PROJECT FINAL REPORT

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4.1 Final publishable summary report

4.1.1 Executive summary

Running from 2010-2013, ESNIP 3 represents the only organised surveillance network for influenza in pigs in Europe. Identification of swine influenza viruses (SIVs) in European pig herds is necessary to further expand the knowledge of the epidemiology of these viruses and to study their antigenic and genetic evolutions. Three work packages (WP 2, 3, 4) aimed to increase the knowledge of the epidemiology and evolution of SIV in European swine through organised field surveillance programmes (WP2). Virus strains detected in these programmes were subjected to detailed characterisation both antigenically (WP3) and genetically (WP4) using standardised methodology. Specifically, this involved timely information on genomic data and generation of antigenic maps using the latest technology. Consortium partners further disseminated information through peer review publication, and attendance and presentations at conferences/meetings (WP5). The viruses isolated though programmes of surveillance in WP2 and characterised in WP3 were submitted by partners to the virus bank maintained for the scientific community (WP5). A database was established in accordance with quality assurance requirements for maintenance of a virus collection, modelled on similar systems already in place for virus archives at P1-AHVLA (formerly VLA at the start of the project). Other project objectives were to harmonize surveillance approaches and diagnostic techniques. Importantly, ESNIP 3 sought to provide insights into the public health risk of influenza in swine, especially in respect of novel and emerging viruses.

Among the 24 participants, 16 partners from 13 different countries were actively involved in SIV surveillance and identification. Inventories of surveillance systems and diagnostic methods in-use by participating countries were organised through questionnaires. This showed that several partners had reinforced their surveillance schemes and that passive surveillance is used in most cases. Minimum datasets have been defined for standardization of the epidemiological analyses. Standard protocols and reference reagents have been established and disseminated to partners for virus detection, isolation and preliminary subtyping. A standard panel of five reference sera and antigens was tested to calibrate the protocol to be used for preliminary antigenic subtyping. To confirm that all participating laboratories were capable of detecting all relevant SIV subtypes, a PCR ring trial was arranged. Twelve partners participated in this ring trial which showed that every laboratory employs diagnostic tests capable of detecting all relevant circulating SIV subtypes in Europe, including the A (H1N1)pdm09 virus. Several laboratories also used several RT-PCR assays for rapid molecular subtyping. Detected viruses include the endemic strains: avian-like swine H1N1, swine H1N2 and human-like swine H3N2, and A (H1N1)pdm09. These viruses continue to co-circulate but at different levels of incidence by country. The H3N2 subtype has not been isolated in some geographic areas whereas it was prevalent in other parts of Europe.

Preliminary antigenic cartography maps revealed substantial geographical and temporal differences. A larger number of circulating SIVs were then tested and added to the map, creating a final 3D map of European H1 viruses including representative strains from different partner countries. This showed marked antigenic diversity among the viruses isolated from European pigs. The antigenic evolution of SI (H1) viruses in Europe is clustered with co-circulation of multiple different antigenic variants through the time period. Antigenic cartography was also used to quantify the evolution of H3 influenza A viruses in European pigs and to assess the antigenic relationships with a reference strain from the H3N2v outbreak in North American pigs. No significant difference was found in the antigenic variants of H3 currently-circulating in pigs in different EU countries. There was some antigenic difference between currently-circulating strains and the vaccine strain, but determining the significance of any potential reduction in vaccine efficacy would require further cross-protection experiments. The

antigenic distance between European H3 strains and H3N2v strains, however, is likely to be large enough to risk incursion into European pigs were it to be introduced.

Virological surveillance conducted by ESNIP 3 partners from November 2010 to October 2013 allowed the detection of 2742 influenza A virus-positive herds over 8977 farms examined in 17 countries (30.5% of herds examined were positive). Over the period, 1885 viruses have been subtyped, either directly in positive clinical samples or after virus isolation. More than 350 isolates were submitted by the partners to the virus bank and from these, 229 full genomes have been sequenced and delivered to the virus database. A further 113 genomes remain to be finalized and deposited. The data showed that while the internal gene cassette of the genome tended to associate together strongly, the external haemagglutinin and neuraminidase segments were shown to frequently reassort. During the previous EU-concerted action ESNIP 2 (SSPCE-CT-2005-022749), five distinct genotypes were detected. However, for samples collected since 2010 under ESNIP 3, a total of 14 distinct genotypes were observed, including reassortants between the Eurasian avian (EA) genotype, ‘ 2009 pandemic’ genotype, and human-origin external segments (H1, H3, N1, and N2). One particular genotype of an isolate from Spain was a triple-reassortant virus containing an EA internal cassette with human-origin H3 and N2 external segments, but with a pandemic-origin matrix-protein (MP) segment. Acquisition of the pandemic MP by an H3N2 SIV in the United States was implicated in recent zoonoses into humans showing the value of this ESNIP programme to provide early knowledge of emerging viruses with veterinary public health significance

Since the emergence of A (H1N1)pdm09, the virus has established in swine across Europe, with complete pandemic genotypes observed across six countries and pandemic reassortants observed in four other countries. The frequency at which the pandemic genotype is observed is not homogeneous across Europe, with mainland Europe showing a lower proportion of infections as pandemic SIV compared to the UK. Since 2010, all sequenced genomes in England were either a complete pandemic genotype or a pandemic reassortant with human-origin H1 and N2 external segments. Continuing and more complex levels of genetic reassortment amongst influenza A viruses co-circulating in European pigs has been demonstrated in ESNIP3providing the first cohesive insights across Europe of genetic diversity. The potential establishment of some of these novel combinations provides important information to a wide range of stakeholders in both veterinary and public health fields.

In conclusion, the epidemiology of SI across Europe continues to differ and vary between regions. The critical output from the project, disseminated through the secure web-based forum FLU-LAB-NET (SSPE-CT-2007-044453), conference attendance and 14 peer-review publications, has been to update the status of the epidemiology of influenza viruses in European pigs and its context in a global setting especially by comparison to the USA and China (achieved with membership through this consortium). Whilst providing an evidence base for decisions relating to veterinary health, dissemination of knowledge coming from surveillance in pigs is also consistent with pandemic preparedness.

4.1.2 Project Context and Objectives

ESNIP 3 has built upon the achievements of the previous EU concerted actions in ESNIP 1 and 2 which were:

1) The standardisation of protocols for SIV isolation, serology, antigenic and genetic typing of SIV isolates.

2) The selection and production of reference virus strains and (hyper-immune) sera. These were made available to all participants for preliminary sub typing of SIV isolates.

3) The establishment of a central SIV bank with a collection of recent isolates from various geographical areas in Europe.

4) The establishment of an electronic database with relevant information on the SIV isolates that were obtained in different countries during the life of the network.

5) The antigenic and genetic characterisation of a number of recent H1N1, H3N2 and H1N2 SIV isolates from different European countries.

6) The organisation of a serological survey to obtain preliminary data on the prevalence of different SIV subtypes in various European countries.

The strategic objectives of ESNIP 3 were:

1) To further expand our knowledge of the epidemiology and evolution of SIVs in Europe through both virological and serological surveillance for influenza in pig populations.

2) Provision of coherent data sets at EU level in relation to SIV.

3) Rapid characterisation of contemporary viruses contributing to better information for authorities concerned with veterinary public health.

4) Harmonisation of surveillance approaches and diagnostic techniques for swine influenza within the network.

5) Application of developments in novel technologies and cutting-edge tools to the study of SIV in European pigs.

6) To provide insights into the public health risk of influenza in swine especially in respect of novel and emerging viruses thereby consistent with pandemic preparedness by conducting surveillance in pigs.

7) Establishing an EU SIV bank for the scientific community.

8) Global dissemination of knowledge and information including strategic partnerships with other networks of influenza in other host species including humans.

The research objectives of ESNIP 3 were grouped into five main tasks:

Task 1: To keep track of major changes in the epidemiology of SIV in Europe

An extensive virological surveillance in the European pig populations was conducted according to systems in place in each participating country. Clinical material was collected from respiratory disease outbreaks on swine farms. Influenza viruses were isolated and preliminary sub typing was carried out using optimised protocols and reagents. Selected strains were used for downstream analysis in WP3 and WP4. A limited serosurveillance was carried out in select regions to investigate enzootic SIV prevalence and evolutionary changes. An inventory of surveillance programs that are currently active in all participating countries was made. A survey of diagnostic tests being carried out in European laboratories was conducted. Ring tests for reverse transcription polymerase chain reaction (RT-PCR) and serological assays were organised. In particular, the use of regional strains in HI tests was evaluated and results were compared to those obtained with strains selected as “European standards”. Epidemiological data was analysed and information for SI in Europe was compared to that in the USA and China.

Task 1 was achieved through the following specific and timed subtasks:

Subtask 1.1: Preparation of an inventory of the surveillance programmes that were active in all participating countries, even in those European countries that are not ESNIP 3 partners. Obtain information from additional European countries through a questionnaire. Define minimum datasets for standardised epidemiological analyses. (Month 1 to Month 12).

Subtask 1.2: Harmonisation of diagnostic tools for virological surveillance, focusing on virus detection by RT-PCR, virus isolation (in cell culture or eggs) and preliminary sub typing (HI tests and/or RT-PCR). This included a survey of which tests are being carried out in European laboratories. One RT-PCR ring test was organized at the beginning of the project. For serology, the use of regional strains in HI tests was evaluated and compared with results obtained using strains selected as "European standards". Two serological ring tests were organized over the three year period of the project. (Month 3 to Month 36).

Subtask 1.3: Collection and testing of material (nasal swabs, lung tissue) from respiratory disease outbreaks on swine farms. Influenza virus isolation and preliminary sub typing using protocols and reagents established during ESNIP 2. Selection of strains for carrying out the tasks outlined in WP3 and WP4. In countries with a more active surveillance, isolation of multiple strains from the same herd over the three year period will be encouraged, allowing new data to be generated about SIV dynamics, especially with respect to possible persistence of virus in herds versus repeated new introductions. (Month 1 to Month 36).

Subtask 1.4: Limited serosurveillance in select regions to obtain data on endemic SIV prevalence and evolutionary changes. (Month 1 to Month 36).

Subtask 1.5: Epidemiological data analyses and comparison of epidemiology of SI in Europe to that in the USA and China. (Month 12 to Month 36).

Task 2: To study the extent of antigenic evolution of SIVs

The antigenic properties of influenza viruses are routinely estimated using the haemagglutination inhibition (HI) assay. The HI assay is a binding assay based on the ability of influenza viruses to agglutinate red blood cells and the ability of anti-sera raised against this or related strains of influenza virus to block this binding. The interpretation of HI data to describe the antigenic properties have provided some insights but sometimes gives inconsistent data because the antigenic distances between strains are determined in an indirect way and do not properly take data below the sensitivity threshold of the assay into consideration. Similarly, production of genetic data has revealed important insights into the evolution of influenza viruses, however, prediction of antigenicity based on genetic data is difficult because there is a huge variation in the antigenic effect of specific amino acid substitutions due to the particular amino acid substitution, the localisation of the amino acid and/or the interaction of multiple substitutions. Thus a single substitution may have profound impact on antigenicity of the protein whereas multiple other substitutions might have no impact.

The overall aim of this task was to generate data on the antigenic variation of circulating European SIVs by the use of antigenic cartography in order to improve the control and prevention of infections and interspecies spread of SIV strains.

Task 2 was achieved through the following specific and timed subtasks:

Subtask 2.1: HI data was performed using the protocols developed in ESNIP 2 and further refined. The protocol developed in WP2 was revisited and validated initially on archived samples. It was then distributed to selected partners together with reference anti-sera and antigens generated during the ESNIP 2 project. For

some viruses, where homologous sera are not available, hyper-immune sera were generated by parental immunisation of specified pathogen free (SPF) pigs under controlled conditions. (Month 1 to Month 12).

Subtask 2.2: Further subtyping of European SIVs. Influenza-positive samples from pigs were identified and initially subtyped (H and N subtype) in WP2. Protocols for further sub typing were compiled, validated and ring tests arranged to validate the protocols among the participating partners. (Month 3 to Month 18).

Subtask 2.3: Antigenic characterisation of virus isolates by HI test. Virus isolates generated in WP2 were tested using the HI assay against a panel of sera raised against diverse and well-characterised viruses. In addition, the anti-sera were tested against a panel of reference viruses as a control with which to test consistency among HI assays, and to more accurately combine data from multiple tests. The HI results were stored in a database together with sequence information and used to complete the antigenic cartography in subtask 2.4. (Month 6 to Month 36).

Subtask 2.4: Antigenic cartography was performed using sequence data generated in WP4 and HI data generated in **subtask 2.1**. HI and genetic data obtained from sera of experimentally-infected pigs from previous projects on SI were provided by the participating partners for calibration of the methods. These antigenic cartography methods have been validated in a variety of influenza systems including human seasonal A (H3N2) (Smith et al., 2004; Russell et al., 2008), pandemic A (H1N1) (Garten et al., 2009), equine A (H3N8) (Lewis et al., 2011), avian A (H5) and A (H7), A(H9), and American swine A (H3N2) and (H1) (de Jong et al., 2007; Lorusso et al., 2011). (Month 6 to Month 36).

Subtask 2.5: Pulling together and consolidation of datasets from WP3 and WP4. (Month 12 to Month 36).

Task 3: To study the extent of genetic evolution of SIVs

With the emergence of H5N1 avian influenza and A (H1N1)pdm09 as important human pathogens, the importance of detailed understanding of influenza viruses in swine has become essential. This is especially true for A (H1N1)pdm09, since knowledge of influenza virus variation in swine and the outcome of introducing A (H1N1)pdm09 into pigs from humans would require investigation into the dynamics of SI evolution and evidence to be gathered for the genesis of A (H1N1)pdm09 in pigs.

WP4 addressed influenza virus variation in swine through characterisation, by whole genome sequencing, of more than 200 virus isolates from ESNIP 3 partners (collected in WP2).

The specific and timed subtasks of **Task 3** were:

Subtask 3.1: Sequence full length influenza genomes from swine using capillary and/or 454 methods. (Month 6 to Month 36).

Subtask 3.2: Produce a data repository for full-length genome sequences and underlying raw sequence data, with timely deposition of finished sequences to GISAID. (Month 12 to Month 24).

Subtask 3.3: Determine the population genetics and evolutionary dynamics of SI and investigate the functional significance of genetic variation. (Month 15 – Month 36).

Task 4: To establish and maintain a European swine influenza virus bank

The existing small bank assembled in ESNIPs 1 and 2 and held at P2-UGent was relocated to P1-AHVLA. Viruses isolated though programmes of surveillance in WP2 and characterised in WP3 were submitted by partners to the bank. The database was established in accordance with ISO 9001, 2008 for maintenance of a virus collection. It contains key information regarding the origin of the virus, the type of material and any brief characteristics associated with the strain generated in WP 3/4. The system is accessible to consortium partners through a secure web-based forum (hosted by FLU-LAB-NET, SSPE-CT-2007-044453) and P1-AHVLA provides a curation service to ensure appropriate maintenance of the collection. This includes the handling of requests from within and out with the consortium for supply of virus strains. A standard MTA was prepared on behalf of the consortium as applicable for sharing of viruses. P1-AHVLA, to partners depositing strains, provided a service if required for supply of viral RNA for sequencing through P14-WTSI to meet the requirements of WP4.

Task 5: Interaction with other influenza networks and the international community

Consortium partners further disseminated information through attendance and presentations at conferences/meetings. The consortium also submitted project data for a number of publications in peer-review journals. P1-AHVLA was well placed to facilitate the coordination of network interaction through its role as an OIE/EU reference laboratory for avian influenza. In particular, we utilised links with colleagues operating in the human and equine health sectors, especially through P23-AHT. Information flow on surveillance programmes in action and their relative relevance to other animal sectors was pursued. The existing small virus bank held by P2-UGent (produced in ESNIPs 1 and 2) was relocated to P1-AHVLA. The viruses isolated through programmes of surveillance in WP2 and characterised in WP3 were submitted by partners to the virus bank. A database was established in accordance with quality assurance requirements for maintenance of a virus collection, modelled on similar systems already in place at P1-AHVLA for virus archives. The database contains key information regarding the origin of the virus, the type of material and any brief characteristics associated with the strain (generated in WP3), and is accessible to consortium partners through a secure web-based forum within FLU-LAB-NET (EU-funded interactive forum for laboratory networks). P1-AHVLA provided a curation service to ensure appropriate maintenance of the collection. This included handling of requests from within and out with the consortium for supply of virus strains. A standard MTA was prepared on behalf of the consortium as applicable for sharing of viruses. Through the FLU-LAB-NET forum, real-time availability of information derived in WP2, WP3 and WP4 was made available. Project partners were encouraged to attend and participate in international meetings to enhance dissemination of results from the project. WP5 promoted the global dissemination of knowledge.

