European Commission

A decade of EU-funded Animal Health Research

Research and Innovation

This publication is dedicated to the memory of Isabel Minguez Tudela who made a major contribution to the Animal Health research sector over many years in the Commission services, but who sadly passed away on 16 April 2011.

EUROPEAN COMMISSION

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Contents

FOREWORD 9

CHAPTER 1. NETWORK, SURVEILLANCE 11

[EMIDA] Coordination of European research on emerging and major infectious diseases of livestock 12

[STAR-IDAZ] Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses 17

[EPIZONE] Network of Excellence for Epizootic Disease Diagnosis and Control 21

[DISCONTOOLS] Development of the most effective tools to control infectious animal diseases 24

[Arbo-Zoonet] Network for Capacity-building for the Control of Emerging Viral Vector-borne Zoonotic Diseases 27

[WildTech] Novel Technologies for Surveillance of Emerging and Re-emerging Infections of Wildlife 31

[ICONZ] Integrated control of neglected zoonoses: improving human health and animal production through scientific innovation and public engagement 36

CHAPTER 2. EPIDEMIC DISEASES OF LIVESTOCK 41

European policies for animal health sustained by EC-funded research project
Introduction by Jim Scudamore and Alberto Laddomada 42

2.1 Foot-and-mouth disease 44

[FMD_ImproCon] Improvement of foot-and-mouth disease control by ethically acceptable methods based on scientifically validated assays and new knowledge on FMD vaccines, including the impact of vaccination 44

[CA FMD/CSF] Foot-and-mouth disease (FMD) and classical swine fever (CSF) coordination action 50

[FMD-Disconvac] Development, enhancement and complementation of animal-sparing, foot-and-mouth disease vaccine-based control strategies for free and endemic regions 54

2.2. Classic swine fever **59**

[CSFVACCINE & WILD BOAR] Epidemiology and control of classical swine fever (CSF) in wild boar and potential use of a newly developed live marker vaccine 59

[CSF-GoDIVA] Improve tools and strategies for the prevention and control of classical swine fever 66

2.3. African swine fever **71**

[ASFRISK] Evaluating and controlling the risk of African swine fever in the EU 71

2.4. Orbivirus **77**

[BTVAC] Improved vaccines for bluetongue disease 77

[Medreonet] Surveillance network of reoviruses, bluetongue and African horse sickness, in the Mediterranean basin and Europe 82

[ORBIVAC] Development of vaccines for bluetongue virus (BTV), epizootoc hemorrhagic disease virus (EHDV) and African horse sickness virus (AHSV) 89

CHAPTER 3. ENDEMIC DISEASES **95**

The economic impact and research synergy resulting from EC-funded projects
Introduction by Alex Morrow and Declan O'Brien 96

3.1. Mycobacterial diseases **101**

[VENoMYC] Veterinary network of laboratories researching into improved diagnosis and epidemiology of mycobacterial diseases 101

[TB-STEP] Strategies for the eradication of bovine tuberculosis 107

3.2. Parasites **112**

[DELIVER] Design of effective and sustainable control strategies for liver fluke in Europe 112

[PARASOL] Novel solutions for the sustainable control of nematodes in ruminants 117

[PARAVAC] Vaccines against helminth parasites of livestock of economic and/or public health significance 121

3.3. Tick-borne diseases **125**

[PiroVac] Improvement of current and development of new vaccines for theileriosis and babesiosis of small ruminants 125

3.4. Porcine circovirus diseases 129

[PCVD] Control of porcine circovirus diseases (PCVD): towards improved food quality and safety 129

[NMSACC-PCVD] PCVD: Towards improved food quality and safety within EU new Member States (NMS) and associate candidate countries (ACC) 136

3.5. Porcine reproductive and respiratory syndrome 141

[PoRRSCon] New tools and approaches to control Porcine Reproductive and Respiratory Syndrome (PRRS) in the EU and Asia 141

CHAPTER 4. INFLUENZA 147

Introduction by Ilaria Capua 148

4.1. Avian flu 150

[AIV-VACC] Vaccine, diagnostic test development and immunology aspects of avian influenza 150

[FLUAID] Generation of information and tools to support the management of the avian influenza crisis in poultry 155

[Novaduck] Novel avian influenza (AI) DIVA recombinant vaccines for duck 158

[Healthy Poultry] Development of new integrated strategies for prevention, control, monitoring of epizootic poultry diseases 163

[FLUPATH] Avian influenza: Impact of virus-host interactions on pathogenesis and ecology 166

[New-Flubird] Network for early warning of influenza viruses in migratory Birds in Europe 172

[FLURESIST] Avian influenza virus survival in poultry commodities, poultry manure and the environment 179

[Rivers] Resistance of influenza viruses in environmental reservoirs and systems 182

[FLUTEST] Improved diagnosis and early warning systems for avian influenza outbreak management 184

[FLU-LAB-NET] Development and enhancement of laboratory networks for avian influenza 189

[FLUTRAIN] Training and technology transfer of avian influenza diagnostics and disease management skills 195

[ConFluTech] Capacity-building for the control of avian influenza through technology transfer and training 198

4.2. Swine flu **202**

[ESNIP 2] European Surveillance Network for Influenza in Pigs 2 202

[ESNIP 3] European Surveillance Network for Influenza in Pigs 3 204

[FLUPIG] Pathogenesis and transmission of influenza in Pigs 211

Foreword

As we move towards a predicted global population of 9 billion by 2050, with an expected 70% increase in world food demand and meat consumption projected to double to 465 million tonnes, food security and animal health are more important than ever.

In February I announced the Commission's proposal for a European Bioeconomy Strategy, a key component of which strives to ensure that the importance of the primary production sector is clearly recognised, reconciling demands for agriculture and food security with the sustainable use of resources, while ensuring environmental protection.

While primary food production is intensified to meet increasing demand, the resulting increased international trade leads to greater movement of animals and animal products and higher risk for animal health. Infectious diseases directly affect livestock production with consequences for food security and food safety, trade, rural development, and the environment, while also affecting the livelihood of farmers. Climate change is expected to influence the spread of certain pathogens; we have recently experienced the rapid appearance across Europe of new diseases such as those caused by blue tongue or Schmallenberg viruses. In addition animal health is also important in preventing the spread of certain potentially dangerous diseases from animals to humans.

Animal disease can have devastating socio-economic effects: in Europe for example the total cost to the rural economy of the outbreak of foot and mouth disease in the UK in 2001 may have been close to € 8 billion as a result of disruption in trade and impacts on industries such as tourism. Bovine Spongiform Encephalopathy is estimated to have cost the UK € 8.5 billion and more recently bluetongue in the Netherlands was estimated to have cost € 155 million. The costs related to endemic conditions such as mastitis or lameness, are also reaching several billion Euros per year for the European dairy industry.

The need to develop innovative and better tools to control disease is now more critical than ever. The lessons learnt from a decade of EU research are shaping the new research and innovation programme (Horizon 2020), which follows the current programme in 2014, as food security is covered by the societal challenge food security, sustainable agriculture, marine and maritime research and the bio-economy.

This catalogue brings together the fruits of European research efforts targeting animal health; it includes forty projects with a total budget close to 100 million euros. The scope of the work is vast, ranging from new vaccine development, enhanced epidemiological models, better surveillance activities and improved diagnostics methods. These research results help the development of European policies to better manage disease surveillance, prevention, control and eradication. There have been several outstanding successes particularly in developing tools for the effective control of swine diseases such as porcine coronavirus disease or classic

Máire GEOGHEGAN-QUINN

Commissioner for Research, Innovation and Science

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swine fever as well as enhancing European expertise on the avian and swine influenza viruses, knowledge which has been widely deployed by industry.

This publication highlights the impact of our research funding in guaranteeing the overall health and welfare of European livestock as well as contributing to the high quality and performance of this sector. I am convinced that new innovation opportunities will arise from sharing this knowledge more widely.

CHAPTER 1.

Network, surveillance

[EMIDA]

Coordination of European research on emerging and major infectious diseases of livestock

Acronym:
EMIDA

Project number:
219235

EC contribution:
EUR 997 218

Duration:
45 months

Start date:
1 April 2008

Instrument:
Coordination and support action

Summary

The disease threats to the livestock industry have increased steadily over the past decades as a result of globalisation, evolving pathogens and climate change. Responding to animal disease threats relies heavily on science; research makes a significant contribution to the development of disease control policy and the translation of policy, and other drivers for improving animal health, into practical effect. Although the legislation that underpins policy for the control of statutory diseases is determined at the EU level, the research that supports policy development and implementation is primarily carried out at the national level and is largely uncoordinated as is the research on other major infectious diseases currently affecting livestock production.

Improved coordination and collaboration of this research activity is therefore vital to ensure the efficient and effective underpinning of EU and national policy and the sustainability of the European livestock and animal health industries and the animal health science capacity. A Collaborative Working Group (CWG) on animal health and welfare under the Standing Committee of Agriculture Research (SCAR) was developed to address this gap, with the objectives of developing a durable, focused network of national research funders in the EU Member States and associated countries for the purpose of sharing information, coordinating activities and working towards a common research agenda and mutual research funding activities.

The aim of the EMIDA ERA-NET is to build on and accelerate the work of the SCAR CWG in the field of animal health. The scope of the project includes emerging and major infectious diseases of production animals, including fish and bees and including those conditions which pose a threat to human health but excluding food safety issues relating to the handling of livestock products and diseases of wildlife except where they act as reservoirs of infection for humans or production animals.

The objectives of the ERA-NET are being delivered through the following four work packages: WP1: Project coordination, management, communication and dissemination; WP2: Mapping and analysis of existing research and current needs and information on the commissioning and management of joint programmes; WP3: Develop, test, evaluate and refine instruments (Pilots) and WP4: Developing a strategic transnational animal health research agenda.

Problem

Most of the funding for research on animal health in Europe comes from the EU Member States and associated countries, involving

government ministries (agriculture and health) or research councils. All these funding bodies have their own agendas/priorities that are set independently of each other thus resulting in fragmentation and potentially duplication of effort.

Aim

To build on, and accelerate the work of, the SCAR CWG in developing a durable, focused network of national research funders in the EU Member States and associated countries for the purpose of sharing information, coordinating activities and working towards a common research agenda and mutual research funding activities in the field of animal health.

Project activities

EMIDA started on 1 April 2008 and currently has 29 partners from 19 countries (15 Member States, Israel, Norway, Switzerland and Turkey) with a combined annual research budget for work on animal health in the region of EUR 270 million. There are also four associated partners. The project partners consist of ministries of agriculture, ministries of health/public health, ministries of education/research councils and agencies under the aforementioned. The varied background of the organisations involved, each with their own agendas/priorities, encompasses the funding of research across the spectrum from basic to strategic and applied science.

Systematic exchange of information

A project website has been established which is linked to the CWG website and associated project database. The EMIDA website contains a password-protected discussion forum allowing exchange of information between project partners. A framework was established under the CWG for the capture of research project information and a database developed which is serving the needs for the collection of information for both EMIDA and the CWG. Details of over 2 000 projects have been uploaded to the project database by the project partners. To complete the mapping of the research landscape, three other databases of supporting information in the form of publications over the past four years, international animal health-related patents and EC-funded animal health-related projects have also been developed using data from international scientific databases. These databases, the methodology behind them and the associated reports on research outputs can be accessed on the project website. A questionnaire survey on current management practices relating to the research programmes of the project partners and their perceived needs and priority topics of interest for inclusion in a common call was conducted and the resulting report is also available on the project website.

Development and pilot of instruments for common calls

Through the aforementioned questionnaire, a large number of topics were suggested as possible subjects for a common research call. A matrix approach based on disease groups and technologies was used to rationalise these and from this, four broad topics were identified and agreed at the second Project Consortium meeting. Analysis of partners' programme management practices provided the basis for those practices and instruments employed in the EMIDA common call. It was agreed that a virtual common pot funding mechanism would be utilised. The pilot common call, with a budget in the region of EUR 20 million, based on the following four topics was opened in early September 2009.

- Vector-borne diseases — Development of underpinning knowledge and tools for early warning, detection and monitoring and novel control strategies.
- Zoonoses and antimicrobial resistance, excluding microbial safety of products — Development of underpinning knowledge and tools for early warning,

detection and monitoring and novel control strategies.
- Major infectious diseases affecting production — Development of underpinning knowledge and tools for early warning, detection and monitoring and novel control strategies, including genetics of resistance.
- Aquaculture — Development of underpinning knowledge and tools for early warning, detection and monitoring and novel control strategies, particularly vaccine-based approaches.

Twelve projects to a total value of over EUR 21 million have been funded, two on Topic 1 (Vector-borne diseases), two on Topic 2 (Zoonoses and antimicrobial resistance, seven on Topic 3 (Major infectious diseases affecting production) and one on Topic 4 (Diseases of fish in aquaculture). Details of these projects can be found online (http://www.emida-era.net/index.php?page=content&id=17). A second common call was launched in March 2011 with a budget in the region of EUR 20 million with the proposal expected to address one of seven activity lines or the associated specific topics.

Strategic research agenda development

A Foresight and Programming Unit (FPU) was established which identified and reviewed all the relevant foresight studies, identifying issues and drivers and a report has been produced which is available on the project website. This work was further developed and refined in a Delphi study, for which participants from a broad range of disciplines were engaged and, following a consensus workshop, a draft long-term strategic research agenda was produced. Terms of reference for the future of the FPU beyond the end of the EMIDA ERA-NET have been developed and are available on the project website. These will be incorporated into the collaborative agreement which is currently being developed, outlining a sustainable structure for the collaborative working group.

Project outputs achieved so far

The major project outputs to date are as follows (links):

- Project website: http://www.emida-era.net
- Methodology For EMIDA publications database
- report on the mapping of European research publications on infectious diseases of livestock
- Report on mapped and analysed data and information from National Programmes FPU Terms of Reference
- Overview of foresight studies
- Pilot Research Call with an online submission system launched on 7 September 2009 http://www.submission-emida-era.net
- Second Common Call with a budget of EUR 20 million launched on 7 March 2011

Expected final results and their potential applications and impact

A sustainable structure will be established for the collaborative working group with sharing of information at all levels, and taking forward a common research agenda developed by the Foresight and Programming Unit using procurement procedures developed and piloted under EMIDA. This will reduce duplication of effort and focus the available funding on addressing the research needs for improved control of emerging and major infectious diseases of livestock.

Project website

http://www.emida-era.net

Keywords

infectious diseases of animals, research coordination, animal health

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[STAR-IDAZ]

Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses

Summary

Animal diseases can cause serious social, economic and environmental damage and, in some cases, also threaten human health. An increasing number of the major disease problems or threats faced by the livestock industry and zoonoses are of a global nature. The overall aim of the global strategic alliances for the coordination of research on the major infectious diseases of animals is to improve coordination of research activities on the major infectious diseases of livestock and zoonoses so as to hasten the delivery of improved control methods. This will be achieved through the establishment of an international forum of R & D programme owners/managers and international organisations for the purpose of sharing information, improving collaboration on research activities and working towards common research agendas and coordinated research funding on the major animal diseases affecting livestock production and/or human health. It will build on the groundwork established by the SCAR Collaborative working group on animal health and welfare research, the EMIDA ERA-NET project and specific INCO-NETs involving partner countries. The scope of the project includes coordination of research relevant to emerging and major infectious diseases of livestock, including fish and managed bees, and those infections of livestock that may carry the risk of disease threat to human health. Diseases of wildlife will also be considered where they are identified as reservoirs of infection with emerging and major infectious diseases of humans or production animals.

These objectives will be delivered through the following five work packages: WP1: Project coordination, management, communication and dissemination; WP2: Sharing information on existing research programmes; WP3: Analysis of and responding to global, regional and industry sector priorities; WP4: Networking of ongoing

Acronym:
STAR-IDAZ

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265919

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EUR 999 130

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Start date:
1 February 2011

Instrument:
Coordination and support action

research activities on major issues; and WP5: Developing a strategic transnational animal health research agenda. Regional networks for (i) the Americas and (ii) Asia and Australasia are being established and it is hoped that a regional network covering the Middle East and Africa will follow.

Problem

An increasing number of the major disease problems or threats faced by the livestock industry and zoonoses are of a global nature. Recent disease threats, such as the global threat from H5N1 avian influenza, H1N1 swine influenza and the spread of bluetongue in Europe, highlight the need for rapid coordinated research to provide the evidence needed for the development of effective control policies. Lack of coordination between the funding bodies internationally can result in duplication of effort in some areas and insufficient attention and funding being given to other areas. This necessitates seeking wider coordination and collaboration, if value for money and rapid progress on disease control is to be achieved.

Aim

The specific objectives of the global network are to:

- strengthen the linkages between, and reduce the duplication of, global research efforts on high priority infectious diseases of animals (including zoonoses); maximise the efficient use of expertise and resources; and accelerate coordinated development of control methods;
- identify and coordinate the response to gaps in research activities for targeted diseases;
- create the necessary critical mass and capacity to address emerging infectious disease threats;
- improve the cost-effectiveness and added value to network partners of current research programmes;
- develop durable procedures for a better coordinated, rapid response to urgent research needs;
- identify unique regions with localised diseases and improve access to research in those areas;
- improve access to, and the use of research results across all partner organisations;
- facilitate the establishment of research management capacity and programmes in those partner countries wishing to develop research activities in this area.

Expected results

Specific project outputs should include a web-based hub for information exchange, a web-based discussion forum, a long-term common strategic research agenda, collaboration on the research activities relating to a number of priority diseases, including networking the research communities concerned, and coordination of new research requirements.

The major expected outcome is a long-term sustainable network allowing exchange of information and coordination of research activities so as to hasten the development of improved control methods.

Potential benefits

- Efficient deployment of national funds for both national and transnational (joint) research, including research procurement in response to emergency situations
- Improved cost-effectiveness of commissioned research, by creating a consensus on the level of funding that should be directed at given priorities of both nationally and internationally funded programmes
- Improved coordination of research priorities suitable for future programme funding
- Improved availability of validated and relevant research data for animal health policymakers, and the animal health and livestock industries (including aquaculture)

- Improved availability of information on national research capacity, including expertise, in the various areas
- Identification of the needs for building capacity and capability in animal health research
- Improved consultation with other international policymakers and animal health organisations (e.g. EFSA, ECDC, OIE, FAO, and WHO)

Keywords

infectious diseases of animals, zoonoses, research coordination, animal health, global network

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[EPIZONE]

Network of Excellence for Epizootic Disease Diagnosis and Control

Summary

EPIZONE brings together expert scientists to combine and harmonise efforts on the development of new strategies and tools to combat future epizootic animal diseases. Epizootic diseases in food-producing animals including aquaculture constitute a major risk for food safety and food security and therefore have become a concern for both animal and human health. 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. To develop new methods for the prevention and control of epizootic diseases, collaborations of scientific institutes are indispensable and international harmonisation and standardisation of procedures is urgently needed.

The objective of EPIZONE is to improve research on preparedness, prevention, detection and control of epizootics by improvement of excellence through cooperation.

EPIZONE has developed a network of more than 350 key scientists with an international reputation, complementary expertise and skills working together within Europe and worldwide. Through increased excellence by collaboration of veterinary institutes from Europe, China and Turkey, the economic and social impact and the public health risk of future outbreaks of foot-and-mouth disease, classical swine fever, avian influenza and other relevant epizootic disease like bluetongue and African swine fever, will be reduced.

EPIZONE has been 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 agriculture administrations and biotechnology companies. EPIZONE includes 18 institutes from 12 countries.

Acronym:
EPIZONE

Project number:
016236

EC contribution:
EUR 14 000 000

Duration:
60 months

Start date:
1 June 2006

Instrument:
Network of Excellence

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 generates durable interactions among partners. EPIZONE includes the FAO as a world-oriented organisation, and also an enterprise (SME) specialised in dissemination of knowledge via the Internet. Within organisational work packages integration activities, including communications, meetings and trainings have been developed. Within scientific work packages, joint research is executed covering four thematic areas: diagnostics, intervention strategies, surveillance, epidemiology and risk assessment. Given the network structure, the technical resources and the scientific excellence, EPIZONE assures strategically driven state-of-the-art research of world-renowned quality.

Problem

In recent years, much time and money has been devoted to fighting and controlling outbreaks of well-known major animal diseases, such as avian influenza, classical swine fever and foot-and-mouth disease. The threat of new emerging and re-emerging epizootic animal diseases seems to increase in all parts of the world. Epizootic diseases constitute a major risk to food production. Such diseases spread very quickly through animals, vectors or animal products especially where susceptible animals are kept at a high density. Outbreaks showed enormous social and economic impact, and need to be addressed across the whole production chain of animal-related food. Animal disease control methods involving the mass culling of livestock are no longer acceptable for the international society. Innovative and rapid prevention and control strategies will be needed and international cooperation becomes more and more important.

Aim

The mission of EPIZONE is: 'To improve research on preparedness, prevention, detection, and control of epizootic animal diseases through cooperation, with extra attention for new and emerging epizootic animal diseases including these which may have zoonotic potential'. This includes the goal to reduce the economic and social impact of future outbreaks of foot-and-mouth disease, classical swine fever, avian influenza (AI) and other relevant epizootic diseases such as bluetongue and African swine fever, through increased excellence by collaboration.

Results

EPIZONE has established an international network for sharing knowledge and expertise on epizootic animal diseases and materials and reagents. Increased excellence by collaboration generated by the project will help to combat future epizootic animal disease more efficiently and more cost-effectively. Improved knowledge on epizootic disease diagnosis, epidemiology, risks and intervention strategies, generated by EPIZONE will contribute to the prevention and control of epizootic animal diseases on a global level. In this way, European experience, knowledge and scientific achievements will gain visibility and will be used to support decision-making and future research.

EPIZONE has achieved the following.

- Created strong collaboration between veterinary institutes and with non-EU partners through staff exchanges, scientific missions, workshops, training and technology transfer, and also bringing together next-generation scientists. After its EU-funded period, which ends 1 January 2012, EPIZONE will continue as the EPIZONE European Research Group.
- Established online databases on reference materials, diagnostic protocols, cell cultures, viruses, and disease outbreaks.
- Accommodated the development, validation and harmonisation of detection methods including new molecular

techniques, pathogenic virus-specific diagnostics and pen-side tests, using test panels generated by partner institutes.
- Provided important data on epidemiology, surveillance and risks of recent epizootic animal disease outbreaks in the EU including bluetongue and avian influenza.
- Produced novel reagents and new methods for development of new vaccine technologies.
- Generated scientific publications in international peer-reviewed journals.

Project website
http://www.epizone-eu.net

Keywords
epizootic diseases, animal diseases control, network of excellence

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[DISCONTOOLS]

Development of the most effective tools to control infectious animal diseases

Acronym:
DISCONTOOLS

Project number:
211316

EC contribution:
EUR 978 660

Duration:
48 months

Start date:
1 March 2008

Instrument:
FP7 — Support actions

Summary

DISCONTOOLS, an ongoing EU-funded project, has three objectives. Firstly, to develop a disease prioritisation methodology enabling the prioritisation of research in order to stimulate the delivery of new or improved diagnostics, vaccines or pharmaceuticals. This will help to improve our ability to effectively control animal diseases which is a key input into meeting the challenges of future food supplies. Once this methodology is agreed with stakeholders, the objective is to establish a reference database ensuring a clear focus on priority research areas leading to more rapid breakthroughs in technology development. Secondly, to develop a gap analysis for each of the prioritised diseases to identify where research is needed. Thirdly, the DISCONTOOLS project will explore how new technologies can be deployed more efficiently in the animal health research area.

Problem

The concept for this proposal arose from of the work of the European Technology Platform for Global Animal Health (ETPGAH) which was launched in December 2004. Since then, the ETPGAH has developed a vision, a strategic research agenda (SRA) and an action plan to implement the recommendations in the SRA.

Recent disease outbreaks have highlighted the necessity for not only producing new vaccines but also for improving existing vaccines and providing marker vaccines.

Effective tools for controlling animal diseases of major social and economic importance are vital not only for Europe but also for the rest of the world. The use of vaccines and diagnostic tests are a key component as they have the potential to support control and eradication and to be highly cost-effective. At present, there are no antiviral medicines for use against the major viral diseases of animals. Consequently, vaccines and diagnostic tools are often the only solution available for control.

New and improved vaccines are required for a range of major animal diseases. In addition, improved diagnostic tests must be developed to enable the early diagnosis and detection of outbreaks along with tests to demonstrate the effectiveness of control programmes. The development of new pharmacological or biocidal solutions to the containment and control of disease outbreaks also needs to be considered.

All these factors underline the need for a coordinated, transparent and multidisciplinary R & D effort from basic sciences through to the emerging technologies and on to product development, production, authorisation and distribution. There is an urgent need to boost research with effective funding so that new or improved veterinary medicines — vaccines, pharmaceuticals and diagnostic tests can be delivered.

It is important to develop — through public and private partnerships — an overview of current research and identify the gaps. Programmes can then be developed to fill these gaps whilst at the same time developing research collaboration and synergies to avoid duplication of research effort. Within the EU, the lack of a formal mechanism to identify research gaps increases the reliance placed on scientific communities, panels and workshops to assess these needs. Assessments are limited and need continuous updating. It is equally important to adopt a global approach to ensure that research is coordinated and rationalised to ensure maximum returns for the investment in research.

Aim

DISCONTOOLS will be carried out over four years. It has three complementary strands for addressing the main objectives of the FP7-KBBE-2007-1-3-03 call. These strands all contribute to the primary objective of the call which is to enable research to be optimised by public and private funders in a more effective manner to enable new and improved tools to be developed and delivered for the control of the major infectious diseases of animals including zoonoses.

Expected results

The first strand will provide a validated database and peer-reviewed methodology in order to prioritise infectious animal diseases. Gap analysis is the second strand and will be carried out to identify those areas where information and knowledge of the disease is deficient and where current tools are lacking, inadequate or could be improved. Information will be collected in a standard format for validation and entry into a specific disease database. A detailed analysis will then be carried out for each of the priority diseases to identify gaps in key areas.

The third strand is to identify current and new technological tools that may be used to improve the ability to control infectious animal diseases. The work will include review of existing arrangements by stakeholders and the development of methodologies to identify and evaluate new technology. Effective identification and technology transfer is essential if new tools for disease control are to be developed.

