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MEDICINE

The Estonian H1N1 influenza 2009 outbreak was highly underestimated

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Abstract. The H1N1 influenza strain Mexico 2009 (H1N1pandemic09) led to mild symptoms (with no or low fever) in Estonia during the 2009–2010 outbreak. Due to the lack of clinical signs, it was difficult to estimate the real spreading of this influenza virus in Estonia and no cases of H1N1 influenza were officially registered in animals either. We used an ELISA method to screen blood sample collections for the presence of anti-H1N1 and anti-H3N2 antibodies. All sera were also tested with the hemagglutination inhibition (HI) assay. Out of the 123 samples from human patients, 23 (i.e. 18.7%) were seropositive for the H1N1pandemic09 virus. In addition, blood samples from six persons were positive for both H1N1 and H3N2 viruses, while according to the data from the Estonian Health Board, people aged 15–65 had a general disease rate of around 3.9%. Almost all of the tested animals from two herds (out of four studied) were seropositive for H1N1pandemic09. The seven HA protein sequences isolated from Estonia were aligned with a consensus sequence of the pandemic H1N1 HA sequences from Mexico using ClustalW, and 12 amino acids substitutions were found.

Key words: influenza H1N1, human, porcine, ELISA, hemagglutination inhibition.

INTRODUCTION

In Europe, the H1N1 influenza strain Mexico 2009 (H1N1pandemic09) [1], also known as 'swine flu', appeared to be a mild to moderate disease affecting preferentially school-age children. Elderly adults were underrepresented in severe cases [2–6]. However, the true proportion of infected persons could not be well assessed due to the lack of serological evidence of asymptomatic cases. Asymptomatic and mild cases are missed by current reporting techniques of influenza and only a few studies provide assessment of seroprevalence during an epidemic [3,7].

Also the H1N1pandemic09 generally led to only mild symptoms in Estonia during the 2009–2010 outbreak. Hence, due to the lack of clinical signs, it was difficult to estimate the real spreading of this influenza virus in Estonia. No cases of H1N1 influenza were officially registered in animals either during this period. However, even a mild H1N1 influenza infection would afford a good level of protection [8,9]. The H1N1 vaccine became available only at the end of the outbreak. Consequently, only 13.4% of the persons belonging to high-risk groups and about 2.7% of the whole Estonian population were vaccinated [10].

The aim of this study was to assess how well the spread of H1N1pandemic09 during the 2009–2010 outbreak in Estonia was estimated. Our hypothesis was that the outbreak was highly underestimated, which would deserve a definitive epidemiologic demonstration. The potential consequences of underestimation are hereby discussed.

MATERIALS AND METHODS

To provide an easy way to test the H1N1pandemic09 influenza virus (IV) seropositivity, we designed an

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ELISA test that identifies the specific reactivity to two different H1N1 viruses and to one H3N2 virus. To get the first insight into the real spread of the H1N1 virus in the Estonian population during this outbreak, we used this ELISA test on serum samples collected from 123 patients, mainly voluntary blood donors, during spring 2010. The same test was also carried out with samples from 95 pigs collected in four different herds. All samples were tested also with the hemagglutination inhibition (HI) assay.

Blood samples

Human blood samples were collected from 27 volunteers (10 pregnant women among them) and from 96 blood donors. Two millilitres of venous blood was coagulated during 2 h at room temperature and centrifuged for 10 min at 800 *g*, after which the serum was isolated. For both human and animal samples, data on age and sex were collected. Information about vaccination was available for human samples Nos 1–19 only. Among those, volunteers No. 4 and No. 6 had been vaccinated against seasonal influenza (H1N1, H3N2, and influenza B). None of the participants had been vaccinated against H1N1pandemic09, since human sera were collected in October 2009–January 2010.

Blood samples were taken from 96 swine from four herds in May 2010. Studies involving humans comply with the principles of the Helsinki Declaration of 1975 and with due subsequent amendments by the World Medical Assembly. Ethics Review Committee (ERC) on Human Research of the University of Tartu, No. 181/T-1, was issued 20.04.2009 to Sirje Rüütel Boudinot.

ELISA

Two commercially available vaccines – inactivated influenza virus either produced in cell culture on Vero cells (Celvapan, H1N1 IV Pandemic09 developed by Baxter) or propagated in chicken eggs (Pandemrix, H1N1 IV Pandemic09 by ClaxoSmithKleine) – were diluted in 50 μL of the Coating Buffer per well (pH 9.6) and used to coat Nunc Maxi-Sorp Immuno Plates. The final concentration of antigens was 3.75 μg/mL. The plates were incubated overnight at 4 °C and washed three times with 0.05% Tween 20 in ddH_2O between every step. Non-specific binding sites were blocked with 200 μL of 2% casein/PBS and incubated for 1 h at room temperature. Both serum samples and controls were diluted by two-fold dilution series up to 1 : 64 000. Of the dilutions 50 μL was added to plates with negative samples (PBS without antigen) and incubated overnight at 4 °C. Then 50 μL of secondary antibody (DAKO Rabbit Anti-Human IgA, IgG, IgM, Kappa, Lambda/HRP; DAKO Rabbit anti-pig antibody 162.5 pg/mL) was added to each well and incubated for 1 h at room temperature. Freshly mixed peroxidase substrate reagent (1 mM tetramethylbenzidine and 2.3 mM H_2O_2 in 0.1 M potassium citrate buffer, pH 4.5) was added to the plates and incubated for 20 min at room temperature. To stop the reaction 1 M H_2SO_4 was added to each well. Optical densities (OD values) were detected by reading the plates on an ELISA plate reader (Labsystems Multiskan MCC/340) at the wavelength of 450 nm. Samples with the absorbance value twice as high as that of the average absorbance of the negative controls were considered positive.