Specific and timed subtasks of **Task 5** were:

Subtask 5.1: Establish foray for dissemination of knowledge both within and out with the consortium. (Month 1).

Subtask 5.2: Formally establish interaction with other networks. (Month 2 to Month 24).

Subtask 5.3: Utilise a web-based forum (FLU-LAB-NET) to ensure timely dissemination of laboratory protocols, methods and data. (Month 6 to Month 36).

Subtask 5.4: Develop a dissemination strategy for the project to include conference attendance and peer-review scientific publication of results. (Month 3 to Month 36).

4.1.3 Results (WORK PROGRESS AND ACHIEVEMENTS)

WORK PACKAGE 2: VIROLOGICAL SURVEILLANCE FOR INFLUENZA IN PIG POPULATIONS

Work package leader: Dr Gaëlle Simon, Anses, France (P3)

WP deputy-leader: Dr Willie Loeffen, CVI, The Netherlands (P12)

Duration: 36 months

Sixteen partners and St. Jude Children's Research Hospital (USA) participated in WP2 actions (Table 2).

Table 1: Participants in WP2

Partner no.	Organisation short name	Country	Contacts
P1	AHVLA	United Kingdom	Ian Brown Scott Reid
P2	UGent	Belgium	Kristien van Reeth Karen van der Meulen
P3	Anses	France	Gaëlle Simon
P4	ISZLER	Italy	Emanuela Foni Chiara Chiapponi
P5	DTU	Denmark	Lars Erik Larsen Solvej Breum
P6	NVRI	Poland	Iwona Markowska-Daniel Andrzej Kowalczyk
P7	LCV	Spain	Montserrat Agüerro Garcia Azucena Sanchez
P8	IDT	Germany	Ralf Dürrwald
P9	EVIRA	Finland	Anita Huovilainen Tiina Nokireki
P10	KVI	Israel	Irit Davidson
P11	CAO	Hungary	Ádám Dan
P12	CVI	The Netherlands	Willie Loeffen
P13	UTH	Greece	Charalambos Billinis Constantinos Kyriakis
P17	FLI	Germany	Christian Grund Timm Harder
P21	Merial	France	Michel Bublot Thaïs Vila
P22	HIPRA	Spain	Jaime Maldonado Garcia
*	St Jude	USA	Richard Webby

***Additional participant**

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Work package 2 objectives:

1) To maintain and expand the detection of SIVs in pig herds in different European countries.
2) To harmonize virological and serological diagnostic tools.
3) To define minimum datasets to standardize epidemiological analysis.
4) To provide information on the epidemiology of SI in pigs in Europe and to compare it with the situation in North America (USA) and Asia (China).

Work package 2 – Description of work

An extensive virological surveillance in the European pig populations was conducted according to systems in place in each country involving participants. Clinical materials were collected from respiratory disease outbreaks on swine farms. Influenza virus was isolated and preliminary sub typing was carried out, using optimised protocols and reagents. Selected strains were used for WP3 and WP4. A limited serosurveillance was carried out in select regions to investigate endemic SIV prevalence and evolutionary changes. An inventory of surveillance programs that are currently active in all participating countries was made, even those not necessarily collecting virus strains. Extra information from additional European countries was attempted through a questionnaire. A survey of diagnostic tests being carried out in European laboratories was conducted. Ring tests for reverse transcription polymerase chain reaction (RT-PCR) and serological assays were organised. In particular, the use of regional strains in HI tests was evaluated and results were compared to those obtained with strains selected as “European standards”. Epidemiological data were analysed and information for SI in Europe compared to that in the USA and China. In general, the work in WP2 proceeded as expected during the course of the project.

Work package 2 - Work breakdown (tasks), Deliverables (D) and Milestones (M)

Task 2.1: Make an inventory of surveillance programs currently active (M1 > M12)

D2.1: Inventory of surveillance programs used in European countries (M12)

Task 2.2: Harmonization of diagnostic tools for virological surveillance (M3 > M36)

D2.2: Ring tests results and proposals for harmonised diagnostic tools and protocols (M36)

M2.2: First ring test results (M12)

Task 2.3: Collection of clinical material (M1 > M36)

D2.3: Collection of preliminary characterized SIV isolates from 13 European countries and selection for further antigenic (WP3) and genetic (WP4) characterization (M36)

M2.1: Collection of first biological samples for SIV isolation and preliminary typing (M3)

M2.3: Transmission of approximately 100 viruses to WP3 and WP4 for further antigenic and genetic characterization (M18)

Work package 2 results

Task 2.1 "Make an inventory of surveillance programs that are currently active" and **Task 2.2** "Harmonization of diagnostic tools for virological surveillance" were both addressed during the first year of the project. Actions (milestones) and results (deliverables) related to these tasks were previously described in the first periodic report but key data are summarized below. **Task 2.3** "Collection of clinical material" was addressed throughout the program and an overview of viruses identified during the three-year period is given below. **Task 2.4** "Serosurveillance" was only addressed in some countries where the virological surveillance was less developed. Results are also summarized here, giving knowledge complementary to that obtained from the virological surveillance. Concerning **Task 2.5** "Epidemiological data analyses", basic epidemiological analyses were not performed as initially expected, as insufficiently datasets were obtained in all countries. Nevertheless, the surveillance performed during this three-year programme provided an updated understanding of the general epidemiology of SI across Europe.

D2.1: Inventory of surveillance programs used in European countries (M12)

An inventory of SI surveillance programmes implemented in the participating countries was carried out by sending questionnaires to partners. The questionnaire asked if the virological surveillance was passive and/or active, if there were serological investigations, who was conducting the different surveillance programmes, who paid for sampling and testing, if there were national networks, who provided sampling kits, who reported clinical influenza-like events, who took the biological samples for the surveillance and which area or regions within the country were investigated.

The inventory of surveillance systems showed that the degree of sophistication and co-ordination of programs varied between countries and that several partners have reinforced and adapted their surveillance schemes since 2009. The core of the ESNIP 3 surveillance was conducted in the UK (P1- AHVLA), France (P3-Anses), Belgium (P2-UGent), Italy (P4-IZSLER), Denmark (P5-DTU), Poland (P6-NVRI), Spain (P7-LCV and P22-HIPRA), Germany (P8-IDT, P17-FLI and P2-UGent), Finland (P9-EVIRA), Israel (P10-KVI), Hungary (P11-CAO), The Netherlands (P12-CVI, P2-UGent and P8-IDT) and Greece (P13-UTH). Samples have been also obtained from Belarus, Slovakia, Lithuania and Russia and analyzed by P6-NVRI.

All countries except Israel used a passive surveillance system based on reporting of clinical acute respiratory disease in pigs. Most often, acute respiratory outbreaks were reported by vets, or by farmers or farm technicians, directly or indirectly through vets. The acute respiratory disease is most often characterized by fever, apathy, anorexia, sneezing, nasal discharge and coughing but in any case, SI must be differentiated from a variety of respiratory diseases of swine with subsequent virological analyses. This resulted in sampling of nasal swabs from sick animals and in some cases of mortality, tissue samples from the lungs and/or the upper respiratory tract were also taken for virus detection. Most of the time, biological samples were taken by veterinarians or medical company employees, but partner laboratory scientists were sometimes forced to go sampling in herds so that the monitoring could be done. Clinical material taken from pigs was sent to local veterinary laboratories and/or institutional partner laboratories, accompanied with information including expected data for epidemiological

analyses, but which at least included the location of the farm at the regional level and the date of collection.

Passive surveillance was effective in all administrative regions in the UK, Belgium, Denmark, Hungary and Greece. It should also cover entire territories in other countries, but pig dense areas remained the most investigated in France (Brittany), Italy (Northern and central parts), Poland, Spain, Germany (Northwest), Finland and The Netherlands. In the UK, France and Finland this passive surveillance was funded and organized at the national level, with private practitioners liaising with specified veterinary laboratories agreed over identification of SIVs, meeting an algorithm that finally resulted in the submission of positive samples to the partner laboratory for virus identification. In other countries, partners collaborated with their own network of voluntary veterinarians. Specific programmes supported by the pig industry also partially contributed to the surveillance in for example, Germany. In addition, vaccine manufacturers participated in the network by enhancing the flow of material from the field to the partner laboratories: IDT in Germany, HIPRA in Spain, Merial SAS in Belgium, France, Italy, The Netherlands, Poland and Hungary.

In some countries, authorities, pig producers and/or research laboratories have also undertaken some active surveillance, especially after the pandemic for detection of A (H1N1)pdm09 virus, but this was rare and use of active surveillance was extremely limited. Information collected through ESNIP 3 indicated that some targeted active virological surveillance programs were occasionally implemented in France, Denmark, Poland, Germany, Israel and Greece. These programs were predominantly structured on an *ad-hoc* basis and, for instance, were conducted in response to a specific question, i.e. about a virus in a particular region in association with a disease problem. Otherwise the programs targeted only animals at slaughter.

The first ESNIP 3 objective was virological surveillance and most of the participating countries offered a limited serological surveillance capability. In some cases, serological surveillance was purely targeted to reach a diagnosis in response to a clinical presentation on a premise, while in other cases it was part of a more structured approach which included a selection of farms in a region and aimed at measuring seroprevalence and addressing particular questions in relation to the husbandry of pigs and potential spread of virus. Thus, during ESNIP 3, targeted serological surveillance programs were punctually implemented in Belgium, Italy, Poland, Greece and Israel, in order to provide additional data about the prevalence of enzootic strains. In other countries, including France, Germany, The Netherlands and the UK, serological investigations were undertaken in response to clinical outbreaks and were linked to the passive virological system.

D2.2: Ring tests results and proposals for harmonised diagnostic tools and protocols (M36)

An inventory of diagnostic methods in use by the partners' laboratories was carried out through a second questionnaire. Partners were asked to indicate the use of molecular tests and/or other protocols to detect influenza A viruses in pig samples, as well as specific molecular assays to subtype the SIVs detected and/or isolated. The questionnaire also intended to list anti-sera in use for preliminary antigenic subtyping by HI or neuraminidase inhibition (NI) tests. This inventory indicated that in most countries initial screening of clinical samples for detection of any influenza A virus was carried out by RT-PCR technology based on the gene for the matrix (M) protein, while one laboratory also ran a nucleoprotein (NP) gene RT-PCR assay. Partners used different protocols that each had developed and/or adapted previously.

To confirm that all participating laboratories were capable of detecting all relevant SIV subtypes, a RT-PCR ring trial was arranged by P3-Anses (see first periodic report). Twelve laboratories participated in this ring trial, which revealed small differences in analytical performance depending on the protocol in use. Briefly, results showed that every laboratory employed diagnostic tests capable of detecting European SIVs of H1N1, H1N2 and H3N2 subtypes. All

methods also detected the A (H1N1)pdm09 virus, except one based on the primers proposed by Spackman et al. (2002). As previously reported, M gene RT-PCR assays used before A (H1N1)pdm09 emergence but not modified according to mutations in H1N1pdm M gene were shown to be not suitable for the detection of A (H1N1)pdm09-like swine viruses. Some differences in sensitivity were seen among the participating labs, most depending on the assay they used. As attempted, real-time RT-PCR assays were more sensitive than conventional RT-PCR. Real-time assays based on TaqMan® or SYBR® Green technology exhibited a similar sensitivity, higher than that of the PriProET technology-based assay. TaqMan®-based RT-PCR assays that specifically amplified conserved sequences of M gene or NP gene from influenza A viruses exhibited similar sensitivity.

Once influenza A virus is detected in a sample following initial screening by M or NP gene RT-PCR, several laboratories run RT-PCR assays specific for HA or NA genes of European SIVs and A (H1N1)pdm09 for a rapid molecular subtyping. Most often, real-time RT-PCR assays that specifically detect H1pdm and N1pdm genes were run first. Then, conventional multiplex RT-PCR assays allowing the amplification of HA and NA genes from European SIVs were implemented on A (H1N1)pdm09-negative samples by some partners. These multiplex RT-PCRs intended to discriminate either H1 from H3, or $H1_{av}$ from $H1_{hu}$ and H3, or N1 from N2. When combined, these two specific RT-PCR assays allowed users to rapidly identify $H1_{av}N1$, $H1_{hu}N2$ and H3N2 viruses, as well as reassortant viruses that would have exchanged HA or NA genes, such as $rH1_{av}N2$ or $rH1_{hu}N2$ viruses.

To assist in subtyping of novel isolates (obtained following propagation in cells or embryonated eggs) by HI and NI assays, a standard panel of five anti-sera was provided to partners. These sera were produced in SPF pigs by P3-Anses and were preliminary tested in five laboratories before dispatching (see periodic report). Three of them were hyper-immune sera against strains previously used as reference antigens during ESNIP 1 and ESNIP 2, *i.e.* A/Sw/Finistere/2889/1982 ($H1_{av}N1$), A/Sw/Flandres/1/1998 (H3N2) and A/Sw/Scotland/410440/1994 ($H1_{av}N2$). The fourth was a hyper-immune serum against a more recent $H1_{av}N1$ SIV isolate, A/Sw/Cotes d'Armor/0388/2009, while the fifth serum was a post-vaccination serum containing antibodies against A (H1N1)pdm09 virus, A/California/04/09.

Following discussions about diagnostic tools, several partners acquired during ESNIP 3 novel methods in their laboratories, when necessary. Indeed, implementation of RT-PCR for virus detection appeared to be very important for a successful virological surveillance, as it is a more sensitive method than virus isolation. This is especially the case for A (H1N1)pdm09 virus that may replicate in MDCK cells producing a cytopathic effect but with low HA titres. As it is possible that an A (H1N1)pdm09-like virus might go undetected by checking virus amplification through HA testing only, it was therefore recommended that a specific RT-PCR is run before (or after) virus isolation to be sure that the A (H1N1)pdm09 virus is detected. Also, molecular methodology appeared to be very useful for rapid subtyping and detection of reassortant viruses, and multiplex RT-PCRs were developed by some partners who did not use these tools prior to ESNIP 3.

P17-FLI has developed a novel real-time multiplex assay for SIV molecular subtyping. This methodology consists of a tetraplex real-time RT-PCR for HA subtyping and a duplex real-time RT-PCR for NA subtyping. P17-FLI sent mixes to four ESNIP 3 partners (P1, P3, P4 and P5) to test some of the recently isolated local strains as part of a multicenter study that would provide a more comprehensive validation of the methods. Initial results showed regional variations in HA subtyping, but also revealed that the NA duplex assay can be used as a common method for subtyping European SIVs in all countries. Further adaptations and developments will continue beyond ESNIP 3 by the five partners involved.

H3N2v

Following the emergence of a novel H3N2 reassortant virus (rH3N2p or H3N2v) in the United States pig population that gave rise to several infections in humans from 2011, two viruses, A/Swine/Iowa/A01203121/2012 and A/Swine/Indiana/A00968373/2012, were submitted to the ESNIP 3 virus bank. These were included in diagnostic analyses to evaluate their detection and identification using the methods employed by two partners, P1-AHVLA and P3-Anses. It was shown that both H3N2v were detected by routinely-used M-gene real-time RT-PCR assays. These were identified as viruses of H3N2 subtype by both molecular and antigenic sub-typing assays. In conclusion, such viruses should be detected as other H3N2 viruses if they would infect European pig herds. However, their belonging to the "American rH3N2p (H3N2v)" lineage and not to the "European human-like reassortant H3N2" lineage cannot be established from this preliminary subtyping. Additional genome sequencing would be necessary to discriminate these viruses from European H3N2 viruses.

D2.3: Collection of preliminary characterized SIV isolates from 13 European countries and selection for further antigenic (WP3) and genetic (WP4) characterization

The clinical material had to be collected from respiratory disease outbreaks on swine farms. Influenza A viruses can be detected in nasal or oropharyngeal swab supernatants, mucosa from the upper respiratory tract, or lung tissues.

During July 2011, partners were asked to send in the numbers of herds investigated, the numbers of positive cases and the different viruses they identified since November 2010. A second overview was asked on March 2012, a third in October 2012 and a fourth in October 2013.

In total, the virological surveillance conducted by ESNIP 3 partners from November 2010 to October 2013 allowed the detection of 2742 influenza A virus-positive herds over 8977 farms examined in 17 countries (30.5% positive) (Table 2).

