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2007

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Luxembourg: Office for Official Publications of the European Communities, 2007

ISBN 978-92-79-05420-4

Printed in Belgium

Printed on white chlorine-free paper

COMMISSIONERS' PREFACE

The emergence and spread of the highly pathogenic H5N1 avian influenza virus and its unusually high death toll in sporadic cases of human infections has drawn the attention of the public to the health threat of a new influenza pandemic. While we know today that the 'Spanish Flu' pandemic of 1918 to 1919 was responsible for far more deaths (up to 50 million) than the First World War, it is easily overlooked that even 'regular' seasonal influenza epidemics are responsible for an estimated 250 000 to 300 000 deaths each year worldwide. In addition to human illness and suffering, the disease has an enormous direct and indirect economic impact on afflicted countries. The cost of a moderate influenza epidemic in France alone has recently been estimated at EUR 16.6 billion.

Similarly, avian influenza represents one of the major concerns to animal health. The disease is listed by the World Organisation for Animal Health (OIE) and is subject to EU legislation. Its incidence has greatly increased in the past 6 years compared to the previous 40 years, in particular due to the H5N1 strain. Outbreaks of highly pathogenic avian influenza cause huge economic losses to the poultry industry and directly affect food security and the livelihood of rural areas in developing countries. It is estimated that hundreds of millions of animals have died or have been culled as a consequence of the H5N1 outbreaks around the world.

Science has already delivered a number of effective tools for protection measures against influenza (such as human and animal vaccines as well as antiviral drugs), but very important questions remain unanswered, and research is of prime importance in the fight against influenza. We need more potent, longer-acting and more broadly protective vaccines for humans (including vaccines against a potential pandemic strain). We need to develop better veterinary vaccines that allow vaccinated and infected birds to be distinguished — crucial for effective surveillance and large scale administration. We need improved rapid diagnostic methods in birds and humans and new molecular targets for better antiviral drugs, as well as research into the effectiveness of containment and mitigation strategies in the case of a pandemic.

We would like to take this opportunity to emphasise that the European Commission's support for influenza research dates back to long before the current media attention to the threat of an H5N1 pandemic. This catalogue gives you an overview of the breadth and the depth of this effort. Since 2001, the Commission has supported specific influenza research projects with over EUR 65 million. In addition, it is currently supporting, with more than EUR 40 million, a number of larger projects on viral or infectious diseases in general, in which influenza plays an important role. This level of commitment arguably makes the Commission the most important funding organisation for influenza research in Europe. As you will discover in this publication, several of these projects have already resulted in major scientific breakthroughs and/or have already led to improvements in public health policy.

This publication cuts across a variety of different activities in the Fifth and Sixth Framework Programmes for Research and also includes projects funded through the Programme of Community Action in the field of Public Health, illustrating the interdisciplinary nature of influenza research. It is our firm conviction that the fight against influenza — like many other challenges — can only be won if research, policy support and the implementation of research results work closely together for the benefit of public health. Many of the projects in this publication are living proof of this inextricable link.

It almost goes without saying that uniting Europe's considerable strength in influenza research and fostering the collaboration of teams with complementary expertise is as important as joining forces in the public and veterinary health measures against a virus that — more than ever in an era of globalisation that includes human travel as well as the poultry trade — does not stop at any border. We are glad to say that we will continue to support all these efforts in the Seventh Research Framework Programme (FP7), which was launched earlier this year and which will run until 2013, and in the context of the new Public Health Programme covering the period 2008 to 2013. For the first time a specific activity within the 'Health' theme of FP7's Cooperation Programme will be dedicated to 'Emerging Infectious Epidemics'. We look forward to a future edition of this publication with the results of the first FP7-funded influenza research projects!

Janez Potočnik
European Commissioner
in charge of Science and Research

Markos Kyprianou
European Commissioner
in charge of Health

INTRODUCTION

Pandemic influenza has become a paradigm of the potential health threat that emerging infectious diseases pose to the world. Today, we could not be further from the optimism of the 1960s and 1970s, when statements like 'the war against infectious diseases has been won' abounded. The 'Spanish Flu' pandemic of 1918 to 1919 remains one of the most deadly infectious diseases of all time, and the recent spread of the highly pathogenic avian influenza virus H5N1 is just the most recent example of the continued threat of emerging zoonoses. Against this background the European Commission's Seventh Research Framework Programme (FP7, 2007-2013) has established for the first time a new dedicated activity — 'Emerging Infectious Epidemics' — and the Council Decision for FP7's Specific Programme 'Cooperation'[1] explicitly mentions highly pathogenic influenza in the description of this activity.

1. *Council Decision 971/2006/EC, OJ L 400, 30.12.2006, p. 124*

The new mandate to build a coherent strategy in this research area is also an opportunity to take stock of past and ongoing EU-funded activities in influenza research. As with previous disease outbreaks, such as BSE (bovine spongiform encephalopathy) and SARS (Sudden Acute Respiratory Syndrome), the Commission has been able to respond rapidly to the spread of bird flu and human sporadic cases of H5N1 with a EUR 28 million dedicated call for proposals, launched in late 2005. The present publication also demonstrates the longer-term track record of Framework Programme funding in this field.

The aim was to include in this catalogue all FP5- (1999-2002) and FP6- (2002-2006) funded projects that are either exclusively dedicated to research on any aspect of influenza (the majority of projects) or address a broader range of diseases, but with a significant part devoted to influenza. A table at the end of this catalogue differentiates these two groups of projects. Also included are four influenza-specific projects from outside the Research Framework Programmes. These are from the Directorate General for Health and Consumer Protection's Programme of Community Action in the Field of Public Health. This valuable addition underlines the coherence of the Commission's policy in this area and the close cooperation between the responsible services. The substantial contribution of a number of other public health projects that take a broader approach to infectious diseases (the training of epidemiologists, horizontal issues in vaccination policy or the improvement of communicable diseases control and communication) was also fully recognised, but it was decided, however, not to include these projects in the present publication in order to keep the focus on influenza research projects.

Given the close link between animal and human influenza, it was an obvious decision to include in this compilation projects on animal, as well as human influenza. Recent calls and future plans in the area of influenza as well as other emerging zoonoses are being coordinated in close collaboration between the units responsible for animal and human infectious diseases within the Research Directorate General.

The Framework Programmes' emphasis on interdisciplinary research seemed to justify an attempt to divide the projects into thematic chapters that cross the border between animal and human influenza. The hope is that this structure might serve as an incentive for interesting comparisons, detecting and questioning parallel or divergent approaches. Even closer collaboration between the animal health and human health specialists will be required in the future in order to successfully combat influenza as well as other zoonoses. Together with the respective scientific communities, the Commission services will also use this compilation to identify strengths and weaknesses in our funding portfolio. Is it defensible, for example, that there is currently only one human project (from the Public Health portfolio) in the Diagnostics and Surveillance chapter? What are the lessons to be drawn from the multitude of vaccine projects? What should be the priorities in this field for future calls?



At the same time, there are of course inherent difficulties in any ex-post classification. Clearly there are projects that fall into more than one category or might in fact include additional areas not covered in the four chapters. Similarly, the 'labels' of those four categories, Vaccines; Diagnostics and Surveillance; Biology; and Networking, Training, Socio-Economic and Legal Issues can certainly be questioned. I apologise in advance for any possible misclassification, but hope that the advantages of this approach for the reader will outweigh its imperfections. On the last pages of the booklet some alternative classifications are provided that group the projects alphabetically, according to animal/human influenza and according to their origin in FP5, FP6 or Public Health.

CORNELIUS SCHMALTZ

Acknowledgements: Many people have contributed to this catalogue. First and foremost of course, my thanks goes to the scientific coordinators (and their staff) of the projects presented. To a very high degree they have been able to accommodate all wishes, have revised and adjusted their abstracts, have provided illustrations and been patient with my requests. Anna Lonnroth and Bernard Mulligan (Deputy Head and Acting Head of the Infectious Diseases Unit) and Tuija Jansson have provided critical support in every phase of the project. Without the enormous contribution and key advice of Isabel Minguez-Tudela, the scientific officer responsible for all animal influenza projects, this publication could never have been realised. My other colleagues from the Research Directorate General, Torbjoern Ingemansson, Annabelle Ascher, Marta Iglesias, Christian Wimmer and Beatrice Lucaroni have been very helpful in identifying and contributing influenza projects from their respective programmes. Similarly, my thanks goes to Michel Pletschette, Head of Unit, Solvejg Wallyn and Cinthia Menel Lemos from the Public Health Executive Agency, as well as to Franz Karcher and Frank van Loock from the Directorate General for Health and Consumer Protection for their crucial support regarding the Public Health projects.

VACCINES

Vaccination is considered the cornerstone of influenza control in humans. Vaccination against seasonal influenza has been in use for more than 50 years, has an excellent safety record and provides a high degree of protection. It is currently recommended in most countries for use in risk groups (mainly the elderly and people with an underlying medical condition), but routine use in children[1] and even universal immunisation programmes exist or are under consideration in some countries/regions.

1. *European Centre for Disease Protection and Control (ECDC): Technical Report of the Scientific Panel on Vaccines and Immunisation: Infant and children seasonal immunisation against influenza on a routine basis during inter-pandemic period, Stockholm, January 2007 (http://www.ecdc.eu.int/documents/pdf/Flu_vacc_18_Jan.pdf)*

Yet, many challenges remain that are further amplified when it comes to the challenge of pandemic vaccines. Longer-acting vaccines that are more broadly cross-protective against heterologous strains and clades of influenza are required, immune responses in elderly and small children need to be optimised, and cell-culture production (which could be scaled up much faster in the case of a pandemic) is about to replace egg-based production facilities (where egg supply might be particularly vulnerable during a pandemic derived from an avian strain of influenza).

The use of vaccination in poultry is complex and only a limited number of countries have implemented a vaccination strategy to date. With currently available vaccines, avian influenza (AI) viruses can still infect and replicate in vaccinated birds. However, they protect against clinical signs and mortality, reduce the level and duration of virus shedding and hence decrease possibilities of transmission. There is evidence that when used adequately and as part of a wider control strategy in combination with other measures, such as increased biosecurity, stamping out and appropriate surveillance, vaccination may be a powerful tool in the control and eradication of AI. A key issue for the use of vaccines is the ability to detect birds exposed to the field virus in a vaccinated population. This is known as the DIVA strategy (differentiating infected from vaccinated animals). The development of new DIVA vaccines with a wider cross-protection and species range that can be administered on a large scale and at low cost is thus an important research task.

Some of the earliest funded projects in this catalogue are vaccine projects that have delivered a number of important results: **MUCADJ** has developed a potent new adjuvant for mucosal (i.e. intranasal) delivery of human influenza vaccines and an H7N1 vaccine developed by the **FLUPAN** consortium is currently in clinical trials. A novel computer algorithm that assesses the relatedness of influenza viruses was developed by **NOVAFLU**, and has already been integrated into the biannual WHO (World Health Organisation) vaccine strain selection process. **FLUAID** works on vaccine candidate strains as well as on diagnostic tests with partners in Asia, Australia and Africa. A remarkably high number of consortia (eight) are led by industry, in particular small and medium-sized enterprises (SMEs). These are **MUCADJ**, **CHIMERIC VACCINES**, **UNIVERSAL VACCINE**, **SARS/FLU VACCINE**, **FLUVACC**, **AIV VACC DIAGNOSIS**, **Intranasal H5vaccine** and **FluVac**. The last two, as well as **PANFLUVAC**, are the result of the most recent (and last FP6) call for proposals. They have just started, and address the special challenges of the clinical development of a pandemic human vaccine through novel adjuvants, delivery methods and genetically engineered vaccine viruses. Similarly, **AIV VACC DIAGNOSIS** and **NOVADUCK**, funded through the same recent call, are both aimed at creating effective live influenza vaccines for poultry that allow the distinction of vaccinated and infected animals through specific markers and could be mass administered. Importantly, the Public Health project **FLUSECURE** is dedicated to establishing a network of public health institutes as a partner for the vaccine industry to promote the production of an effective pandemic vaccine.

EFFICACIOUS VACCINE FORMULATION SYSTEM FOR PROPHYLACTIC CONTROL OF INFLUENZA PANDEMICS

Acronym: **PANFLUVAC**
EC contribution: €3 334 798
Duration: 48 months

Starting date: 01/01/2007
Instrument: STREP

Key words: Pandemic H5N1 influenza, intranasal delivery, virosomal vaccine, lipopeptide adjuvants, dendritic cell targeting, pre-clinical to clinical evaluation strategy

SUMMARY:

Influenza epidemics remain a burden for both human health and national economies, as witnessed by the recent advance of the pathogenic avian H5N1 influenza virus. While the numbers of human deaths in Europe has remained relatively low, the presence of such cases in Turkey demonstrates the danger posed by this virus. The avian H5N1 virus has now been detected in wild birds in numerous European countries, and the PANFLUVAC consortium is committed to creating an efficacious vaccine against this virus, to provide strong protection in a pandemic situation.

The overall aim of PANFLUVAC is to construct vaccine delivery systems for intranasal and parenteral vaccines. New H5N1 vaccines are to be based on well-established virosome technology — proven its worth for efficacious interpandemic vaccines — as well as whole virus vaccines. This will permit comparison of the intranasal virosomal vaccine with the whole virus vaccine. The vaccine potency will be enhanced by novel adjuvants targeting dendritic cells, offering both antigen-sparing potential and immunopotentiation characteristics. Both ISCOMs and lipopeptide adjuvants are already proven immunopotentiators, biosafe for humans. Certain of these have been employed with experimental influenza vaccines which allow the new H5N1 vaccines to be fast-tracked in their development. Accordingly, PANFLUVAC will generate the first H5N1 vaccine within the first 18 months of the project.

The PANFLUVAC project is also designed to facilitate rapid modification of the vaccine in the face of virus drift. Within the preclinical evaluation, the new vaccines will be tested for the degree of heterotypic cross protection they offer. PANFLUVAC offers a generic vaccine development system to provide safe and efficacious vaccines against influenza, fitting in with the European Commission's Working Paper on Community Influenza Pandemic Preparedness and Response Planning.

PROBLEM:

The challenge

The avian H5N1 influenza virus is now spreading among the wild bird population in Europe, and infection of humans has already reached the borders of continental Europe. For a truly efficacious vaccine, one must consider the route of virus entry into the host (the respiratory tract), and host requirements for protective immune defences. That is, an ideal vaccine should induce both local (mucosal) and systemic (serum) immunity.

Currently, parenterally administered inactivated influenza vaccine is the best prophylactic control measure. However, parenteral vaccination does not ensure induction of local immunity in the respiratory tract — the route by which the virus infects humans — and from where it transmits to other individuals. Inducing mucosal immunity by vaccination would enhance control of both disease and transmission. Although mucosal immunisation has been studied, no acceptable intranasal vaccine against influenza is yet available. A major problem for the intranasal vaccine has been the adjuvant, which is required for efficient induction of immunity.

WHO recommendations

The WHO assessment of the risk to human health from the H5N1 avian influenza virus states: 'The disease in humans has no vaccine to confer protection and no specific treatment once illness becomes severe.' The WHO warning that 'outbreaks in birds pose a significant threat to human health', and that H5N1 'has the potential to ignite a global influenza pandemic in humans', has led to an urgent requirement for H5N1 pandemic influenza virus vaccines. The PANFLUVAC vaccines fit this requirement and allow for a rapid response to the entry of H5N1 into Europe, enforcing the WHO recommendation that 'trials of experimental influenza pandemic vaccines for humans be accelerated'.

Antigen-sparing strategies and effects of immunomodulatory molecules

PANFLUVAC is designed to construct a vaccine delivery and formulation to meet current and future influenza pandemics. The proposed H5N1 vaccine will be prepared with regard to the immediate needs, as identified above by the WHO. In addition, the project will generate the 'mock-up' library of vaccine reagents to meet the permanent threat from pandemic influenza. The overall aim is to construct the efficacious H5N1 vaccine, enhancing the capacity to protect the people of Europe both now and in the future, rather than just in the short term. The rapidly ageing European population, which is particularly vulnerable to influenza complications and influenza-related deaths, is a clear reminder of the need for better interpandemic as well as pandemic vaccines.

The project allows for 'robust scale-up of vaccine production', being coordinated by the leading group in virosomal technology – Crucell (partner 2 CRU). CRU holds the intellectual property rights for the application of ISCOMs for influenza vaccines. To this end, the project employs the latest advances in reverse genetics (partner 3 - National Biological Standards Board) — referred to in the Commission Working Paper (COM(2004)201 final). This allows for novel vaccines to be provided within a short time. Vaccine efficacy will be enhanced through application of novel formulations and adjuvants, in response to the requirement in the call for antigen-sparing strategies and the application of novel immunopotentiating agents. The proposed adjuvants, both current and under development, will provide the formulated vaccine with a potent immunogenicity. Moreover, these selected adjuvants target the dendritic cells, which are critically important for the activation of an efficacious immune response. This allows for the development of more efficacious vaccination and better usage of the vaccine available.

By promoting the vaccine to reach the target organs and cells more efficiently, lower doses of vaccine are required to maintain immunogenic concentrations. This increases the likelihood of manufacturing potential reaching demand,

and reduces the risk of adverse side effects due to incorrect interaction with organs. It also responds to the Commission Working Paper (COM(2004)201 final), on development of a safe vaccine.

The proposed solution

PANFLUVAC will create an efficacious vaccine inducing potent local and systemic immune responses to protect the host and prevent viral transmission. This is based on virosomal technology, together with a novel lipopeptide adjuvant targeting the critical cells of the immune system – dendritic cells. Consequently, PANFLUVAC will provide a particularly efficacious and safe vaccine against influenza virus (H5N1). In addition, the basis for efficacious intranasal and parenteral vaccines will be established, for future demands.

AIM:

Considering the evolution of the avian H5N1 influenza virus and the threat it poses for initiating a human pandemic, an efficacious vaccine against this virus is urgently required. Accordingly, the PANFLUVAC project will construct an efficacious vaccine formulation to meet immediate and future needs for controlling influenza epidemics and pandemics. PANFLUVAC will deliver both an efficacious H5N1 vaccine and an inactivated whole virus vaccine within the first 18 months of the project. The vaccine is based on well-proven virosomal technology, together with a novel promising ISCOM and lipopeptide adjuvants. By investigating novel adjuvants, PANFLUVAC will provide the basis for the generation of efficacious and safe vaccines to combat both interpandemic seasonal influenza and influenza pandemics.

EXPECTED RESULTS:

a) A formulated H5N1 pandemic vaccine developed from existing technologies and material for preclinical evaluation.
b) Virosomal and whole virion vaccines (existing technologies) compared in pre-clinical evaluations.
c) Intranasal and parenteral administration evaluated.
d) A pandemic influenza vaccine to meet the current need for required vaccines in the face of imminent pandemic influenza threat.
e) The efficacy of the formulated H5N1 pandemic vaccine generated from existing technologies assessed by Phase I Clinical Trial evaluation.
f) Studies in vaccinated subjects performed to generate a knowledge platform on immunological correlates and correlates of protection challenged.
g) An erudite link formulated, joining preclinical and clinical evaluations.
h) A dossier prepared on proposed H5N1 vaccines formulated with new, novel adjuvants.

POTENTIAL APPLICATIONS:

Many of the techniques being employed - including the adjuvants, virosomal vaccine delivery system and evaluation strategies - are applicable in a generic manner. The *in vitro* and *ex vivo* tests to be employed for immunological correlates of protection can be adapted easily for application to other vaccines. Certain of the formulations are patented, while others developed by the consortium will be subject to patent application. With the involvement of a number of SMEs in the project, patent application and Intellectual Property availability is at a high level. Overall, the main potential applications are the following:

a) An efficacious H5N1 vaccine for parenteral administration.
b) An efficacious H5N1 vaccine for mucosal administration.
c) ISCOMs as adjuvants for influenza vaccines.
d) Safe adjuvants enhancing mucosal and systemic immunity.
e) *In vitro* and *ex vivo* tests for vaccine evaluation, reducing animal experimentation.
f) Immunological correlates of protection for rapidly identifying new efficacious vaccines.
g) Promoting human health and well-being, and population confidence in the face of influenza.

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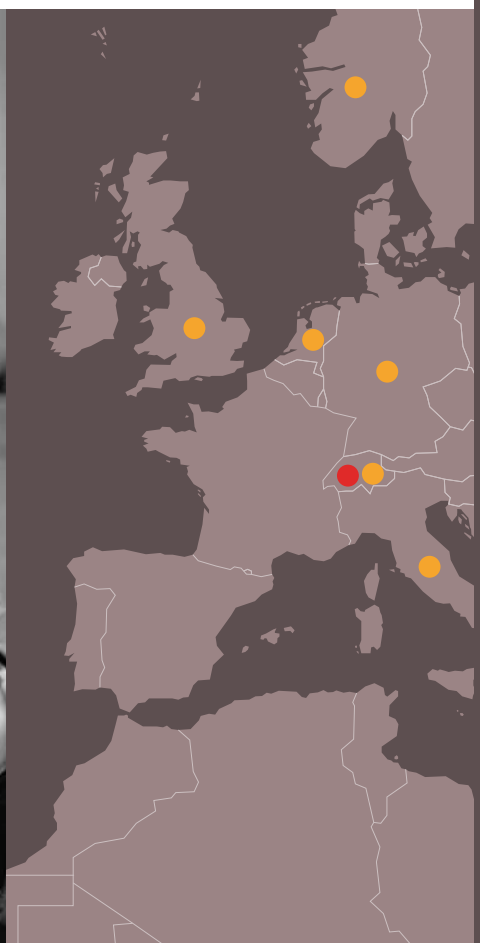

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PANFLUVAC

IMMUNOGENICITY AND PROTECTIVE EFFICACY OF INTRANASAL DELNS1(H5N1) INFLUENZA VACCINE

Acronym: **Intranasal H5vaccine**
EC contribution: €2 680 400
Duration: 36 months

Starting date: 01/01/2007
Instrument: STREP

Key words: NS1, H5, avian influenza, vaccine, replication deficient, intranasal

SUMMARY:

In collaboration with six partners in four European countries and in Russia, Green Hills Biotechnology is developing a novel vaccine against avian influenza, within the framework of a research project subsidised by the European Union. The project budget totals EUR 2.6 million.

This project is being carried out by an international consortium over a three-year period. Besides Green Hills Biotechnology, a total of three other companies and three universities are participating in the development and evaluation of an intranasal H5 vaccine. The proposed vaccine is based on proprietary technology from Green Hills Biotechnology. Immunological properties will be assessed by the Goethe University in Frankfurt and preclinical experiments will be carried out at Biotest s.r.o. in the Czech Republic. Novel, sensitive methods developed for assessment of the antibody response against viral proteins are being established in cooperation with the Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry in Moscow. These assays will allow the selection of an optimal vaccine candidate that will be subsequently evaluated in clinical trials at the Medical University, Vienna, and at Retroscreen in the UK.

PROBLEM:

Mankind is threatened by avian influenza. For the period of December 1, 2003, to February 3, 2006, the WHO reported 161 confirmed human cases of avian influenza A (H5N1); of these 86 (or 53%) were fatal. It is not clear how many amino acid substitutions are still needed to convert avian viruses into a new human pandemic strain. Old human influenza viruses of the H1N1 or H2N2 subtypes could also return in humans, causing a new pandemic. Sooner or later there will be an influenza pandemic unless new and more efficient vaccines are developed. Therefore, the current situation with the H5N1 avian flu could be considered as a challenge for global science and the biotech industry.

AIM:

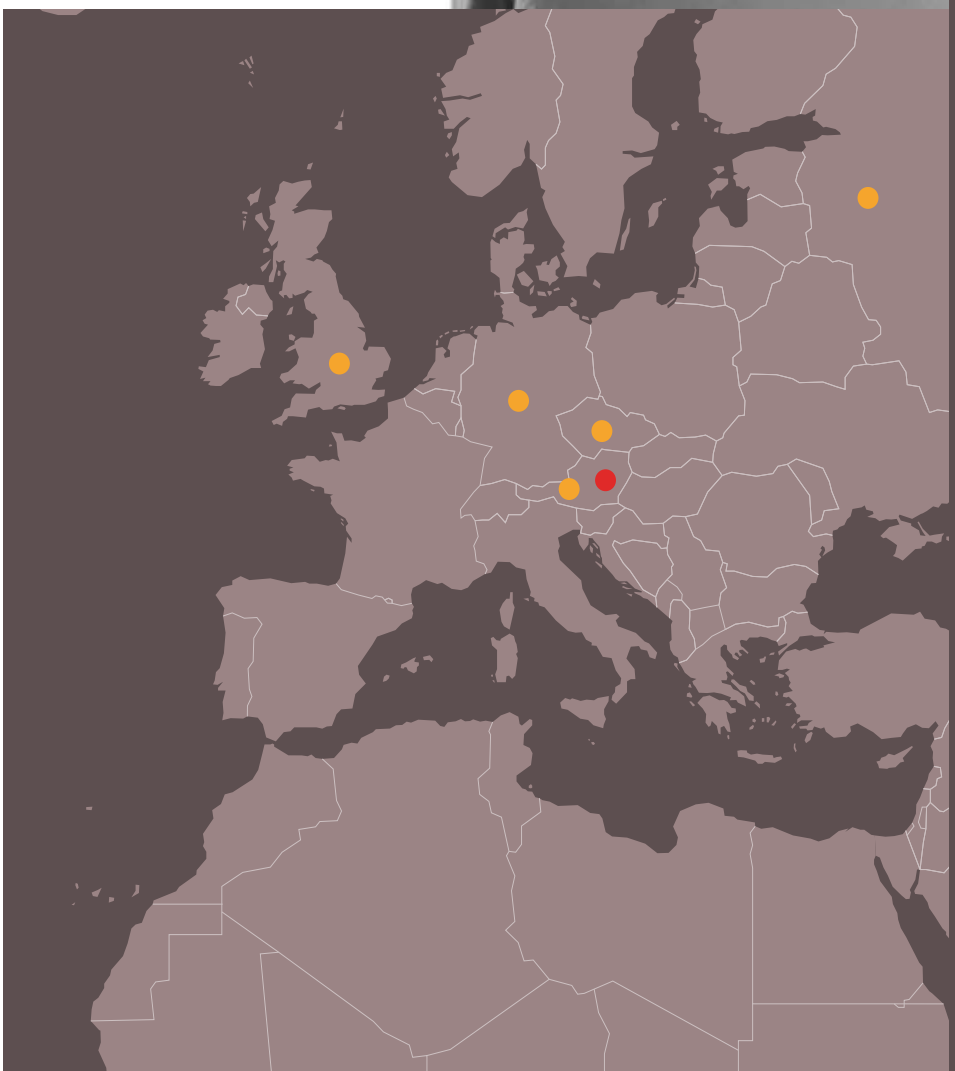
The main objective of the current project aims to define an intranasal H5N1 pandemic vaccine with enhanced capacity to evoke a strong, long lasting local and systemic immune response in humans. The replication-deficient vaccine is based on the deletion of the NS1 protein (DelNS1 vaccine). First, several vaccine candidates will be screened for parameters including antigenic properties, receptor specificity, attenuation and immunogenicity in animal models. Studies on elucidation of mechanisms responsible for protection will comprise parameters of innate and adaptive immunity. Several standardised immunological techniques such as haemagglutination inhibition (HI) and ELISA will be used for evaluation of vaccine candidates. In addition, novel sensitive methods based on synthetic sialic receptor analogues will be developed for assessment of the antibody response against the haemagglutinin (HA) and neuraminidase (NA).

EXPECTED RESULTS:

Protective properties of the vaccine against homologous and heterologous influenza strains will be tested in ferret challenge experiments using different H5 strains. To close the gap between the ferret model and human studies we will use the macaque model to evaluate the immune response. These pre-clinical studies will allow the selection of the most potent vaccine candidate, which will be produced according to cGMP guidelines on Vero cells. After toxicological evaluation of the vaccine, clinical Phase I/II studies will be performed. If permitted by authorities, a human challenge study using attenuated H5 virus will be carried out. Alternatively, for proof of principle of the delNS technology, an H1-delNS1 vaccine will be produced and used in an H1 challenge study.

POTENTIAL APPLICATIONS:

The proposed clinical evaluation of delNS1 (H5N1) vaccine should be considered as a component of European systemic efforts to prevent and control potential pandemics of avian influenza. This is a severe respiratory infection that poses a major health threat on a worldwide scale. The proposed vaccine will reduce mortality and morbidity rates, lost workdays, and hospitalisations in case of H5N1 outbreak.



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DOSE SPARING AND INCREASED IMMUNOGENICITY FOR VACCINATION AGAINST PANDEMIC INFLUENZA WITH COVACCINE HT

Acronym: **FluVac**
EC contribution: €3 500 000
Duration: 48 months

Starting date: Planned for 01/07/2007
Instrument:STREP

Key words: Adjuvant, vaccine, dose-sparing, efficacy, influenza, pandemic, emergency vaccination

SUMMARY:

The risk of a new influenza pandemic is emphasised by a WHO report documenting several hundreds of recent cases of human infection with a new virus strain and approximately 50% mortality. Control or prevention of a pandemic by emergency vaccination depends on availability of products with high efficacy and broad protection. The combination of an effective adjuvant and cell culture technologies for antigen production meets the requirements of a pandemic influenza vaccine.

The consortium of this FluVac project, among them proven and renowned experts in human vaccine development and influenza research, plans to deliver a prototype vaccine with proof-of-concept within 48 months. Optimal doses of inactivated, cell culture-derived whole influenza virus (H5N1) and CoVaccine HT as adjuvant will be tested in pre-clinical and clinical studies. In comparison to the widely used production system in eggs, production in cell culture offers the advantages of immediate and reliable availability of antigen of constant quality and with reduced risk of contamination.

The use of whole virus in a vaccine instead of subunits, is considered to induce broader protective immunity. The novel adjuvant CoVaccine HT has been shown to elicit high humoral and cellular responses against different types of antigens, including inactivated influenza virus, and in different animal species (mice, rats, rabbits, pigs, horses, primates, etc.). It is considered to be a promising candidate for a pandemic influenza vaccine. A Phase I trial, human challenge study and extensive immunological evaluation of the vaccine prototype will provide the information required for further development and registration (Scheme 1).

PROBLEM:

The impact of an influenza pandemic can be enormous as illustrated by the 'Spanish Flu' from 1918 to 1919 - one of the most dramatic events in human history with 20 to 40 million casualties worldwide. Influenza pandemics were caused by influenza virus A subtypes. Wild birds as the natural hosts can easily spread the virus and infect other animals and humans. Recently, a new highly pathogenic H5N1 strain of avian influenza A virus has become epidemic in birds in Asia and has been transmitted to European and African countries. Mortality rates among infected humans exceeded 50%, which is alarming. This H5N1 virus did not spread between humans, but once an influenza virus acquires the ability to transmit through respiratory droplets from one human to another it could spread within a few months around the world. The consequences of a pandemic outbreak could be enormous as it could disrupt daily life with tremendous social and economic effects and put a large burden on the health care system. High costs of anti-influenza drugs and limited availability thereof, implicates an urgent need for an affordable vaccine for the prevention and control of a pandemic influenza outbreak.

Currently, most human influenza vaccines are produced in embryonated chicken eggs, which demands strict logistics. In case of a pandemic influenza outbreak, the availability of eggs might be a critical factor. Therefore, this project exploits cell culture technology for antigen production as this method is more flexible, independent of supply of animal-derived materials and more consistent. With a master and working cell-bank system, production and up-scaling can be immediately started in case of emergency.

Schematic representation of the programme.

Explorative studies	Pre-clinical studies	Clinical studies
Immunogenicity (adult, neonatal, elderly mice)	GMP manufacturing Validation methods Optimisation formulation Efficacy (ferrets) Toxicity (ferrets, rabbits)	Phase I trial Safety Immunogenicity Human Challenge study
Extensive immunological characterisation of humoral and cellular responses		

An adjuvant may compensate the shortcomings of the seasonal influenza vaccines for application during a pandemic. Firstly, an adjuvant may reduce considerably that antigen dose required to establish a certain level of immunity. Secondly, it may have a beneficial effect on the type, level and kinetic of the immune response. Thirdly, it may decrease the number of low-/non-responders thereby affording a higher degree of immunity in a susceptible population. Fourthly, it may enhance cross-protection to distinct influenza virus strains. Feasibility studies with CoVaccine HT, a sucrose fatty acid sulphate ester incorporated in a submicron emulsion of squalane-in-water exerted the ability to induce strong humoral and cellular immune responses against a wide range of antigens in various animal species and high efficacy in combination with low antigen dose (Fig. 1).

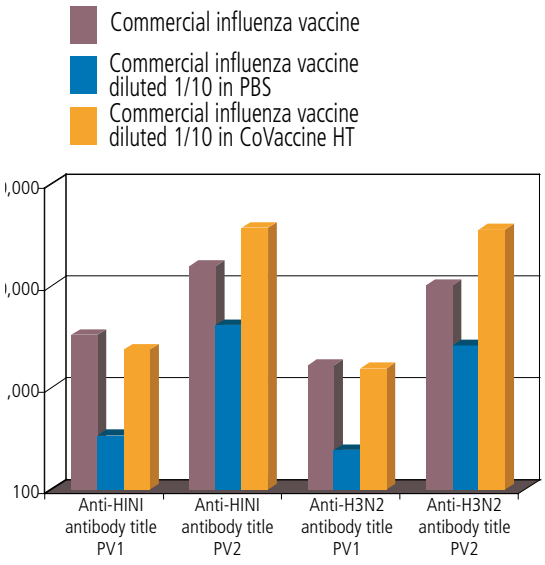
The combination of these technologies opens unique opportunities for preventing and controlling an influenza pandemic and the knowledge and expertise gained might be applicable to the design of other (types of) vaccines.

AIM:

The consortium aims at a novel influenza vaccine formulation by combining a novel adjuvant CoVaccine HT and an inactivated, cell culture-derived, whole influenza virus (H5N1). Feasibility studies with the adjuvant indicated that CoVaccine HT is a promising candidate for emergency vaccines to establish high levels of immunity and to compensate for the limited availability of antigen.

The ultimate goal of the FluVac project is to prove safety and efficacy of a CoVaccine HT adjuvanted pandemic whole H5N1 virus vaccine in humans and to gain insight in its performance in animal models. After extensive preclinical evaluations, a vaccine with the optimal composition will be studied in a Phase I clinical trial and subsequently in a human challenge study. In parallel, the vaccines will be investigated in mouse models for

neonates and elderly. The ambitious preclinical and clinical development plan addresses all known aspects of protective immunity against influenza.



Effect of 1/10 dilution in PBS or CoVaccine HT of a commercial influenza vaccine on ELISA antibody titres in pigs (data kindly provided by CoVaccine BV).

EXPECTED RESULTS:

Successful completion of the FluVac project and subsequent development and registration of the vaccine will have great benefits for the health of the European population. In addition, the project will contribute to the development of a novel generation of adjuvants and of improved vaccines to combat infectious diseases. The consortium is supported by the complementary know-how and technology of individual partners. The participants are renowned international experts in such areas as human (influenza) vaccine development, adjuvant development, neonatal immunity and influenza research i.e. Protherics (United Kingdom), OrganonBiosciences (the Netherlands), Retroscreen (United Kingdom), Landspitali University Hospital (Iceland) and Erasmus Medical Centre (the Netherlands).

A number of defined objectives will be pursued during the proposed project:

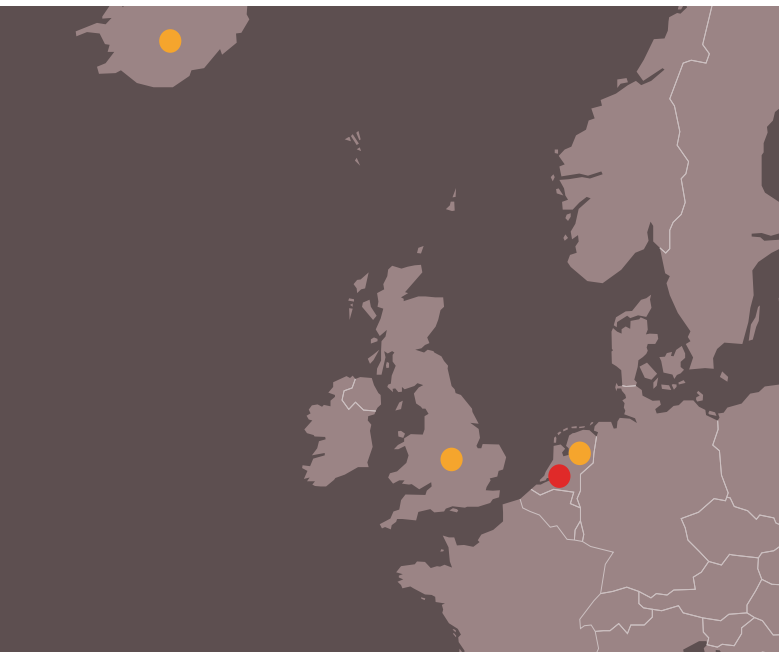
a) Delivery of non-GMP and cGMP grade CoVaccine HT and H5N1 antigen for use in preclinical, toxicology and clinical studies.
b) Evaluation of dose sparing approaches by incorporating CoVaccine HT, by analyses of humoral and cellular responses in mice and ferrets.
c) Proof of efficacy of the prototype vaccine in protection models in ferrets with current pandemic influenza virus strain(s).
d) Determination of kinetic and quality of humoral, cellular and mucosal immune responses especially in high-risk populations in mouse models for elderly and neonates.
e) Development of a stable vaccine using validated methods.
f) Achievement of pre-clinical proof of safety in animal toxicology studies.
g) Evaluation of safety and immunogenicity in human clinical Phase I trial.
h) Evaluation of protection and immunogenicity in a human challenge study induced by the CoVaccine HT-adjuvanted pandemic influenza vaccine.

POTENTIAL APPLICATIONS:

The FluVac consortium proposes a novel product for (pre-)pandemic vaccination by combining Protherics' proprietary adjuvant CoVaccine HT with OrganonBiosciences' H5N1 viral antigen produced in cell culture. Upon successful completion of clinical proof-of-concept studies, the consortium will pursue further clinical development.

Currently there are no adjuvanted Influenza vaccines licensed and marketed in the USA. MF59 and virosomes are licensed in Europe for epidemic and Al(OH)3 for pandemic influenza vaccine.

The consortium envisages that this project will contribute to the urgent need of improved adjuvants for emergency vaccines. Knowledge and expertise gained will be useful to other types of vaccines.



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NOVEL AI DIVA RECOMBINANT VACCINES FOR DUCK

Acronym: **NOVADUCK**
EC contribution: €1 416 380
Duration: 36 months

Starting date: 01/01/2007
Instrument: STREP

Key words: Avian influenza, recombinant vaccines, vectored vaccines, ducks, DIVA, immune response

SUMMARY:

The NOVADUCK project brings together both private (two companies and one SME) and public sector stakeholders (European reference laboratories and agencies on avian influenza) from four European countries with the aim of developing and evaluating new highly protective and cost-effective avian influenza live vaccines for ducks, based on live viral vectors and in line with the DIVA strategy (Differentiating Infected from Vaccinated Animals).

Viral vectors will be engineered to optimise both their immunogenicity and protective capacities. Vaccine candidates will be pre-screened for safety and immunogenicity using newly developed duck-specific immunological tools. The best vaccine candidates will be evaluated for efficacy in an HPAI H5N1 challenge-model in ducks. A serological DIVA test, able to detect infection in vaccinated duck flocks, will be generated, and the effect of vaccination on genetic and antigenic drift of H5N1 will be assessed.

PROBLEM:

The ongoing outbreak of H5N1 HPAI has recently spread from Asia to Africa and Europe, posing a real public threat as HPAI can occasionally infect humans. Ducks play a major role in the epidemiology of avian influenza because wild waterfowl, including ducks, constitute the natural reservoir of all subtypes of influenza A virus. Experimental infection of ducks with recent isolates indicates a longer shedding period and a selection for lower virulence variants, suggesting that duck has become the 'Trojan horse' of Asian H5N1 AI.

Although biosecurity is the first line of defence against HPAI, strategic use of vaccination is clearly recognised as a tool to help eradicate HPAI in an infected country. Most studies evaluating the efficacy of AI vaccines have been performed in chickens, and duck studies have been relatively rare. Existing inactivated AI vaccines are less immunogenic in ducks than in chickens and must generally be administered twice to be fully efficient; furthermore, there is no commercially available DIVA test to monitor AI infection in birds injected with this type of vaccine. Therefore, highly efficient, cost-effective, DIVA-compatible AI vaccines for ducks are still greatly needed.

In this specific context, live vector-based vaccines hold the greatest promise and are one of the most effective options. Indeed, some live recombinant vector-based AI vaccines have shown excellent results in chickens, but they are not necessarily adapted for use in ducks. Expected advantages of this type of vaccine include administration at a younger age, mass administration, rapid onset of immunity, and compatibility with the DIVA strategy.

The NOVADUCK project is designed to demonstrate and exploit the potential of live vector vaccines to develop a new generation of highly efficient and cost-effective AI vaccines for ducks and therefore could contribute to eradicating AI from the ecosystem.

AIM:

The NOVADUCK project aims to develop and evaluate new highly protective and cost-effective avian influenza live vaccines for ducks based on live vectors and in line with the DIVA strategy. More specifically, the NOVADUCK project will:

a) identify the optimal AI immunogenic sequence to be inserted into the selected live vectors;
b) generate and optimise three types of live recombinant vector-based vaccines;
c) develop reliable and cost-effective duck-specific immunological tools to measure the immune response induced by the different vaccine candidates and to detect infection in a vaccinated duck (DIVA strategy);
d) assess the safety and immunogenicity of the new vectored vaccine candidates and compare it with those of existing vaccines to select the best vaccine candidate(s);
e) set up a challenge model in ducks for vaccine evaluation of efficacy;
f) measure the efficacy of the most immunogenic vectored vaccine candidates against recent HPAI H5N1 and compare it with existing vaccines;
g) study the effect of vaccination on genetic/antigenic drift;
h) select the best candidate(s) to be developed based on its (their) immunogenic and protective properties as well as its (their) estimated cost of production and administration mode flexibility (e.g. individual versus mass administration).