One of the main features of the project is the involvement of a wide range of stakeholders who will actively participate in the governance of the project. This will

ensure that the stakeholders involved from research through to delivery of new control tools will be able to contribute to the project. Dissemination of information from all three strands of work will be essential if the project is to be successful. This will be achieved through the communication strategy which will include interactive web systems and databases as an integral part of the project.

To date, 12 sets of disease information have been placed on the public website (http://www.discontools.eu). Expert groups have completed their work in relation to an additional 18 diseases and this information is going through the approval process. It is anticipated that the majority of expert groups will have completed their work by the end of June 2011. In examining the results, it has become clear that the information is quite complex and so we are developing an interpretation guide that will be placed on the website to clarify the use of the results. In addition, each disease will be accompanied by a short summary of the main findings assisting all concerned in interpreting the data.

As the database is populated with the 51 diseases and as we receive input through the public website, the data on the website will become quite definitive in terms of the state of play concerning research prioritisation focused on delivering new and improved diagnostics, vaccines and pharmaceuticals. In addition, as the model matures, we can then move on to a new phase of adding further diseases. Via the ongoing consultation process, the model will remain up to date and prioritisation will change over time as gaps are filled and as new information becomes available including on emerging and re-emerging diseases.

Potential applications

Our wish is to create a definitive source of data on prioritisation of research in the field of animal diseases, driven by stakeholders, that will facilitate public and private funders in determining the most efficient way of deploying research funding.

References/publications

- Brochure (http://www.discontools.eu/documents/1380_DISCONTOOLSbrochure.pdf).
- DISCONTOOLS methodology is available on the website under 'Working Groups/Documents of interest'.
- DISCONTOOLS interactive disease database (http://www.discontools.eu/home/disease_home).

Project website

http://www.discontools.eu

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[Arbo-Zoonet]

Network for Capacity-building for the Control of Emerging Viral Vector-Borne Zoonotic Diseases

Summary

Arboviruses are arthropod-borne viruses, which include West Nile fever virus (WNFV), a mosquito-borne virus, Rift Valley fever virus (RVFV), a mosquito-borne virus, and Crimean-Congo hemorrhagic fever virus (CCHFV), a tick-borne virus. These arthropod-borne viruses can cause disease in different domestic and wild animals and in humans, posing a threat to public health because of their epidemic and zoonotic potential. In recent decades, the geographical distribution of these diseases has expanded. Outbreaks of WNFV have already occurred in Europe, especially in the Mediterranean basin. Moreover, CCHFV is endemic in many European countries and serious outbreaks have occurred, particularly in the Balkans, Turkey and Southern Federal Districts of Russia. In 2000, RVFV was reported for the first time outside the African continent, with cases being confirmed in Saudi Arabia and Yemen. This spread was probably caused by ruminant trade and highlights that there is a threat of expansion of the virus into other parts of Asia and Europe. In the light of global warming and globalisation of trade and travel, public interest in emerging zoonotic diseases has increased. This is especially evident regarding the geographical spread of vector-borne diseases. A multidisciplinary approach is now imperative, and groups need to collaborate in an integrated manner that includes vector control, vaccination programmes, improved therapy strategies, diagnostic tools and surveillance, public awareness, capacity-building and improvement of infrastructure in endemic regions.

Problem

The virgin soil-epidemic raises the threat of expansion into other parts of Asia and Europe. The general public concern regarding emerging zoonotic disease has gained interest and relevance in light of global warming. This is especially true regarding the spread of the arboviruses such as RVFV, CCHFV and WNFV, which are transmitted by mosquitoes or ticks. It is therefore imperative to work out integrated control measures that include vector control, and vaccination programmes, which improve therapeutic strategies, as well as diagnostic tools and surveillance, public awareness, capacity-building and the infrastructures in endemic regions.

Aim

The Arbo-Zoonet project aims at creating common knowledge of these diseases, as well as sharing and exchanging data, expertise, experiences and scientific information. The surveillance systems will be maintained and expanded, monitoring

Acronym:
Arbo-Zoonet

Project number:
211757

EC contribution:
EUR 998 470

Duration:
36 months

Start date:
1 May 2009

Instrument:
coordination and support action

disease occurrence and vaccine use. The disease detection and control tools will be introduced, distributed and harmonised. The consortium will also disseminate knowledge and train staff of relevant third countries. The project partners think it is also important to interlink different scientific disciplines which approach the problems from different angles.

Work plan

The work plan of Arbo-Zoonet foresees a number of interrelated tasks, with measurable deliverables and milestones. The following are the specific aims of the work plan.

(a) Identifying risk areas and undertaking the necessary preparatory work for updated risk maps on RVFV, WNFV and CCHFV introduction and/or spread throughout the EU territory. Efforts will focus on understanding the ecology of host, vectors and disease reservoir. Moreover, this task will produce maps and estimate the numbers of vectors in order to prepare models for policymakers.

(b) Create a pathogen database open to the scientific community that will contain information on where live samples of a given pathogen are available. This database will include other biological material such as serum and genetic material from different geographical areas where the relevant diseases are endemic.

(c) Surveillance networks will be established for the collection of global data on the occurrence of RVFV, WNFV and CCHFV. An essential task is the reporting on the analysis of the RVFV, WNFV and CCHFV surveillance systems for the EU and for affected areas in countries outside the EU. These analyses will be used to establish adequate georeferenced data and to derive spatial conclusions. The assessment will address significant aspects of the surveillance and control activities (monitoring approaches, diagnostic methods and capabilities, established information systems, data analysis capabilities, geographic distribution of virus strains, vector competence studies, entomological expertise and surveillance methods applied, protocols for vaccine use) to serve as a framework for shared data sets.

(d) Moreover, working groups will be established to assess data focused on vector control, vaccination and therapy. The project will act as a platform to bring together those participants who are actively involved in molecular vaccine development. Emphasis will be given to integrated vaccine strategies using vaccines based on pathogen and vector components and development of appropriate delivery systems. In this context, studies on molecular characterisation the interaction between host, vector and pathogen will be promoted, primarily through scientific exchange visits. Therapeutic options will also be examined. This will be done either by working on existing pharmaceuticals or by developing new ones.

(e) The principal focus of the project is the transfer of knowledge and technology between the members of the consortium, which includes partners from relevant countries outside the EU. In this context, links will be established to the national and international organisations (WHO, FAO, OIE, or the International Regional Organisation for Plant and Animal Health (OIRSA)), institutions and laboratories located in the different areas in order to disseminate and transfer technologies needed to develop strategies for integrated control measures in endemic regions such as diagnostics, epidemiology and economic dimension of a number of endemic as well as epizootic animal diseases.

(f) Arbo-Zoonet will play a coordinating role a within the EU's animal health strategy by bringing together interested members of other EU consortia that share the focus on zoonoses caused by vector-borne arboviruses, such as the

Emerging Diseases in a changing European eNvironment (EDEN) project, the network of excellence for epizootic disease diagnosis and control (EPIZONE), and the Environmental Vulnerability Assessment (EVA) project.

Expected results

The coordinated research programme — comprising key laboratories in Europe and neighbouring countries — will address questions of joint interest, thus enabling the development of effective control measures that will improve the EU's response to disease outbreaks.

Potential applications

The Arbo-Zoonet consortium hopes this network will be continued beyond the time frame of the project, and will be extended to address other public and animal health problems.

Activities

A number of activities such as scientific meetings and technical workshops both on national and regional levels were organised. In summary, a total of six scientific meetings and technical workshops for epidemiology and diagnostic tools were held in the following countries: Algeria, France, Germany, South Africa and Turkey.

In the context of collaboration, the Arbo-Zoonet project established the following links:

- FAO (North Africa)
- EPIZONE
- International Consortium on Ticks and Tick-Borne Diseases (ICTTD-3)
- ConFluTech
- ASEMDialog
- Society for Tropical Veterinary Medicine
- European Meeting on Viral Zoonoses, Saint Raphaël, France, September 2009

Dissemination activities

The dissemination activities of the Arbo-Zoonet project comprised:

- Publication and distribution of a biannual newsletter: Arbo-Zoonet news
- Publication of an article in *Eurosurveillance*, Vol. 14, Issue 12, 26 March 2009
- Distribution of CDs containing presentations and protocols of the meetings and workshops conducted
- Maintenance of a specific website for this purpose (http://www.arbo-zoo.net)

Keywords

Crimean-Congo hemorrhagic fever, Rift Valley fever, West Nile fever, arbovirus, hemorrhagic fever, encephalitis, mosquito, tick

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[WildTech]

Novel Technologies for Surveillance of Emerging and Re-emerging Infections of Wildlife

Summary

For many reasons, the health of wildlife is of major concern throughout the world. Apart from important influences on the health of many wildlife species, infectious diseases of wildlife have significant impacts on public health and health of livestock. Effective disease surveillance is essential in order to inform control strategies and this depends critically on the development and application of methods of disease diagnosis which are both accurate and rapid. The WildTech project has been established specifically to address these problems and to set up a technology centre that may be exploited in Europe and elsewhere as a basis for high throughput disease diagnosis in wildlife.

The project combines

- technological development to enable high throughput nucleic acid- and peptide-based array screening of samples from a wide variety of wild animals;
- surveillance of terrestrial, aerial and marine wild animal species within Europe and from countries which act as portals of disease entry into the EU;
- epidemiological analysis and risk assessment using data generated during the project and from other sources;
- development and proposal of a model framework for disease surveillance within Europe.

Acronym: WildTech

Project number: 222633

EC contribution: EUR 5 996 822

Duration: 48 months

Start date: 1 July 2009

Instrument: Collaborative project

The project will place the EU at the centre of wildlife disease surveillance and enables the translation of high throughput array-based technologies to human and veterinary medicine.

Problem

WildTech addresses the problem of the increasing prevalence of new and emerging diseases arising from wildlife which has clear implications for disease spread to domestic animals and humans both across Europe and globally. The reasons for this alarming trend are multifactorial and have been well documented in the literature. In brief, the continued increase in the human population results in habitat fragmentation caused by factors such as deforestation and increasing levels of pollutants. These issues inevitably impact on host-pathogen relationships and the spread of pathogens into geographical areas previously unaffected. Alterations in land use and livestock rearing practices and the rapid global movement of humans, animals and other organisms are also key factors. Finally, the evolution of viral pathogens adds another factor into this alarming and complex equation.

WildTech is focused on wildlife as a reservoir of disease. It is reported that 61 % of known pathogens infect multiple animal species and 75 % of all diseases which have emerged in the last two decades are of wildlife origin. It is therefore clear that the surveillance of disease in wildlife not only impacts on communities that rely on healthy domestic animals but is also an essential tool for the protection of human health. Despite this alarming situation, surveillance for infectious diseases in wildlife is far from satisfactory. Until now, there has been no coordinated effort to monitor the spread of infection within and between different countries in the EU. Surveillance of wildlife infectious disease has been largely passive in structure rather than a proactive attempt to predict and manage future disease threats across Europe.

Aim

The key objectives of WildTech are as follows.

- The application of microarray technology for the detection of known infectious agents in wildlife populations.
- The application of microarray technology to the detection and identification of novel and unknown infectious agents in wildlife populations.
- The application of microarray technology to the development of high throughput serological screening of wildlife populations for infectious disease.
- The utilisation of these technologies to assess the spread of selected diseases (proof of concept) using historical samples and those collected during the project. We will monitor and model patterns of wildlife disease spread and the risks associated with it. Ultimately, this epidemiology framework will be used to reduce the risk of further potential epidemics by producing a generic action plan in case of emerging epizootics among wildlife.
- The development of a state-of-the-art wildlife disease data management system with mapping capability for use in Europe and beyond.
- The establishment of a framework for pan-European surveillance of wildlife diseases.

Expected results

- Effective and validated high throughput microarray technology, both generic and adapted to a commercial platform, for the detection of nucleic acid of a focused list of up to 20 infectious agents (viruses, bacteria and parasites) from wild animal samples. We will, in addition, develop generic arrays for 200 infectious agents which will be incompletely validated.
- Effective and validated high throughput serological array technology, both generic and partially adapted to commercial platform for detection of specific antibodies in serum/blood against approximately 20 infectious agents from selected wild animal hosts, in addition to incompletely validated tests for further infectious agents.
- Information on the spatial and temporal distribution of a focused list of up to 20 infectious agents in wild animal species in selected European countries/regions and countries outside Europe that represent potential sources of introduction into Europe.
- Information on the risk to human and domestic animal health from the presence and evolution of infectious agents in selected wild animal populations.
- Established management systems for wildlife disease information, which are accessible to national and international animal and human health organisations, the international wildlife disease community and policymakers.
- Proposal for surveillance system for wildlife diseases in Europe, which will contribute to protecting European wildlife, domestic animal and human health.

Potential applications

Benefits to the European economy include the following.

1. The technology proposed for this project together with the strengthened European wildlife community network working in collaboration with international reference laboratories under the aegis of the OIE will make a substantial contribution to preventing major outbreaks of infectious disease in Europe.

2. The benefits to the European economy also include short-term direct economic benefits from the exploitation and application of the technologies to be developed during this project. The exploitation of the high throughput microarray technology will enable a European company to be a major focus for high throughput disease-screening technology. This microarray technology will expand the current income stream from within and outside Europe.

3. The exploitation of the serology array will enable expansion of the existing market in serological detection of food-borne zoonoses and will facilitate the development of new markets in surveillance of wildlife and other animal and human diseases. The size of this market is impossible to predict and will depend on the extent to which large-scale surveillance of diseases in wildlife, man and domestic and companion animals becomes a reality within Europe and beyond. Initial markets are likely to be in Europe and North America.

Social impact

1. The enormous financial losses from emerging animal diseases hide the intense personal impact that such diseases have on rural and other communities, both through the destruction of animals and livelihoods and, in the case of zoonoses, through the potential for large-scale human morbidity and mortality with the associated economic and personal damage inflicted on the continent. The proposed combination of new generation technologies together with an improved framework for monitoring and surveillance are central to

ensuring that major outbreaks do not occur with the potentially devastating effects on the European and world economy and to developing a mechanism that can effectively identify and respond to the threat presented by new pathogens.

2. The European effects will be mirrored by reduced mortality and morbidity and associated improved welfare in domestic animals beyond Europe. In poorer third world countries, outbreaks of infectious disease in domestic animals can have far-reaching consequences for the well-being of entire human communities. Thus, improved surveillance and subsequent intervention strategies will have dramatic effects on the quality of human life in more deprived areas within and outside Europe.

3. The project results will also have indirect impact on human health as diseases coming either directly or indirectly through wildlife are likely sources of zoonotic infection. By improving our detection of these pathogens, this would enable a rapid and effective response to an emerging infection, which would minimise the impact on the human population.

Project website

http://www.wildtechproject.com

Keywords

wildlife disease surveillance, high throughput array technologies, epidemiology, data management systems

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[ICONZ]

Integrated control of neglected zoonoses: improving human health and animal production through scientific innovation and public engagement

Acronym:
ICONZ

Project number:
221948

EC contribution:
EUR 6 million

Duration:
5 years

Start date:
April 2009

Instrument:
Collaborative project

Summary

ICONZ is a five-year, 21-partner EU FP7 project undertaking studies to evaluate the epidemiology, socioeconomic and policy implications of eight 'neglected' zoonotic diseases across seven African countries.

Problem

Neglected zoonoses are major causes of ill health in many developing countries worldwide. Both production and companion animals can act as significant reservoirs for transmission of zoonotic disease to man. This societal burden of disease is often compounded by the effect of zoonotic disease on livestock productivity, hence inflicting a further burden on affected communities who depend on their livestock as a source of food and income.

Aim

ICONZ is a collaborative project involving 21 European and African partners, with the integrated control packages of eight key 'neglected' zoonoses, namely anthrax, bovine tuberculosis, brucellosis, cystic echinococcus, porcine cysticercosis, leishmaniasis, rabies and zoonotic trypanosomiasis. Through a series of complementary actions, ICONZ aims to improve human health and animal production to alleviate poverty and contribute to the Millennium Development Goals (MDGs). New technologies and research outcomes are being used to quantify burdens of neglected zoonoses on communities in Africa through a series of case studies in the International Partner Cooperation Countries (ICPCs); Mali, Morocco, Mozambique, Nigeria, Tanzania, Uganda and Zambia. It is expected that through these field-based case studies, improved control and awareness of neglected zoonotic disease at both community and national/regional policy levels will occur through the development and utilisation of novel intervention strategies. It is expected that intervention strategies will be communicated through a well researched dissemination strategy to both communities and policymakers in affected regions.

Expected results

It is widely accepted that current estimates of the prevalence and burden of neglected zoonotic diseases are inadequate. ICONZ anticipates and predicts a major impact

on the understanding, implementation and efficacy of control strategies for neglected zoonoses in developing countries, through (i) increased understanding of their burden and significance and (ii) through the testing and refinement of practical, cost-effective strategies for control. To support this, ICONZ is currently in the process of undertaking surveys across seven countries in Africa, in order to increase understanding of the societal burden of neglected zoonoses, and help develop informed control strategies, taking into consideration the socioeconomic status of affected communities, and the wider national and regional policy agendas that govern these communities.

Potential applications

The ongoing overview of current worldwide research activities aims to reduce duplication of effort, leading to more effective use of limited resources and funds, whilst at the same time identifying major 'gaps' within international research agendas. ICONZ is currently also in the process of cataloguing current tools for surveillance, diagnosis and treatment of neglected zoonoses, which will help identify areas where such tools are inadequate or unavailable, and subsequently lead to recommendations for improved inputs. The development of cost-effective diagnostic and control tools which are field-friendly and easily available cannot be underestimated: effective tools employed within simple strategies may preclude the expense of emergent tools, vaccines and improved veterinary medicines.

Project website

http://www.iconzafrica.org/

Keywords

neglected zoonoses, one health, livestock productivity, human health

Logo

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Integrated Control Of Neglected Zoonoses

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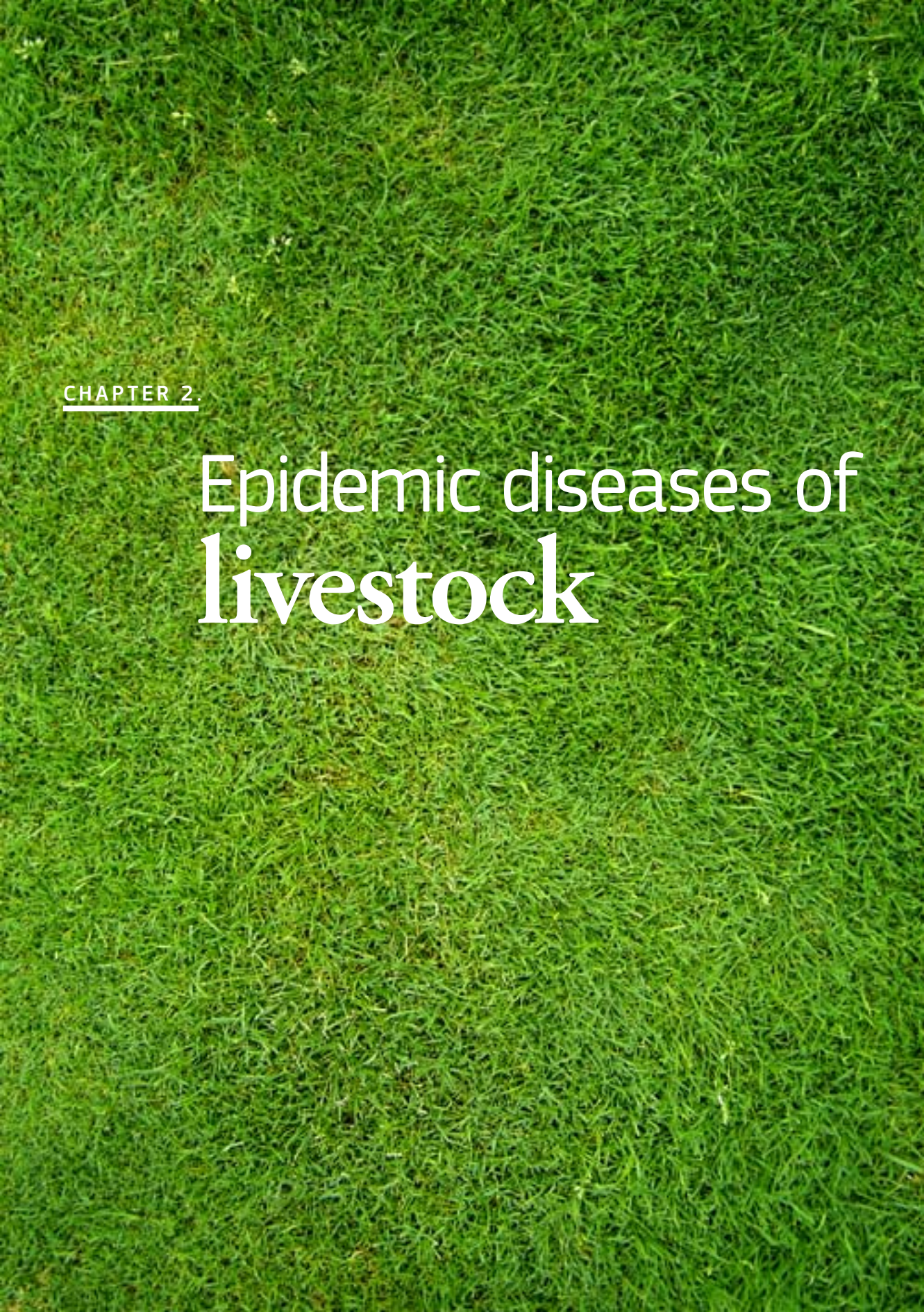

CHAPTER 2.

Epidemic diseases of **livestock**

European policies for animal health sustained by EC-funded research projects

Research to support animal health is critical for the development of evidence-based policies and the introduction of legislative measures to govern disease surveillance, prevention, control and eradication. These policies must be based on the best scientific evidence, some of which is provided from the portfolio of projects funded through the EU seventh framework programme for research (2007–13).

Animal diseases of public importance remain a priority area but research funding is not unlimited. The DISCONTOOLS project provides a mechanism to prioritise diseases and to identify the gaps in current knowledge and available control tools such as vaccine, diagnostic tests and pharmaceuticals. The coordination of research funding between the EC projects and those funded by the Member States is essential to obtain maximum benefits, from the available funding. The EMIDA and STAR-IDAZ projects will both make important contributions by coordinating European research and developing EU-wide and global networks of funding organisations.

Some overarching projects seek to develop networks to maximise the use of expertise, increase excellence through collaboration and ensure that Europe has the capacity to deal with emerging problems. Networks and collaboration are vital as it is no longer feasible for one group alone to undertake complex and expensive research.

Even for targeted diseases such as foot-and-mouth (FMD), much of the research currently underway requires an interdisciplinary and collaborative approach. It is no longer the preserve of one discipline and a wide range of multi- and interdisciplinary research activities are required to solve complex problems. In addition to researchers and scientists, it is increasingly important to imply the whole spectrum of expertise involved in animal health including producers, advisors, veterinarians and scientists. This is encouraged by a number of EC-funded projects which will generate durable interactions among partners with complementary expertise and skills.

Research is needed to ensure that the facilities and expertise necessary for the investigation and identification of new and emerging diseases is available. WILDTECH develops more effective surveillance methods for emerging diseases from wildlife. Another forward-looking project, ARBO-ZOONET, builds capacity so that Europe would have the expertise and facilities to deal with the potential risks of disease outbreaks as a result of emerging viral vector-borne diseases.

It is also quite important to support research in neighbouring countries and developing countries especially for exotic diseases of high prevalence which have the potential to enter the EU. There are many benefits from the range of EC-funded projects which involve research institutes from countries outside the EU.

New or improved tools for the prevention and control of animal diseases are an essential component of EC research programmes.

Diagnostic tests with high sensitivity, specificity and reproducibility are requested for use on the front line, local laboratories and in abattoirs. New technologies enable the development of pen-side tests for a range of diseases with one sample along with remote transfer of data. Projects such as CSFVACCINE & WILD BOAR, CSF-GoDIVA or FMD-Disconvac have resulted in the development of diagnostic tests which could also discriminate between vaccinated and infected animals. This is critical information for the development of new control policies.

The development of new or improved vaccines for a wide range of epidemic and endemic diseases was the topic of a number of research projects. In many cases, the research aimed to understand the immune response, to develop improved vaccines which would provide protection against a number of serotypes and to use third generation technology to develop marker vaccines. Research projects were funded to develop vaccines for bluetongue (BT-VAC ORBIVA), classical swine fever (CSFVACCINE & WILD BOAR and CSF-GoDIVA), FMD (FMD Disconvac) and African horse sickness (ORBIVAC). In a number of these projects, the potential for control measures using vaccines was also studied in order to reduce the need to slaughter animals during an outbreak of an epidemic disease.

Projects such as those for African swine fever, avian influenza, bluetongue, classical swine fever and FMD have significant impacts. The scientific outputs often result in improved measures being adopted in EU Member States and in non-EU countries engaged in trading of livestock and products of animal origin. Evidence from EU research is often used by international bodies such as the World Organisation for Animal Health (OIE) to develop international standards for disease control, animal welfare and trade.

Much of the research into animal diseases is carried out in support of policies by providing the scientific evidence and technologies to those responsible for policy development. This is a particularly important pathway in animal health and welfare and has been used very effectively over the past decade. The EC-funded research projects have made major contributions to European policies for animal health with a comprehensive portfolio in relation to policy needs. The programme has also been characterised by the anticipation of research needs in advance of these becoming clear in policy circles. This is a major achievement and demonstrates the benefit of long-term strategic portfolio management by an individual Dr Isabel Minguez-Tudela who had the foresight to identify future research needs and to ensure continuity of research.