All samples were also tested for trivalent influenza vaccine Vaxigrip (3.75 μg/mL), which is composed of H1N1-A-Brisbane 07, H3N2-A-Brisbane 07, and B-Brisbane 08 virus subtypes to exclude the prevalence of antibodies to those subtypes.

Hemagglutination inhibition assay

Human and swine sera were inactivated by incubation at 56 °C for 30 min and HI assays of human and swine sera were conducted following a standard protocol [11]. Briefly, two-fold serial dilutions of human or swine sera were mixed and pre-incubated in 96-well plates for 30 min at room temperature with 8 HA units of the virus antigen per well. Chicken red blood cells were added at a final concentration of 0.25%, and the plate was incubated at room temperature for 30 min. HI titres were determined at the highest dilution that displayed hemagglutination activity. Specific HI activity of sera was calculated as the lowest concentration of sera that displayed hemagglutination activity. Samples with HI titre higher than 40 were considered positive.

RESULTS

In spring 2009, of the 123 human samples 23, that is 18.7% (samples Nos 1, 5, 7, 14, 17, 25, 28 31, 34, 36, 45, 49, 54, 60, 63, 76, 82, 83, 88, 91, 107, 108, and 123), were seropositive for the H1N1 virus (Fig. 1). During sampling, patient 1 had acute viremia and high temperature, and the antibody titre was still rather low. Additionally, blood samples from six persons were highly positive for both H1N1 and H3N2 (Figs 1 and 3) viruses, indicating that these patients had been most probably infected by both viruses (Fig. 1, patients Nos 28, 36, 60, 76, 82, and 83). The titres measured in these double seropositive patients were significantly higher than those observed after vaccination against seasonal influenza with Vaxigrip trivalent influenza vaccine (see samples Nos 4 and 6 from vaccinated patients aged 51 and 63, respectively). Human sera Nos 12, 15, 39, 40, 44, 69, and 77 showed positive values to Vaxigrip and not to H1N1pandemic09 (Fig. 3). Among the 123 tested patients only 2 persons