EXPECTED RESULTS:

The NOVADUCK project is designed to identify the optimal AI immunogenic sequence to be inserted into a vector. New specific tools to evaluate humoral, cellular and mucosal immunity induced in ducks will be developed. Three types of vector-based vaccines containing the optimal immunogenic sequence will be generated, and their immunogenicity using different administration modes will be compared to that of existing inactivated AI vaccines using the newly developed immunological tools. A challenge

model using the most recent and representative AI isolates in which clinical signs, oral and cloacal shedding can be significantly detected in all challenged birds will be set up in ducks and will be used to compare the efficacy induced by the newly selected vector-based vaccine candidates with that induced by existing vaccines. Studies will be designed to verify that the newly developed vaccines show clear advantages over existing vaccines. The compatibility of these vector-based vaccines with newly developed DIVA tests will also be verified. In addition, data will be generated on the effect of vaccination of ducks on genetic/antigenic drift of the challenge virus.

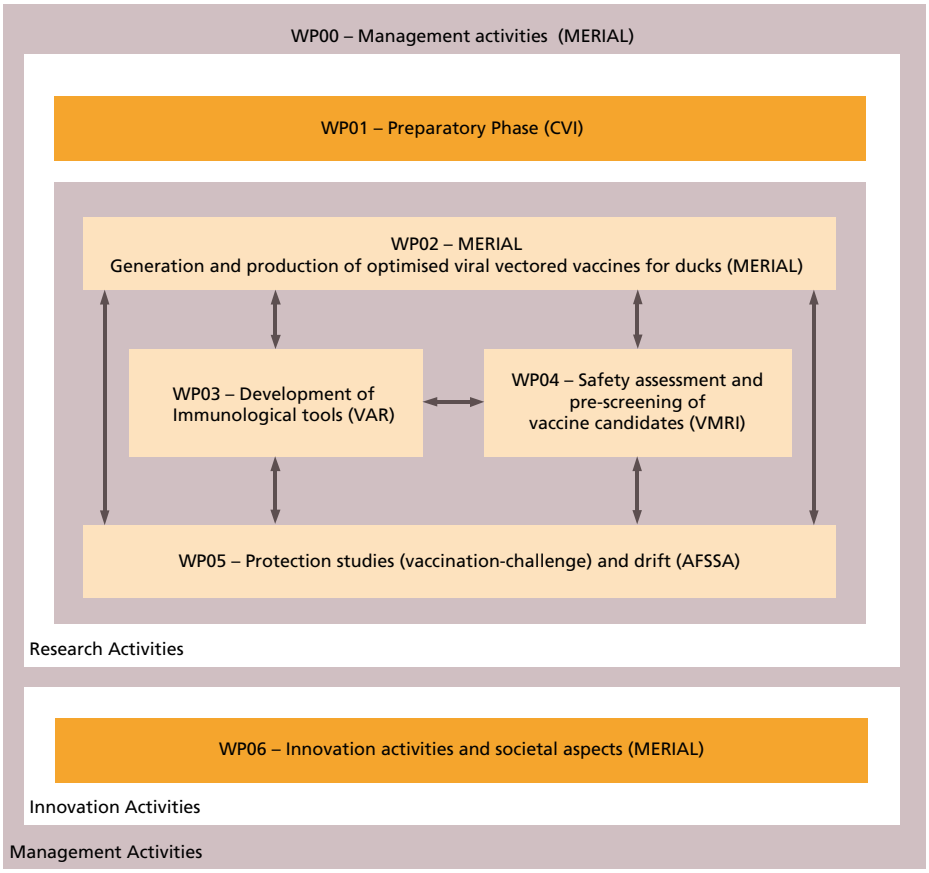
POTENTIAL APPLICATIONS:

The newly developed vector-based AI vaccines for ducks generated during the NOVADUCK project could be available for vaccination of ducks in countries in which the biosecurity measures are not sufficient to control AI H5 infection. The selected vaccine candidates should show clear advantages over existing vaccines, such as DIVA-compatibility, low cost, early onset of immunity, adjuvant-free, and/or ease of administration (e.g. mass vaccination, duckling administration at the hatchery). These new types of vaccines would be suitable for emergency vaccination, thereby limiting the mass slaughtering of poultry around an infected area, or for preventive vaccination in high risk or enzootic area.

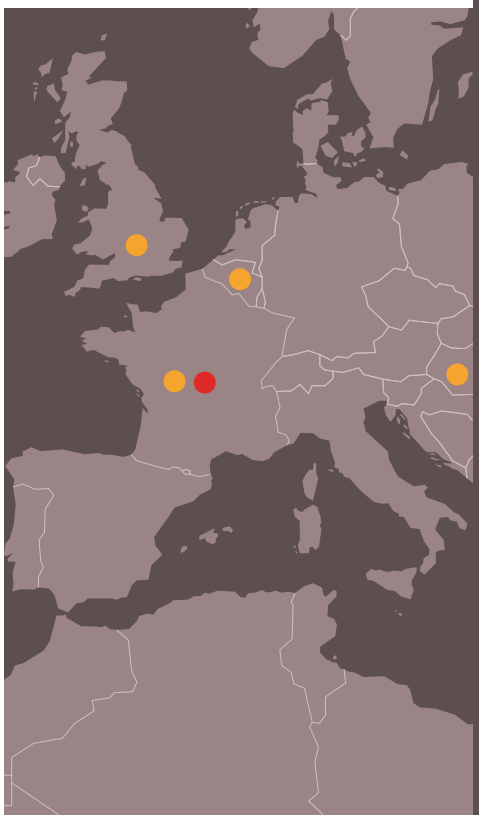
Some of the newly developed vaccine candidates may also be safe and efficacious in other species, including other poultry (e.g. chickens, turkeys and geese), but also mammals (e.g. cats, dogs, pigs, horses), making them ideal multi-species AI vaccine candidates. Additionally, if efficacy can be obtained after administration by the oral route, the potential exists to use such vaccines to vaccinate wild ducks and thus potentially contribute to the eradication of AI from the ecosystem.

The duck-specific immunological tools that will be developed during the NOVADUCK project would also be essential to evaluate the immunogenicity of any existing, as well as future, duck vaccines. In particular, the DIVA test could be used to monitor infection in vaccinated flocks.

The development of a reproducible AI challenge model in ducks will be useful to define the standard for evaluation of efficacy of duck AI vaccine. The protection data generated during the project would also help to define the minimal level of protection that should be expected for an acceptable AI duck vaccine. Overall, the project should give new tools to control AI infection in a species playing a significant role in AI epidemiology.



NOVADUCK PROJECT STRUCTURE



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SSPE-CT-2006-044217

NOVADUCK

VACCINE, DIAGNOSTIC TEST DEVELOPMENT AND IMMUNOLOGY ASPECTS OF AVIAN INFLUENZA

Acronym: **AIV VACC DIAGNOSIS**
EC contribution: €1 372 890
Duration: 36 months

Starting date: 01/12/2006
Instrument: STREP

Key words: Avian influenza viruses, Newcastle disease viruses, vaccines, diagnostics, immunology, DIVA

SUMMARY:

Avian influenza is a zoonotic disease and is seen as one of the most important emerging diseases with serious economic consequences. Although some vaccines for poultry are available, all vaccines have considerable drawbacks with regard to dose and application methods (injection), onset of immunity, efficacy or costs of production and application which limit their use.

The primary aim of this project is to develop better avian influenza vaccines through live or vector vaccines that could be mass applicable through spray, drinking water or eye drop. These vector vaccines would offer considerable advantages - mass applicable, less labour intensive and animal friendly application, protection by local and systemic immunity and less interference with eventual maternal antibodies, more complete protection through cellular and humoral immunity, faster onset of immunity when used in face of an outbreak and cheaper production methods.

The project exploits recently acquired knowledge concerning the molecular characterisation of the viruses resulting in the construction of candidate strains with highly interesting efficacy and safety profile. Safety and efficacy with Newcastle disease (NDV) vectors and infectious laryngotracheitis (ILT) vectors both for H5 and for H7 inserts have already been demonstrated in vivo.

A system in which gene cassettes for the foreign proteins can easily be constructed and exchanged will be developed and will be able to respond very quickly to a change in antigenicity of the field virus. Further optimised additional candidate strains will be constructed and extensively tested. Experiments on genetic *in vitro* and *in vivo* stability, immunological responses, virulence testing, spreading, and transmission studies in chickens, ducks and other avian species will be performed.

The vaccines to be developed would also have marker aspects which will allow the differentiation of infected from vaccinated animals (DIVA principle). The development of sensitive, specific and easy to use marker diagnostic tests that will be compatible with the vaccines is another goal of this project.

PROBLEM:

The highly pathogenic H5N1 avian influenza (AI) currently circulating in Asia, and recently in northern and western Africa and Europe, has led to the deaths of more than 150 million birds and over 150 humans. Due to the seriousness of this threat, some countries are taking steps to vaccinate their entire poultry population. Currently, a consensus is emerging that vaccination of birds at risk could be a critical part of a control strategy in averting a human pandemic.

Although very useful in the fight against avian influenza, all currently available influenza vaccines have considerable shortcomings; several vaccines developed over the past two decades to protect poultry against the highly pathogenic H5 or H7 are based on inactivated whole virus vaccines. Apart from the challenge of setting up a robust diagnostic test for differentiating vaccinated from infected animals, these vaccines have to be administered by labour intensive and expensive parenteral injections.

In view of the worrying spread of epidemic avian influenza H5N1 and the large undertaking to vaccinate billions of birds in some parts of the world, development of efficacious vaccines that could be administered by mass application routes, such as spray or drinking water, is urgently needed. In the endeavour to develop improved vaccine against AI, recombinant DNA technology was employed to generate vectored, subunit or DNA vaccines. Although a wide range of these vaccines has been experimentally shown to be effective against AI, only a fowl pox-vectored vaccine with H5 gene insert is commercially available. This recombinant vaccine, however, also requires administration by parenteral injection.

In order to better control avian influenza, more effective mass applicable vaccines are needed. For better design of future vaccines and strategies to combat avian influenza more insight is also required into the immunological mechanisms and characterisation of immune responses after vaccination and infection.
An easy to use, sensitive and specific serological test allowing the DIVA principle, which can be used in conjunction with the use of inactivated or vectored vaccines, is needed.

AIM:

An ideal vaccine to be added in controlling AI should be: i) efficacious in reducing virus transmission; ii) genetically close to the circulating virus; iii) serologically distinguishable from wild type virus; iv) applicable by mass administration routes; and v) inexpensive.

Recent achievements showed that safe and efficacious NDV vectors could be constructed that would make development of a vaccine with the above characteristics possible. NDV vectors carrying H7 respectively H5 gene inserts were recently constructed and 100% protection against clinical disease as well as considerable reduction in virus shedding was observed after challenge with NDV and AI. However, the H7 and H5 ORF used for the construction of these vectors was not derived from recent isolates and it can be expected that protection will be enhanced when using vector vaccines carrying recent H5 or H7 genes. This knowledge will be used to construct optimised NDV vector vaccines that will have advantages in terms of their improved efficacy against recent H5N1 isolates. The NDV parent strains that will be used for the construction of the recombinants are a vaccine strain that is widely used in the field for years and with a well established safety record.

Objectives of the vector vaccines constructed in the project:

a) H5 genes genetically close to the currently circulating high pathogenic Asian H5N1 viruses.

b) Safe and highly efficacious against lethal challenge with recent highly pathogenic H5N1 strains. Protection against clinical disease and reduction in excretion of the challenge virus.
c) Earlier onset of immunity and protection, because of local protection as compared to the currently available inactivated vaccines.
d) Administration via mass applicable methods such as drinking water, spray or eye drop.
e) Serologically distinguishable from wild type virus by use of an easy-to-use accompanying Elisa.

EXPECTED RESULTS:

This project is expected to deliver the first mass applicable live vaccine against highly pathogenic H5N1 avian influenza. The vaccine would offer major technical advantages, including production aspects, over the currently existing vaccines.

An easy to use, sensitive and specific serological test allowing the DIVA principle, which can be used in conjunction with the use of inactivated or vectored vaccines, will considerably enhance optimal avian influenza vaccination and control strategies.

Better knowledge of the critical pathways involved in influenza immunity and immunopathology may eventually contribute to improved vaccine design, and optimised immunisation or other intervention strategies.

The development of molecular biological tools will allow in the future a quicker response to changes in antigenicity of the field-virus. The construction of gene cassettes that will allow fast integration of the H region of future genetically and antigenically different influenza strains in the ND vector will allow a much faster response for construction of updated vaccine strains.

POTENTIAL APPLICATIONS:

Live vaccines with much easier methods of application will result in less labour and thus lower costs of vaccination. Methods of application via natural route (drinking water, spray, eye drops) are easier to perform and in certain areas could offer considerable advantages over parenteral vaccination. Less costly and easier vaccination methods will also increase compliance with vaccination campaigns and policies, especially in developing countries.

Vaccination by more natural application routes will also result in a much more animal friendly procedure, as stress due to vaccination will be considerably reduced and animal welfare greatly improved. The project will also deliver fast, sensitive, robust and specific ELISA tests. This will enable the DIVA concept. These tests can be used in conjunction with the newly developed vaccines or with existing vaccines.

The availability of easy to perform, fast and reliable test systems that could be used for mass-testing is a prerequisite for the development and successful implementation of control measures and eradication policies.

The successful use of the live vaccine and DIVA tests will offer major possibilities and tools in the fight against avian influenza and thus will have a major contribution to economical poultry farming and human health.

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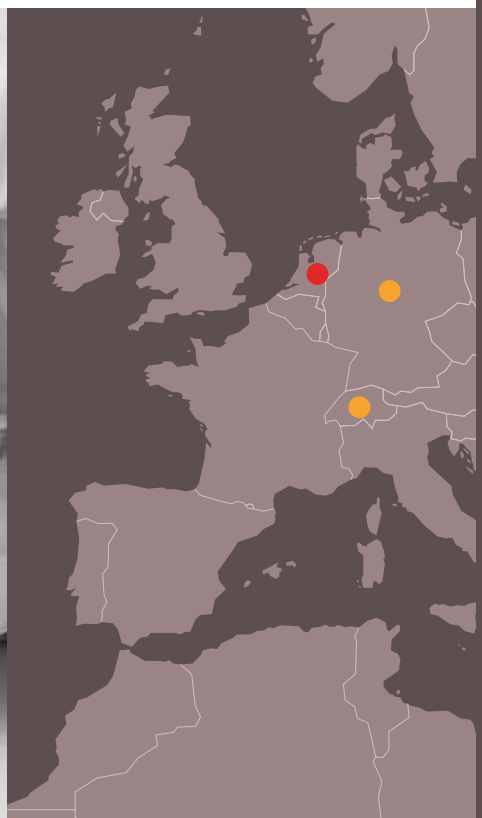

WWW.AIV-VACC-DIAGNOSIS.COM
SSPE-CT-2007-044141
AIV VACC DIAGNOSIS

DEVELOPMENT OF A COMBINED INFLUENZA/SARS VACCINE

Acronym: **SARS/FLU VACCINE**
EC contribution: €1 607 500
Duration: 36 months

Starting date: 01/01/2005
Instrument: STREP

Key words: Vaccine, SARS, influenza

SUMMARY:

The spontaneous appearance of SARS in humans in 2003 and the regular yearly appearance of influenza provoke an obvious question: could it be possible to produce a single vaccine that protects against both diseases?

A project led by an Austrian SME aims to do exactly this. Green Hills Biotechnology is well acquainted with influenza: it currently has several products in development based on genetically modified versions of the virus.

The company is now using its expertise in viral engineering to develop a combined SARS/flu vaccine. It has teamed up with three other SMEs (in Austria, the Czech Republic and Slovenia) which have added their expertise in antigen identification, preclinical testing and protein purification. The project also includes two renowned university research institutions that bring additional expertise in virology and immunology.

PROBLEM:

In 2002, an atypical pneumonia, characterised by progressive respiratory failure, emerged in the Guangdong Province in Southern China. The causative agent was rapidly identified as a new coronavirus which was designated as severe acute respiratory syndrome-associated virus SARS-CoV. The disease swept rapidly to neighbouring regions and led to several cases even in Toronto, Canada. By the end of the epidemic in July 2003, about 8 000 SARS cases and almost 800 deaths due to SARS had been recorded worldwide. Since then, the world is in an inter-epidemic period, as no new cases were reported.

AIM:

The project uses Green Hills' engineered influenza virus as its starting point. This modified virus is unable to replicate, but still expresses immunogenic influenza proteins on its surface. The sequences for relevant antigens are identified by bioinformatic methods and were verified by immunological methods. Promising antigens from the SARS-associated coronavirus will be expressed by the influenza vector so that it expresses SARS proteins too. Several constructs are tested for immunogenicity: the one that provokes the best immune response without compromising safety in animal testing is selected for a full preclinical testing programme.

EXPECTED RESULTS:

The particular properties of the modified influenza virus – apathogenicity due to abortive replication while being highly immunogenic due to its IFN-inducing properties – make it not only attractive as a safe live attenuated vaccine against influenza virus but also an attractive candidate as a vector for the expression of antigens of foreign pathogens such as the SARS-CoV. Such a SARS/flu vaccine as developed in this project has the major advantage that it has the potential to induce protective immune responses against SARS-CoV and influenza virus with one immunisation.

POTENTIAL APPLICATIONS:

The vaccine developed in this project will be a major step towards an immunisation concept against SARS-CoV and influenza virus with one immunisation. The technologies that are used in this project will have the potential to be applied to other relevant pathogens as well.

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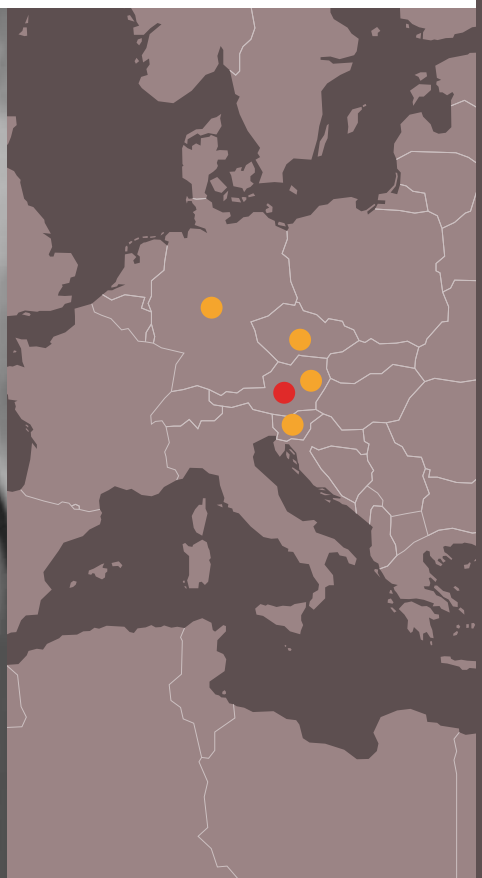

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LSHB-CT-2004-512054

SARS/FLU VACCINE

NOVEL ANTIGEN-ADJUVANT VEHICLE AS AN EFFECTIVE INFLUENZA VACCINE

Acronym: **Universal Vaccine**
EC contribution: €1 154 717
Duration: 24 months

Starting date: 01/06/2005
Instrument: SMEs - Cooperative Research Projects

Key words: Antigen-adjuvant vehicle, universal influenza vaccine, mucosal immunity

SUMMARY:

One of the biggest challenges concerning influenza vaccination is trying to keep up with the virus's mutational variation. The currently approved vaccines work by stimulating the body's immunity against the haemagglutinin and neuraminidase proteins on the virus's surface. As these proteins are prone to mutation, vaccines only induce immunity against specific subtypes of the virus. However, the influenza virus has a third protein in its outer coat, M2, and the extracellular domain of this protein, M2e, has been remarkably conserved in the amino acid sequence since human influenza virus was first isolated in 1933. If this protein could stimulate an adequate immune response it might be possible to develop a broad-spectrum vaccine against all influenza A subtypes.

Previous research has shown that when the extracellular domain of M2 (M2e) is linked to appropriate carrier particles, such as the hepatitis B virus core, it becomes highly immunogenic, inducing antibodies that fully protect mice against a potentially lethal influenza infection. Swedish biotech company Biovitrum AB has teamed up with researchers at the Flanders Interuniversity Institute for Biotechnology in Ghent (BE), who initially worked on the M2 protein, European SMEs Pepscan (the Netherlands), Proxima (UK) and Göteborg University to develop what could become the first universal vaccine for influenza. It could provide lifelong immunity against the virus and thus provide far greater protection in the event of a pandemic. It may even help to eradicate the disease in humans.

Another feature of this vaccine is its nasal administration. For respiratory diseases like influenza it is beneficial to bring the vaccine to where it is most needed to stimulate a local immune response, and nasal vaccination may help to induce stronger or more lasting immunity in recipients. Furthermore, needle-free nasal sprays are safer and easier to administer, reduce the risk of contamination – and are far less likely to deter people from participating in vaccination programmes.

The unique combination of the consortium for the rational design of a mucosal influenza vaccine is unprecedented in European vaccine research. If the universal vaccine proves successful in clinical trials it will not only help to diminish the social and economic costs of influenza, but also secure the growth and development of the European vaccine industry in the global market.

PROBLEM:

Influenza is a recurrent global threat and affects millions of people in the world every year. Vaccination constitutes undoubtedly the most cost-effective preventive measure against morbidity and death from infectious diseases such as influenza. Current influenza vaccines are based on the major influenza glycoproteins haemagglutinin (HA) and neuraminidase (NA) as antigenic determinants. However, these proteins are subject to mutation (drift) and gene re-assortment (shift) generating seasonal variations in the circulating influenza strains thereby rendering this type of vaccine less efficient in long term protection against viral infection and necessitating annual updates of the vaccine components. Furthermore, most vaccines today are injectable and use aluminium salts as their adjuvant component. However, there is general agreement that needle-free vaccines are preferable for reasons of improved patient comfort and ease of administration, as well as reducing the risk of contamination and other adverse effects while promoting compliance and increasing the safety of vaccination. Moreover, for respiratory diseases like influenza, nasal delivery brings the vaccine to where it is most needed to stimulate a local immune response, namely the respiratory tract.

AIM:

The Universal Vaccine project strives to meet this medical need by employing a highly innovative strategy that represents a conceptually novel way of rationally designing a vaccine against influenza. The aim of the Universal Vaccine project is to develop a powerful, new, safe and easily-administered nasal vaccine for humans that provides lifelong protection against influenza.

The scientific excellence and technologies within the consortium will be combined to design, develop, formulate and evaluate novel M2e-based influenza vaccine candidates with the aim of achieving improved efficacy, longer lasting protection and broad-spectrum immunity against the diversity of influenza strains, exceeding the performance of current annual influenza vaccines. In the longer perspective the project may help reduce or even eradicate influenza infections in humans.

EXPECTED RESULTS:

The development of a universal influenza vaccine is based on the identification of an extracellular domain of the minor influenza protein M2 protein, referred to as M2e. The sequence of the M2e domain has been highly conserved since the first isolation of human influenza virus in 1933, despite numerous epidemics and at least two major pandemics. Therefore, by utilising the M2e peptide as antigenic determinant a vaccine will be created that could provide long term protection against a broader spectrum of influenza strains.

The development of needle-free vaccines has been hampered by the lack of effective mucosal adjuvants. Cholera toxin (CT) and the closely related Escherichia coli heat-labile toxin (LT) are powerful mucosal adjuvants but have proved unsuitable in the clinical setting due to their inherent toxicity and potential association with Bell's palsy (paralysis of the facial nerve). A novel mucosal adjuvant, CTA1-DD, has been developed which combines the enzymatic activity of the A1 unit of cholera toxin with a dimer of the Ig-binding element of Staphylococcus aureus Protein A, thereby targeting the adjuvant specifically to B-cells and other Ig-binding cells of the immune system. This strategy has proved successful in that the CTA1-DD adjuvant is completely non-toxic and, despite its more selective binding properties, has retained potent mucosal and systemic immunoenhancing functions making it a highly suitable adjuvant for development of new, efficient mucosal vaccines.

The operational goals of the project are to:

a) covalently fuse the M2e-peptide with the CTA1-DD adjuvant, creating a strongly immunogenic influenza vaccine suitable for mucosal delivery;
b) further improve vaccine efficacy by incorporating the fusion protein in proprietary liposomes;
c) increase the *in vivo* maintenance of the antigen by using blocked or constrained peptides or peptidomimetics;
d) determine the *in vivo* mechanism of action of the mucosal influenza vaccine;
e) demonstrate the safety and efficacy of the vaccine in animal challenge models.

For reasons of safety, efficacy and cost, mucosal administration of vaccines is today a priority for immunising against mucosal as well as systemic infections. The ease of administration of needle-free vaccines facilitates their distribution to larger populations, particularly in the developing world, while improving vaccination safety and compliance. This will have great positive impact on global health and immense societal gains. The long term effect of a conceptually novel influenza vaccine will contribute to reducing and perhaps even eradicating influenza infections in humans.

POTENTIAL APPLICATIONS:

The Universal Vaccine project will play a pivotal role in the future development and competitiveness of the SMEs involved. A successful novel mucosal vaccine against influenza would have significant impact on the global market and secure growth and development of the European vaccine industry. The results of the project will be investigated for patentable knowledge and it is anticipated that the SMEs could establish themselves firmly on the market for mucosal vaccines. Their extended and potentially stronger IP would allow expansion of their respective business areas and open up new opportunities for exploitation and commercialisation.

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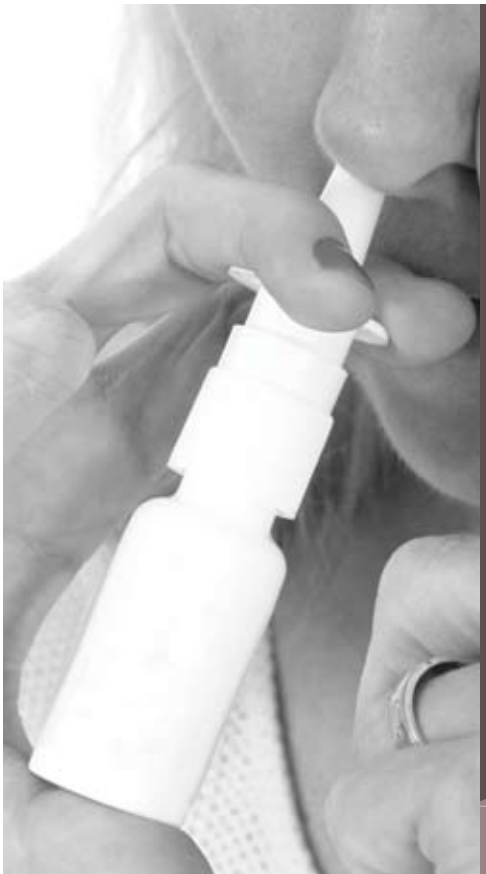

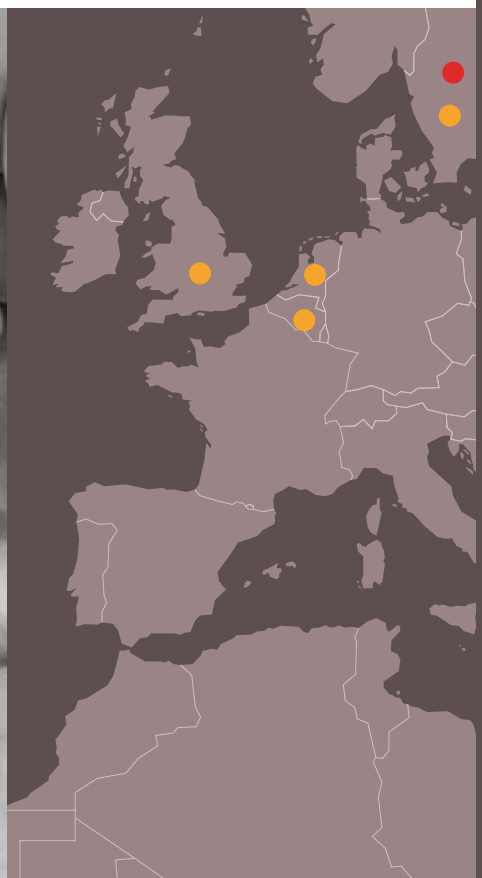

WWW.UNIVERSALVACCINE.ORG

COOP-CT-2005-017749

UNIVERSAL VACCINE

GENERATION OF INFORMATION AND TOOLS TO SUPPORT THE MANAGEMENT OF THE AVIAN INFLUENZA CRISIS IN POULTRY

Acronym: **FLUAID**
EC contribution: €1 200 000
Duration: 30 months

Starting date: 01/01/2006
Instrument: STREP

Key words: Avian influenza, vaccines, diagnostics

SUMMARY:

Avian influenza (AI) has become a great risk both for animal and human health. In five years, over 200 million birds have been affected by this disease, including estimations of the ongoing H5N1 epidemic. By bringing together both European and non-European laboratories, the FLUAID project aims to generate data on significant issues linked to AI outbreak management, about which scientific knowledge is currently lacking.

PROBLEM:

Avian influenza outbreaks have recently caused severe losses to the poultry industry, its stakeholders and ultimately to the EU taxpayer. It is estimated that since 2000, 200 million birds have died or have been culled following infection with influenza viruses subtypes H5 or H7. Approximately 50 million of these birds were from Europe. Most importantly, human infections have also been reported in several of these outbreaks. The ongoing H5N1 outbreaks are a serious concern for food security and human health on a global level, with the crossing of the species barrier representing a serious potential risk of a new human pandemic virus emerging. The increased relevance of AI in the fields of animal and human health, has highlighted the lack of scientific information on several aspects of the disease. This has hampered the adequate management of some of the recent crises thus resulting in millions of dead animals and concern over loss of human lives and over management of the pandemic potential.

AIM:

The primary goal of this proposal will be the joint development and application of novel technologies to combat AI infections. This goal will be achieved through the interaction of leading European institutes along with the active collaboration of laboratories in Indonesia, Pakistan, South Africa, Thailand and Vietnam.

EXPECTED RESULTS:

FLUAID will make available specific guidelines for the use of vaccination, including candidate strains for an EU vaccine bank and a validated companion diagnostic test. It will also develop novel, rapid and sensitive pen-side tests which will be validated by both European and non-European partners. This, coupled with improved knowledge on pathogenesis and transmission of the AI virus, will contribute to the development of more effective control measures for the disease to be applied globally. In this way European experience, knowledge and scientific achievements will gain visibility and will be used to support decision making and further research.

POTENTIAL APPLICATIONS:

The primary applications developed during this project will be related to vaccination and rapid diagnosis of AI.

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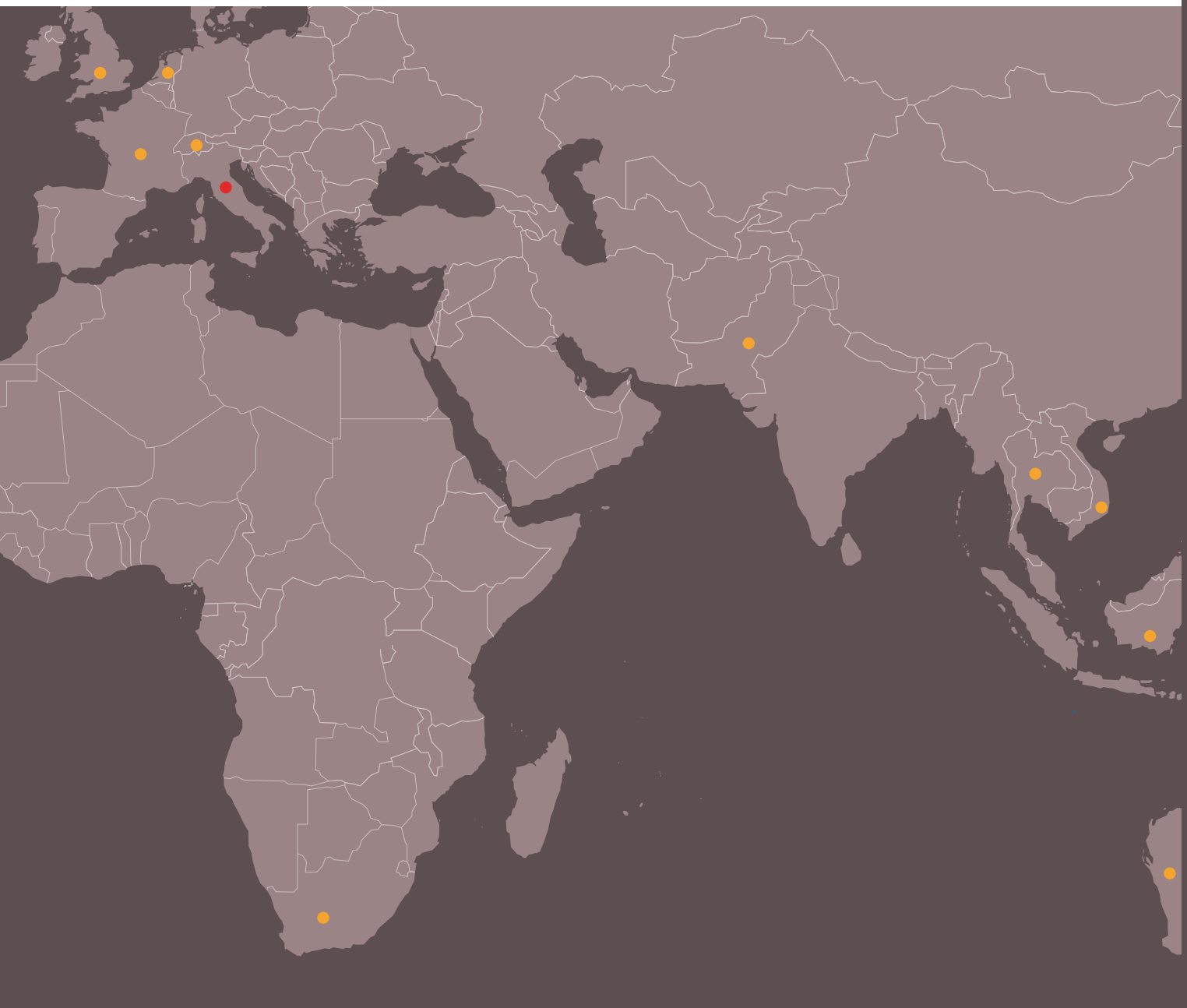


WWW.FLUAID.EU

SSPE-CT-2005-022417

FLUAID

LIVE ATTENUATED REPLICATION-DEFECTIVE INFLUENZA VACCINE

Acronym: **FLUVACC**
EC contribution: €9 200 000
Duration: 60 months

Starting date: 01/09/2005
Instrument: Integrated Project

Key words: Replication, deficient, vaccine, influenza

SUMMARY:

Green Hills Biotechnology is developing a novel vaccine against influenza in a joint project that brings together the expertise of eight different partner institutions both from academia and biotech industry in four European countries and in Russia. The ambitious project is being carried out by the international consortium over a five-year period. The FLUVACC vaccine is a novel component of European systemic efforts to prevent and control influenza, based on a replication deficient virus that is generated by a specialised technique called reverse genetics. The vaccine will be produced in cell culture.

PROBLEM:

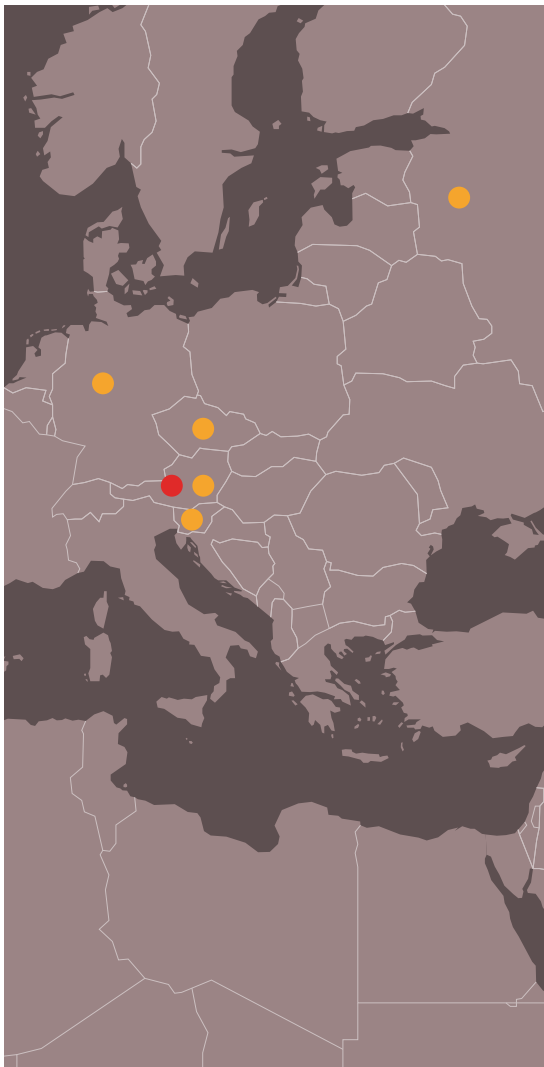
Industrial production of influenza vaccine still relies on traditional techniques. Essentially, chicken eggs are used as mini vaccine factories. They are injected with live influenza virus and incubated for several days so the virus can multiply. The egg is then opened, the virus harvested, purified and inactivated. Unfortunately, highly pathogenic avian viruses do not grow well in eggs as they tend to kill the embryo.

There are many other problems associated with egg-based production. The whole process is time intensive and hard to scale up, so that during a pandemic it may be difficult for supply to meet demand. In addition, the combination of vaccine with egg proteins can lead to allergic reactions in some people.

FLUVACC aims to shift vaccine production away from the traditional methods by generation of live attenuated-replication deficient vaccines that can be produced in cell culture. Instead of using egg-produced viral proteins, the live attenuated vaccines developed by the FLUVACC consortium contain whole replication deficient viruses that generate a strong immune response but are non-pathogenic.

AIM:

FLUVACC has improved its core technology for live attenuated vaccine production, using a technique called reverse genetics. Together with the project partners, Green Hills Biotechnology has developed a 'master strain' that is lacking the NS1 gene which is essential for productive viral replication. Candidate vaccines for emerging influenza subtypes can be quickly produced by inserting their genes into this master strain so that they express the immunogenic surface proteins, but remain replication-deficient. This master strain was adapted to grow to high titers in tissue culture, making it possible to produce large quantities in the case of a pandemic.



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EXPECTED RESULTS:

With this project the FLUVACC partners have developed, evaluated and produced several vaccine candidate strains for endemic and pandemic influenza. The vaccine candidates were evaluated in mice and ferrets, and a GMP production process based on Vero cells was developed. Clinical Phase I studies will be performed in the first half of 2007.

POTENTIAL APPLICATIONS:

The FLUVACC vaccine is as a novel component of European systemic efforts to prevent and control influenza. Influenza is a severe respiratory infection that poses a major health threat on a worldwide scale. The proposed vaccine will reduce mortality and morbidity rates, lost workdays, and hospitalisations.

FLUVACC
LSHB-CT-2005-518281
WWW.GREENHILLSBIOTECH.COM/EU_PROJECTS.HTML

DEVELOPMENT OF INFLUENZA DEL NS1 VIRUS AS A VECTOR FOR FOREIGN ANTIGENS

Acronym: **CHIMERIC VACCINES**
EC contribution: €1 384 945
Duration: 30 months

Starting date: 01/11/2004
Instrument: SMEs – Co-operative Research Projects

Key words: Influenza, avian, vaccine, adjuvant, chimeric

SUMMARY:

The project CHIMERIC VACCINES is being carried out by an international consortium over a 30-month period. The consortium aims at developing a novel vaccine that will provide protection against avian influenza and seasonal influenza. The technical basis is a replication deficient influenza virus that was modified to express foreign antigens.

CHIMERIC VACCINES is led by the Austrian SME Green Hills Biotechnology and consists of three partner companies in Austria, Slovenia and the Czech Republic and two renowned university partners in Austria and Germany. In order to bring these vaccines to clinical use, one of the partners is the Russian WHO reference laboratory for influenza in St Petersburg, which has abundant experience in testing new vaccines.

PROBLEM:

Avian influenza is an infectious disease of birds caused by type A strains of the influenza virus. The disease occurs worldwide. While all birds are thought to be susceptible to infection with avian influenza viruses, many wild bird species carry these viruses with no apparent signs of harm. A highly pathogenic form was first identified in Italy in 1878 and can lead to a mortality rate in infected animals of 100% within 48 hours.

Influenza viruses are species-specific and only rarely cause infection in other species. Since 1959, instances of human infection with an avian influenza virus have been documented on only 10 occasions. Of the hundreds of strains of avian influenza A viruses, only four are known to have caused human infections: H5N1, H7N3, H7N7 and H9N2. In general, human infection with these viruses has resulted in mild symptoms and very little severe illness, with one notable exception - the highly pathogenic H5N1 virus.

Currently, the H5N1 virus is of greatest significance as it has crossed the species barrier to infect humans on at least three occasions in recent years leading to the current outbreaks that began in December 2003. Currently, no vaccine against avian influenza is available.

AIM:

This consortium has developed a novel approach for vaccination against avian influenza. The technology is based on the insertion of selected epitopes into a genetically modified influenza virus that is apathogenic. To achieve these results, both a stable vector and promising antigens were identified. Antigen selection was based on bioinformatics methods that were substantiated by experimental validation. The properties of the backbone vector in terms of safety and stability were assessed in preclinical experiments and gave highly satisfactory results. To bring the proposed chimeric vaccine into clinical trials, a production process in small scale was established using a novel purification technology. Finally, preparations for a clinical trial in Russia were made by the Russian Institute of Influenza.