Table 2: Numbers of investigated herds, positive herds and subtyped viruses in each of the three 12-month periods of the ESNIP 3 programme and in total from November 2010 to October 2013

Period	Herds investigated	Positive herds	Frequencies of positive cases	Subtyped viruses
November 2010-October 2011	2589	772	29,8	504
November 2011-October 2012	2716	890	32,8	676
November 2012-October 2013	3672	1080	29,4	703
November 2010-October 2013	8977	2742	30,5	1885

Numbers of herds investigated by ESNIP 3 partners increased through the project, especially during the third 12-month period of the programme (Table 2). However, the mean frequency of positive herds maintained at around 30%. The total number of subtyped viruses also slightly increased from one year to the next.

The intensity of the surveillance programmes varied greatly across the European countries involved in ESNIP 3. Areas with intensive production and national programmes in place inevitably resulted in a greater number of submissions than regions where production is less intensive and/or where surveillance programmes are less well organized or formalized. Thus,

the number of investigated outbreaks was highly variable depending on partners/countries (Table 3).

Table 3: Numbers of investigated herds, positive herds and subtyped viruses (at the time of writing) in the different European countries from November 2010 to October 2013

Country	Numbers of investigated herds	Numbers of positive cases	Frequency of positive cases (%)	Numbers of subtyped viruses
Belarus	20	2	10,0	0
Belgium	96	29	30,2	29
Denmark	1171	488	41,7	254
Finland	62	4	6,5	3
France	818	433	52,9	350
Germany	3427	1099	32,1	874
Greece	52	9	17,3	3
Hungary	102	35	34,3	38
Israel	5	3	60,0	2
Italy	2098	360	17,2	179
Lithuania	1	0	0,0	0
Netherlands	87	42	48,3	39
Poland	185	56	30,3	29
Russia	3	2	66,7	0
Slovakia	3	1	33,3	1
Spain	371	81	21,8	27
United Kingdom	476	98	20,6	57
Total	**8977**	**2742**	**30,5**	**1885**

The objective was not only to detect the viruses, but also to identify their lineages and subtypes, and to provide novel isolates to the virus bank (WP5) for further antigenic and genetic characterization (WP3 and WP4, respectively).

As there was some confusion and discordance in strain nomenclature, it was agreed during the first annual meeting that all partners will name viruses according to international nomenclature (A/Swine/Place of isolation (country level)/laboratory number/year) and will provide the virus subtype (H1N1, H3N2 or H1N2) and, if possible, the lineage as follows:

* European avian-like sw H1N1 (H1avN1)
* European human-like reassortant sw H3N2 (H3N2)

* European human-like reassortant sw H1N2 (H1huN2)
* European reassortant sw H1N1 (with HA of human origin) (rH1huN1)
* European reassortant sw H1N2 (with HA of avian origin) (rH1avN2)
* pandemic-like sw H1N1 (H1N1pdm)
* Other reassortant pandemic-like sw HxNy
* Other viruses

Thus, from November 2010 to October 2013, 1885 viruses have been subtyped, either directly in positive clinical samples or after virus isolation (Tables 2 and 3). Individual results of subtyping (molecular and/or antigenic) per country are given in Table 4.

Table 4: Numbers of viruses identified within the different subtypes and lineages in 14 countries from November 2010 to October 2013

Country	Number of subtyped viruses	Influenza A subtypes and lineages within subtypes						
		H1N1			H3N2	H1N2		Others
		$H1_{av}N1$	$rH1_{hu}N1$	H1N1pdm	H3N2	$H1_{hu}N2$	$rH1_{av}N2$	reass. pdm-like sw HxNx
United Kingdom	57	4	?	32	0	6	?	15
Belgium	29	16	?	0	10	3	?	?
Denmark	254	68	0	79	0	0	89	18
Finland	3	0	0	3	0	0	0	0
France	350	240	4	7	1	88	10	0
Germany	874	536	6	40	88	94	23	87
Greece	3	0	?	0	3	0	?	?
Hungary	38	19	0	12	4	1	0	2
Israel	2	0	?	1	1	0	?	?
Italy	179	82	2	10	38	39	7	1
The Netherlands	39	19	?	0	11	9	?	?
Poland	29	15	0	10	3	1	0	0
Slovakia	1	1	0	0	0	0	0	0
Spain	27	10	0	0	12	5	0	0
Total	**1885**	**1010**	**12**	**194**	**171**	**246**	**129**	**123**
%		**53,6**	**0,6**	**10,3**	**9,1**	**13,0**	**6,8**	**6,5**

Globally, the three old European enzootic SIVs (of H1N1, H1N2 and H3N2 subtypes), as well as A (H1N1)pdm09, constituted the four dominating lineages of influenza A viruses circulating in the European pig population from November 2010 to October 2013 (Table 4).

In line with results obtained within the previous ESNIP 1 and ESNIP 2 actions between 2000 and 2008, the "avian-like swine H1N1" ($H1_{av}N1$) lineage that emerged in 1979 was still the most prevalent lineage in many countries, reaching more than 53% of relative prevalence with regard to other subtypes identified during the period.

In contrast, the enzootic "human-like reassortant swine H3N2" lineage that emerged in 1984 counted for only 9% of the virus identifications. As already reported during ESNIP 2, this virus was no more isolated in some regions with high pig density whereas it was still prevalent in other parts of Europe (Table 4). Thus, H3N2 circulated widely in many of the main pig producing regions such as Belgium, Germany and The Netherlands, as well as in the Low Countries, Italy, Spain and Greece. In other areas of intensive pig production such as the UK, France (Brittany), Denmark and Poland, the H3N2 virus has been almost exclusively absent for many years. Only one isolate was obtained in France in early 2012, in the Northern part close to the Belgian border. Interestingly, these areas where H3N2 virus did not circulate had still relatively high levels of H1N2 virus circulation.

The enzootic "human-like reassortant swine H1N2" ($H1_{hu}N2$) lineage that emerged in 1994 represented the second most prevalent lineage of SIVs in circulation in Europe from 2010 to 2013, being unidentified in 13% of cases. However, a slight decrease in its relative frequency has been observed over time, from 2010 to 2013 (data not shown), a decrease that could be correlated with the appearance of the A (H1N1)pdm09 virus and it’s derivatives (reassortants) in many countries.

Indeed, A (H1N1)pdm09 viruses were isolated at an increasing incidence over time (from 2010 to 2013) in many countries, especially in the UK, Germany, Denmark, France and Italy, but also in Poland where the virus was first detected in 2012. Detections were also reported in Finland and Hungary, where circulation is suspected. Altogether, A (H1N1)pdm09 detections counted for more than 10% of the viruses identified. These data likely indicated that A (H1N1)pdm09 has become established in the European pig population, whereas it has not been detected and/or identified in Belgium, The Netherlands and Spain during the ESNIP 3 programme (Table 3).

Furthermore, reassortant viruses within the three enzootic SIV subtypes represented 7.5% of the viruses. They were identified in several countries, with evidence of further spread through the swine population for one of them in Denmark. In Denmark, a reassortant H1N2 virus of second generation ($rH1_{av}N2$), bearing a H1 gene coming from the H1avN1 lineage, supplanted the initial H1huN2 virus and has become the only H1N2 virus in circulation in that country.

Co-circulation of A (H1N1)pdm09 with European enzootic H1N1, H1N2 and H3N2 SIVs has also resulted in various reassortment events, leading to the detection of viruses that have mainly exchanged HA and/or NA genes. Finally, reassortants between A (H1N1)pdm09 and seasonal human strains have been also punctually detected in pigs in Italy. In Germany, reassortant viruses consisted in A (H1N1)pdm09 viruses that acquired an N2 gene from European SIVs. In Hungary, a reassortant was identified as an A (H1N1)pdm09 that acquired an N1 gene from the H1avN1 lineage. In UK, reassortant H1N2 viruses exhibit HA and NA genes from the enzootic H1huN2 lineage but internal genes from A (H1N1)pdm09. Thus, 6.5% of the viruses were identified as reassortant viruses that have acquired genes from the A (H1N1)pdm09 virus.

Altogether, A (H1N1)pdm09 viruses and viruses that contain one or more genes from A (H1N1)pdm09 represented nearly 17% of the viruses identified within the 3-year period of the programme.

When comparing the relative frequencies of the different virus lineages at the country level, very strong regional variations could be observed. Thus, countries can be grouped according to their profile in circulating strains. Interestingly, one can observe that countries within each group also shared common features related to surveillance programmes, SIV infection prevalence and perhaps diagnostic tools in use.

Countries that reported the highest diversities in SIVs lineages, including A (H1N1)pdm09 viruses and reassortants, belonged to Group A, subdivided into groups A1 and A2.

Group A1 comprised Germany, France, Denmark and Italy, countries where very high numbers of herds were investigated (>800), where high numbers of herds were found positive (> 350) and where high numbers of viruses were subtyped (> 170) using molecular (real-time and/or conventional RT-PCRs) and antigenic tools. In Group A2 (UK, Hungary and Poland) the numbers of investigated herds were lower than in Group A1 and were considered intermediate (100-500), as well as numbers of positive herds (35-100) and numbers of viruses that were subtyped (30-60), but in these cases viruses were also identified using molecular tools, in addition to antigenic subtyping by HI tests.

In countries from Group B (The Netherlands, Belgium and Spain), a lower diversity was observed than in Group A. The three old European enzootic SIV lineages were detected, but not the A (H1N1)pdm09 viruses and not any reassortant virus, either between enzootic SIVs or between enzootic SIVs and A (H1N1)pdm09. In this group the numbers of investigated herds were intermediate (80-400) as in Group A2 yet, as well as numbers of positive herds (55-80) and numbers of subtyped viruses (25-40). However, it has to be noted that in this group, viruses were mostly identified only using antigenic subtyping.

In Group D, an intermediate number of herds were investigated (60-70), whereas lower than in Group B, but very few herds were detected positive (4), showing a lower prevalence of SIV in pig population from these regions. As a consequence, very few viruses were identified (3), and molecular subtyping revealed they all belong to the same lineage. As it happens, it was here A (H1N1)pdm09 in Finland, a country that was approximately free of European enzootic SIVs before 2009.

Finally, in Group E were grouped countries where the virological surveillance was very limited, i.e. Greece, Israel and Slovakia. Thus, a low number of herds were investigated (<55), a low number of herds were detected positive (<10), and a low number of viruses were subtyped (<5) (using molecular and/or antigenic subtyping). In these cases, results of virological surveillance gave rise to relative frequencies that are probably not relevant to SIVs that circulate in these regions.

Among the 1885 viruses that were identified in WP2, 356 novel isolates were submitted to the ESNIP 3 virus bank for further antigenic (WP3) and genetic (WP4) characterizations (see WP5).

D2.4: Seroprevalence of European endemic SIVs in selected European countries (M36)

In Greece and Israel, passive virological surveillance was limited (see above). However, serological surveys conducted in these countries confirmed the circulation of influenza A viruses within pig herds.

Greece:

1) November 2010 to October 2012
   476 sera taken from in 28 farms > 245 positive (51.5 %) [HI tests (H1N1, H1N2, H3N2, H1N1pdm)] Antibodies were detected to H3N2, but also H1N1 and H1N1pdm.
2) November 2012 to May 2013
   291 sera from 24 farms > 182 positive (62.5 %) [ELISA (Ingenasa Swine Influenza ELISA Kit)]

Israel:
775 sera from 52 herds: around 90% were positive by ELISA.
The antibody subtype-specificity was determined on 407 sera from 27 herds by haemagglutination inhibition with four viruses, A/sw/Flandre/1/98(H3N2), A/sw/Scotland/410440/94(H1N2), A/sw/Cotes d'Armor/0388/09(H1N1) and (A/ck/Israel/1525/H9N2). All herds had antibodies to SIV H1N2 and H3N2 while only 10 herds had antibodies to SIV H1N1. The highest HI titers were towards SIV H1N2, SIV H3N2 HI titers were of intermediate values, while SIV H1N1 exposure produced the lowest titers. No antibodies to AIV subtype H9N2 were detected.

In Poland, an extensive and comprehensive serological survey has been conducted yearly for some time. Since 2009, HI tests detected antibodies to SIV H1N1 and H3N2 predominantly, as well as to A (H1N1)pdm09 and H1N2, in accordance with results from the virological surveillance.

Year	Number of sera	Number / % of positive sera			
		H1N1	H1N2	H3N2	pH1N12009
2010	5250	1583 / 30,1	626 / 11,9	815 / 15,5	≈10% Σ =510
					126 / 24
2011	3379	996 / 29,5	862 / 25,5	875 / 25,9	600 / 18,2
2012	5081	776 / 15,3	439 / 8,6	791 / 15,6	451 / 8,9

D2.5: An understanding of the epidemiology of SI across Europe

At the beginning of ESNIP 3, minimum datasets, to include such essential information as date and location of sample collection, but also epidemiological data at the animal and farm levels, have been defined for standardization of potential epidemiological analyses. Data sheets for samples, epidemiological information and results of analyses have been proposed to partners for supporting data collection (see periodic report). Epidemiological analyses at the European level would provide information on the incidence of influenza in different production systems across Europe. Indeed, when the detailed knowledge of herd structure, size, population characteristics, vaccination strategies are fully known, regional variations in SIV epidemiology should become more fully understood. However, such detailed epidemiological analyses, other than at the virological level, appeared rather difficult to collect on field. Apart from basic information (herd location and date of sampling), full additional data were regularly obtained only in the UK and France where national surveillance programmes were implemented and provided veterinarians with epidemiological data sheets together with sampling kits.

ESNIP 3 WP2 contribution to the FLURISK Project "Development of a risk assessment methodological framework for potentially pandemic influenza strains" (EFSA)

The main objective of the FLURISK project is the development and validation of an influenza risk assessment framework (IRAF) for the ranking of animal influenza A strains in their potential to cross the species barrier and cause human infection. ESNIP 3 contributed with data to the following three FLURISK activities:

1. The survey on influenza surveillance and control systems in animals: Information on 14 SIV surveillance components was shared by ESNIP 3; seven of them could be included in the survey. This allowed FLURISK to add five countries to the analysis that had not responded to the survey.
2. The FLURISK Epidemiological Report: ESNIP 3 partners shared information on 520 swine influenza virus isolates from their SI surveillance activities, of which 51 isolates (31 A (H1N1)pdm09 isolates from swine in Europe and 20 European H3N2 SIV) were selected for the

analysis in the epidemiological report and integrated into the overall dataset to produce graphs and maps (since this report provides details of FLURISK validation strains only).

3. The validation of the virus score, one parameter in the Influenza Risk Assessment Framework (IRAF): ESNIP 3 provided a H3N2 validation strain, with its full genome sequenced, to be run through the IRAF virus score and compared with H3N2v viruses from North America.

Comment on problems and corrective actions undertaken for WP2

No corrective actions were required.

WORK PACKAGE 3: ANTIGENIC CHARACTERIZATION OF SWINE INFLUENZA VIRUS

WP leader: Prof Lars Erik Larsen, DTU, Denmark (P5)
WP deputy-leader: Prof Iwona Markowska-Daniel, NVRI, Poland (P6)
Duration: 36 months

WP3 participants are listed in Table 5.

Table 5: Participants in WP3

Partner no.	Organisation short name	Country	Contact name
1	AHVLA	United Kingdom	Ian Brown Scott Reid Sharon Brookes
3	Anses	France	Gaëlle Simon
4	IZLER	Italy	Emanuela Foni
5	DTU	Denmark	Lars Erik Larsen Ramona Trebbien
6	NVRI	Poland	Iwona Markowska-Daniel
8	IDT	Germany	Ralf Dürrwald
9	EVIRA	Finland	Anita Huovilainen
10	KVI	Israel	Irit Davidson
12	CVI	The Netherlands	Willie Loeffen
13	UTH	Greece	Charalambos Billinis Constantinos Kyriakis
15	UCAM	United Kingdom	Nicola Lewis

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Work package objectives:

1) To compile archived influenza anti-sera and generate sera from pigs infected with well-characterized strains.

2) To test and validate aspects of the standardised HI assay protocol prior to antigenic cartography.

3) To perform cross-HI studies with selected influenza strains and sera.

4) Use antigenic cartography to visualise the antigenic properties of European SIVs.

5) To develop and validate assays for characterisation of intra-subtypic variation of strains.

6) To develop harmonised protocols for producing robust data sets for antigenic analysis.

7) To investigate the antigenic characteristics of European SIVs using antigenic cartography.

8) Determine the genetic basis for the antigenic differences seen among European SIVs.

Work package deliverables:

D3.1 Produce a standard protocol for detailed antigenic characterisation using the HI test (month 12).

D3.2 Complete antigenic cartography on first trance of approximately 50 viruses (month 18).