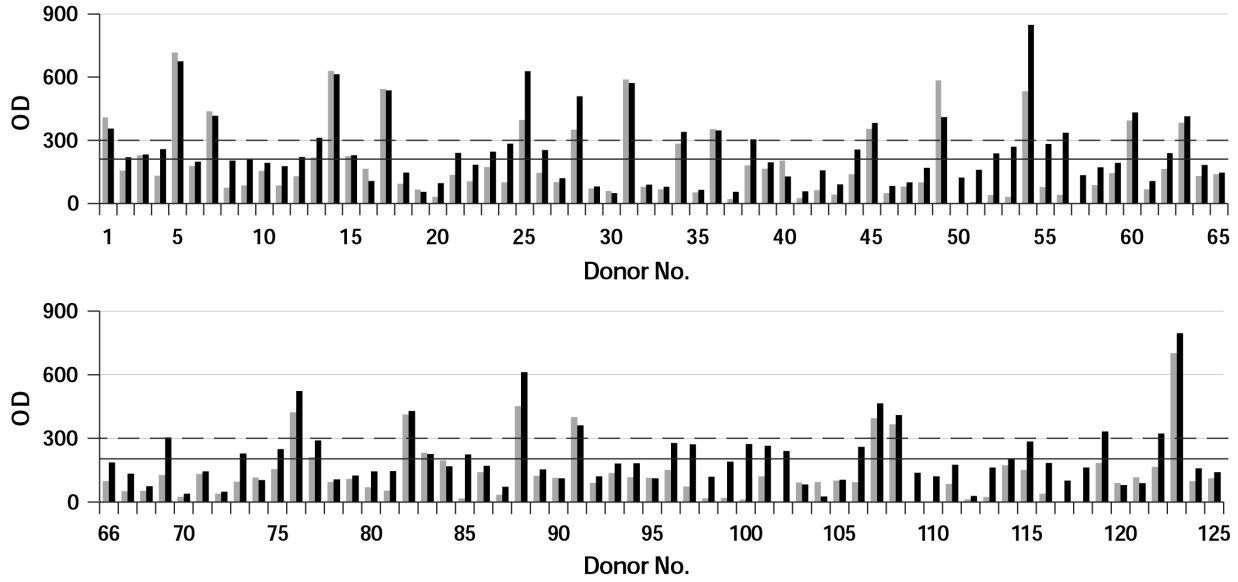

Fig. 1. Optical densities (OD) of 1 : 16 000 serum dilutions from 123 human patients showing reaction to Celvapan (grey bars) and Pandemrix (black bars) H1N1pandemic09 antigens. Human sera Nos 124 and 125 from spring 2008 were used as negative references. Sera Nos 5 and 17 from patients with confirmed cases of H1N1pandemic09 were used as positive references. Sera Nos 5 and 17 were taken one month and four months after confirmed infection, respectively. A human serum was considered as positive for H1N1pandemic09 when 1 : 16 000 dilution gave twice higher OD values than negative controls. The cutoff values for Celvapan and Pandemrix ELISA are represented as solid and dashed lines, respectively.

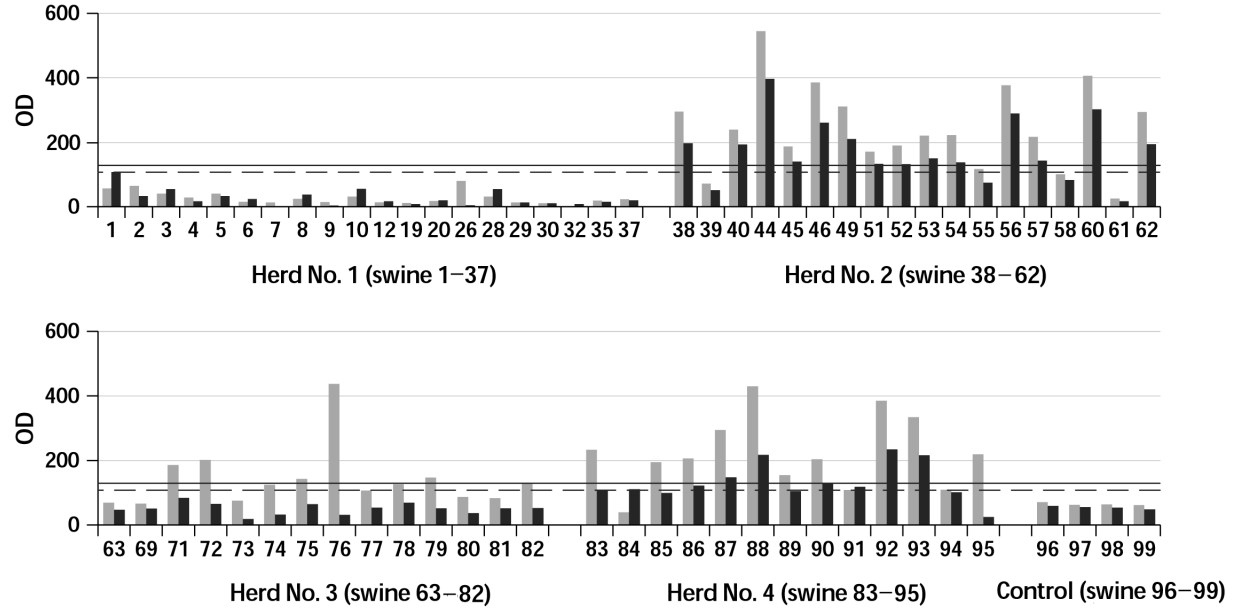

Fig. 2. Optical densities (OD) of 1 : 16 000 serum dilutions from 95 swine showing reaction to Celvapan (grey bars) or Pandemrix (black bars) H1N1pandemic09 antigenes. Swine sera Nos 96 and 97 from the year 2007 and swine sera Nos 98 and 99 from the year 2008 were used as negative references. Swine serum was considered as positive to H1N1pandemic09 when 1 : 16 000 dilution gave twice higher OD values than negative control porcine sera Nos 96–99, which were taken before the H1N1 pandemic 2009/2010 (OD 100 or higher). The cutoff values for Celvapan and Pandemrix ELISA are represented as solid and dashed lines, respectively.