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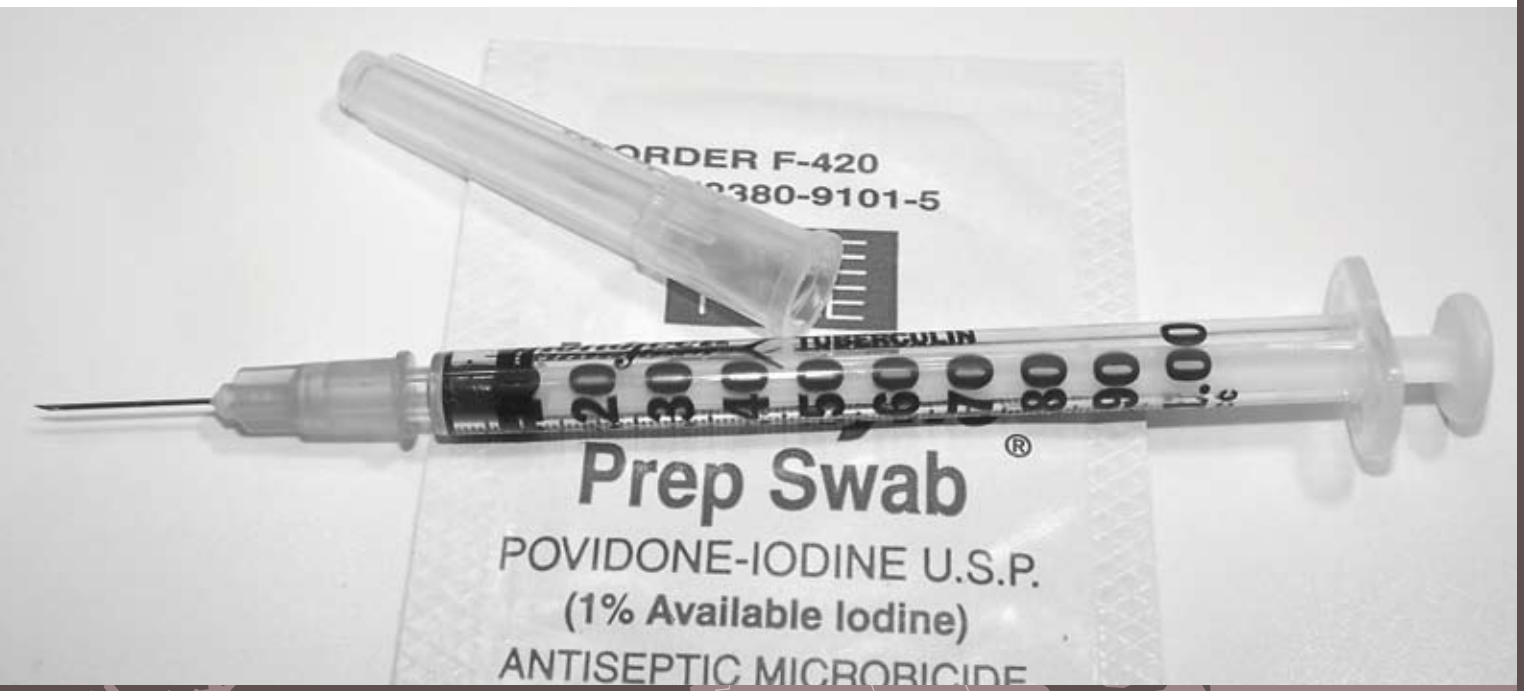
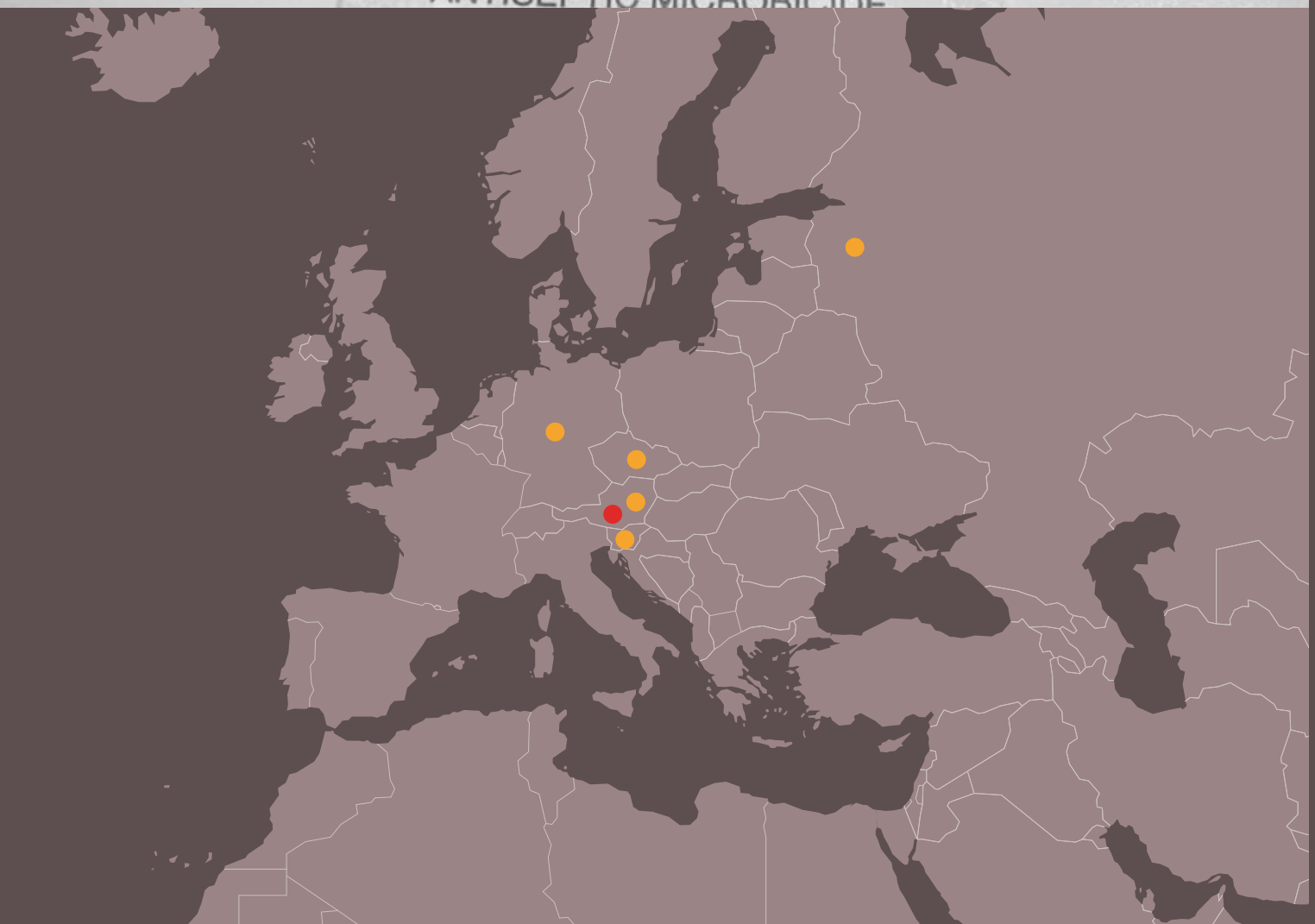
EXPECTED RESULTS:

Results of this project will shed light on important aspects in the development of novel vaccination strategies and will help to further develop the concept of a chimeric vaccine. In addition, important information regarding immunogenicity and safety will be generated and a process for the production of purified viruses will be established.

POTENTIAL APPLICATIONS:

The vaccine developed in this project will be an important step towards a vaccine that can induce an immune response against foreign antigens of pathogens. The technologies that are used in this project will have the potential to be applied to other relevant pathogens as well.





NOVEL VACCINATION STRATEGIES AND VACCINE FORMULATIONS FOR EPIDEMIC AND PANDEMIC INFLUENZA CONTROL

Acronym: **NOVAFLU**
EC contribution: €1 679 211
Duration: 36 months

Starting date: 01/10/2002
Instrument: Shared Cost Action

Key words: Influenza, vaccine, strain selection, computer modelling, cell culture, MVA, ISCOM, defective viruses, CTL, animal model

SUMMARY:

Annual influenza epidemics and pandemics cause a significant disease burden and death rates in human beings, and have a high economical impact on the EU. The major drawbacks of influenza vaccines are their limited efficacy and production methods that are hard to adapt to requirements of continuously changing influenza viruses. We aim to improve the efficacy and production methods of epidemic and pandemic influenza vaccines through:

a) Improving epidemic vaccine strain selection based on retrospective and prospective analyses.
b) Improving vaccine strain selection and reference reagents for pandemic influenza.
c) Implementation of reverse genetics technologies to increase flexibility and yield of influenza vaccines.
d) Use of cell substrate for vaccine production.
e) Use of novel antigen delivery systems.
f) Detailed examination of correlates of protection. Promising strategies were tested in appropriate animal models.

PROBLEM:

Annually occurring influenza virus epidemics, associated with antigenic 'drift' of the surface glycoproteins haemagglutinin and neuraminidase (HA and NA) of the influenza A and B viruses, cause a significant disease burden and high death rates in human beings which exceed deaths due, for example, to road accidents in Europe. Less frequent pandemic outbreaks of influenza (three in the last century) associated with the introduction of a new influenza A virus subtype from an animal reservoir - antigenic 'shift' - are even more serious in this respect, causing millions of deaths in a short period of time.

Although recently a new generation of antiviral compounds (neuraminidase inhibitors) with promising anti-viral effects have been developed, the most cost-effective and efficient way of controlling influenza remains preventive vaccination. The major drawbacks of the currently used multi-component influenza vaccines, produced in embryonated chicken eggs, are their limited efficacy and classical production methods which are hard to adapt to requirements related to 'drift' and 'shift' phenomena.

AIM:

The aim of the project was the development of more effective strategies for the vaccination of human beings against epidemic and pandemic influenza. Divided over the respective work packages four objectives were identified. These were:

a) Development of optimal strategies for vaccine selection: Computer algorithms were developed which are used for the interpretation of complex serological data and allow the easy assessment of antigenic relatedness of influenza viruses. These tools have been implemented and fully integrated in the bi-annual WHO vaccine strain selection process. In addition, these newly developed tools can be used for the antigenic characterisation of the potentially pandemic H5N1 strains. Thus in the case of a pandemic outbreak, the prior knowledge of the antigenic properties of these viruses can aid in the selection of the best vaccine strains to provide the broadest protection possible. This scenario was exercised with the avian H7N7 viruses that caused an outbreak of fowl plague in the Netherlands in 2003 (see c). Extensive surveillance of avian influenza viruses amongst wild birds resulted in a library of virtually all subtypes of HA and NA, which were used for vaccine preparation and the preparation of reference reagents to aid in the adequate diagnosis and identification of avian influenza virus infections in man and animals.

b) Development of alternative approaches for vaccine production with the recent registration of MDCK-cells as an influenza vaccine production platform and the advent of reverse genetics technology which allows the genetic manipulation of viruses. It was the objective to engineer influenza viruses and develop reverse genetics technology that would allow the rapid generation of reassortant strains that give high yields of the relevant viral proteins, the HA and the NA.

In Work Packages 2 and 3 the development of these reverse genetic systems was addressed. For the production of viruses in embryonated chicken eggs or chicken embryo fibroblasts, the avian POL1 promoter was cloned which can drive the transcription of viral RNA in avian cells. It was not possible to optimise the extremities of the HA gene segments for enhanced vaccine production yield since with a reporter gene expression system it was demonstrated that these non-coding regions are already compatible with the RNA complexes of various viruses including that of the vaccine back-bone strain A/PR/8/34. Furthermore, a universal reverse genetics system was developed based on the T7 polymerase and the T7 promotor system. This would allow the transcription of RNA in any cell type independent of the species from which the cells originated (e.g. MDCK cells).

A patent application was filed on the T7 reverse genetics system. The availability of an alternative reverse genetic system constitutes a very valuable alternative that would prevent the use of existing systems and for which the intellectual property rights lie outside the EU. In addition the use of the T7 system is better tailored for the use in MDCK cells to which EU companies have access.

c) Development of novel vaccine candidates: This was addressed in three different ways. First, recombinant MVA vectors were developed and evaluated as a novel delivery system for influenza viral proteins. MVA vectors were constructed that express the HA of two different strains of H5N1 virus. These recombinant H5N1 poxvirus vector vaccines

were evaluated in a mouse model and it was demonstrated that with these vaccine preparations protective immunity could be induced against the lethal challenge of H5N1 virus. Based on these data a patent application was filed. Furthermore, MVA were constructed expressing the nucleoprotein (NP) gene of various influenza viruses, including the H5N1 viruses. It is anticipated that recombinant MVA vaccine candidates, of which the production is independent of existing influenza vaccine production capacity, can contribute to overcoming the envisaged vaccine shortage in the face of pandemic outbreaks of influenza.

The second approach that was evaluated was the use of immune stimulating complexes (ISCOMS) as an alternative antigen delivery system. Using a combination of reverse genetics technology, avian influenza surveillance and novel vaccine strain selection procedures an H7N7 vaccine strain was prepared for the production of the viral antigens HA and NA. This preparation was adjuvanted with ISCOMS and used for the immunisation of mice, which developed virus specific antibodies and were protected against infection with a lethal H7N7 virus.

The third approach for the development of novel candidate vaccines is the use of defective influenza virus particles. Again, reverse genetics technology was exploited for the production of defective virus particles. Viruses were produced that lack one functional gene that is essential for its replication. It proved possible to produce defective particles by trans-complementation and a patent application was filed on this procedure. A vaccine based on defective virus particles is considered promising, since it is expected that it will not only induce antibody responses but also cell-mediated immunity.

d) Definition of correlates of protection: For the determination of correlates of protection a lot of emphasis was put on virus specific cytotoxic T lymphocytes (CTL). A detailed analysis was made of the interaction between influenza viruses and the human CTL response. First, it was observed that epitopes recognised by human CTL are under selective pressure, which is indirect evidence that CTL are important immune correlates in the control of influenza virus infections. The impact of variation in individual epitopes was studied on the virus specific human CTL response and it was found that a single amino acid change in an epitope affected the CTL response significantly, indicating that this variation is advantageous to the virus.

In one case a number of co-mutations was observed with a mutation in an epitope and it proved impossible to rescue virus by reverse genetics technology with a single substitution in the epitope. It was found that the co-mutations were necessary to functionally compensate for the detrimental effect of the amino acid substitution in the epitope. Also, the extent of variation of CTL epitopes was assessed and some more examples of point mutations associated with escape from CTL were identified.

Although the variation of CTL epitopes could complicate the development of vaccines that aim at the induction of virus specific CTL response, it was also found that certain conserved epitopes are under functional constraints. In a mutational analysis it proved impossible to introduce amino acid substitutions without the loss of viral fitness. This may indicate that the induction of T cell immunity against these conserved epitopes may be a feasible approach for the induction of broad-spectrum immunity, also against pandemic strains of influenza.

EXPECTED RESULTS:

Results obtained during the project were presented at national and international conferences on 91 occasions. A total of 75 publications in international peer-reviewed journals emanated from the project and three patent applications were filed.

POTENTIAL APPLICATIONS:

a) The computer algorithm for the antigenic characterisation of influenza viruses is already applied in the annual influenza vaccine strain selection process and could be used for pandemic vaccines strain selection.
b) A library of HA/NA subtypes could be used for the preparation of prototype vaccines.
c) Novel reverse genetics technology can be used for the preparation of vaccine strains in MDCK cells and CEF cells.
d) Novel antigen delivery systems (MVA, defective virus particles and use of ISCOMS) can be used for the production of effective vaccines and may aid in overcoming the envisaged shortage of vaccines in the face of a pandemic.
e) The induction of virus-specific CTL may be a way to prepare broadly protective vaccines.

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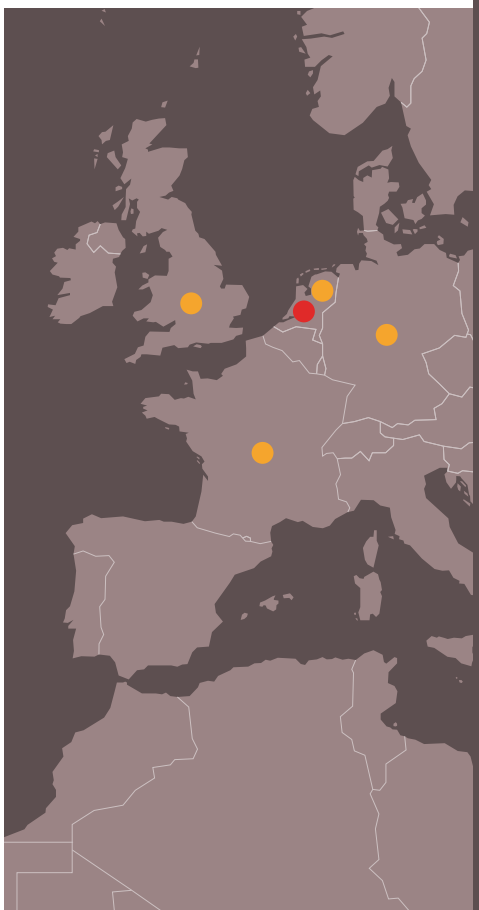

QLK2-CT-2002-01034
NOVAFLU

PREPARING FOR AN INFLUENZA PANDEMIC

Acronym: **FLUPAN**
EC contribution: €2 100 000
Duration: 36 months extended to 48 months

Starting date: 01/12/2001
Instrument: Shared-Cost Action

Key words: Influenza, pandemic, vaccine, bird flu, reverse genetics

SUMMARY:

Although the threat of avian influenza has only recently made major media headlines, Europe began developing its defences many years ago. In September 2001, a team of scientists from the UK, Italy and Norway collaborated with vaccine researchers from Sanofi Pasteur in France on the FLUPAN project. Funded through the Fifth Framework Programme, the project aimed to develop a candidate vaccine for human pandemic flu.

The partners decided to target the H7N1 avian influenza subtype which caused lethal outbreaks in Italian poultry in 1999. In 2003, 80 people in the Netherlands were infected with the related H7N7 subtype caught from poultry; one person died of the disease.

Although recent media attention has been devoted to the H5N1 subtype, researchers believe that H7 subtypes could also cause a pandemic. The deadly H7N1 strain is too dangerous for standard influenza vaccine production, so the FLUPAN scientists used a technique called reverse genetics to alter the H7 protein and make the virus safe. This process also modified the virus so that it could be grown in a mammalian cell line as well as the more usual poultry eggs. The use of a mammalian host for the virus makes large-scale production of a human vaccine easier and safer. The resulting vaccine will be the first influenza pandemic vaccine produced entirely in mammalian cells.

But the FLUPAN project has done more than produce a potential vaccine against H7 avian influenza. In other strands of this research, surveillance of avian influenza viruses in Italy has enabled the partners to build up a library of reagents which will be a valuable resource for pandemic vaccine development in the future; new tests to monitor antibodies induced by avian influenza viruses have been developed which should improve our ability to detect emergence of new pandemic viruses.

Status (January 2007):
A vaccine candidate against the H7N1 bird flu virus has been developed using reverse genetics technology and egg-free vaccine production technology. This new vaccine, called RD-3, went into pre-clinical immunogenicity studies in early 2006, and safety/efficacy Phase I clinical trial with 60 volunteers at the end of 2006. The protocols and techniques for monitoring human infections with avian influenza and carrying out clinical trials, which the FLUPAN consortium has developed will be important tools in pandemic preparedness and will provide methods that can be used to develop vaccines also against H5N1 in the event of a pandemic.

PROBLEM:

At the start of the FLUPAN project, we were ill-prepared to react to the emergence of highly pathogenic avian influenza viruses in man. Reverse genetics technology had never before been used to produce influenza vaccine viruses and there was little experience in producing and testing potential pandemic vaccines. It was vital to rehearse our pandemic preparedness by involving not only research scientists and vaccine specialists but also health and safety regulators and vaccine licensing agencies.

AIM:

To diagnose more effectively, the emergence and spread of potential pandemic influenza viruses from animals to man and to be able to respond rapidly by producing safe, effective vaccines.

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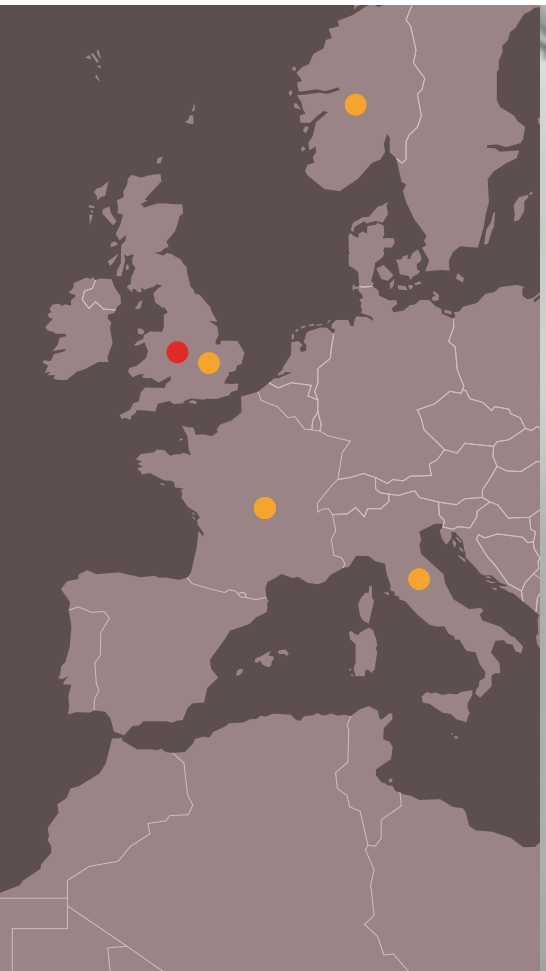
EXPECTED RESULTS:

a) Improved understanding of influenza viruses circulating in domestic and wild animals in the EU.
b) Use of reverse genetics to derive a safe vaccine virus from a highly pathogenic H7N1 avian virus.
c) Production of cell culture H7N1 vaccine for preclinical and clinical trial.
d) Demonstration of H7N1 vaccine immunogenicity and efficacy in mouse model.
e) Demonstration of H7N1 vaccine safety and immunogenicity in a Phase I/II clinical trial.
f) Development and validation of new H7N1 antibody tests.

POTENTIAL APPLICATIONS:

As the tools and techniques developed by FLUPAN can be adapted to develop new vaccines quickly and efficiently, for example against H5N1, the project has made an important contribution to Europe's pandemic preparedness. The experience gained in developing safe working practices for genetic modification of highly pathogenic influenza viruses and in devising a testing protocol for demonstrating safety of vaccine viruses derived by reverse genetics has been invaluable in producing WHO biosafety risk assessments for H5N1 vaccine development.



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QLK2-CT-2001-01786
FLUPAN

PROVE THE MUCOSAL ADJUVANTICITY OF LT MUTANTS WITH INFLUENZA ANTIGENS FOR INTRANASAL IMMUNISATION

Acronym: **MUCADJ**
EC contribution: €1 003 359
Duration: 36 months

Starting date: 01/02/2000
Instrument: Shared Cost Action

Key words: Control of infectious diseases, pre-clinical development of vaccines, transdisease vaccinology

SUMMARY:

Mucosal administration of antigens is a global priority. Many approaches have been adopted to develop vaccines that can be delivered without using syringes and that induce immune response at the mucosal sites that are often the portal of entry of pathogens. Here we propose to manufacture GMP lots of the mucosal adjuvants LTK63 and LTR72, two non-toxic derivatives of E. coli enterotoxin obtained by site-directed mutagenesis, and to test them in a clinical setting in human adult volunteers to obtain proof of concept that these molecules can be effectively used to adjuvant mucosal vaccines for human use. As a model, for this demonstration project we have chosen the influenza vaccine for intranasal administration.

PROBLEM:

Delivery of vaccines by mucosal route is one of the major goals of today's research. The advantage of easier delivery combined with the possibility of neutralising pathogens at their portal of entry have made mucosal immunisation one of the major targets of the European Union, WHO and the US NIH. Furthermore, mucosal delivery by eliminating the use of syringes would increase compliance and decrease the risk of spread of infectious diseases that has been reported by improper use of syringes during vaccination.

In spite of the obvious advantages offered by mucosal vaccination, and the success obtained in this area in animal models in recent years, mucosal vaccines are not yet a reality, and it remains unclear whether they can be used for human vaccination. It is therefore mandatory to obtain proof of concept of the feasibility in humans of mucosal vaccines.

Most vaccines are unable to induce an immune response when delivered at mucosal sites. In order to make them immunogenic, strong mucosal adjuvants are required. In animals, the most potent mucosal adjuvants are the E. coli enterotoxin (LT) and cholera toxin (CT). Unfortunately, these two toxins cannot be used in humans and therefore their use as mucosal adjuvants has been restricted to animal studies.

Recently, by site-directed mutagenesis, mutants of LT have been obtained which are either completely non-toxic or have greatly reduced toxicity. Preclinical studies have shown that these mutants still act as mucosal adjuvants when used in animal models, and that the LT mutants are generally more active than the CT mutants. Two particular LT mutants, LTK63 and LTR72, both of which are completely non-toxic or with very low residual activity showed, respectively, a good safety and adjuvanticity profile in preclinical studies, suggesting that they could be proposed for human studies.

AIM:

This project was officially initiated on February 1, 2000, and concluded its technical and experimental activities on January 31, 2003.

The key objectives of the project were to demonstrate:

a) that the genetically detoxified mutants of E. coli enterotoxin act as mucosal adjuvants and can be safely administered to humans by intranasal route;
b) that subunit influenza antigens formulated with the LT mutants induce an increased immune response at the mucosal sites and a systemic response similar to that of conventional vaccines for parenteral administration;
c) the role in humans, if any, of appropriate delivery systems to increase the immunogenicity of intranasally administered flu vaccines adjuvanted with LT mutants.

RESULTS:

The following project milestones have been achieved:

a) The LTK63 adjuvanted influenza vaccines formulations, with and without delivery system, for intranasal administration, have been developed.

b) The demonstration of safety and potency in the animal model of these vaccine formulations has been achieved by means of ad hoc toxicological and immunogenicity studies in different animal species.

c) The demonstration of safety and immunogenicity of mucosal influenza vaccines for intranasal administration in humans has been achieved by means of an ad hoc proof of concept clinical trial.

d) The demonstration in the proof of concept clinical trial that mucosal influenza vaccines enhance the immune response at the mucosal level compared to currently available vaccines in humans.

Overall, our studies have indeed shown that LTK63 can be safely used as a mucosal adjuvant for intranasal administration in humans and in addition it enhances the immune response at the mucosal level.

POTENTIAL APPLICATIONS:

The results deriving from this project will be useful to potentially open the way to the clinical development of many other mucosal vaccines, including the development of vaccines against traveller's diarrhoea, which could be targeted by recombinant LT mutants produced in E. coli. The knowledge and results produced in the course of the MUCADJ project will also be used for the development of vaccines for future pandemics such as the H5N1 influenza. To this purpose, several studies have already been started by most MUCADJ participants using a non-pathogenic variant influenza A/Duck/Singapore/97 (H5N3) virus as leading vaccine candidate combined with the adjuvant emulsion MF59TM . The key preclinical and clinical results obtained in the MUCADJ project have already been published in the following papers:

- Peppoloni S, Ruggiero P, Contorni M, Morant M, Pizza MG, Rappuoli R, Podda A and Del Giudice G. Mutants of the Escherichia coli heat-labile enterotoxin as safe and strong adjuvants for intranasal delivery of vaccines. Expert Rev. Vaccines. 2003, 2 (2), 285- 293.
- Stephenson I, Zambon M, Rudin A, Colegate A, Podda A, Bugarini R, Del Giudice G, Minutello A, Bonnington B, Holmgren J, Mills K, Nicholson K. Phase I Evaluation of intranasal trivalent inactivated influenza vaccine with non-toxigenic Escherichia coli enterotoxin and novel biovector as mucosal adjuvants, using adult volunteers. Journal of Virology 2006; Vol 80 (10): 4962-4970.

On the whole, these findings have important implications for the rational design of vaccines for future pandemics.

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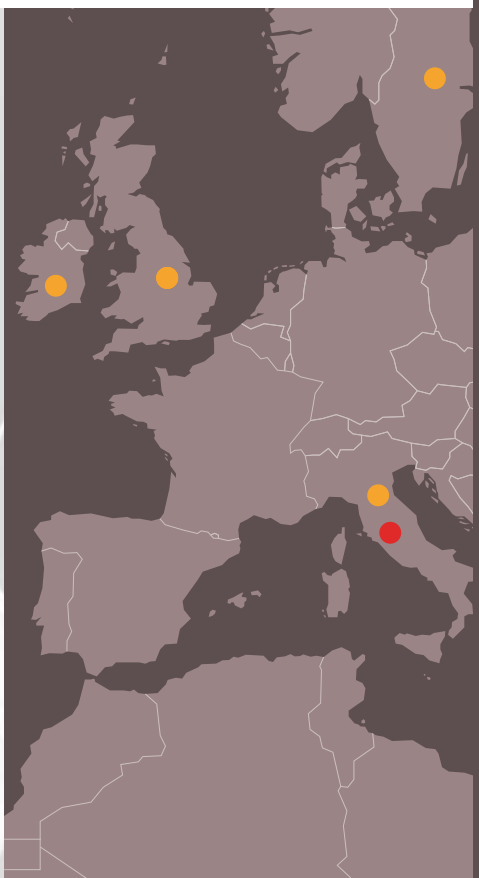

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QLK2-CT-1999-00070

MUCADJ

COMBATING FLU IN A COMBINED ACTION BETWEEN INDUSTRY AND THE PUBLIC SECTOR IN ORDER TO SECURE ADEQUATE AND FAST INTERVENTION IN EUROPE

Acronym: **FLUSECURE**
EC contribution: €3 749 685
Duration: 36 months

Starting date: 01/02/2006
Instrument: Public Health Programme

Key words: Pandemic influenza, public-private partnership, cross protection, reassortants, adjuvants, vaccine efficacy, clinical network

SUMMARY:

New or deliberately released viruses are a continuing serious health threat for which no effective countermeasures are available. The main problem is that no vaccine can be produced in advance as the exact nature of the virus is not known until the outbreak starts. Pandemic influenza is now felt to be the major health threat, especially with respect to the difficulties in effective preparedness planning. As pandemic influenza is a cross-border problem, it needs a cross-border solution. This requires that European industry and public health authorities collaborate to gain control over it. As an overall objective this project aims to 'enable the manufacture of the most effective pandemic vaccine in the shortest possible time in sufficient quantity for the EU population, by setting up a European network of public health institutes as the public sector input for a public-private partnership'.

PROBLEM:

Pandemic influenza currently is the greatest infectious diseases threat to public health. The establishment of a public consortium is essential for assisting manufacturers in the timely production and testing of influenza vaccines for the European population.

AIM:

a) To enable the production of the most effective pandemic vaccine in the shortest possible time in sufficient quantity for the EU population.

b) To establish a European network of public health institutes for a public-private partnership on European pandemic influenza vaccines.

c) To enhance vaccine preparedness in the case of an influenza pandemic.

EXPECTED RESULTS:

a) The establishment of a process of continuous dialogue with vaccine manufacturers to ensure that the assets of the public and private sectors are used to the best advantage to increase the availability of vaccines to the European population.

b) A decrease in the lead time of vaccine production by establishment of a library of safety-tested reference influenza strains available to industry.

c) An improvement in the efficacy of pandemic influenza vaccines in terms of formulation, adjuvants and dosing regimens.

d) Quantification of vaccine efficacy by establishment of cross protection and advanced correlates of protection.

e) Evaluation of the pandemic vaccines in use by providing a strategy to set up a multi-centre clinical trial network and post marketing surveillance. This will assist industry in producing efficacy and surveillance data in the event that vaccines are produced and utilised under the mock-dossier scheme.

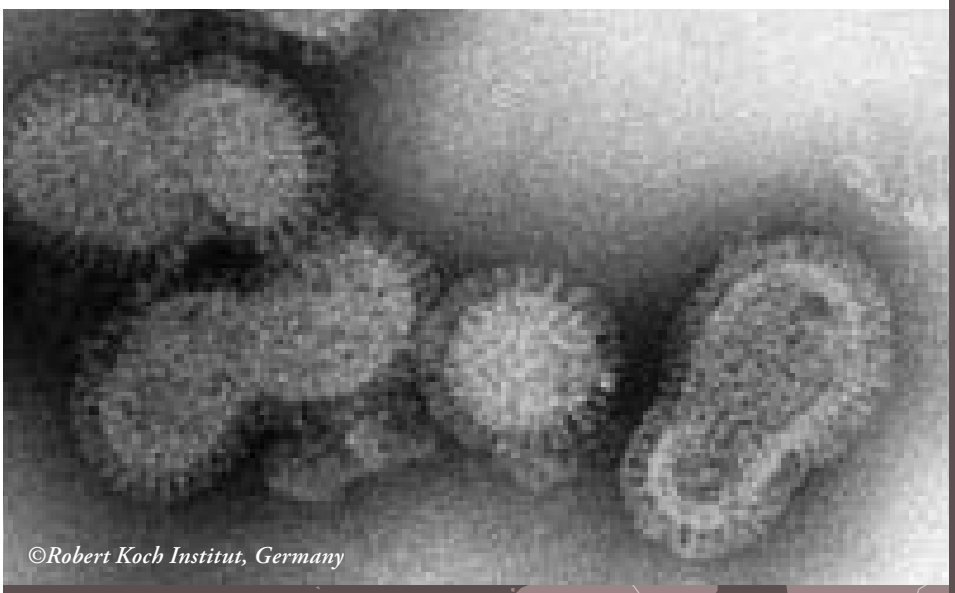
POTENTIAL APPLICATIONS:

a) Creation of a library of tested reassortants and reagents which will be available for direct vaccine production.

b) Optimised vaccine formulation for influenza vaccines in terms of formulation, adjuvants and dosing regimen.

c) Quantification efficacy assays for cross protection and advanced correlates of protection.

d) Multi-centre clinical trial network and post marketing surveillance network available for pandemic vaccine studies in Europe. Provides a platform for public (governments and academia) and private (vaccine manufacturers) studies on pandemic vaccines.



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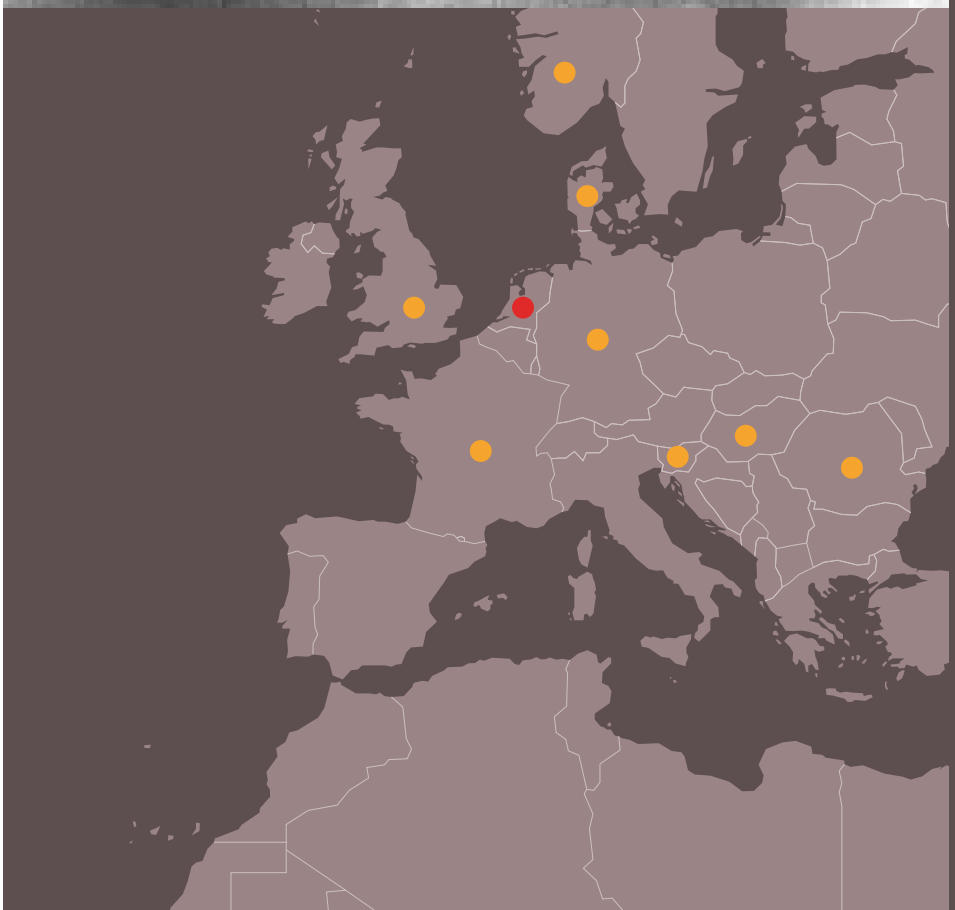

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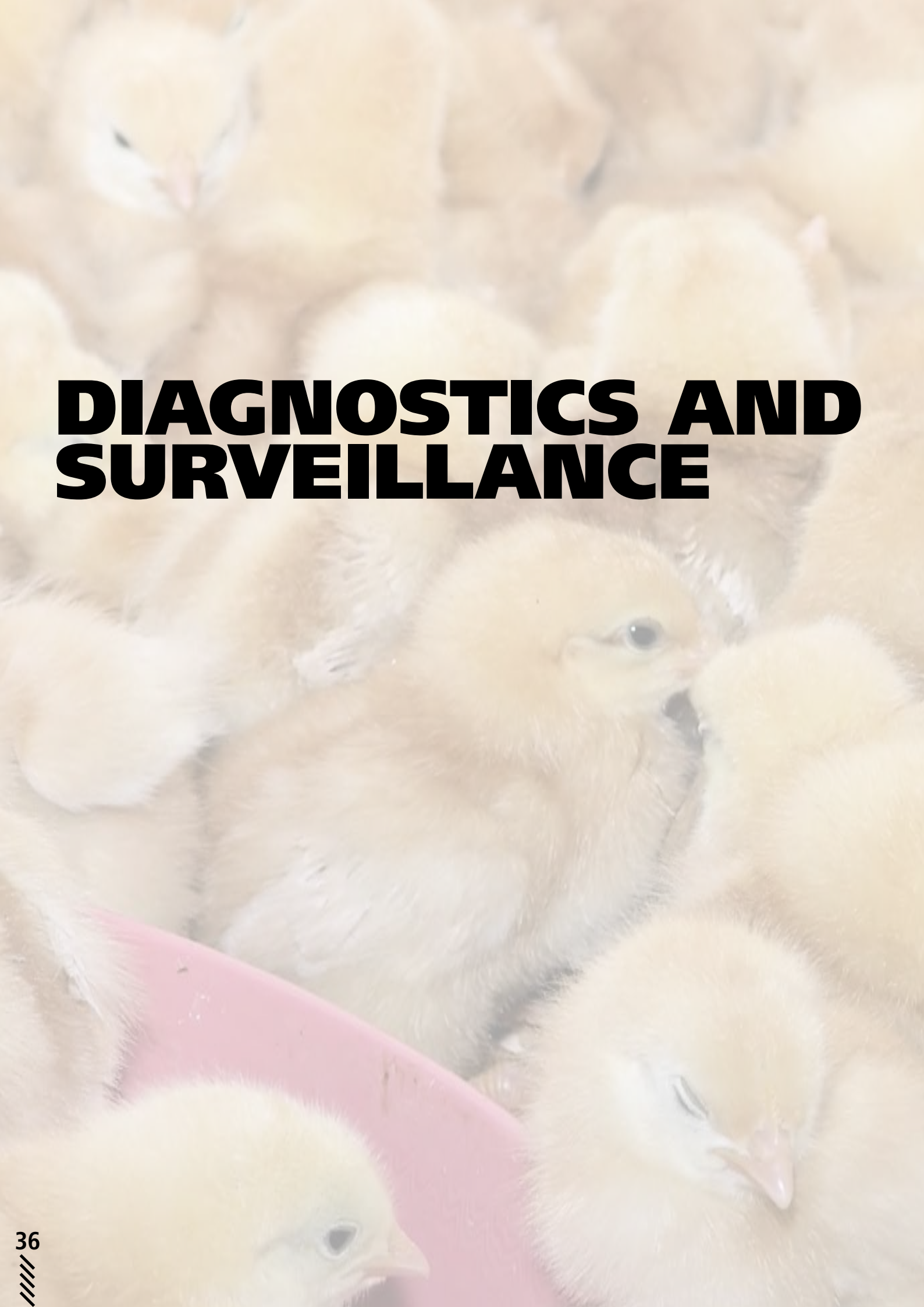

DIAGNOSTICS AND SURVEILLANCE

Development and standardisation of methods to detect and identify influenza viruses are essential to ensure the efficient diagnosis of influenza and to monitor spread of the virus in animal and human populations. This knowledge is, in turn, essential for the earliest possible intervention steps in control of the disease.

A broad range of different diagnostic tools is already available, ranging from direct antigen-detection and molecular assays such as (real-time) PCR to a variety of different serological tests that detect the response of the infected host. Two important problems that need to be tackled by researchers, however, are a) the adaptation of these methods to the strain H5N1, which has pandemic potential and is not well detected by several routine methods and b) the robustness and high-throughput use of these tests in 'field' or point-of-care conditions. These problems apply to both the animal and human health field. While during a true pandemic, health services are likely to have to rely on clinical diagnosis alone (also called 'syndromic triggering'), robust diagnostic tests are crucial in the early phase of a pandemic for surveillance purposes.

Mapping the spread of an epizootic (epidemic in animals) is a key part of targeting control measures such as culling or vaccination of animals as well as specific awareness-raising and training of the population in the affected area. Similarly, epidemiological surveillance in humans is a prerequisite not only to monitor potential outbreaks, but also as a basis for a number of important research questions, such as the seasonality of 'normal' influenza epidemics or its spread into different population and age groups. Sophisticated multi-parameter modelling becomes increasingly important in linking existing data and developing different scenarios for future outbreaks.

The vast majority of projects funded to date in the field of diagnostics and surveillance originate in the animal health community – and it will be the task of future calls for proposals to fill the respective gaps in the area of human health: **NEW-FLUBIRD** unites the expertise of virologists and ornithological organisations worldwide to study the contribution of migratory birds to the spread of avian influenza. **FLURESIST** and **RIVERS** examine the key public health question of how long influenza viruses can survive and be detected in animal carcasses, commodities and the environment. **FLUTEST**, **LAB-ON-SITE** (which also considers a number of other animal diseases) and **AVIFLU** (which also includes vaccine research) all focus on the development of sensitive and robust diagnostic tests. **ESNIP** and **ESNIP2** are making important contributions to the diagnosis and surveillance of influenza infections in pigs – taking into account their role as potential 'mixing vessels' for human and avian viruses. The European Influenza Surveillance Scheme (**EISS**), funded through the Public Health Programme, has established an important Europe-wide surveillance network for human influenza infections.

IMPROVED DIAGNOSIS AND EARLY WARNING SYSTEMS FOR AVIAN INFLUENZA OUTBREAK MANAGEMENT

Acronym: **FLUTEST**
EC contribution: €1 502 880
Duration: 36 months

Starting date: 01/02/2007
Instrument: STREP

Key words: Avian influenza, poultry, highly pathogenic, diagnostic

SUMMARY:

The primary goal of this proposal will be the joint development and application of technologies to combat avian influenza (AI) infections. This goal will be achieved through the interaction of leading European institutes along with the collaboration of non-EU laboratories experienced in AI outbreak control and management.