D3.3 Complete database with HI titre linked to genetic data on the specific viruses for antigenic cartography (month 36).

D3.4 Compare antigenic and genetic data sets to define any relationships (month 36).

Work package 3 – Description of work

The haemagglutinin (HA) is one of two viral glycoproteins and is the primary target of the protective immune response to SIV. Changes to the antibody binding sites of the HA protein can allow viruses to evade the host's immune response; a process called antigenic drift and can lead to vaccine failure in the field. Antigenic changes in SIVs are routinely measured using the HI assay. Antigenic cartography is a computational method that enables high-resolution quantitative analyses and visualizations of HI assay data. It is now routinely deployed to analyse influenza A viruses in the World Health Organization vaccine composition group.

Building on the virological surveillance carried out as part of WP2, it is critical to fully characterise the antigenic or phenotypic properties of the SIVs circulating in Europe and understand the antigenic inter-relationships among different co-circulating viruses of the same subtype.

Firstly, the existing HI protocols were harmonised among the seven WP3-participating laboratories, including testing the effect of using RBCs in the HI assay acquired from different species, such as turkeys or chickens, as without validation, variability would be introduced that might have a biasing effect on the antigenic distances seen among SIVs when using a technique such as antigenic cartography. In addition, testing and validation was undertaken to determine which method of raising anti-sera to use in the HI assay was most appropriate. After completion of this validation step, a standard HI protocol has been approved and made available to all partners through the project web-page. A survey of participating laboratories was carried out to ascertain the production methods used to generate hyper-immune SIV sera and the strain, genotype and volumes available for use in WP3. Partners then selected a panel of reference European H1 subtype hyper-immune SIV sera and antigens. This panel contained

representatives of all currently identified genotypes circulating in Europe. Preliminary antigenic maps were generated to determine that the sera were well-distributed in the antigenic map, this would likely position viruses robustly when new data was subsequently added, and that there was no duplication where two sera reacted similarly in the HI assay. Cross-HI tests were performed on more than 60 H1 viruses against the reference serum panel, and the first draft 3D map of the antigenic inter-relationships among H1 SIVs in Europe has been generated. Furthermore, reagents for antigenic characterization of SIV have been exchanged between the major participants in WP3 in anticipation of the isolation and characterization of H1 SIV throughout the project duration, and made available for all partners by uploading at the project web-page. Finally, a web-based database for the handling of HI and sequence data was developed and validated. In two separate subtasks, more than 30 H1 (subtype) viruses isolated in Denmark and 21 viruses isolated in the UK (swH1N2) during a 13 and 19 year period were run against the standard panel. Based on the results of these HI tests combined with sequence data from the HA gene, separate maps were generated showing significant genetic as well as antigenic drift of these viruses in Denmark as well as in the UK. In general, the work in WP3 proceeded as expected during the course of the project and all milestones were fulfilled.

The significant results/major achievements of WP3 were:

1) Validation of HI protocols by analysing the same samples in five different laboratories.
2) Development of a panel of hyper-immune sera covering all European subtype H1 viruses.
3) Exchange of reagents between the major participants in WP3.
4) Protocols have been made available for all partners through uploading at the project webpage.
5) Completion of the first draft antigenic 3D map of swine influenza A (H1) viruses.
6) Development of a web-based database for the handling of HI and sequence data.
7) Testing of more than 60 H1 viruses against the reference panel.
8) Completion of a final antigenic 3D map of swine influenza A H1 viruses in Europe.
9) Analysis of antigenic and genetic drifts of subtype H1 viruses in the UK and Denmark.
10) Development of a panel of hyper-immune sera covering all European subtype H3 viruses.
11) Completion of a final antigenic 3D map of swine influenza A H3 viruses in Europe.

There were no major deviations from the planned WP3 schedule.

WP3 objectives were addressed through **Task 2** (**Subtasks 2.1, 2.2, 2.3, 2.4 and 2.5)**. Detailed descriptions relating to **Task 2** and the **5 subtasks**, linked with the corresponding objectives and deliverables (D) are given below.

In order to predict the impact of specific substitutions in drifted viruses there is a need to generate data on the antigenic variation of circulating European SIVs. The antigenicity of these viruses is poorly defined including contemporary viruses circulating in European pigs. The overall aim of **Task 2** was to generate data on the antigenic variation of circulating European SIVs to improve the control and prevention of infections and interspecies spread of SI strains. To accomplish this, influenza viruses isolated throughout Europe were subtyped (WP2 and WP3) and characterized antigenetically by HI tests. Detailed cartograms were created for diverse circulating SI strains, including A (H1N1)pdm09 of potential swine origin. Monitoring the independent antigenic evolution of these viruses in pigs is important in assessing risks to public health as well as contemplation of intervention strategies such as voluntary vaccination. This element of the project is strengthened by partners from the veterinary pharmaceutical industry.

Subtasks 2.1 Generation of HI data for the cartography using standardized protocols and 2.3: Antigenic characterisation of virus isolates by HI test

-Objective 1 of WP3: To compile archived influenza anti-sera and generate sera from pigs infected with well-characterized strains

-Objective 2 of WP3: To test and validate aspects of the standardised HI assay protocol prior to antigenic cartography

-Objective 3 of WP3: To perform cross-HI studies with selected influenza strains and sera

-D3.1: Produce a standard protocol for detailed antigenic characterisation using the HI test (month 12)

The protocol developed in ESNIP 2 has been tested and validated among participating laboratories, initially by testing a selection of samples in seven different laboratories (P3-Anses; St. Jude; P2-UGent, P17-FLI, P5-DTU, P7-LCV and P22-HIPRA). This activity overlaps with activities previously described in WP2. The results showed that the different laboratories obtain comparable titres in the HI test even when using different pre-treatments of serum. Furthermore, at P1-AHVLA, the species from which RBCs were derived for use in the HI assay were also compared by characterising influenza viruses circulating in other hosts, variability can occur when using RBCs derived from either chicken or turkey. Turkey RBCs were chosen for the remainder of work in WP3 based on more robust results with the range of viruses used, particularly relating to the inclusion of the recent A (H1N1)/pdm09 viruses. The updated protocol was uploaded at the project website (FLU-LAB-NET) and distributed to interested partners.

Subtask 2.1: Generation of HI data for the cartography using standardized protocols (generation of hyper-immune sera to HI viruses)

-Objective 1 of WP3: To compile archived influenza anti-sera and generate sera from pigs infected with well-characterized strains

-Objective 2 of WP3: To test and validate aspects of the standardised HI assay protocol prior to antigenic cartography

-Objective 3 of WP3: To perform cross HI studies with selected influenza strains and sera a selected panel of using hyper-immune SIV sera

-D3.1: Produce a standard protocol for detailed antigenic characterisation using the HI test (month 12)

Some of selected sera for the reference panel were not hyper-immune sera but were obtained from vaccinated or infected animals. Previous experience with antigenic maps has shown that it is necessary to obtain relatively high titres with the homologous virus and this might be difficult using post-infection sera alone. Furthermore, it is uncertain if the origin of the sera may reflect the titres obtained in cross-HI tests. Based on these considerations, it was decided to develop hyper immune sera against those of the selected viruses from which hyper immune sera were not available. Different protocols for obtaining hyper-immune sera were discussed. Comparisons were carried out at P1-AHVLA to determine the optimum host species for antiserum. Both chicken and swine sera were assessed with the latter being chosen as the most relevant and reliable in the assay using swine viruses.

The use of hyper-immune and post-infection sera were compared, the former were selected based on titre and available quantities to share with partners. The protocols developed by P3-Anses and P1-AHVLA were selected for generation of sera for the serum panel using living and inactivated virus, respectively. Selected protocols were subsequently uploaded at the project web-based forum (FLU-LAB-VET). Hyper-immunisations with 10 different viruses were set up at

P8-DTU. Briefly, two pigs were immunised by each virus by intramuscular infection four times spanned by 2 weeks. The virus was inoculated intramuscularly. The amount of virus varied between 7-9 log10 EID50/ml. Blood samples were taken prior to the immunisation and again two weeks following the third immunisation. The titres against the homologous virus varied between 40 and 320 and therefore a fourth immunisation was performed. The animals were killed and bled two weeks later. Based on the obtained titres and availability of serum, the final standard panel was selected consisting of the sera listed below in Table 6. The panel consisted of seven sera and viruses previously developed by P3-Anses and one of the new sera developed at P5-DTU within the framework of ESNIP 3.

Table 6: Final standard serum panel

Strain/Hyper-immune serum	Subtype
A/Sw/Cotes d'Armor/0070/2010 (rH1N1)	Hu-like Sw H1N1
A/sw/Finistere/2889/1982 (H1N1)	Av-like sw H1N1
A/sw/Cotes d'Armor/0388/2009 (H1N1)	Av-like sw H1N1
A/sw/Denmark/19126/1993 (H1N1)	Av-like sw H1N1
A/sw/Scotland/410440/1994 (H1N2)	Hu-like swH1N2
A/sw/ Cotes d'Armor /0113/2006 (H1N2)	Hu-like swH1N2
A/sw/ Cotes d'Armor/0186/2010 (rH1N2)	Av-like sw H1N2
A/Sw/Sarthe/0155/2010 (H1N1)	pdm09-like sw H1N1

Subtasks 2.3 Antigenic characterization of virus isolates by HI test and 2.4: Antigenic cartography (selection of standard panel of sera)

- **Objective 1 of WP3:** To compile archived influenza anti-sera and generate sera from pigs infected with well-characterized strains
- **Objective 2 of WP3:** To test and validate aspects of the standardised HI assay protocol prior to antigenic cartography
- **Objective 3 of WP3:** To perform cross-HI studies with selected influenza strains and sera
- **D3.2:** Complete antigenic cartography on first trance of approximately 50 viruses (month 18)

Antigenic cartography techniques allow multiple HI assay tables from different laboratories to be combined into one dataset and used to generate an antigenic map. To do this, there needs to be a degree of commonality among individual HI tests. Thus, we selected a reference serum and antigen panel of eight strains/sera, which subsequently were included in each HI assay test as a control, to confirm consistency among different experiments and to allow data to be merged. The selected reference sera covered the full spectrum of antigenic diversity and were well distributed throughout preliminary antigenic maps, which ensured that points in the map can be reliably positioned (Figure 1). After serum panel selection, a validation procedure was performed in which two different technicians ran the standard panel against the homologous and heterologous viruses four times each on different days with different batches of blood and plates to validate the robustness of the test. The results showed that the deviation between repeated tests with the same serum/virus pair were within two-fold dilutions. Subsequently, initial cross-HI tests were performed on 21 serum samples and 12 virus isolates from Europe and USA which together represented all known genotypes of H1 SIVs circulating worldwide. These data were combined with data from cross-HI tests previously performed at P3-Anses and P1-AHVLA and a preliminary antigenic map was created (see Figure 2).

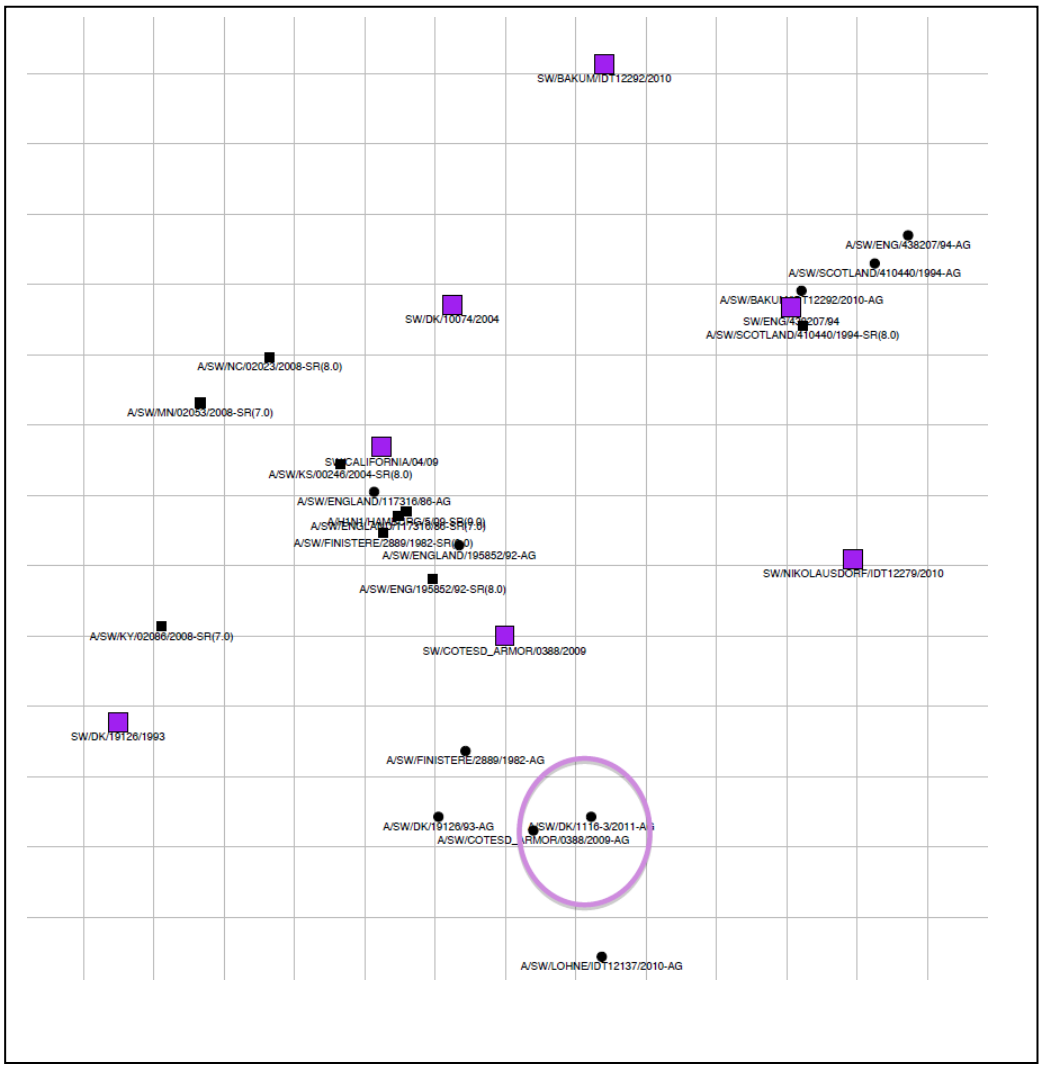


***Figure 1*: Antigenic map of swine influenza A (H1) viruses.** *The relative positions of strains (dots) and swine hyper-immune anti-sera (squares) were computed such that the distances between strains and anti-sera in the map with the least error represent the corresponding HI measurements (Smith et al., 2004). The large purple sera squares have been selected for the ESNIP 3 swine influenza A virus reference panel. In addition, another serum has been added raised to a strain representative of the antigenic group highlighted by the purple circle. One grid square represents one antigenic unit, or a two-fold difference in HI assay titre.*

Subtask 2.4: Antigenic cartography

- **Objective 4 of WP3:** Use antigenic cartography to visualise the antigenic properties of European SIVs
- **D3.2:** Complete antigenic cartography on first trance of approximately 50 viruses (month 18)

Antigenic cartography of swine influenza A (H1) viruses

Based on the preliminary HI data from selected H1 viruses including European, US and Canadian-type viruses and sequence data of the H1 gene, the first 3D antigenic map was created (Figure 2).