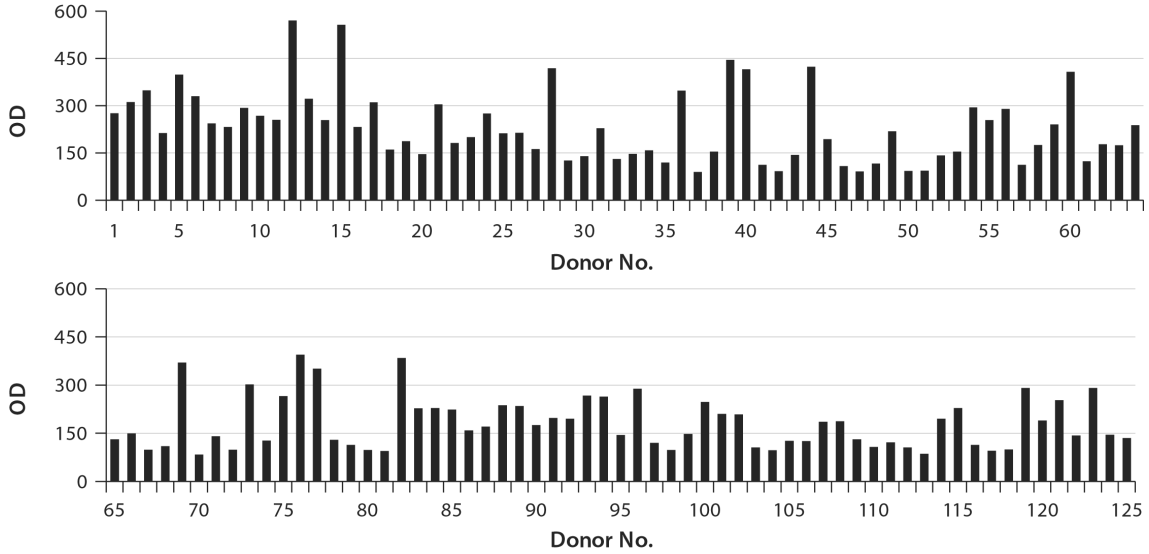

Fig. 3. Optical densities (OD) of 1 : 16 000 serum dilutions from 123 human patients showing reaction for Vaxigrip (H3N2) antigens. Human sera Nos 124 and 125 from spring 2008 were taken before the H1N1 pandemic 2009–2010. Sera Nos 12, 15, 39, 40, 44, 69, and 77 show positive values to Vaxigrip but not to H1N1pandemic09. Samples with absorbance value twice higher than that of the average absorbance of the negative controls (samples 124 and 125) were considered positive.

(Nos 5 and 17, aged 40 and 36, respectively) had been infected with the H1N1pandemic09 virus (confirmed cases) and had exhibited high fever and other typical acute clinical signs. Patients Nos 5 and 17 suffered the infection one month and four months before blood sampling, respectively. Both of them had a similarly high level of specific anti-H1N1 antibodies. It is noteworthy that two other patients who had not shown any signs of acute respiratory disease during the previous year had even higher titres of anti-H1N1 antibodies (patients 7 and 14). Patient No. 14 could only indicate that she had had a bit sore throat a month before blood sampling.

We adapted the test to measure the titres of anti-H1N1pandemic09 in pigs because these animals were regularly infected worldwide during the H1N1 epidemic in 2009–2010 [12,13]. To get a first insight into H1N1pandemic09 seropositivity in Estonian pig herds, blood samples from four different locations were analysed. Farms Nos 1 and 4 were located in the vicinity of Tartu in south-east Estonia and farms 2 and 3 were located in north-east Estonia. The results were homogeneous within each farm, suggesting that the virus was well spread in a herd when present at a given site (Fig. 2). Animals from farm No. 1 had no anti-H1N1 antibodies and most probably never met the virus. This farm is a closed breeding farm, which does not take in animals from abroad. In contrast, the animals from farms 2 and 4 were almost all seropositive, most individuals having high titres of antibodies directed against the H1N1 virus. A few animals from farm 3 were seropositive, with especially high titres against Celvapan, indicating that the virus H1N1pandemic09 was present in this site. The results obtained with the two antigens used (Celvapan from Vero cells and Pandemrix produced in eggs) were fully consistent. The observations indicate that the H1N1 virus spread in these three farms, and that most of the animals had been infected. Remarkably, most of the tested humans and animals from the four herds showed high titres of anti-H3N2 antibodies (Fig. 4).

All results from the ELISA were confirmed with hemagglutination inhibition assay. The results of the assay were also consistent with the ELISA. Serum dilutions from 32 up to 512 showed specific inhibition of hemagglutination (HI titres ≥128; Figs 5 and 6).

The seven Estonian H1N1 HA protein sequences (Appendix 1) and a consensus sequence of the pandemic H1N1 HA sequences from Mexico (Appendix 2) were aligned using ClustalW [14]. As a result, 12 sites were found where some of the sequences differed from one another (Table 1, Appendices 1 and 2). In addition, 5216 H1N1 sequences were obtained from the GenBank and aligned using the built-in ClustalW algorithm of MEGA4. Analysis of the obtained alignment showed that while some of the changes in the amino acid composition of the H1N1 HA in Estonia had been commonly detected worldwide, others were quite rare (Table 1). Position 179 where two of Estonian sequences had amino acid substitution from serine (S) to asparagine (N) belongs to immunodominant (S179N) epitope (pos. 168–182) [15]. A similar change was found in porcine sequence ACH69547.1. Position 154 with amino acid substitution P → S belongs to another immunogenic region, where the change of amino acid alters the antigenic properties of the protein [16].

