2		H1N2 Scotland/94		H1N2 England/96			
		No.	%	No.	%	No.	%	No.	%	No.	%
Belgium	100	97	97·0	86	86·0	89	89·0	69	69·0	1	1·0
Germany	93	87	93·5	85	91·4	70	75·3	54	58·1	2	2·2
Italy	76	63	82·9	52	68·4	27	35·5	15	19·7	9	11·8
Spain	100	65	65·0	67	67·0	87	87·0	68	68·0	4	4·0
Czech Rep.	84	32	38·1	4	4·8	13	15·5	7	8·3	47	56·0
Ireland	98	41	41·8	16	16·3	2	2·0	0	0	44	44·9
Poland	100	9	9·0	0	0	0	0	0	0	91	91·0

Table 4. Prevalence of antibodies to multiple swine influenza viruses subtypes in sows in different European countries

Country	% of animals with antibodies to						
	H1N1 only	H3N2 only	H1N2 only	H1N1 + H3N2	H1N1 + H1N2	H3N2 + H1N2	All 3 subtypes
Belgium	16·16	6·67	2·83	13·33	21·17	3·67	30·17
Germany	21·33	10·23	1·60	21·45	3·58	2·47	24·41
Italy	14·91	13·16	1·97	20·61	3·95	0·88	7·02
Spain	6·83	10·00	18·17	6·67	13·33	9·67	11·67
Czech Rep.	8·65	0·18	0·36	0·18	2·16	0·18	0·18
Ireland	16·70	3·42	0	0·57	0·19	0	0·19
Poland	7·96	0	0	0	0	0	0

since the 1980s.[13] This is in line with the isolation of all three subtypes from acute respiratory disease outbreaks in these countries during the study period (ESNIP1, unpublished data). In the Czech Republic, Ireland and Poland, in contrast, both H1N2 and H3N2 are either undetectable or found at very low levels. The dominant subtype in these countries is clearly H1N1 but its prevalence is lower than in the first series of countries.

The interpretation of HI test results for SIV is more complicated now than in the 1980s, when only H1N1 and H3N2 were present in European swine populations. The three subtypes examined in this study have antigenically distinct HA and serological cross-reactions in the HI test are rare but cannot be excluded. In an experimental study, only eight of the 160 sera collected after infection of pigs with one or two SIV subtypes showed cross-reactive antibodies to another subtype in the HI test.[15] As an example, some pigs that had been infected sequentially with H1N2 and H1N1 showed low (10–20) HI antibody titres to H3N2 without having been exposed to this subtype. In the present study, the few positive sera to H3N2 in the Czech Republic and to H1N2 in Ireland may result from such serological cross-reactions. This is emphasised by the fact that, with one exception, all these sera were also positive to one or both other subtypes in the HI test. A second important variable is the antigenic and genetic diversity among SI H1N2 viruses in Europe. The heterogeneity within H1N2 viruses is reflected by the higher seroprevalence rates with the Scotland/94 isolate than with the antigenically distinguishable England/96 isolate. More importantly, novel H1N2 reassortants comprising an HA of avian-like swine H1N1 virus origin have been detected in France, Italy and Denmark.[8,16,17] This second generation of H1N2 viruses is antigenically unrelated to the older H1N2 viruses like sw/Scotland/94 and sw/England/96, which derived the HA gene from human H1N1 viruses circulating during the early 1980s. The prevalence of this antigenic variant of H1N2 viruses remains unknown and it cannot be deduced from our study, because the HI test cannot distinguish between such second generation H1N2 viruses and avian-like H1N1 viruses. HI tests will also fail to discriminate between infection with the first generation of H1N2 viruses and novel H1N1 reassortants with a human-like HA, which have been reported occasionally.[8,17] Nevertheless, the high infection rates with SIV possessing human HA in Western Europe indicate that inclusion of a human-like H1N2 virus in current vaccines would be justified. The current commercial SIV vaccines contain only H1N1 and H3N2 components and they confer little if any protection against the human-like H1N2 virus.[18,19]