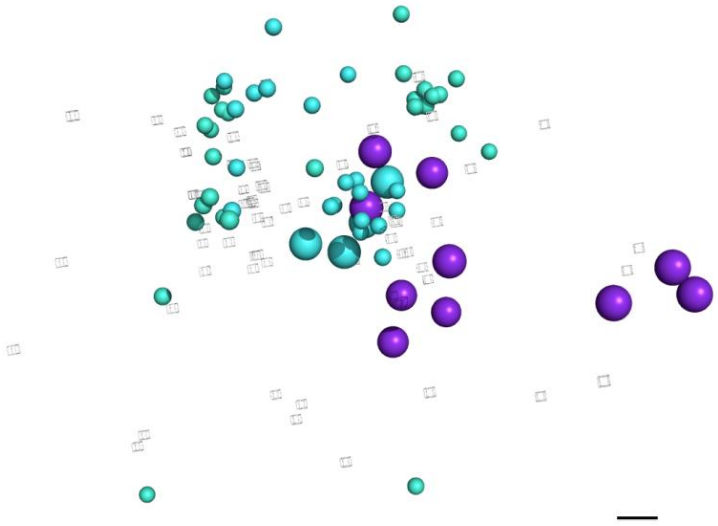
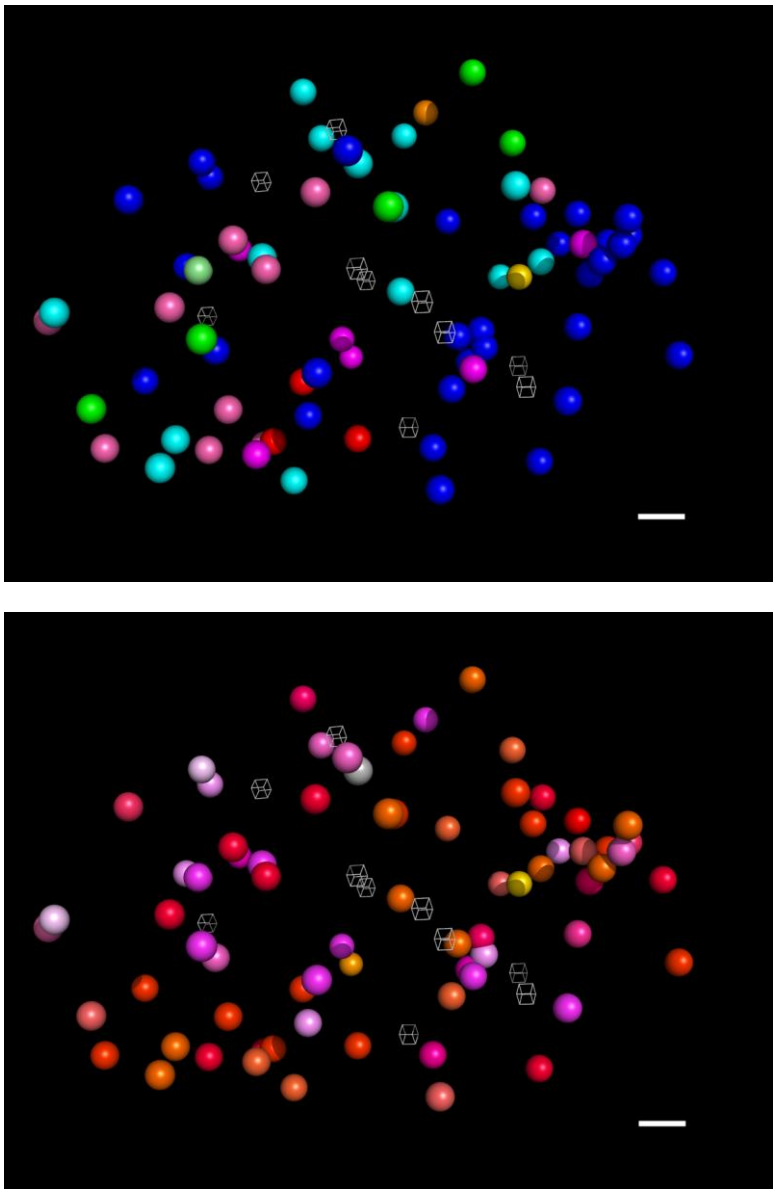


***Figure 2*: 3D Antigenic map of swine influenza A (H1) viruses.** *The relative positions of strains (spheres) and swine hyper-immune anti-sera (cubes) were computed such that the distances between strains and anti-sera in the map with the least error represent the corresponding HI measurements (Smith et al., 2004). Cyan spheres indicate strains from North America and Canada, and purple spheres indicate strains from Europe. Large spheres are HI data generated by ESNIP 3 partners. The black scale bar represents one unit of antigenic distance, corresponding to a two-fold dilution of anti-serum in the HI assay.*

Based on the preliminary map, a larger number of circulating SIVs were tested and added to the map, creating a final 3D map of European H1 viruses including representative strains from different partner countries (Figure 3a).

There is marked antigenic diversity among the viruses isolated from European pigs. The antigenic evolution of SI (H1) viruses in Europe is clustered with co-circulation of multiple different antigenic variants through the time period. Figure 3b shows the same antigenic map as Figure 3a, but coloured according to year of isolation, with earlier strains collected as part of ESNIP 1 and 2 shown in pink and viruses contributed to ESNIP 3 shown in dark orange and red. Although there is marked diversity, initial analyses of the genetic basis for antigenic variation indicate that the variation is not wholly predicted by the genetic clade to which the HA belongs.



Figures 3a and 3b*: 3D antigenic map of swine influenza A (H1) viruses isolated from European pigs.** *In* ***Figure 3a *(upper map) the viruses (spheres) are coloured according to EU country of isolation: Denmark (pink), France (cyan), Belgium (dark pink), Germany (red), Italy (green), England, Ireland and Scotland (blue), Czech Republic (light green), Poland (orange) and Spain (gold). In* ***Figure 3b*** *(lower map) the viruses (spheres) are coloured according to relative year of isolation from 1986 in light pink through to dark pink and orange to bright red in 2013.The white scale bar represents a two-fold difference in HI assay titre or one antigenic unit.*

Focussing on the H1N2 viruses currently circulating in the UK, we found that there are two antigenic clusters which have co-circulated over time (Figure 3c), but with more recent strains predominantly but not exclusively in the right antigenic cluster.

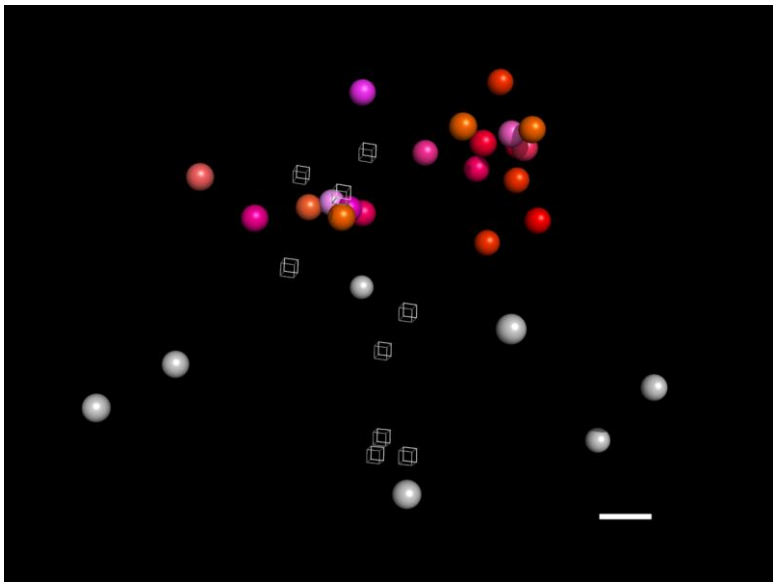


Figure 3c: *Antigenic map of a subset of strains from the EU swine influenza A H1 virus dataset. Strains (spheres) are coloured according to their year of isolation with older strains in light pink and currently circulating strains isolated during ESNIP 3 in orange and red. Reference panel strains are shown as grey spheres and swine sera used in the HI assay are shown as grey cubes.*

Antigenic cartography of swine influenza A (H3) viruses

A reference panel of swine serum raised to SI A (H3) viruses was selected in collaboration with P2-UGent. Selected European influenza A (H3) viruses were characterized in HI assays using this panel. As above, antigenic cartography was used to quantify the evolution of H3 influenza A viruses in European pigs and assessed the antigenic relationships between these viruses and a reference strain from the H3N2v outbreak in North American pigs (Figure 4).

There was no significant difference in the antigenic variants of H3 currently-circulating in pigs in different EU countries. There was some antigenic difference between currently-circulating strains and the vaccine strain, but determining the significance of any potential reduction in vaccine efficacy would require further cross-protection experiments. The antigenic distance between EU H3 strains and H3N2v strains, however, is likely to be large enough to risk incursion into EU pigs were it to be introduced.

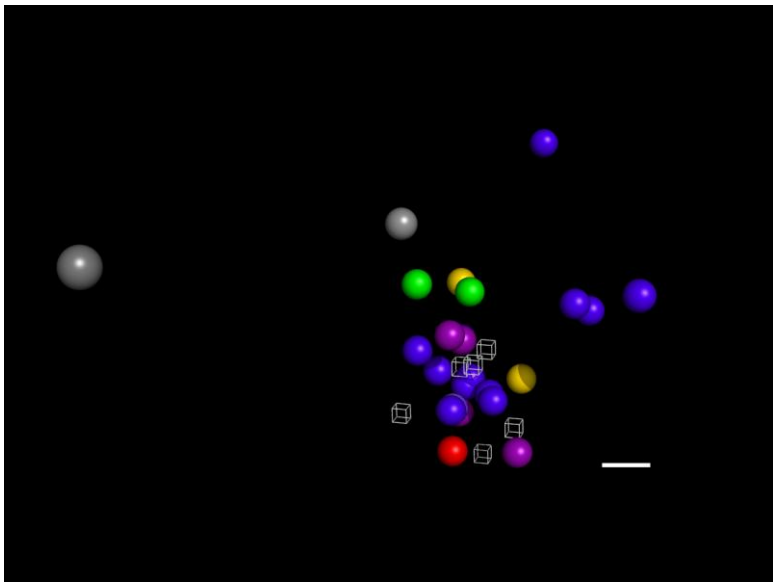


***Figure 4*: 3D antigenic map of swine influenza A (H3) viruses isolated from European pigs.** *The viruses are coloured according to EU country of isolation: France (cyan), Belgium (dark pink), Germany (red), Italy (green), England and Scotland (blue), and Poland (gold). The H3N2v strain A/Indiana/08/2011 is shown as a larger light grey sphere and the vaccine representative A/Port Chalmers/73 as a smaller light grey sphere. The white cubes represent the positions of the swine sera. The bar represents one antigenic unit, or a two-fold difference in HI assay titre.*

Subtasks: 2.1 and 2.5: Database

- **Objective 5 of WP3:** To develop and validate assays for characterisation of intra-subtypic variation of strains
- **Objective 6 of WP3:** To develop harmonised protocols for producing robust data sets for antigenic analysis
- **Objective 7 of WP3:** To investigate the antigenic characteristics of European SIVs using cartography
- **Objective 8 of WP3:** Determine the genetic basis for the antigenic differences seen among European SIVs
- **D3.3:** Complete database with HI titre linked to genetic data on the specific viruses for antigenic cartography (month 36)
- **D3.4:** Compare antigenic and genetic data sets to define any relationships (month 36)

As discussed in WP5, a system was designed for electronic management of the ESNIP 3 virus bank, including all isolates being donated by the consortium members as well as those previously collected from ESNIPs 1 and 2. The system stored information relating to the isolates, including the location of isolation, the animal from which they were obtained and details of the viruses themselves. The system holds antigenic data, which was used by P15-UCAM to generate the antigenic maps. Links to these were made available via the system and were used to handle HI data.

Comment on problems and corrective actions undertaken for WP3

No corrective actions were required.

WORK PACKAGE 4: GENOMIC CHARACTERIZATION OF SWINE INFLUENZA VIRUS

WP leader: Prof Paul Kellam, WTSI, UK (P14)

WP deputy-leader: Dr Ralf Dürrwald, IDT, Germany (P8)

Duration: 36 months

Participants in WP4 are listed in Table 7.

Table 7: Participants in WP4

Partner no.	Organization Short name	Country	Contact names
1	AHVLA	United Kingdom	Ian Brown Scott Reid
3	Anses	France	Gaëlle Simon
4	IZSLER	Italy	Emanuela Foni
5	DTU	Denmark	Lars Erik Larsen
6	NVRI	Poland	Iwona Markowska-Daniel
13	UTH	Greece	Charalambos Billinis
14	WTSI	United Kingdom	Paul Kellam Simon Watson
16	UOXF.AT	United Kingdom	Oliver Pybus

Address of work package leader:

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Telephone number:

Tel: +44 1223 494940

Work package objectives:

1) Sequence full-length influenza genomes from swine using capillary and/or 'next generation sequencing' methods.
2) Produce a data repository for full-length genome sequences and underlying raw sequence data with deposition of finished sequences to GISAID.
3) Determine the population genetics and evolutionary dynamics of swine influenza and investigate function significance of genetic variation.

Work package deliverables:

D4.1 Repository of virus RNA and cDNA samples for whole genome sequence analysis and associated data tracking software (month 30).

D4.2 Whole genome consensus sequence of anticipated 200 swine influenza viruses (month 24).

D4.3 Sequence data for defined segments (HA, NA) using next generation sequencing methods (month 24).

D4.4 Report and protocols for indexed next generation sequencing methods for SIV at the WTS (month 24).

D4.5 Data repository for the distribution of whole genome consensus and segment-specific sequencing (month 24).

D4.6 Alignment, editing and analysis of sequence data (month 33).

D4.7 Deposition of data in GISAID and FLU-LAB-NET (month 36).

D4.8 Provide phylogenetic data concerning the evolutionary dynamics and epidemic history of swine influenza (month 36).

Work package 4 – Description of work

With the emergence of H5N1 and H7N9 avian influenza and A (H1N1)pdm09 virus as important human pathogens, the detailed understanding of influenza viruses in swine has become essential. Importantly, knowledge of influenza virus variation in swine and the outcome of reintroduction of A (H1N1)pdm09 virus into pigs requires investigation into the dynamics of SI evolution. This work package addresses influenza virus variation in swine from the extensive sampling of the ESNIP 3 consortium and makes the genetic data available to ESNIP 3 partners for genetic, antigenic and surveillance purposes.

Specifically this work package was tasked with characterising, by whole genome sequencing, up to 200 virus isolates from ESNIP 3 partners. A total of 466 samples were actually processed and delivered 256 SIV genomes have been sequenced to yield 179 virus genomes, 210 have recently been processed and assembled to yield a further 164 genomes.

The significant results and major achievements of WP4 were:

1) Selection of platform technology underpinning efficient and high quality delivery of the project sequencing requirements.

2) The 'sample to sequence to database' population cycle was established with 12 older ESNIPs 1 and 2 full genomes submitted with automated programmes.

3) Delivery of 229 full genomes into the ESNIP 3 bank database.

4) Deposited and shared with other work packages 237 HA and 118 NA segments.

5) Observation of 17 unique SIV genotypes.

6) Re-sequencing the samples on Illumina allows genome assembly without the need of a reference (*de novo* assembly), which is now possible due to technology improvements, and to confirm the other gaps/polymorphisms.

There were no major deviations from the planned WP4 schedule.

WP4 objectives were addressed through **Task 3** (**Subtasks: 3.1, 3.2 and 3.3)**. Detailed descriptions relating to **Task 3** and the **3 subtasks**, linked with the corresponding objectives and deliverables (D) are given below.

Subtask 3.1: **Sequence full length influenza genomes from swine**

- **Objective 1 of WP4:** Sequence full-length influenza genomes from swine using capillary and/or 'next generation sequencing' methods
- **D4.1:** Repository of virus RNA and cDNA samples for whole genome sequence analysis and associated data tracking software (month 30)

This deliverable was achieved in partnership with P1-AHVLA where primary samples from ESNIP 3 partners were received into the virus bank, processed as virus isolates by culture in eggs and processed into virus RNA from cell-free virus supernatants. This RNA was transported to P14-WTSI where cDNA synthesis and eight segment virus genome amplification was performed. P14-WTSI compiled a resource of 466 isolates, processed to yield virus RNA.

- **D4.2:** Whole genome consensus sequence of anticipated 200 swine influenza viruses (month 24)

- **M4.1:** Collection and pre-processing of 100 influenza nucleic acid genomes (month 22)

P14-WTSI have delivered >100 genomes into the ESNIP 3 virus database.

- **M4.2:** Sequencing of 200 influenza virus genomes (month 34)

- **M4.3:** Compile sequence data from first 50 viruses and distribute to partners (month 12)

P14-WTSI has delivered 229 full genomes to the ESNIP 3 virus database. A further 113 genomes remain to be finalized and deposited.

- **D4.3:** Sequence data for defined segments (HA, NA) using next generation sequencing methods (month 24)

P14-WTSI has deposited and shared with other work packages 237 HA and 118 NA segments.

- **D4.4:** Report and protocols for indexed next generation sequencing methods for SIV at the WTSI (month 24)

P14-WTSI has published a method for NGS assembly of influenza data (Watson et al., 2013). The methods have been further refined to produce a robust assembly pipeline. Essentially, data were first quality-controlled using QUASR version 7.01 by removing primer sequences and applying a read quality cut-off of 30.0 for 454, and 35.0 for MiSeq data. Reads shorter than 200 nucleotides for 454, and 145 for MiSeq, were discarded. Sample-specific references were generated for each readset using an in-house script. Briefly, an influenza-specific BLAST

database was generated from all the unique influenza virus segments in the Influenza Virus Resource containing a complete coding sequence. In total, the database contained 74,340 sequences. A Python script utilizing BioPython's BLAST functionality was used to BLAST a subset of the reads against this database to determine the closest reference for each segment. The quality-controlled reads were mapped against this sample-specific reference using SMALT version 0.5.3 (www.sanger.ac.uk/resources/software/smalt/) and converted to a pileup format using SAMtools version 0.1.8. QUASR was then used to create the consensus sequence from this pile-up file (Figure 5).