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2.1. Foot-and-mouth disease

[FMD_ImproCon]

Improvement of foot-and-mouth disease control by ethically acceptable methods based on scientifically validated assays and new knowledge on FMD vaccines, including the impact of vaccination

Acronym:
FMD_ImproCon

Project number:
503603

EC contribution:
EUR 2 399 907

Duration:
60 months

Start date:
1 January 2004

Instrument:
Specific Targeted Research or Innovation Project (STREP)

Summary

There is a strong desire to reduce reliance on large-scale culling of animals to control future outbreaks of FMD in EU Member States. As an alternative, it is proposed to use emergency vaccination and then to screen for residual infection using tests for antibodies to the non-structural proteins of FMD virus. It is intended to amend the policy on FMD control to enable such an approach to be used in the very near future. In reality, this means that current contingencies must be based on the use of existing vaccines. Therefore, this project seeks to address the specific gaps in our knowledge and technological ability with respect to the implementation of a vaccinate-to-live policy. The availability of adequate discriminatory diagnostic tests is the keystone of the new EU FMD control policy. The project is focused on the validation of NSP-based tests to discriminate unequivocally between infected and vaccinated animals, in order to allow the implementation of the new policy in the immediate term. Validation of existing and new NSP tests as confirmatory tests will be a major output of this project. The experimental design will also provide expected outputs in the field of the impact of vaccination on the carrier state and on virus dissemination, the onset of vaccinal protection, vaccine potency in relation to emergency use, vaccine strain selection and new marker vaccines. This project focuses on marker vaccines to induce durable protection against FMD. Conventional and marker vaccines will be targeted to dendritic cells with particular attention to promote dendritic cell mucosal homing (from parental immunisation), because mucosal immunity can prevent FMD virus establishing local infection and the carrier status.

Problem

As there is a strong desire to reduce reliance on large-scale culling of animals to control future outbreaks of foot-and-mouth disease (FMD) in EU Member States, the European Commission (EC) amended its policy and has changed its directive on Community measures for the control of foot-and-mouth disease (Council Directive 2003/85/EC), making the use of emergency vaccination easier when combined with screening for residual infection using tests for antibodies to non-structural proteins (NSPs). In reality, this means that current contingencies must be based on the use of existing vaccines. Therefore, this project addressed the specific gaps in our knowledge and technological ability with respect to the implementation of a vaccinate-to-live policy.

Aim

The aim of the project was to support the EC policy to reduce reliance on large-scale culling of animals for controlling future FMD outbreaks in EU Member States and to increase the possibility for implementing the vaccinate-to-live policy through:

- validation and development of (confirmatory) NSP tests;
- improved FMD virus detection;
- better knowledge on the impact of vaccination on FMD virus dissemination and the carrier state;
- improved vaccine strain selection;
- the development and evaluation of FMD marker vaccines;
- better knowledge on mucosal immune responses and dendritic cell targeting of the FMD vaccines.

Results

WP1: Validation of existing NSP tests

WP1 of the project has contributed significantly to the availability of validated assays able to discriminate unequivocally between vaccinated and infected animals. Knowledge (advantages and limitations) on their sensitivity and specificity, as well as on application of such tests in outbreak and post-outbreak situations, is essential in light of the requirements laid down in the new Council Directive on Community measures for the control of foot-and-mouth disease (Council Directive 2003/85/EC) and their application to substantiate freedom from FMD has been discussed.

A tool for validating NSP tests without 'Gold Standard' was provided by: (i) the development of at least four tests based on different detection methods for the virus antigen (for instance an antigen-detection ELISA, virus isolation, PCR and a RCA-ELISA (see below)); or by (ii) the use of state-of-the-art statistical methods such as Latent Class analysis or Bayesian analysis.

WP2: Development and validation of confirmatory and new NSP tests

The work performed in WP2 was aimed at developing and validating new NSP assays as confirmatory tests or as alternative primary assays, in order to improve on the diagnostic performance of the NSP ELISAs validated under WP1 and to create kit production opportunities of NSP assays with a view to facilitate the distribution of the product to all interested countries within the European Union and the rest of the world. Some of the developed prototype assays have already been taken up by kit manufacturers and others might do this in the future.

WP3: Improved FMD virus detection

Improving on existing FMD detection methods in WP3 has sometimes proven difficult, but by better understanding the basic principles of FMD growth on cell culture, progress has been made.

A real-time RT-PCR system consisting of two independent FMDV PCR protocols (plus one backup protocol) and two independent SVD PCR protocols were established and validated.

Furthermore, a new goat cell line has been identified to enhance FMD virus detection that also enables a quicker diagnosis.

WP4: Impact of vaccination on virus dissemination and the carrier state

WP4 provided new insights in virus transmission and the impact of vaccination, which will prove crucial when faced with new FMD outbreaks. The value of vaccination in reducing virus transmission has been demonstrated in different FMD susceptible species (cattle, sheep and pigs) with different FMDV strains and serotypes. However, the results of WP4 also clearly show that vaccination will not be able to prevent infection when it occurs within a week following the administration of the vaccine. Nevertheless, these experiments were successful in measuring the efficacy of vaccines, the dynamics of NSP seroconversion and its relationship to virus replication, persistence and clinical signs. Mathematical analysis of the experimental data has been used to predict carrier numbers in cattle herds and sheep flocks after emergency vaccination, as an aid to determining the feasibility of using NSP serosurveillance to substantiate FMD freedom.

WP5: Improved vaccine strain selection

The data provided on vaccine strain selection in WP5 will help decision-makers in their difficult choice of vaccine use and in identifying relevant strains for inclusion in FMDV antigen banks. The advantage of using high potency vaccines when no matching vaccines are readily available in antigen reserves has been shown. High potency vaccines enhance the probability of achieving adequate levels of cross-protection even when faced with low r-values and sequence homology. Furthermore, the limits of the existing *in vivo* FMD vaccine potency tests have been demonstrated (high level of between test variability) which point towards more international acceptance of alternative *in vitro* approaches avoiding live viral challenge (e.g. serology, interferon-gamma responses and antigen payload).

Mouse monoclonal Antibody (MAb) antigenic profiling data proved to be a very effective tool for monitoring how effectively a vaccine strain protects against field viruses. However, mapping data combined with full capsid sequences of field strains would provide more information which helps to resolve the crucial epitopes and develop vaccine strategies focusing on these epitopes. Moreover, further research is required to better understand the contribution of different viral epitopes to cross-protection and thereby to improve predictive methods of vaccine efficacy.

It is well known that neutralising antibody titres are important in protecting against FMDV infection. However, it has often been shown that the humoral antibody titre is not always fully predictive of vaccine-induced protection against FMD. Therefore, a correlation between cell-mediated immune responses, humoral immune responses and post-vaccination protection against FMDV infection was investigated. It is concluded that T cell stimulation assays such as the whole blood IFN-γ assay along with VNT are potential candidates for vaccine evaluation and could reduce the need for *in vivo* challenge in the future.

WP6: Development of a marker vaccine

Progress has been made in WP6 to enhance mucosal immunity. Moreover, a new generation vaccine based on a serotype A chimera vaccine in which the GH-loop region was replaced with that of another serotype proved fully protective in cattle against challenge from the unsubstituted parental serotype A virus. Furthermore, the presence of a heterologous GH-loop could be exploited to discriminate serologically between vaccinated and vaccinated-and-challenged cattle. Subsequently, a spontaneous loop-deleted mutant vaccine virus was discovered and shown to elicit an

antibody response that was similarly cross-reactive compared to antibodies elicited by a loop-undeleted vaccine virus. Follow-up funding has been granted to further pursue the marker vaccine potential of this virus.

Dendritic cells (DC), essential for inducing and regulating immune defences and responses (systemic and mucosal), represent the critical target for vaccines against pathogens such as FMDV. The interaction of FMDV vaccine antigen with DC was studied and showed that following internalisation of FMDV antigen, these DC were efficient antigen presenting cells, observed in terms of their ability to stimulate specific lymphocyte proliferation and virus-specific antibody production. These results are advantageous for conventional FMD vaccines, which will be composed cell-culture adapted HS-binding variants of FMDV. Furthermore, immuno-modulatory factors targeting DC for promotion of antibody and mucosal immune responses, like the vitamin A derivative all-trans-retinoic acid and the *E. coli*-derived heat-labile enterotoxin (LT), were identified. Moreover, the use of TLR ligands as additional immunostimulating molecules to promote systemic and mucosal immune responses was characterised *in vitro* and *in vivo*. Nevertheless, caution is required when translating findings from mouse models to a natural host of FMD.

Applications

New diagnostic experience and new knowledge about the epidemiology and virus properties were transferred in different ways. Results open to the public were placed on the website (http://www.fmdimprocon.org) and presented not only at scientific meetings of the project group, but also at the annual meeting of the EU reference laboratories for vesicular diseases and at the open sessions of the FAO-EUFMD RG that brings together, every two years, 32 European countries and reference laboratory representatives from all other continents. Workshops have been organised in collaboration with the Directorate-General for Research and Innovation and the Directorate-General for Health and Consumers for the EU reference laboratories, in collaboration with other FP6 projects (EPIZONE and CA FMD/CSF), FAO-EUFMD, OIE and TAIEX for candidate Member States. Results will further be discussed with the Directorate-General for Research and Innovation and the Directorate-General for Health and Consumers. If results are deemed appropriate to change the control policy, they could be presented to the EC Scientific Committee. Through close collaboration between some of the partners and the EU, FAO-EUFMD and the OIE, possible changes can be communicated to these organisations.

WP1 has contributed to the availability of validated assays to be applied in the substantiation of freedom from FMD when vaccination is performed and clear advice for the control policymakers on the application of NSP tests has been expressed.

Some of the prototype assays developed within WP2 have already been taken up by kit manufacturers and others might be in the future.

A new goat cell line has been identified within WP3, to enhance FMD virus detection that also enables a quicker diagnosis.

The animal experiments performed in WP4 and WP5 provided indispensable serum sample collections from vaccinated and/or infected animals that enabled validation and development of existing and new DIVA (differentiation of infected from vaccinated animals) tests. These sera can readily be shared with other institutes and organisations, especially since reference serum panels have been developed for the purpose of assessing FMD DIVA tests.

The data provided on vaccine strain selection in WP5 will help decision-makers in

their difficult choice of vaccine use and in identifying relevant strains for inclusion in FMDV antigen banks. Furthermore, the limits of the existing *in vivo* FMD vaccine potency tests have been demonstrated (high level of between test variability) which point towards more international acceptance of alternative *in vitro* approaches avoiding live viral challenge (e.g. serology, interferon-gamma responses and antigen payload).

Progress has been made in WP6 to enhance mucosal immunity. Moreover, a new generation vaccine based on a serotype A chimera vaccine in which the GH-loop region was replaced with that of another serotype proved fully protective in cattle against challenge from the unsubstituted parental serotype A virus.

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Keywords

foot-and-mouth disease, non-structural proteins, vaccine, cross-protection, validation, mass culling

Project website

http://www.fmdimprocon.org

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[CA FMD/CSF]

Foot-and-mouth disease (FMD) and classical swine fever (CSF) coordination action

Acronym:
CA FMD/CSF

Project number:
513755

EC contribution:
EUR 1 400 000

Duration:
42 months

Start date:
1 January 2005

Instrument:
Coordination action

Summary

This coordination action gathered and shared information relevant to the control of two of the most important OIE Notifiable diseases — foot-and-mouth disease (FMD) and classical swine fever (CSF), both of which pose serious threats to our livestock industries. The project has strengthened the networks of national reference laboratories (RL) for these diseases by providing coordination, web-based network resources, disease management manuals and mechanisms. The involvement of the EC, OIE and EUFMD/FAO has enhanced and expanded European networks with inputs from experts in other countries. The project focused on the coordination of research, global disease surveillance, risk analysis, vaccine reserves, diagnostics, laboratory preparedness, control policies including vaccination and wild boar issues. It has initiated and strenthened the EU and International scientific networks of FMD reference laboratories, vaccine banks, the Community Reference Laboratories for both FMD and CSF, harmonised disease control tools, fostered trust and facilitated collaboration through information exchange and provided information to the wider stakeholder community.

Problem

FMD and CSF are the subject of continuous eradication efforts in Europe and other parts of the world where fully susceptible, densely populated and highly mobile livestock population exist. This difficulty has led to the reappraisal of the stamping out approach, a control method that is increasingly abhorrent to the public. In the case of FMD, a new EU directive places a greater emphasis on the use of vaccination rather than slaughter to control future outbreaks, but this poses veterinary and social problems.

Ideally, better vaccines and better diagnostic tests are needed, more information is required on many aspects of vaccine performance, better global surveillance and risk analysis would help us to choose which vaccines to stockpile, and new modelling tools could help with the difficult decisions as to which variant of a particular control policy to use. Ultimately, the best way of protecting Europe against FMD and CSF is to control them elsewhere at their endemic sources. In Turkey, where FMD is still endemic, vaccination is being used to help bring about the eradication of the disease. There is pressure to stop CSF vaccination campaigns in several new Member States as they are about to join the EU, but the disease is still endemic in the wild boar populations of some of these countries. Without a more global approach, it is most unlikely that these diseases can be controlled, let alone eradicated. A global approach would benefit both Europe and other countries and would build a framework for the control of other significant livestock diseases.

The critical mass of scientific effort dedicated to improving the control of FMD and CSF is small and highly fragmented

and, consequently, scientific expertise is highly dispersed. Each nation tends to support its own limited laboratory facilities without sufficient international interaction. Further afield, the same uncoordinated, and often shallow, effort may be duplicated in other parts of the world, whilst cutting-edge research activities may be almost completely lacking or ignored by the official bodies. Where expertise already exists on these diseases, it is not readily accessible. It is obvious that this is not an efficient model for internationally effective use of resources with regard to research to support EU policy. Improved coordination of FMD and CSF research and other activities is therefore urgently needed.

Aim

To strengthen and coordinate existing initiatives for collaborative actions involving:

- the Community Reference Laboratory/ National Reference Laboratories network;
- the Research Group of FAO's European Commission for the Control of FMD;
- the network of reference laboratories of the Office International des Epizooties;
- other international stakeholders involved in FMD and CSF research.

To initiate new collaborative action on:

- research activities and needs;
- global surveillance and risk management/research;
- diagnostic harmonisation and laboratory preparedness;
- vaccine/antigen banks;
- the problem of CSF in wild boar;
- refinement of disease management and control options.

Results

The following are the key achievements of the project.

- The formation and strengthening of the global network of OIE and FAO FMD reference laboratories that provides better coordination, cooperation and collaboration across a variety of surveillance and diagnostic activities between the European Commission and national reference laboratories, and international animal health |organisations.
- The formation and continuation of the International Foot-and-Mouth Disease Vaccine Strategic Reserves Network that provides a platform for a coordinated approach to antigen/vaccine bank activities around the world facilitating the harmonisation of standards for vaccine bank antigens, ensuring better control of FMD in the event of an outbreak, and reducing some of the costs attributing to the maintenance of such reserves with a concept of sharing resources.
- The project has helped to overcome long-lasting problems in coordination of CSF research as well as in harmonisation of diagnostic methods and disease control tools. It supported strategic planning in the development of new vaccines and cooperation with another EU programme, EPIZONE, the more effective use of available resources.
- The project activated and boosted the collaboration between CSF reference laboratories and promoted research cooperation. It helped to develop improved methods and techniques for diagnostic. CSF diagnostics and vaccine performance were assessed and published in a scientific article. In addition a workshop on 'Classical swine fever — Assessment of control tools and research gap' was held in Hannover in cooperation with the American Agricultural Research Service, United States. As a result, the report 'Future needs and goals of CSF research' was provided to funding bodies.
- An agreement was reached between the CSF reference laboratories on contemporary CSF diagnostic techniques and standards. The results were reviewed in the 'Diagnostic Manual establishing diagnostic procedures, sampling

methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever'. To ensure proper and effective diagnostic in cases of emergency like an outbreak of CSF in fully susceptible and densely populated livestock, guidelines for laboratory contingency planning and real-time exercises were established. They will help laboratories to develop their own contingency plans and facilitate real-time exercises.

- The partners developed guidelines for the harmonisation and improvement of international comparison testing. Thereupon an advisory board for comparison tests was established.
- The collaboration of researchers from different countries reviewed control tools, control strategies and risk analyses for CSF in pigs and wild boar and distributed their results in several scientific publications. During the workshop 'Refinement of disease management and control options', experiences were exchanged between scientists, policymakers, representatives of the OIE and FAO, retail organisations, and agrarian associations on the pros and cons of the use of currently available (marker) vaccines in the eradication of CSF, more specifically the options for trade of vaccinated animals and their products. The conclusions and recommendations of this meeting were summarised and forwarded to the EU commission.
- The provision of a publicly open and interactive web-based disease awareness, communication and education facility for FMD scientists and stakeholders (http://www.foot-and-mouth.org/) and a restricted but interactive CSF database for scientists (http://viro08.tiho-hanover.de/eg/csf).
- Open publications of guides and templates for the preparation of Laboratory Contingency Planning documents for FMD and CSF (available at 'Laboratory Preparedness', http://www.foot-and-mouth.org/fmd-csf-ca).

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Project website

http://www.foot-and-mouth.org/fmd-csf-ca

Keywords

foot-and-mouth disease, classical swine fever, FMD, CSF, network, coordination, collaboration, cooperation

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[FMD-Disconvac]

Development, enhancement and complementation of animal-sparing, foot-and-mouth disease vaccine-based control strategies for free and endemic regions

Acronym:
FMD-Disconvac

Project number:
226556

EC contribution:
EUR 2 998 863

Duration:
39 months

Start date:
1 April 2009

Instrument:
Collaborative project

Summary

Foot-and-mouth disease (FMD) is one of the most infectious diseases of livestock and continues to pose a significant threat to endemic and free regions alike. The impact of FMD on society and international trade is high, thereby demanding stringent prevention, surveillance and control plans taken up in crisis preparedness plans. On the other hand, there is a global increased demand for animal welfare and ethical considerations necessitating a decreased reliance on eradication of animals to control FMD virus (FMV) spread, and on the use of animals for the regulatory testing of veterinary products. The project seeks to balance these apparently contrasting viewpoints by addressing specific gaps in our knowledge on all aspects of FMD control to enable implementation of enhanced animal-sparing vaccine-based control strategies tailored to the needs of free and endemic settings. Consequently, four main objectives have been identified, including: (i) the improvement of the quality of existing FMD vaccines and diagnostics; (ii) the refinement and replacement of *in vivo* FMD vaccine quality tests; (iii) the development of new generation FMD vaccines and diagnostics by applying cutting-edge technologies; and (iv) the enhancement of our knowledge on FMD spread and transmission following the use of high-potency monovalent or multivalent vaccines. The role of wildlife (buffalo, gazelles and wild boar) in FMDV maintenance and transmission will also be investigated. The project consists of seven different, yet interlinked, work packages (WP) each addressing one of the items listed in the work programme topic KBBE-2008-1-3-02, and led by renowned WP leaders with years of relevant experience in the field of FMD. As such, significant progress towards the objectives of the EU's Animal Health Strategy (2007–13), the European Technology Platform for Global Animal Health, and the Global Roadmap for improving the tools to control FMD in endemic settings will be achieved.

Problem

Although the EU's Animal Health Strategy (2007–13) recognises that significant scientific and logistic advances in FMD prevention, diagnosis, surveillance and

control have been made in recent years (e.g. through the sixth framework programme projects FMD_ImproCon (SSPE-CT-2003-503603), EPIZONE (FOOD-CT-2006-016236) and the coordination action FMD & CSF (SSPE-CT-513755)), some drawbacks regarding the implementation of animal-sparing, FMD vaccine-based control strategies for free and endemic regions persist and need to be tackled to improve food safety, as well as economic and animal welfare aspects.

Aim

The proposed consortium seeks to build on expertise gained in the FMD_ImproCon project (SSPE-CT-2003-503603) to address the remaining gaps, as identified by ETP-GAH, in current knowledge and expertise regarding the implementation of animal-sparing, FMD vaccine-based control strategies for free and endemic regions, thereby combating the disease at source.

By building on existing knowledge and through project-generated data, the project aims to:

(i) improve the quality of existing FMD vaccines and diagnostics;
(ii) refine and replace *in vivo* vaccine quality tests;
(iii) develop new generation FMD vaccines and diagnostics by cutting-edge technology;
(iv) and to increase/enhance our knowledge on FMDV spread and transmission following the use of high-potency monovalent or multivalent vaccines in free and endemic settings.

The role of wildlife (buffalo, gazelle and wild boar) in FMDV maintenance and transmission will also be investigated.

Expected results

Apart from the management tasks described in WP1, six priority work packages have been identified towards which scientific and technological efforts will be directed:

WP2: Reduction and refinement of in vivo *vaccine quality tests by* in vitro *methods*

This WP aims to replace the current *in vivo* 'Gold Standard' tests for vaccine efficacy (potency), purity and safety, in light of the 3R principle, by validated *in vitro* laboratory tests. More specifically by: (i) the determination and validation of correlation models between *in vitro* laboratory tests and *in vivo* protection based on experimental and field data; (ii) the development of *in vitro* immunoassays to monitor vaccine purity by the reduction of FMDV non-structural proteins content during vaccine purification and in the final vaccine; and (iii) the development of alternative methods to quantify the antigen payload content in the final vaccine.

WP3: Assessment and improvement of heterologous protection by FMD vaccines

This WP aims to predict how well a vaccine will protect against a challenge virus of another strain within the same serotype. Comparisons will be made between results of cross-challenge and homologous challenge tests. Correlations will be made between observed cross-protection, predicted serology and amino acid homology of the respective virus capsids. The work will focus on serotypes O and A.

Moreover, r-value determination between vaccine strains and FMDV field isolates will be improved by harmonising test methodologies and drafting guidelines for the reliable selection of reagents to include in *in vitro* vaccine matching studies, thereby avoiding future *in vivo* cross-protection studies.

The depth of our knowledge and expertise regarding vaccine spectrum coverage will be increased as well.

WP4: Development of vaccines and alternatives (antivirals) with rapid onset of immunity and based on safer production methods

This WP aims to increase our knowledge by investigating approaches for reinforcing the mucosal immune response in order to prevent FMDV infection at the primary portal for virus entry. Methods will be evaluated to elicit and measure mucosal immunity against FMDV in cattle. Ways to stimulate innate (rapid) and adaptive (lasting) mucosal immune responses will be investigated, using novel delivery systems, adjuvants and viral vectors. Other new generation vaccines will also be developed and efficacy tested, avoiding the need for virus culture, thereby making the production of FMD vaccines environmentally safer.

Moreover, the use of potent and selective anti-FMDV antiviral compounds, that rapidly and completely prevent FMDV replication, will be investigated in order to decrease the post vaccination immunity gap.

WP5: Improvement in FMD diagnostics

This WP aims to: (i) increase the availability of FMD diagnostics; (ii) improve standardisation and harmonisation of FMD diagnostic results; and (iii) develop new and, possibly better, diagnostic tools for confirmatory tests and/or test systems for NSP-serology. Therefore, a panel of stabilised, validated and reliable diagnostic kits for FMD serology and antigen typing, ready for commercial exploitation, will be developed and/or validated (i.e. confirmatory NSP test, IgA in saliva ELISA, assays in which serum reaction profiles are obtained simultaneously against a number of antigens — multiplexing).

WP6: Improving knowledge on FMDV transmission between species and in recently vaccinated animals

This WP aims to obtain previously unavailable quantified knowledge on FMDV transmission within and between different FMDV susceptible species in the period shortly after applying emergency vaccination, and to study transmission dynamics in real-time outbreak situations to set up early warning systems for FMDV penetration. The effect of vaccination in preventing FMD transmission through contact exposure to the virus will be studied by carefully designed FMD transmission experiments. A newly developed infection model will be used to study the ability of the Asian buffalo to transmit FMDV infection and to investigate the efficacy of vaccination to prevent this. The role of wildlife in FMDV maintenance and transmission, and quantified knowledge on the presence of FMDV in viral secretions and excretions in different species will be studied as well.

WP7: Development or adaptation of computerised FMD spread models to optimise vaccination schemes

This WP aims to study the applicability and feasibility of modifying existing simulation models (InterSpread Plus model, Davis model, NAADS model and other models within the consortium) for FMD spread to suit the exploration of vaccination strategies in the EU and other Western European countries where FMD is considered an exotic threat.

Computer models developed to enable vaccination strategies to be designed for high-risk regions within countries belonging to this consortium, would be relevant stepping stones to model vaccination strategies for truly endemic regions of the world.

Potential applications

The reduction and refinement of *in vivo* vaccine quality tests by *in vitro* methods will guarantee the overall quality of the vaccine batch in a verifiable form to end-users and other stakeholders, strengthening the position of the EU on the global market when it comes to the implementation of the 'vaccinate-to-live' policy. Consequently, reliance on animals for regulatory testing of vaccine

batch release control will be decreased and animal welfare increased (WP2).

The assessment and improvement of heterologous protection by FMD vaccines will help decision-makers in their difficult choice of which vaccine to use in future outbreaks and in their responsibility to update and reinforce FMD vaccine/antigen banks (WP3).

The development of new generation vaccines and antiviral compounds, based on safe production methods and specifically aimed to reduce the immunity gap shortly after vaccination, will (i) supplement the existing control tools to combat FMD and (ii) allow the enhancement of emergency contingency plans enabling a better, quicker and animal-sparing response to FMD outbreaks (WP4).

The improvement in FMD diagnostics will help the OIE/EU to better interpret the dossiers submitted to demonstrate/substantiate FMD-freedom and, by facilitating and accelerating the development and distribution of the most effective diagnostics for FMD in Europe and the rest of the world, could contribute to the vision of the ETPGAH. Knowledge on performance characteristics of available DIVA (differentiation of infected from vaccinated animals) diagnostics on a global scale will help to understand the FMD situation in other regions of the world, resulting in an increased awareness of the potential threats to the European Union (WP5).

Knowledge on FMDV transmission between species and in recently vaccinated animals can be used to adapt and improve computerised FMD spread models to optimise FMD vaccination programmes in free and endemic settings alike (WP6).

Computerised FMD spread models developed within this project could enable the design of vaccination strategies for high-risk regions within countries belonging to this consortium and could be relevant stepping stones to model vaccination strategies for truly endemic regions of the world (WP7).