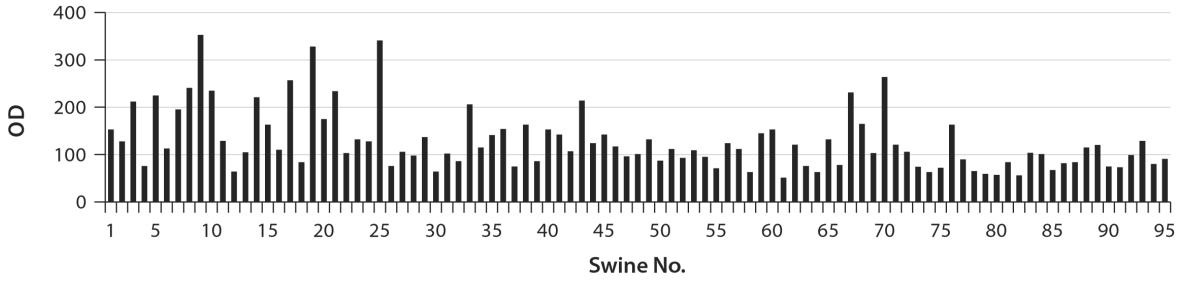

Fig. 4. Optical densities (OD) of 1 : 16 000 serum dilutions from 95 swine showing reaction to Vaxigrip (H3N2) antigens.

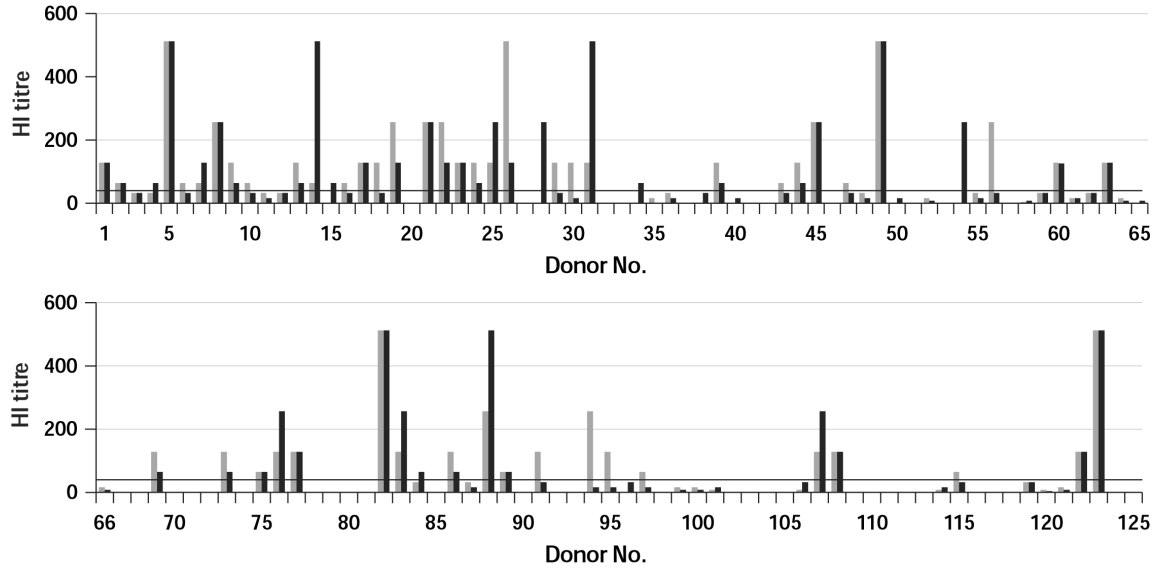

Fig. 5. Highest human serum dilutions that gave hemagglutination activity in the presence of H1N1pandemic09 antigens from Celvapan (grey bars) and Pandemrix (black bars). Sera Nos 124 and 125 from spring 2008 were used as negative controls. The cutoff value for the hemagglutination assay is represented as solid line.