A study will be conducted to establish the effectiveness of the current EU surveillance and early warning systems for AI and then to develop blueprints for improvements to these programmes in disease-free periods and during outbreaks. The model will include criteria for harmonised diagnostic tests for on-farm outbreak investigation.

To complement this study a range of diagnostic tools will be developed, evaluated and validated alongside the evaluation of a range of commercially available tests. This will include sophisticated laboratory based methods, high throughput techniques for molecular and serological testing, penside testing and simplified tests for use in laboratories with limited resources or experience. Efforts will particularly focus on the validation of tests for use on clinical materials derived from Anseriformes, other wild bird species and some selected mammalian species.

PROBLEM:

In recent years, Avian influenza (AI) outbreaks have caused severe losses to the poultry industry, its stakeholders and, ultimately, to the EU taxpayer. In addition, the ongoing Asian H5N1 outbreak is a serious concern for food security and human health. It is estimated that since 2000, more than 200 million birds have died or have been culled following infection with influenza viruses subtypes H5 or H7. Approximately 50 million of these birds were from Europe. Importantly, human infections have also been reported in several of these outbreaks. In Asia, due to both social conditions and the particular characteristics of the H5N1 virus, the crossing of the species barrier represents a serious potential risk of a new human pandemic virus emerging.

AI is a highly contagious trans-boundary animal disease, able to spread in a susceptible population in a short period of time. Therefore, the prompt identification of infected animals is crucial for control and eradication purposes. Surveillance must be targeted to appropriate areas and species, and diagnostic tests must be appropriate for the setting in which they will be used, be properly validated and 'fit for purpose'.

AIM:

This project aims to generate data on significant issues linked to AI surveillance and outbreak diagnosis and management, on which scientific knowledge is currently lacking. To complement this we will also develop and validate laboratory tests that can be used as tools in early warning systems and surveillance programmes for AI, in the presence and absence of vaccination. Protocols will be harmonised and applicable to surveillance of wild birds and to different areas of the poultry industry.

EXPECTED RESULTS:

We will establish comprehensive, harmonised, validated AI surveillance, 'early warning' and diagnosis protocols, based on an intelligent framework, spanning different poultry populations and industry sectors in the EU. This will provide an appropriate surveillance system model for the EU, with capabilities extending beyond the immediate remit of establishing optimal surveillance strategies.

Such a dynamic surveillance model can also be utilised to provide decision-support mechanisms for disease control policies and enhanced analysis of novel, emerging test technologies and emerging threats or variables that may be encountered in

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the future. A report will be prepared describing the quality of the current EU surveillance programme and blueprints for improvement of surveillance programmes. A description of the poultry population and production sector demographics across European Union Member States will be provided and the average time between virus introduction and detection and the average number of infected flocks at the time of detection, given the current EU surveillance programme, will be established. Then we will determine the influence that factors such as vaccination, sensitivity and specificity of the test, sample sizes, sampling interval and frequency of clinical inspection have on these parameters. In addition, factors such as the efficient capture and use of data in the face of an outbreak and the optimum distribution of resources for surveillance and for action on disclosure of the first detected case will be evaluated. This will enable us to establish the optimal lay-out of surveillance programmes, developed 'fit-for-purpose' for different conditions between EU Member States, using different tests available and/or developed in FLUTEST.

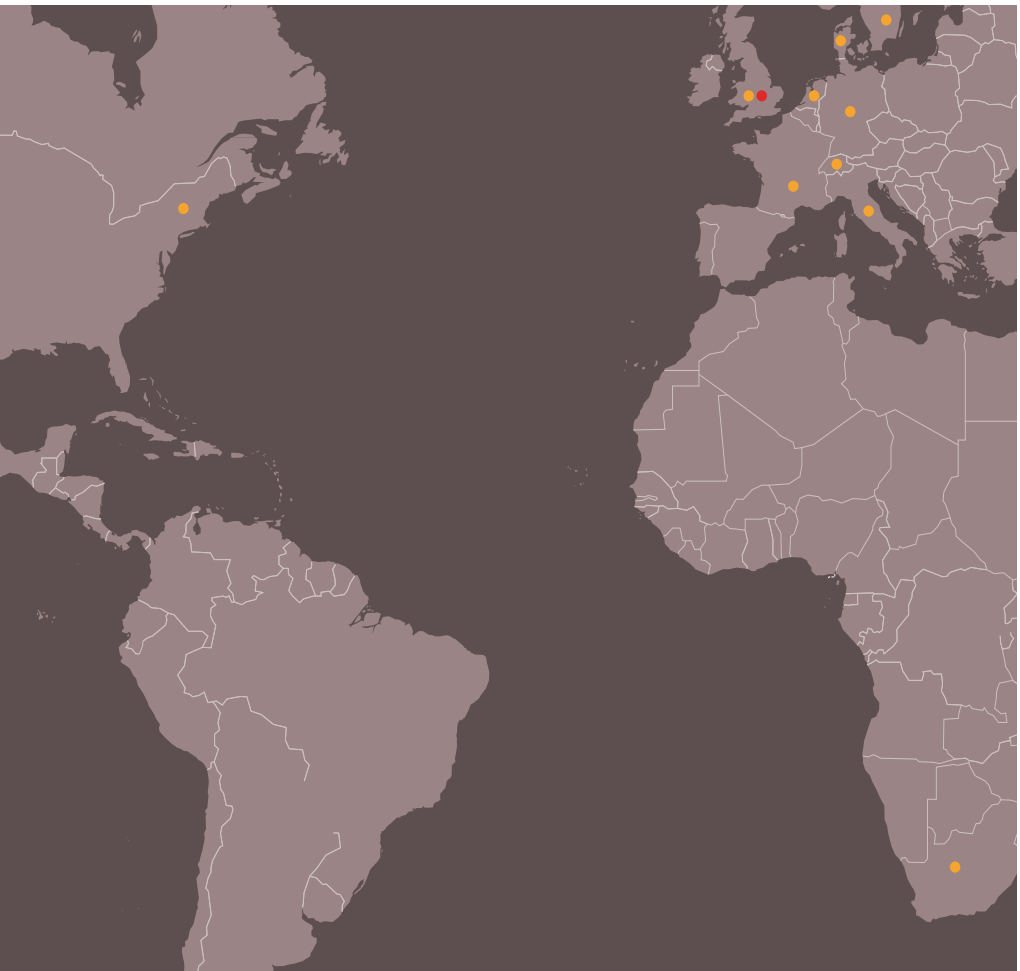
FLUTEST will address the issues of molecular diagnostics, antigen detection and novel technologies. The purpose is to focus efforts on the development, evaluation, application and harmonisation of these novel molecular techniques, for detection and differential diagnosis of AI virus infections in domestic and free-living avian populations. The assays will be internationally standardised and validated, by following the OIE rules of assay validation, in order to harmonise and standardise the diagnosis of avian influenza across the EU and to provide tests according to to the standard specified in the EU diagnostic manual. Where appropriate, the assays developed will be transferred to portable formats, trialled between partners and 'in field'.

Experience has shown that introduction of HPAI strains to poultry may not be reported immediately and the situation is exacerbated for LPAI strains. Serological surveillance programmes are not primarily suited to detect an infected flock at a very early stage based on the time lag for detectable seroconversion to occur following exposure and challenge. However, a high-test frequency can counter this disadvantage. Using blood samples at high frequency of testing is impractical and not cost-effective, but the measurement of AI antibodies in egg yolk could offer many advantages. A laboratory test to detect AI antibodies in eggs will be developed, validated and automated to make it cost-effective for use in surveillance programmes.

Many laboratories experience difficulties when subtyping AI isolates with success, influenced by the reagents and the expertise required when interpreting the test results.

A competitive lateral flow device (LFD) will be developed that will subtype the virus isolates. This will involve the use of recombinant antigens and phage display antibodies. An LFD capable of detecting virus specific antibodies will be developed in conjunction with an antigen sub-typing LFD, as both benefit from utilisation of the same recombinant antigens/peptides. This test could have applications for DIVA surveillance when vaccination strategies are employed.

We will also explore innovative technologies for their applicability as avian influenza diagnostic tests. DNA microarrays are powerful tools for the detection and comprehensive characterisation of viruses. They enable multi-agent detection and hence differential diagnosis. However, the optical detection is expensive and difficult to implement in portable instrumentation. An AI biosensor based on microarrays, but using label-free electrical detection of DNA hybridisation, and a novel sensing mechanism employing low-temperature polycrystalline silicon-thin film transistors (LTPS-TFTs) technology will be developed. The advantages of this technology are cost (two to three euro per device), portability and rapid results, typically in less than 15 minutes. Thus it would be ideal for field use and point-of-care diagnosis.



POTENTIAL APPLICATIONS:

FLUTEST specifically addresses areas of knowledge which are currently lacking and unexplored and that are crucial to the attainment of high levels of animal health and welfare and consumer protection. In addition, the outcomes of the research will support some of the unanswered questions that are of pivotal importance in the management of the globally expanding impacts and threat of avian influenza.

The data generated will aid decision makers within the European Commission with regard to the prevention and control of epizootic disease of poultry. The project addresses the needs within the EU for sustained improvements in animal health and welfare standards, particularly for a disease which has resulted in high economic losses and poses potential risks to human health.

A key strategic impact of FLUTEST will be the development and provision of a range of validated diagnostic tests tailored to complement surveillance and outbreak management programmes. Techniques will include novel, simple, user-friendly and inexpensive pen-side tests to provide cost-effective, biosecure, point-of-care surveillance and diagnostic test capabilities, tools which can be readily used in the field by veterinarians, abattoirs and small laboratories globally. FLUTEST will therefore provide for validated, front line diagnosis of potential disease. These tests will be complemented and supported with 'high throughput' and rapid assays for use in well equipped laboratories for surveillance and differential diagnosis.

This presents a strategically fundamental factor in the fight against AI. With the availability of such tools, rapid diagnosis is made possible, spread of infection can be limited and all necessary control and/or eradication procedures can be implemented more rapidly.

NETWORK FOR EARLY WARNING OF INFLUENZA VIRUSES IN MIGRATORY BIRDS IN EUROPE

Acronym: **NEW-FLUBIRD**
EC contribution: €1 855 350
Duration: 36 months

Starting date: 01/02/2007
Instrument: STREP

Key words: Influenza, pandemics, vaccines

SUMMARY:

NEW-FLUBIRD will establish a European network of virologists and ornithologists, data managers, epidemiologists and modellers, in order to provide 'early warning and risk assessment systems' in real time for the threat posed to animal and human health by avian influenza (AI) viruses from migratory birds. The network will largely build on and extend existing collaborations between AI virologists in Europe and international ornithological organisations active within and outside Europe including Africa, the Middle East and Eastern Europe.

Epidemiological assessments will thus cover the major flyways of migratory birds over Europe and the areas from which birds in Europe migrate. Furthermore, it will focus on experimental infection of selected migratory bird species with HPAI virus H5N1 and possibly other relevant HPAI viruses, to determine pathogenesis and excretion profiles. In turn, the ornithological studies will construct migratory route maps and set up systematic sampling from healthy wild migratory birds thus providing insight in volume and timing of migration as well as key sites of those migratory bird species that pose the highest risk of transmitting HPAI viruses to poultry in Europe. Finally, NEW-FLUBIRD will seek integration with global early warning systems developments like GLEWS of FAO and WHO (Global Early Warning System) and GNAIS of WCS (Global 3 Network for Avian Influenza Surveillance).

PROBLEM:

The threat posed to animal and human health by AI viruses from migratory birds.

AIM:

The NEW-FLUBIRD project, initially based on the work carried out in the framework of the FP5 NOVAFLU project and also on the FP6 EDEN and the French MigrAv project networks, will be extended with additional sampling sites positioned within Europe and along migratory routes to Europe in areas that represent a relatively high risk to Europe since they harbour migratory birds that migrate to Europe, like in Russia, the Middle East and Africa. This will be implemented by a network of institutes involved in water bird monitoring and research under the lead of Wetlands International, involving Station Biologique Tour du Valat (Camarque, France), Wildfowl and Wetlands Trust (Slimbridge, UK), Oiseaux Migrateurs du Palearctique Occidental (OMPO, France), CIRAD (France) and offices of Wetlands International in Wageningen, the Netherlands (HQ), Kiev (Ukraine), Moscow (Russia) and Dakar (Senegal).

In addition, the network will interconnect with other networks covering similar activities in the Americas and Asia though collaborative activities of participants of the NEW-FLUBIRD network. The implementation of the project has a practical general target: the urgent development of 'early warning and rapid response systems' to be used by the relevant EU and other international organisations as well as policymakers in EU Member States involved in combating animal and human influenza threats posed by AI viruses from migratory birds.

Scientific and technological objectives of the project

The scientific objective of the project is to establish a multidisciplinary network for 'early warning and risk assessment' in real time for influenza viruses in migratory birds in Europe. The project aims to do this by gaining insight into the role of migratory birds in the possible spread of AI viruses to and among poultry and mammals in Europe with a strong emphasis on the current threat posed by the HPAI virus H5N1.

To this end the NEW-FLUBIRD network of influenza virologists, ornithologists, data managers, epidemiologists and modellers will be established. Specific attention will be paid to the selection of surveillance sites on the basis of geographical distribution, risk estimation, the volume of migrating birds of different species and behavioural aspects of birds related to possible contacts with domestic poultry. Surveillance sites will be provided with the appropriate equipment needed to expand wild bird surveillance for AI and standardised techniques related to sampling and laboratory testing for AI viruses including quantitative real-time PCR and sequence analyses, as well as reporting bird morbidity and mortality.

In addition, wider scale mortality monitoring will be implemented on the basis of existing logistics in the framework of the International Waterbird Census (IWC), coordinated by Wetlands International. In Europe alone this global scale monitoring scheme for water birds results in over 25 million water birds counted from over 10 000 sites annually. Systems will be put in place for real time reporting of mortality from this network. Observations on interaction between wild birds and poultry in and around the various poultry management systems and situations in Europe will be obtained as a contribution to the assessment of risks. Data managers, epidemiologists and modellers will use well-established and standardised tools and techniques. Standardisation of techniques will be achieved by targeted training courses for all participants involved in the respective areas, and by providing the laboratories and sites with standardised materials and equipment.

In addition, on the basis of the currently available and emerging data from the project, experimental infections with a recent HPAI H5N1 virus isolate (e.g. turkey isolate from Turkey) will be carried out in a high security laboratory setting (BSL 3+) in at least nine wild bird species considered to pose a possible risk to domestic poultry in Europe. Clinical signs, (histo)pathology, immune histochemistry (IHC) and H5N1 virus excretion profiles will be monitored in these species.

Collectively, the data generated by the bird surveillance activities and by the experimental bird infection experiments will form the scientific basis for the main objective of this project: creation of real time early warning and risk assessment

system as regards the role of migratory birds in spreading AI viruses to and among poultry and mammals in Europe.

EXPECTED RESULTS:

The NEW-FLUBIRD network will establish 'early warning and risk assessment systems' in real time for the threat posed to animal and human health by AI viruses from migratory birds.

Exploitation and dissemination of the results of these systems will be communicated on real time basis to EU organisations like DG-SANCO, ECDC, WHO (regional office, Copenhagen) UNEP and European chief veterinary officers as well as international organisations like OIE (Paris) and FAO (Rome). In addition, ministries of agriculture and public health of Member States will also be directly informed about the threats and risks as they emerge. To this end, these organisations will be part of a direct AI early warning and risk assessment mailing system and the data will be accessible by all the participants and the organisations via the shielded part of the NEW-FLUBIRD website that will be created specifically for this purpose. All the data generated by the project will be published in the peer reviewed international scientific literature, after securing intellectual property rights as appropriate. In addition, the public part of the website will inform people about the outcome of those parts of NEW-FLUBIRD that, according to the project's intellectual property management rules, can be made public.

The added value of carrying out the work at the European level is illustrated by the participation of scientific groups from nine EU Member States and motivated by the specific expertise of each of the partners in the areas of virology and/or ornithology or in the areas of data management, epidemiology and modelling. In addition, the relevance of the sampling sites of migratory birds in Europe and in areas from where birds migrate to Europe has motivated the inclusion of several of the partners. Furthermore, several of the partners are involved in other projects covering the same subject in the Americas, Africa, the Caucasus and Eurasia, thus allowing the NEW-FLUBIRD network to integrate global 'early warning and risk assessment systems' as they emerge.

POTENTIAL APPLICATIONS:

Early warning and risk assessment systems will be used by policy-makers, as input for decisions and control measures for AI viruses coming from migratory birds.

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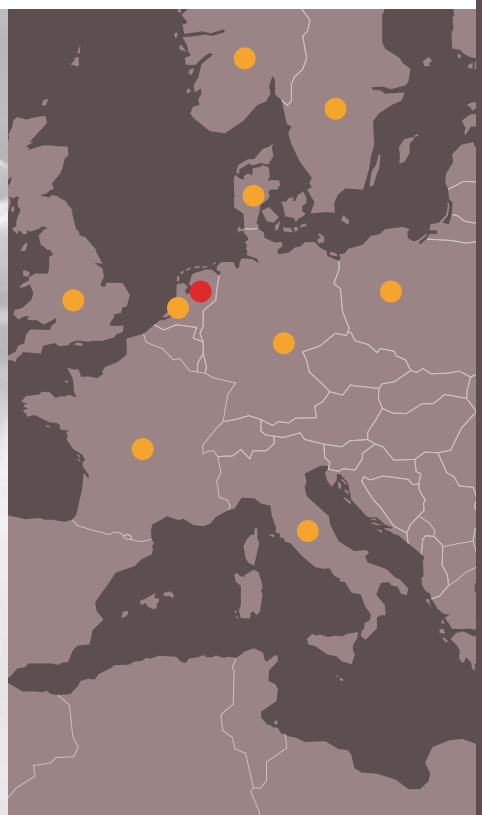


SSPE-CT-2007-44490

NEW-FLUBIRD

AVIAN INFLUENZA VIRUS SURVIVAL IN POULTRY COMMODITIES, POULTRY MANURE AND THE ENVIRONMENT

Acronym: **FLURESIST**
EC contribution: €870 000
Duration: 36 months

Starting date: 01/03/2007
Instrument: STREP

Key words: Avian influenza, poultry commodities, virus survival, bio-security measures, disinfection, cleansing operations, public health risk

SUMMARY:

Avian influenza (AI) outbreaks have recently caused severe losses to the poultry industry, its stakeholders and, ultimately, to the EU taxpayer. In addition, the ongoing Asian H5N1 outbreak is a serious concern for food security and human health worldwide.

In Asia, due to both social conditions and the particular characteristics of the H5N1 virus, the crossing of the species barrier represents a serious potential risk of a new human pandemic virus emerging. Evidence is growing that HPAI H5N1 is not only spreading by trade but is also carried by wild birds. H5N1-infected wild birds, mainly water fowl, have recently been detected in the European Union in Italy, Austria, Germany, France, Greece, Sweden and Poland. These findings are raising our awareness that H5N1 is becoming more and more endemic in wild birds. The finding of a cat, stone marten and raptors that died as result of infection with H5N1 has uncovered the consequences of this development.

More questions are being raised about the risk of contamination of surface water in relation to the health of other animals and humans. To answer these questions and to be able to assess the risks involved in trading in poultry commodities and litter, more knowledge about virus content of commodities, the stability of the virus in these products, in litter and the environment is needed.

PROBLEM:

The circulation of the HPAI virus in Asia and now also in the Middle East and Africa could represent the origin of a pandemic virus for humans, and a great number of questions have been raised with a view to finding a way to combat the ongoing AI crisis. Due to the lack of field and experimental data certain questions on virus survival in the environment and in poultry and other avian commodities are not yet answered and these knowledge gaps should be filled following the results of the ongoing and new research efforts of the scientific community.

AIM:

The aim of the project is to obtain data and provide knowledge about the presence of influenza viruses in commodities and litter of infected poultry. In order to develop validated protocols for cleansing, disinfection and treatment of litter and to be able to assess the risk of carcass disposal, treated litter, and poultry commodities such as meat, feathers and eggs, the virus survival will be determined in a standardised manner in different environments. The project also aims to create knowledge about environmental factors that influence virus stability. Data collected will be used for proper risk assessment of the trade in treated and fresh poultry commodities, poultry litter and the contaminated environment.

EXPECTED RESULTS:

Research will provide data on survival of different avian influenza viruses, and the effect of physical parameters such as pH and temperature on survival. Virus concentrations in poultry commodities such as meat, feathers and eggs will be determined. Studies will generate quantitative data on survival of viruses in these commodities at ambient temperatures and at temperatures used to treat poultry products.

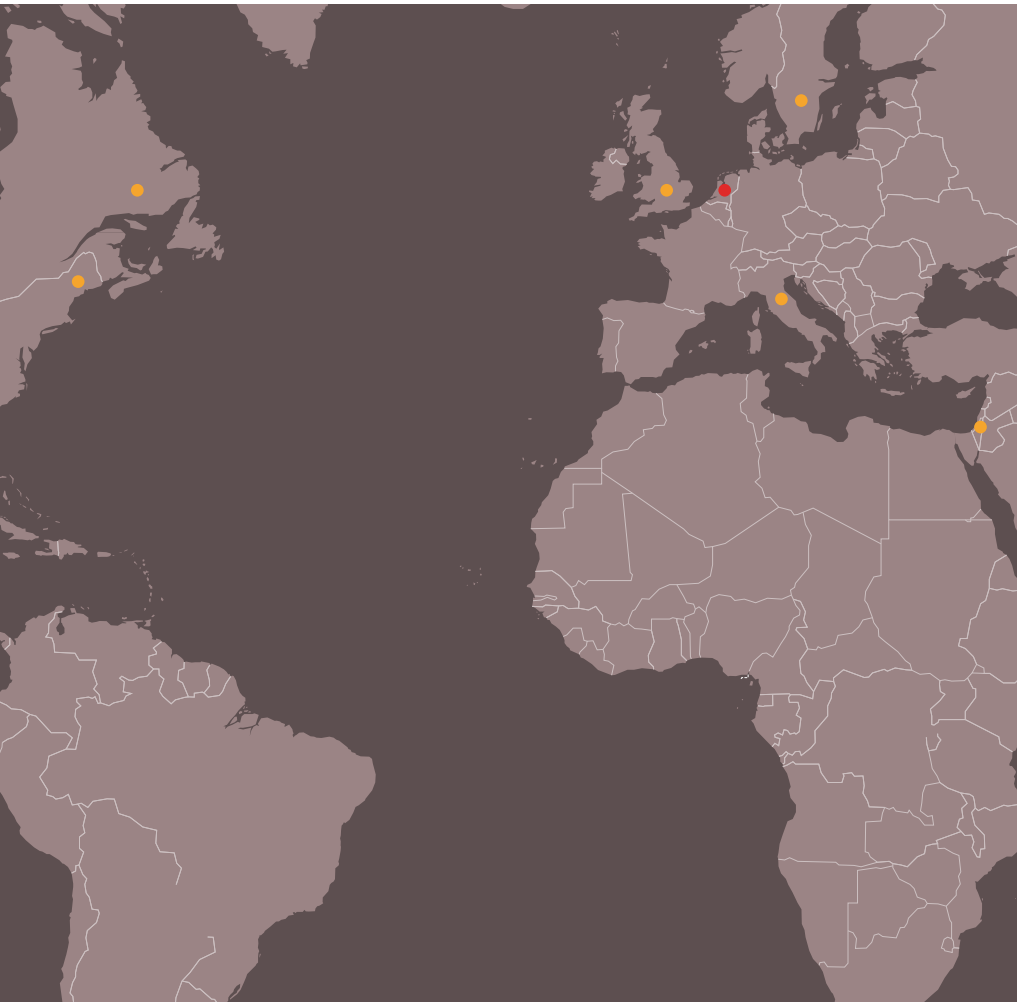
Furthermore, knowledge will be obtained on whether certain soils, lake silts and living organisms, enteric factors of waterfowl, sterile faeces or gut flora and sewage pollution increases or decreases virus survival. Based on the results protocols for waste treatment, carcass disposal and disinfection will be adapted and or validated. Data will be used to make proper risk assessments.

POTENTIAL APPLICATIONS:

The current threat from avian influenza poses serious threats to the poultry industry, and perhaps eventually to man. In view of these threats, society faces a number of problems and this project is designed to provide the scientific data to underpin the formulation of any response and a basis for the development of further measures of bio-security. Any such response must be driven by an understanding of the behaviour of the virus both in its host and the environment and particularly in relation to the way in which humans interact with both.

Threats to industry include the introduction of virus from wild populations or trade; this in turn threatens the competitiveness of the industry: an outbreak would certainly restrict a nation's trading capacity and have severe consequences for those engaged in the industry. Similarly, consumer misgivings over the safety of poultry, and even meat or eggs of vaccinated poultry, have to be addressed even for consumption at home.

Finally, in the event of an outbreak there would be a need to dispose of a large quantity of carcasses and litter and the virological consequences of this have to be considered if we are to avoid potential contamination problems for ground and surface water and even possibilities of virus survival in soil. For these reasons this project is designed to provide data on the levels and stability of virus in different materials, particularly avian materials and products, and thus help calm public concerns.



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SSPE-CT-2007-044311

FLURESIST

RESISTANCE OF INFLUENZA VIRUSES IN ENVIRONMENTAL RESERVOIRS AND SYSTEMS

Acronym: **RIVERS**
EC contribution: €1 395 000
Duration: 36 months

Starting date: 01/02/2007
Instrument: STREP

Key words: Influenza, avian, pathogenic, zoonotic, resistance, inactivation, virus, environment, reservoirs, systems, models, restocking

SUMMARY:

The surge of the global avian influenza epizootic mainly caused by the genotype Z high pathogenic avian influenza virus (HPAIV) has posed numerous questions, in particular to risk managers and policymakers. Scientific knowledge is thin on many aspects of the ecology and environmental properties of HPAIVs, in particular H5N1. Virus survival, a key element in control strategies, is an illustration of this paucity of knowledge. Data from the literature on AIV survival is rather limited, often very old and sometimes not confirmed from one study to another or even contradictory.

The results obtained with various sub-types of influenza A viruses cannot be extrapolated to the current A (H5N1) viruses before careful consideration. Furthermore, little information is provided regarding the survival of IVs in the air and surfaces - no standardised protocols exist to detect AIVs in waters, in the air or in/on solid matrices. Ideally, the virus detection technique to be used should be sensitive, quantitative, rapid and applicable in routine before or after a standardised sampling method, including or not concentration.

Under this project, nine institutions directly involved in AIV, three from Asian countries, have joined forces in order to investigate the prevention and control of influenza outbreaks in the animal population at present and at time of restocking. More specific objectives are:

a) to understand the basis of virus survival from a virological viewpoint;
b) to understand the impact of physical and chemical elements on virus survival;
c) to evaluate the role of environmental reservoirs;
d) to propose standardised protocols for the concentration and detection of AIVs in waters, including waste waters, and in different matrices including food;
e) to provide a database together with analytical tools to allow the generation of evidence-based guidelines for the prevention and control of influenza outbreaks in animal and human populations, especially at times of restocking.

PROBLEM:

Highly pathogenic avian influenza epizootics associated with zoonotic human cases.

AIM:

The overall aim of this project is the prevention and control of avian influenza A(H5N1) in the animal population through the following specific objectives:

a) Gathering data on the survival of avian influenza viruses (AIV), in natural environments.
b) Generating scientific knowledge about the survival of avian influenza viruses in experimental settings.
c) Providing figures about the effect of various treatments either chemical (e.g. disinfectants) or physical (e.g. UV light) on influenza virus survival.
d) Providing figures on the effect of various types of food processing on influenza virus survival.
e) Elaborating models about the survival of AIV in natural environments to demonstrate, in connection with other EU projects in this area, their perpetuation in nature both in biological and environmental reservoirs.

EXPECTED RESULTS:

a) Criteria for bio-equivalence in relation to virus survival between IV strains.
b) Method of virus viability assessment other than virus titration on cell culture, approvable and standardised protocols for influenza virus recovery from various surfaces, approvable and standardised protocols for testing the effect of chemical and physical treatments of different types of water on influenza virus survival, Standard Operating Procedures for virus disinfection/inactivation in different settlements.
c) Data on the prevalence of AIVs in waters (lakes, ponds and rivers) in the course of time throughout the year and along the stream of rivers; data on gastropods and bivalve molluscs regarding their potential role as concentrators and reservoirs of AIVs in aquatic biotopes.
d) Data (database) on IV survival in the air and on various kind of surfaces and in various conditions, data on the prevalence of AIVs in the surroundings of farms with present and past outbreaks in the course of time throughout the year.
e) Descriptive, data driven, low-level simulation models of AIVs perpetuation, viability and deactivation in (i) various water environments; laboratory-controlled and natural; (ii) in air at laboratory-controlled environments; (iii) in avian faeces and farm manure.
f) Multi-scale agent-based simulation model of possible determinants for AIV's stability, perpetuation and deactivation.

POTENTIAL APPLICATIONS:

a) Recommendations for the prevention and control of current and future avian influenza outbreaks in wild and domestic birds with a pandemic potential in Europe and the rest of the world will be drawn from the data obtained through RIVERS in a final report to the EC to allow evidence based policymaking.
b) International guidelines for the control and prevention (through virus inactivation and disinfection for example) of outbreaks in domestic birds but also in humans will benefit from the data generated by the project.

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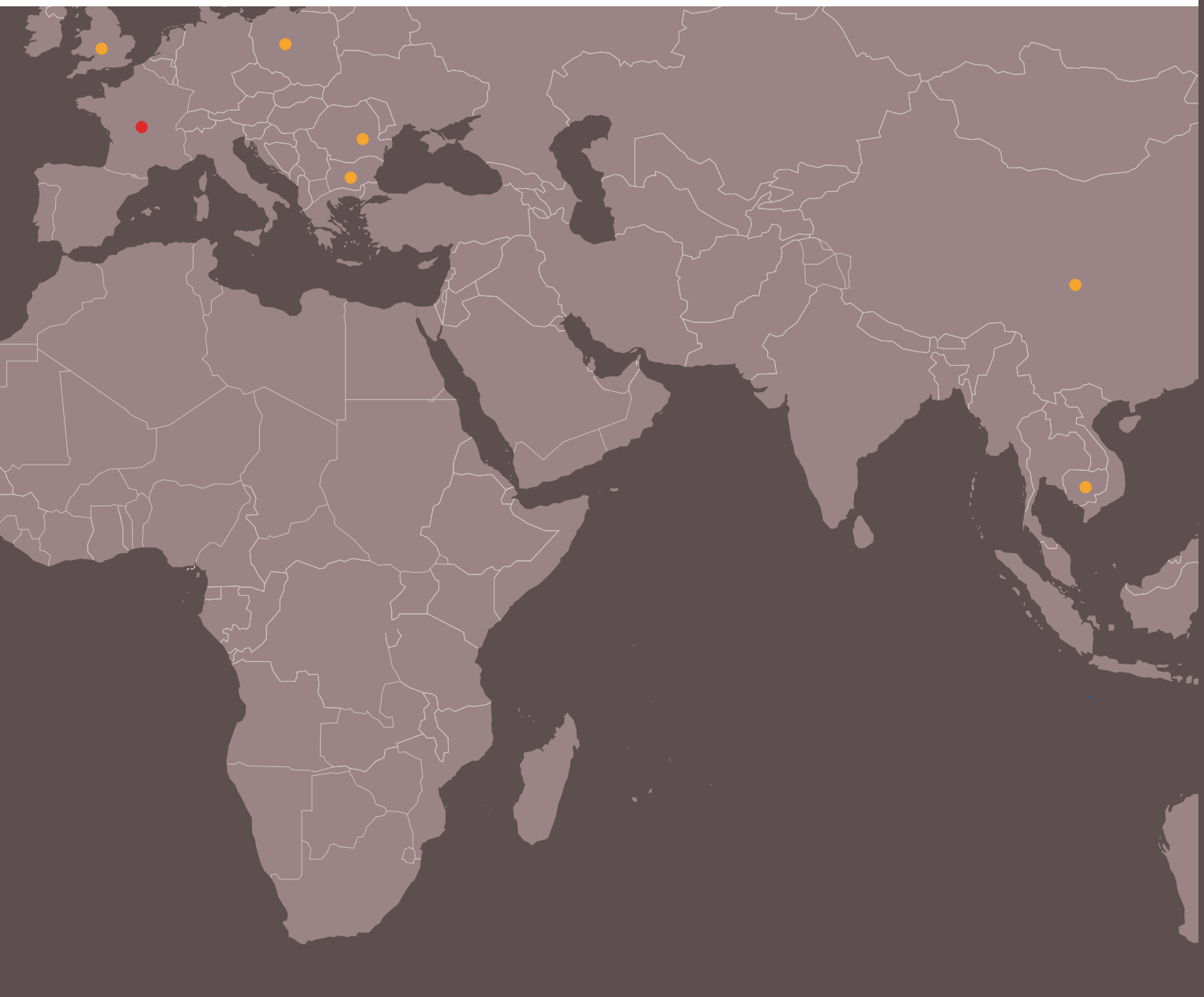

WWW.RIVERS-PROJECT.EU

SSPE-CT-2006-044405

RIVERS

NEW AND EMERGING TECHNOLOGIES: IMPROVED LABORATORY AND ON-SITE DETECTION OF OIE LIST A VIRUSES IN ANIMALS AND ANIMAL PRODUCTS

Acronym: **LAB-ON-SITE**
EC contribution: €1 500 000
Duration: 39 months

Starting date: 01/11/2004
Instrument: STREP

Key words: Diseases notifiable to OIE, transboundary animal diseases, viruses, diagnosis, diagnostic tests, dipstick, emerging technologies

SUMMARY:

This project is improving the diagnosis of nine major transboundary animal diseases (TADs) — foot-and-mouth disease, swine vesicular disease, vesicular stomatitis, classical swine fever, African swine fever, bluetongue, African horse sickness, Newcastle disease and highly pathogenic avian influenza — by addressing the recommendations of the Scientific Committee on Animal Health and Animal Welfare (SCAHAW). Regarding its epidemiological importance, swine influenza is also included in the project. The programme is focusing on new and emerging technologies, specifically the development, validation and dissemination of robust, specific and sensitive diagnostic tests.

The project is combining the development of improved 'front line' diagnostics, such as dipstick tests that can be used by veterinarians in the field with development of robust and simple nucleic acid and antigen-antibody detection methodologies to be applied in local laboratories and in abattoirs. In addition, the most recent approaches in real-time PCR and in microarray technologies are applied for the improved diagnosis of the nine TADs, notifiable to OIE.

PROBLEM:

TADs are regularly emerging and re-emerging, threatening animal and human health worldwide (www.oie.int/). To combat these diseases more effectively, the development of rapid, highly sensitive and specific novel diagnostic methods is necessary, including simple and powerful on site tests.

AIM:

The aim is the complex diagnosis of nine TADs notifiable to OIE. The project is focusing on the development, validation and dissemination of robust, specific and sensitive diagnostic tests, including 'front line' diagnostics, such as dipstick tests.

EXPECTED RESULTS:

a) Improved laboratory methods for sensitive diagnosis of nine TADs notifiable to OIE.
b) Improved, commercially available dipstick tests for on site diagnosis.
c) Methods to analyse animal products for the presence of the targeted viruses.
d) Sample banks, standardisation, validation, dissemination of results, SOPs, international training.

WORK PERFORMED ON AVIAN INFLUENZA VIRUSES (AIV):

1. Rapid detection of AIV with light upon extension primers and a duplex RT-PCR

A real-time RT-PCR assay utilising light upon extension fluorogenic primers (LUX RT-PCR) was developed for the rapid and efficient detection of AIV (Kiss et al., 2006). The LUX RT-PCR technology provided a highly specific and sensitive novel means of AIV detection. Besides its simplicity (use of only two oligos), the LUX technology efficiently reduces the likelihood of false positive results by the use of melting point analysis upon amplification.

A duplex reverse transcription-polymerase chain reaction (dRT-PCR) assay has been developed for the simultaneous, rapid and specific detection of AIV and Newcastle disease virus (NDV) in clinical specimens (Farkas et al., in press). The assay is robust in a sense that it is capable of detecting a broad range of AIV and NDV variants in the same reaction. The speed, simplicity and complexity of the assay facilitate the immediate and front line diagnosis of AI and ND close to outbreak cases.

The assay was shown to be capable of detecting either virus in faecal samples. That makes it an appealing tool among the diagnostic procedures used in monitoring programmes. Therefore, the described dRT-PCR provides a useful and practical supportive method for the effective diagnosis of avian influenza and Newcastle disease.

In order to produce an internal control for the AIV PCR, a 'mimic' has been constructed, which is assuring the reliability of the diagnosis.
Rapid one-step real-time PCR (RT-PCR) for the detection (pathotyping and sub-typing) of HPAIV using SYBR Green and TaqMan chemistries is under development.

2. Development of rapid, simplified method for the on-site detection of highly pathogenic AIV (HPAIV) antigens (dipstick tests)

Two monoclonal antibodies (MAbs 5F10, and HB-65) were evaluated in dipstick tests. Devices for lateral chromatography were prepared using either of the MAbs and specificity testing was carried out. The obtained results show that only MAb 5F10 gave a positive result using the HPAIV strains while MAb HB-65 gave failed to react. MAb 5F10 was able to detect all three HPAIV strains and the negative controls gave a negative result in the experiment.

3. Development of ELISA assays for HPAIV typing and antigen detection

The objective is the development of ELISAs to identify H5 and H7 viruses using monoclonal antibodies specific for the H5 and H7 haemagglutinin antigens, respectively. Two MAbs out of 42 generated hybridomas specific to H7 and 20 specific to H5 were selected. Sandwich ELISAs were designed for H5 and H7 antigen typing, using a unique MAb, coated to microplate wells as antigen capture antibody, and conjugated with peroxidase as tracer. MAbs 5D8 (H5-specific) and 7A4 (H7-specific) were used.

Analytical sensitivity. Allantoic fluids containing each of four H5 and three H7 subtypes were examined. The detection limit of H5- and H7-ELISA corresponded respectively to 104,5/105 EID50/0,1ml and 104/104.5 EID50/0,1ml.

Analytical specificity. To evaluate the analytical specificity the number of AIVs

mentioned above was tested in both H5 and H7 ELISAs. All the H5 subtypes exhibited strong reactivity in the H5-ELISA, in contrast with any other AIV that reacted negative. Similarly, all H7-subtypes, including the equine strain Praga 1/56 H7N7, were positive in the H7-ELISA, in contrast with subtypes displaying other H antigens.

In conclusion, the H5 and H7 ELISAs developed showed a very high specificity, conferred by the two MAbs selected, combined with a broad intra-subtype reactivity that enabled the identification of all the H5 and H7 AIVs examined. Concerning analytical sensitivity, a significant concentration of antigens is needed to produce a positive signal, as expected for antigen detection ELISAs. Then, H5 and H7 typing ELISAs are suited to identify viruses isolated in SPF chicken embryonated eggs and in clinical specimens during acute infection.

Monoclonal antibody-based ELISAs for the detection of antibodies elicited to H5 and H7 influenza viruses were developed as well. Total 813 serum samples were tested by HI assays using the H5 and H7 subtypes of AI strains as antigens. The 5D8 (H5) and 7A4 (H7) MAbs selected for antibody-detection ELISAs have HI activity and recognise an epitope present on all strains tested with the respective H subtype.

H5 and H7 competitive ELISAs. Sensitivity and specificity of the two ELISAs were evaluated using haemagglutination inhibition (HI) test as reference test on both natural and experimental positive sera. For specificity analyses, both naive samples (n=220) and sera originating from animals infected with heterologous subtypes of AIVs were tested. All 173 sera positive in H5-HI scored positive also in H5-ELISA whilst 2 out of 417 sera positive in H7-HI were not detected by H7-ELISA. Two missed sera samples from vaccinated turkeys showed a threshold titre in HI test. Concerning specificity, all 396 sera negative in H7-HI were negative also in H7-ELISA, including 176 sera strongly positive to AIVs of subtypes different than H7.

In conclusion, the correlation between the competitive ELISA and the HI test for the detection of antibodies specific to H5 and H7 AIVs was strongly significant, so that sensitivity and specificity performances of the ELISA evaluated with respect to HI test are very high.

ELISA assays for the detection of antigens and/or antibodies of AIV with main focus on serotypes H5 and H7 described above were developed and improved during the first and second year of the project. At present, they represent rapid, robust, simple tests, which can be used as first line diagnostic test procedures and also in laboratories not equipped with more sophisticated instruments. Improvement of assays in terms of standardisation and diagnostic application was performed and specific monoclonal antibodies are now available.

POTENTIAL APPLICATIONS:

The developed new diagnostic methods will be disseminated to partner institutes and animal health authorities both in and outside the EU. One of the most important issues in the control of OIE-listed diseases in countries outside the EU is the availability of very simple, easy to use and inexpensive tests like the dipstick tests or diagnostic tests based on portable real-time PCR instruments to be developed in this project. Field veterinarians, simply equipped laboratories and abattoirs can easily use such methods. Simple and effective methods will be provided for the 'first line' diagnosticians.

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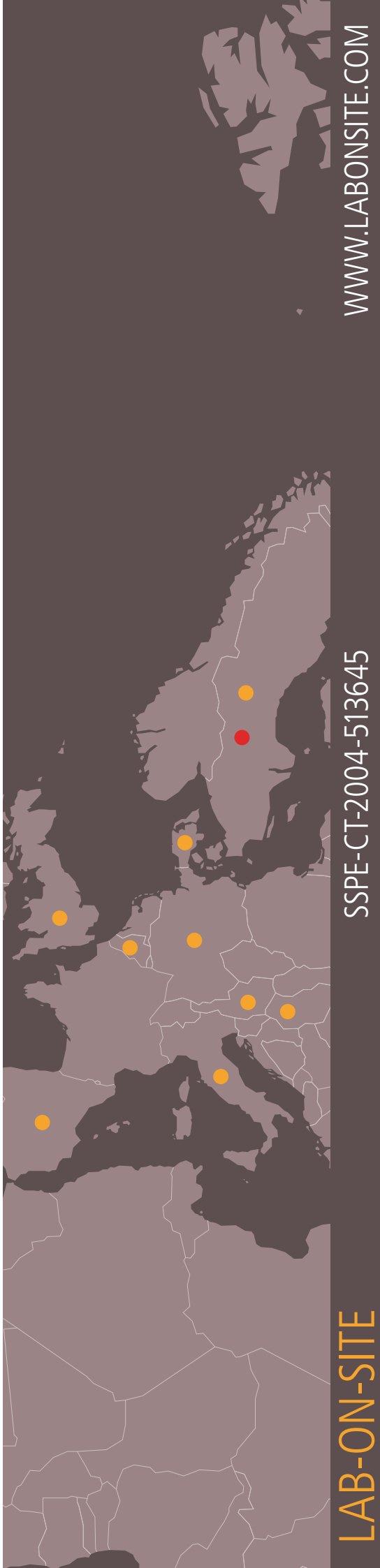

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PATHOGENESIS AND IMPROVED DIAGNOSIS AND CONTROL OF AVIAN INFLUENZA INFECTIONS

Acronym: **AVIFLU**
EC contribution: €1 839 294
Duration: 48 months

Starting date: 01/10/2002
Instrument: Shared Cost Action

Key words: Avian influenza, poultry, highly pathogenic, pathogenicity

SUMMARY:

In recent years there has been a global increase in the number of outbreaks of highly pathogenic avian influenza (HPAI). Two devastating outbreaks have occurred in Europe - in Italy and the Netherlands - both resulting in major economic losses. In Asia an ongoing epizootic is causing major concern due to the number of human infections associated with a particular strain of H5N1 AI virus, sparking fears and considerable planning for a potential influenza pandemic. Millions of birds have been culled to control these outbreaks.