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Project website

http://www.fmddisconvac.net

Keywords

foot-and-mouth disease, disease control, vaccines, diagnostics, transmission, vaccination strategies

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2.2. Classic swine fever

[CSFVACCINE & WILD BOAR]

Epidemiology and control of classical swine fever (CSF) in wild boar and potential use of a newly developed live marker vaccine

Summary

Classical swine fever virus (CSFV) is a recurring infection of domestic pigs and wild boar. Notwithstanding the relative success in eradicating the disease in certain parts of Europe and despite intensive efforts on a national as well as on an international level, the complete eradication of CSF in Europe has proved to be elusive. Additionally, the disease is still endemic in some new EU Member States (Bulgaria and Romania) and sporadic outbreaks still occur in free areas. Although the specific role of wild boars as CSFV reservoirs needs to be further clarified, the CSFV infection can persist in wild boar populations and is therefore probably responsible for CSF re-emergence. The latter is very important as in the last decade, the wild boar population has increased all over Europe and wild boar density is recognised as one of the most relevant risk factor enabling endemic infections. Direct and indirect contacts between domestic and wild species and illegal swill feeding practices have been demonstrated as the main source for the spreading of the infection in domestic pigs. The relevant risk of introduction of CSFV from wild boar is clearly demonstrated by the facts that about 80 % of primary CSF outbreaks in domestic pigs have occurred in regions where CSF in wild boar is endemic. Therefore, any programme aimed at a permanent eradication or control at long term of CSF should include methodologies which allow not only an efficient approach in domestic animals but also in wild boar. However, the latter was hampered by the lack of knowledge regarding wild boar population dynamics and structure as well as

Acronym:
CSFVACCINE & WILD BOAR

Project number:
501599

EC contribution:
EUR 1 656 760

Duration:
4 years and 9 months

Start date:
1 January 2004

Instrument:
specific targeted research or innovation project (STREP)

epidemiological parameters influencing the course of the infection within it. By using existing literature, data from previous outbreaks (such as Varese (Italy), Luxembourg and Mecklenburg-Western Pomerania (Germany)) or the development of new applications, different parameters were obtained and subsequently used to design a new mathematical model based on a metapopulation principle. The mathematical development of this model was validated using field data for outbreaks in Italy and Switzerland. The major benefit of this model is that it allows a better understanding of CSFV epidemiology in wild boar populations and the evaluation of the impact of control strategies (such as hunting and vaccination) on the course of the infection. Hunting showed to be an ineffective way to control the infection as only unrealistically intensive hunting efforts (> 70 %/year) could eradicate the infection. Although in small populations (< 1000 animals) a non-intervention policy revealed to be successful, vaccination was demonstrated to be an effective tool in controlling the CSFV infection as it always reduces the epidemic peak. The chance of a successful eradication of the infection is determined by the percentage of the susceptible population that is vaccinated within a short range of time (approximately 15 days). While a 60 % vaccination rate of the susceptible animals will lead to prompt eradication, 20 % will increase the probability of endemic stability of the infection. The latter clearly shows that when the decision is made to vaccinate, it should be done in such a manner that a very large portion of the population is reached.

On the domestic pig level, the current control and eradication strategies for CSF management are based on stamping out the infection combined with pre-emptive culling. Notwithstanding the efficacy of this approach, there is an increasing ethical concern and public resistance, as numerous healthy, uninfected animals are killed and destroyed. Furthermore, this stamping-out policy is not suited for management of the infection in wild boar populations. Vaccination can provide an alternative for this current strategy. However, the contemporary available live vaccines such as C-strain, although potent and oral applicable, do not allow serological differentiation from infected animals. In contrast, such a differentiation is possible with the commercial E2-marker subunit vaccine. However, this commercial E2-marker subunit vaccine needs to be administrated parenterally as oral application is not possible and can therefore not be used for CSFV management in wild boar. Due to the shortcomings of the current available E2-marker subunit vaccine there is a need for a new type of vaccine which is not only efficient but also has DIVA (differentiation of infected from vaccinated animals) capacity and can be administrated orally. This was addressed during this project by the development of a live marker vaccine, whereby the E2-region of BVDV (CP7) and CSFV (C-strain) were replaced by the corresponding sequence of CSFV (*Alfort 187*) and BDV (*Gifhorn*) respectively. A select number of chimerical candidates, displaying the desired *in vitro* characteristics (such as replication kinetics, cell specificity and DIVA potential), were subjected to extensive *in vivo* evaluation. Although the CSFV-based candidates (e.g. pRiems_ABCgif) were safe to use, as no adverse effects were noted in the inoculated animals irrespective of the method of application, the protection was not always complete, especially when given orally, or differentiation with wild type CSFV was not unequivocally possible using commercial ELISA systems. Conversely, CP7_E2alf was found to protect domestic pigs as well as wild boar completely against a lethal CSFV challenge when given intramuscularly, oronasally or by the newly developed baits. This complete protection was already achieved after 7 and 14 days following intramuscular or oronasal vaccination respectively. Initial potency studies have indicated that a dose of $10^{3.5}$–$10^{4.5}$ $TCID_{50}$ is sufficient for clinical protection against a lethal challenge when administrated intramuscularly, with 100 %

of the animals reaching a protective VNT of 1:20 at 21dpv. Using oronasal application, the potency of CP7_E2alf was similarly confirmed and was found to be comparable to that of a commercial C-strain vaccine. Furthermore, the immunity granted by CP7_E2alf was sterile, as no evidence was found of shedding, excretion or transmission to contact animals in any of the animals experiments performed, and could be achieved at a dose of approximately $2 \times 10^{5.5}$ $TCID_{50}$. Furthermore, a long-lasting antibody response was observed at least up to 98 days dpv, even when a suboptimal dose was used of $2 \times 10^{4.5}$ $TCID_{50}$. The latter is important as such a dose may occur when there is incomplete bait uptake or where vaccine titres are reduced in the field or during the production process. CP7_E2alf is not only highly efficient, it is also safe to use. The latter was demonstrated as in none of the performed animal trials were any significant adverse effects observed in domestic pigs and wild boar. Similarly, no effects on farrowing or the birth performance of the piglets were observed on vaccination of pregnant sows and no evidence of transplacental transmission was found. One of the goals of this project was to develop a vaccine that can be used for CSF management in wild boar. A number of specific safety issues are linked to the field application of a vaccine, such as the transmission of the vaccine to other species. This is even more important due to the BVDV-background of CP7_E2alf. As previously stated, the immunity achieved by CP7_E2alf is sterile (no shedding, no excretion and no contact transmission), meaning that transmission to other species by animal to animal contacts is very unlikely. Indirect transmission, by uptake of the baits (filled with vaccine) is equally unlikely because no clinical, serological or virological evidence could be found for the presence and shedding of the CP7_E2alf in young ruminants and rabbits on oral application, and even after intramuscular application to cattle and sheep. The latter confirmed the changed cell tropism of CP7_E2alf from bovine to porcine previously observed *in vitro*. The field applications during CSFV vaccination campaigns in wild boar have encountered another problem, namely the limited uptake of baits by young animals. The latter is important because independently of the efficacy of the vaccine, this can lead to a part of the wild boar population, already more vulnerable to infection, remaining unprotected. This could contribute to the persistence of the infection. For this purpose new small spherical and cuboid baits were designed and constructed. The new 3 cm spherical bait clearly showed an improved uptake rate in young animals up to three-and-a half months. However, even this new small bait was not taken up by animals younger than three months, probably due to the fact that they prefer suckling. This has important implications in any vaccination strategy as it has to be kept in mind that these young animals cannot be immunised in this way. Repeated baiting or baiting at a time point sufficiently long after farrowing (when all the gruntlings are older than three months) is therefore desirable. In summary, based on *in vitro* and *in vivo* results, it can be clearly stated that the CP7_E2alf is the most suited vaccine candidate coming forth from the used dual approach and the resulting numerous chimerical constructs. The great benefit, however, of the used strategy is that not only a very good candidate has been obtained but that a backup is available, which is advantageous if at a later stage problems should occur with the CP7_E2alf, and that additional constructs are at hand which can be used to study the different aspects of live marker vaccines, such safety features, growth and immunogenic enhancers and so on.

Following the development and testing of the vaccine candidates, production on an industrial scale of the final vaccine candidate CP7_E2alf and the backup candidate pRiems_ABC_gif was established and resulted in a 'master' and 'working cell stock' of the sk-6 cell line and a 'master'

and 'working seed virus'. All the stocks were prepared in full compliance with European Pharmacopoeia guidelines regarding sterility and freedom of extraneous contaminants. In order to be able to use as much as possible of the data generated during the animal trials for future licensing/registration, 125 vials (volume: 20 ml per vial) at $10^{6.4}$ $TCID_{50}$/ml of the pilot vaccine CP7_E2alf were produced following good manufacturing practice (GMP) and the requirements of European Pharmacopoeia requirements regarding sterility.

Simultaneously with the development of a new vaccine, the necessary serological and virological diagnostic tools were developed and subsequently evaluated in an interlaboratory evaluation (ILE). At the start of the project, it was decided to evaluate the DIVA potential of existing commercial ELISA systems. Similar to earlier initial results, differentiation between vaccinated and infected animals was possible for CP7_E2alf and, to a lesser degree, for the CSFV-based candidate, using existing commercial systems (E^{RNS}-antibody ELSIA). However, diagnostic sensitivity and specificity should be improved and batch-to-batch conformity should be addressed as variable results were obtained with different batches. Notwithstanding the fact that the focus was placed on the use of existing ELISA systems, the potential of creating adapted versions or the development of new ELISAs was analysed by searching for Mabs with DIVA potential. The latter not only resulted in an increased insight into the epitopic structure of E2 and E^{RNS} proteins of both CSFV and BVDV, which will be beneficial for better understanding the immunogenic characteristics of vaccines, but also in the identification of multiple Mabs that can be used for the development of DIVA ELISAs for either CP7_E2alf or pRiems_ABC_gif

During the last decade, PCR-based techniques have become more and more important as a diagnostic tool, especially with the development of real-time RT-PCR. These techniques combine high throughput capacity with a superior sensitivity and reduced labour requirements, compared to virus isolation (VI). Such advantages are important during outbreaks because they allow an earlier detection of the infection (in this project, real-time RT-PCR detected CSFV one to two days earlier than VI), and permit a larger number of samples to be analysed within a shorter time. The latter is further accentuated by the ability to pool at least five samples for real-time RT-PCR evaluation without loss of sensitivity as demonstrated in this project. However, early detection is influenced by the choice of tissue used for analysis. The detection of the virus in tonsil preceded that of other tissues, including blood, and is therefore the most suited tissue for early as well as long-term detection. On the other hand, blood can be used as an alternative at herd level if a larger number of samples are analysed to compensate for the lower probability of detecting the virus. The availability of accompanying genetic diagnostic tools is an important criterion for a more generalised use of a CSFV vaccine. Therefore, classic gel-based and real-time RT-PCRs were developed that are able to differentiate between CSFV and the new vaccine candidate CP7_E2alf (genetic DIVA). As expected, the real-time RT-PCR has a slightly higher sensitivity compared to the classical systems with a detection limit of 50 (CSFV) and 500 copies (CP7_E2alf) of cRNA per reaction for the classical system and 20 (CSFV) and 50 (CP7_E2alf) copies of synthetic cRNA for the real-time RT-PCR. Although single-tube formats have been developed for both, the two-tube format is preferable as its sensitivity is 10 to 100 times higher. The applicability and DIVA capacity of the newly developed real-time RT-PCR was confirmed in the interlaboratory evaluation where it was found to be functioning with very good robustness and reproducibility, although some optimisation and additional testing is required for the CP7_E2alf detection. Despite the diagnostic potential of PCR-based methods, some issues have arisen regarding samples which

were scored positive by PCR but negative by VI. Although research in the field of CSFV was one of the first to report this problem, it is not restricted to it as it is now also described for other viruses. In addition to differences in detection sensitivity and antibody complexation, it was demonstrated, by using a newly developed RT-PCR panel, that the presence of viral genome fragments in the sample can equally be the cause of discrepant PCR and VI results.

Not only DIVA diagnostic tools to be used in combination with the new vaccine candidate have been developed during this project but also on-site or field detection tools. The latter further enhances the early detection of CSFV. A new lateral flow detection prototype, incorporating a specific CSFV Mab, has been developed following numerous screenings. Although further specificity and sensitivity testing is required, the clear and specific signal obtained clearly underlines its promising potential.

In summary, the work performed during this CSFVACCINE & WILD BOAR resulted not only in an enhanced insight into the epidemiological situation of CSF in wild boar but also to a validated model allowing the evaluation of the impact of different control strategies on the infection course. In addition, with the development and the production under GMP conditions of a novel potent and safe live marker vaccine and its accompanying genetic and serological DIVA tools, an interesting alternative can be presented to the current control and eradication strategies.

Aim

The main objectives of the project were the:

- development of an epidemiological and economic model for CSFV eradication in wild boar;
- adaptation of the C-strain vaccine baits for use in wild boar with special attention to young animals;
- development of a marker vaccine and accompanying diagnostic assays and protocols.

Results

Development of an epidemiological model for CSF eradication in wild boar

A number of epidemiological parameters were either determined by using existing literature, data collection or by developing new applications for estimating them (such as transmission coefficient β and the minimum wild boar number). For the latter, a user-friendly Microsoft Office Excel sheet was developed based on the hunting bag. Subsequently, a new mathematical model based on a meta-population principle was designed and validated using data from previous outbreaks. This new model showed that hunting is an ineffective way to control the infection as only unrealistically intensive hunting efforts could eradicate the infection. Although in small populations (< 1 000 to 1 500 animals) a non-intervention policy revealed to be successful, vaccination was demonstrated to be an effective tool in controlling the CSFV infection as it always reduces the epidemic peak. The chance of successful eradication of the infection is determined by the percentage of the susceptible population that is vaccinated within a short range of time. While a 60 % vaccination rate of the susceptible animals will lead to prompt eradication, 20 % will increase the probability of endemic stability of the infection.

Adaptation of the C-strain vaccine baits for use in wild boar with special attention to young animals

New small spherical and cuboid baits were designed and constructed. The new 3 cm spherical bait clearly showed an improved uptake rate in young animals up to three-and-a-half months. However, even this new small bait was not taken up by animals younger than three months, probably due to the fact that they prefer suckling. This has important implications in any

vaccination strategy as it has to be kept in mind that these young animals cannot be immunised in this way. Furthermore, it was demonstrated that lyophilization increased vaccine stability under field conditions and is therefore a promising method to increase bioavailability during vaccination campaigns.

Development of a marker vaccine and accompanying diagnostic assays and protocols

A new live marker vaccine was developed whereby the E2-region of BVDV (CP7) was replaced by the corresponding sequence of CSFV (*Alfort 187*). Based on *in vitro* and *in vivo* results, it can be clearly stated that CP7_E2alf is the most suited vaccine candidate as it not only provides complete sterile immunity, independently form the application method, it is also very safe to use. In none of the animal experiments were any adverse effects noted on health or farrowing with normal birth performance of the piglets. Notwithstanding the BVDV background of CP7_E2alf, no serological or virological evidence could be found for the presence of the CP7_E2alf in young ruminants and rabbits on oral application, and even intramuscular application to cattle and sheep did not result in detectable vaccine virus replication or shedding. In addition to the safety of this candidate, animals immunised with CP7_E2alf can be differentiated from wild-type infected animals using either commercial ELISA system or by a real-time RT-PCR developed during this project. The robustness of the developed real-time RT PCR was confirmed during an interlaboratory evaluation.

Potential applications

The development of an epidemiological and economic model for CSF eradication in wild boar in combination with the development of a live marker vaccine and accompanying diagnostic assays, to adapt this vaccine for use in wild boar with special attention to young animals will enhance European excellence in strategies to prevent or limit the introduction of an exotic agent (OIE List A) and to avoid transmission of pathogens between species (wildlife to farmed animals). It will reinforce considerably the competitiveness of the European pig production. By developing a vaccine with accompanied diagnostic assays the societal problem of mass killing of healthy animals can also be reduced.

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Project website

http://www.csfvaccine.org

Keywords

classical swine fever, wild boar, live marker vaccine, DIVA, epidemiology, diagnostics

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[CSF-GoDIVA]

Improve tools and strategies for the prevention and control of classical swine fever

Acronym:
CSF-GoDIVA

Project number:
227003

EC contribution:
EUR 2 999 983

Duration:
48 months

Start date:
1 March 2009

Instrument:
Collaborative Project

Summary

Although classical swine fever (CSF) has been eradicated in wide areas within the EU, the disease is endemic in some new Member States particularly in backyard pigs. In order to improve eradication strategies, the project aims are: (a) the final development and testing of a live marker vaccine candidate for the prevention and improved control of CSF, both orally and intramuscularly applicable; (b) the development and optimisation of accompanying discriminatory diagnostic tests; (c) the production of an effective, oral delivery system for the marker vaccine for use in wild boar and backyard pigs; (d) the easy selection of diseased animals. The improved knowledge on immunological reactions and pathogenesis will support a more efficient vaccine application and provide data for the epidemiological models. Epidemiological studies of CSF in domestic and backyard pigs and in wild boar including molecular epidemiology intend to increase insight into CSF transmission and persistence. Epidemiological models will be developed to support risk assessment both for conventional eradication strategies as well as for new strategies using the new vaccine and diagnostic tools including the role of CSF reservoirs. The results concerning antiviral treatment will be evaluated and compared with the traditional eradication strategies.

Problem

CSF is notifiable in the EU and has been eradicated from the domestic pig population in most EU countries. Nevertheless, and despite intensive efforts at national and international level, sporadic outbreaks still occur in areas which are free of CSF. Additionally, the disease is still endemic in some countries that recently joined the EU Bulgaria and Romania; CSFV is widespread among wild boar populations on the European continent. Furthermore, CSFV is present in the backyard pig population in Bulgaria and especially Romania. The epidemiology of CSF in this type of pig holding which is novel within the European Union, has not yet been fully investigated. Therefore, a strong need exists to improve knowledge and intervention strategies for backyard pigs.

Currently, a CSF non-vaccination policy, exists within the EU. However, due to problems with CSF in wild boar and in domestic pigs in some Member States, derogations from this non-vaccination policy have been granted and vaccination is applied in certain areas of Europe. For the oral vaccination of wild boar, the modified live C-strain vaccine is used. This vaccine is very efficacious under field conditions but its major disadvantage is that no differentiation can be made between vaccinated and infected animals. Baculovirus produced E2 subunit vaccines are available but data suggest that these vaccines are less efficacious than conventional modified live vaccines and multiple vaccinations are needed before protection is obtained. Moreover, subunit vaccines are not suitable to be used in baits for oral vaccination

of wild boar. The control of CSF outbreaks in domestic pigs as well as in wild boar and backyard herds would be significantly enhanced if a safe and efficacious marker vaccine with an accompanying DIVA (differentiation of infected from vaccinated animals) diagnostic assay would be available.

Aim

The main goal of this project plan is complex and multidisciplinary: (a) the final development and testing of a live marker vaccine (LMAV) candidate for the prevention and improved control of classical swine fever (CSF), both orally and intramuscularly applicable; (b) the development and optimisation of accompanying discriminatory diagnostic tests; (c) the production of an effective, oral application system for the marker vaccine for use in wild boar and backyard pigs; (d) the easy selection of diseased animals; (e) epidemiological studies of CSF in domestic and backyard pigs and in wild boar, including molecular epidemiology and the study of alternative methods of suppression of viral replication.

Results

The expected results of this project are:

(i) availability of new standardised diagnostic methods applicable to domestic and backyard pigs and wild boar;
(ii) validated new methods for the easy selection of suspicious animals;
(iii) a better risk analysis including molecular epidemiology;
(iv) a marketable third generation live marker vaccine for intra muscular and/or oral application in domestic and backyard pigs and wild boar;
(v) new methods for the easy oral application of the vaccine;
(vi) better insight into the role and pathogenesis of CSF virus reservoirs;
(vii) new epidemiological models for CSF in domestic and backyard pigs and in wild boar;
(viii) new models for the evaluation of the wild boar population and baiting;
(ix) proof of principle for the use and registration of antiviral treatment.

The following is a summary of the results achieved so far.

(i) The final live marker vaccine candidate for intramuscular and oral application was selected among two chimeric pestivirus vaccine candidates.
(ii) A commercially available CSFV antibody ELISA was identified for use in conjunction with the new marker vaccine. New antigens and monoclonal antibodies for the development of alternative DIVA ELISAs were also characterised.
(iii) New small spherical baits were produced and their uptake evaluated in a field study.
(iv) An existing CSFV spread model for wild boar was further developed to assess the impact of oral mass vaccination of wild boar and to assess the effect of landscape structure and population dynamics including hunting on the size of outbreaks and on virus persistence.
(v) A backyard pig database was developed for Bulgaria.
(vi) For domestic pigs, a management-orientated epidemiological model was developed as a tool to evaluate

the relative performance of different emergency control strategies in domestic pigs.

(vii) The current surveillance strategies for CSF in wild boar and in domestic pigs were reviewed.

(viii) The potential of an antiviral molecule to reduce the transmission of CSFV from treated to untreated pigs was demonstrated.

Potential applications

The combination of the final development and validation of a third generation LMAV with the discriminatory tests, the development of simple pen-side tests and the epidemiological evaluation of CSF in both non-vaccinated and vaccinated domestic pigs, backyard pigs and wild boars will result in a better understanding and better tools in order to fight this highly devastating disease. Improved immunisation and baiting procedures as well as new LMAV and new procedures (DIVA, easy selection, sampling) should allow the efficient immunisation of pigs, and should enable an improved diagnosis including a discrimination of vaccinated from infected animals (marker vaccines principle). The new available knowledge concerning CSF epidemiology in particular in backyard pigs, risk assessment, molecular epidemiology, control, immune response as well as the new diagnostic methods will help the animal health authorities inside and outside the EU in their policymaking.

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Project website

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classical swine fever, live marker vaccine, antivirals, backyard pigs, wild boar, DIVA diagnostics, epidemiology, thermography, molecular diagnosis, RT-PCR

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2.3. African swine fever

[ASFRISK]

Evaluating and controlling the risk of African swine fever in the EU

Summary

African swine fever (ASF) in EU Member States is currently confined to Italy (Sardinia), it was recently introduced to Caucasian countries and Russia and it is highly prevalent in sub-Saharan African countries. Serological surveys during outbreaks occurring previously in eastern and southern African regions have shown that although the average prevalence of viral infection in the domestic pigs is high, in many cases serum conversion was not detected by OIE and commercial ELISA tests. These events suggest that complex epidemiological patterns for ASF are established in those regions in that African swine fever virus (ASFV) isolates of higher virulence coexist with others inducing moderate to chronic forms of the disease, as observed in the past in Europe.

These epidemiological scenarios, further complicated by the incremented mobility of people, animals and goods across the globe, emphasise the serious threat ASF presents to the growing pig farming sector in Africa and in disease-free EU Member States. Work developed allows the characterisation and a better understanding of the epidemiological situation of ASFV in Africa, Sardinia and the Caucasus region as well as the main risk factors that are contributing to the persistence and spread of the disease in those territories and provided new tools and strategies for the control of ASF and to reduce the risk of importation and/or spread of the disease in EU Member States. Current ASF serological and molecular diagnosis procedures were evaluated and new antibody and nucleic acid-based diagnostic tools were developed, including front-line and pen-side tests, which will be supplied to diagnostic facilities in Africa and to animal health laboratories in the EU and others for the early detection of potential ASFV incursions. Additionally, studies on ASFV-host interactions and the achievement of attenuated recombinant virus strains opened new insights for the characterisation of pig immune mechanisms relevant for survival following infection with ASFV and for vaccine development. The new strategies and the tools developed within this project have been transferred to ICPC partners and other countries through local training courses, traineeships and technology transfer actions.

Aim

The project has been organised into four main tasks aims: (i) to evaluate the current ASF epidemiology in Africa, to develop and validate a generic risk assessment for the introduction of ASF into EU countries and subsequent control strategies; (ii) to develop and validate new antibody and nucleic acid-based diagnostic tools for ASF, including front-line and pen-side tests, which will be supplied to diagnostic

Acronym: ASFRISK

Project number: 211691

EC contribution: EUR 2 984 712

Duration: 42 months

Start date: 1 April 2008

Instrument: Collaborative project

facilities in Africa and to animal health laboratories in the EU for the early detection of potential ASFV incursions, in particular by newly emerging strains; (iii) to characterise pig immune mechanisms relevant for survival and to identify mechanisms of virus-encoded 'evasion' genes towards the development of candidate vaccines and to assess the use of antiviral molecules as complementary methods for the control of the disease; and (iv) to transfer the new strategies and the tools developed to ICPC partners and to others through training, workshops and technology transfer activities.

Results and potential applications

The project has substantially contributed to increase the knowledge of the epidemiology, risk factors and best control strategies to better prevent and control ASFV. There was extensive work carried out to characterise and better understand the epidemiological situation of ASFV in Africa, Sardinia and the Caucasus region as well as the main risk factors that are contributing to the persistence and spread of the disease in those territories. One of the most significant outputs of this project was the development of a generic risk assessment framework that is available to evaluate the risk of introduction of ASFV into each EU country. This framework incorporates all concepts and methods developed during the project in an easy-to-use Excel file that will be distributed and may be potentially used for any EU country/policymaker to evaluate the specific risk of ASFV entrance in the country and the most important risk pathways that contribute to that risk. The idea is to provide policymakers with a useful decision support tool that may be easily accessible, implemented and updated. Moreover, participatory methods have been used to perform risk mapping in endemic countries (Africa and Caucasus region) that will support more cost-effective preventive and control strategies in the affected territories. Results obtained will support better preventive and control strategies for ASF. For example, the identification of the risk factors and routes of potential introduction and spread of ASF will allow the implementation of risk reduction measures in those countries that have the disease or are currently ASF-free.