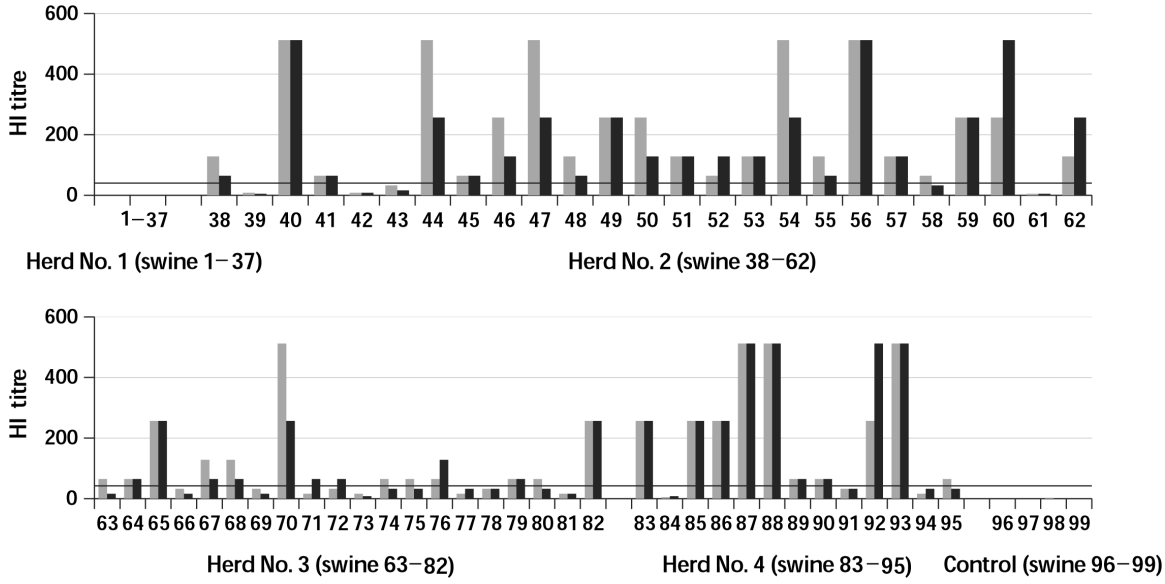

Fig. 6. Highest swine serum dilutions that gave hemagglutination activity in the presence of H1N1pandemic09 antigens from Celvapan (grey bars) and Pandemrix (black bars). Sera Nos 96–99 from the year 2008 were used as negative controls. The cutoff value for the hemagglutination assay is represented as solid line.

Table 1. Amino acid differences between seven Estonian 2009 pandemic H1N1 HA sequences and a consensus of the pandemic H1N109 HA sequences from Mexico (H1N1pan09). For comparison, amino acids in these positions of an H1N1 strain isolated in 2007 from swine in Europe (GenBank ID ACH69547.1) and in a strain isolated in 1977 from humans in the USSR (GenBank ID ABD60933.1) are listed in a separate section

GenBank accession number	Similarity to H1N1pan09 consensus, %	Amino acid position											
		16	49	154	179	220	239	303	338	391	477	521	537
H1N1pan09	**100.0**	**N**	**L**	**P**	**S**	**S**	**D**	**I**	**I**	**E**	**I**	**K**	**V**
ADG42533.1	99.5	K	L	P	S	T	D	I	V	E	I	K	V
ADG42543.1	99.3	N	L	S	S	T	E	I	V	E	I	K	V
ADG42553.1	99.1	N	L	S	N	T	E	I	V	E	I	K	V
ADM12971.1	99.6	N	L	P	S	T	D	I	I	K	I	K	V
ADM12981.1	99.8	N	L	P	S	T	D	I	I	E	I	K	V
ADM31858.1	99.9	N	L	S	N	T	E	I	V	E	I	E	V
ADM86399.1	99.1	N	I	P	S	S	D	V	V	E	V	K	A
ACH69547.1	94.0	N	L	S	N	S	D	I	I	G	I	K	V
ABD60933.1	79.9	D	L	S	S	S	G	I	I	G	I	K	V

DISCUSSION

In this study, we used an ELISA method to screen blood sample collections for the presence of anti-H1N1 and anti-H3N2 antibodies. We established that in 2009–2010 both humans and pigs had been infected with the H1N1 virus in Estonia at surprisingly high rates. Considering that the majority of the seropositive patients had not exhibited typical signs of influenza, the virus that spread in Estonia was probably attenuated compared to the strains that were characteristic of the 2009–2010 pandemy in many other countries. It is well known that the seasonal H1N1 influenza spreads easily within human population and may induce a partial protection via cross-reaction. As the 1977 influenza outbreak in the former USSR was due to an H1N1 virus [17], the elevated number of cases observed in Estonia that year [18] was most likely due to H1N1 viruses. Several younger blood donors had probably not been exposed to this seasonal H1N1.

No mortality or abnormal respiratory diseases were reported in the studied swine herds during the first half of 2010. Since the health status of the tested pigs was followed by a veterinarian in the studied farms, the fact that no signs of influenza were observed indicates that the H1N1 virus present was also apperantely attenuated. In fact, the last laboratory-confirmed case together with influenza virus isolation from a pig in Estonia was registered in 1957 [19]. Interestingly, almost all the animals analysed in 2010 were seropositive for the H3N2 influenza virus, but with antibody titres lower than against the H1N1pandemic09 virus, suggesting that they had recently encountered the H1N1pandemic09 virus. Considering that the sampling was carried out in May 2010 and the serum antibody level is typically high for four months after infection [8], the high rates observed in many animals probably indicate an infection during spring 2010. Reports from Scandinavia, Poland, and even New Caledonia suggest that in 2009–2010 several previously clean farms turned positive for the H1N1pandemic09 virus [20–24]. However, with the increasing numbers of human infections, a spillover of this virus to pigs during the 2009–2010 pandemic was quite plausible also in Estonia like it was in the case of the above-mentioned countries.