Much attention has focused on determining the best control strategies for dealing with outbreaks and new introductions of HPAI viruses in poultry. Successful control strategies depend upon a number of factors including the use of rapid and sensitive laboratory tests appropriately applied; knowledge about the infecting virus, e.g. the host species affected; rates and mechanisms of transmission between animals of the same or different species; and determinants for pathogenicity and the use of vaccination.

We have addressed some of these issues in the AVIFLU project: Rapid and sensitive laboratory tests for avian influenza (AI) for use in surveillance and controls programmes were developed and validated. A transmission model was used to study the efficacy of vaccination in poultry and ducks, AI marker vaccines were developed and evaluated, and reverse genetics was used to study the effect of molecular pathogenicity markers *in vitro* and *in vivo.*

PROBLEM:

Control of an infectious disease that can spread as rapidly as HPAI requires a rapid and harmonised response. It is critical that veterinarians and animal keepers recognise the clinical signs of the the disease and report suspected cases quickly. Suspected outbreaks must be backed up by rapid, sensitive and highly specific laboratory tests before control measures can be implemented.

Early warning systems such as surveillance in wild bird reservoirs and in poultry flocks also play an important part in the control of AI. This requires the testing of high volumes of samples in a timely manner and may require a different testing strategy to that of index cases. It is also crucial that we understand the mechanisms and markers for the emergence of HPAI and the impact vaccination can have on the spread of disease.

AIM:

a) To develop rapid and sensitive laboratory tests for avian influenza (AI) for use in surveillance and controls programmes.
b) To develop a transmission model to elucidate the dynamics of influenza A virus infection in chickens and waterfowl and to use this model to examine the emergence of highly pathogenic viruses from those of low pathogenicity, and to measure the efficacy of vaccines and vaccination strategies.
c) To develop and evaluate the use of AI marker vaccines.
d) To study the pathogenesis of AI in animal models and the molecular basis of virulence.
e) To study AI pathogenicity, transmission and the efficacy of vaccination in ducks.

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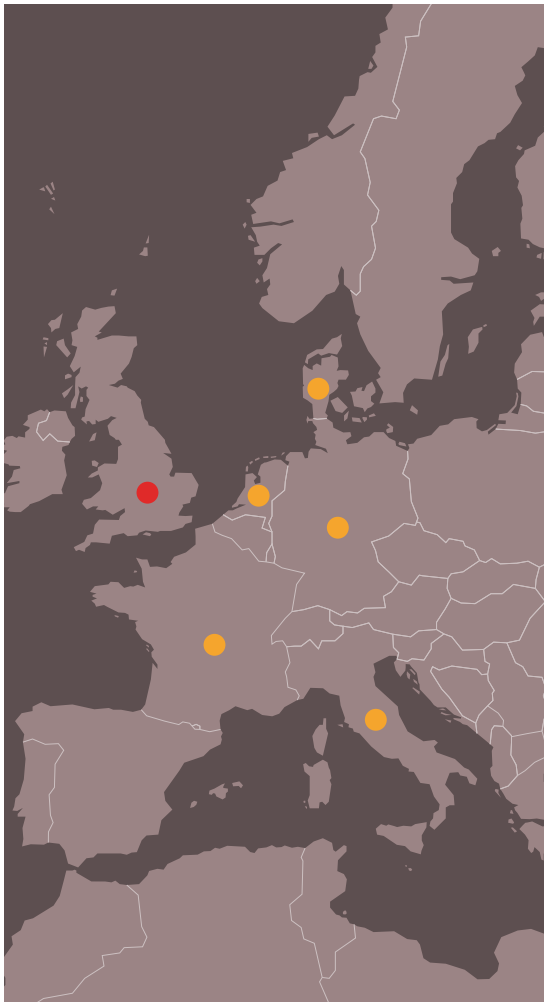

EXPECTED RESULTS:

The AVIFLU project has now been completed and some elements of the work are now being explored further in the follow-on project, FLUAID. Highlights of our findings are summarised below:

DIAGNOSTIC TESTS

Two ring trials were conducted that involved six EU national AI laboratories and a number of other invited laboratories, with the aim of establishing an agreed approach to AI diagnostic RT/PCR in the EU. The trials were designed to evaluate RT/PCR and real time RT/PCR protocols for the detection of H5 and H7 AI viruses that had been developed by each participating laboratory. The best performing protocols for sensitivity and specificity were then validated and circulated to all EU AI national laboratories. They are now included in the EU diagnostic manual which supports the new AI directive.

A generic AI antigen capture ELISA had been developed using as a key reagent AI Nuclear Protein (NP) specific monoclonal antibodies (Mabs) developed within the AVIFLU project. These NP Mabs have been evaluated for their potential as reagents in a penside antigen detection test, in the form of a Lateral Flow Device. Prototype devices were produced, tested and compared with other devices available commercially. The prototypes performed well in comparison with other devices for cloacal and oropharyngeal swabs collected from chickens and Pekin ducks.

VACCINATION

An infection model was developed enabling the quantification of different intervention strategies on the transmission of avian influenza viruses. A stochastic susceptible, latently infected, infectious, recovered (SEIR) epidemic model was developed and used to analyse data generated with the infection model. The transmission experiments offer a way to measure the spread of virus under experimental conditions and also to quantify the effect of vaccination on the transmission characteristics of the viruses. The model was used to investigate the transmission characteristics of LPAI and HPAI viruses of the H7 and H5 subtypes in vaccinated and non vaccinated chickens, Pekin ducks, teal and golden pheasants.

The major findings are:

a) Vaccination with heterologous vaccines - suitable for use in a DIVA (differentiation of infected from vaccinated animal) strategy protected chickens against disease and mortality but also reduced the spread of virus within a group of birds.

b) In ducks HPAI H5 and H7 viruses were more often shed via the respiratory route.

c) H7 HPAI infection of teals did not cause clinical signs and vaccination prevented spread of the virus.

d) H7 HPAI infection of golden pheasants caused mortality and vaccination protected birds from clinical signs but spread of the virus still occurred.

e) Following infection of Pekin ducks with HPAI A/duck/Vietnam/12/2005 H5N1 in naïve birds, clinical signs, with a predominance of nervous signs, were observed starting on day two post infection. The overall mortality rate was 70%, indicating that Pekin ducks are clinically susceptible and may die following infection with certain strains of HPAI H5N1. In situ hybridisation showed a strong neurotropism for this virus in ducks.

f) Vaccination of Pekin ducks with a single heterologous dose of commercial inactivated H5N2 vaccine and challenged with HPAI H5N1 (Asian lineage) vaccination prevented death and reduced the shedding of virus but not sufficiently to prevent spread of the infection to other animals. In contrast, a two-dose vaccination programme commencing at one day old, prevented shedding, clinical signs and mortality. This evidence suggests that the perpetuation of the infectious cycle may be interrupted by optimum vaccination.

MOLECULAR BASIS OF PATHOGENICITY

A reverse genetics system was established based on viruses from the 1999-2000 Italian H7N1 LPAI and HPAI outbreaks. This was used to investigate the biological relevance of various mutations observed between these viruses. The findings were that additional glycosylation of the haemagglutinin near the receptor binding site promotes the spread of infection by HPAI virus, apparently due to increased virus release from cells and not due to changes in receptor specificity. Also, a shortened neuraminidase stalk is a prerequisite for adaptation to chickens. Viruses with a reconstituted full NA stalk had a loss of infectivity and grew to lower titres.

Mutations found in the NS1 proteins of high and low pathogenic viruses seem to be a critical determinant for host adaptation.

POTENTIAL APPLICATIONS:

All the diagnostic tests and reagents (including monoclonal antibodies) developed in this project are now recommended for use in the national avian influenza reference laboratories of the EU. The protocols are available in the EU diagnostic manual. This effort has harmonised the approach taken by EU national AI laboratories and enabled the best molecular tests currently available to be used throughout the EU. The experience gained in running the ring-trials is now used by the AI Community Reference Laboratory to conduct the EU national AI laboratory annual proficiency panel testing for AI molecular tests. The strategy adopted for these trials will also ensure that improved tests will be identified, thus keeping our testing regimes up to date with advances in molecular diagnostics.

Monoclonal antibodies produced in this project have been evaluated for their potential as reagents in a penside antigen detection test, in the form of a Lateral Flow Device. The test is now being developed further and validated within the current FLUAID project. It is likely that this test will be marketed commercially in the future.

Differences in the transmission of highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) strains may play a role in the mechanism of selection by which HPAI viruses arise in the field. The mathematical infection model will contribute to an understanding of these processes. It is a tool that enables the effect of different intervention strategies to be measured, e.g. vaccination.

Our results indicate that vaccination of Pekin ducks with an inactivated, conventional product is successful in preventing clinical signs and mortality and in suppressing shedding of viable virus. This suggests that the perpetuation of the infectious cycle may be interrupted by optimum vaccination.

These findings have significant implications on practical aspects of AI control. It appears that following field challenge a vaccinated flock will not shed enough virus to infect either vaccinated or unvaccinated flock-mates. As the amount of virus shed in the environment is not sufficient to infect unvaccinated ducks, it could be speculated that such a practice would presumably reduce the risk of infection spilling over into the wild bird population, and possibly to mammalian hosts.

The vaccination protocol under study was also developed in order to evaluate a system that could be compatible with husbandry practices. Vaccination at one and 30 days old appears to be compatible with Pekin duck husbandry methods in Asia. The results indicate that such a programme could be used in the field to prevent primary introduction and secondary spread in naïve Pekin ducks. A duration of immunity above threshold levels for more than five months was recorded, which appears to be longer than the economic life of a meat duck, approximately four months. Vaccination may also prevent the establishment of viraemia and therefore prevent viral colonisation of internal organs, positively influencing the food security of duck products.

The reverse genetics system has already given some surprising insights into the determinants for host range and pathogenicity. Some of the markers identified may be relevant for the development of new diagnostic tests and for the surveillance of avian influenza.

EUROPEAN SURVEILLANCE NETWORK FOR INFLUENZA IN PIGS

Acronym: **ESNIP**
EC contribution: €269 984
Duration: 36 months

Starting date: 01/01/1999
Instrument: Concerted Action

Key words: Influenza, swine, surveillance, epidemiology, evolution, diagnosis, public health risk

SUMMARY:

A network of laboratories working in the field of swine influenza was established and harmonised swine influenza diagnostic tests. Using these tests a preliminary surveillance of influenza in pigs was performed, and swine influenza viruses isolated in the last decennia were further characterised antigenically and genetically.

PROBLEM:

Swine influenza viruses (SIVs) are enzootic in swine dense regions of Europe and they are a major cause of respiratory disease in fattening pigs. Until recently, however, there was no organised surveillance for influenza viruses of swine, as is the case for human and equine influenza viruses. In addition, there was no standardisation of diagnostic techniques or of the techniques used for antigenic and genetic characterisation of swine influenza virus (SIV) strains. Because of this lack of organisation and harmonisation of SIV surveillance, it was difficult to make recommendations for the control of SIV in Europe, and for the selection of vaccine strains in particular. These needs have led to the submission of a proposal for an EC concerted action by researchers from several European countries.

AIM:

The main objective of this concerted action was to standardise protocols used for the diagnosis and characterisation of SIVs used in different laboratories and to define standard reagents and make these available to all participants. Other aims were:

a) finalised requirements for antigenic characterisation;
b) finalised requirements for genetic characterisation;
c) database and data registration;
d) evaluation and SI epidemiology, and recommendations for SI control.

RESULTS:

The objective has been achieved. Selected SIV isolates from different countries were compared antigenically and genetically. The use of standardised protocols and reagents allows the comparison of SI viruses isolated during the project period and in the future.

One of the problems was that only a low number of SIV isolates was available from some countries, because of few diagnostic submissions for SIV.

A comparative serosurveillance study for SIVs in different countries was performed, but not all partner countries were able to participate. To get a more complete picture of the SIV epidemiology in the major swine producing countries of Europe, researchers from Spain (Laboratorios Hipra, Girona) and Germany (Impfstoffwerke Dessau, Rodleben), who were not official partners of the ESNIP project, were also involved in the serosurvey and/or collection of SIV isolates. This allowed us to get a more complete picture of the SIV epidemiology in Europe.

A website (www.esnip.wur.nl) is hosted by one of the partners. All protocols and a summary of the SI isolates made in the period 2000-2003 is available to the public on this website. A more detailed electronic database is password protected and accessible only to the partners.

POTENTIAL APPLICATIONS:

a) A network of laboratories involved in the laboratory diagnosis for swine influenza viruses in different European Member States was established. Because participating laboratories standardised the methods for antigenic subtyping and for further genetic characterisation, new variants can be easily recognised.

b) If future funding is available, the network will provide valuable information about the prevalence of swine influenza viruses. This information is important because swine influenza viruses can potentially contribute to the evolution of new pandemic influenza strains in mammals including humans.

c) The emergence of a new H1N2 pig subtype that is not covered by current vaccines was demonstrated.

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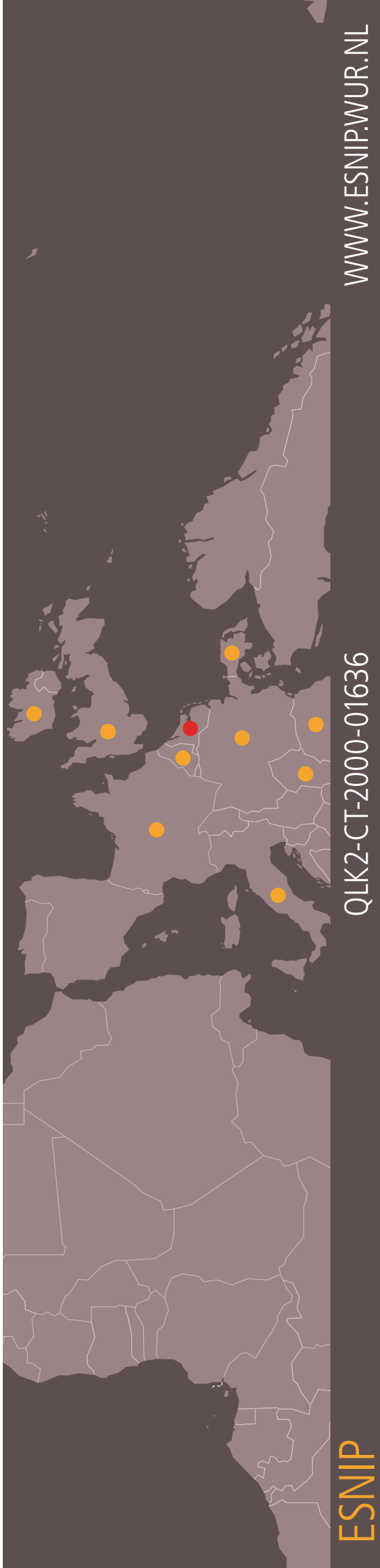

EUROPEAN SURVEILLANCE NETWORK FOR INFLUENZA IN PIGS 2

Acronym: **ESNIP 2**
EC contribution: €300 000
Duration: 36 months

Starting date: 01/01/2006
Instrument: Coordination Action

Key words: Influenza, swine, surveillance, evolution, diagnosis, public health risk

SUMMARY:

The European Surveillance Network for Influenza in Pigs (ESNIP) 2 will maintain and expand a surveillance network that was established during a previous EC concerted action (ESNIP, QLK2-CT-2000-01636). Three work packages (WP 1, 2, 3) aim at a better understanding of the epidemiology and evolution of swine influenza virus (SIV) in different European countries, through an organised surveillance programme together with antigenic/genetic characterisation using standardised methodology. These data will be used to improve the diagnosis of SIV by updating the reagents used in classical techniques (WP 4) and by the development of a rapid, molecular test for SIV detection (WP 5).

The virus bank and electronic database that were established during ESNIP will also be expanded with relevant SIV isolates and information (WP 6). Beyond this, we will be better able to define the public health risks of influenza in swine. This will be achieved by serological monitoring of swine for avian influenza viruses (WP 7) and by comparison of the influenza viruses that are currently circulating in swine, avian species and humans (WP 8). ESNIP 2 represents the only organised surveillance network for influenza in pigs and the first attempt to establish formal interactions with human and avian surveillance networks.

These initiatives and interactions are entirely consistent with improved pandemic preparedness and planning for human influenza. The consortium consists of 11 participants who are actively working with SIV. Eight participants are from seven different EU Member States, five of which have been actively involved in the ESNIP network whilst one participant is from Bulgaria, a candidate Member State (at the time of the project start date). Participation from third countries (Hong Kong, USA) will facilitate greater global interaction and worldwide understanding of the epidemiology of SIV. Comparisons of swine populations in Europe and in southern China, the classical epicentre for influenza, for the circulation of avian influenza viruses will prove invaluable and permit technology and knowledge exchange.

PROBLEM:

During the last decade, the epidemiology of swine influenza in Europe has become particularly complex. At least 3 SIV subtypes – H1N1, H3N2 and H1N2 – are currently circulating and new reassortant viruses between these subtypes have been occasionally detected. The heterogeneity in swine influenza has important implications for the diagnosis and control. Indeed, the strains used in serodiagnostic tests need to be matched to the current epidemic viruses, and inactivated SIV vaccines should contain all of the prevailing subtypes for a broad protection. Two significant difficulties, however, are that there is little surveillance for SIVs and that our understanding of the SI epidemiology is far from complete.

So far there have been rather limited attempts at detailed characterisation of SIVs. In addition, there has been confusion about the extent of antigenic evolution of these viruses, i.e. considerable antigenic drift of H1N1 and H3N2 SIVs was reported in some regions of Europe (de Jong et al. 1999, 2000), but not in others (Campitelli et al. 1997). One difficulty was a lack of standardised reagents and protocols for the subtyping and antigenic/genetic characterisation of SIV, which greatly contributed to discordant results between different laboratories.

Virus isolation followed by haemagglutination inhibition (HI) and neuraminidase inhibition (NI) with reference sera is the standard procedure for subtyping SIVs. The HI test is also most widely used for the serologic diagnosis and for serologic studies of SIV. The reagents used in such tests must be updated if relatively minor antigenic changes in the circulating SIVs should occur, and failure to do so may result in incorrect subtyping of SIVs and false seronegatives in the HI test. There is also a strong need for alternative, more rapid and simple tests for detection and subtyping of SIVs.

Finally, pigs are known to be susceptible to infection with both human and avian influenza viruses and there are concerns that avian influenza viruses or human-avian reassortants may transmit to humans via the pig. However, the true public health risk of the pig remains unknown, because there is no screening of pigs for influenza virus from other hosts.

AIM:

The strategic objectives of the current Coordination Action are:

a) to further expand our knowledge of the epidemiology and evolution of swine influenza viruses in Europe and to apply this knowledge to optimise diagnostic techniques for swine influenza;
b) to provide insights into the public health risk of influenza in swine by monitoring swine for avian influenza viruses and by comparison of influenza viruses in swine and in human populations.

The research objectives can be grouped into six major tasks:

a) To keep track of major changes in the epidemiology of SIV in Europe.
b) To further study the extent of antigenic and genetic evolution of SIVs.
c) To improve the diagnosis of SIV.
d) To expand the SIV bank and electronic database which were established during ESNIP.
e) To screen European swine populations for the circulation of avian influenza viruses.
f) To compare the influenza situation in swine with that in humans and birds and ensure dissemination of information to human and avian influenza researchers.

EXPECTED RESULTS:

Scientific:

a) Maintenance of the surveillance network for various influenza A subtypes in swine in Europe.
b) Data about the prevalence and circulation patterns of different SIV subtypes in swine in Europe and comparison with the situation in southern China and in the USA.
c) Antigenic and genetic characterisation of SIVs

in pig herds in different European countries.
d) Data about the prevalence of avian influenza viruses in swine in Europe.

Technical and policy support:

a) Recommendations about the type of reagents to be used in classical diagnostic tests for SIV.
b) Standardisation and validation of novel, more rapid tests for the diagnosis and characterisation of influenza viruses in swine.
c) Maintenance and expansion of the SI virus bank and electronic database.
d) Development and validation of a serologic assay for avian influenza viruses in swine.
e) Formal interaction between the SIV surveillance network and avian and human networks.

POTENTIAL APPLICATIONS:

In a broader context, this Coordination Action supports the EU animal health policy, because it will contribute to a high status of swine health throughout the EU. Indeed, SI is an economically important disease for the swine industry. Investigations in the Netherlands and Belgium have shown that SIV is involved in up to 50% of the acute respiratory disease outbreaks in fattening swine. In the UK, the financial loss resulting from reduced weight gain in fattening pigs due to SIV alone has been estimated at approximately £7 per pig, equivalent to a total loss in the UK per annum of £60 million.

The project will improve and facilitate the diagnosis of SI and the subtyping of SIVs. This is essential for a rational design of vaccination strategies on individual swine farms. In addition, we will obtain a clear picture of the SIV subtypes that are currently circulating in Europe and their antigenic characteristics in comparison with vaccine strains. This will indicate whether changes in the vaccine strain composition may be required. In this way the ESNIP 2 project will contribute to the welfare of swine and the profitability of swine farmers.

The objectives of the project are also in line with the OIE missions, namely:

a) to guarantee the transparency of animal disease status worldwide;
b) to collect, analyse and disseminate veterinary scientific information to help Member States to improve the methods used to control and eradicate diseases;
c) to provide expertise and promote international solidarity for the control of animal diseases.

Finally, our research will have an impact on food quality and safety. We will determine the prevalence of avian influenza viruses in swine in Europe, which will reveal whether there is a potential risk for pig meat to be contaminated with such potentially hazardous viruses.

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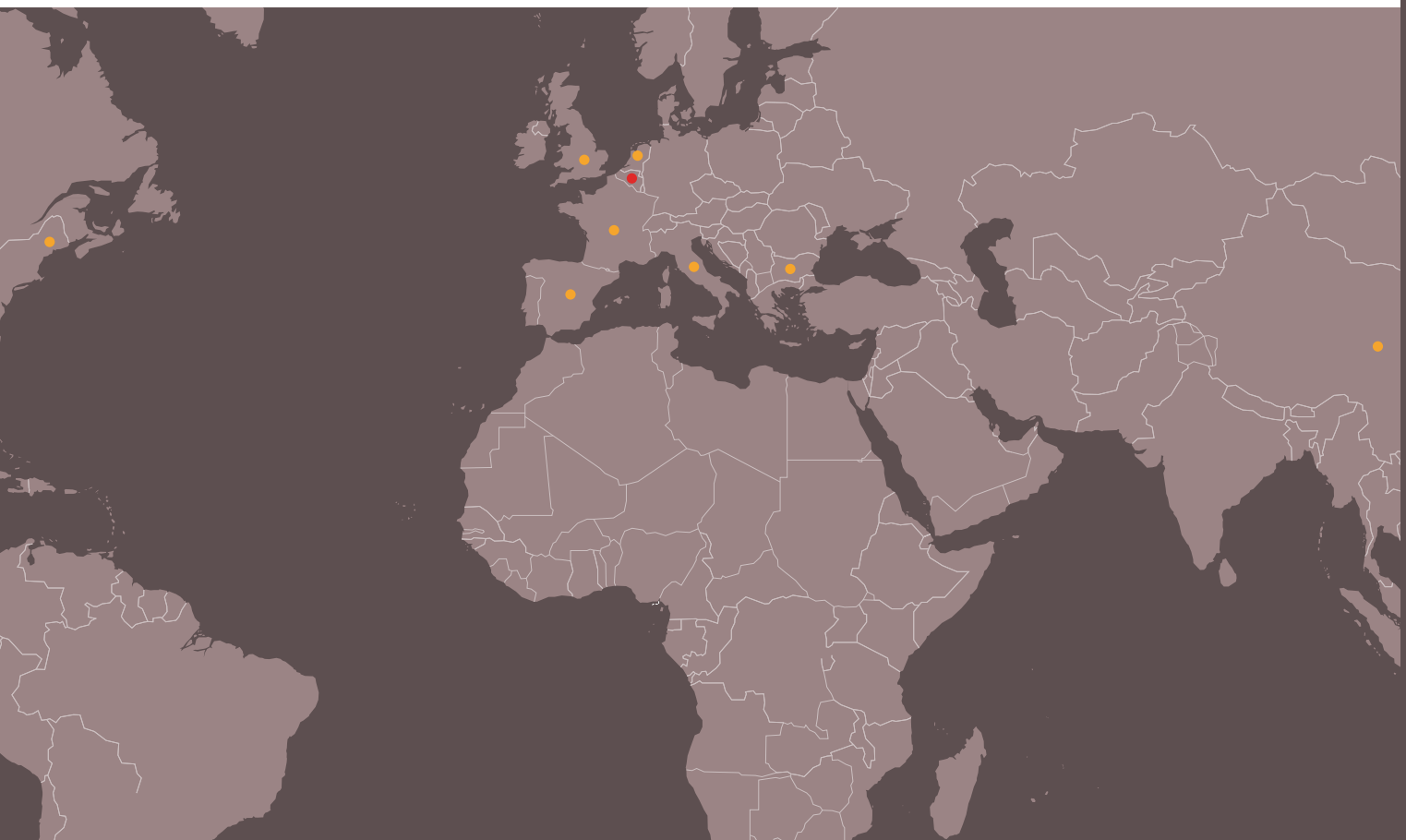

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ESNIP 2

EUROPEAN INFLUENZA SURVEILLANCE SCHEME

Acronym: **EISS**
EC contribution: €1 439 790
Duration: 36 months

Starting date: 1/09/2003
Instrument: Public Health Programme

Key words: Surveillance, influenza, epidemiology, virology

SUMMARY:

The European Influenza Surveillance Scheme (EISS) publishes a weekly surveillance report on influenza activity during the winter. The report covers a total population of 484 million people in 30 countries and is based on data reported by roughly 13 000 sentinel physicians.

EISS also operates the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL). The CNRL includes 38 laboratories across Europe and its objective is to provide high quality reference services for human influenza surveillance, early warning and pandemic preparedness.

PROBLEM:

Influenza is a major public health problem in Europe. It is associated with higher general practice consultation rates, increased hospital admissions and excess deaths. It must also be considered in terms of health care planning, increased days lost due to absence from work and influenza pandemic planning. Worldwide influenza pandemics in the twentieth century occurred in 1918-20 (more than 20 million deaths), 1957-58 and 1968-70.

AIM:

The general aim of the EISS project is to contribute to a reduction in the burden of disease associated with influenza in the EU Member States.

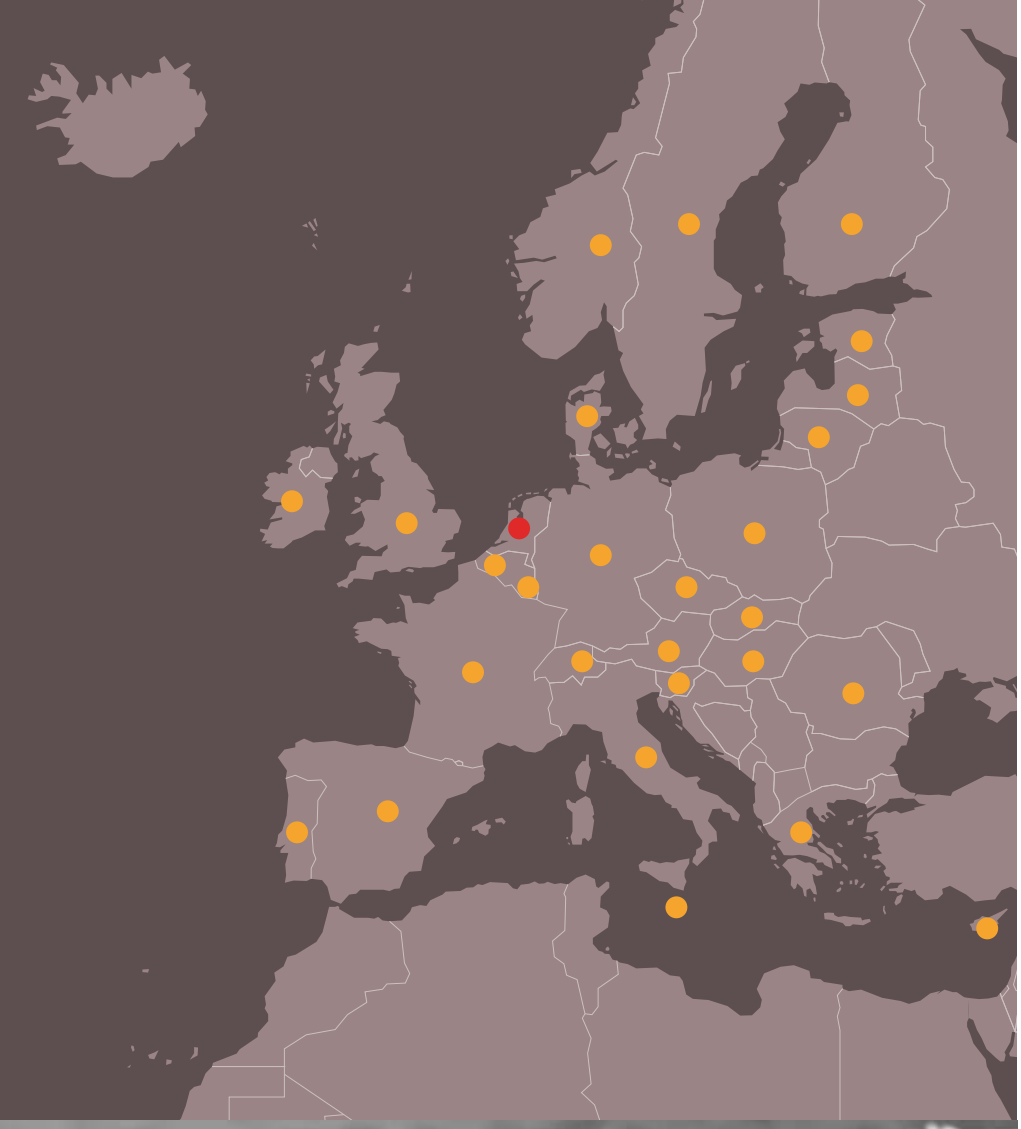
EXPECTED RESULTS:

The collection and exchange of timely information on influenza activity, which will help contribute to the annual determination of the influenza vaccine content and European influenza pandemic preparedness activities.

POTENTIAL APPLICATIONS:

The data provided by the EISS can be used in a number of ways:

a) To provide information about influenza to health professionals and the general public.
b) To contribute to the annual determination of the influenza vaccine content.
c) To support health care planning associated with seasonal influenza activity (e.g. hospitals and nursing homes).
d) To establish surveillance structures in preparation for a pandemic.



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26 EU Member States participate in EISS, plus Norway and Switzerland. Each country has a contact institute for their virological and epidemiological surveillance data.
For a detailed list, see www.eiss.org/cgi-files/wiw_members_display.cgi

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EISS

BIOLOGY

It is becoming increasingly clear that the distinction between 'basic' and 'applied' research is an outdated classification system with little relevance in today's health sciences – and research into the biology of influenza viruses and influenza infection is a prime example of this paradigm. Research that addresses a number of fundamental questions about the molecular and structural biology of these viruses is crucial for the development of advanced solutions to a number of very practical problems in the prevention and treatment of influenza or in public health efforts to stop an epidemic.

The dysregulated innate immune response of the host is increasingly being recognised as a key factor in the severity of the clinical disease. While many of the projects assembled in this chapter include some research on this important aspect, the detection of virus and host determinants of this non-specific response is the major focus of the recently launched **FLUINNATE** project. **EUROFLU**, **FLUPATH** and **INNFLU** all tackle – in addition to the innate immune response – several other issues related to the pathogenicity of viruses: They examine the important question of how easily viruses are transmitted from one species to another, including the crucial jump from birds to humans, and which factors are responsible for this species-specificity as well as for the clear tropism of the virus for only a very limited range of cell types within a given organism. Related to this question are studies of virus-host receptor interactions, the contribution of haemagglutinin and neuraminidase proteins to virulence in different species as well as research on cellular mechanisms that regulate the viral replication within the infected cell.

FLUPOL (targeted exclusively on influenza) and **VIZIER** as a broad-based effort aimed at a number of RNA-viruses (but including influenza as well as other medically relevant viruses), both aim to characterise the structure of core enzymes of the viral replication machinery with a view to finding new targets for antiviral drugs. The **RespViruses** consortium takes a different approach and studies the innate and acquired immune response of elderly patients to known and newly discovered respiratory viruses also with the goal of developing innovative therapeutics, such as antiviral siRNA-based approaches and strengthening the host's immune response.

HOST-SPECIFIC VARIANTS OF THE INFLUENZA VIRUS REPLICATION MACHINERY

Acronym: **FLUPOL**
EC contribution: €1 973 450
Duration: 36 months

Starting date: 01/01/2007
Instrument: STREP

Key words: Influenza virus polymerase, viral replication, X-ray crystallography, cryo-electron microscopy, host-dependent mutations, ribonucleoprotein particle, high throughput screening

SUMMARY:

We aim to understand how the influenza virus replication machinery adapts during interspecies transmission and to use this knowledge to provide new tools to combat potentially pandemic influenza outbreaks.

PROBLEM:

Currently circulating H5N1 avian influenza viruses are lethal to man and could cause a devastating pandemic if they became transmissible between humans. It is therefore crucial to understand the mechanisms whereby influenza virus adapts from avian to human hosts. The best understood factors in inter-species transmission are certain characteristics of the surface glycoprotein, haemagglutinin. However, several recent studies have highlighted the importance for transmissibility of mutations in the proteins of the viral replicative machinery, in particular the polymerase which transcribes and replicates the viral RNA. We propose a comprehensive study of the molecular structure and function of the influenza virus polymerase with the aim of understanding how it adapts during inter-species transmission.

AIM:

We will focus on determination of the atomic structure of polymerase domains as well as the complete trimeric complex by state-of-the-art methods such as X-ray crystallography, nuclear magnetic resonance and cryo-electron microscopy. This will provide the detailed framework required to understand polymerase function and the effect of specific point mutations in inter-species adaptation.

We will also undertake biochemical, cellular and animal functional studies of the replication machinery and identification of host cell factors interacting with the polymerase using advanced functional genomics methods. In parallel, candidate mutations that may be important for inter-species transmission and virulence will be identified by bioinformatics analysis of influenza genome sequences, updated with sequences of new H5N1 isolates, as well as from studies of laboratory strains adapted from one host to another (e.g. avian to mouse).

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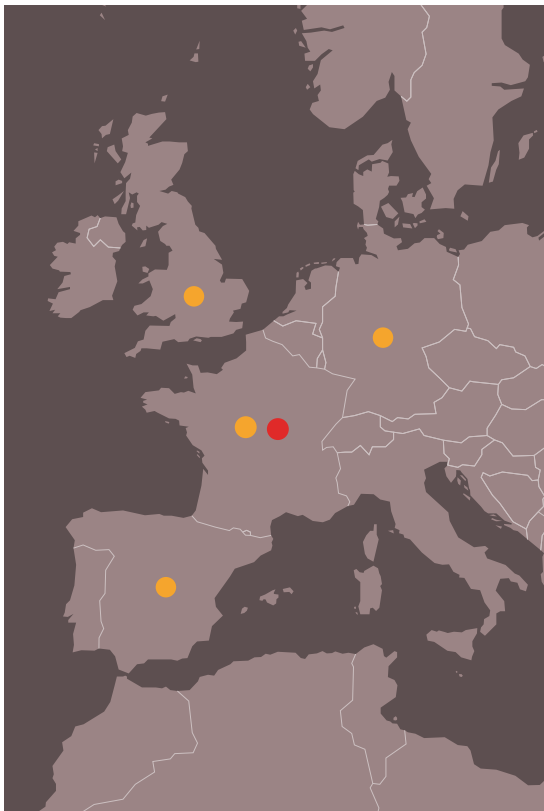

EXPECTED RESULTS:

We will systematically identify independently folded and soluble domains of polymerase subunits and nucleoprotein (NP) and determine their atomic structures. We will elucidate the structure of the polymerase-viral RNA-nucleoprotein complex by cryo-electron microscopy. We will provide a structural interpretation of species specific mutations. Using yeast two-hybrid method and *in vivo* tagging of complexes we will identify host factors required for transcription and/or replication of the influenza genome. We will use *in vitro* and cell-based *in vivo* characterisation (e.g. polymerase activity, replication efficiency) to assess the effects of mutations in polymerase and NP associated with inter-species transmission.

All these studies, combined with a systematic bioinformatic analysis of viral sequences and mathematical modelling will potentially contribute to elaboration of a comprehensive model of evolution of lead to elaboration of new strategies for development of anti-viral compounds targeting polymerase or polymerase-host cell factor interactions.

POTENTIAL APPLICATIONS:

The influenza virus polymerase complex is an excellent target for new anti-viral drugs, since it is essential for viral replication and contains several functional active sites likely to be significantly different from those found in host cell proteins. However, the lack of a detailed structure based understanding of polymerase function, in particular the structure of the target active sites, has hindered progress in this direction. Our structural and functional studies on polymerase aim to rectify this situation, by providing atomic resolution detail of the mechanism of action of this complex machine, including interactions with host cell partner proteins. Based on our biochemical and structural results we aim to develop new high-throughput assays to screen for anti-viral compounds. Other applications include new molecular biology tools such as specific monoclonal antibodies which could be used for diagnostic purposes.

SP5B-CT-2007-044263

INNATE IMMUNITY IN INFLUENZA VIRUS INFECTION OF MAMMALIAN AIRWAYS

Acronym: **FLUINNATE**
EC contribution: €1 436 130
Duration: 36 months

Starting date: 01/01/2007
Instrument:STREP

Key words: Emergent diseases, emerging influenza A virus, viral pathogenicity factors, interferon, innate immunity, human airway epithelium, host range, antiviral response

SUMMARY:

Emerging influenza A virus (FLUAV) infections pose a considerable health threat to mankind. The molecular determinants governing increased virulence of emerging virus strains in humans are presently not well understood. FLUINNATE proposes to identify and study the essential viral and host factors that determine the outcome of infection. FLUAV enters the human respiratory tract and must replicate in the face of multiple innate immune defence mechanisms to establish infection *in vivo.*

Successful viruses must adapt to intrinsic cellular restriction factors and evolve the capacity of circumventing the antiviral interferon (IFN) response, either by limiting IFN production or by blocking IFN actions. We will test the hypothesis that the speed and efficiency by which a given virus circumvents these early host responses are critical determinants in its host range and pathogenicity. In this respect, the crucial role of the virus polymerase and its cellular interactors will be analysed. Likewise, the importance of the IFN-inducible Mx GTPase as a major anti-FLUAV effector molecule will be evaluated.

Virus-induced inflammatory cytokines and chemokines exert powerful effects against FLUAV in lung epithelial cells. However, they may be detrimental to the host, causing accelerated influenza pathogenesis in the human respiratory tract. We will analyse viral factors governing the innate antiviral cytokine response and determine the impact of these factors on virus growth, cell survival and pathogenicity. Human, avian and porcine FLUAV will be used in animal models and in cell culture systems, such as human airway epithelium. The present studies should generate important information that will help to better understand the processes involved in the emergence of lethal influenza viruses and to develop efficient control measures against these devastating pathogens.

PROBLEM:

Influenza is a highly contagious, acute respiratory illness that is still a major health problem. Epidemics caused by influenza A viruses occur regularly, often leading to excess mortality in susceptible populations. In addition, influenza A viruses can cause devastating pandemics in humans. An avian influenza A virus originating from Asia and currently circulating among domestic birds in Europe and neighbouring countries makes headlines because of its potential to infect and kill people. If further adaptation to humans occurs, this virus strain might become the origin of a future pandemic.

Although influenza viruses belong to the best studied viruses, the host adaptation processes which enable influenza viruses to jump from one species to another are largely unknown. Likewise, the properties required for host-to-host transmission are presently not understood. Moreover, efficient control of influenza virus infections is still not possible. Immunisation regimes are continually being confronted with the extreme antigenic variability of influenza A viruses brought about by antigenic drift and shift. It is evident that new approaches and reagents to control influenza are urgently needed.

AIM:

The FLUINNATE objectives focus on the identification of influenza A virus genes and gene products which contribute to virulence/pathogenicity in experimental animal and tissue culture models. The required animals are available and will consist of mice with the wild-type Mx1 gene as part of the full innate immune response, various strains with targeted mutations in specific genes and pigs as natural hosts and 'mixing vessels' for influenza A viruses. In addition, human airway and porcine epithelial cell cultures will be established and characterised. Human airway epithelial cell cultures are a rare but most precious substrate to study the biology of influenza virus infection.

The influenza viruses used will be human, avian and swine strains, some of which will be generated by reverse genetics entirely from plasmids. Stock viruses and single and multi-segment reassortants will be produced and fully characterised together with the parental strains with respect to growth kinetics in tissue culture and *in vivo*, the capacity to induce or respond to interferon, and the capacity to induce disease or death in experimental animals. The technology for expressing, purifying and analysing the viral RNA polymerase complex will be established and further refined, as well as biochemical and biophysical approaches to identify co-purifying host cellular factors. Advanced tests for protein-protein interactions such as the yeast three-hybrid system will be set up and candidate interactors evaluated in functional tests, based on transfection experiments. The real-time RT-PCR methodology will be optimised for the analysis of interferon and cytokine responses and gene array data will be generated.