The current epidemiological situation of ASF worldwide has highlighted the need to evaluate the existing serological and molecular diagnostic techniques towards the development of adequate diagnostic tools of the disease. As no vaccine is available, the presence of ASFV antibodies is indicative of previous infection and, as antibodies are produced from the first week of infection and persist for long periods, they are a good marker for the diagnosis of ASF. The work developed aimed at confirming the use of current ELISA diagnostic tests, the development of new ELISA, the development of pen-side tests for anti-ASF antibody detection. Findings obtained have confirmed the current serological tests are able to detect antibodies induced against ASF in all epidemiological situations confirming the robustness of the current antibody detection techniques. However, new ELISA based on ASFV recombinant proteins targeting, *inter alia*, the detection of ASFV-anti-IgM antibodies for the early detection of infection were developed, standardised and validated to be further developed as ELISA commercial prototypes. A rapid, one-step immunochromatographic strip (INGEZIM PPA CROM ®) serological pen-side test capable of specifically detecting anti-ASF antibodies in serum specimens has been developed, validated and is now commercially available. The immunochromatographic test provides a reliable method for detection of anti-ASF antibodies with 99 % of sensitivity and 100 % of specificity and confidence where laboratory support and skilled personnel are limited. This test will be very useful as a simple, robust and cheap tool for rapid in-farm ASF diagnosis in countries that are endemically infected. In particular, it will be essential for African countries

where the delay for the shipment of samples to expert laboratories can be long and the use of more sophisticated tests sometimes problematic. In parallel with this work, the question of the sample preservation was addressed during the project. Indeed, in tropical countries, a cold chain is sometimes difficult to maintain during the shipment of the samples. Therefore, the use of filter paper for blood collection, drying and storage at high temperature (> 30 °C) was evaluated. This support was proved to be suitable for virus and nucleic acids preservation. It can be also used with the newly developed molecular tests described below.

Further to the use of antibody detection tests, the molecular diagnosis of ASF relies so far on a limited number of PCR methods. The updating of current molecular diagnostic techniques has been a main issue. A real-time PCR method using a commercial Universal Probe Library (UPL-PCR) probe, a Linear-After-The-Exponential PCR (LATE-PCR) assay, and a Loop-mediated isothermal amplification (LAMP) system have been developed and evaluated for their application in the molecular diagnosis of ASF. All assays enable the detection of different ASFV p72 genotypes tested, showing as well significant levels of sensitivity and specificity. The developed molecular methods, and specifically the UPL-PCR and the LAMP techniques, have been adapted as commercial diagnostic kits and are under evaluation for their upcoming launching in the market.

So, a range of valuable molecular and serological tools has been produced within the ASFRISK project, being now offered to improve and complement the available ASF diagnostic tools, suitable for use in well-equipped international and national reference laboratories, in basic regional and local laboratories, or even for rapid on-site application. This will assist African countries and others to improve their diagnostic capacities and will contribute to improve knowledge on the epidemiology of the disease.

The project aimed also towards the implementation of a new strategy for the development of vaccines against ASF. Development of vaccines against ASF using classical approaches through viral inactivation or viral attenuation has shown to be unsuccessful since the disease was first reported. None of the treatments applied to produce inactivated immunogens afforded levels of protection acceptable for a vaccine. The attempts to obtain ASFV attenuated by either serial passage in cell cultures or alternate passages in swine and rabbits have produced immunogens able to protect swine against the fully virulent homologous virus. However, all the protective attenuated ASFV obtained showed a residual level of virulence unacceptably high to be used as a vaccine. Two main areas were considered in this project as a major strategy for the development of immunogens against ASF:

- the characterisation of viral host interactions through the study of the role of virus and host genes in infection (modulating host defences, immune evasion, virus productivity and virulence) towards the obtainment of attenuated virus strains;
- the *in vitro* and *in vivo* characterisation of pig immune mechanisms relevant for animal survival against ASFV.

This work revealed further insights into the ASFV protection model selected for this study: two ASFV isolates of different virulence (ASFV/L60 and ASFV/NHV/P68, referred to as L60 and NHV respectively), in which NHV has previously shown to induce pig survival against L60 infection, but with a residual virulence that must be eliminated for vaccination purposes.

Several cell models have been optimised for their use in growing and titrating many ASFV strains (both from the field or laboratory) and can be used to infect directly with the field isolates in an established cell line to amplify virus samples either for diagnosis or infection studies, and it was the

basis to generate and purify virus deletion mutants from the NHV virus model by homologous recombination. A number of new recombinants from different parental viruses deleted of specific genes were constructed and used in *in vitro* studies to analyse the role of the virus genes in the evasion of the antiviral response.

Another achievement was the initiation of the cloning of the ASFV genome into a Bacterial Artificial Chromosome (BAC), an important tool to facilitate the manipulation of virus genes and the generation of recombinant viruses with specific genes deleted by a non-conventional approach. The sequences of many ASFV genes including the open reading frames and flanking regulatory regions in the NHV and L60 isolates, were obtained during this project and used for the generation of the ASFV deletion mutants.

Regarding the control of the host antiviral response, we have confirmed the role of different ASFV genes involved in immune response (A238L, EP153R, K205R, I329L, etc.) and in the control of apoptosis and cell cycle (A224L, EP153R, g5R, etc.). Specifically, the following have been determined: (i) the immunomodulatory role of A238L gene, confirming the inhibition of NF-KB and NFAT transcription factors through a novel mechanism involving protein kinase-C-mediated p300 transactivation; (ii) the effect of EP153R gene in the modulation of the SLA-I expression, including 3D modelling of the interaction between the viral lectin and the MHC-I molecules, and the analysis of the critical regions involved in the inhibition; and (iii) the role of K205R, I329L and MGF-360-18R virus genes in the inhibition of IFN induction and signalling and in the NF-kB activation, including the analysis of different structural regions of the viral proteins in the modulation of these processes. The modulation of specific cytokines, chemokines and inflammatory molecules, induced during virus infection of porcine macrophages, has also been analysed.

A major tool expected to be delivered by the project is the development of an attenuated model for protective immunity. NHV recombinants deleted of specific genes (A238L, A224L and EP153R) were obtained in order to reduce the residual virulence of the parental NHV isolate and are currently used in an *in vivo* experiment to determine the possible protection induced against other virulent ASFV isolates. This eventually should lead to a strategy to identify the best attenuated virus model to construct an effective vaccine against ASF.

As an alternative approach to the vaccine development, a number of antiviral drugs were studied to be used to contain the virus spreading from the ASFV outbreaks. Several antivirals have been demonstrated to be effective in the inhibition of *in vitro* ASFV infection: (i) chemicals directed against viral DNA polymerase; (ii) siRNA against different virus components (A151R gene or vp72); and (iii) non-conventional antivirals like lauryl gallate, affecting cellular factors required for the productive infection. All of them were found to be selective and non cytotoxic at the concentrations used to inhibit ASFV infection, and several of them have been selected to be tested in an *in vivo* experiment to analyse the possible protection of pigs pre-treated with the antivirals after a challenge with virulent ASFV isolates.

ASFRISK was also dedicated to the organisation of training and technology transfer activities coordinated by EU partners in collaboration with the African and Asian partners of the consortium. Under 'Local training/workshops and technology transfer', five training courses on clinical and laboratory diagnosis and ASF epidemiology-modelling were organised in China, Ivory Coast, South Africa, Spain and Uganda. These courses were very successful both for training on relevant topics for the diagnosis, prevention and control measures of ASF in different scenarios and for the reinforcement or establishment of working links between the EU partners in the project and countries

in other parts of the world (different countries in Africa, Belarus, China, Russia and Vietnam) for which ASF is a great matter of concern. Technology transfer mainly based on the most recent validated laboratory diagnostic tests and on epidemiological tools developed within the ASFRISK project was included in the different training courses, traineeships and on a ring trial on the easy and rapid LAMP PCR recently performed.

Further to the above, an interactive CD on ASF diagnosis available in English, French, Russian and Spanish was produced by the Faculty of Veterinary Medicine of Madrid (OIE reference laboratory) with the collaboration of CISA-INIA (EU reference laboratory for ASF). This CD has been used for teaching purposes during training activities of the project and distributed to several institutions worldwide. 'Individual Long-Term Training' offered one MSC training in South Africa, three PhD trainings in Ivory Coast, Portugal and South Africa and another one long-term training (six months) for a Chinese fellow in Spain. In parallel, four short-term trainings were organised for two Chinese and two Vietnamese fellows in Sweden and Spain.

The work achieved under the different tasks of the ASFRISK project has contributed to the reinforcement of EU scientific and technological capacities and to the establishment of links with African, Asian and eastern European countries that will also benefit from the achievements of the project.

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Project website

http://www.asfrisk.eu

Keywords

ASF, ASFV, Africa, epidemiology, risk assessment, molecular diagnostics, antibody detection, vaccine development, immunity

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2.4. Orbivirus

[BTVAC]

Improved vaccines for bluetongue disease

Summary

Bluetongue is a viral disease of livestock, particularly sheep that is endemic in many tropical and sub-tropical countries and can result in high morbidity and mortality. Outbreaks have been frequent in Southern Europe since 1998, and in 2006 the virus emerged as far north as France, Belgium, Holland and Germany, reaching the UK in 2007. Existing vaccines, based on attenuated strains of the causative virus, are protective but cause viraemias and teratological effects when pregnant animals are vaccinated.

The primary aim of this project was, therefore, to generate completely safe, genetically inert vaccines for BTV, initially for serotypes that are identified in Europe with a future aim to generate a portfolio of vaccines that will protect against all BTV serotypes. All partners made significant scientific progress in meeting the aims and objectives of the project. The effect of the inactivated BTV vaccine on pregnant ewes and lambs has been determined with no *clinical side effect recorded*. Substantial progress has also been made in understanding the immune response to the single BTV VLP vaccine in sheep and demonstrating that it provides *protection against clinical symptoms and viraemia*. The BTV VLP vaccines with more than one serotype, have also been successful in a clinical trial with the animals protected from clinical symptoms and viraemia. This indicates that there is *no interference* in the immunological response to the VLPs. Both the BTV VLP and inactivated BTV vaccines are promising candidates. Preliminary results from field

Acronym:
BTVAC

Project number:
044211

EC contribution:
EUR 840 000

Duration:
45 months

Start date:
1 January 2007

Instrument:
Specific Targeted Research or Innovation Project (STREP)

sera highlight the potential use of a *NS1 competitive ELISA* developed within this framework and VP7 ELISA as a *DIVA test*. Real time RT-PCR detections of BTV have also been developed, all showing highly sensitive detection methods.

Problem

Bluetongue virus (BTV) is the cause of bluetongue (BT) disease, an insect-vectored emerging pathogen of wild ruminants and livestock causing disease in sheep, goats, and cattle with mortality reaching 70% in some breeds of sheep. BTV infection occurs throughout many of the temperate and tropical regions of the world, coincident with the distribution of specific species of *Culicoides* biting midges that act as biological vectors of the virus. Bluetongue virus is the prototype member of the genus *Orbivirus*, family *Reoviridae*.

Bluetongue virus has a complex multilayered architecture (Figure 1). Virions contain non-equimolar amounts of 7 proteins organised into two capsids. The outer capsid, composed of two major structural proteins (VP2 and VP5), is involved with cell attachment and virus penetration during the initial stages of infection. The cellular attachment protein, VP2, is also the serotype determinant of BTV and is the most variable protein in the virus. After entry into cells, the outer capsid (VP2, VP5) is removed to release a transcriptionally active core particle composed of two major structural proteins (VP7 and VP3) and the transcription complex of three enzymatic proteins (VP1, VP4 and VP6) in addition to the segmented dsRNA genome.

Like almost all other RNA viruses, the BTV genome shows a rapid rate of sequence mutation. In addition, each virus particle contains 10 different RNA segments and these segments reassort when two different viruses infect the same host. Thus, BTV reassorts freely and exchange readily occurs between different serotypes.

On the basis of serotype-specific virus neutralization assays, 24 distinct serotypes of BTV have been described to date, although the Toggenburg virus isolated from goats in Switzerland, is probably a 25th serotype. BTV is endemic in many tropical and sub-tropical countries. Since 1998 there have been separate and repeated incursions of bluetongue into Europe and 6 serotypes (BTV-1, -2, -4, -8, -9 and -16) have been introduced into mainland Europe and the Mediterranean region. 2006 was a landmark year as it saw the emergence of BTV (serotype 8) as far north as France, Belgium, Holland and Germany and subsequently across the English Channel to the UK in 2007. Climate change may have contributed to the emergence of BTV in Europe through the increased distribution and size of insect vector populations. It is also evident that BTV is being transmitted by novel vector species, which are abundant in central and northern Europe. Thus, BTV now represents a considerable threat in all European countries including the UK.

The European incursion of BTV has had a considerable negative economic impact, partly due to high mortality and associated high morbidity but, more importantly, as a result of the ban of trade between

BTV-infected and non-infected areas. Based on the number of ruminants, the annual turnover of an efficient BTV vaccine is estimated to be as much as €16m within Europe. To limit direct losses and in an effort to minimize the circulation of BTV, European countries have vaccinated livestock with available vaccines.

The threat of BT disease to European agriculture has stimulated the development of new and safer vaccines against BTV. The purpose of this EC funded project, specifically, was to test the safety of inactivated vaccines in pregnant animals and develop new types of sterile (protein based) vaccines to Bluetongue.

Aim

The aims of this project were to generate completely safe genetically inert vaccines for BTV, particularly for European serotypes, either by inactivation of the infectious virus or by recombinant technology that completely avoids infectious virus. The specific objectives of the project were:

1. To establish the safety of vaccination with inactivated vaccines in pregnant animals.
2. Validated the protective efficacy of VLP vaccine in European sheep, cattle and goat breeds against virulent virus challenge.
3. To characterise the type of immune response elicited by vaccination with inactivated and VLP vaccines.
4. To determine the breadth of protection afforded by inactivated vaccine and VLP vaccine in sheep.
5. To determine the degree of cross-neutralisation afforded by a mixture of VLPs to all current European serotypes against homologous and heterologous serotypes.
6. To develop a test, based on BTV NS1 and VP7, capable of distinguishing vaccinated versus naturally infected animals.

Results

In order to achieve the aim and objectives of the project, Merial (Partner 2) developed an inactivated virus-based BT vaccine, effective against a number of BTV strains (BTV-2, -4 and -8), that has been used successfully during the 2006-2008 European outbreaks, although not in all potential ruminant hosts. As there was no data on the safety and efficacy of these vaccines in pregnant animals, a clinical trial was undertaken. The data obtained clearly demonstrated that these killed-virus vaccines are also safe in pregnant ewes, in contrast to live virus vaccines. In parallel, LSHTM (Partner 1) developed novel complex protein-based recombinant virus-like particle (VLP) vaccines for a number of European strains (BTV-1, -2, -4, -8 & -9). VLPs have been proven to substantially improve immunogenicity and elicit stronger and longer-lasting immune responses (both B- and T-cell). Moreover, because the VLPs contain only the protein, and not the viral genome, there is no chance of reversion to virulence, reassortment or incomplete inactivation. Several of these VLPs were tested by UCM, ANSES and AUTH (Partners 3, 4 & 5 respectively) in three different European breeds of sheep that are susceptible to BTV. Vaccination with monovalent, divalent or multivalent (VLPs of two or more different serotypes) VLPs were capable of protecting sheep successfully from BT disease when challenged with virulent viruses. In each case viraemia was completely suppressed. Moreover, there was no interference observed from mixed serotype vaccines, when comparing the monovalent and polyvalent VLP vaccines. The VLP vaccinated sheep showed complete protection against virulent BTV strains. Furthermore, there was no interference in protection when sheep were vaccinated with more than one type of VLPs. Thus, demonstration of the effectiveness of VLPs as a vaccination strategy in European animals has been successfully achieved. Since it is essential to develop a test that is capable

of distinguishing vaccinated and infected animals (*DIVA test*), IDVET (Partner 6) focused on the development of serological diagnostic tests for discriminating vaccinated versus naturally infected animals as well as developing real time RT-PCR detections of BTV together with VAR (Partner 7). Within this EC project, LSHTM also constructed VLPs for the eastern (Greece isolate; GRE) and western (South African isolate; RSA) lineages of BTV-1, BTV-2 and BTV-4 which were validated in clinical trials using European breeds of sheep. In addition, the degree of cross-neutralisation achieved from VLPs vaccines containing mixtures of serotypes was investigated. Tests capable of distinguishing vaccinated and infected animals were also developed and supplied to partners for the comparative vaccine trials.

Potential applications

The proliferation of BTV severely limits sheep production and the live sheep and cattle export business; countries free from virus will not accept animals from countries where the disease has broken out or is endemic with substantial economic implications. As a result of the BTVAC project, the following Outcomes have been achieved;

1. A better knowledge of the type of immunity elicited by the vaccines in the study.
2. Production of reagents for the rapid generation of a marketable novel VLP vaccine for all the circulating European serotypes of BTV.
3. Evidence that single and/or multiple serotype VLP vaccines are highly efficacious against a virulent virus challenge.
4. There was no interference in the generation of neutralizing antibodies or protection afforded by a multiple serotype VLP vaccine.
5. Demonstration that VLPs afford protective efficacy (no clinical sign or viraemia) in 3 different European sheep breeds.
6. Important data on the safety of inactivated vaccine in pregnant animals.
7. A prototype kit for BT capable of distinguishing vaccinated and infected animals based on NS1.and VP7
8. New patent for new technology to generate BTV VLPs.
9. VLPs production technology has been successfully transferred to a vaccine manufacturing company.
10. Further development and validation of real time RT-PCR detection and quantification of BTV.
11. A strengthening of the ties between the research and commercial partners in the project promoting the transfer of technology from a research to a production phase.

In conclusion, the project has been extremely successful both scientifically and for practical purposes and substantial achievements towards the objectives of the project have been obtained.

Keywords

bluetongue, non-live vaccine, VLPs, DIVA, diagnostics

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[Medreonet]

Surveillance network of reoviruses, bluetongue and African horse sickness, in the Mediterranean basin and Europe

Acronym:
Medreonet

Project number:
044285

EC contribution:
EUR 460 000

Duration:
42 months

Start date:
1 January 2007

Instrument:
coordination action

Summary

Bluetongue virus (BTV) and African horse sickness virus (AHSV) are reoviruses transmitted by vectors species belonging to the *Culicoides* genus that affect respectively ruminants and Equidae.

BT disease has occurred sporadically in the Mediterranean region in the past, involving relatively short-lived epizootics. Since 1998, large BT outbreaks affected different countries around the Mediterranean. The virus has extended further north than ever. This geographical expansion is mainly due to the northern extension of the main afro-tropical BT vector *C. imicola*. During summer 2006, BT outbreaks due to serotype 8 were recorded in Belgium, Germany, France and the Netherlands with European *Culicoides* species probably involved in this emergence. This episode highlighted the potential of BT to further establish in Europe and presents a major risk to the livestock industry. Two new serotypes appeared in northern Europe: BTV-6 and BTV-11 in Germany and the Netherlands in November 2008, and in Belgium in early 2009 respectively. Several other serotypes coming from eastern Europe (mainly Israel) such as serotypes 5, 15 (outbreaks in 2009) and serotype 24 (outbreaks in 2009 and 2010) are a threat to Europe since only serotype-specific vaccines are available (http://www.reoviridae.org/dsRNA_virus_proteins/ReoID). A new orbivirus named BTV-25 (Hofman et al., 2009; Hofman et al., 2010; Chaignat et al., 2009) was described in Switzerland in 2008. The importance of implementing surveillance networks has to be noted in the way it helps the countries to detect bluetongue occurrence even if the clinical signs were moderate or even absent for the newly detected serotypes.

AHSV outbreaks have occurred in southern Europe in the past, especially in Spain from 1987 to 1991. It causes one of the most severe diseases in horses. It is closely related to BTV and is transmitted by the same *Culicoides* vectors; hence regions at risk of BTV can be regarded as at risk of AHSV. Recent outbreaks of AHSV serotype 2 occurred in Nigeria and Senegal in 2007 and, more recently, in 2010 in Ghana. Apart from BT and AHS, another *Culicoides*-borne virus, Epizootic hemorrhagic disease virus (EHDV) has been detected in 2004 in Morocco, in 2006 in Israel and the Maghreb northern countries (Algeria, Morocco and Tunisia) and on La Réunion Island in 2009 and 2010. Dairy cattle (Holstein and Brown Swiss) were suspected to

have EHD on the basis of clinical investigation between early July and the last week of August 2007 in several cities in the western part of Turkey, the cases were confirmed (Temizel et al., 2009). The coordination action will gather and share information on BT, AHS and EHD to: (i) promote regional studies on the risks of introduction of new strains and spread with inclusion of neighbouring areas (North Africa, Turkey) as an early warning; (ii) survey the expansion of *C. imicola* in new northern territories taking into account the potential novel vectors group in Europe; and (iii) improve information technology for storage, communication and sharing of vector and sentinel surveillance and vaccination data. The consortium is bringing together national and international reference laboratories working on vectors, detection of infection, and surveillance and risk assessment around the Mediterranean.

Problem

A total of 21 partners with two giving us a hard time by not returning the administrative documents and one which did not provide any input to the project.

Aim

The strategic objectives of Medreonet are:

- to further expand our knowledge of the epidemiology of bluetongue, African horse sickness and Epizootic Haemorrhagic Disease in the Mediterranean basin and Europe;
- to apply this knowledge to optimise the surveillance of these *Culicoides*-borne diseases.

The research objectives of Medreonet are:

- to further study the ecology of *Culicoides* vectors and their expansion;
- to further study the behaviour of bluetongue and Epizootic Haemorrhagic Disease after their emergence in the Mediterranean basin and in the north of Europe.

The aim of this project is to promote the coordination of efforts directed at improving knowledge relevant to the control of bluetongue (BT), African horse sickness (AHS) and Epizootic Hemorrhagic disease (EHD). It is intended to strengthen the surveillance of these three *Culicoides*-borne diseases by providing a framework for interactions between research institutions and national veterinary services. The project will focus on the coordination of research, disease surveillance at the Mediterranean level, risk analysis through network resources, information exchange on technical issues and dedicated studies.

In order to answer the objectives of the call, six work packages have been identified divided into vertical activities dealing with either tools or data needed for surveillance, and horizontal or transversal activities dealing with more integrative activities.

The vertical activities are:

- regional surveillance of virus activity and vaccination — WP1;
- regional surveillance of vectors — WP2;
- molecular Epidemiology — WP3.

The transversal activities are:

- databases, web design and GIS — WP4;
- risk assessment — WP5;
- meetings — WP6.

Results

1. Scientific

- Improvement of the surveillance network for BT, AHS and EHD in the Euro-Mediterranean region
- Data on vector distribution
- Data on viral strain distribution
- Data on potential threatening viral strain around the region

2. Technical and policy support

- Recommendations on implementation of national surveillance systems
- Better documentation of disease status
- Harmonisation of diagnostic tests and case definition
- Development and maintenance of Euro-Mediterranean database on viral strain and disease situation

The potential impacts are to strengthen and coordinate existing initiatives for collaborative actions involving national reference laboratories, OIE/EU reference laboratories and other international stakeholders involved in BT or AHS research and to initiate new collaborative action on research activities and needs, regional surveillance and risk management/research, diagnostic harmonisation and laboratory preparedness; to establish a website acting as a front-end for Euro-Mediterranean situation towards BT, AHS and EHD and a European research group on BT, AHS and EHD threats; to provide a management structure to improve the outputs from collaborative actions and to involve stakeholders in the scientific and technical developments.

More specifically, for bluetongue, the potential impacts are:

- facilitate decision-making based on previous experience in Mediterranean countries and Europe;
- enhance the knowledge on BT in zones at risk;
- disseminate information about AHS and EHD in the Euro-Mediterranean countries and Europe;
- increase the rapidity of early warning when a new strain is introduced in one area;
- spread knowledge and diagnostic techniques relevant to strain threatening the Mediterranean basin and Europe;
- better advice on the use of vaccination against BT and the choice of vaccine serotype to be included.

For African horse sickness and Epizootic Hemorrhagic Disease, the potential impacts are:

- enhance the knowledge on AHS and EHD in zones at risk;
- disseminate information about AHS and EHD in the Euro-Mediterranean countries and Europe;
- increase the rapidity of early warning when a new strain is introduced in one area;
- assess the risk from source of AHS and EHD virus from areas surrounding the Mediterranean.

Potential applications

The potential applications of the different deliverables that have been achieved during the project are described by WP:

WP1: Regional surveillance of virus activity

The general objectives of this WP1 consisted in the strengthening and the harmonisation of surveillance of BTV/AHSV/EHDV infection in Europe. The specific objectives are the evaluation and the harmonisation of surveillance protocols and description of available tools for surveys and viral. This general objective was pursued by evaluating the following three different components: (i) surveillance protocols; (ii) available tools for surveys and strain identification and (iii) vaccination strategies and helping all the partners to have a European point of view of the vaccination programmes, case definition and laboratory diagnosis of each of the participating countries.

A questionnaire was sent out to all the participants to address several questions about BT, AHSV in 2007 in terms of case definitions, laboratory diagnosis and surveillance of these diseases in each country.

At the end of 2007, in order to avoid duplications, it was decided to take advantage

of the EUBTNet system and obtain from EUBTNet the surveillance data required by the WP1 with the following justifications:

- data in the EUBTNet system belong to the national competent authorities (i.e. the involved ministries of the Member States);
- it would not have been difficult for the institutions involved in the project to be granted the permission to use aggregated data for the aims and scopes of the project;
- data in the EUBTNet system is the official information available from the Member States, therefore the use of EUBTNet data guarantees the consistency with the official situation of member countries;
- since the Medreonet project requires only aggregate data on the basis of administrative units, the proper management of sensible data can be easily assured.

WP2: Regional surveillance of vectors

The general objectives of this WP2 consisted in the strengthening of *Culicoides* entomological surveillance in Europe and neighbouring countries. The specific objectives are the evaluation and the harmonisation of:

(i) surveillance protocols; and
(ii) available tools for trapping, identification of vectors and modelling of vector habitat.

The various deliverables that have been achieved could all be considered as potential applications, particularly:

- the recommendation of the optimal trap design for sampling *Culicoides*;
- the development of a guide for the identification of *Culicoides* vectors and potential vectors;
- the recommendation of optimal surveillance protocols for *Culicoides*;
- the development of standardised protocols for detection and estimation of abundance of *Culicoides*;
- the development of a reference collection of *C. imicola* specimens for DNA analysis.