In parallel, each sample was assembled *de novo* using SMALT version 0.7.4. Contiguous stretches of assembled reads (contigs) generated from this assembly were BLASTed against the influenza-specific BLAST database mentioned previously to remove those contigs that were not of influenza-origin. For the remaining contigs, the segment to which each contig belongs was determined, and the sequence compared against the relevant reference-based approach by using a Python script. If the sequences did not match, the ambiguities were resolved by looking at the underlying reads in the reference-based pileup file.

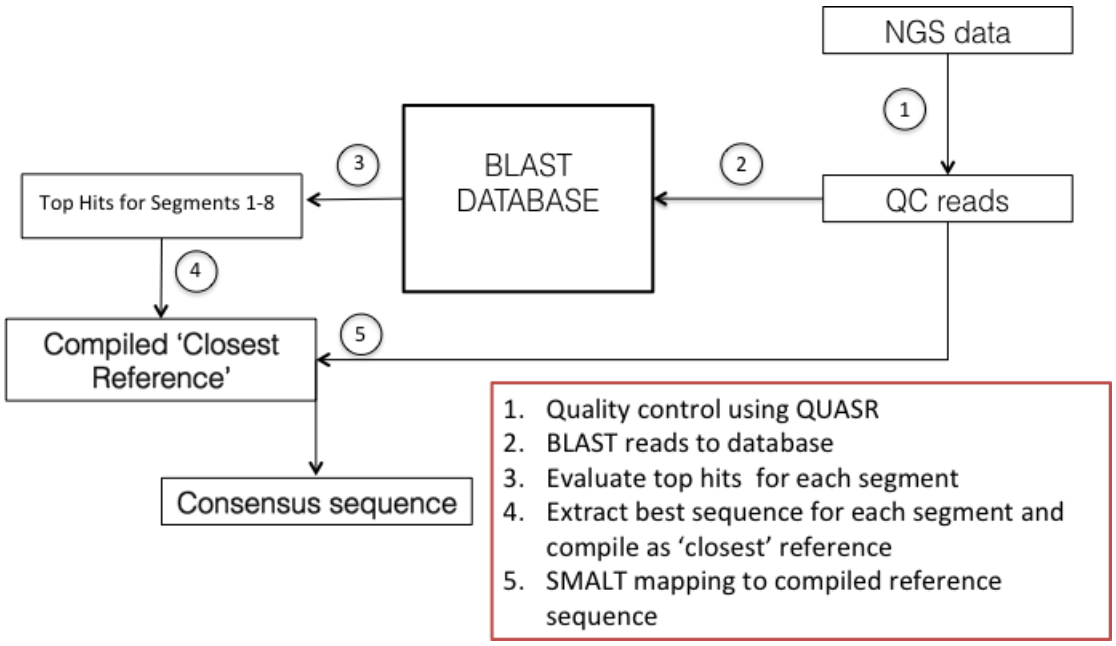


***Figure 5*: NGS assembly of influenza data.**

Subtask 3.2: Produce a data repository for full length genome sequences and underlying raw sequence data, with timely deposition of finished sequences to GISAID

- **Objective 2 of WP4:** Produce a data repository for full-length genome sequences and underlying raw sequence data with deposition of finished sequences to GISAID

- **D4.5:** Data repository for the distribution of whole genome consensus and segment specific deep sequencing (month 24)

Sequence data was uploaded by P14-WTSI into the virus database for access by consortium partners.

Subtask 3.3: Determine the population genetics and evolutionary dynamics of swine influenza and investigate the functional significance of genetic variation

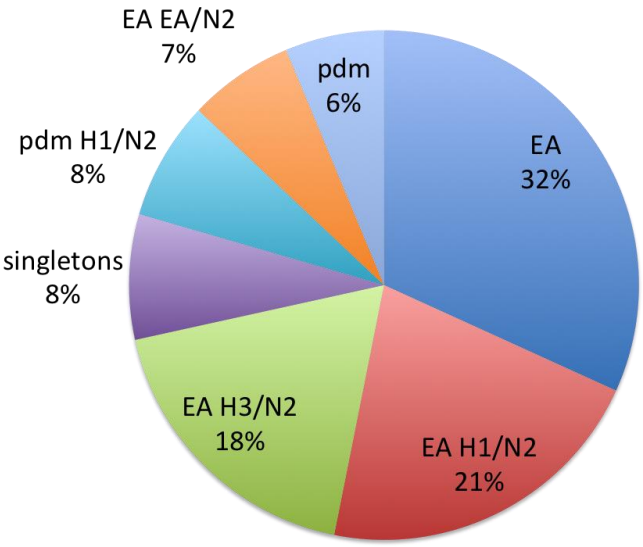
- **Objective 3 of WP4:** Determine the population genetics and evolutionary dynamics of swine influenza and investigate function significance of genetic variation
- **D4.6:** Alignment, editing and analysis of sequence data (month 33)

P14-WTSI has analyzed the reassortment structure of the full virus genomes. The genotypes of an initial 180 European SIVs were determined by characterizing each segment's lineage of origin from their phylogenetic trees (Figure 6). The most frequent genotype was the complete EA swine genome, which was observed in 32% of the samples across 10 countries (Germany, France, Italy, The Netherlands, Belgium, Denmark, Spain, Czech Republic, Poland, and the UK [England]). The next-most commonly observed genotype was a reassortant with an EA internal cassette and human-origin H1N2 external segments, observed in 21% of samples across eight countries (Germany, France, Italy, The Netherlands, Belgium, Spain, the UK [England, and Scotland]). The EA internal cassette with human H3N2 external segments was observed in 18% of samples across seven countries (Germany, Italy, The Netherlands, Belgium, Spain, France, and Hungary). A pandemic genome which had acquired human-origin H1 and N2 segments was observed in 8% of samples, but was geographically-restricted to England, while a reassortant of an EA genotype with an acquired human N2 segment was present in 7% of the samples across three countries (France, Denmark, and Finland). Lastly, the complete pandemic H1N1 genotype was observed in 6% of samples across six countries (Germany, England, Denmark, France, Hungary, and Finland). These six genotypes comprised the majority of those observed in swine, with the remaining 8% of swine containing singleton genotypes that were seen only in a single country, and not shown to persist across time.

Multiple circulating reassortants: Of the 17 unique genotypes that were observed in these data (Figure 6), only 14 were seen contemporaneously. Thirty-one of the samples were collected before 2010, and were included as a control for the whole-genome sequencing. Of these, three samples contained viral genotypes that were not subsequently observed: A/swine/England/117316/1986 contained a complete classical swine (CS) genotype, A/swine/Ireland/Eire89/1996 contained a complete Eurasian/Oceanic avian (EAav) genotype, and A/swine/England/163266/1987 contained a complete human-origin H3N2 virus.

While the internal gene cassette of the SIV genome tends to associate together strongly, the external haemagglutinin and neuraminidase segments are shown to frequently reassort. For samples collected since 2010, a total of 14 distinct genotypes were observed across the different countries, including reassortants between the EA genotype, pandemic genotype, and human-origin external segments (H1, H3, N1, and N2). One particular genotype of note is that of A/swine/Spain/28778/2012, which is a triple-reassortant virus containing an EA internal cassette with human-origin H3 and N2 external segments, but with a pandemic-origin matrix-protein (MP) segment. Acquisition of the pandemic MP by an H3N2 SIV in the United States was implicated in recent zoonoses into humans.

Since the emergence of the pandemic H1N1 genotype in 2009, the virus has established in swine across Europe, with complete pandemic genotypes observed across six countries (Denmark, England, Finland, France, Germany, and Hungary), and pandemic reassortants observed in four further countries (Belgium, Italy, The Netherlands, and Spain). The frequency at which the pandemic genotype is observed is not homogeneous across Europe, with mainland Europe showing a lower proportion of infections as pandemic SIV compared to England. Since 2010, all sequenced genomes in England were either a complete pandemic genotype or a pandemic reassortant with human-origin H1 and N2 external segments.



17 unique genotypes observed

Green = Eurasian avian-like
Red = pandemic
Purple = Human H1/H3 N1/N2
Blue = Classical swine
Grey = Eurasian/Oceanic avian

PB2	PB1	PA	HA	NP	NA	MP	NS
pdm	pdm	pdm	pdm	pdm	pdm	pdm	pdm
pdm	pdm	pdm	H1	pdm	N2	pdm	pdm
pdm	pdm	pdm	H3	pdm	N2	pdm	pdm
pdm	pdm	pdm	pdm	pdm	N2	pdm	pdm
pdm	pdm	pdm	EA	pdm	N2	pdm	pdm
pdm	pdm	pdm	EA	pdm	EA	pdm	pdm
EA	EA	EA	H3	EA	pdm	EA	EA
EA	EA	EA	H3	EA	N2	pdm	EA
EA	EA	EA	H3	EA	N2	EA	EA
EA	EA	EA	H1	EA	N2	EA	EA
EA	EA	EA	H1	EA	EA	EA	EA
EA	EA	EA	EA	EA	N2	EA	EA
EA	EA	EA	EA av	EA	N2	EA	EA
EA	EA	EA	EA	EA	EA	EA	EA
hu	hu	hu	H3	hu	N2	hu	hu
CS	CS	CS	CS	CS	CS	CS	CS
EA av	EA av	EA av	EA av	EA av	EA av	EA av	EA av

***Figure 6*: Distribution of the observed SIV genotypes.**

- **D4.7:** Deposition of data in GISAID and FLU-LAB-NET (month 36)
- **M4.4:** Deposition of sequence data in GISAID and FLU-LAB-NET quarterly (quarterly from month 12)

The data has been deposited in relevant data resources for the project and partners.

- **D4.8:** Provide phylogenetic data concerning the evolutionary dynamics and epidemic history of swine influenza (month 36)

P14-WTSI has undertaken an initial phylogenetic analysis (Bhatt et al., 2013). More detailed analysis is now ongoing.

Comment on problems and corrective actions undertaken for WP4

No problems or corrective actions were required.

WORK PACKAGE 5: INTERACTION WITH OTHER INFLUENZA NETWORKS AND THE INTERNATIONAL COMMUNITY

WP leader: Prof Ian Brown, AHVLA, UK (P1)

WP deputy-leader: Dr Scott Reid, AHVLA, UK (P1)

Duration: 36 months

The participants in WP5 are listed in Table 8.

Table 8: Participants in WP5

Partner no.	Organisation short name	Country	Contact names
1	AHVLA	United Kingdom	Ian Brown Scott Reid
2	UGent	Belgium	Kristien Van Reeth
3	Anses	France	Gaëlle Simon
5	DTU	Denmark	Lars Erik Larsen
14	WTSI	United Kingdom	Paul Kellam
17	FLI	Germany	Martin Beer Timm Harder
18	IZSV	Italy	Giovanni Cattoli
19	USDA	USA	Amy Vincent
20	HVRI	China	Hualan Chen
21	MSS	France	Michel Bublot
22	HIPRA	Spain	Jaime Maldonado Garcia
23	AHT	United Kingdom	Debra Elton
24	AFBI	United Kingdom	Michael Welsh

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Work package objectives:

1) Ensure regular exchange of information between Europe, America and Asia.
2) Facilitate interaction between networks working with influenza in the avian, equine and human sectors.
3) Promote greater understanding of the epidemiology of SIVs at a global level.
4) Establish, curate and maintain a European SIV bank.

Work package deliverables:

D5.1 Establish fora for dissemination of knowledge both within & out with the consortium (month 3)

D5.2 Formally establish interaction with other networks (month 3)

D5.3 Utilise web-based forum (FLU-LAB-NET) to ensure timely dissemination of laboratory protocols, methods and data (month 6)

D5.4 Establish and maintain a European swine influenza virus bank (month 12)

Work package 5 – Description of work

Within the project consortium and through both the project delivery committee meetings and formal project meetings, an exchange of information was facilitated between colleagues in Europe, America and Asia (China). In addition, extra contacts were made with institutes and laboratories in both avian and human influenza fields including the European Centre to Disease Control (ECDC; who became an associate member of the consortium) and with scientists working in the public health field (mediated through the Health Protection Agency London as part of the network of Community Reference Laboratories for Human Influenza in Europe). This involved an exchange of information on the epidemiology of influenza in respective sectors. Formal interactions at meetings of these networks including presentations at the EU national laboratories for avian influenza annual meeting in April 2011, May 2012 and September 2013 (attended by partners P1-AHVLA, P9-EVIRA, P11-CAO and P17-FLI) plus to the CNRL lead in London, May 2012. This also facilitated an exchange of knowledge into the ESNIP 3 consortium from other respective fields.

Technical support and advice was provided to the CNRL to develop a ring trial to test the capability of human influenza laboratories in Europe in their ability to detect swine strains should they appear in the human population. In addition, two members of the project consortium (P1-AHVLA and P2-UGent) are members of the OFFLU SI group together with P19-USDA and P20-HVRI, representing other global sectors. This forum has provided an opportunity for harmonisation of methodology, exchange of knowledge on virus characteristics, development of laboratory tools, analysis of epidemiological data and the identification of global surveillance gaps. Data from ESNIP 3 has been instrumental in informing this group.

Dissemination of information to stakeholders and policy makers has been primarily facilitated in Europe through advice and expert opinion to the ECDC especially when reviewing the current status of SI in European pigs and providing specific information on risk assessments for infection of humans with these viruses. This has been particularly urgent and high profile task given the detection of numerous zoonotic infections with SIVs in the USA. In addition, advice has been provided directly to DG SANCO Animal Health.

In total, 410 novel isolates were submitted to the virus bank held and curated by P1-AHVLA (including 54 isolates collected as part of ESNIPs 1 and 2) and the first and second phases of the web-based management system for the physical virus bank were implemented. The first phase allowed members to record details of isolates each was donating to the virus bank, and to enable them to see what isolates other partners had donated. The second phase focused on adding capacity to capture genomic sequencing, antigenic cartography and phylogenetic analysis data, produced in tandem with the respective partner organisations (P14-WTSI, P15-UCAM and P16-UOXF.AT).

In order to support the use of data through ESNIP 3 within the larger EU research programme, an interaction took place with ‘RTD2farm’ for collection of results from EU-funded projects for

technology transfer to pig producing farms within the framework of CEPS (Centre of Excellence of Pig Science). Furthermore, epidemiology and surveillance data, especially in relation to systems in place were shared with FLURISK (an EFSA-sponsored project) informing the risk of emergence of pandemic influenza from the animal reservoir.

Fourteen peer-review publications were generated within the lifetime of the project. Two non-peer review articles were also published; the first of these appearing in Pan-European Networks: Science & Technology (June 2012).

Throughout the project, ESNIP 3 information was disseminated at a number of international scientific meetings on influenza or other diseases of swine, or at other relevant international conferences attended by consortium partners.

The significant results and major achievements of WP5 were:

1) Establishment of an interactive electronic database – phases 1 and 2; enabling data upload and linking data from WP2 and WP3.

2) Establishment of a virus bank in the laboratory of P1-AHVLA during ESNIPs 1 and 2. The virus bank has been expanded with new SIVs (n=356) collected during ESNIP 3.

3) Establishment of an ESNIP 3 project website (http://www.esnip3.eu/index.html).

4) Establishment of interaction with other networks.

5) Dissemination of information through a secure web-based forum (hosted by FLU-LAB-NET, SSPE-CT-2007-044453), conference attendance and peer review scientific publication of results.

There were no major deviations from the planned WP5 schedule.

WP5 objectives were addressed through **Task 4 and Task 5** (**Subtasks: 5.1, 5.2, 5.3 and 5.4)** during the first 18 month period. Detailed descriptions relating to these tasks/subtasks, linked with the corresponding objectives and deliverables (D) are given below.