EXPECTED RESULTS:

- Generation of reassortant influenza viruses containing genes from high and low virulent PR8 strains by reverse genetics.
- Generation of recombinant influenza viruses expressing various tagged polymerase complexes.
- Generation of recombinant viruses with distinct receptor specificity.
- Identification of viral gene constellations responsible for high pathogenicity in Mx1+/+ mice.
- Sequence comparisons of newly isolated H5N1 viruses with emphasis to the polymerase subunit PB2, the nucleoprotein NP, and the interferon-antagonistic protein NS1.
- Optimisation of the co-immunoprecipitation assay using recombinant influenza viruses expressing a tagged polymerase complex.
- Construction and expression of polymerase clones from human and avian influenza viruses.
- Testing the functionality of these polymerase clones in human and avian cells.
- Reconstitution of active viral polymerase complexes from subunits.
- Set-up of a yeast two-hybrid systems with viral polymerase subunits as a bait.

- Establishment of a three-hybrid system in yeast using PB1 + PB2 or PB1 + PA as a bait.
- Screening for host factors by using a co-purification strategy followed by identification of co-purifying host factors by mass spectrometry.
- Identification of polymerase-interacting host factors involved in high polymerase activity and enhanced virulence.
- Gene array data of human airway epithelial cells upon influenza virus infection.
- Data on the consequences of viral receptor specificity for gene expression and function of human immunocompetent cells (macrophages, dendritic cells, T- and B-lymphocytes, neutrophils, NK-cells).
- *In vivo* pathogenesis studies in pigs to examine the exact role of IFN during an infection with swine influenza virus.
- Comparative data of IFN induction and susceptibility to IFN for different swine influenza viruses *in vivo* in a porcine system.
- Results from *in vitro* studies on the molecular basis of IFN induction by swine influenza virus in porcine cells.
- Comparison of different avian influenza viruses with swine influenza viruses for IFN induction and susceptibility to IFN in porcine cells.
- Assessment of the spectrum of influenza virus recognition by PTX3 and other pentraxins.
- Detection of PTX3 levels in infected mice.
- Susceptibility of PTX3 gene targeted mice to influenza virus infection.
- Identification of the critical influenza virus components recognised by PTX3. Susceptibility of TIR8-deficient mice to influenza virus infection.
- Susceptibility of D6-deficient mice to influenza virus infection.
- Susceptibility of TLR-deficient mice to avian influenza viruses.
- Formation of a network for collaboration on avian influenza viruses with Chinese research institutions, agencies and hospitals.

POTENTIAL APPLICATIONS:

It is expected that FLUINNATE will provide innovative vistas on the immunopathology of influenza infection as well as candidate new markers and possibly therapeutic agents. Using cutting-edge technology (such as reverse genetics systems) useful recombinant viruses will be produced and provided to the scientific community for research purposes. Results on antiviral host restriction factors and viral virulence determinants will be of great interest to epidemiologists and healthcare authorities and may have an impact on future pandemic planning.

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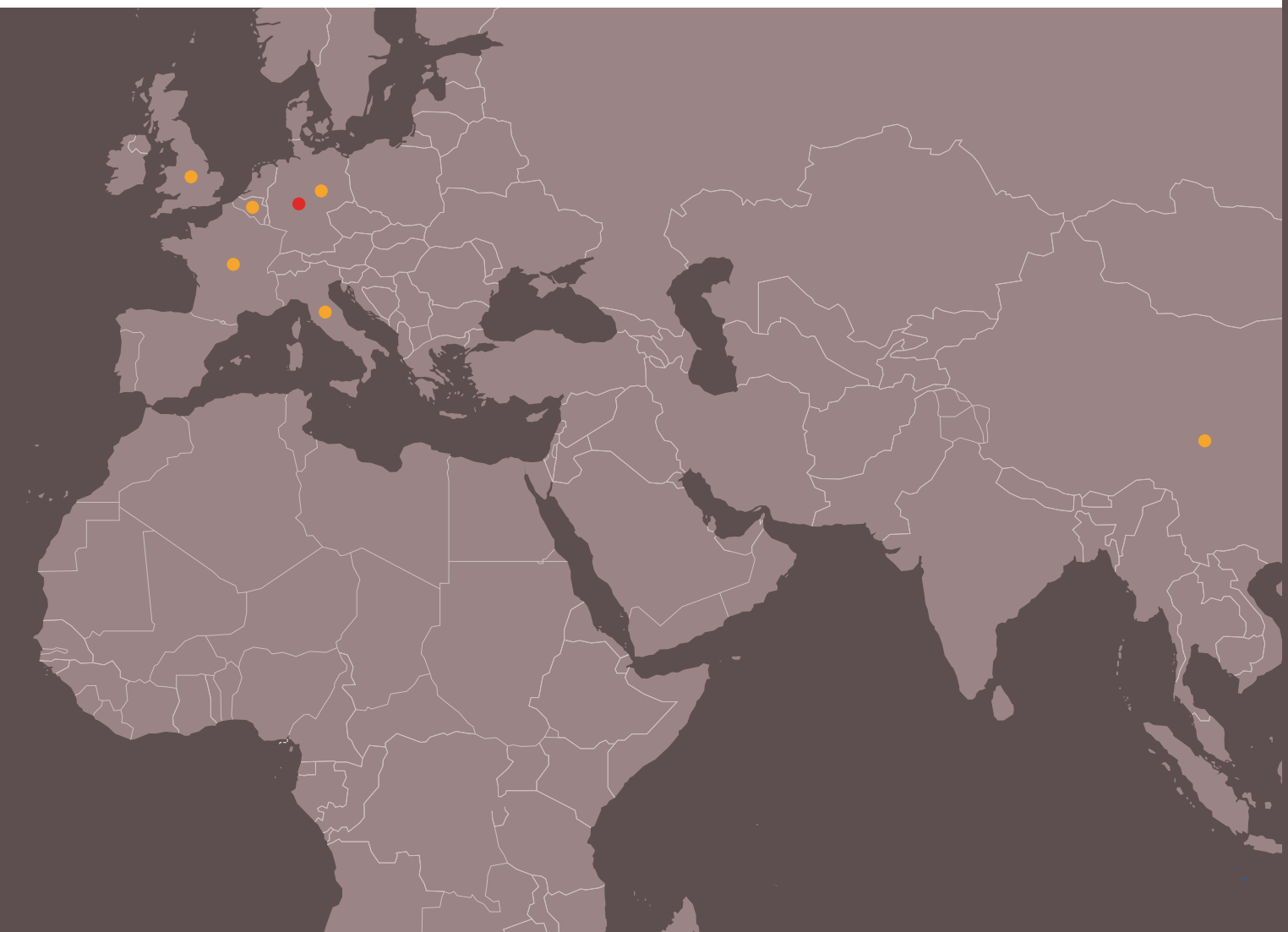

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SP5B-CT-2006-044161

FLUINNATE

MOLECULAR FACTORS AND MECHANISMS OF TRANSMISSION AND PATHOGENICITY OF HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS

Acronym: **EUROFLU**
EC contribution: €1 355 811
Duration: 36 months

Starting date: 01/01/2007
Instrument: STREP

Key words: Avian influenza viruses, pathogenicity, transmission, replication, virulence, vaccines, drugs, diagnosis

SUMMARY:

Epidemics caused by highly pathogenic avian influenza viruses (HPAIV) are a continuing threat to human health and to the world's economy. This now becomes clearly evident after the emergence of the HPAIV H5N1 subtypes that have infected and killed humans in Asia and Europe. The global dimension of current HPAIV infections of birds and humans highlights an urgent need to increase international and multidisciplinary research effort collaborations to develop new diagnostics, vaccines and drugs.

The EUROFLU consortium integrates interdisciplinary experimental and computational research approaches carried out by 11 partners from four EU Member States and from one Associated Member State. The overall objective of EUROFLU is to study the molecular factors and mechanisms of HPAIV transmission and pathogenesis. In order to build a profound scientific platform that will help to support the European policy makers in the fight against HPAIV, EUROFLU will focus on three major research tasks:

a) Identifying, characterising and validating HPAIV factors that are involved in the recognition and targeting of the virus to the cellular host receptor, thereby defining host range through advanced computational and biochemical analyses. Special focus will be put on the viral haemagglutinin protein, which binds to sialic-acid coupled cell receptors of human and avian hosts.
b) Revealing viral and cellular factors and mechanisms that regulate virus replication within the infected cell (and which can therefore determine cell tropism and host specificity) employing diverse virology, molecular- and cell-biology methods. Focus will be on virus/host interactions between viral factors of HPAIV and their crosstalk to cellular factors.
c) Using chicken and mouse models for *in vivo* analysis of HPAIV infections of birds and mammals to monitor HPAIV transmission and pathogenicity within organisms. The *in vivo* characterisation of immune responses and investigation of mechanisms of neurotropism in chicken and in mice upon HPAIV infection will allow the correlation and verification of EUROFLU's experimental and computational results in natural and in experimental host species, respectively.

PROBLEM:

Influenza is currently considered one of the most severe threats to human health and animal welfare. During the past 10 years outbreaks of HPAIV have occurred mostly in Central and East Asia, and since 1999 these viruses have also been found in the Middle East as well as in Eastern and Southern Europe. The unprecedented spread of the H5N1-type HPAIV in poultry and wild waterfowl throughout Asia, Europe and more recently in West Africa, has been associated with at least 241 transmissions to humans with a case fatality rate (CFR) of more than 50%. The virus also spread to several other mammalian species including cats. Should these viruses acquire the capacity for easy human-to-human transmission, the outbreak of a new influenza pandemic with a considerably high CFR could be expected.

Several infections of humans with the recent HPAIV H5N1 viruses have been characterised by an unusually strong cytokine response in the host ('cytokine storm') that appears to contribute to viral pathogenicity. There are also first indications of a neuro- and an endothelial-tropism of recent HPAIV in mammals. The molecular basis of the latter findings that may determine virulence and host cell tropism is not known and needs to be urgently defined.

To date there is no vaccine against H5N1 and variants resistant to current antiviral drugs are emerging. This highlights the urgent need for fast and specific diagnosis, an effective vaccine and new efficient antiviral drugs.

AIM:

The overall objective of the EUROFLU consortium is to understand the molecular determinants and the mechanisms responsible for virulence, host specificity, inter-species transmission and pathogenicity of HPAIV, which will eventually accelerate the development of effective diagnostic tools, anti-HPAIV vaccines and drugs.
To achieve this goal an interdisciplinary consortium of experts in virology, immunology and bio-computing has been established. EUROFLU will provide European authorities, policy makers and the community with advanced scientific knowledge supporting a successful control of avian influenza. The specific research aims are:

a) Defining virus/cell surface-interactions: This section of the scientific work aims to characterise the specific interaction between domains of the haemagglutinin subunit 1 (HA1) and the host-cell receptor (termed as recognition and targeting markers (RTMs)) as one of the main factors determining host range. This task will be approached by advanced computational and biochemical analysis. Focus will be on human and avian isolates of the H5 and H7 types of HPAIV and results will be compared with data gained from human H1 and H3 virus strains including the 1918 'Spanish Flu'.

b) Analysing intra-cellular virus/host-interactions: This part of the projects aims to understand the molecular mechanisms of interaction between HPAIV and host-cell factors and the role of these interactions for viral pathogenesis. The analyses will involve reverse genetics, genomic and proteomic techniques and will elucidate the similarities and differences of how HPAIVs replicate in avian and mammalian host cells and how they evade cellular defences. Furthermore, the current knowledge on HPAIV's misuse of cellular activities to support their propagation will be extended.

c) Determining virus/host-interactions in animal hosts. The studies in this section of the project will use mouse and chicken model systems for: (i) the characterisation of immune responses after HPAIV infection (ii) the investigation of mechanisms of viral neurotropism and neuropathogenicity in mammals and in birds.

EXPECTED RESULTS:

a) VIRUS/CELL SURFACE INTERACTIONS:
The expected outcome is enhanced knowledge of HPAIV cell specificity and tropism by the identification and characterisation of RTMs and through the investigation of HPAIV-RTM/host cell receptor interaction. This will lead to the identification of viral and cellular structures that can be used for diagnosis and for the generation of new vaccines and might be interesting targets for drugs designed to block virus/cell interaction.

b) INTRA-CELLULAR VIRUS/HOST INTERACTIONS:
This analysis is expected to increase the understanding of: (i) HPAIV replication in avian and in mammalian host cells; (ii) the viral mechanisms aimed at evading cellular defences; (iii) the HPAIV usage of cellular mechanisms for their propagation.

The knowledge gained will allow the characterisation of viral conditions that define cell tropism (avian or human) of HPAIV and will also pave the way for new anti-HPAIV drugs interfering with virus replication.

c) VIRUS/HOST INTERACTIONS IN ANIMAL HOSTS:
The results of this analysis will elucidate basic virulence mechanism(s) in the studied mouse and chicken models and they will also give important insights into the course of H5N1 infections in humans, which will help to adjust the medical treatment of patients.

POTENTIAL APPLICATIONS:

Results gained in the EUROFLU project will provide the knowledge base for new therapeutic approaches in the development of improved diagnostic tools and of vaccines to block HPAIV infections. It will set the molecular basis for new anti-viral drugs against influenza viruses independent of the specific virus strain and circumvent the constant risk of selecting resistant viral variants that make current drugs ineffective. The new understanding of virulence mechanisms will help to develop new strategies for the treatment of patients.

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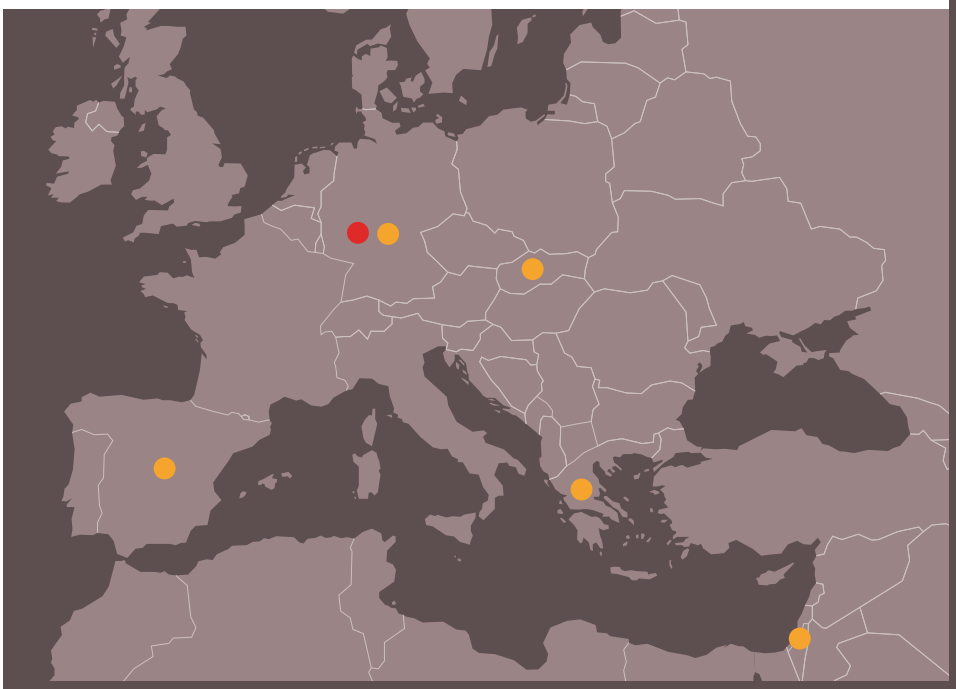
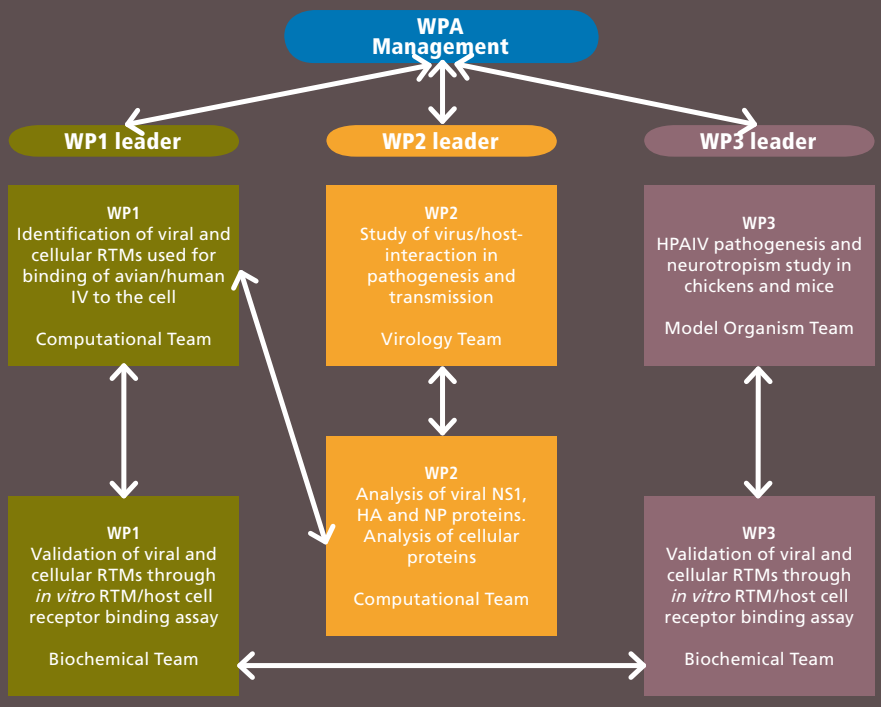


The EUROFLU STREP is composed of four Work Packages (WP1-4) and four Scientific Teams (Computational, Virology, Biochemical and Model Organism). The management in WP4 is headed by the Coordinator who will be assisted by the Administrative Manager and a Management Board, which includes members of the consortium. Each WP is headed by a Work Package Leader (WPL), who will implement the scientific and organisational decisions of the management. The scientific teams will work on the indicated topics and will strongly interact within the consortium.



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SP5B-CT-2007-044098
EUROFLU

AVIAN INFLUENZA: IMPACT OF VIRUS-HOST INTERACTIONS ON PATHOGENESIS AND ECOLOGY

Acronym: **FLUPATH**
EC contribution: €1 915 800
Duration: 36 months

Starting date: 01/01/2007
Instrument: STREP

Key words: Avian influenza virus, virus-host interactions, virus-receptor interactions, host-specificity, expression profiling, virulence, pathology, ecology, innate immunity

SUMMARY:

Highly pathogenic avian influenza viruses (HPAIV) have acquired the unprecedented and alarming capacity to infect humans. By establishing a permanent ecological niche in wild birds, HPAIV will pose a continuous risk for poultry and fatal human infections, especially if these birds excrete HPAIV without showing any clinical signs of disease. These changes in the ecology of the disease and behaviour of the virus may create opportunities for a pandemic virus to emerge.

Attempts to avoid or contain HPAIV outbreaks have been largely unsuccessful. This can be directly linked to our lack of fundamental knowledge. Therefore, it is essential to increase our knowledge of the ecology and pathology of avian influenza virus infections in poultry and other species.

Full understanding of the ecology and pathogenesis of HPAIV requires a multi-disciplinary approach determining host-pathogen interactions and the role played by the host immune response. To this end, the FLUPATH consortium was established.

FLUPATH is composed of 13 partners, six of which are National Reference Laboratories for avian influenza. The consortium further includes five academic institutions and two institutions that specialise in animal science and health. The participants, with expertise in chicken genomics, micro array technology, pathology, receptors, innate immunity and chicken immunology will use multidisciplinary and complementary approaches to address key problems and unanswered questions with respect to the ecology and pathogenesis of avian influenza.

FLUPATH will provide knowledge and tools for new strategies which will be tailored for the control and management of avian influenza at the European and international level. This will limit the impact of the disease both in terms of human health and losses to the poultry industry. The accompanying reduction in animal slaughter and financial and economic losses will place a significantly lower demand on EU and Member States' budgets.

PROBLEM:

Lack of sufficient fundamental knowledge: avian influenza represents one of the major concerns for public health that has recently emerged from the animal reservoir. The increased relevance of avian influenza in the fields of animal and human health has highlighted the lack of scientific information on the disease. This has hampered the management of some of the recent crises, resulting in millions of dead animals, concern over loss of human lives and management of the virus's pandemic potential. For this reason, and for the devastating effects on the poultry industry, international organisations such as WHO, OIE and FAO have worked together and established a coordinated set of guidelines and action plans to combat the ongoing Asian epidemic.

Due to the low profile of avian influenza until 1997, a significant amount of information and the specific tools necessary to manage avian influenza epidemics adequately are lacking. This includes both the EU situation and the ongoing H5N1 crisis. Recent outbreaks of HPAI have affected avian species that are showing a reduced susceptibility to this virus. If HPAI infection of the wild bird host becomes compatible with normal behavioural patterns and migration, the result will be the development of an endemic cycle in wild birds. The consequences of such a situation are unpredictable and potentially very dangerous.

Retrospective analysis of recent outbreaks has permitted the identification of weak points in the management system that represent areas of uncertainty for which improvement is required. Several of these weak points can be directly linked to our lack of fundamental knowledge about the importance of both viral as well as host factors in determining the outcome of infection. Therefore, it is essential to increase our effort to enhance our knowledge about the ecology and pathology of avian influenza virus infections in poultry and other species.

AIM:

This proposal aims to generate data on significant issues linked to AI outbreak management on which scientific knowledge is currently lacking. These issues are all related to virus-host interactions. Four major tasks (work packages) have been identified which address the objectives of task 3 of the EU call SSP5-B INFLUENZA: ecology and pathogenesis of avian influenza infections:

Work Package 1 (WP1) addresses the issue of pathogen-host interactions and virulence determinants at the molecular level. Little is know about the host-response following infection with viruses that differ in virulence and gene constellation. The contribution of specific viral genes — or gene sequences — to pathogenesis, host- and tissue-tropism as well as their role in interference with host defence mechanisms will be examined *in vivo* in different species in animal experiments as well as *in vitro* using professional antigen presenting cells such as dendritic cells and macrophages (see also WP4 below). State-of-the-art technology such as gene-expression profiling using DNA micro-arrays and identification of differentially expressed genes using gene libraries obtained by suppression subtractive hybridisation (SSH), will be used to identify specific host genes which are involved in the reaction to infection by specific avian influenza virus genotypes. The involvement and importance of viral genes as well as host genes will be evaluated by cross-validation and *in vitro* experiments.

Work Package 2 (WP2) is mainly concerned with studying the ecology and pathology of different avian influenza strains in different host species. These studies will evaluate and compare host-pathogen interactions of different HPAI and LPAI viruses. These evaluations include histopathological descriptions of virus infection, quantification of viral loads in different organs and tissues by real-time RT-PCR and confocal immunohistochemistry. Apart from chickens, specific attention will be given to water birds such as ducks (mallard) which seem to be clinically less

susceptible to highly pathogenic avian influenza and therefore are implicated in transmission of the virus. Furthermore, experiments on pigs will be conducted in order to assess their role in maintenance and transmission of avian influenza since pigs may serve as a 'mixing vessel' for avian and human viruses. The significance of several adaptive mutations such as the NS1 truncations and lys 627 PB2 mutations will be examined in a chicken and mouse model system using either natural isolates or specific mutants generated by means of reverse genetics. Finally, the capacity of avian influenza to cross the species barrier will be examined by using mammalian-adapted viruses.

Work Package 3 (WP3) will address the issue of virus-receptor interactions with the goal of determining the role of receptor specificity and neuraminidase activity of avian viruses in interspecies transmission, pathogenicity and emergence of potentially new pandemic strains. Different receptor phenotypes might provide the virus with an enhanced potential for interspecies transmission to pigs and humans. Molecular mechanisms for avian-to-avian, avian-to-pig and avian-to-human transmission will be studied by using different cells and tissues including tracheal explants from chickens, turkeys and ducks, and cultures of human airway epithelium.

Work Package 4 (WP4) is concerned with the identification of virulence factors that determine avian influenza virus pathogenesis and transmissibility, focusing on the interaction of HPAI and LPAI viruses with the innate immune system. These studies will analyse the requirements of the virus to adapt to its host and to interact with the different cellular compartments of the innate defence system, in particular dendritic cells (DC), macrophages (MΦ) and natural killer (NK) cells. The role of the HA protein in eliciting *in vivo* and *in vitro* cytokine responses will also be investigated.

EXPECTED RESULTS:

The FLUPATH proposal will improve our understanding of the origins of HPAIV, the patterns of its evolution, and its behaviour in avian and mammalian species. Work on currently circulating viruses will allow us to track changes in the present situation and thus issue precise warnings, should the threat of a pandemic increase.

It is anticipated that this approach will permit a more complete understanding of the immunological, cell biological and molecular basis of the threat posed by HPAIV such as the current H5N1. Essential information on critical viral and host factors, which determine the transmissibility of the virus to mammals, will be determined. This knowledge will clearly be of high value when implemented in novel strategies to combat avian influenza.

POTENTIAL APPLICATIONS:

FLUPATH will ultimately provide knowledge and tools for new strategies which will be tailored for the control and management of AI at both a European and international level. This should result in a noticeable reduction in the impact that this disease has had in the past.

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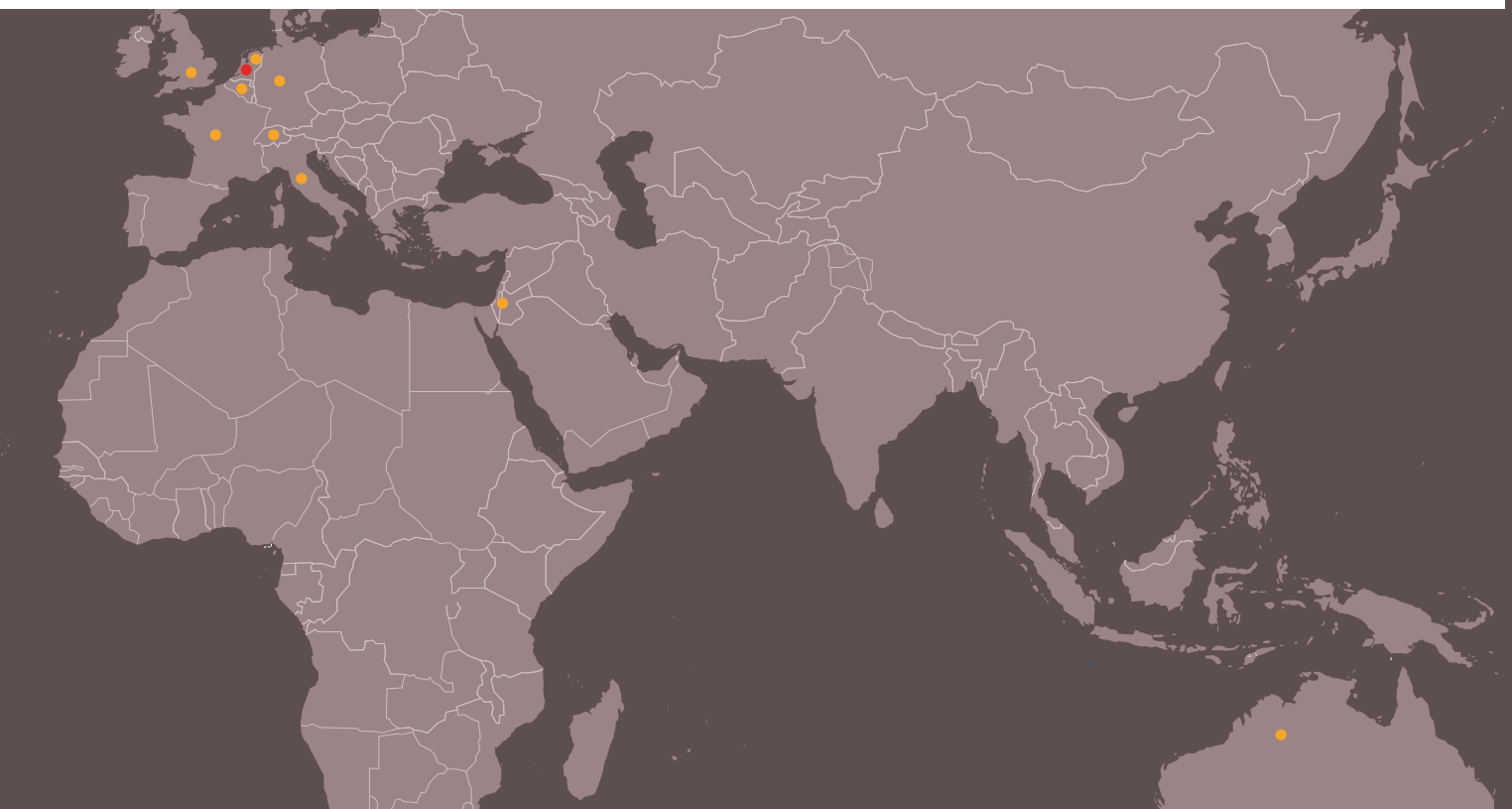

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SSPE-CT-2007-044220

FLUPATH

INFLUENCE OF VIRAL PROTEINS OF AVIAN INFLUENZA VIRUS ON THE INNATE IMMUNE RESPONSE OF BIRDS

Acronym: **INN-FLU**
EC contribution: €1 800 000
Duration: 36 months

Starting date: 01/01/2007
Instrument: STREP

Key words: Avian influenza virus, innate immunity, pathogenesis, susceptibility, transmission, wild bird species, monitoring

SUMMARY:

We are currently facing a continuing infection cycle caused by a highly pathogenic avian H5N1 influenza virus (hpH5N1) in several countries in Asia. This virus may have the potential to become a pandemic virus. There have been outbreaks of the H5N1 virus in poultry in several countries in Asia since 1997. In 2005 hpH5N1 was first observed outside of Asia in European poultry, which was followed by a fast spread in Europe during February 2006.

The most dangerous property of this hpH5N1 virus is its ability to infect and cause disease in other species than birds including cats, tigers, dogs and stone martens. The INN-FLU consortium integrates nine partners from six EU Member States (Germany, Belgium, United Kingdom, Poland, France and Greece). The project will contribute to an understanding of virus pathogenesis and virulence determinants in chicken, determine host-pathogen interactions and define the molecular basis for host specificity. It is based on four areas of research:

a) Characterisation of the virulence of hpH5N1 in several species.
b) Determination of host factors influenced by AIV infection during the very early phase of infection.
c) Research on whether different lines of chicken breeds show differences in resistance to hpH5N1 infection.
d) Investigation of contact points between wild birds and domestic free range poultry and migratory behaviour of birds based on satellite tracking.

Experiments will address the question of whether HA molecules from non-H5 and non-H7 strains can give low pathogenic viruses the potential to become highly pathogenic. Studies on the host-immune response will focus on the antiviral innate immune response in birds, the role of the influenza virus protein NS1 in contributing to virulence in chickens and expression of genes related to the interferon pathway including Mx.

PROBLEM:

The continuing outbreaks of disease caused by hpH5N1 in poultry and other birds and of human cases in Asia, Europe and Africa are cause for serious concern. Unlike in the past for hpAIV, there are increased concerns about the potential of this hpH5N1 virus to initiate a human pandemic. It is, however, not possible to predict with certainty if and when this might occur.

The spread of the hpH5N1 virus during the last year was not to be predicted in the observed dimensions, but also emphasised the need for additional knowledge of the disease in poultry, specifically water fowl. In February 2006 the virus entered the European Union with the first description of hpH5N1 in Greece. Whether this occurred via migratory birds is not clear.

It is becoming increasingly evident that hpH5N1 can spread via infected ducks and probably also via other migratory species. The hpH5N1 virus was subsequently isolated from more stationary living birds (e.g. swans) in many EU Member States. This raised great concern with regard to the potential of migratory birds for propagating and spreading these viruses and it underlined the important role of wild birds in viral spread. In addition, these H5N1 viruses become less pathogenic to domestic ducks but remain pathogenic to other domestic poultry.

Host factors which influence the outcome of the hpH5N1 infection and the function of viral elements which influence the virulence of the virus are poorly understood. Research and technological developments will further increase the knowledge of AIV and influenza virus in general. Basic research on virus structure and function, viral pathogenesis, and the host response due to infection will lead to more effective approaches to controlling influenza virus infections.

AIM:

The principal objectives of the INN-FLU consortium are to use synergies between the partners in order to improve knowledge about the susceptibility of different bird species for AIV, the innate immune response in AIV infected animals and the ecology of AIV, important for the transmission of the infection between wild and domestic birds. The work plan includes exclusively bird species due to the importance of the disease in these species. To achieve this goal an interdisciplinary consortium of experts in molecular virology, genetics, immunology and ornithology has been established. INN-FLU will provide European authorities, policy makers and the community with scientific knowledge enabling a better prediction of potential risks and a successful control of avian influenza.

The scientific aims of the consortium are:

a) comparing different hosts for the susceptibility to HPAI H5N1 infection. Investigation on the susceptibility of different bird species including the pathogenesis, immune response and clinical picture will yield important data about the time of infection and disease symptoms which can be observed and looked at in the field. Observed differences in virulence between bird species can possibly be correlated to differences in tissue distribution of the virus (tissue tropism) and the local immune response;

b) defining the role of genetically-determined innate immunity in susceptibility and resistance to HPAI H5N1 infection. The results will inform us whether there are differences in susceptibility to hpAIV in certain chicken lines and if this difference can be related to specific components of the innate immune response. A special emphasis will be laid on the interferon response during the very early phase of infection;

c) defining the influence of NS1 protein of AIV on the innate immune response of chickens. Available data indicates that NS1 influences

mechanisms in the cell which directly or indirectly reduce the cytokine responses of infected hosts. As AIV field isolates with truncations in the NS1 genes have been described it will be important to analyse their significance. Tools to study the avian cytokine system have only recently become available and a significant number of these tools have been established by partners of the INN-FLU consortium;

d) analysing the function of several AIV genes for their contribution to virulence in chicken. The work is focused on the viral proteins haemagglutinin (HA) and neuraminidase (NA). Several mechanisms have been hypothesised that only the HA gene of the H5 and H7 can acquire the property to become the highly pathogenic pathotype of AIV. The aim of this task is to determine whether only the introduction of a polybasic cleavage site would be sufficient to lead to the hpAIV phenotype and whether something other than H5 and H7 genes can also support the generation of hp AIV;

e) determining the ecology and transmission of avian influenza. This will involve the investigation of contact frequencies between wild birds and domestic fowl in open pens. Monitoring of wild birds and recording of contact points between wild birds and domestic poultry in free-range pens will yield important epidemiological data for future models applicable for wild life monitoring programmes.

EXPECTED RESULTS:

It is expected that the results of the INN-FLU project will contribute significantly to a better understanding of why the new hpH5N1 isolates are able to kill different wild bird species (including water fowl). Infection studies with the hpH5N1 AIV of different species of birds will answer the questions about the induction of disease symptoms as well as the virus excretion in these animals. Also the age dependency for the occurrence of disease symptoms will be explored. This is important since the first line of defence against avian influenza worldwide is surveillance. The results obtained from the infection experiments in combination with the data on bird surveillance during their migration and possible contact points with free range poultry will give a profound basis for an objective, knowledge-based risk assessment. Experiments with the different chicken lines will determine different susceptibility patterns between certain chicken breeds.

Consequently, an increase in the level of genetic resistance provides a possible means of enhancing protection of flocks against AIV infections. The analysis of the innate immune response which is important as the first line of defence against influenza viruses will increase the knowledge about crucial molecular virus-host interactions.

POTENTIAL APPLICATIONS:

Outbreaks of hpH5N1 in domestic poultry, wild birds and humans have caused serious concerns. The unpredictable speed of the spread of the virus during the last years has left questions about how the virus became present in a number of European countries in a very short time. The INN-FLU project will add useful information for surveillance studies on which birds should be predominantly monitored and which clinical signs can be expected. This will have great impact on monitoring programmes and their design. The knowledge that will be gained in the INN-FLU project with regard to the molecular determinants important for innate immune response and disease pathogenesis will improve and support vaccine developments to control AIV.

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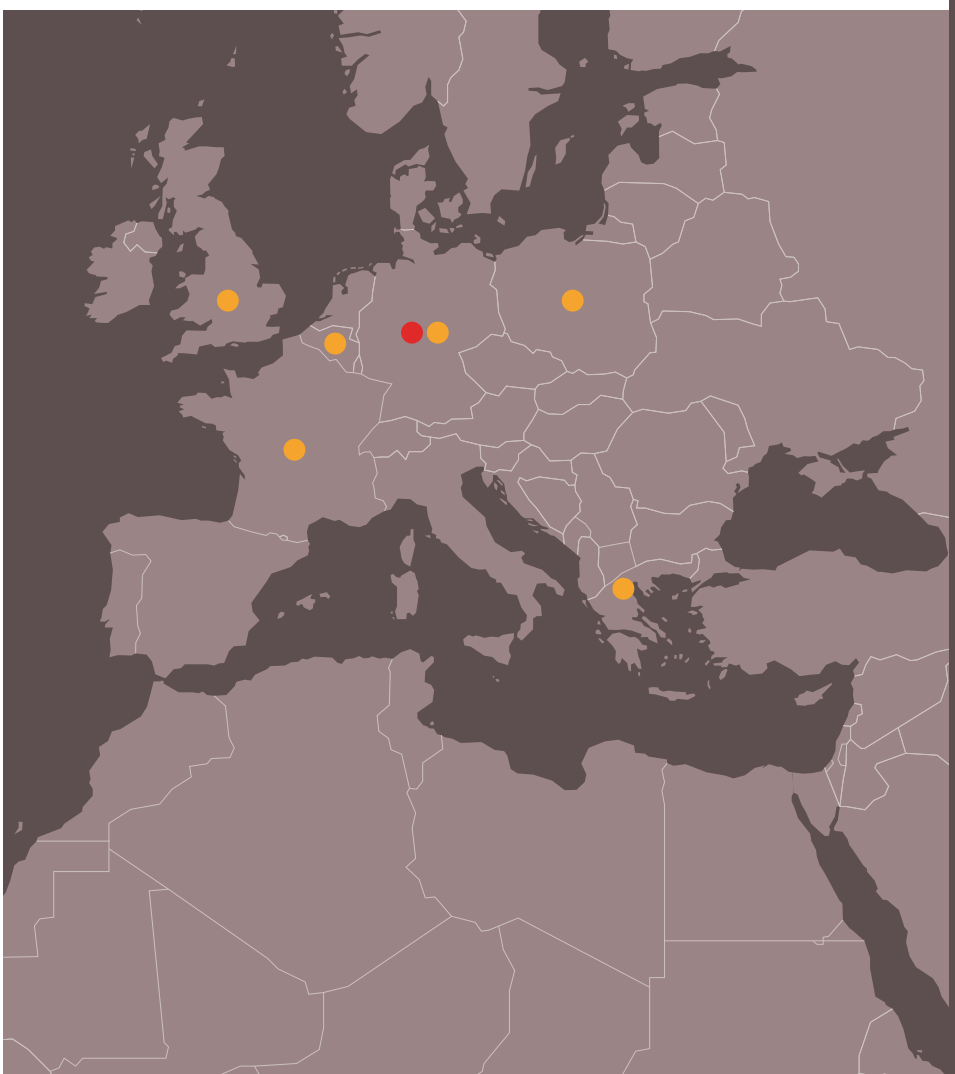

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SSPE-CT-2006-044372

INN-FLU

COMPARATIVE STRUCTURAL GENOMICS ON VIRAL ENZYMES INVOLVED IN REPLICATION

Acronym: **VIZIER**
EC contribution: €12 905 986
Duration: 48 months

Starting date: 01/11/04
Instrument: Integrated Project

Key words: RNA viruses, genomics, structural genomics, antiviral drugs, crystal structure, bioinformatics, protein production, high-throughput, screening

SUMMARY:

This project aims to impact the antiviral drug-design field through the identification of potential new drug targets against RNA viruses and their use in a comprehensive structural characterisation of a diverse set of viruses. The common strategies used for the development of antiviral drugs are mainly based on the knowledge accumulated through studies of virus genetics and structure. The VIZIER project proposes to fill the existing gap between the necessary scientific characterisation of emerging viruses and pre-clinical drug design.

PROBLEM:

RNA viruses include more than 350 different major human pathogens and most of the etiological agents of emerging diseases: viruses of gastroenteritis (more than 1 million deaths annually), measles (more than 45 million cases and 1 million deaths annually), influenza (more than 100 million cases annually), dengue fever (approximately 300 million cases annually), enteroviruses and encephalitis (several million cases of meningitis annually), hepatitis C virus (more than 150 million infected people in the world). The SARS outbreak has dramatically demonstrated how high the economic cost of an epidemic caused by an emerging virus could be. This negative impact is actually widening every day, as many governments are forced to make costly arrangements to cope with the threat of bio-terrorism, which lists some deadly RNA viruses in its arsenal.

To meet these challenges, science needs to look for new therapeutic and prophylactic substances active against RNA viruses since those currently available are scarce and of poor potency. The common strategies used for the development of antiviral drugs are mainly based on the knowledge accumulated through studies of virus genetics and structure. Yet, it is a strange paradox that genomic and structural characterisation of RNA viruses was not accepted as a priority until very recently. The VIZIER project proposes to fill the existing gap between the necessary scientific characterisation of emerging viruses and pre-clinical drug design.

AIM:

To address society's needs, scientists need to anticipate potential threats in order to be ready should they arise. The participants of the VIZIER project have created a team that brings together the leading authorities on RNA viruses available in the EU or elsewhere as well as many leading European structural biologists. This team includes three partners with P4 facilities, as well as leaders in the field of structural genomics. The development of protocols for high-throughput (HTP) protein production means that a concerted programme of structure determination is now appropriate and feasible. Because they are both the most likely to emerge and the most prone to genetic variability, the VIZIER consortium will characterise RNA viruses that do not include a DNA stage in their replicative cycle. These virus classes employ profoundly different replicative mechanisms driven by poorly characterised replication machineries. Although virus-specific, the latter are the most conserved and essential viral components and, thus, the most attractive targets for antiviral therapy.

In the framework of this project, the core enzymes/proteins of the replication machinery carefully selected among 300 different RNA viruses, including strains of medical interest, will be characterised. One unique feature of VIZIER, compared to other structural genomics projects, is the integration of major structural effort within a broad multidisciplinary study, having virology upstream and target validation (candidate drug design) downstream. As a result, the implementation plan of the VIZIER project is structured into six interacting scientific sections:

a) Bioinformatics, for genome annotation, target selection and data integration.
b) Virus production and genome sequencing.
c) HTP protein production.
d) HTP crystallisation and structural determination.
e) Target validation to assess the function of enzymes and design strategies for virus inhibition.
f) Training, implementation and dissemination.

This organisation will allow in record time, the full characterisation of a viral target that can quickly be used to design drugs, either by the pharmaceutical industry through the VIZIER Industrial Platform or by any R&D institution.