WP3: Molecular epidemiology

The general objectives of this WP3 consisted in the distribution, origins and movement of the different BTV and EHDV strains, by characterisation of well documented isolates of different serotypes.

- Expanding the existing sequence databases for genome segments 2, 6 and others of BTV and related orbiviruses, including high quality data for representative and well documented isolates of different virus serotypes from different geographical locations is one of the application of this project and is very useful in terms of awareness when new outbreaks occur in orbiviruses free countries.
- The characterisation of novel and existing BTV/EHDV strains from Europe and the Mediterranean basin is helping in the determination of their geographical distribution, movement, potential for reassortment and their original sources (topotypes).
- The identification of diagnostic tools such as molecular probes and primers for the rapid/early identification of new strains-serotypes is also one of the major applications for all of the participants.

WP4: Database, web design and GIS

The general objective of this WP4 was to develop a *Culicoides*-borne virus network through a website which will provide a platform of Internet-based tools to be used by partners and other interested parties, to enable:

(i) effective information exchange between partners on technical and administrative CA-related bluetongue AHS and EHD activities (restricted-access website);

(ii) information related to the CA to be imparted to the wider scientific community and to other stakeholders with an interest in the control of bluetongue and AHS (open-access website);
(iii) a forum for discussions on the vertical work packages:

- to develop a web-enabled geographic information system (GIS) to present epidemiological data and to allow a rapid spread of information related to the diseases;
- to provide a real-time interactive mapping system of the main epidemiological aspects to facilitate the decision-making process and management of control activities at central and local level.

The online procedure is an application developed through an ASP page providing a form that participants can fill in when needed. The online updating system has been implemented in all the web-GIS services developed: BT outbreaks, BT entomological surveillance, AHS outbreaks, EHD outbreaks.

Through a username and password, users can be identified and authorised and they can insert all the information related to their own country. At the moment, data on presence/absence of the disease can be added, but any additional information required by the participants can be easily added in the form and the database including disease distribution, viral and serological activity, entomological activity, control measures.

WP5: Risk assessment

The general objective of this WP5 was to standardise methods for the geographical assessment of the risk of BT/AHS/EHD spread in the Mediterranean basin and Europe.

In order to obtain a concept for geographical risk assessment of BT/AHS/EHD, several analyses were performed and aimed at the following potential applications.

- Transmission pathways from currently affected areas have been developed using international standards for risk assessments. The pathways cover both vector-related transmission as well as spread related to trade.
- A review has been conducted with respect to published models that can be used for the assessment of infection probabilities of specified regions with a methodological guideline to be used in order to classify specific regions or Member States with respect to BT risk.
- Validation of the risk assessment using case studies in selected countries (Algeria, Bulgaria, Tunisia and Turkey) with the collection of samples to identify exotic strains of BT/AHS/EHD and trapping graphically presented as maps.
- Based on the epidemiology of BT/AHS/EHD, risk-based surveillance approaches have been developed. Risk-based surveillance is defined as a surveillance programme that includes risk factors for increased probability of infection in specified populations and/or regions as well as the outcome of risk assessments conducted. Thus, the results of the risk assessments will be used as input for the planning of surveillance. It is anticipated that sampling intensity, target population and sampling interval in surveillance programmes are dependent on the risk category of the Member State or region. Different risk analysis models have been built, to determine the risk of overwintering and introduction of bluetongue virus. The results of the models determine the factors that have more importance in the maintenance/introduction of the virus and, as a consequence, the sub-populations where surveillance can be more sensitive in detecting the infection: quantitative assessment of the probability of bluetongue virus overwintering by horizontal transmission, quantitative assessment

of the probability of bluetongue virus transmission by bovine semen and effectiveness of preventive measures, quantitative assessment of the probability of bluetongue by *Culicoides* introduced via transport and trade networks. A paper was recently published by Napp. et al., 2010, on the quantitative assessment of the probability of bluetongue virus transmission by bovine semen and effectiveness of preventive measures in theriogenology.

WP6: Meetings and dissemination

One tool for the evaluation of the CA is the dissemination of knowledge. In this coordination action, major scientists and laboratories in bluetongue and AHS, representatives of private companies and international organisations as well as political decision-makers were brought together within each work package. Many of the participants and project associates are also members of various national and international societies for virology, entomology and epidemiology. These organisations were used as dissemination platforms. Dissemination and exploitation of the results was therefore guaranteed.

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Project website

http://medreonet.cirad.fr/

Keywords

orbiviruses, Bluetongue (BT), African horse sickness (AHS), Epizootic Hemorrhagic Disease Virus (EHDV), diagnostic, surveillance, risk assessment

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[ORBIVAC]

Development of vaccines for BTV, EHDV and AHSV

Acronym:
ORBIVAC

Project number:
245266

EC contribution:
EUR 2 999 729

Duration:
36 months

Start date:
1 February 2010

Instrument:
collaborative project

Summary

Orbivirus diseases, particularly bluetongue (BT), African horse sickness (AHS) and Epizootic Hemorrhagic Disease (EHD) are serious current (BT) and potential future (AHS, EHD) challenges facing European agriculture. Bluetongue virus (BTV) is the cause of BT disease, an insect-vectored emerging pathogen of wild ruminants and livestock causing disease in sheep, goats, and cattle. BTV infection occurs throughout many of the temperate and tropical regions of the world, coincident with the distribution of specific species of *Culicoides* biting midges that act as biological vectors of the virus. Since AHSV and EHDV are genetically closely related to, and are transmitted by the same insect vectors as BTV, there is a clear risk of the potential introduction of these other orbiviruses, and indeed other arboviruses, into Europe.

Although there are effective inactivated vaccines for some of the individual BTV serotypes, which are currently used in Europe, these are not currently available for all serotypes. In addition, no effective inactivated vaccines are currently licensed and available for use in Europe, for either AHSV or EHDV. Another issue with the current vaccines is that they generate an antibody response to all of the viral proteins, making assays that can differentiate infected from vaccinated animals (DIVA) difficult or impossible to develop.

Therefore, the major outstanding challenge of orbivirus vaccine research is to develop vaccines that can afford a broad protective immune response against as many serotypes of each virus as possible alongside a high throughput DIVA assay (e.g. an ELISA). This proposal will use a coordinated multi-partner approach to address these issues, to develop new experimental prototype vaccines and diagnostic approaches.

Problem

Since 1998, there have been more than 12 separate introductions of BTV into Europe, involving at least 10 different virus strains belonging to eight different serotypes (types 1, 2, 4, 6, 8, 9, 16 and a new serotype, type-25). These events have resulted in the deaths of more than two million animals and have caused substantial economic losses to the agricultural economies of Europe. The outbreak caused by BTV-8, which started in the Netherlands and Belgium in 2006, is, by itself, the largest single outbreak of bluetongue ever recorded. New introductions of the virus into Europe, which have been linked to climate change, have occurred almost every year since 1998, with the identification of four new virus strains in 2008 alone. New *Culicoides* species responsible for virus spread have also been identified in central and northern Europe, confirming that the whole of the EU is now at high risk from incursion of these diseases.

Since AHSV and EHDV are genetically closely related to, and are transmitted by the same insect vectors as BTV, there is a clear risk of the potential introduction of these other orbiviruses and other related viruses into Europe. The recent detection of BTV-9 in North Africa, EHDV in Turkey, and

two different strains of AHSV in West Africa, provide further indications of increased risk from these diseases. The continued appearance of new BTV strains in southern, central and northern Europe raises the question what virus and what serotype will arrive next.

Two types of orbivirus vaccine are currently commercially available, based on either attenuation or inactivation of the live virus. Each is effective at controlling disease but there are concerns over incomplete attenuation of the live vaccines in the field and their ability to readily reassort with field strains. Both vaccines only confer serotype-specific protection and the inactivated vaccines require two doses for effective protection of sheep and cattle. Moreover, both types of vaccine generate an antibody response to all of the viral proteins, making assays that can differentiate infected from vaccinated animals) (DIVA) difficult to develop.

Aim

The overall aims of the project are threefold. The first aim is to develop multivalent vaccines using different approaches for orbiviruses responsible for livestock diseases, in particular, BTV (25 Serotypes), AHSV (9 Serotypes) and EHDV (7 Serotypes). The second is to understand the best vaccination strategy to elicit multi-serotype protection for these viruses in livestock and analyse immune responses for each of the novel vaccines developed for breadth of protection against multiple serotypes. Finally, the project aims to develop DIVA compatible diagnostics that will work with the new vaccines developed in order to differentiate between vaccinated and infected animals.

The project will use a coordinated multi-partner approach to achieve these aims. It builds on specific expertise and reagents that are only available within the consortium and links out to other international efforts in South Africa and the United States to develop improved vaccines for these diseases where these viruses are also a threat. The consortium includes a number of industrial partners (Merial, Pfizer, Deltamune, Boehringer) who are already active in vaccine manufacture for these and other veterinary diseases, in order to ensure that the findings of the research are transferred as soon as possible into commercial vaccines for European livestock. The project also includes two SMEs (IDVET and INGENASA), who will be specifically involved in the development of DIVA compatible diagnostic tests.

Expected results

Based on the latest developments in vaccine and orbivirus research, there are a number of exciting approaches that offer the potential to produce effective multivalent vaccines to orbivirus diseases. In terms of eliciting immune responses, two complementary approaches will be followed. One is based on proven observations that attenuated virus, VLPs and the VP2 protein of BTV and AHSV, are protective. The second strategy will attempt to map cross neutralising domains within BTV and AHSV epitopes, and then use a single immunogen to elicit cross neutralising protection.

One of the consortium partners (Partner 1 — LSHTM) is a leader in orbivirus reverse genetics and will specifically generate a new class of disabled single cycle (DISC) vaccine for BTV that promises improved immunogenicity over non-replicating vaccines and avoids the problems associated with replicating attenuated vaccines. A complementary, protein-based, AHSV multivalent subunit vaccine will also be produced. Partner 1 will also develop VLP vaccines for EHDV, which have not previously been reported for this orbivirus. Other partners have specific expertise in the use of canine adenovirus, canarypoxviruses and capripoxviruses as delivery systems for orbivirus antigens and will test the

use of these systems to deliver optimised multivalent immunogens. Another partner, in collaboration with one of the industrial partners, will use Parapox as an expression system for the serotype determining VP2 protein of AHSV. Each of the vaccine systems that will be tested in the project offers its own advantages either in terms of ease of transfer to manufacturing, safety or degree of long-term immune response elicited. The project will compare the new and established systems for the ability to stimulate a multivalent immune protection against multiple virus serotypes.

The project will incorporate new reagents and methods for rapid diagnosis and typing of orbivirus outbreaks and to distinguish infected and vaccinated animals.

Current typing of orbivirus diseases is based on RT-PCR approaches. The consortium plans to develop real-time RT-PCR assays for all 25 BTV serotypes as well as microarrays to provide higher throughput and improved sensitivity for the detection and rapid typing of diagnostic BTV samples. The new molecular (array) assays will be independently validated against current RT-PCR based systems and reference samples from previously identified BTV strains for speed and accuracy.

The new vaccine approaches developed during the project should all be suitable for serological tests that differentiate infected from vaccinated animals (DIVA). The consortium will develop serological tests that allow DIVA to be completed for vaccinated animals. The consortium will also develop ELISA-based tests that allow serotype determination and differentiation between BTV and EHDV. Sero-group-specific ELISA tests that can be used to distinguish animals vaccinated against one serotype but exposed to a second serotype of BTV as part of a DIVA strategy will also be developed. These tests will be directed at serotypes currently circulating in Europe. The possibility of using virus non-structural proteins as a basis for DIVA diagnostics will also be investigated, as well as a quantitative real-time PCR assay for EHDV and new serological DIVA tests for AHSV.

Potential applications

New generation multivalent vaccines and accompanying tests that are compatible with DIVA will be developed. None of the current commercial vaccine approaches, inactivated and attenuated virus vaccines, are compliant with DIVA principles, and therefore it is necessary for a new generation of vaccines that overcomes this limitation to be developed. The primary expected outcome is that stable, multivalent, new generation, DIVA-compliant vaccines will be produced.

The project will produce and test new prototype vaccines to BTV (DISC and multiepitope-conserved antigen types), AHSV (multi-epitope-virus vectored, and protein based multivalent subunit) and EHDV (virus-like particles). All of the new vaccines are multivalent and designed to elicit cross protection against multiple strains of virus. Major research findings of the project are anticipated to be the degree of cross protection that is afforded by combined vaccines and by new immunogens where multiple epitopes from different serotypes have been engineered into the same immunogen.

In parallel to each of the new vaccines, DIVA-compatible diagnostic reagents have been developed which will allow routine testing of vaccinated and imported animals. These have specifically been designed to be compatible with the new vaccines (above). The project will also address the outstanding issues in the diagnosis of orbivirus diseases by developing microarray and improved real-time PCR reagents for serotyping of BTV and by producing a new group specific ELISA test that for the first time provides an immunological test for distinguishing EHDV and BTV.

The consortium comprises several industrial partners, which include the major industrial companies currently manufacturing vaccines for BTV in Europe. Inclusion of these partners in the same consortium will facilitate commercialisation of any of the new vaccine approaches developed by the consortium. The consortium also includes active participation from third countries (South Africa and the United States), with partners who bring specific expertise to the consortium and enhance its scientific and technical excellence.

Project website

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CHAPTER 3.

Endemic diseases

The economic impact and research synergy from EU-funded projects

The context

In exploring the economic impact from EU-funded research projects, it is necessary to place the research in the context of the cost of animal diseases, which can have devastating socioeconomic effects both in developed and developing countries. Infectious diseases directly affect livestock production, food security and food safety, trade, rural development, environment, livelihood of farmers and in some cases are of public health concern. Infectious diseases also have major consequences on animal welfare.

The most obvious costs are those associated with epidemic disease outbreaks. The outbreak of foot-and-mouth (FMD) disease in the United Kingdom in 2001 is estimated to have cost EUR 3.5 billion, including costs associated with the culling of 0.76 million cattle, 4.9 million sheep and 0.43 million pigs, without mentioning other species. However, the total cost to the rural economy may have been in the region of EUR 8 billion as a result of disruption in trade and impacts on industries such as tourism. BSE is estimated to have cost the United Kingdom EUR 8.5 billion between 1996 and 2010. Bluetongue in the Netherlands in 2006–07 was estimated to have cost EUR 155 million. In the case of zoonotic conditions such as avian influenza or West Nile fever, the costs would also include those associated with human morbidity and mortality. Table 1 summarises

Table 1: Cost of epidemic disease outbreaks

(million EUR)

	FMD TAIWAN 1997	CSF NETHERLANDS 1997/98	FMD KOREA 2000	FMD JAPAN 2000	FMD URUGUAY 2000/01
Government costs					
Compensation	141	887	283	0.5	—
Control	50	103	50	11	15
Private costs					
Agricultural sector	1 652	318	—	—	—
Related industries	2 409	447	—	—	45
Other	712	—	—	—	—
Total	**4 964**	**1 755**	**333**	**11.5**	**60**

Source: FAO/OECD: FMD = foot-and-mouth disease; CSF = classical swine fever

the cost of a range of epidemic disease outbreaks.

Costs that are not so obvious relate to endemic diseases. Costs associated with liver fluke amount to EUR 2.5 billion globally per year and the disease is spreading, with increasing costs, due to climate change. It is also estimated that mastitis costs the European dairy industry in the region of EUR 3.5 billion per year or EUR 150 per year for each cow.

Improved awareness of, preparedness for, and response to outbreaks are needed for the effective management of the threats of animal diseases and depend on sound science. Whilst we have good tools to control many diseases, the situation is constantly evolving. The objective of the research is to provide tools for control where none exist today or to develop better tools where this is necessary. The economic impact of research comes in the form of reducing the impact of disease or eliminating it altogether from a region or globally — it is to be noted that rinderpest was officially classified as eradicated globally in 2011. It is estimated that the cost of eradication/control was USD 4 billion from 1945 to 2010. Rinderpest previously caused devastation to cattle farmers in Africa, the Middle East and India and its eradication was greatly assisted by the development of a heat stable vaccine. With FMD, one of the goals of research is to develop a multivalent heat stable vaccine that would allow us to fight this disease much more effectively as in the case of rinderpest.

Human capacity, future planning, continuity and impact

Considering the research work recently completed or ongoing, the theme of developing human capacity, planning for the future, continuity of the research effort and focusing on the diseases with greatest impact while reacting to evolving situations is obvious.

The PARASOL project ensured the generation of a cohort of new specialists in the area of internal parasite control. Previously, research in this area had become less popular because of the effectiveness of the then available control methods and many new areas of research, such as genomics, had opened up. However, with the development of resistance to anthelmintics, it became clear that there was need for a rethink of control methods and to stimulate research in this area to ensure that the expertise was not eventually lost. The DELIVER, PARASOL and PARAVAC projects continue to ensure that expertise.

Bluetongue virus (BTV) is spread by midges (*Culicoides* species) and had been threatening or present in southern Europe when projects were commenced to investigate arboviruses. BTVAC was launched in 2007 and Arbo-Zoonet followed on in 2009 as a means of spreading knowledge concerning arboviruses in the EU and outside. BTV spread to northern Europe in 2006 and it was a rush against time to produce inactivate vaccines. However, vaccines were deployed to prevent a major outbreak of the disease in 2009 that would have been

expected to lead to very considerable morbidity and mortality in sheep and cattle populations in Europe. The arbovirus work has spread knowledge about these types of diseases and has stimulated expertise in the veterinary entomology area — something that was greatly needed as very few experts previously existed.

The VENoMYC project was launched in 2004 creating a large network of laboratories working on mycobacterial diseases (tuberculosis and paratuberculosis). The aim was to lead to improved diagnostics, a better understanding of the diseases, research on the use of vaccines, study of natural resistance, public health implications and an identification of further research needs. The TB-STEP project followed in 2008, focusing only on tuberculosis to get a better understanding of the epidemiology of the disease, the role of other species — especially wildlife — in the maintenance and transmission of the disease, utility of vaccines including in wildlife, better diagnostics and to propose control strategies. It is important that this type of research continues as we struggle to control tuberculosis.

The PCVD project in 2004 recognised the need to respond to the threat of porcine circovirus (PCV). The project achieved great strides in terms of the use of vaccines to control this very damaging disease. It was very interesting to see the synergistic role of nutrition as a factor in controlling the disease.

Later on, the NMSACC-PCVD project facilitated the communication, networking and training of all parties concerned in relation to the control of PCV. There was also a particular focus on young scientists, new Member States and accession countries.

Synergy from EU-funded projects

In many situations, there is considerable research effort at the national level and the EU has recognised the added benefit that can be achieved through coordination of this activity using various instruments. The European Technology Platform for Global Animal Health (ETPGAH) was launched in 2004 as a stakeholder-driven initiative with the objective of bringing a focus on the research effort across stakeholders. A vision was developed and strategically important issues identified. An action plan then followed identifying 28 major actions.

The action plan identified the need for a European research area network (ERA-NET) in the animal health area to coordinate activity at the level of the research funders, and EMIDA was subsequently launched in 2009. This brought the funders of research from 19 countries together, leading to two calls for collaborative research across international borders, including some public-private partnerships. This represented a tremendous step forward in terms of research synergy.

The EMIDA ERA-NET is being followed by a second ERA-NET with the short title of ANIWHA, launched early in 2012.

Another project that emerged from the ETPGAH is DISCONTOOLS. This project focuses on disease prioritisation, with the model being developed on the basis of the analysis of 51 diseases. Key information is agreed leading to the identification of gaps in knowledge or tools (diagnostics, vaccines, pharmaceuticals) and the diseases are then prioritised by looking at our knowledge of the disease and its impact on animal health and welfare, public health, wider society, trade and the availability of control tools. By having this prioritisation, we can focus the research effort on the most critical gaps in the most important diseases.

Following the success of EMIDA, the STAR-IDAZ project takes the concept of collaboration between funders on to a wider international level. STAR-IDAZ was launched in 2011, bringing Argentina, Australia, Brazil,

Canada, China, Europe, India, Mexico, New Zealand, Russia and the United States to the table. The enthusiasm to collaborate exists as the big prize is the ability to control disease, making everybody a winner in any advances that are achieved. This represents a tremendous synergy in the research effort.

Neglected zoonoses and wildlife

The ICONZ project, launched in 2009, recognises that a number of diseases cause a significant burden on poor and marginalised communities in Africa. The project focuses on endemic zoonotic diseases which have been identified by the World Health Organisation (WHO) as neglected. The diseases in scope include anthrax, brucellosis, bovine tuberculosis, cystic echinoccosis, leishmaniasis, rabies, cysticercosis and trypanosomiasis. The project plans to add knowledge, test culturally acceptable and cost-effective control interventions and engage with policymakers to seek better control strategies.

Research on animal diseases is expensive, especially where high containment facilities are required. The added advantage of linking existing research facilities and its importance in underpinning the programme of research funded through the FAFB work programmes was recognised in the funding of the European Network for Animal Diseases and Infectiology Research Facilities (NADIR) project by the Capacities, Research Infrastructures programme. This project unites the major experimental animal facilities with capacity to undertake research on zoonoses, emerging diseases and other animal infectious diseases requiring bio-containment at level 3 with the aim of sharing resources and a coherent development of high bio-containment facilities.

Conclusions

Given the fact that animal diseases impose a high impact on society in terms of lost production due to endemic diseases, extremely high costs due to epidemic diseases and an ongoing threat to the human population due to zoonoses, investment in research in this area makes good sense. We aim to reduce the impact of endemic diseases — this adds value not only in terms of avoiding production losses but also contributes to climate change mitigation by using less inputs (feed, water, energy, etc.) and producing less waste (manure, CO_2, methane, etc.). By preventing epidemic diseases via better control tools, we can avoid significant costs to society as per Table 1. Naturally, preventing zoonoses via the development of better control tools has tremendous benefits to human health. The practical elimination of rabies across Europe via vaccines including vaccines delivered to wildlife via baits is a very good example of a tremendous benefit to human health.

By launching projects that ensure we have the correct human capacity, by looking to the future threats, by ensuring continuity in the effort to develop solutions and by focusing on high-impact diseases, the research programme over the past 10 years has been very well focused.

The initiation of the technology platform, the launch of networks and the development of ERA-NETS has ensured that the research effort becomes even more focused and that it is approached in a collaborative manner, building on previous work and avoiding overlap of effort.

The lateral thinking of also looking at wildlife reservoirs as a potential source of previously unknown diseases is an inspiring example of future planning. In addition, the focus on neglected zoonoses ensures that the appropriate tools are provided to control these diseases of significant impact in societies least able to respond.

The research effort needs to continue. Diseases continue to impact on animal health

and welfare and society at large. As we move to a predicted global population of 9 billion by 2050, the time to develop new and better tools to control disease is now. We are making progress and continue to do so. We must continue on the path that we are on, moving towards an ever greater focus on the priority research targets and maximising synergy across all parties concerned to make the research effort most productive.

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3.1. Mycobacterial diseases

[VENoMYC]

Veterinary network of laboratories researching into improved diagnosis and epidemiology of mycobacterial diseases

Summary

Tuberculosis and paratuberculosis of livestock are mycobacterial diseases that represent a threat not only to public health but also have a high economic impact due to mortalities, condemnations, decreases in weight and fertility, and drop in productions. Countries of the European Union are under eradication programmes for bovine tuberculosis and/or paratuberculosis. To date, eradication has not been achieved in the EU due to several problems and, therefore, tuberculosis and paratuberculosis remain major concerns in livestock production. The most relevant problems (lack of appropriate methods of diagnosis, the role played in the epidemiology of the diseases by other domestic and wild animals, difficulties in the laboratory work with these pathogens, and lack of adequate vaccines that do not interfere with diagnosis) were addressed in this coordination action. In addition, the application of new systems was discussed (i.e. use of functional genomics to detect new molecular markers and/or to develop new vaccines).

The collaboration between the partners was structured through a multidisciplinary network made up of 37 partners from 17 countries. The partners was selected on basis of active researching on mycobacterial infections. The work focused on seven tasks devoted to specific concerns, and were divided into 11 work packages grouped into three categories: (i) devoted to dissemination of knowledge to the Community; (ii) dedicated to specific harmonisations; and (iii) focused on topics that will have a deep impact on the understanding and control of the diseases in the near future. Available tools for partners were general meetings, workshops (lectures and hands-on training in the laboratory) and the exchange of personnel for training purposes. The main approach of this coordination action was to share technology and expertise in order to both avoid research fragmentation and obtain a common knowledge on mycobacterial diseases. The final target was to develop harmonised recommendations and/or procedures for their potential transposition into EU policies.

Problem

The main approach of this coordination action is to share technology and expertise

Acronym:
VENoMYC

Project number:
501903

EC contribution:
EUR 796 201

Duration:
60 months

Start date:
1 September 2004

Instrument:
Coordination action

in order to both avoid research fragmentation and obtain a common knowledge on mycobacterial diseases. The final target was to develop harmonised recommendations and/or procedures for their potential transposition into EU policies.

The project brought together partners with expertise in a number of different technologies for evaluation of the different diagnostic tests, control measures, molecular characterisation protocols, etc., for mycobacterial diseases. The project took advantage of the partners' experience in new technological developments to study the application for tuberculosis and paratuberculosis diagnosis.

Aim

The key objective of this coordination action project was to develop a multidisciplinary European network of laboratories researching into mycobacterial diseases of veterinary interest. The strategic objective was the translation of the research results into EU policies.

The project was divided into seven tasks and 11 work packages (WPs), according to the different technologies employed by the participating laboratories. Some of these tasks (WPs) were an extension of tasks developed under the project Concerted Action FAIR6-CT98-4373. The selected 11 work packages were:

WP1: Development and support of VENoMYC website;
WP2: Scientific audiovisual presentation;
WP3: General meetings;
WP4: Laboratory diagnosis of *Mycobacterium* spp.;
WP5: Direct detection of *M. a. paratuberculosis* in dairy products;
WP6: Immunology-based tests for mycobacterial infections;
WP7: VNTR/MIRUs and DVR-spoligotyping for *Mycobacterium bovis* typing;
WP8: Standardisation of molecular techniques for epidemiological studies;
WP9: Wildlife reservoirs of mycobacterial infections;
WP10: Application of functional genomics results on mycobacterial diagnosis;
WP11: Use of vaccines in the control of tuberculosis and paratuberculosis.