Task 4: Establish and maintain a European swine influenza virus bank

- **Objective 4 of WP5:** Establish, curate and maintain a European SIV bank
- **D5.4:** Establish and maintain a European swine influenza virus bank (month 12)

The ESNIP 3 virus bank was developed by the internal IT Unit of P1-AHVLA to electronically manage and record information on all isolates submitted to the bank. The first phase of the work was made live on 1st November 2011; allowing members of the consortium to record details of isolates being donated and to view the details of isolates donated by fellow consortium partners. A presentation showing the functionality of the first phase was given by P1-AHVLA at the first annual meeting hosted by P3-Anses. The second phase was delivered on 1st May 2012. This allowed consortium members to record genomic sequencing data for isolates donated by them to the bank. It also facilitated management of the dispatch of: (i) RNA to P14-WTSI for full genomic sequencing; (ii) isolates to P5-DTU for HI assay results to be recorded and (iii) the resulting information to be forwarded to P15-UCAM for antigenic cartography. Importantly, the ability to forward genomic sequence data to P16-UOXF.AT was also added to enable downstream phylogenetic analysis to take place.

Submission of clinical material (virus isolates) to the bank

The small virus bank of isolates (n = 54) held by P2-UGent (produced in ESNIP coordination actions 1 and 2) was relocated to P1-AHVLA. During the project, 356 novel isolates were

submitted by partners to the bank for further antigenic (WP3) and genetic (WP4) characterizations (Table 9). Prior to further characterization and donation to interested parties, the viability of each submitted virus was checked by P1-AHVLA through detection of a haemagglutinating agent in embryonated SPF fowls' eggs (OIE, 2008).

Subtask 5.1: Establish foray for dissemination of knowledge both within and out with the consortium and Subtask 5.3: Utilise a web-based forum (FLU-LAB-NET) to ensure timely dissemination of laboratory protocols, methods and data

- **Objective 1 of WP5:** Ensure regular exchange of information between Europe, America and Asia
- **Objective 2 of WP5:** Facilitate interaction between networks working with influenza in the avian, equine and human sectors
- **D5.1:** Establish fora for dissemination of knowledge both within & out with the consortium (month 3)
- **D5.3:** Utilise web-based forum (FLU-LAB-NET) to ensure timely dissemination of laboratory protocols, methods and data (month 6)

The project website (http://www.esnip3.eu/index.html) was established. Hosted through the FLU-LAB-NET portal this contains a) general information on SI; b) general information on the previous ESNIP co-ordination actions (objectives, partners involved); c) standard operating methods for the diagnosis of SI; d) an electronic database with all SIV isolates in the virus bank; e) a complete electronic database with all SIV isolates obtained during ESNIPs 1 and 2 and relevant information; f) ESNIP meeting reports and g) links to relevant websites. While parts a), b), c), d) and g) are public, parts e) and f) are confidential (password-protected) and are accessible to ESNIP 3 consortium members only.

The diagnostic tests and techniques developed by this co-ordination action in WP2 and WP3 are readily made available between participants through the downloading of documents on the project website. Training of personnel in harmonized diagnostic methods through a specific workshop was held immediately prior to the ESNIP 3 kick-off meeting. A two-day workshop was held at P1-AHVLA to introduce the diagnostic tools for virological surveillance for SI as a prelude to the kick-off meeting. The two-day workshop, from 16-17 November 2010, focussed on detection of SIV by real-time RT-PCR (RRT-PCR), virus isolation in embryonated fowls' eggs and preliminary subtyping (HI tests and RRT-PCR) through lectures and practical classes. Nine participants from national laboratories concerned with surveillance and diagnosis of SI in Belgium (P2), Denmark (P5), Spain (P7), Greece (P13), Poland (P6) and a colleague from the Harbin Veterinary Institute in China (P20) attended.

Reagents, including selected SIV strains and hyper-immune sera for SIV subtyping, are available to veterinary institutions and laboratories beyond those in this co-ordination action upon request to P1-AHVLA who will obtain permissions from all participants (consortium agreement).

Subtask 5.2: Formally establish interaction with other networks

- **Objective 2 of WP5:** Facilitate interaction between networks working with influenza in the avian, equine and human sectors
- **D5.2:** Formally establish interaction with other networks (month 3)

ECDC could not directly become a member of the consortium but became an associate member. A representative of ECDC attended both the ESNIP 3 kick-off and first annual meetings.

With the public health and medical fields, a formal agreement was made for the exchange of data and viruses which resulted in the donation of three SIV isolates to the Health Protection Agency, Colindale, London, on 20 February 2012 for use in a proficiency panel. One SIV was donated from each of P1-AHVLA, P4-ISZLER and P8-IDT after the necessary permissions were

granted in support of CNRL.

Interaction with 'RTD2farm' took place during March-April 2012. RTD2farm were collecting results from EU-funded projects for technology transfer to pig producing farms within the framework of CEPS (Centre of Excellence of Pig Science). The aim of CEPS is the acquisition, compilation and exchange of knowledge and research results. Through CEPS these research results can be made easily accessible to farmers, multipliers and other interested people. The ESNIP 3 project was identified to be of great interest for CEPS who wanted to know more. An information template was filled in and returned. This information could then be made publicly-available through the RTD2farm website from August 2012, thereby providing an additional opportunity for dissemination of ESNIP 3 results.

Subtask 5.4: Develop a dissemination strategy for the project to include conference attendance and peer-review scientific publication of results

- **Objective 3 of WP5:** Promote greater understanding of the epidemiology of SIVs at a global level
- **D5.1:** Establish fora for dissemination of knowledge both within & out with the consortium (month 3)

Template A1 lists the 14 scientific peer review articles relating to the foreground of the project, published during the lifetime of the project. Additionally, four articles are, at the time of writing, in preparation for peer-review publication. An article by P1-AHVLA was published (non-peer review) in Pan-European Networks: Science & Technology in 2012 (Template A2). This was followed by the publication of a report by P5-DTU.

Key scientific contacts from partners P1-AHVLA, P9-EVIRA, P11-CAO and P17-FLI attended the Joint Annual Meetings of the EU National Laboratories for Avian Influenza and Newcastle Disease in Brussels (April 2011), Dublin (May 2012) and Helsinki (September 2013). On each occasion, an update of the project update was given by P1-AHVLA. An introduction to ESNIP 3 was also provided by P1-AHVLA at the joint annual meeting held during May 2010 in Vienna.

Where applicable, scientific information was disseminated at international scientific meetings on influenza or other diseases of swine, or at other relevant international conferences attended by partners. Template A2 lists the meetings and dissemination activities attended by consortium members. Furthermore, P2-UGent presented date at OFFLU Technical Meetings held during November 2010 (Rome) and April 2011 (Paris).

The contribution of WP2 to the FLURISK Project was further highlighted by the update provided by P3-Anses on influenza A viruses identified in European pig herds by ESNIP 3 partners at the FLURISK Workshop, EFSA, from 3rd - 4th April 2013 in Parma, Italy.

Comments on problems and corrective actions undertaken:

The old website was renewed and expanded but the new website was made available for access.

There was a technical delay with the new website and database but priority was given to each to provide external access to the scientific community (website) and internal access to members of the consortium (database), respectively.

Table 9: Numbers and subtypes of isolates submitted by partner countries to the ESNIP 3 virus bank curated by P1-AHVLA from November 2010 to October 2013

Partner No.	Organization short name	Country	TOTAL	Swine influenza virus subtype								
				H1N1	H1avN1	rH1huN1	H1N1pdm	H3N2	H1N2	H1huN2	rH1avN2	UNTYPED
ESNIP 1 & 2	ESNIP 1 & 2		54	22				16	16			0
P1	AHVLA	UK	65	7	3		10	2	43			0
		TOTAL	**52**	**29**				**15**	**8**			**0**
P2	UGENT	BELGIUM	27	16				10	1			0
		NETHERLANDS	24	13				5	6			0
		GERMANY	1	0				0	1			0
P3	ANSES	FRANCE	52	20	5	2	3	1	14	3	4	0
P4	ISZLER	ITALY	37	15			1	7	14			0
P5	DTU	DENMARK	19	7				0	6			6
P6	NVRI	POLAND	38[a]	15			10	3	1			9
P8	IDT	GERMANY	30	11			1	7	11			0
P9	EVIRA	FINLAND	4	4				0	0			0
P10	KVI	ISRAEL	7	0				0	0			7
P11	CAO	HUNGARY	7	4				3	0			0
P12	CVI	NETHERLANDS	13	4				3	6			0
P13	UTH	GREECE	5	0				0	0			5
P19	USDA	USA	7	0				7	0			0
P22	HIPRA	SPAIN	20	6				10	4			0
	TOTALS		**410**	**144**	**8**	**2**	**25**	**74**	**123**	**3**	**4**	**27**

[a] Including two isolates submitted from Belarus and three isolates from Russia

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4.2 Use and dissemination of foreground

Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[2] (if available)	Is/Will open access[3] provided to this publication?
1	Phylogenetic analysis of the first Polish H1N2 swine influenza virus isolate.	Kowalczyk A. et al. (P6-NVRI)	Bull. Vet. Inst. Pulawy	Volume 56	Vet. Inst. Pulawy	Pulawy, Poland	2012	419-424		
2	Serological evidence of co-circulation of different subtypes of swine influenza virus in Polish pig herds.	Markowska-Daniel I. et al. (P6-NVRI)	Bull. Vet. Inst. Pulawy	Volume 56	Vet. Inst. Pulawy	Pulawy, Poland	2012	425-429		

[2] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[3] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

3	The genome sequence of monoreassortant H1N1 swine influenza virus isolated from a pig in Hungary.	Bányai K. et al. (P11-CAO)	Journal of Virology	Volume 86 (23)	American Society for Microbiology	Washington, USA	2012	13,133		
4	Emergence and characterisation of pandemic H1N1 influenza viruses in Hungarian swine herds	Bálint A. et al. (P11-CAO)	Acta Veterinaria Hungarica	Volume 61 (1)	Akadémiai Kiadó	Budapest, Hungary	2013	125-134		
5	Epidemiosurveillance of swine influenza in France from 2005 to 2012: programs, viruses and associated epidemiological data.	Simon G. (P3-ANSES)	Bulletin Epidémiologique Santé Animale et Alimentation Anses-DGAl	Volume 56	Anses & DGAL	France	2013	17-22	http://www.anses.fr/bulletin-epidemiologique/Documents/BEP-mg-BE56.pdf	
6	Expanded cocirculation of stable subtypes, emerging lineages, and new sporadic reassortants of porcine influenza viruses in swine populations in northwest Germany.	Harder T.C. et al. (P17-FLI)	Journal of Virology	Volume 87	American Society for Microbiology	Washington, USA	2013	10, 460-10,476		

7	Distinction of subtype-specific antibodies against European porcine influenza viruses by indirect ELISA based on recombinant hemagglutinin protein fragment-1.	Zhao N. et al. (P17-FLI)	Virology Journal	Volume 10	BioMed Central	London	2013	246		
8	Influenza pandemics: does the greater threat come from pigs, not birds?	Van Reeth K. (P2-UGent)	Pig Journal	Volume 69	Pig Veterinary Society	England	2013	17-24		
9	The first detection of influenza in the Finnish pig population: a retrospective study.	Nokireki T. et al. (P9-EVIRA)	Acta Vet. Scand.	Volume 55	Veterinary Associations of the Nordic Countries	Finland	2013	69		
10	Full-Genome Sequence of a Reassortant H1N1 Swine Influenza Virus Isolated from Pigs in Italy.	Chiapponi C. et al. (P4-ISZLER)	Genome Announcements	Volume 1	American Society for Microbiology	Washington, USA	2013	e00778-13		
11	First case of the isolation of the H1N2 swine influenza virus in Polish pig farm.	Markowska-Daniel I. et al. (P6-NVRI)	Bull. Vet. Inst. Pulawy	Volume 57	Vet. Inst. Pulawy	Pulawy, Poland	2013	9-14		
12	Emergence of the pandemic H1N1 2009 influenza A virus in swine herds in Poland.	Markowska-Daniel I. et al. (P6-NVRI)	Bull. Vet. Inst. Pulawy	Volume 57	Vet. Inst. Pulawy	Pulawy, Poland	2013	293-300		
13	Influenza A Virus with a Human-Like N2 Gene Is Circulating in Pigs.	Breum S.Ø. et al. (P5-DTU)	Genome Announcements	Volume 1(5)	American Society for Microbiology	Washington, USA	2013	e00712-13		

14	Review of Influenza A Virus in Swine Worldwide: A Call for Increased Surveillance and Research.	Vincent A. et al. (P19-USDA)	Zoonoses Public Health		Wiley-Blackwell	Oxford, UK	2013	doi:10.1111/zph.12049. [Epub ahead of print]		
15	Seroepidemiological survey of swine influenza viruses from sub-clinical infections in pigs.	Davidson I. et al. (P10-KVI)	Epidemiology and Infection	Manuscript submitted	To be decided	To be decided	To be decided			
16	European surveillance network for influenza in pigs: Surveillance programs, diagnostic tools and swine influenza viruses identified in 13 European countries from 2010 to 2013.	Simon G. et al. (P3-Anses)	To be decided	Manuscript in preparation	To be decided	To be decided	To be decided			
17	Interlaboratory comparison of RT-PCR protocols for the detection of Influenza A viruses in swine samples: a ring trial from the European surveillance network for influenza in pigs.	Simon G. et al. (P3-Anses)	To be decided	Manuscript in preparation	To be decided	To be decided	To be decided			
18	Genotypic variation of contemporaneous influenza viruses circulating within European swine	Watson S.J. et al. (P14-WTSI)	Eurosurveillance	Manuscript in preparation	To be decided	To be decided	To be decided			

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

NO.	Type of activities[4]	Main leader	Title	Date/Period	Place	Type of audience[5]	Size of audience	Countries addressed
1	Oral presentation at Joint 16th Annual Meetings of the National Laboratories for Avian Influenza and Newcastle Disease of European Union Member States 2010	Reid S.M. (P1-AHVLA)	European Surveillance Network for Swine Influenza in Pigs 3 (ESNIP 3).	26 May 2010	Vienna, Austria	Scientific Community (higher education, Research)		All 27 EU NRLs for avian influenza and Newcastle disease.
2	Oral presentation at OFFLU Technical Meeting	Van Reeth K. (P2-UGent)	European Surveillance Network for Influenza in Pigs: ESNIP – Experiences, concerns and considerations.	16 – 17 November 2010	Rome, Italy	Scientific Community (higher education, Research)		International audience.
3	Oral presentation at information meeting for pig farmers and veterinarians	Simon G. (P3-Anses)	Update on swine flu: results of surveillance for influenza A/H1N1 pandemic virus in pigs in France.	10 December 2010	Ploufragan, France	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias)		National external dissemination (France).

[4] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[5] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

4	Oral presentation at "One Flu" Strategic Retreat (organized by the Centers for Disease Control and Prevention and the Istituto Zooprofilattico Sperimentale delle Venezie)	Van Reeth K. (P2-UGent)	The public health risk of influenza in pigs – recent insights, key knowledge gaps.	1 – 3 February 2011	Castelbrando, Treviso, Italy	Scientific Community (higher education, Research)		International audience.
5	Oral presentation and Proceedings of the International Meeting on Emerging Diseases and Surveillance, pp.61.	Van Reeth K. (P2-UGent)	Influenza transmission: pigs to people and back.	4 – 7 February 2011	Vienna, Austria	Scientific Community (higher education, Research)		International audience.
6	Proceedings of Journées de la Recherche Porcine, Vol. 43, pp. 273-280.	Simon G. et al. (P3-Anses)	Pandemic influenza virus H1N1 2009 in pigs: main issues, development of novel diagnostic tools and results of the monitoring conducted in France in 2009-2010.	15-16 February 2011	Paris, France			National external dissemination (France).
7	Oral presentation at Joint 17th Annual Meetings of the National Laboratories for Avian Influenza and Newcastle Disease of European Union Member States 2011	Reid S.M. (P1-AHVLA)	ESNIP3: Update.	5 April 2011	Brussels, Belgium	Scientific Community (higher education, Research)		All 27 EU NRLs for avian influenza and Newcastle disease.
8	Oral presentation at OFFLU Technical Meeting	Van Reeth K. (P2-UGent)	Benefits and drivers of swine influenza virus (SIV) surveillance.	6 – 7 April 2011	Paris, France	Scientific Community (higher education, Research)		International audience.
9	Oral presentation at OFFLU Technical Meeting	Van Reeth K. (P2-UGent)	The European Surveillance Network for Influenza in Pigs: ESNIP.	6 – 7 April 2011	Paris, France	Scientific Community (higher		International audience.

						education, Research)		
10	Oral presentation at 6th International Symposium on Emerging and Re-emerging Pig Diseases	Larsen L.E. et al. (P5-DTU)	Pandemic Influenza A H1N1v circulates in Danish pigs.	12-15 June 2011	Barcelona, Spain	Scientific Community (higher education, Research)		International audience.
11	Oral presentation and Proceedings of ESWI conference	Van Reeth K. (P2-UGent)	Why is swine flu so dangerous for humans? What is the importance of surveillance for swine flu?	11 – 14 September 2011	St. Julian's, Malta	Scientific Community (higher education, Research)		International audience.
12	Oral presentation at Merial Flu Dating	Simon G. (P3-Anses)	Epidemiology of swine influenza viruses (SIV) in France.	20 October 2011	Ploufragan, France	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias		International audience.