EXPECTED RESULTS:

VIZIER will produce an unprecedented wealth of data on replicases of RNA viruses with a window into the antiviral drug development. A representative set of RNA-based viruses that belong to three major classes, profoundly different in their replicative strategies, will be characterised by a concerted and multidisciplinary effort unparalleled to date. At the end of this programme, the percentage of sequenced genomes of RNA virus species that infect vertebrates will virtually double from 30% to 55%. As an example, the genomic characterisation of Flavivirus (ssRNA+) and Arenavirus (ssRNA-) genera, which include a large number of human pathogens, will be systematically achieved.

A dramatic advancement is expected in the number and diversity of 3D structures of the replicative subunits, now in the one-digit range. VIZIER will aim at the identification of lead molecules inhibiting the replicative enzymes, but will not enter into the broad field of drug development. Offers of cooperation will be made to the pharmaceutical and biotechnology industry for further drug development, on a contractual basis, and through the VIZIER Industrial Platform, which connects upfront scientific results to the pharmaceutical industry.

POTENTIAL APPLICATIONS:

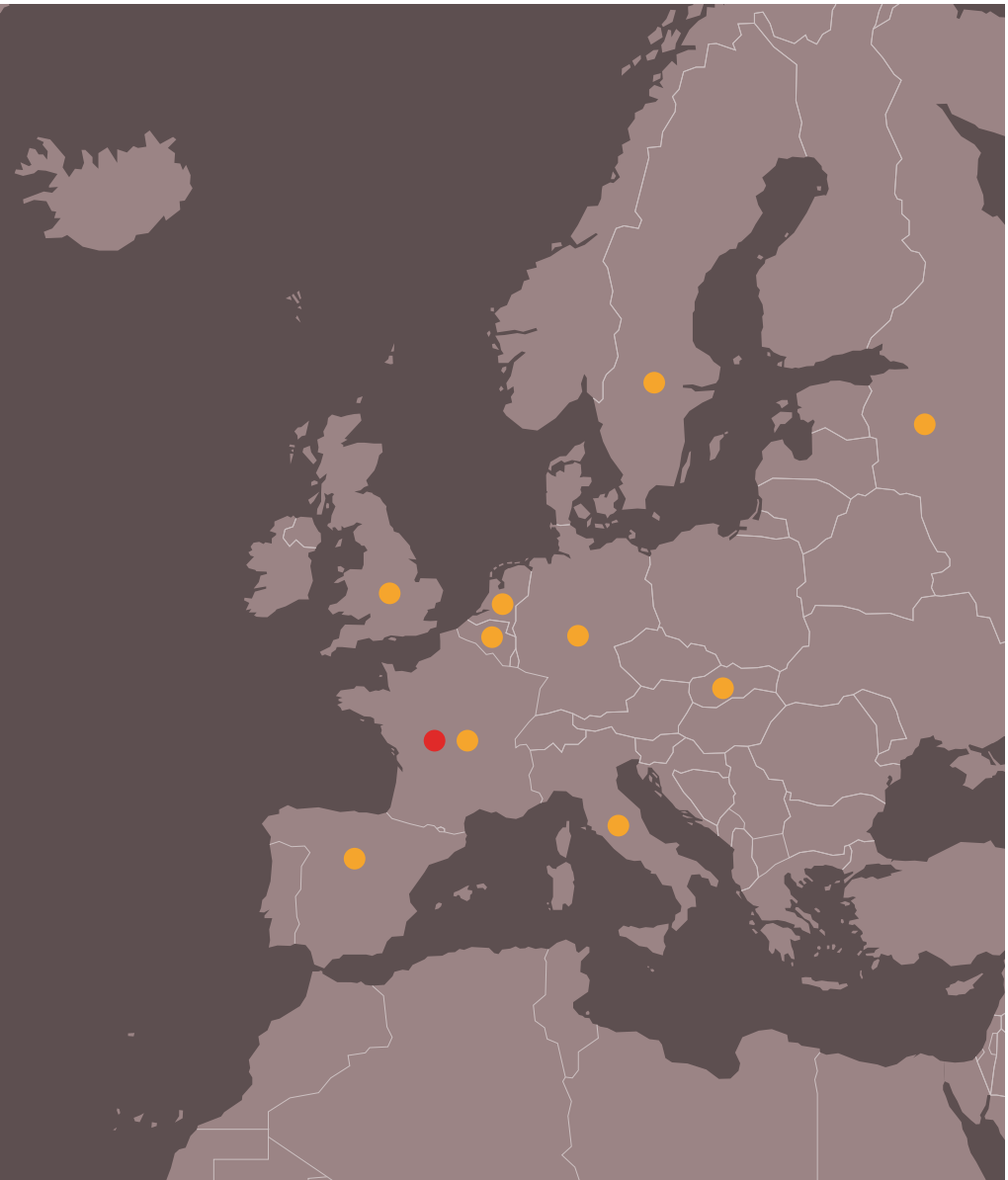
With no equivalent integrated programme in the world, the VIZIER project will undoubtedly have a profound impact on the field of structural

genomics of emerging viruses. In particular, it is expected that VIZIER will contribute very significantly to the sequencing of new viruses (viral genomics) as well as to the deposition in the Protein Data bank of new crystal structures of viral proteins. These viral proteins can then be considered as targets for drug design. The scientific impact on drug design is expected to be considerable through concepts and methods implemented up to the drug design step. Indeed, current drug discovery still often relies on screening compounds in a blind manner.

Thousands or millions of compounds are screened on infected cells or purified enzymes, and 'hits' are selected. However, initial discovery of the inhibitor activity is followed by a lengthy and costly process of toxicology and other confirmatory studies. It is widely believed that structural biology is capable of speeding up the whole process, and providing the anticipatory power for future emerging viruses. HTP crystallography coupled to a strong validation section such as that proposed in VIZIER, will undoubtedly reinforce this trend by leading the field. Indeed, the concept of finding a drug together with its target, and the putative bottlenecks to further improvement is scientifically challenging, innovative, and promising.

VIZIER will develop new products, technologies and strategies. The products are RNA virus genomic sequences, soluble viral protein domains, their 3D structures, assigned protein functions, and inhibitors or ligands for selected protein targets (drug leads). All the above products will have a substantial impact on our (currently limited) understanding of the RNA viral replication machinery. They will also identify entirely new targets for the development of specific drugs, with a high level of detail.

Collectively such information is seen as being of strong strategic value, not only for the health issues described, but also for the development of industrial enterprises. Although diverse DNA-based cellular and viral parasites are also responsible for a large fraction of different human infections, none of them is so poorly controlled by drugs as the RNA viruses are. Recent viral outbreaks (H5N1, SARS, Chikungunya) confirm the difficulty of designing drugs in a timely fashion and the need for scientific anticipation. Consequently, drug development against RNA viruses - the ultimate goal of the VIZIER project - is becoming a top priority for global healthcare programmes.



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LSHG-CT-2004-511960

VIZIER

IMMUNE RESPONSE TO VIRAL RESPIRATORY INFECTIONS AND VACCINATION IN THE ELDERLY

Acronym: **RespViruses**
EC contribution: €1 770 361
Duration: 36 months

Starting date: 01/01/2007
Instrument: STREP

Key words: New respiratory viruses, immune response of the elderly, mouse models, diagnosis, siRNA, EGS

SUMMARY:

During the last years some new respiratory viruses (HMPV/hCoVNL63/SARS/ Bocavirus/flu H5N1) have emerged. In addition to other viruses, such as RSV, EBV and Paramyxoviruses, they are able to induce severe respiratory diseases in high-risk patients, in particular young children and the elderly.

PROBLEM:

We will study the innate and acquired immune response of the elderly against the new and known respiratory viruses. Clinical and basic research aspects will be addressed equally, and new diagnostic assays to evaluate the immune status of the elderly will be developed. Within the project an animal model for investigation of elderly people's immune response will be established. In this model antiviral agents will be tested for their ability to support the patient's immune response. Up to 40 000 elderly sera collected during the last nine years will be tested for antibodies to emerging respiratory viruses (HCoV-NL63/HMPV/RSV/EBV/fluH5N1), providing a wide view of the epidemiology of these viruses. For a precise view of elderly people's immune response to the viruses, a defined and uniform panel of relevant clinical data related to respiratory infections will be collected. Internationally standardised prospective data mining and a new multi-lingual software tool will be developed within the project and used by the partners.

EXPECTED RESULTS:

We expect to gain detailed knowledge on the innate and acquired immune response to emerging respiratory viruses in the elderly. A thorough basic investigation of emerging respiratory viruses will provide further handles to develop antiviral strategies based on siRNA and external guide sequences (EGSs). Furthermore, known antivirals will be evaluated with the final goal of supporting the elderly's immune response whilst reducing mortality in the elderly caused by respiratory infections.

Coordinator Oliver Schildgen, UKB, working on virus growth in cell culture © *European Commission*

POTENTIAL APPLICATIONS:

New diagnostic assays; new antiviral therapies; optimised treatment and vaccination strategies; new software tool for surveillance and clinical data mining.

Small and medium enterprise (SME) contribution: Three European SMEs are partners in RespViruses, and are based in Belgium, Germany and Spain. These SMEs will receive approximately 50% of the project budget. Having INGENASA, skilled in enzyme immunoassays, monoclonal antibodies production, nucleic acid cloning and recombinant protein expression as a partner, will help to develop new prototypes for diagnostic assay development in respiratory diseases. INGENASA is an SME biotechnology company dedicated to the research, development, production and commercialisation of products for the diagnostic sector and it has a 25-year history in this field, mainly for viruses that affect livestock animals or pets. INGENASA is also active in areas of prevention of animal diseases (vaccines) and has participated in several funded European projects (BRIDGE, BIOTECH, FAIR, FP5 and FP6 programmes).

The Belgian SME RNA-TEC, with its profound knowledge and expertise in oligonucleotide chemistry plays a crucial role in the project by designing and synthesising suitably stabilised siRNAs and external guide sequences that target highly conserved RNA sequences of the respiratory viruses that are the subject of the study. The partners will test these compounds *in vitro* and also in suitable animal models. A long-term goal of the project is to identify potent lead compounds that can be further exploited as potential antiviral therapeutics using appropriate delivery systems that we can access.

As software developer and consulter in IT departments, SME Mattes Hamann will cover two areas of the project - the internal communication and presentation of the project information and collection and evaluation of medical data. Therefore, multilingual software for acquiring relevant medical data around respiratory disease will be developed. Mattes Hamann already has experience in surveillance tools. They will create and develop the project website, a contact management system and a software tool for acquiring project information, and will also consult any team member if necessary.

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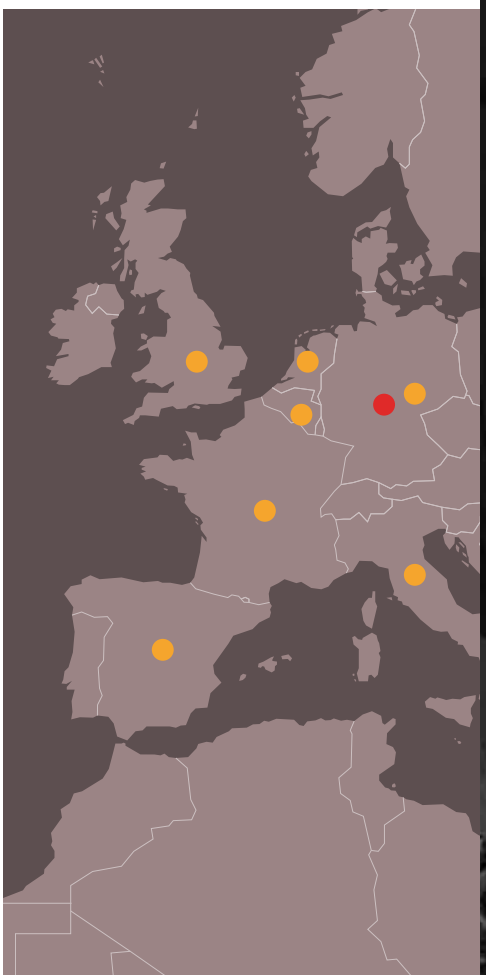

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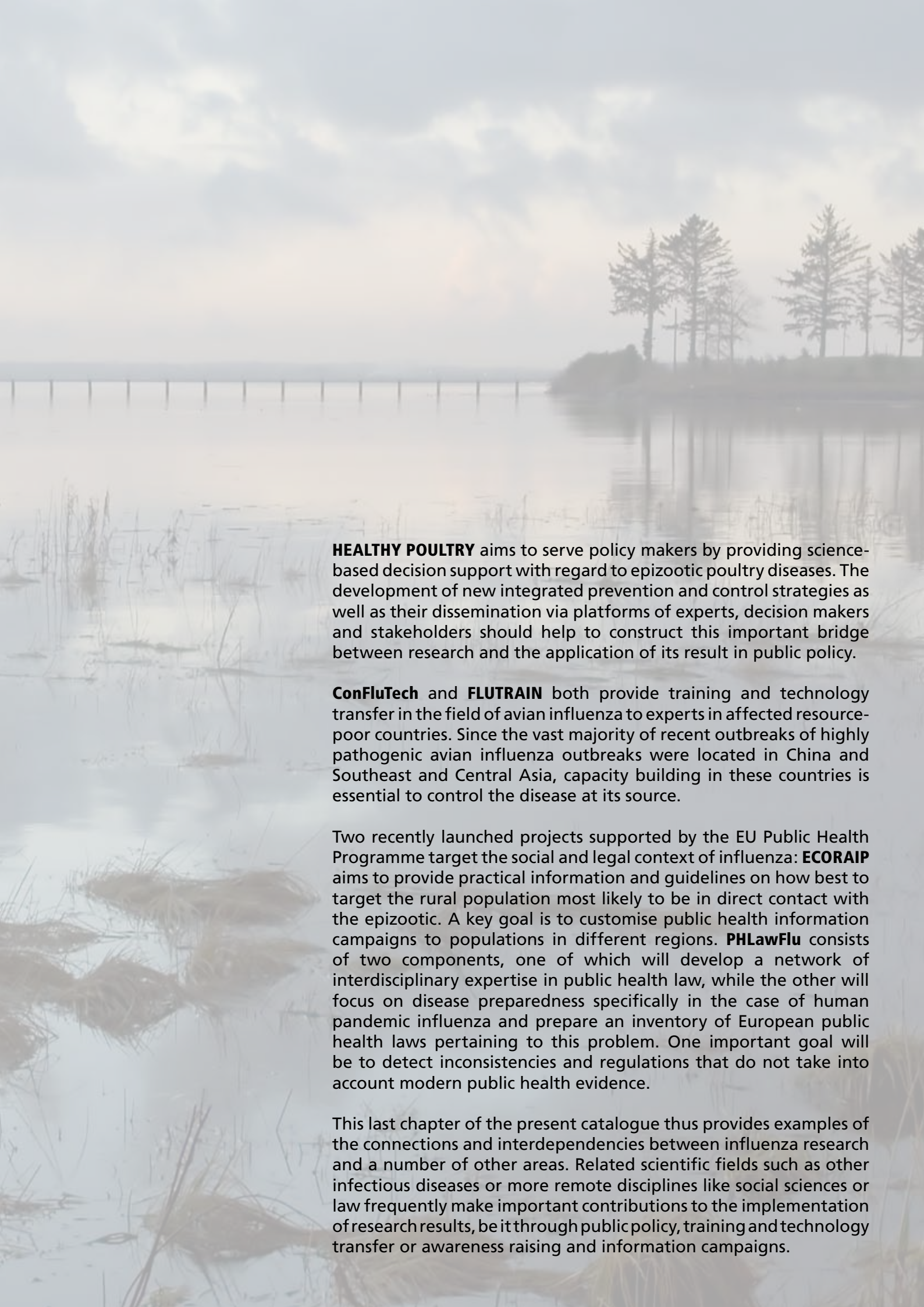
LSHM-CT-2006-037276
RESPVIRUSES

NETWORKING, TRAINING, SOCIO-ECONOMIC AND LEGAL ISSUES

This heterogeneous chapter includes projects that put influenza research into a wider context – be it the context of other related diseases, the development of networks, the link between science and policy and/or the social and legal context of the disease.

EPIZONE and **VIRGIL** are large Networks of Excellence with a broad-based mission to integrate Europe-wide efforts to 'improve research on preparedness, prevention, detection and control of epizootics' and to 'achieve common research objectives in the area of antiviral drug resistance', respectively. Both networks tackle highly important and topical problems and include major components of influenza: in **EPIZONE** as one of the most important epizootics, and in **VIRGIL** as a disease for which resistance to common antiviral drugs is increasingly being reported and might present a major problem in the case of a pandemic. **FLU-LAB-NET** is a smaller Coordination Action exclusively focusing on influenza with the goal of strengthening the network of Community Reference Laboratories and National Reference Laboratories for this disease. Improving the sharing of methodological, virological, genetic, epidemiological and clinical information on influenza will deliver a much needed research base for these laboratories with the key task of providing fast and reliable information to public health authorities, policy makers and citizens in the case of outbreaks.



HEALTHY POULTRY aims to serve policy makers by providing science-based decision support with regard to epizootic poultry diseases. The development of new integrated prevention and control strategies as well as their dissemination via platforms of experts, decision makers and stakeholders should help to construct this important bridge between research and the application of its result in public policy.

ConFluTech and **FLUTRAIN** both provide training and technology transfer in the field of avian influenza to experts in affected resource-poor countries. Since the vast majority of recent outbreaks of highly pathogenic avian influenza outbreaks were located in China and Southeast and Central Asia, capacity building in these countries is essential to control the disease at its source.

Two recently launched projects supported by the EU Public Health Programme target the social and legal context of influenza: **ECORAIP** aims to provide practical information and guidelines on how best to target the rural population most likely to be in direct contact with the epizootic. A key goal is to customise public health information campaigns to populations in different regions. **PHLawFlu** consists of two components, one of which will develop a network of interdisciplinary expertise in public health law, while the other will focus on disease preparedness specifically in the case of human pandemic influenza and prepare an inventory of European public health laws pertaining to this problem. One important goal will be to detect inconsistencies and regulations that do not take into account modern public health evidence.

This last chapter of the present catalogue thus provides examples of the connections and interdependencies between influenza research and a number of other areas. Related scientific fields such as other infectious diseases or more remote disciplines like social sciences or law frequently make important contributions to the implementation of research results, be it through public policy, training and technology transfer or awareness raising and information campaigns.

DEVELOPMENT AND ENHANCEMENT OF LABORATORY NETWORKS FOR AVIAN INFLUENZA

Acronym: **FLU-LAB-NET**
EC contribution: €930 000
Duration: 36 months

Starting date: 01/05/2007
Instrument: Coordination Action

Key words: Avian influenza, laboratory, network, FLU-LAB-NET

SUMMARY:

FLU-LAB-NET provides new opportunities for enhancement and reinforcement of the Community Reference Laboratory and National Reference Laboratory network for avian influenza (AI) within the EU. This will strengthen harmonisation and development of laboratory and diagnostic methods, coordination of research efforts and sharing of expertise. Rapid responses to national and global emergencies with data sharing will be key areas of exploitation, contributing to a European laboratory task force capability for AI in animal species. Rapid, formal interactive communications will be addressed through web-based forums.

Laboratories involved in influenza research on domestic mammals will also participate. FLU-LAB-NET will also foster formal links and coordinate with corresponding human, swine and equine influenza networks. FLU-LAB-NET provides opportunities for identification and development of the complementarities of global, multi-disciplinary influenza research programmes. Strategically important third country and INCO partners are also included in this network, in order to raise laboratory standards and benefit from knowledge sharing. This will promote greater trust, understanding and early access to information that may be of importance to both veterinary and public health in the EU.

PROBLEM:

The current global H5N1 crisis has resulted in a significantly increased demand for laboratory capacity and capabilities for AI diagnosis and surveillance. The rapidly expanding growth in AI work has highlighted the benefits of closer collaboration within existing AI laboratory networks, which we propose to address. The spread to humans has also highlighted real concerns of the pandemic potential of H5N1, reinforcing the need for closer integration between veterinary and public health laboratories.

AIM:

To share and exchange methodological, virological, genetic, epidemiological and clinical information on influenza. The network will present up-to-date, quality information on influenza activities for scientists, policy makers, professionals and the public. It will also encourage the identification of duplicate areas of work including surveillance and research projects at a European level.

EXPECTED RESULTS:

a) Enhancement and reinforcement of the existing Community Reference Laboratory and National Reference Laboratory network for avian influenza within the European Union Member States.
b) Development and implementation of web-based, global interactive communities facilitating rapid, formal interactive communications forums.
c) Strengthening harmonisation and development of laboratory and diagnostic methods, coordination of research efforts and sharing of expertise.
d) Facility for rapid responses to national and global emergencies, with data sharing.
e) Extension of knowledge sharing and laboratory support to strategically important third country and INCO partner laboratories.
f) Participation of laboratories involved in influenza research in domestic mammals.
g) Fostering of formal links and coordination with corresponding human, swine and equine influenza networks.

POTENTIAL APPLICATIONS:

By reinforcing and enhancing the existing Community Reference Laboratory and National Reference Laboratories network for avian influenza within the European Union, FLU-LAB-NET will facilitate improvements and harmonisation of laboratory and diagnostic methods. Following the development and implementation of FLU-LAB-NET as a web-based, global interactive community, the EU Member State, third country and INCO partner laboratories will have facilities to optimise rapid, formal interactive communications forums. This will in turn be extended to participating laboratories involved in influenza research in domestic mammals, and provide a hub to allow fostering of formal links and coordination with corresponding human, swine and equine influenza networks.

In addition, FLU-LAB-NET will allow for the coordination of research efforts and data exchange, as well as development and sharing of expertise. Rapid responses to national and global emergencies, with data sharing, will also be a key area of exploitation, contributing to a European laboratory task force capability for AI in animal species. FLU-LAB-NET provides opportunities for identification and development of the complementarities of both EU and global, multi-disciplinary influenza research programmes. This will promote greater trust, understanding and early access to information that may be of importance to both veterinary and public health in the EU.

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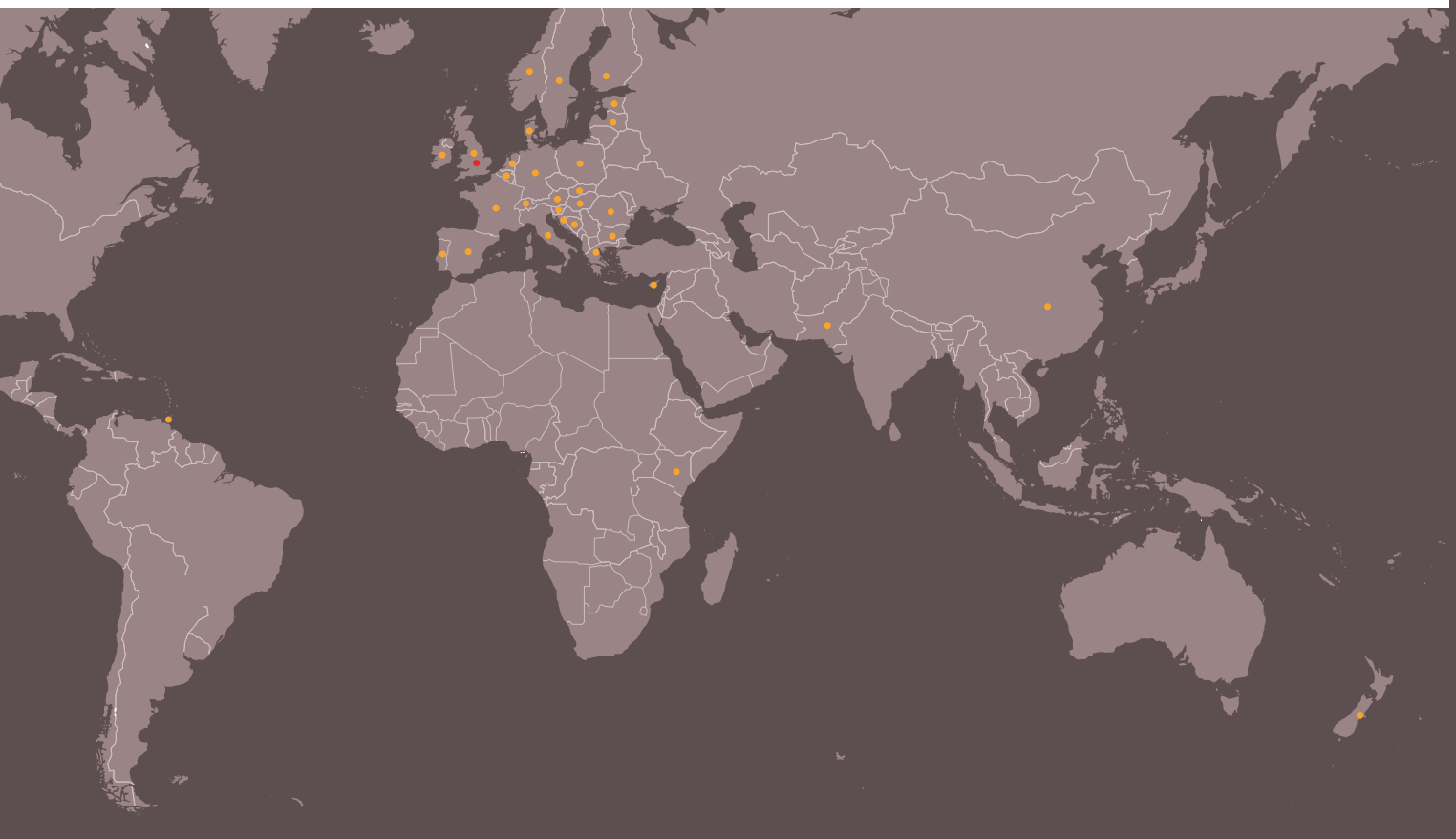

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SSPE-CT-2007-044453

FLU-LAB-NET

TRAINING AND TECHNOLOGY TRANSFER OF AVIAN INFLUENZA DIAGNOSTICS AND DISEASE MANAGEMENT SKILLS

Acronym: **FLUTRAIN**
EC contribution: €1 809 133
Duration: 36 months

Starting date: 01/03/2007
Instrument: Coordination Action

Key words: Avian influenza, training, diagnostic tools, exchange

SUMMARY:

The present worldwide avian influenza (AI) crisis has highlighted the need for comprehensive training and the transfer of technology to EU accession and international cooperation target (INCO) countries with the clear goal of aiding these countries in combating AI with the most up-to-date diagnostic and disease management procedures.

The FLUTRAIN project will first approach the need for training by providing two workshops over the duration of the project that will call on experts in the AI field to pass on their expertise in the diagnosis and management of AI to participants from accession and INCO countries. Training opportunities will also be provided in partner laboratories in order to consolidate the information and practical experience gained during the workshops. In addition, a CD-ROM will be prepared containing essential information provided during the workshops. A website will be developed which will enable participants and the general public to access the training programmes and will include on-line discussion forums between trainees and trainers.

Another goal of FLUTRAIN will be the transfer of technology to accession and INCO countries. This will include the provision of new, simplified and cost-effective diagnostic methods and reagents. It will also involve the transfer of deliverables, both for serological and virological diagnosis, that have been (or will be) developed in three European projects - AVIFLU, Lab-on-Site and FLUAID. Finally, FLUTRAIN will identify and supply funds, in a bilateral manner, for one-off specific support missions that will target specific AI problems in recipient countries.

PROBLEM:

Avian influenza infections have become of increasing relevance both from the animal and human health perspectives. With the extension of the H5N1 epidemic from Asia to Eastern Europe and Africa, notwithstanding the efforts of international organisations, there is clear evidence that in the current situation the attempts to stop the infection's progress are insufficient. There are several factors that are contributing to the spread, an important one being the delay in identifying primary outbreaks in a given area in a timely manner. This has highlighted the need for comprehensive training and the transfer of technology to accession and INCO countries with the clear goal of aiding these countries in combating AI using the most up-to-date diagnostic and disease management procedures.

AIM:

Avian influenza infections caused by several subtypes are endemic in vast areas of the world, particularly in developing countries and countries where poverty is widespread. Under these circumstances AI infections are very difficult to control, due to the lack of funds to train staff, produce or purchase diagnostic reagents or apply modern diagnostic technology. The objective of FLUTRAIN is to bridge the gap of knowledge between EU scientists and colleagues in AI affected countries. This project has the objective of supporting countries affected by AI infections through training and the transfer of knowledge and technology.

Training in avian influenza techniques at the FLUTRAIN © *coordinator's institute (IZSVe, Italy –2006)*

EXPECTED RESULTS:

a) Generation of classical and molecular, cost effective diagnostic tools (reagents, PCR, ELISA) that can be exported to accession and INCO countries.
b) Two workshops addressing general and specific topics related to AI.
c) Generation of a group of trainers that can pass their knowledge on to other scientists\ laboratory diagnosticians.
d) Generation of a CD-ROM containing the material of the workshops that will be distributed to trainees and partners.
e) Generation of an interactive website.

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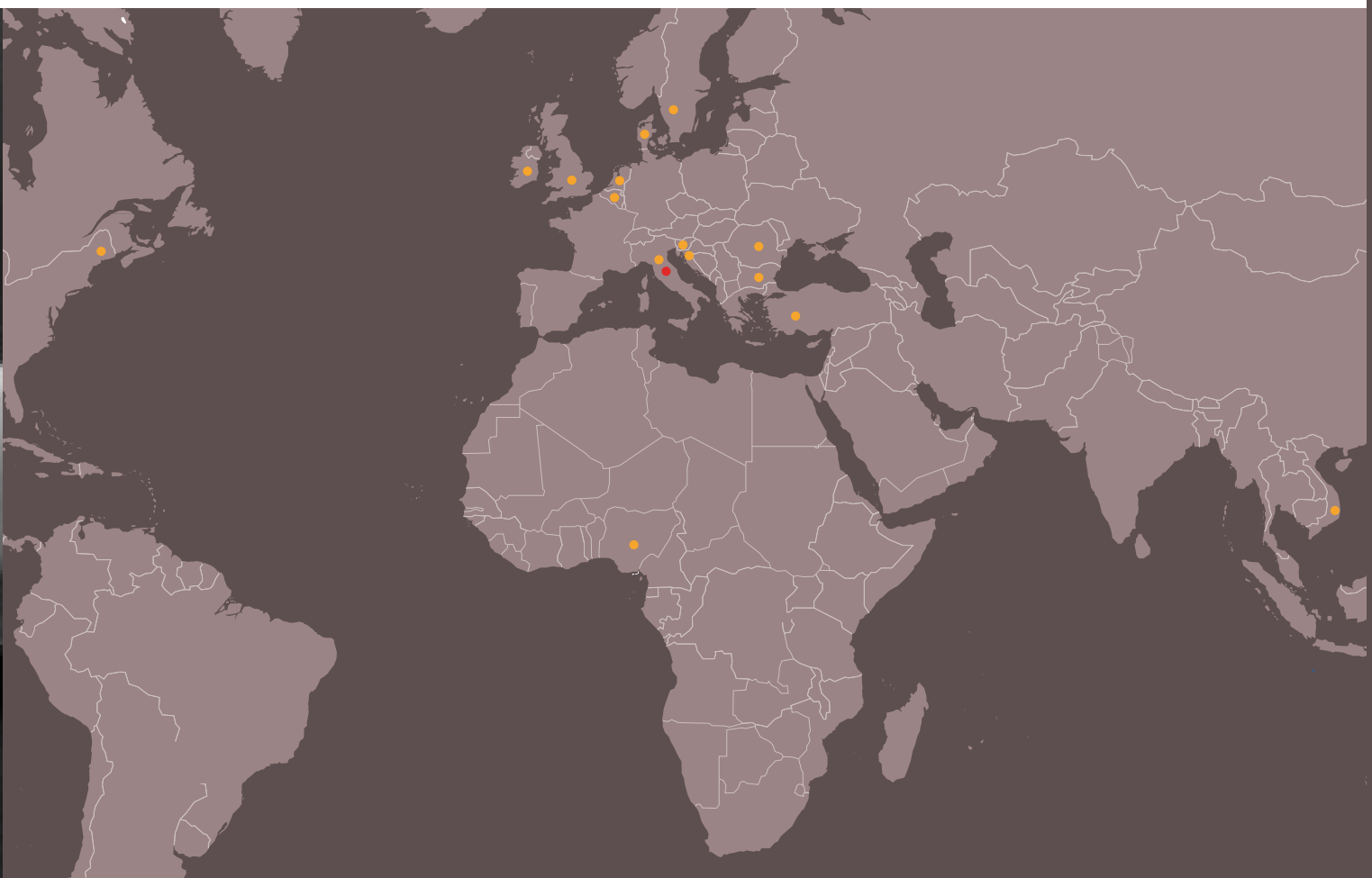

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SSPE-CT-2006-044212
FLUTRAIN

CAPACITY BUILDING FOR THE CONTROL OF AVIAN INFLUENZA THROUGH TECHNOLOGY TRANSFER AND TRAINING

Acronym: **ConFluTech**
EC contribution: €547 255
Duration: 36 months

Starting date: 01/07/07
Instrument: SSA

Key words: Avian influenza, polymerase chain reaction, epidemiology, disease outbreak

SUMMARY:

Avian influenza (AI) or 'bird flu' is a highly contagious viral infection which can affect all species of birds and can manifest itself in different ways depending mainly on the pathogenicity of the virus involved and on the species affected. The highly pathogenic avian influenza (HPAI) virus causes serious disease with high mortality (up to 100%) - notifiable to the OIE. Worryingly, HPAI has been shown to infect and cause death in humans. Up to now, a total of 103 deaths have been recorded due to HPAI infection in a number of countries such as Vietnam, Turkey and Iraq (WHO, 21 March 2006).

Beside the fact that a number of countries were surprised by the outbreaks, an even greater number of developing countries do not have adequate tools to detect and differentiate HPAI and are lacking experience to manage the outbreak of the disease. Thus, there is an urgent need for technology transfer and training. To fulfil these gaps, the partners of this consortium will:

a) organise technical workshops to facilitate technology transfer particularly in the field of molecular diagnostic tools for pathogen detection and differentiation, to reinforce epidemiological analysis for monitoring and modelling of avian influenza especially and to respond to outbreaks of infectious diseases of livestock in general;

b) provide training through organisation of seminars and short-term courses in well qualified laboratories of a number of EU Member States;

c) organise technical workshops, courses and training in the INCO target countries to improve the technical experimental level of the staff and laboratories in charge of livestock infectious diseases.

PROBLEM:

Avian influenza is a highly contagious viral infection which can affect all species of birds and can manifest itself in different ways depending mainly on the pathogenicity of the virus involved and on the species affected. The highly pathogenic avian influenza (HPAI) virus causes serious disease with high mortality (up to 100%). Worryingly, HPAI has been shown to infect and cause death in humans in a number of countries such as Vietnam, Turkey and Iraq. Besides the fact that a number of countries were surprised by the outbreaks, an even greater number of developing countries do not have adequate tools to detect and differentiate HPAI and are lacking experience to manage the outbreak of the disease. Thus there is an urgent need for technology transfer and training.

AIM:

The overall aim of this proposal is to facilitate technology transfer and training to promote capacity building in INCO (International Cooperation) target countries, with a particular emphasis on countries that border the EU, for better control of avian influenza and general outbreaks of infectious diseases in livestock. This will be achieved through the organisation of technical workshops and training courses in the following fields:

a) Molecular diagnostic tools for pathogen detection and differentiation.
b) Standardisation and validation of diagnostic tools according to OIE instruction.
c) Epidemiological tools for monitoring and modelling avian influenza outbreaks.
d) Management of disease outbreaks.

EXPECTED RESULTS:

The project will result in the transfer of technologies required for specific and rapid diagnosis of diseases, disease surveillance and management of disease outbreak. The impact of the project lies in the introduction of techniques required for the implementation of the required control measures. In addition, the project will serve as a platform for technology transfer for the improvement of livestock disease control measures and will contribute to reducing the risk of the spread of transboundary diseases to Europe. The objectives of the project contribute to the realisation of EU policy regarding food security and food safety in the developing countries. Moreover, they contribute to reducing the risk of diseases spreading to Europe and to protecting Europe's livestock.

POTENTIAL APPLICATIONS:

The following technical and scientific advances are expected:

a) Transfer of knowledge and technology for surveillance systems to exploit and understand disease transmission pathways, establishment of reliable indicators and tools for disease monitoring activities (analytical and modelling) to describe the present situation and predict the future course of AI as well as for a better response to diseases.

b) Standardised diagnostic tools and methods.

c) Tools in decision making, communication and information systems.

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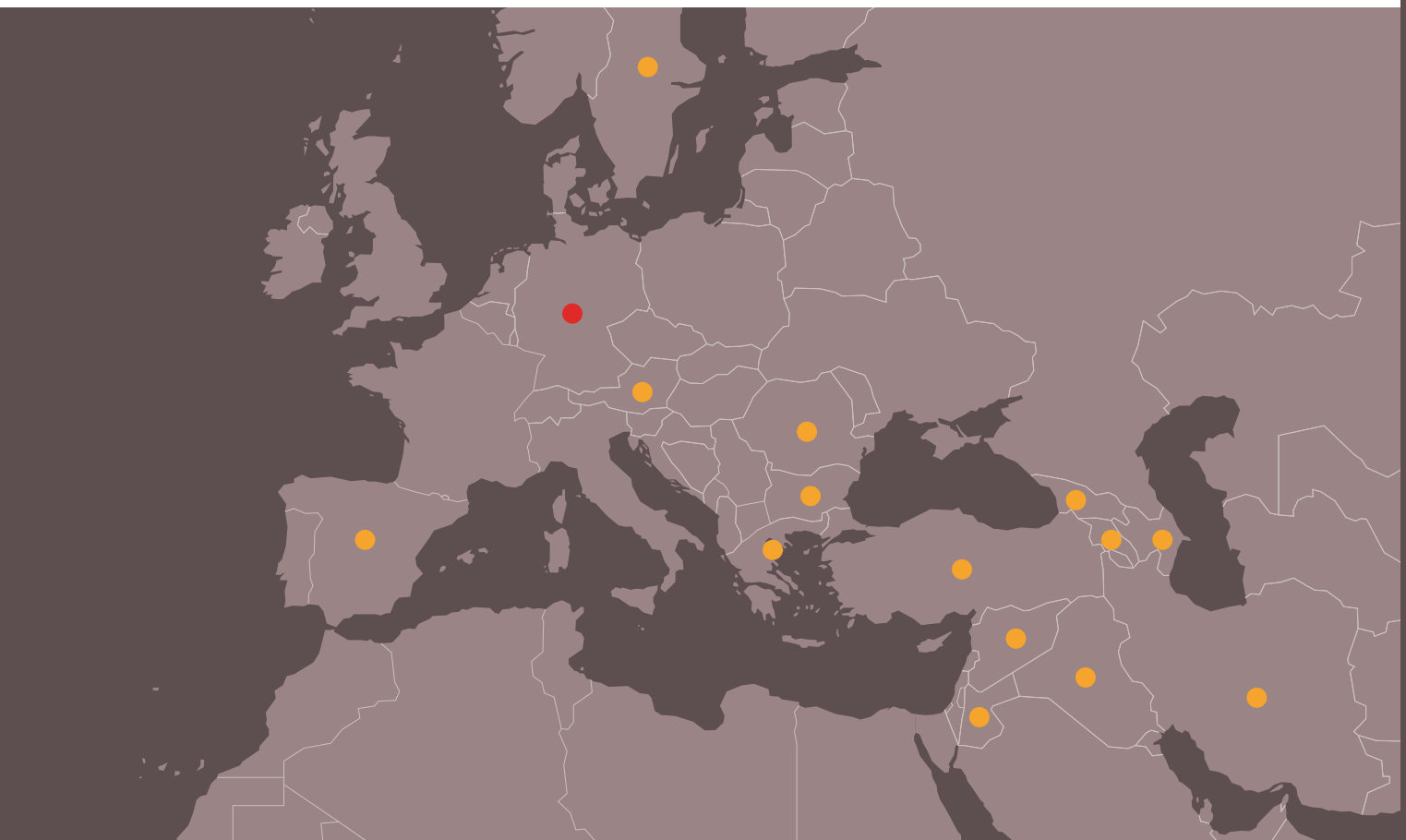

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SSPE-CT-2006-044462

CONFLUTECH

EUROPEAN VIGILANCE NETWORK FOR THE MANAGEMENT OF ANTIVIRAL DRUG RESISTANCE

Acronym: **VIRGIL**
EC contribution: €9 000 000
Duration: 48 months and beyond

Starting date: 01/05/2004
Instrument: Network of Excellence

Key words: Antiviral, drug, resistance, hepatitis, influenza, flu, HCV, HBV, virus

SUMMARY:

VIRGIL (for Vigilance against Viral Resistance) is the first European surveillance network capable of addressing current and emerging antiviral drugs resistance developments in the field of influenza and viral hepatitis. Coordinated by Inserm (the French Institute for Health and Medical Research) and supported by a grant from the Priority 1 Life Sciences, Genomics and Biotechnology for Health programme in the Sixth Framework Programme of the EU, the network's activities started in May 2004 with the initial task of integrating the fragmented European capacities and major expertise in the field into a single coherent Network of Excellence. VIRGIL initially gathers 60 organisations, including more than 60 academic laboratories and seven companies from 14 European countries and beyond.

VIRGIL is structured into seven integrated platforms centred on the patients with each focusing on a topic contributing towards the project objectives, from surveillance of resistant viruses in Europe, innovation in diagnostic tools, analysis of the virological mechanisms of antiviral treatment efficacy and resistance development and pharmacology to studies on host (patient-related) factors and the socio-economic impact of antiviral drug resistance. VIRGIL benefits from a central management unit that offers industrial partners a single contact point as well as integrated services for conducting large-scale European clinical trials. Finally, the network is also committed to training students and researchers within and outside of the participating organisations and to spreading VIRGIL's practices of excellence and the resulting knowledge throughout the medical community and the general population.

PROBLEM:

The development of new antiviral therapies over recent decades constitutes major progress in the treatment of viral infections with a considerable impact on life expectancy and quality for patients. In the case of highly contagious viruses such as influenza, antiviral drugs can also be used to control epidemics, and, in the case of the emergence of a pandemic virus could even contribute to slowing or preventing its propagation.