The strategic milestones of the VENoMYC coordination action were:

- accreditation of laboratories for the diagnosis of mycobacterial diseases and approval of internal standards;
- description of a standard procedure to implement the gamma-interferon test, taking into account the current situation of European livestock (i.e. dual infection, vaccination);
- application of typing methodology to disclose the relative importance of the sources of infection to understand the epidemiology of the diseases;
- recommended strategies to control transmission of infections from reservoirs to livestock;
- possible uses of vaccines in the control of tuberculosis and paratuberculosis;
- understanding the advantages of application of functional genomics results on diagnosis;
- evaluation of the public-health implications of mycobacterial infections in animals.

Expected results

The expected outputs from this permanent network of researchers were: (i) improvement of the sensitivity of current tests and/or development of new diagnostic tests using new technologies which allow the early detection of subclinical infections; (ii) molecular typing of mycobacteria; (iii) epidemiology studies; (iv) standardisation of techniques; (v) application of vaccines; (vi) study of natural resistance of certain species; (vii) potential public health

implications; and (viii) identification of research needs.

Throughout the duration of the project, two general meetings were organised: a first general meeting in 2004 and a final meeting in 2009. Moreover, a total of eight workshops were organised (Table 1). In the five years of the project, 31 short training mobilities were carried out. The objectives of the short training mobility funded within the VENoMYC project were to facilitate the inter-laboratory comparison of techniques and to provide training in specific methodologies. Attendance at international colloquiums is an excellent opportunity to acquire updated knowledge on all aspects related to mycobacterial infections. For this reason, partners had also the opportunity to attend the international congresses on paratuberculosis and *M. bovis* with sponsorship from VENoMYC.

Potential applications

The project was best carried out at the Community level because it brought together expertise and exchange of techniques and methodologies that would not be available within any one single Member State. For example, a European dimension is needed to standardise procedures for molecular characterisation techniques, and to obtain the appropriate variety of strains required to carry out epidemiological studies at a European level. This allowed the direct comparison of results obtained, reducing fragmentation between research and diagnostic centres and reference laboratories.

Paratuberculosis and tuberculosis not only represent a significant disease of domestic livestock but also of wildlife. The close communication between partners working in different ecosystems enabled a greater level of understanding of the epidemiology of the disease, and also represented a diversity of countries with a wide variety of wild animals.

Another advantage was that countries with a low prevalence in a mycobacterial infection, and therefore without active research or specific facilities, received updated information on the diagnosis and control of such infection that can be applied immediately in the event of an outbreak: this type of event is frequently associated with international trade of infected animals.

The outputs of WP7 (VNTR/MIRUs and DVR-spoligotyping for *Mycobacterium bovis* typing) and WP8 (Standardisation of molecular techniques for epidemiological studies) contributed to the definition of VNTR *loci* that are recommended for typing of *M. bovis*, spacers for an enhanced discriminative typing for *M. bovis* and enhanced use of databases for mycobacterial typing.

The results obtained in WP6 (Immunology-based tests for mycobacterial infections) contributed directly in the field implementation of the IFN-γ assay by the European

***Table 1**: List of workshops organised within the VENoMYC project*

DATE	PLACE	WORKSHOP	WORKSHOP
26–29 April 2006	Jena, Germany	WP4	Laboratory diagnosis of *Mycobacterium* spp.
13–16 September 2006	Maynooth, Ireland	WP9	Wildlife reservoirs of mycobacterial infections
19–21 October 2006	Toledo, Spain	WP7	VNTR/MIRUs and DVR-Spoligotyping for *Mycobacterium bovis* typing

DATE	PLACE	WORKSHOP	WORKSHOP
9–12 November 2006	Laguardia, Spain	WP11	Use of vaccines in the control of paratuberculosis and tuberculosis
10–13 May 2007	Athens, Greece	WP5 and WP8	'Detection of *M. a. paratuberculosis* in dairy products' and 'Standardisation of molecular techniques for epidemiological studies'
16–19 September 2007	Cambridge, United Kingdom	WP10	Application of functional genomics results on mycobacterial diagnosis
30 and 31 May 2008	Belfast, United Kingdom	WP6	Immunology-based tests for mycobacterial diseases
23–25 March 2009	Madrid, Spain	WP7	VNTR/MIRUs and DVR-Spoligotyping for *Mycobacterium bovis* typing — Results of the VNTR-DVR Spoligotyping Ring Trial

Commission. Moreover, the description of transmission of tuberculosis between wildlife and livestock derived from WP9 (Wildlife reservoirs of mycobacterial infections) has an impact on the control of the disease if staff of Ministries of Agriculture and Fisheries and Environment take them into account for the design of eradication campaigns.

References/publications

The VENoMYC coordination action did not fund research activities but at each general meeting and workshop organised within the VENoMYC project, a book of proceedings was handed to all participants (downloaded from the project website). Moreover, standardised protocols for typing mycobacteria were also available for all partners.

Project website

The website (http:///www.venomyc.com) was named after the acronym of the project: VENoMYC (Veterinary European Network of Mycobacteria).

Information on the website includes:

- objectives of the coordination action;
- work packages;
- a list of partner institutions;
- contact details;
- meeting plans (workshops information and reports);
- news; and
- links to international organisms on animal health, databases, etc.

Keywords

tuberculosis, paratuberculosis, *M. avium* complex, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium avium* subsp. paratuberculosis, standardisation, epidemiology, wildlife, vaccines, diagnosis, epidemiology, genomics

VENoMYC logo

Partnership

The consortium was designed to include at least one Institution from each European country to ensure a representative image of problems caused by mycobacterial infections. The fact that some countries Spain, Italy and the United Kingdom) were over-represented reflects a higher impact of mycobacterial diseases and active research. The needed link with human impact was obtained with integration of partners P19 (RIVM, Netherlands) and P24 (AGU, Greece).

The scientific component of the Consortium (37 partners) created a multidisciplinary

team integrating disciplines such as disease diagnosis, epidemiology, typing and control over 17 European countries. The partnership included a combination of universities, research institutes and reference laboratories. The partners complemented one another to ensure collaboration with groups in Europe working with mycobacterial diseases.

Interaction between 60 % of the participant laboratories began with the previous EU programme FAIR6-CT98-4373, 'Concerted action for the setting-up of a European veterinary network on diagnosis, epidemiology and research of mycobacterial diseases'.

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MRI	Dr Karen Stevenson	Moredun Research Institute, United Kingdom
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SSI	Dr Peter Andersen	Statens Serum Institut, Denmark
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NVI	Dr Berit Djønne	National Veterinary Institute, Norway
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ACRONYM	PARTNER LEADER	ORGANISATION
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LNIV	Dr Alice Amado	Laboratório Nacional de Investigação Veterinária, Portugal
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[TB-STEP]

Strategies for the eradication of bovine tuberculosis

Summary

Tuberculosis is an infectious disease caused by microorganisms of the *Mycobacterium tuberculosis* complex. This infection affects domestic and wild animals and represents a major concern worldwide because of its high economic impact due to mortalities, condemnations, decreases in productions, and its zoonotic potential.

Eradication programmes based on a test-and-slaughter policy have been implemented in the European Union, and have proved successful in some countries. However, they have been unable to eradicate the infection in others despite the use of vast economical resources. A relevant problem is the existence of infected wildlife: the best known examples are the European badger (*Meles meles*) in United Kingdom and Ireland, and the wild boar (*Sus scrofa*) in Spain. Besides this fact, there is only a limited knowledge about other potential underlying causes, such as: (i) the real contribution of cattle-to-cattle transmission at the same area (neighbouring farms and communal pastures) or after movement of animals; (ii) the role played in the epidemiology by other domestic animals; or (iii) the effect of interferences in the diagnosis tests. The weight of these causes may also differ depending on the farming system and ecological factors. It is unlikely that there can be a single solution as it is unlikely that there is a single cause.

Aiming at the eradication of this infection, this TB-STEP project has planned a multifaceted battlefront. The consortium is made up of 12 partners from eight countries that will carry out research on seven work packages devoted to improved tools and to developing strategies for the eradication of bovine tuberculosis in areas where the disease is present in both domestic and wildlife populations. It will include: (i) vaccination of bovine animals and wildlife, (ii) control of populations, to reach numbers compatible with animal welfare, and strategies to limit contact between domestic and wild species; and (iii) development of improved diagnostic tools for detection of infected animals.

Acronym:
TB-STEP

Project number:
212414

EC contribution:
EUR 2 894 759.00

Duration:
39 months

Start date:
1 October 2008

Instrument:
Collaborative project

Problem

The *Mycobacterium tuberculosis* complex causes pulmonary, gastrointestinal and disseminated disease in mammals. M. bovis and M. caprae are the most relevant in animal health. M. bovis has an exceptionally large host range, including domestic animals and wildlife. Tuberculosis transmitted between wildlife, livestock and humans presents major challenges for the protection of human and animal health, the economic sustainability of agriculture and the conservation of wildlife. The potential for non-ruminants and wildlife to act as reservoirs has opened up new lines of research in the epidemiology of mycobacterial infections. Therefore, it is important to determine the role of wildlife in the epidemiology of tuberculosis in domestic livestock to establish control strategies that are pertinent to the range of agricultural systems employed in the EU. The European badger (*Meles meles*) is implicated in the transmission of M. bovis to cattle and

constitutes the most important reservoir of infection in the United Kingdom and Ireland. The wild boar (*Sus scrofa*) seems to be the main reservoir for tuberculosis in Spanish Mediterranean habitats. M. bovis is also a threat to valuable wildlife species and exotic animals.

Thus, the eradication of tuberculosis would require eradication from domestic animals and control (or eradication) of the infection in wildlife. However, to date this has been hampered by a number of reasons: (i) the limited sensitivity and specificity of the *in vivo* diagnostic test in cattle under some circumstances; (ii) the lack of diagnostic tests in wildlife, making difficult it to estimate the prevalence and the high-risk areas; (iii) the difficulties in using vaccines regarding the cross-reactions (cattle) and delivery (wildlife), as well as the limited protection; and (iv) the insufficient understanding about the relative contributions of each (domestic and wild) species and the impact of the management practices. The four major points presented above are addressed by this TB-STEP project.

Aim

The project aims at the design of strategies to achieve the eradication of tuberculosis from livestock and wildlife. The acronym of the project derives from the two keywords in the idea: the design of strategies based on a sound understanding of the epidemiology (TB-STEP). To approach the eradication of this infection, this project plans a multifaceted battlefront because the hypothesis is that a combination of methodologies would be needed to achieve the task. Thus, research is focused on topics that will have a deep impact on the understanding and control of the diseases in the near future. The work packages have been designed to share technology and expertise in order to both avoid research fragmentation.

More specifically, the project will:

- identify the relative impact of the different potential causes in the epidemiology;
- understand the role of other species in the epidemiology (maintenance and transmission);
- study the effect of vaccination in cattle and goats;
- assess the safety and immunogenicity of vaccine in wildlife;
- study the suitability of vaccines in wildlife;
- develop affordable serological tests to use in wildlife to estimate prevalence;
- improve cattle diagnosis by detecting interferences;
- develop a model system to assess the risk, taking into account the ecological and farm management factors;
- design the optimal strategy for control of infection in wildlife.

Expected results

The final target is to develop rational strategy/ies and/or procedures for their potential transposition into EU policies. For that reason, the project develops a multidisciplinary approach ranging from vaccination and immunology, molecular epidemiology, diagnosis to ecology. TB-STEP contributes to the scientific, technical, social and policy objectives (Cooperation

Theme 2 Food, Agriculture and Fisheries, and Biotechnology (European Commission C(2006) 6839)). The results from the TB-STEP project will lead to greater efficiency and cost savings which will improve the competitiveness of the European livestock and dairy industries in internal and global markers. Furthermore, it would contribute to the demand for safer food, the sustainable use of and production of bio-resources, the risk of zoonoses diseases and concern on animal welfare.

The project is also contributing to building a European knowledge-based bio-economy as it brings together science (the research organisations), industry (the SMEs) and stakeholders (policymakers).

Potential applications

The project will deliver strategies to be applied in the tuberculosis eradication campaigns in the affected EU countries where the disease is present in both domestic and wildlife populations. Potential applications include: (i) vaccination of bovine animals, wildlife and feral reservoirs; (ii) control of populations to reach numbers compatible with animal welfare; (iii) improved diagnostic tools for detection of infected animals; (iv) strategies to limit the contact between domestic and wild species. The diversity of wild species (some legally protected) and farming systems are also taken into account.

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Project website

http://www.vigilanciasanitaria.es/tb-step/

Keywords

tuberculosis, *Mycobacterium bovis*, *Mycobacterium caprae*, strategies, wildlife, vaccines, diagnosis, epidemiology

TB-Step Logo

Partnership

The collaboration between the partners will be structured through a multidisciplinary network made up of 12 partners from seven European countries (Hungary, Ireland, Italy, the Netherlands, Spain, Switzerland, and United Kingdom), and a third country (South Africa). The fact that some countries (ES, UK) are over-represented reflects a higher impact of mycobacterial diseases and also the reported role of wildlife as reseroirs of the infection. In total, 10 research organisations and two SME (Ingenasa and Prionics) are involved in the Consortium. The partnership includes a combination of universities, research institutes and Reference Laboratories.

The partnership will establish collaboration with researchers from developing countries (from the ICPC). Potential candidates have been identified from countries located in Africa and South America, based on known interest in these areas of animal health.

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3.2. Parasites

[DELIVER]

Design of effective and sustainable control strategies for liver fluke in Europe

Acronym:
DELIVER

Project number:
023025

EC contribution:
EUR 3 540 000

Duration:
40 months

Start date:
1 February 2006

Instrument:
Specific targeted research or innovation project (STREP)

Summary

The DELIVER project aimed to improve methods for controlling liver fluke disease. The project started in 2006 in response to growing concerns in Europe's agricultural industry about the impact of the liver fluke parasite on productivity. This parasite, *Fasciola hepatica*, infects cattle, sheep, goats and other exotic farmed species that eat grass. It has a complex life cycle that is dependent on suitable temperature and rainfall and involves an intermediate snail host. The main areas of concern are: (i) changing climate that better suits the parasite's life cycle and allows it to spread to new areas; (ii) resistance to treatment with the widely used flukicide drug triclabendazole; (iii) the parasite's ability to make animals more susceptible to other bacterial infections such as salmonellosis and bovine tuberculosis. The DELIVER project has been successful in bringing together 15 research groups from Europe and Latin America to tackle these problems and provide solutions.

Problem

Liver fluke disease causes annual losses of over EUR 2.5 billion to livestock production and the food industry worldwide. The prevalence of fasciolosis is dramatically increasing; in recent years, for example, increases of up to twelvefold have been recorded in EU Member States. In the United Kingdom, the prevalence of infection in cattle ranges from 45 % to 84 % and in Ireland alone, annual losses have been estimated at over EUR 60 million. Control of the disease in livestock is based on the use of anthelmintic drugs, with accompanying risks of chemical residues in foodstuffs. Moreover, anthelmintic resistance is emerging as a problem in many areas of Europe and globally. Fasciolosis is also an emerging human disease in many INCO countries, with an estimated 17 million people infected.

Current control of fasciolosis in ruminants is based on prophylactic/therapeutic use of flukicidal (anthelmintic) drugs, with the risk that residues of these drugs may contaminate food and the environment. The DELIVER project proposes to enhance the safety and quality of the food supply available to European consumers by developing improved, environmentally-friendly methods for the control of this infection in domestic livestock. Improved control will also decrease the potential for transmission to humans of this infection via plant products.

Aim

The main objective of the project was to develop novel control methods for fasciolosis in livestock, thus enhancing

food safety and allaying consumer concerns. This objective included three areas of work.

1. **Epidemiology:** The aim of the work in this package was to improve our understanding of the epidemiology of fasciolosis by determining farm-specific risk factors for infection, the role of wildlife in transmission, improving the sensitivity of diagnosis of patent infection and improving our understanding of how genetic variation in populations of fluke affect their interaction with the intermediate snail host and their infectivity and pathogenicity for farmed livestock. Ultimately, these results will lead to web-based predictive models which farmers can use to assess the seasonal and year-on-year risk of infection and disease in their herds and flocks and assist in decision-making to assess if treatment is necessary, when to treat and which drug to use. This work package also determined the significance of fluke infection in humans within the EU.
2. **Anthelmintic resistance:** The main aims were: standardisation of field and *in vitro* assays for detecting anthelmintic resistance and determining the extent of triclabendazole resistance in Europe and in INCO partner countries; and determination of mechanisms of drug action and resistance in order to formulate new strategies for conservation of drug efficacies. This work package also aims to develop protocols for the measurement of drug residues in food products and in the environment.
3. **Immunoprophylaxis:** This work aims to provide alternative, immunoprophylactic means of controlling fasciolosis. Work towards this aim encompasses studies of the basic immunology of infection as well as vaccine trials with new and existing recombinant antigens, and with DNA, carried out under controlled conditions.

Results

Epidemiology: Over 4 000 samples of milk and serum were collected from England, Greece, Spain and Wales to determine the prevalence of infection in livestock in these countries. The large-scale survey in England and Wales has provided a complete coverage of these countries and enabled us to construct a GIS showing the spatial distribution of *F. hepatica*. A risk analysis showed that climatic variables, particularly rainfall, temperature, soil type and presence of sheep are significantly correlated with a high prevalence of exposure to *F. hepatica* at a postcode area level. Interestingly, the data suggests that climatic variables occurring in the previous year or over a five-year period can be used to determine the risk of *F. hepatica* infection. However, farm-specific factors have yet to be identified to account for variation between individual farms in close proximity.

Characterisation of the *F. hepatica* soluble, juvenile and egg proteomes has been completed. A novel GST has been identified and characterised and, in addition, an antigenic high molecular weight HSP (FhHEA1) identified in eggs. A host trypsin inhibitor complex has been identified as a biomarker of infected animals. A diagnostic PCR has been developed that can detect a single egg per gram of faeces in naturally infected animals. An analysis of a panel of sera from a group of farmworkers considered to be a high risk population, showed no evidence that they had been exposed to *F. hepatica* infection.

RFLP analysis of mitochondrial DNA from flukes has provided markers that have been used to assess the genetic diversity of flukes of known provenance, isolated from different regions of the EU. Initial results suggest that there is extensive diversity between isolates that is not geographically restricted. This raises questions about potential differences in the virulence and infectivity between different isolates and also the potential for the emergence and spread of drug resistance through Europe.

Drug Action: Anthelmintic drugs are still the commonest means of controlling fluke infection. Over the last 20 years, triclabendazole has been the drug of choice for many livestock farmers, but cases of resistance have been reported in veterinary journals in the United Kingdom and other European countries. Research groups from Europe and Argentina have been collaborating through the DELIVER project to try and understand how triclabendazole kills fluke and how resistance develops.

One success has been the finding that the effectiveness of drug can be increased by co-treatment with appropriate metabolic inhibitors. We now have a much better understanding of how the parasite resists drug action and this may lead to new therapies to improve the efficacy of the drug.

Measuring drug resistance: Surveys in Ireland, the Netherlands and the United Kingdom have provided data on how widespread resistance to triclabendazole is. In Ireland, resistance was present on three out of six farms investigated. A test to measure reduction in fluke egg count following treatment is being developed and standardised by the Veterinary Laboratories Agency in England and Wales, and in Ireland at the Teagasc Sheep Research Farm.

Immunoprophylaxis: During the project, significant advances were made in understanding the immune response, the mechanisms by which the parasite exerts a regulatory effect on the immune system, and the relevance of this effect for livestock health and infection with other pathogens. Signifcant advances were also made; particularly through the use of proteomics and using mouse models where it was demonstrated that tegumental antigen of *F. hepatica* actively suppressed Th1 responses, through interaction with dendritic cells. These findings are relevant to the compromised ability to control bacterial infections seen in fluke-infected animals. Evidence of the 'real-life' importance of this effect was obtained using a model system of co-infection with *F. hepatica* and *Mycobacterium bovis* in cattle.

The difficulties inherent in carrying out vaccine trials, and the compounding factors that can affect the interpretation of results, became clear during this period. Preliminary evidence of differences in innate susceptibility to infection in sheep was obtained, and an 'adjuvant effect' using Quil A, again in sheep, was demonstrated. Several vaccine trials, using various combinations of antigens, did not demonstrate any consistent protective effect. A field trial in cattle indicated that direct neutralisation of CL1 –activity by antibodies from immunised animals is not likely to be a major protective mechanism. Overall, the results of this period emphasise the difficulties of vaccine trials in target species, while highlighting some aspects of the nature of the host-parasite relationship. These findings ill be important in the path to an effective vaccine.

Potential applications

The DELIVER project has been very successful in meeting its objectives by improving our understanding of the parasite in these key areas. This is important because once a parasite population has acquired drug resistance, there is no reversion back to susceptibility even if the drug is withdrawn.

If the liver fluke affects the susceptibility of cattle and sheep to other infections such as bovine TB, control of the parasite will have benefits for the control of other infections as well. Progress has been for developing a protective vaccine in cattle.

Finally, we have identified key risk factors both at the individual farm level and at an area level that can be used to measure the likely risk and severity of liver fluke disease occurring each year. These will form the basis of an online forecasting system that will be available to farmers within the course of the next few years. The effect of climate change,

the spread of drug resistance and changing patterns in EU agriculture are likely to make the economic impact of liver disease even more significant in the next decade.

The research results have been used to produce guidelines for liver fluke control that has been distributed to farmers and veterinarians organisations to help curb the spread and severity of the disease leading to healthier animals and a better return for farmers.

References/publications

Publications supported by the project can be obtained from the project website (http://www.deliver-project.eu/index.php?page=information-for-researchers).

Project website

http://www.deliver-project.eu/

Keywords

liver fluke, *Fasciola hepatica*, epidemiology, anthelmintic resistance, vaccine, immune response

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[PARASOL]

Novel solutions for the sustainable control of nematodes in ruminants

Summary

The overall objective of PARASOL was to create lowinput and sustainable strategies for the control of gastrointestinal nematode infections of ruminants. These parasites pose the greatest current threat to agricultural productivity and animal welfare.

Problem

Gastrointestinal nematodes pose the greatest current threat to agricultural productivity and animal welfare. Current conventional methods for worm control involve repeated dosing of whole herds with synthetic anthelmintics. This approach is not sustainable since it promotes the spread of anthelmintic resistance by failing to leave untreated parasites *in refugia* and there are concerns with regard to food residues and environmental impact. Nevertheless, effective chemical anthelmintics remain irreplaceable for worm control, and their elimination is not practical on animal welfare and economic grounds.

Aim

The lack of a sound scientific basis for targeting anthelmintic treatments has meant that producers have become over-reliant on intensive chemoprophylaxis. With the PARASOL project, we hope to replace current practice with targeted selective treatments (TSTs), where only animals showing clinical symptoms or reduced productivity are given drugs. We assessed several innovative methods, under various farming conditions, for identifying animals that require treatment, and produced and standardised tests for anthelmintic resistance to ensure that the drugs remain effective.

We produced clear guidance and protocols for sustainable, low-input, user- and consumer-friendly nematode control. To do this, we:

(i) assessed the effect of TSTs on productivity, animal welfare and the spread of anthelminthic resistance (AR) genes under a range of farming conditions;
(ii) determined the best methods of identifying animals and herds requiring anthelmintic intervention;
(iii) standardised existing *in vivo* and *in vitro* tests for detecting AR and developed new tests where previous ones were inadequate;
(iv) tested the potential for optimising the efficacy and bioavailability of anthelmintics by modulating parasite P-glycoprotein detoxification systems;
(v) communicated with farmers, veterinarians, advisors and trained animal health technicians throughout the participating countries, to produce and disseminate guidance to ensure good uptake and implementation of the protocols produced.

Expected results

The overall objective of PARASOL work packages 1 and 2 was to provide a scientific

Acronym:
PARASOL

Project number:
022851

EC contribution:
EUR 2 940 000

Duration:
40 months

Start date:
1 February 2006

Instrument:
Specific Targeted Research or Innovation Project (STREP)

rationale for deciding which animals to treat. PARASOL studies have confirmed the value of the TST approach in maintaining acceptable levels of productivity and conserving anthelmintic efficacy. The ability to target treatments developed in the PARASOL project has greatly facilitated the development of decision support systems and enabled researchers to make regionally appropriate recommendations and guidelines.

These are invaluable for farmers, advisors and policymakers who are concerned with improving sustainability, minimising tissue residues and the environmental impact of anthelmintic control strategies. As expected, the research conducted within PARASOL has confirmed the need for recommendations that are tailored to fit the local prevalence of the different economically important parasites, the situation with regard to anthelmintic resistance, and regional production systems. The findings from work packages 1 and 2 have been disseminated locally to farmers and more widely to advisors, the major research providers and policy-makers. Work package 3 was specifically designed to provide standard tests for the detection of anthelmintic resistance as an invaluable adjunct to work packages 1 and 2. Field surveys using the Faecal Egg Count Reduction Test (FECRT) in cattle have shown macrocyclic lactone-resistant *Cooperia* are present in Belgium, Germany, Sweden and the United Kingdom and *Ostertagia* in the United Kingdom. Because of the importance of the cattle industry to the EU, continued surveillance for anthelmintic resistance is recommended and strategies to slow the development and spread of resistance are required. The research in work packages 4 and 5 was ultimately aimed at providing additional standard methodologies for the detection of anthelmintic resistance, particularly to anthelmintics in the macrocyclic lactone family. These tests will be used as the basis of standardised protocols for conducting surveys into the extent of anthelmintic resistance. It was shown that the egg hatch test can be used for surveillance for benzimidazole resistance in bovine nematodes but further research is required before the larval development test is reliable for use with cattle worms.

Protocols for the running of FECRTs in sheep have been drawn up and combined with advice on the conducting field trials for resistance surveys. Work package 6 examined the possibility of enhancing drug bioavailability by targeting the mechanisms of drug transport and metabolism, in an effort to conserve the efficacy of our current anthelmintic families.