According to the Estonian Health Board, 124 000 cases of H1N1pandemic09 were estimated in Estonia in 2009–2010 and 21 deaths were associated with the H1N1 influenza virus during that period. The first case of H1N1 was confirmed on 29 May 2009. Until September, only occasional cases were registered, mostly linked to travellers. The influenza epidemic in Estonia started in October–November 2009, with twice as many patients with signs of respiratory disease compared to the previous years in the same period. The virus did not appear to be more virulent than in the previous autumns. From December, the number of patients decreased, but the number of respiratory disease recorded was still high in March. At the beginning of 2010, several confirmed cases of H1N1 in adults were associated with low fever and mild clinical signs. The highest disease rate – 19.5% – was among children aged 0–14, while people aged 15–65 had a much lower disease rate of around 3.9%, and those over 65 years a still lower rate (0.95%), probably due to the protection afforded by the previous encounter with H1N1 viruses [10,25].

Considering that the analysed patients were 15–65 years old, 23 seropositive persons among 123 appears to be an unexpectedly high proportion. However, it is well compatible with the spread of an attenuated virus causing no clinical disease. Our results deserve to be confirmed by an epidemiologic study to assess the true rate of infection of the Estonian population by the H1N1 virus. We could find only seven sequences of H1N1 hemag-

glutinin from Estonia in the databases, which do not provide a comprehensive description of the viral diversity present in the population in the period. However, analysis of the obtained alignment showed that while some of the changes in the amino acid composition of the H1N1 HA in Estonia had been commonly detected worldwide, others were quite rare (Table 1). For example, a study from Finland shows that one or two amino acid changes (N125D and/or N156K) in the major antigenic site of the hemagglutinin of the influenza A (H1N1)2009 virus may lead to significant reduction in the ability of the patient and vaccine sera to recognize influenza A(H1N1)2009 viruses [16]. Such studies are important to find out whether or not the H1N1 seropositivity and protection rate are indeed significantly underestimated in Estonia due to frequent asymptomatic H1N1 infections. This knowledge has significant practical importance because it determines the planning of the vaccination against the H1N1 influenza virus with social and financial consequences.

CONCLUSIONS

Our results indicate the need for a better characterization of the status of the influenza circulating in Estonia and other countries during epidemic episodes. Better links with clinics and coordination with comprehensive surveys of the disease among farms would also bring a lot more understanding of the basic mechanism of the spreading of the viruses. ELISA and HI methods were used to screen both Estonian human and porcine blood samples of different origin for the presence of anti-H1N1 antibodies. We could establish that both humans and pigs had been infected with the H1N1 virus with often having no clinical signs, which may indicate that an attenuated virus spread in the Estonian population during that period. We also show here for first time that several herds in Estonia were infected with the pandemic H1N1 virus in spring 2009. We could establish that in Estonia, like in other countries, both humans and pigs were infected with the H1N1 virus at surprisingly high rates in 2009–2010.

ACKNOWLEDGEMENTS

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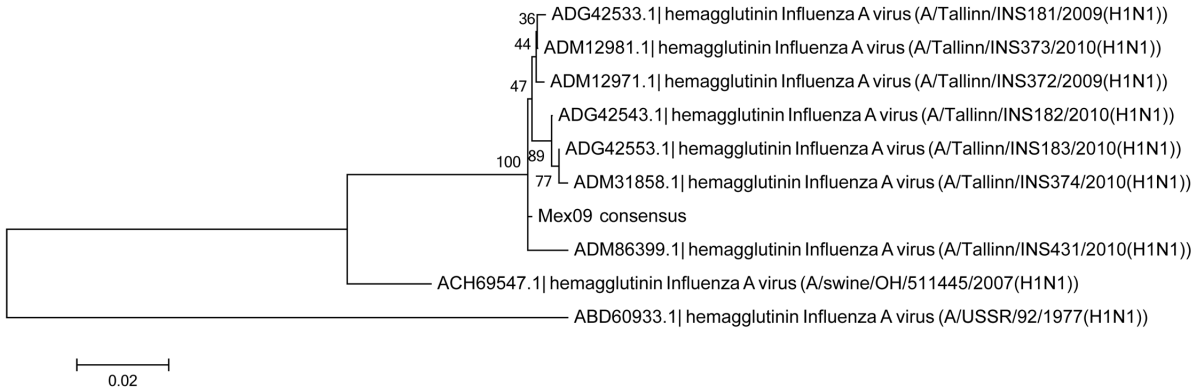
APPENDIX 1

MOLECULAR PHYLOGENETIC ANALYSIS BY THE MAXIMUM LIKELIHOOD METHOD



The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model [26]. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed [27]. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [27]. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of the common sites was <100 or less than one fourth of the total number of the sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 10 amino acid sequences. Evolutionary analyses were conducted in MEGA5 [28].