One of the consequences, however, of this success is the high frequency of drug resistance, which may be due to the patient (host), or to the virus, or to a combination of both. In particular, the emergence of resistant viral strains during treatment (Darwinian selection), in the absence of adequate follow-up, can lead to treatment failure, or even epidemic diffusion of the resistant strains. It has been estimated, for example, that after four years of hepatitis B antiviral therapy with lamivudine, 70% of patients present resistances. Rational and logical use of antiviral drugs is essential to minimise the clinical and epidemiological impact of resistance. Treatment strategies must therefore be adapted in real time to changes in viral populations. This monitoring requires a multidisciplinary approach, as it is based on epidemiology, clinical follow-up, diagnostics and basic research and involves public institutions as well as private laboratories.

AIM:

The resources and skills allowing control of the various aspects of resistance already exist in European private and public laboratories, but until recently they were fragmented, constituting a major obstacle to progress of knowledge. The primary goal of VIRGIL is to gradually integrate resources and skills dispersed throughout Europe to achieve common research objectives, including the study of the socio-economic dimension of antiviral drug resistance. VIRGIL was therefore designed to be a virtual institute organised into seven collaborative platforms able to deal with all aspects of antiviral drug resistance, regardless of the type of virus concerned (surveillance, diagnosis, basic virology and modelling, host-inherent factors, pharmacology, technological innovation, socio-economic impact). It currently comprises more than 70 laboratories, including six biotech small and medium enterprises (SMEs), in more than 16 Member States of the European Union and beyond.

The other goals of VIRGIL are to:

a) provide the pharmaceutical industry with centralised management and standardised computer tools to conduct large-scale clinical trials on viral hepatitis and influenza;
b) rationalise the use of antiviral drugs for the benefit of patients and health systems and to contribute to the development of new more effective and less expensive drugs;
c) transpose these concepts of excellence to other regions of the world, including developing countries where the viruses studied are endemic;
d) constitute the basis for a future European Virology Foundation.

In the first phase, three disease models were selected (influenza, hepatitis B and hepatitis C) for the development and testing of working tools. Chronic hepatitis (15 million people infected in Europe) is responsible for two thirds of all cases of cirrhosis and liver cancer, and 50% of patients present drug resistances. Influenza, with its annual epidemic waves, is responsible for 21 000 deaths per year in France and 114 000 hospitalisations. The network is also reactive and can respond to emergency situations such as influenza pandemics.

RESULTS:

Less than three years after the creation of VIRGIL, the preliminary results obtained on antiviral drugs used to treat influenza and hepatitis B and C demonstrate the pioneer role in Europe of an integrated approach linking basic research and clinical research.

VIRGIL teams were the first to precisely characterise resistances to newly marketed antiviral drugs (adefovir, entecavir and multi resistant strains) for the treatment of hepatitis B. As a result of the links between VIRGIL and scientific societies such as EASL (European Association for the Study of the Liver), these results could be adopted by health authorities for the establishment of official guidelines. In the case of hepatitis C, several *in vitro* studies have identified synergies and antagonisms between antiviral molecules, new more effective interferons, as well as new viral targets for treatments. This will allow the design of clinical trials using combinations of these molecules whose synergy and absence of cross-resistance have been demonstrated *in vitro*.

VIRGIL has initiated a collaboration with the European Influenza Surveillance Scheme (EISS), and the WHO network of national influenza reference laboratories to study, over several consecutive seasons, the conditions of emergence of drug-resistant influenza viruses, in susceptible subgroups of the population (children, immunosuppressed patients).

The VIRGIL approach will allow combined analysis of the characteristics of resistance strains (genetics, morphological), their associated clinical features (symptoms, infectious property) and their epidemiological impact. Prof. Alan Hay, director of one of the four WHO world influenza reference centres (London, UK), says: 'By integrating the efforts of people working on various aspects of influenza, VIRGIL plays a key role in the real-time surveillance, study and diffusion of knowledge on resistance to Neuraminidase Inhibitors (NIs).' According to him, this initiative arrives 'just at the time when knowledge on NI resistance needs to be developed'.

A training programme on NI resistance tests for directors of WHO national influenza reference centres has also been set up by the VIRGIL team directed by Dr Maria Zambon (Health Protection Agency, London, UK). The objective is to allow each European country to autonomously develop the necessary skills to monitor resistant strains circulating in its territory.

Apart from their immediate benefit on seasonal influenza, the skills and infrastructures developed in the context of this programme could be mobilised in the event of an influenza pandemic. The H5N1 virus continues to circulate in Asia and Africa, inducing numerous epizootic infections in wild and domestic birds, and the risk of emergence of a pandemic still remains high, according to the WHO. At the present time, all countries of the EU have set up stocks of oseltamivir. These drugs will only be useful if they are used rationally, and if the emergence of resistant viral strains is controlled by continuous surveillance set up throughout the EU according to a sufficiently dense network.

Making Europe an important place for the conduct of large-scale clinical trials

During its first two years of existence, VIRGIL has established a number of standardised criteria for data collection in clinical trials. This is a crucial step, as adoption of these criteria by all members of VIRGIL will allow comparison of the results obtained in various trials. This standardisation process has allowed:

a) the elaboration of an informed consent form to perform research on biological samples taken from hepatitis B or C patients. Translated into 10 languages and downloadable from VIRGIL's intranet site, this form was designed to ensure that this research strictly complies with the ethical requirements of current EU legislation;
b) the adoption by all members of VIRGIL of standardised definitions of primary and secondary drug resistance to hepatitis B and C antiviral drugs based on viral load cut-off values;
c) the design and development of on-line databases allowing standardised and secure exchange of information on patients (profile, composition of treatment, follow-up, primary or secondary resistance etc.). They will also allow selection of patients of interest as a function of the questions raised by particular studies.

Several clinical trials have been initiated on these databases by VIRGIL to characterise resistances of HBV to new molecules such as entecavir or tenofovir, and resistances of HCV to dual therapy with peg-interferon and ribavirin.

VIRGIL plans to develop centralised clinical trial services with major pharmaceutical groups and promote the integration of SMEs (biotechs) in the European economic tissue by linking them with various regions of excellence represented by VIRGIL's partners. According to Dr Jerome Weinbach, logistic coordinator of VIRGIL (INSERM Transfert, Paris), 'access to the international market is an essential condition for the sustainable growth of SMEs'. Installation of VIRGIL in the Rhône-Alpes competitiveness pole in France therefore constitutes a major advantage.

WWW.VIRGIL-NET.ORG
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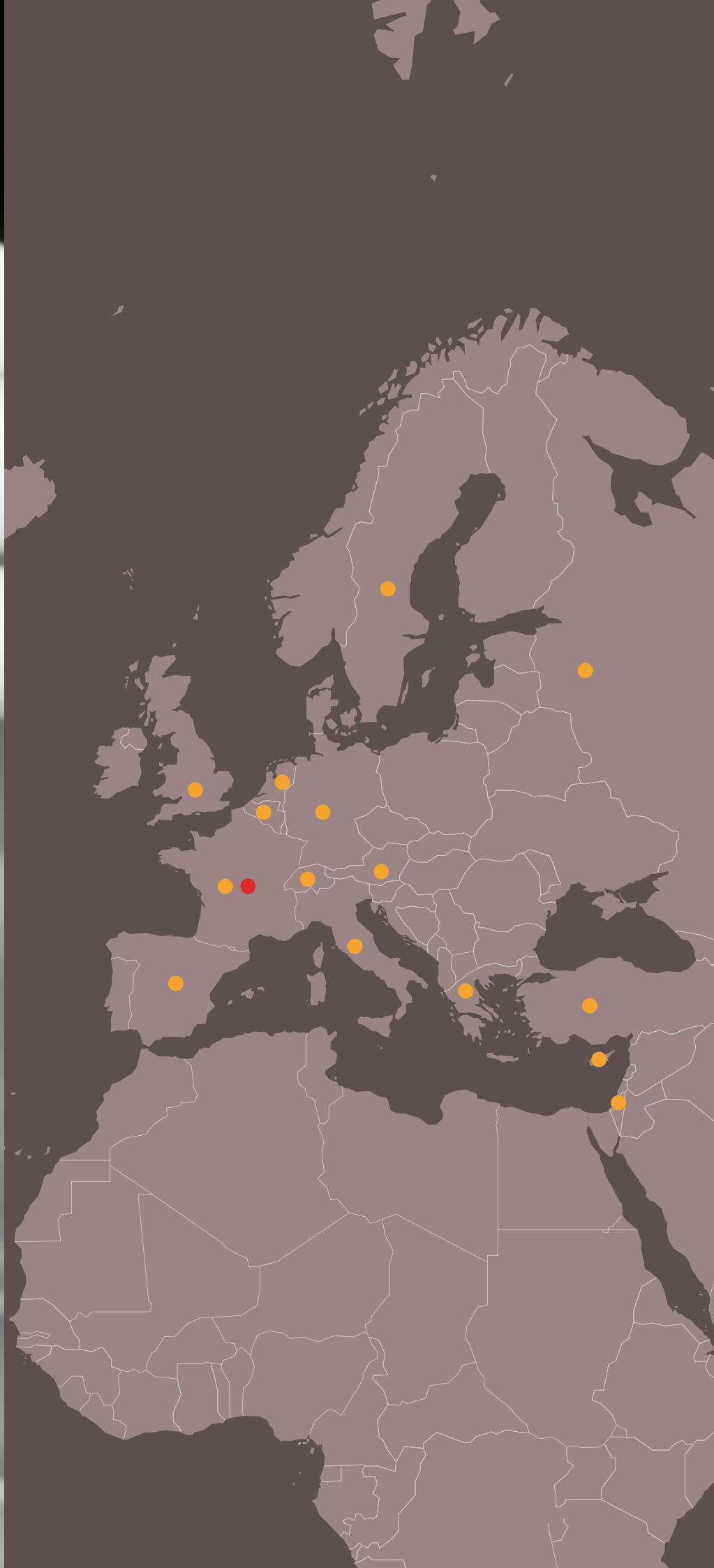

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DEVELOPMENT OF NEW INTEGRATED STRATEGIES FOR PREVENTION, CONTROL AND MONITORING OF EPIZOOTIC POULTRY DISEASES

Acronym: **HEALTHY POULTRY**
EC contribution: €1 119 404
Duration: 36 months

Starting date: 01/11/2004
Instrument: STREP

Key words: Avian influenza, epizootic poultry diseases, disease control and prevention, policy and decision making

SUMMARY:

When highly pathogenic strains of influenza break out in poultry, the consequences can be devastating (around 30 million chickens had to be killed during the avian influenza (AI) outbreak in The Netherlands in 2003). Destruction of infected birds is the primary means of control today but, as the Dutch crisis proves, even such drastic measures cannot totally avoid disaster. Given the global spread of the H5N1 AI subtype, policy makers across Europe are looking at revising their strategies to better prepare for future outbreaks of epizootic diseases in poultry. They want to know how best to avoid infection and, when infections do occur, how best to limit their spread and impact.

HEALTHY POULTRY brings together seven academic institutions from Italy, the Netherlands, Germany and Hungary to assess current scientific understanding of epizootic AI. Researchers will then use this scientific basis to suggest new strategies for their prevention, control and monitoring. They will analyse different approaches and then provide guidelines for the implementation of these strategies in EU Member States for specific situations at the regional level. The partners will complement their recommendations with a 'risk assessment toolkit'. A geographic information system (GIS) system will allow policy makers to evaluate the possible consequences of different strategies on the health of poultry flocks, the geographical spread of a disease and economic outcomes of particular interventions.

The results of this project will be disseminated through two important groups: a platform of experts and decision-makers (the 'users' of the project results), and representatives of major stakeholders in the poultry business who would be most affected by the implementation of new policies. Close co-operation between these panels and the project research teams could quickly lead to new epizootic disease policies that could circumvent disaster if H5N1 ever gets into poultry stocks.

PROBLEM:

The European Union aims at assuring a high level of animal health and animal welfare without compromising the functioning of the internal market. Nevertheless, in the last decade, several epizootics of AI occurred throughout the EU. These had a devastating veterinary and economic impact. Moreover, fear amongst the population increased because of a possible impact on human health as well, particularly during the last couple of years. Finally, control of AI currently coincides with severe problems related to socio-ethical issues and animal welfare.

Intensive trade contacts (of animals and poultry products) between Member States pose considerable risks to poultry in the EU once a single Member State is struck by AI.
Quite obviously, strategies and measures for prevention and control of AI need improvement to fulfil the EU objectives. Future prevention and control of AI should be more efficient, ethically acceptable and less costly. Self evidently, because of the single market context of EU livestock production, only a comprehensive approach at the level of the EU is likely to be successful. HEALTHY POULTRY aims at addressing these issues.

AIM:

The primary aim of the project is to provide scientifically-based support to decision makers in the field of epizootic poultry disease prevention and control.
The objectives of the project are:

a) to develop new integrated strategies for prevention, control and monitoring of epizootic poultry diseases;
b) to analyse these strategies in a comprehensive way;
c) to provide guidelines for the implementation of these strategies in EU Member States;
d) to develop user friendly toolboxes for strategy evaluation;
e) to disseminate project results to a broad relevant audience.

EXPECTED RESULTS:

The results of this project will provide a comprehensive, EU-wide scientific basis for the formulation of future EU policies with regard to prevention and control of epizootic poultry diseases, particularly AI.

The guidelines for implementation of these policies will result in strategies which are more tailor made with regard to specific regional conditions, e.g. with regard to the density of animals and holdings (i.e. herds), organisation and trade and structure of livestock production. In this way, the veterinary impact of epizootic poultry diseases will be reduced. In turn, the financial and economic losses will be largely reduced, as well as the risks for human health.

Implementation of prevention and control strategies which comply more with general public demands on public health, socio-ethical and animal welfare issues will lead to an increase in acceptance by the general public of the EU policies in this respect. In turn, it will improve the livestock sector's 'licence to produce', i.e. its social sustainability.

The delivery of user friendly toolboxes and decision support systems (DSS) will enable epizootic livestock disease decision makers to evaluate strategy options at the earliest moment possible, i.e. during the time of development of ideas.

POTENTIAL APPLICATIONS:

The primary field of application of the results is policy — and decision — making with regard to prevention and control of epizootic poultry diseases, i.e. avian influenza, particularly at the level of the EU and of Member States. Part of the results will also be valuable for other stakeholders within the poultry production chains, e.g. integrated production chains, animal health services, product boards etc.

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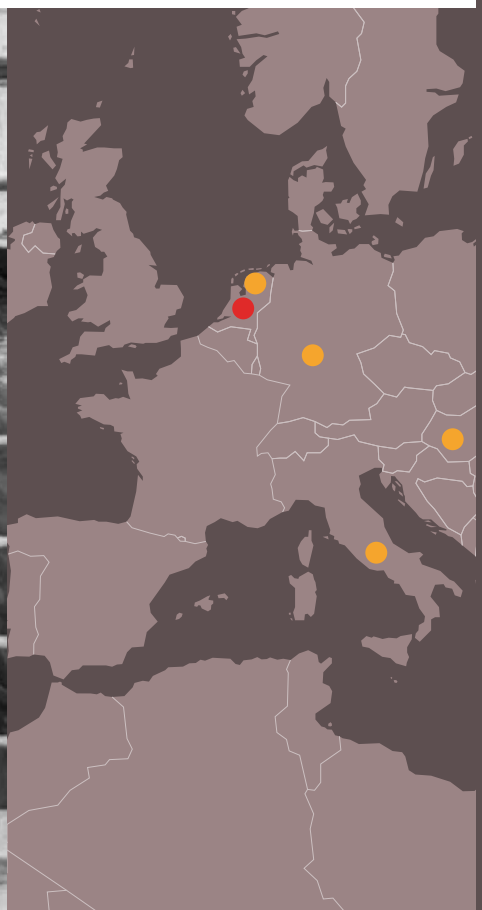

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SSPE-CT-2004-513737

HEALTHY POULTRY

NETWORK OF EXCELLENCE FOR EPIZOOTIC DISEASE DIAGNOSIS AND CONTROL

Acronym: **EPIZONE**
EC contribution: €14 000 000
Duration: 60 months

Starting date: 01/06/2006
Instrument: Network of Excellence

Key words: Epizootic diseases, network of excellence

SUMMARY:

Epizootic diseases in agriculture and aquaculture animals constitute major risks for food production. Such diseases spread very fast in high densities of susceptible animals through animals, vectors or animal products. Outbreaks in Europe have had enormous social and economic impact, and need to be addressed across the whole production chain of animal-related food.

The objective of EPIZONE is to improve research on preparedness, prevention, detection, and control of epizootics by improvement of excellence through collaboration. EPIZONE will be developed for the integration of scientists in health and production of animals, at the European level.

The benefits of EPIZONE primarily concern consumers and stakeholders throughout the food supply chain but also the agriculture administrations and biotechnology companies. EPIZONE includes 18 institutes from 12 countries. Partners maintain networks worldwide, linked to EPIZONE, and most have (inter)national reference lab-based tasks for control of epizootics. The management structure of EPIZONE will generate durable interactions between partners. Initially more than 250 key scientists with international reputations, complementary expertise and skills are identified within the partners.

EPIZONE will generate a worldwide network of institutes contributing to available expertise and spreading of excellence. EPIZONE includes the FAO as a world oriented organisation, and an SME specialised in dissemination of knowledge via the Internet. Organisational work packages will develop integration activities, including communications, meetings, and training/ continuous professional development. Scientific work packages will undertake jointly executed research on epizootics selected on importance in Europe and cover four thematic areas: Diagnostics; Intervention Strategies; Surveillance and Epidemiology; Risk Assessment. Given the network structure, the technical resources and the scientific excellence, EPIZONE will assure strategically driven state-of-art research of world-renowned quality.

PROBLEM:

Epizootic diseases in agriculture and aquaculture animals constitute major risks for food production. Such diseases spread very fast in high densities of susceptible animals through animals, vectors or animal products. Outbreaks in Europe showed enormous social and economic impact, and need to be addressed across the whole production chain of animal-related food.

AIM:

The mission of EPIZONE is to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks of foot-and-mouth disease, classical swine fever, avian influenza and other relevant epizootic diseases like bluetongue and African swine fever, through increased excellence by collaboration.

EXPECTED RESULTS:

EPIZONE will be developed for integration of scientists in health and production of animals, at the European level. Benefits of EPIZONE primarily concern consumers and stakeholders throughout the food supply chain but also the agriculture administrations and biotechnology companies.

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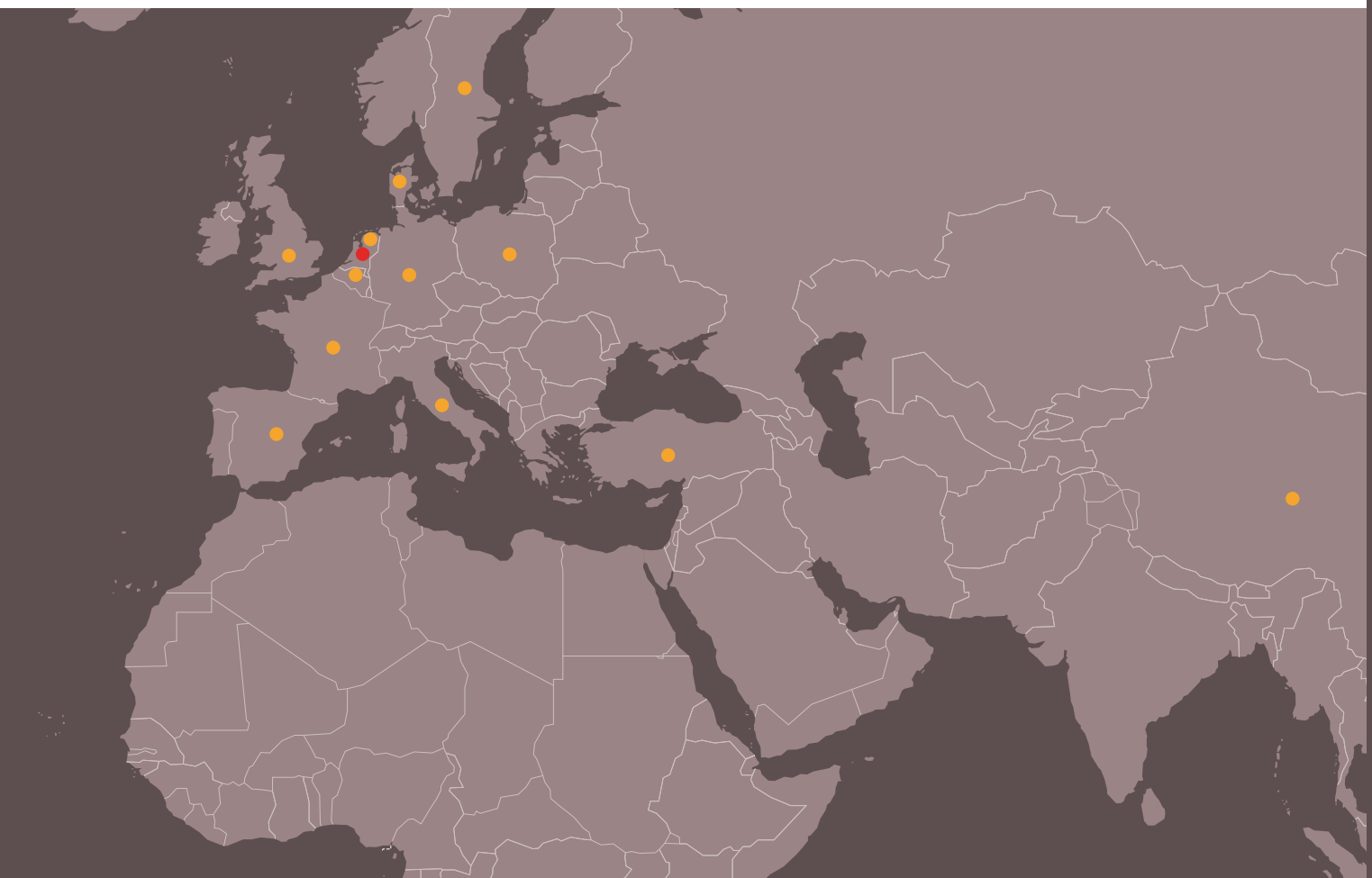

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EPIZONE

PUBLIC HEALTH LAW TO SUPPORT PANDEMIC INFLUENZA PREPAREDNESS

Acronym: **PHLawFlu**
EC contribution: €500 000
Duration: 36 months

Starting date: Planned for 07/2007
Instrument: Public Health Programme

Key words: Law, disease, human rights, public health, generic preparedness, pandemic influenza, expertise, network

SUMMARY:

The project consists of two substantive components. Component 1 will develop a network of interdisciplinary expertise in public health law. This will be achieved by establishing a Platform for European Public Health Law Expertise by means of a website to facilitate exchange of data and expertise. The research team will identify expertise in public health law in Member States, establish a network of expertise and facilitate communication and exchange by means of conferences, seminars and web contact. Component 2 will develop a profile of public health law supporting disease preparedness across Europe, by means of case studies focusing on a defined list of public health measures related to communicable disease control and interventions for the purposes of comparison.

While our analysis of Member States' legislative frameworks will inform the control of generic disease threats, the focus of the case studies will be on pandemic influenza control. The objective of the cases studies will be to identify commonalities, inconsistencies and gaps in disease control regulation across states. A database of European laws addressing pandemic influenza will be developed in English and will be available through the website.

PROBLEM:

Human pandemic influenza poses global challenges to human health protection. Law is an important tool in the armoury of states in disease control, but our preliminary research suggests that national laws across Europe are disparate and inadequate in addressing disease control and prevention. We have shown that, in their pandemic preparedness planning, a substantial number of countries anticipate the need to reform their public heath laws. Given that the framing of laws by Member States varies considerably, the opportunity exists for national public health law reforms to result in greater coherence. Preparations related to adoption of the International Health Regulations also offer an incentive to reform. The objective of this project is to strengthen Member States' legal frameworks in support of pandemic influenza preparedness in a timely manner.

There is considerable potential for the use of law as a tool in disease control and prevention. WHO noted that effective control of the SARS outbreak in 2003 relied more on traditional control measures embedded in legislation such as contact tracing and quarantine, than on modern medical technology. Our preliminary research suggests that there are marked inconsistencies in laws within Europe. Laws may not be sufficiently or uniformly grounded in the emerging public health evidence base. Consequently the public health benefits which should accrue through the judicious application of laws may not be fully realised.

Much national public health law addresses nineteenth century perceptions of public health threats and is grounded in nineteenth century mores. Many national disease control laws fail to recognise contemporary issues of human rights and health ethics. We have earlier demonstrated that a substantial number of EU Member States have identified the need to update their domestic legislation in response to the threat of pandemic influenza. This project aims to provide knowledge and build expert capacity which will enable European states to frame laws which assist public health programmes in relation to pandemic influenza, and in so doing to strengthen a coherent Europe-wide approach to pandemic influenza preparedness.

AIM:

a) To strengthen legal tools in support of disease preparedness.
b) To support the development of coherent European legal responses to the human pandemic influenza threat.
c) To develop and support an interdisciplinary network of expertise, knowledge and shared experience in public health law in relation to communicable disease control, including human pandemic influenza control, across the European Union.

EXPECTED RESULTS:

a) A multi-disciplinary maintained European network of expertise on public health law linked to a Platform for European Public Heath Law Expertise. The project aims to develop and co-ordinate a European network of public health and legal professionals whose work has relevance for public health law.

b) Development of a profile of laws supporting human pandemic influenza control across the European Union. The framework for cooperation in generic preparedness planning in the EU includes sharing national plans and making comparisons and evaluations of plans, identifying the contribution and role of existing EU legislation and ensuring that national plans fully take EU law into account.

The profile of disease control laws will provide an important preparedness resource for this task. Analysis of national legal approaches to disease control measures will assist in the formulation of a co-ordinated and coherent EU-wide legal response to the pandemic threat. The study will focus on defined public health measures and interventions, for example vaccination, school closures, healthcare workers' duties, border control measures and quarantine, across 32 states and will identify differences and commonalities in national laws.

c) Dissemination of knowledge and expertise on public health law in support of influenza preparedness planning across Member States. Dissemination, including through the network developed under Component 1, will ensure improved sharing of knowledge on pandemic influenza responses across EU states, assist in building scholarly capacity allied to pragmatic public health challenges in public health law, and support coherent policy development and public health law reform. The network may also, over time, provide expertise to assist other global regions as they seek to develop coherent legal responses to pandemic threats.

POTENTIAL APPLICATIONS:

The use of law as a public health tool in relation to all communicable and non-communicable disease threats to health.

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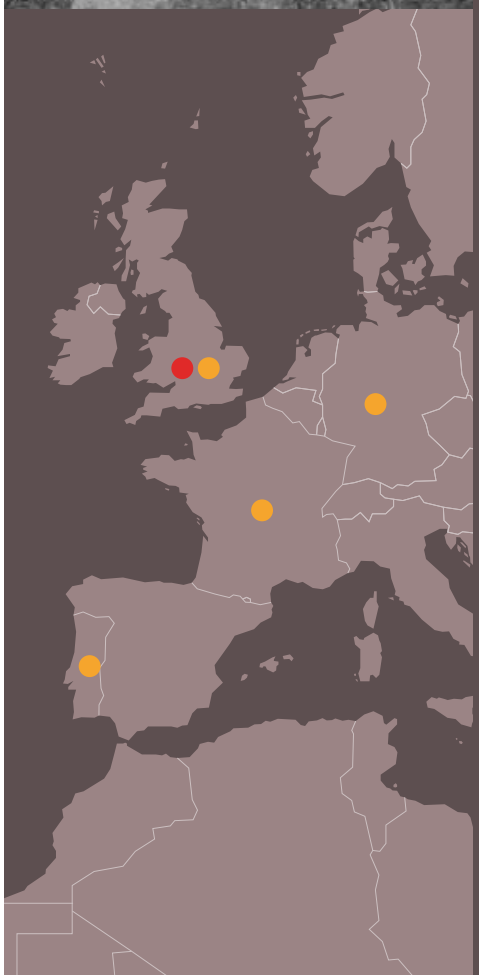

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PHLAWFLU

EUROPEAN CONTENT FOR PUBLIC HEALTH AWARENESS OF RURAL POPULATION ON AVIAN INFLUENZA PREVENTION

Acronym: **ECORAIP**
EC contribution: €199 956
Duration: 18 months

Starting date: 01/04/2007
Instrument: Public Health Programme

Key words: Avian flu, awareness campaigns, rural population

SUMMARY:

The population living in the rural areas of Europe is crucial to the potential transformation of avian influenza into a deadly pandemic influenza. This project aims to provide practical information and guidelines on preventing and managing these potential threats, aiming specifically at the rural population, where a gap of information can be observed.

The project will take into account the special needs of the rural population and its different characteristics across three European regions which are representative of central-north, south, and eastern Europe. Up-to-date scientific knowledge will be evaluated and criteria will be defined to integrate or customise the available material of public health campaigns, so that this can be disseminated in a feasible and effective way to the population. The channels and networks used for the dissemination of the public health material will also be assessed, reviewing existing efforts targeting the rural population. Following this review, the most effective communication strategies and information material will be identified and proposed as a best practice model.

PROBLEM:

In the past decade outbreaks of avian influenza infection among poultry have been reported worldwide and have in some cases also led to sporadic cases in humans. In Europe a 2003 outbreak in The Netherlands affected more than 80 people. Avian influenza infection in humans can be severe and life-threatening. Highly pathogenic avian influenza is a threat to public health because it may evolve into an efficient and dangerous human pathogen. WHO has expressed concern that the avian influenza virus may re-assort its genes with those from a human influenza virus, thereby acquiring the ability to move easily from human to human and thus triggering a pandemic.

AIM:

The general objectives of this project are:

a) to reduce the risk of human infection in rural areas and thus control a pandemic at its onset, by promoting awareness on its risk to the rural population and proposing preventive measures targeting the rural population's specific needs. The measures proposed will be customised to correspond to the particularities of the three European areas (central-north, south, eastern);
b) to empower European/international/national/local initiatives with more efficient tools that set the focus on the population living in the rural areas of Europe, providing guidelines for health information campaigns on the preparation and prevention of avian influenza pandemics. These guidelines will be developed to be specific for the rural population's characteristics (including animal farming practices) and needs;
c) to increase public health awareness on the topics of avian flu, and more specifically to fill the existing information gap by promoting the preparedness of those who are involved in small-scale poultry and general animal farming, and all those who are more likely to be exposed to wildlife;
d) to use this project as a pilot for implementing similar projects which might aim at developing a model for public health campaigns targeting the rural population for health hazards, such as the avian flu pandemic.

EXPECTED RESULTS:

a) A checklist of rural life characteristics related to avian influenza and the table of rural life characteristics which could be related to the spread of avian flu epidemic in three European regions (central-north, south, eastern).

b) A list per country of the identified public health campaigns and guidelines targeting the rural population with regards to avian flu.

c) Collection, examination and evaluation of the material of public health campaigns. Although all identified material will be read and evaluated by experts who can comprehend the corresponding language, no formal translation will take place. Moreover, a formal analysis of the impact of identified material will not be done since it is outside the scope of the project and the limited budget does not allow this.

d) Model guidelines and material for public health campaigns on the topics of avian flu epidemic control and prevention, specific for the rural population in each European region (central-north, south, eastern). The model guidelines and material will be designed following the results of the review and evaluation of the collected material of public health campaigns and of the relevant guidelines, against the checklist of rural life characteristics.

e) Pilot testing of the model material and production of a relevant report comprising the results from the pilot testing experience and the results of the evaluation questionnaires' analysis.

POTENTIAL APPLICATIONS:

The model of guidelines that will be developed will include:

a) the content of the campaign;

b) issues to be addressed by a campaign on avian flu prevention;

c) suggestions for the different types of material that can be used (printed, audio, visual) and criteria on what to choose (i.e. for audiovisual presentations scenarios will be developed while the actual presentation will not be produced);

d) suggestions for different modes of dissemination, including criteria on how to choose among these (i.e. special radio broadcasting, seminars at the municipality level). These could be used by such a campaign in order to successfully access the target population.

The model guideline will be made available to key stakeholders, including public health authorities, regional and municipality authorities, municipality leaders, communicable disease experts, agronomists and veterinarians, who are expected to use this in designing their campaigns for the prevention of avian flu.

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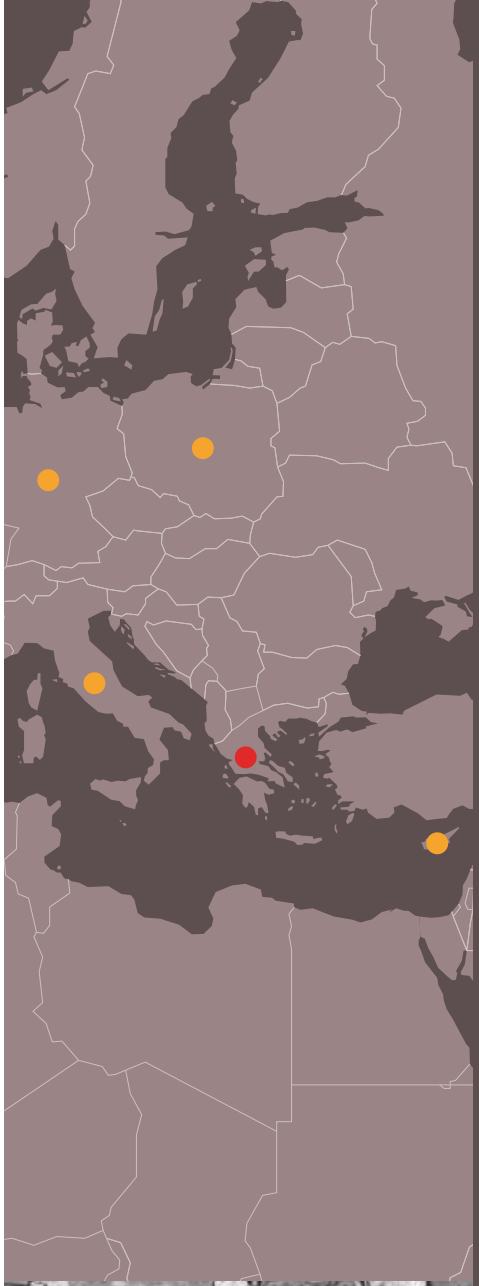

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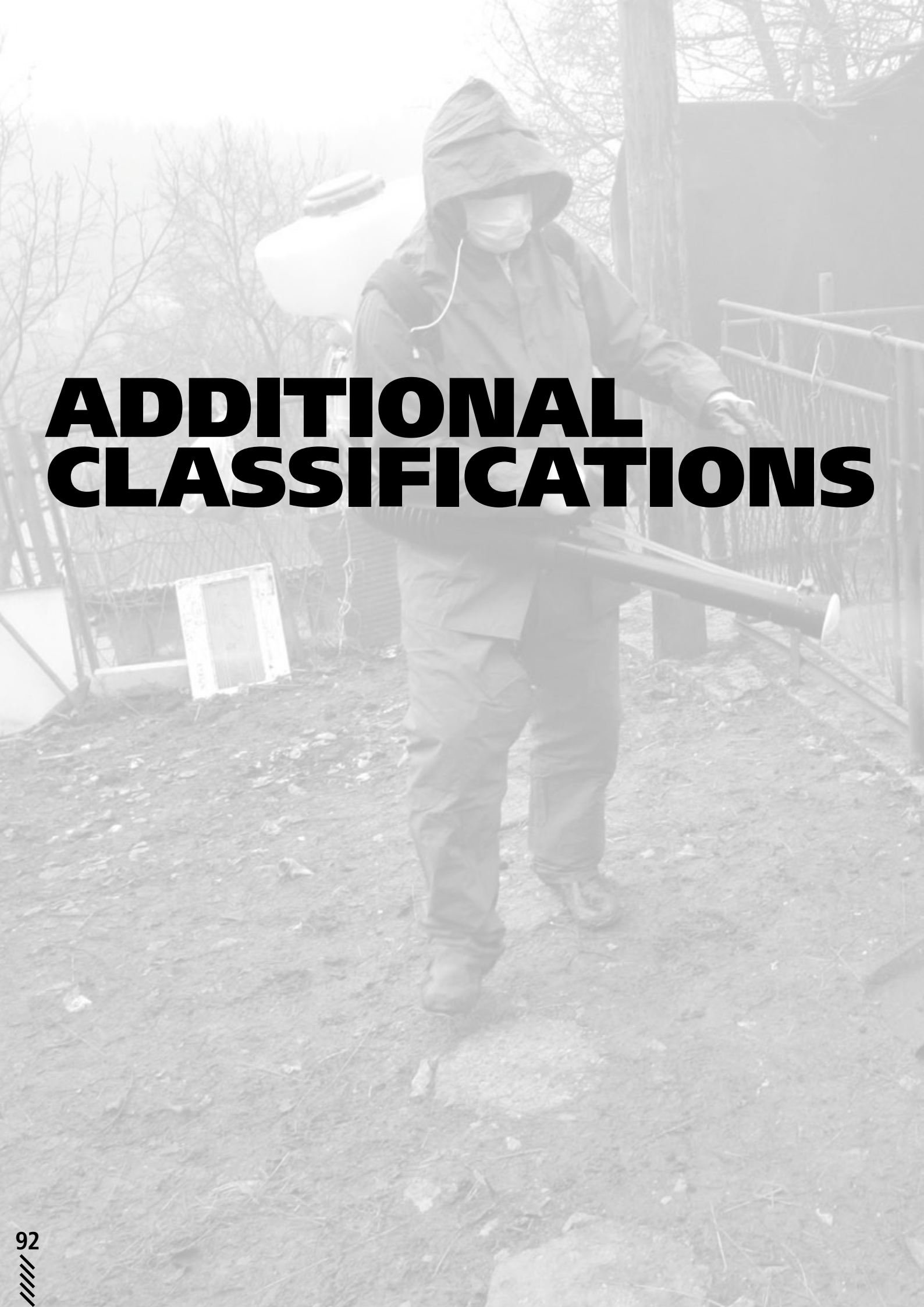

ADDITIONAL CLASSIFICATIONS

PRIMARILY ANIMAL HEALTH PROJECTS:

NOVADUCK, FLUTEST, FLUPATH, NEW-FLUBIRD, FLURESIST, FLU-LAB-NET, FLUTRAIN, AIV VACC DIAGNOSIS, INN-FLU, RIVERS, ConFluTech, HEALTHY POULTRY, LAB-ON-SITE, FLUAID, EPIZONE, AVIFLU, ESNIP, ESNIP 2, ECORAIP

PRIMARILY HUMAN HEALTH PROJECTS:

FLUPOL, FLUINNATE, PANFLUVAC, Intranasal H5vaccine, FluVac, EUROFLU, VIRGIL, VIZIER, SARS/FLU VACCINE, UNIVERSAL VACCINE, FLUVACC, RespViruses, CHIMERIC VACCINES, NOVAFLU, FLUPAN, MUCADJ, FLUSECURE, EISS, PHLawFlu

RESEARCH FRAMEWORK PROGRAMME 5 (FP5):

MUCADJ, ESNIP, FLUPAN, AVIFLU, NOVAFLU

RESEARCH FRAMEWORK PROGRAMME 6 (FP6):

FLUPOL, FLUINNATE, PANFLUVAC, Intransasal H5vaccine, FluVac, EUROFLU, NOVADUCK, FLUTEST, FLUPATH, NEW-FLUBIRD, FLURESIST, FLU-LAB-NET, FLUTRAIN, AIV VACC DIAGNOSIS, INN-FLU, RIVERS, ConFluTech, VIRGIL, HEALTHY POULTRY, LAB-ON-SITE, VIZIER, SARS/FLU VACCINE, UNIVERSAL VACCINE, FLUVACC, FLUAID, EPIZONE, RespViruses, CHIMERIC VACCINES, ESNIP 2

PROGRAMME OF COMMUNITY ACTION IN THE FIELD OF PUBLIC HEALTH:

FLUSECURE, EISS, PHLawFlu, ECORAIP

PROJECTS FOCUSING (ALMOST) EXCLUSIVELY ON INFLUENZA:

FLUPOL, FLUINNATE, PANFLUVAC, Intranasal H5vaccine, FluVac, EUROFLU, NOVADUCK, FLUTEST, FLUPATH, NEW-FLUBIRD, FLURESIST, FLU-LAB-NET, FLUTRAIN, AIV VACC DIAGNOSIS, INN-FLU, RIVERS, ConFluTech, UNIVERSAL VACCINE, FLUVACC, FLUAID, CHIMERIC VACCINES, NOVAFLU, AVIFLU, FLUPAN, ESNIP, ESNIP 2, MUCADJ, FLUSECURE, EISS, ECORAIP

PROJECTS ADDRESSING A BROADER RANGE OF (VIRAL OR OTHER INFECTIOUS) DISEASES, BUT WITH A SIGNIFICANT PART DEVOTED TO INFLUENZA:

VIRGIL (large network dealing with antiviral drug resistance in influenza, hepatitis B and hepatitis C), HEALTHY POULTRY (dedicated to control of all epizootic poultry diseases), LAB-ON-SITE (diagnostic tests for nine animal diseases, including avian influenza), VIZIER (structural characterisation of a diverse set of viruses, including influenza), RespViruses (immune response of the elderly to respiratory viruses) and EPIZONE (large network dealing with epizootic diseases), SARS/FLU VACCINE (combined vaccine against SARS and influenza), PHLawFlu (general public health law but with a focus on pandemic influenza)

INDEX

European Commission

EUR 22822 — Influenza Research — EU funded projects 2001-2007

Luxembourg: Office for Official Publications of the European Communities

2007 — 96 pp. — 21.0 x 29.7 cm

ISBN 978-92-79-05420-4

KI-NA-22822-EN-C

Every year seasonal influenza is thought to be responsible for more than 500 000 deaths worldwide. In addition, major changes in the surface antigens of the circulating viruses have the potential to lead to much more dangerous pandemic outbreaks, such as the 'Spanish Flu' which took more than 50 million lives in 1918-1919. Birds are natural reservoirs of influenza viruses and highly pathogenic variants of these avian influenza viruses (HPAI) can cause rapidly spreading epizoonotics in poultry populations, leading to high economic losses. The increasing number of human infections with an H5N1 HPAI virus makes pandemic influenza a paradigm of the potential health threat that emerging infectious diseases pose to the world.

The present catalogue assembles 38 projects funded between 2001 and 2007 mostly through the European Commission's Framework Programmes for Research, but also through its Public Health Programmes. It demonstrates the scope and long term track record of EU research funding in this field, as well as its rapid response to the more recent developments. The inclusion of both animal and human health projects underlines the importance of interdisciplinary cooperation in the area of zoonoses and emerging infectious diseases.

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ISBN 92-79-05420-1
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