The relatively low SI activity in Ireland and the two central European countries can in part be explained by the lower pig density and/or differences in the structure of the pig industry. As is the case with other respiratory viruses, high regional pig density, larger herd size, shorter distances between herds and a larger size of neighbouring herds all increase the risk of infection with SIV.[20] Ireland has fewer pigs than the other Western European countries examined[21] and an isolated geographic position. The human-like H1N2 viruses, which are likely to have their origin in the UK,[8] have seemingly not yet spread to Ireland and this is consistent with the lack of H1N2 isolations. Approximately 19 million pigs are produced per year in Poland and as such Poland is the largest producer of pigs of the central European countries that have joined the EU since 2003. The number of pigs produced per year and per farm, however, is significantly lower (approx. 16) than in an average farm in Western Europe (approx. 94).[22] Clearly, avian-like H1N1 SIV are circulating in both Poland and the Czech Republic and this is supported by serological and virological investigations.[23–27] In contrast, H3N2 or H1N2 SIV, have never been isolated in Poland or in the Czech Republic to date and our present data support the belief that they are absent or epidemiologically unimportant. The four H3N2-positive sera in the Czech Republic are believed to be false positives and this may also apply to the 17 H1N2-positive sera, of which 13 were also positive to H1N1. Despite the usual lack of serological cross-reactions between the H1N1 and H1N2 strains used in HI tests, further investigations of the H1N2 status in pig populations in the Czech Republic would be invaluable. In another serological study in other regions of Poland in 2001, 3·9% of 3576 pigs tested positive to H3N2 and 6·5% were positive to H1N2, compared with 17·7% positives for H1N1.[23] This suggests that H3N2 and H1N2 may circulate exclusively in the east of Poland and not in the west, where the samples for the present study were collected, but further confirmation is needed.

In the four countries with high SIV seroprevalences 25·44–38·17% of the sows had antibodies to two subtypes and 7·02–30·17% were positive for all three subtypes. Thus, H1N1, H1N2 and H3N2 viruses with antigenically distinct HA are not only circulating concurrently, but they also frequently cause consecutive or mixed infections. This therefore provides an opportunity for genetic reassortment to occur between these viruses and this has led to the emergence and maintenance of phylogenetically diverse SIV in pigs in Europe.[8,16,17] While antigenic drift within a given virus subtype is less important in pigs than in horses or humans,[3] more influenza virus genotypes are apparently circulating in pigs compared with other mammalian species. Humans are also susceptible to H1N1 and H3N2 viruses that are antigenically distinguishable from their counterparts in pigs, and H1N2 reassortants have been isolated from humans since 2001.[28] However, the human H1N2 viruses still have a similar HA to contemporary human H1N1 strains.[28,29] The epidemiology of SI, unlike that of human influenza viruses, is entirely different in different continents. In the USA H1N1, H3N2 and H1N2 SIV are also enzootic, but their origin and evolution differs from that of the European viruses and several different genotypic variants of reassortant viruses have also been isolated from pigs in North America.[1] The extensive circulation of SIV in swine dense regions and the occurrence of co-infections are facilitated by the continual availability of young non-immune pigs that have lost their maternal immunity. Indeed, the vast majority of pigs are killed at the age of 6 months and the feeder and fattening pig population has a rapid turn over, so that susceptible individuals are continuously available.

The aim of this serosurvey was to gain preliminary insights to the epidemiology of SI in Europe and many questions remain unanswered. The future epidemiology through cocirculation of H1N1, H3N2 and H1N2 viruses remains uncertain, potentially including the emergence of a dominant subtype or genotype. The importance of the new, second generation reassortant viruses requires careful monitoring. Determination of the SIV prevailing in other central European countries is important together with epidemiological impacts resulting from greater integration of production enhanced through cooperation with companies and investors in Western Europe. This could result in a higher prevalence of SIV and establishment of new SIV subtypes in the future in these countries. These and other issues are being addressed through the 2nd European Surveillance Network for Influenza in Pigs (ESNIP2, SSPE-CT-2005–022749, 01/06 until 12/08). This latter co-ordination action also includes partners from the US and Asia, which will facilitate greater global interaction and worldwide understanding of the epidemiology of SIV. We also intend to interact with human and avian influenza surveillance networks to gain insights into the antigenic and genetic relationships of influenza viruses of different species.

Addendum

R. Dürrwald, E. Foni, G. Labarque, P. Lenihan, J. Maldonado, I. Markowska-Daniël and Z. Pospisil contributed to the organisation of the study and to the supervision of serological tests in the lab. K. Van Reeth and G. Labarque analysed all data. K. Van Reeth wrote the paper with the help of M. Pensaert and I. Brown. G. Koch was the co-ordinator of ESNIP1. All authors were involved in the planning and design of the study, interpretation of the data and revision of the manuscript, and they agreed to its final form.

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