13	Poster presentation and Proceedings of 4th European Symposium of Porcine Health Management, P048, pp. 135.	Reid S.M. et al. (P1-AHVLA)	European surveillance network for influenza in pigs (ESNIP 3).	25-27 April 2012	Bruges, Belgium	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias		International audience.
14	Oral presentation at Joint 18th Annual Meetings of the National Laboratories for Avian Influenza and Newcastle Disease of European Union Member States 2012	Reid S.M. (P1-AHVLA)	ESNIP3: Update.	22 May 2012	Dublin, Republic of Ireland	Scientific Community (higher education, Research)		All 27 EU NRLs for avian influenza and Newcastle disease.
15	Oral presentation and Proceedings of 4th Merial Swine Forum, pp. 58-62.	Simon G. et al. (P3-Anses)	Swine Influenza in Europe: Epidemiological data, an update.	30 May - 1 June 2012	Berlin, Germany	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias		International audience.

16	Oral presentation and Proceedings of 22nd International Pig Veterinary Society (IPVS) Congress, pp. 165.	Larsen L.E. et al. (P5-DTU)	European surveillance network for influenza in pigs 3 (ESNIP 3).	10-13 June 2012	Jeju Island, Korea	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias		International audience.
17	Poster presentation and Proceedings of 22nd International Pig Veterinary Society (IPVS) Congress	Kyriakis C. et al. (P13-UTH)	Surveillance for influenza viruses in pig farms in Greece.	10-13 June 2012	Jeju Island, Korea	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias		International audience.
18	Poster presentation and Proceedings of 22nd International Pig Veterinary Society (IPVS) Congress, s. 430.	Kowalczyk A. et al. (P6-NVRI)	Phylogenetic analysis of swine influenza viruses isolated from two acute outbreaks in the same herd.	10-13 June 2012	Jeju Island, Korea	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias		International audience.

19	Oral presentation	Markowska-Daniel I. (P6-NVRI)	Why to vaccinate against SIV?	June 2012	Pulawy, Poland	Scientific Community (practitioners and top pig producers)		Poland, Ukraine, Belarus, Lithuania, Latvia, Russia, Slovakia
20	Publication (non-peer review) in Pan European networks: Science & Technology 03, 1-2. www.paneuropeannetworks.com	Brown I.H. (P1-AHVLA)	Protecting Veterinary Public Health from Swine Influenza.	2012		Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias		
21	Report	Breum SØ, Hjulsager CK., Larsen L.E. (P5-DTU)	Overvågning af influenza i svin 2011/Surveillance of influenza virus in swine in 2011.	2012	Technical University of Denmark			
22	Poster presentation and Proceedings of the IXth International Congress of the European Society for Veterinary Virology (ESVV), pp. 169	Simon G. et al. (P3-Anses)	Swine influenza in Europe: Virological and epidemiological data from the European surveillance network for influenza in pigs 3 (ESNIP3).	4-7 September 2012	Madrid, Spain	Scientific Community (higher education, Research)		International audience.
23	Oral presentation and Proceedings of the XIV Congress of the Polish Society of Veterinary Sciences	Markowska-Daniel I. (P6-NVRI)	Occurrence of infections with swine influenza virus in Poland.	14 September 2012	Wroclaw, Poland	Scientific Community (higher education, Research)		National external dissemination (Poland).
24	Poster presentation and	Markowska-	Occurrence of infections with	13 – 15	Wrocław,	Scientific		National external

	Proceedings of XIV Congress of Polish Society of Veterinary Sciences, s. 441.	Daniel I. (P6-NVRI)	swine influenza virus in Poland.	September 2012	Poland	Community (higher education, Research), Industry, Civil Society, Policy makers, Medias		dissemination (Poland).
25	Oral presentation at Antigone One Health Course	Van Reeth K. (P2-UGent)	Epidemiology of swine influenza and risk of transmission to humans.	28 September 2012	Erasmus University, Rotterdam, The Netherlands	Scientific Community (higher education, Research)		International audience.
26	Oral presentation and Proceedings of In Influenza2012: One Influenza, One World under Podium, pp. 17-18.	Larsen L.E. et al. (P5-DTU)	New influenza A virus reassortments have been found in Danish swine in 2011.	1-4 October 2012	St. Hilda's College, Oxford, UK	Scientific Community (higher education, Research)		International audience.
27	Oral presentation and Proceedings of Influenza2012: One Influenza, One World under Podium, pp. 55-56	Larsen L.E. et al. (P5-DTU)	Genetic drift of HA and NA in Danish swine influenza virus from the period 2003-2012.	1-4 October 2012	St. Hilda's College, Oxford, UK	Scientific Community (higher education, Research)		International audience.
28	Oral presentation at information meeting for pig farmers and veterinarians	Simon G. (P3-Anses)	Surveillance and research work on swine flu in France.	20 November 2012	Ploufragan, France	Scientific Community (higher education, Research), Industry, Civil Society, Policy		National external dissemination (France).

						makers, Medias)		
29	Oral presentation and Proceedings of the Annual Congress of the French Pig Veterinary Society (AFMVP), pp. 1-9.	Simon G. (P3-Anses)	Epidemiosurveillance of swine influenza in France from 2005 to 2012: programs, viruses and associated epidemiological data.	6 – 7 December 2012	Paris, France	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias)		National external dissemination (France).
30	Oral presentation at information meeting for swine practitioners in Israel, organized by Israel Veterinary Services	Davidson I. (P10-KVI)	Swine influenza in Israel 2012.	23 December 2012	Bet Dagan, Israel	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias)		National external dissemination (Israel).
31	Poster presentation and Proceedings of International Meeting on Emerging Diseases and Surveillance (IMED), pp. 135-136	Kaartinen L. et al. (P9-EVIRA)	European surveillance network for influenza in pigs 3 (ESNIP 3) – Expanding our knowledge of the epidemiology and evolution of swine influenza viruses.	15-18 February 2013	Vienna, Austria	Scientific Community (higher education, Research)		International audience.
32	Oral presentation and Proceedings of the 2nd	Brown I.H. et al.	Coordinated Surveillance of Influenza Viruses in European	7-8 March 2013	Dublin, Republic of	Scientific Community		International audience.

	International Symposium on Neglected Influenza Viruses, pp.19	(P1-AHVLA)	Pigs: Enhanced Virological and Epidemiological Analysis From The European Surveillance Network For Influenza In Pigs (ESNIP 3)		Ireland	(higher education, Research)		
33	Oral presentation and Proceedings of the 2nd International Symposium on Neglected Influenza Viruses.	Van Reeth K. (P2-UGent)	Pigs and influenza pandemics: what did we really learn since the 2009 pandemic?	7-8 March 2013	Dublin, Republic of Ireland	Scientific Community (higher education, Research)		International audience.
34	Poster presentation and Proceedings of the 2nd International Symposium on Neglected Influenza Viruses, pp. 43	Simon G. et al. (P3-Anses)	Organization of the French national surveillance network for influenza in pigs.	7-8 March 2013	Dublin, Republic of Ireland	Scientific Community (higher education, Research)		International audience.
35	Oral presentation at BIT World Congress of Vaccines	Harder T.C. et al. (P17-FLI)	Dynamics of influenza virus infections in pig populations – a challenge for vaccines and vaccination strategies.	18-20 March 2013	Hangzhou, China	Scientific Community (higher education, Research)		International audience.
36	Oral presentation at information meeting on swine flu and its surveillance, organized by the association of pig farmers from Aquitaine region (INPAQ-AREPSA)	Simon G. (P3-Anses)	Epidemiosurveillance of swine flu in France; Virological and serological diagnostic tools: advantages and limitations.	26 March 2013	Arzacq, France	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias)		National external dissemination (France).

37	Oral presentation at FLURISK Workshop	Simon G. (P3-Anses)	European Surveillance Network for Influenza in Pigs (ESNIP): Update on influenza viruses identified in European pig herds by ESNIP3 partners	3-4 April 2013	EFSA, Parma, Italy	Scientific Community (higher education, Research)		International audience.
38	Oral presentation and abstract at XV^{èmes} Journées Francophones de Virologie. Abstract in *Virologie*, 17-S1, p. S64.	Hervé S. et al. (P3-Anses)	Epidémiosurveillance de la grippe chez le porc en France : dispositifs, virus détectés et données épidémiologiques associées.	18 -19 April 2013	Paris, France	Scientific Community (higher education, Research)		National external dissemination (France).
39	Oral presentation and Proceedings of 5th European Symposium of Porcine Health Management. Paper in the *Pig Journal* 2013, 69, pp. 17-24.	Van Reeth K. (P2-UGent)	Influenza pandemics: does the greater threat come from pigs, not birds?	22 – 24 May 2013	Edinburgh, UK	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias)		International audience.
40	Oral presentation and Proceedings of Med-Vet-Net Association International Scientific Conference, p. 26	Larsen L.E. et al. (P5-DTU)	One health – One flu: Surveillance in pigs and mink has revealed extensive exchange of influenza A virus genes and viruses among animals and humans.	24 – 25 June 2013	Lyngby, Denmark	Scientific Community (higher education, Research)		International audience.
41	Oral presentation at Virology and Human Pathology Laboratory, National Reference Center for Flu	Simon G. (P3-Anses)	Influenza viruses and flu in pigs: impacts in animal health and veterinary public health.	9 July 2013	Lyon University, Lyon, France	Scientific Community (higher education, Research)		National external dissemination (France).

42	Poster presentation and Proceedings of Options for the Control of Influenza VIII, pp. 191	Watson S.J. et al. (P14-WTSI)	Genotypic variation of contemporary swine influenza viruses circulating in Europe.	5-10 September 2013	Cape Town, South Africa	Scientific Community (higher education, Research)		International audience.
43	Oral presentation and Proceedings of Options for the Control of Influenza VIII	Lewis N.S. et al. (P15-UCAM)	Quantifying the global antigenic and genetic evolution in swine influenza A viruses and evaluating the relative zoonotic potential to humans	5-10 September 2013	Cape Town, South Africa	Scientific Community (higher education, Research)		International audience.
44	Oral presentation and Proceedings of Options for the Control of Influenza VIII, pp. 486	Brookes S.M. et al. (P1-AHVLA)	Coordinated surveillance of influenza viruses in European pigs: Enhanced virological and epidemiological analysis from the European Surveillance Network for Influenza in Pigs (ESNIP3)	5-10 September 2013	Cape Town, South Africa	Scientific Community (higher education, Research)		International audience.
45	Poster presentation and Proceedings of the 5th European Congress of Virology, ® *Virologie*, 17-S2, S207	Bonin E. et al. (P3-Anses)	Characterization of pandemic influenza A (H1N1) 2009 viruses isolated from pig and cat in France since 2009.	11 – 14 September 2013	Lyon, France	Scientific Community (higher education, Research)		International audience.
46	Poster presentation and Proceedings of Influenza2013: One Influenza, One World, One Health, pp. 32	Brown I.H. et al. (P1-AHVLA)	Coordinated surveillance of influenza viruses in European pigs: Enhanced virological and epidemiological analysis from the European Surveillance Network for Influenza in Pigs (ESNIP3).	17-19 September 2013	St. Hilda's College, Oxford, UK	Scientific Community (higher education, Research)		International audience.
47	Oral presentation and	Bruem et al.	Novel reassortant swine	17-19	St. Hilda's	Scientific		International

	Proceedings of Influenza2013: One Influenza, One World, One Health, pp. 23	(P5-DTU)	influenza viruses are circulating in Danish pigs.	September 2013	College, Oxford, UK	Community (higher education, Research)		audience.
48	Oral presentation at Joint 19th Annual Meetings of the National Laboratories for Avian Influenza and Newcastle Disease of European Union Member States 2013	Reid S.M. (P1-AHVLA)	ESNIP3: Update.	24 September 2013	Helsinki, Finland	Scientific Community (higher education, Research)		All 28 EU NRLs for avian influenza and Newcastle disease.
49	Oral presentation at the Institute of Biomedical Science conference	Lewis N.S. (P15-UCAM)	Emerging/reemerging infectious disease modelling.	24 September 2013	Birmingham, UK	Scientific Community (higher education, Research)		International audience.
50	Oral presentation at Annual meeting of the Association of Veterinarians working in Organized Productions (AVPO)	Simon G. (P3-Anses)	Influenza A viruses identified in pigs in France.	7 November 2013	Rennes, France	Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias)		National external dissemination (France).

Section B (Confidential[6] or public: confidential information to be marked clearly)

Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[7]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)

[6] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[7] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Please complete the table hereafter:

Type of Exploitable Foreground[8]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[9]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
	Ex: New supercond uctive Nb-Ti alloy			*MRI equipment*	*1. Medical 2. Industrial inspection*	*2008 2010*	*A materials patent is planned for 2006*	*Beneficiary X (owner) Beneficiary Y, Beneficiary Z, Poss. licensing to equipment manuf. ABC*

In addition to the table, please provide a text to explain the exploitable foreground, in particular:

- Its purpose
- How the foreground might be exploited, when and by whom
- IPR exploitable measures taken or intended
- Further research necessary, if any
- Potential/expected impact (quantify where possible)

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[9] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

4.1 Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	**259949**
Title of Project:	**European Surveillance Network for Influenza in Pigs 3**
Name and Title of Coordinator:	**Professor Ian Brown**

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	√***Yes*** *0No*
2. Please indicate whether your project involved any of the following issues (tick box) :	
RESEARCH ON HUMANS	
• Did the project involve children?	No
• Did the project involve patients?	No
• Did the project involve persons not able to give consent?	No
• Did the project involve adult healthy volunteers?	No
• Did the project involve Human genetic material?	No
• Did the project involve Human biological samples?	No
• Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	No
• Did the project involve Human Foetal Tissue / Cells?	No
• Did the project involve Human Embryonic Stem Cells (hESCs)?	No
• Did the project on human Embryonic Stem Cells involve cells in culture?	No
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
• Did the project involve tracking the location or observation of people?	No
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	No
• Were those animals transgenic small laboratory animals?	No
• Were those animals transgenic farm animals?	No

• Were those animals cloned farm animals?	No
• Were those animals non-human primates?	No
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	No
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	N/A
DUAL USE	
• Research having direct military use	0 Yes √ No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	0	1
Work package leaders	1	4
Experienced researchers (i.e. PhD holders)	21	21
PhD Students	4	0
Other		

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	
Of which, indicate the number of men:	None

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?

○ Yes
√ No

6. Which of the following actions did you carry out and how effective were they?

	Not at all effective				Very effective
❑ Design and implement an equal opportunity policy	○	○	○	○	○
❑ Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑ Organise conferences and workshops on gender	○	○	○	○	○
❑ Actions to improve work-life balance	○	○	○	○	○

○ Other: Did not carry out any of these

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

√ No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

√ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

√ Yes- please specify: Website

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

○ Main discipline[10]:

○ Associated discipline[10]:

○ Associated discipline[10]:

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*

○ Yes
√ No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

√ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[10] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- √ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- √ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and		Food Safety		Research and Innovation	
Monetary Affairs		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- √ Local / regional levels
- √ National level
- √ European level
- √ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	14
To how many of these is open access[11] provided?	
How many of these are published in open access journals?	
How many of these are published in open repositories?	
To how many of these is open access not provided?	
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[12]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		None
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	None
	Registered design	None
	Other	None
17. How many spin-off companies were created / are planned as a direct result of the project?		None
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑ Increase in employment, or	❑ In small & medium-sized enterprises
❑ Safeguard employment, or	❑ In large companies
❑ Decrease in employment,	❑ None of the above / not relevant to the project
√ Difficult to estimate / not possible to quantify	

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*

[11] Open Access is defined as free of charge access for anyone via Internet.
[12] For instance: classification for security project.

Difficult to estimate / not possible to quantify	❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

√ Yes ○ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes √ No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

√	Press Release	√	Coverage in specialist press
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	❑	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
√	Brochures /posters / flyers	√	Website for the general public / internet
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

√	Language of the coordinator	√	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of

interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

2. FINAL REPORT ON THE DISTRIBUTION OF THE EUROPEAN UNION FINANCIAL CONTRIBUTION

This report shall be submitted to the Commission within 30 days after receipt of the final payment of the European Union financial contribution.

Report on the distribution of the European Union financial contribution between beneficiaries

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
1.	
2.	
n	
Total	