APPENDIX 2

A CONSENSUS SEQUENCE OF THE PANDEMIC H1N1 HA SEQUENCES

Protein Name:	*HA Hemagglutinin*	*HA Hemagglutinin*	*HA Hemagglutinin*	*HA Hemagglutinin*
Organism Name:	*Influenza A Virus*	*Influenza A Virus*	*Influenza A Virus*	*Influenza A Virus*
Strain Name:	IRD: A/Tallinn/INS181/2009(H1N1) GenBank: A/Tallinn/INS181/2009	IRD: A/Tallinn/INS182/2010(H1N1) GenBank: A/Tallinn/INS182/2010	IRD: A/Tallinn/INS183/2010(H1N1) GenBank: A/Tallinn/INS183/2010	IRD: A/Tallinn/INS372/2009(H1N1) GenBank: A/Tallinn/INS372/2009
Subtype:	H1N1	H1N1	H1N1	H1N1
2009 Pandemic H1N1-like?:	Positive	Positive	Positive	Positive
Host:	IRD: Human GenBank: human; gender F; age 25Y	IRD: Human GenBank: human; gender M; age 30Y	IRD: Human GenBank: human; gender F; age 24Y	IRD: Human GenBank: human; gender F; age 18Y
Collection Date:	12/21/2009	01/04/2010	01/07/2010	12/23/2009
Flu Season:	9-10	9-10	9-10	9-10
Isolation Source Gender:	F	M	F	F
Isolation Source Age:	25	30	24	18
Isolation Country:	Estonia	Estonia	Estonia	Estonia
Lab Host:	P0 passage(s)	P0 passage(s)	P0 passage(s)	P0 passage(s)
GenBank Submission Date:	05/07/2010	05/07/2010	05/07/2010	08/16/2010
NCBI Taxon ID:	747889	747890	747891	856499
Genbank Source Sequence Accession:	CY062995	CY063003	CY063011	CY071063
UniProtKB Accession:	D5XKJ9	D5XKK9	D5XKL9	E0UYG4
GenBank Protein Accession:	ADG42533.1	ADG42543.1	ADG42553.1	ADM12971.1

Protein Name:	*HA Hemagglutinin*	*HA Hemagglutinin*	*HA Hemagglutinin*
Organism Name:	*Influenza A Virus*	*Influenza A Virus*	*Influenza A Virus*
Strain Name:	IRD: A/Tallinn/INS373/2010(H1N1) GenBank: A/Tallinn/INS373/2010	IRD: A/Tallinn/INS374/2010(H1N1) GenBank: A/Tallinn/INS374/2010	IRD: A/Tallinn/INS431/2010(H1N1) GenBank: A/Tallinn/INS431/2010
Subtype:	H1N1	H1N1	H1N1
2009 Pandemic H1N1-like?:	Positive	Positive	Positive
Host:	IRD: Human GenBank: human; gender F; age 33Y	IRD: Human GenBank: human; gender F; age 25Y	IRD: Human GenBank: human; gender M; age 34Y
Collection Date:	01/07/2010	01/07/2010	02/05/2010
Flu Season:	9-10	9-10	9-10
Isolation Source Gender:	F	F	M
Isolation Source Age:	33	25	34
Isolation Country:	Estonia	Estonia	Estonia
Lab Host:	P0 passage(s)	P0 passage(s)	P0 passage(s)
GenBank Submission Date:	08/16/2010	08/24/2010	09/07/2010
NCBI Taxon ID:	856500	856501	856542
Genbank Source Sequence Accession:	CY071071	CY072526	CY073733
UniProtKB Accession:	E0UYH4	E0V3B6	E0V7K5
GenBank Protein Accession:	ADM12981.1	ADM31858.1	ADM86399.1

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2009. aasta H1N1 pandeemilise gripi levik Eestis oli tugevalt alahinnatud

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Mehhikost pärinev 2009. aasta pandeemiline gripp (H1N1pandemic09) põhjustas Eestis 2009. aasta lõpul ja 2010. aasta algul suhteliselt väheste sümptomitega haiguspuhanguid. Kliiniliste tunnuste puudumise tõttu oli haiguse reaalset levikut Eestis raske hinnata, samuti ei olnud sel perioodil ametlikult registreeritud ühtki juhtumit H1N1 gripi esinemise kohta loomadel.

Käesolevas töös kasutasime ELISA meetodit, et teostada vereproovide sõeluuring gripitüvede H1N1 ja H3N2 vastaste antikehade esinemise osas. Kõiki proove testiti ka hemaglutinatsiooni inhibitsiooni meetodil.

Uuritud 123-st inimese seerumist olid 23 (18,7%) H1N1pandemic09 viiruse suhtes positiivsed. Lisaks sellele olid kuue inimese vereseerumid positiivsed nii H1N1 kui ka H3N2 suhtes, samas kui Eesti Terviseameti andmetel oli haigestunute arv sel perioodil kõigest 3,9%. Neljast uuritud seafarmist olid kahes peaaegu kõik loomad H1N1pandemic09 suhtes positiivsed.

Seitset Eestist eraldatud gripiviiruse HA valgu järjestust võrreldi pandeemse viiruse konsensusjärjestusega ja leiti, et 12 aminohapet olid asendunud.

Põhinedes nendele tulemustele võime järeldada, et Eestis oli H1N1pandemic09 gripiviirus laialt levinud nii inimeste kui sigade hulgas.