The research conducted within PARASOL has highlighted the problem of anthelmintic resistance in ruminants and has lead to the production of regionally specific recommendations, based on the following recommendations.

- Targeted selective treatment (TST) and targeted treatment (TT) strategies should be promoted to enable effective and sustainable worm control in ruminants.
- Anthelmintic efficacy should be routinely monitored.

Evidence from the PARASOL research findings highlighted the need for an ongoing research effort to:

- develop new *in vitro* and molecular tests for monitoring efficacy and potential resistance;
- provide a better understanding of the mechanisms of drug resistance;
- develop solutions for reversal of resistance and improved drug efficacy.

Potential applications

The findings and outputs from all of these work packages will have a considerable strategic impact on livestock management in Europe and selected INCO countries. The dissemination of these results to farmers and their advisors will allow them to make

rational decisions regarding the optimal use of anthelmintics within their region and production systems. Overall, the PARASOL project has provided an improved understanding of the development and detection of anthelmintic resistance and the mechanisms that underpin it. This knowledge will contribute to other investigations into AR, particularly human parasites.

References/publications

During the course of PARASOL, we produced 58 publications in peer-reviewed journals, 74 abstracts in conference proceedings, two book chapters and seven PhDs. In addition, several farming publications and leaflets were produced and talks were given to farmers and veterinarians in order disseminate the knowledge acquired through the Parasol-project and promote the TST approach in the control of nematodes in ruminants.

Project website

http://www.parasol-project.com

Keywords

gastrointestinal nematodes, ruminants, anthelmintic resistance, target selective treatment, nematode control, anthelmintic resistance mechanisms

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[PARAVAC]

Vaccines against helminth parasites of livestock of economic and/or public-health significance

Summary:

Livestock production efficiency is impaired by worm infections which are ubiquitous in cattle, sheep and goats worldwide. These cause severely debilitating gastro-intestinal, respiratory or liver disorders, dependent on the infecting species. Control of these parasites relies almost exclusively on the use of drugs, a solution threatened by the global emergence of worm strains which are no longer affected by these chemicals. An alternative, greener and more sustainable approach is to control these infestations by vaccination, but, with one exception, there are no commercial vaccines available for any of these parasites. Members of the present consortium, with participants from the EU and Switzerland, North and South America, and Africa, including three SMEs and one major animal health company, have collaborated to develop prototype vaccines with the efficacy predicted to control several of the most important of these livestock parasites as well the tapeworm *Echinococcus granulosus* in dogs, which can also cause fatal disease in man.

Problem:

As stated above, control of these parasites relies almost exclusively on the use of drugs, a solution threatened by the global emergence of worm strains which are no longer affected by these chemicals. An alternative, greener and more sustainable approach is to control these infestations by vaccination but, with one exception, there are no commercial vaccines available for any of these parasites.

Aim:

This proposal aims to deliver at least one of these prototype vaccines to the point of uptake by the commercial sector or through government/philanthropic agencies. This goal will be addressed by:

(i) developing effective native or synthetic vaccines, the latter using novel, molecular expression systems;
(ii) defining the protective immune responses induced by these vaccines to order to optimise the structure of the antigens and the method of their delivery;
(iii) defining vaccine efficacy with trials in both housed and grazing livestock;
(iv) providing a platform for training and knowledge exchange, which includes participation in training programmes, short exchanges of staff, workshops, and website provision;
(v) interacting closely with computer modellers, the animal health industry, farmer organisations and other stakeholders to define required vaccine characteristics;

Acronym:
PARAVAC

Project number:
265862

EC contribution:
EUR 8 944 185

Duration:
48 months

Start date:
1 April 2011

Instrument:
Collaborative project

(vi) knowledge exchange/dissemination to policymakers, scientists, government departments and the general public.

Expected results

At least one vaccine to reach commercialisation.

Potential applications

Any vaccines developed will be applied for the control of animal health in grazing livestock either globally or regionally. In addition, progress in vaccines against *Haemonchus* and fluke will have application in human medicine for the development of vaccines against fluke and schistomiasis.

Project website

http://paravac.eu

Keywords

livestock, sheep, cattle, helminth disease, vaccine

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3.3. Tick-borne diseases

[PiroVac]

Improvement of current and development of new vaccines for theileriosis and babesiosis of small ruminants

Summary

PiroVac is a major international research project designed to develop control measures to combat two major tick-borne diseases of small ruminants, namely theileriosis and babesiosis. This EU-funded research programme aims at improving existing vaccines, designing new vaccines, and capacity-building in partner laboratories both in Europe and in endemic areas.

Small ruminant piroplasmosis is a major threat to livestock production in many areas of the developing world. Theilerosis and babesiosis, caused by the protozoan parasites *Theileria lestoquardi*, *T. uilenbergi* and *Babesia ovis*, infect sheep and goats causing disease, production loss and sometimes death. Consequently, these diseases have a major impact on animal welfare and stock-holder prosperity throughout the world.

By developing effective measures to control these serious diseases, the PiroVac project represents a major contribution to achieving the United Nation's millennium development goals of food security, food safety, poverty alleviation, animal welfare and environmental sustainability.

PiroVac is a collaborative effort among a number of established research groups working on theileriosis and babesiosis. The consortium also encompasses laboratories involved in malaria research in order that scientific and technological knowledge in that field can be translated into tools and reagents for small ruminant piroplasms. Industrial expertise in vaccine development and delivery systems has also been incorporated in order to maximise the potential for translational application.

Acronym:
PiroVac

Project number:
245145

EC contribution:
EUR 3 million

Duration:
48 months

Start date:
1 April 2010

Instrument:
Collaborative project

Problem

Management of ticks and tick-borne diseases (TTBDS) is primarily through the control of the tick vector using acaricides, although this is unsustainable due to increasing acaricide resistance and food safety concerns. In some endemic regions, attenuated live vaccines have been developed for bovine piroplasmosis (*Theileria* and *Babesia*) however; there has been limited development of vaccines for small ruminant piroplasmosis.

Aim

The PiroVac project was developed as an integrated approach, encompassing

immunology, molecular biology, bioinformatics and genetic engineering, together with pathogen genomics and host genetics, and is directed at addressing two broad aims:

(i) development of effective and reliable vaccines for use in disease control campaigns for sustainable livestock development;
(ii) capacity-building for the sustainable implementation of integrated control measures required for disease control and/or eradication through increasing scientific knowledge, training and improvement of infrastructure.

PiroVac will exploit technology to improve the existing *T. lestoquardi* vaccine and design new vaccines focusing on malignant theileriosis and babesiosis in small ruminants. A wealth of scientific information will be generated that will facilitate the upgrading of production systems using more productive, but disease-susceptible, breeds for improving the genetics of local flocks. The overall objective of the project is to ensure food security and to improve food safety by improving control measures for small ruminant piroplasmosis caused by *T. lestoquardi*, *T. uilenbergi* and *B. ovis*. To achieve these goals, the project will assess parasite diversity and identify molecules associated with attenuation of parasite virulence to be included in the development of safe and efficacious live vaccines. For the improvement of the attenuated *T. lestoquardi* vaccine, a combination of the existing vaccine with subunit vaccines will be examined for synergistic effects. The specific goals of the project are:

(a) improvement and development of live attenuated vaccines for the control of small ruminant theileriosis and babesiosis through determining the effectiveness of attenuation, using:
  (i) *in vivo* assessment of attenuation, analysing clinical and immunological criteria (both humoral and cellular responses) of immunised and challenged animals;
  (ii) subtractive libraries and microarray analysis for the identification of attenuation markers;
(b) subunit vaccine design through:
  (i) identification of suitable antigens using a combination of genomics, bioinformatics and gene expression analysis coupled with experimental confirmation of antigen localisation and presentation — to facilitate antigen discovery, parasite molecules involved in host cell invasion, activation of cytokine-producing CD4+ T cells and NK cells and activation of cytotoxic T-lymphocytes involved in killing of *T. lestoquardi*-infected leucocytes will be identified;
  (ii) immunological characterisation of the identified antigens as potential vaccine candidates.
(c) vaccination trials using:
  (i) live attenuated vaccines;
  (i) recombinant protein and DNA vaccines.

Expected results

The project will contribute to an understanding of the immunological and molecular mechanisms involved in host-pathogen interaction. As important by-products of

the project, reagents required for the characterisation of the innate and adaptive immunity of small ruminants will be generated together with the genome sequences of the three pathogens under study.

The PiroVac project is based on the conviction that the interface between genomics, immunology and vaccinology offers the best prospect for major breakthroughs in vaccine discovery and development. Overall, vaccine development will contribute to reducing losses in animal production due to piroplasmosis of small ruminants and improve the life quality of both farmers and consumers.

Potential applications

- Generation of knowledge and tools required for vaccine production.
- Vaccines useful for upgrading schemes using more susceptible but productive breeds.
- Progress into policy innovations and strategies that will meet critical millennium development goals: food security, food safety, poverty alleviation (e.g. improvement of small farmer income), animal welfare and environmental sustainability.
- Exploitation of the data for commercial purposes by collaborating with the industry (arrangements have been made with the industrial participant for the commercial exploitation of the products).

Project website

http://www.theileria.org/pirovac/

Keywords

small ruminants, piroplasmosis, *Theileria lestoquardi, Theileria uilenbergi, Babesia ovis*, vaccine

Logo

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3.4. Porcine circovirus diseases

[PCVD]

Control of porcine circovirus diseases (PCVDs): towards improved food quality and safety

Summary

This PCVD project was initiated on 1 December 2004. It was a 51-month, research-based FP6-funded project with the specific objectives of providing sound scientific information which could be used to promote food quality and safety through the control of porcine circovirus diseases (PCVDs) within EU Member States and regions. The project brought together a multidisciplinary scientific team of 15 partners representing diagnostic institutes, universities and industry. This project established an EU-led multidisciplinary consortium containing expertise in epidemiology, pig genetics, pig nutrition, pathology, molecular biology, immunology, vaccinology, bacteriology and PCV virology to generate scientifically sound information on the aetiology and early pathogenesis of PCVDs. The project met all the objectives and the information generated has been used to generate control measures for PMWS/PCVD, resulting in the reduction of the use of antibiotics and of secondary zoonotic bacterial infections, meeting consumer concerns for quality/safety of pork products. The results can be summarised as follows:

1. successful development of effective and consistent control measures for PMWS/PCVD that are being applied across all EU Member States and third countries;
2. a reduction in the load of secondary bacterial infections in pig herds, accompanied by a consequential reduction in the use of antibiotic therapy and risk of acquired antibiotic resistance;
3. an increase in the quality and safety of food products derived from pigs;
4. establishment of a common standardised and harmonised reagent bank for the diagnosis and study of PCVD;
5. determination of the molecular mechanisms of PCV2 replication and pathogenesis and the early replication sites of PCV2 in pigs;
7. elucidation of the role of nutrition and other environmental factors in the full clinical expression of PCVD;
8. elucidation of the early interactions of PCV2 with the porcine immune system relevant to susceptibility or resistance to PCVDs.

Acronym:
PCVD

Project number:
513928

EC contribution:
EUR 3 450 000

Duration:
51 months

Start date:
1 December 2004

Instrument:
Specific Targeted Research or Innovation Project (STREP)

Problem

In 1997, the first reports of a 'mystery wasting disease' in pigs in Canada and France started to appear in conference proceedings and the farming press and eventually in peer-reviewed journals. Following these

preliminary reports the disease, named post-weaning multisystemic wasting syndrome (PMWS), quickly spread around the world as a global epizootic causing severe economic losses to pig producers and restricting the movement of pigs and pig products. EU researchers working within projects funded under the fifth and sixth framework programmes (FP5 and FP6), in collaboration with industry and colleagues from North America, have been at the forefront of studies on PMWS and porcine circoviruses that have defined the disease and helped develop commercial diagnostics and vaccines. At the initiation of this FP6 project in 2004, PMWS was the most serious global disease affecting the swine industry. Surveys at the time showed that the prevalence of PMWS and PCVD were high in all parts of the world and had both a strong sanitary and economical impact. Wasting disease was the predominant clinical sign, and mortality, other infections and their direct consequences and the use of antibiotics were universally increased. At the beginning of the outbreaks, mortality rates peaked at 35–40 %, sometimes higher in some batches, and mainly occurred between 6 and 14 weeks of age. In 2004, no commercial vaccine products were available for control of PMWS.

Aim

The strategic objective of this project was to generate information and control measures for porcine circovirus diseases that would have a positive impact on the health and welfare of pigs and meet consumer concerns for quality and safety of pork products. This overarching objective was achieved by completing the nine minor objectives outlined below:

1. to apply the information generated to the elimination and/or control of PCVD;
2. to initiate and maintain a proactive information dissemination programme aimed at all relevant stakeholders, including consumers, producers and policymakers;
3. to identify the common co-factors/triggers in epizootic PCVD scenarios necessary for the full development of clinical disease;
4. to determine the role of nutrition in the susceptibility/resistance to PCVD;
5. to determine the sites of replication of PCV2 and early pathogenesis of PCVDs;
6. to elucidate the early interactions of PCV2 virus with the host immune system;
7. to elucidate the role of porcine genetics in susceptibility/resistance to PCVD;
8. to determine the molecular processes of PCV2 replication (18 months) and virulence;
9. to standardise and harmonise and distribute reagents, and SOPs for use within the consortium.

Results

This project has been successfully completed and all objectives were met.

A classical commercial vaccine was successfully tested in trials in Canada, Denmark and Sweden and successful trials on disease prevention using combined vaccine/nutritional intervention were completed. Vaccination is now a major tool to prevent disease, good nutrition is very efficacious and vaccination and good nutrition are synergistic. In the hands of the consortium, more advanced experimental vaccines did not appear superior to a classical marketed product. Based on the data generated, it can be concluded that piglet vaccination prevents PMWS and improves the growth performance of the piglets but was limited by maternal immunity. Sow vaccination improves reproduction parameters, prevents PMWS mortality and decreases global mortality and improves growth and performance of the piglets and pigs.

Within the project, there was an active programme of knowledge transfer and interaction with stakeholders throughout the EU and internationally. The aim of the

knowledge transfer programme was to ensure good communication of the results of the programme at both the scientific level and at a less technical level to producers, veterinary surgeons and general stakeholders so that relevant results could be applied as quickly as possible to improve the health and welfare of pigs. Close collaboration with the sixth framework specific support action PCVD-SSA helped to transfer both knowledge and skill sets to new Member States, accession countries and beyond. The project website was central to the knowledge transfer effort and it is intended to maintain this as a source of information from the project beyond the lifetime of the project so that the knowledge transfer will be ongoing.

Within the project a complementary feedstuff comprising short-, medium- and long-chain fatty acids was shown to improve growth rates and FCR in field trials on units with pigs diagnosed with PCVD. Pigs offered the feedstuff had lower levels of PCV2 excretion than pigs not offered the complementary feedstuff. The complementary feedstuff did not work by improving digestibility but by improving utilisation of the feed.

It was also shown within the project that porcine embryos and foetuses are susceptible to PCV2 infection and that infection leads to foetal mortality irrespective of genotype or clinical background of the virus strain. Sequential studies have elucidated that mucosal tissues are sites of early replication and these findings have been corroborated by evidence of replicative forms of virus in epithelial and endothelial cells in clinical cases of PCV2 infection. Effects of PCV2 on intestine and intestinal cells have been particularly addressed. Altered transcriptome patterns after PCV2 infection have been shown *in vitro* and *in vivo* and are suggestive for functional changes in these cells. By microarray analysis, information has been generated of differentially regulated gene expression early after PCV2 infection and also during clinical manifestation, which enables future bio-informatic analysis. Based on these studies, the early interactions of PCV2 with the immune system have been elucidated. The mode of PCV2 interacting with different cell subsets as well as with production of antibodies and cytokines has been described in detail in scientific publications.

PCV2 is a small virus with a simple genomic organisation (expressing only few proteins), which shows a high similarity to PCV1. At the initiation of this project, we did not have an understanding, at the molecular and cellular level, of how infection of swine with PCV2 caused a complex and severe disease such as PMWS. By investigation of the entry and the replication of the virus in different cell lines, highly deviating types of infection were found. We have focused on the molecular interaction of PCV1 and PCV2 with cellular components and the identification of up- or down-regulated genes after PCV2 infection. This process has led to the identification of several highly interesting genes that may be involved in PCV2-induced pathogenesis.

At the initiation of this project, consortium members were using a wide range of different reagents and biologicals to diagnose PCVD and carry out experimental studies. A work package (WP) was designed to standardise and harmonise these reagents across the consortium in an attempt to ensure that results generated by consortium members were comparable. In addition, the WP distributed SOPs, reagents and biologicals to researchers and diagnosticians outside the consortium in an attempt to standardise diagnostics and research findings across EU Member States and ACCs. Reagents and procedures were standardised and harmonised by ring testing and distributed to consortium members and a large number of laboratories outside the consortium. In addition, a number of SOPs were written and posted on the PCVD webside and a number of consortium

recommendations for diagnostic procedures were also posted and presented at international meetings.

Throughout the lifetime of the consortium, we have focused on the characterisation of new PCV2 isolates and new biologicals produced by consortium members. To this end, the consortium was the first group to recognise and characterise new PCV2 genotypes (partners 1 and 10) recovered from pigs in Sweden. This was quickly followed by the characterisation of new genotypes from pigs in Denmark and Spain. The association of genotypes of PCV2 with virulence and disease progression is still unclear and a matter of debate.

Potential applications

This collaborative project successfully brought together scientists in 15 institutes from 10 countries to apply a multidisciplinary approach to the understanding and eventual control of an economically important disease of swine.

The project has been successfully completed within the agreed budget, with the vast majority of the anticipated milestones and deliverables being met. The project has significantly contributed to the understanding of porcine circovirus diseases (PCVDs), with 53 publications in the peer-reviewed literature, and maintained and expanded the established internationally recognised expertise in these diseases in Europe. Importantly the project has directly contributed to the control of these diseases around the world by application in the field of results generated within the project on vaccines, epidemiology and nutritional intervention. The practical application of results generated from studies associated with this project has also resulted in improved diagnostic procedures for PCVDs being applied around the world.

The association and interactions of the PCVD STREP with the NMSACC-PCVD SSA have enabled successful technology transfer and training of young scientists within EU Member States and third countries.

Results generated from this project on control measures and vaccinations against PCV2 have therefore contributed significantly to:

- improved welfare for swine;
- protection of the environment;
- protection of the customer, with much less antibiotic use;
- very significant economical benefits to farmers.

The strategic objective of the consortium was fully achieved by 'generating information and control measures for porcine circovirus diseases that will have a positive impact on the health and welfare of pigs and meet consumer concerns for quality and safety of pork products'.

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Project website

http://www.pcvd.org

Keywords

porcine circovirus, virology, swine, wasting disease, postweaning multisystemic wasting syndrome

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[NMSACC-PCVD]

PCVD: Towards improved food quality and safety within EU new Member States and candidate countries

Acronym:
NMSACC-PCVD

Project number:
518432

EC contribution:
EUR 397 340

Duration:
48 months

Start date:
1 January 2006

Instrument:
specific support action

Summary

The NMSACC-PCVD specific support action (SSA) was initiated on 1 January 2006. This SSA was designed to support information dissemination to, and training of, young scientists and various groups of stakeholders and end-users from new Member States (NMSs) and acceding and candidate countries (ACCs) in a wide range of aspects related to porcine circovirus diseases that were being explored and researched within the PCVD STREP. This SSA allowed scientist and other end-users and stakeholders in NMSs and ACCs to benefit from the consolidation and expansion of reagents, information and training opportunities on PCVD and other pig diseases important in food safety. The SSA specifically addressed the linking of NMSs and ACCs to the PCVD STREP, resulting in a multidisciplinary network of over 37 partners. The harmonisation of reagents, dissemination of information and training of young researchers in a network linked to an already established PCVD project of international standing consolidated and strengthened the globally acknowledged centre of expertise on PCVD within the EU. The interactions established within this SSA with colleagues within the NMSs and ACCs assisted preparations for future Community research activities. The project met, and exceeded, all the objectives. Reagents and protocols for diagnosis and control of PCVD were harmonised in a range of NMSs and ACCs, and information dissemination across NMSs and ACCs was achieved through a series of five workshops (two additional workshops over and above those outlined in the original technical annex). A network of training opportunities for young scientists across EC Member States and ACCs has been established.

The results can be summarised as follows

1. Reagents and protocols for the study of PCVD have been harmonised across a number of NMSs and ACCs through a series of ring tests. Reagents have been distributed to 21 institutes in 15 countries.
2. A series of five workshops, successfully completed in NMSs, incorporated delegates from diagnostic and research laboratories, field veterinarians and swine producers. A workshop in Russia was included in this project.
3. An information dissemination network was established that included a specific website. Funding was made available for 16 young scientists to attend international meetings.
4. A successful extensive training and exchange programme for young scientists from NMSs and ACCs and the Russian Federation was completed. In this

programme, 32 young scientists from 11 countries received training in STREP laboratories.
5. Young scientists from NMSs and ACCs have been trained and empowered in a number of aspects of organisation and management of an EC project.

Problem

In 1997, the first reports of a 'mystery wasting disease' in pigs in Canada and France started to appear in conference proceedings and the farming press and eventually in peer-reviewed journals. Following these preliminary reports the disease, named postweaning multisystemic wasting syndrome (PMWS), quickly spread around the world as a global epizootic causing severe economic losses to pig producers and restricting the movement of pigs and pig products. At the initiation of the FP6 STREP on PMWS and PCVD (Control of PCVD: Towards improved food quality and safety: FOOD-CT-2004-513928) in 2004, PMWS/PCVD was the most serious global disease affecting the swine industry. Surveys at the time showed that the prevalence of PMWS and PCVD were high in all parts of the world, including NMSs and ACCs, and had both a strong sanitary and economical impact. It was also clear from these surveys that there was an urgent need for information dissemination and training platforms for scientists, field veterinarians and swine producers in NMSs and ACCS be set up to facilitate transfer of knowledge gained within the PCVD STREP and to help combat this devastating disease.

Aim

The strategic objective of the NMDACC-PCVD SSA was to establish an EU network of researchers and end-users that linked 21 institutes and stakeholders in NMSs and ACCs to an established EU STREP on the control of PCVD. The SSA focused on the harmonisation of reagents, training of young researchers, dissemination of information to end-users and assisting NMSs and ACCs with preparations for future Community research activities. The objectives of the SSA were:

1. to harmonise across NMSs and ACCs the reagents and procedures;
2. to establish by means of a programme of three joint workshops a common understanding of the state-of-the-art knowledge of the pathogenesis, prevention and control of PCVDs; these workshops were to include participation by end-users, industry and young researchers;
3. to disseminate information and knowledge on PCVD across NMSs and ACCs;
4. to develop a scientist exchange programme between NMSs/ACCs and PCVD STREP members, including former USSR countries;
5. to complete and distribute a comprehensive information dissemination package on outcomes and achievements of PCVD SSA/STREP.

Results

This project has been successfully completed and all objectives were met. Some objectives were exceeded.

Harmonisation of reagents

At the initiation of the SSA, work within the PCVD STREP had already begun on the harmonisation of reagents and procedures within laboratories in Western Europe and Canada. From 2006, this work was expanded within the SSA, and NMSs and ACCs were included. Partner 5 (NVRI, National Veterinary Research Institute, Poland) was responsible for further harmonisation studies, ring trials within SSA-affiliated institutes and the circulation of standardised reagents and protocols to NMSs and ACCs. At the termination of the SSA, 21 institutes in 15 countries in NMSs and ACCs had been supplied with reagents and procedures.

Workshops

At the initiation of this project, three workshops were envisaged within the duration of the three-year project. As a result of savings made within the initial budget, a total of five workshops and a meeting for young PhD students were successfully completed. These workshops were all held in NMSs or ACCs countries, with the exception of Workshop 5 which was held in Russia. In all of these workshops, scientists were encouraged to interact with field veterinarians and swine producers to facilitate two-way communication between the 'field' and the laboratory. Written feedback from the workshops supplied by delegates has indicated that this format was very successful, with an average approval rating of over 90 % for each workshop.

Information dissemination

Knowledge dissemination was achieved at all levels within this project and was coordinated by Partner 4 (Veterinary Research Institute, Czech Republic). A dedicated website was set up within the Veterinary Biotechnology, Epidemiology and Food Safety Network CENTAUR (http://centaur.vri.cz) providing access to PCVD news, training courses in the EU and third countries, a handbook on EU funds 2007–13, workshops and translated versions of the STREP newsletter for downloading in Croatian, Czech, English, Hungarian, Lithuanian, Macedonian, Polish, Romanian and Slovak. The website also provided a database of new papers published with abstracts (37 in 2007; 526 since 1985). The database was updated every time a new publication appeared on the Web of Science. The records (authors, title and source) are available on the web page, listed according to the authors or the year of publication. Importantly, within this work package, a funded programme was available to help young researchers attend international meetings to present results on their PCVD-related research.

Within the lifetime of the SSA, 16 young scientists from 10 countries availed themselves of this opportunity, and attended international meetings around the world.

Scientific exchange programme

At the heart of this SSA was the development, training and social interaction of young scientists from across EU Member States and ACCs. Throughout the lifetime of the project, special attention was given to trying to ensure that these young scientists were engaged and developed at all levels of the project. WP4 focused on an exchange programme for young scientists from NMSs and ACCs to visit, interact with and learn from their colleagues in the STREP institutes. Reciprocal visits from STREP institutes to NMSs and ACCs were also arranged.

A total of 32 young scientists (in excess of the original target of 20) from 15 east European countries participated in this programme. The success of this SSA can, in part, be measured by the enthusiastic participation of young scientists from across EC Member States and ACCs in all aspects of the programme and their constructive interactions with one another, both scientific and social, across cultural and political divides.

Management of the SSA

The SSA was successfully managed by the coordinator and the project management team. This can be seen by the savings that were made within the original costings of the project and the additional training of young scientists through two extra workshops and 12 extra technology transfer visits that was achieved without additional cost to the EC.

Potential applications

The SSA linked to the PCVD STREP has focused on information dissemination, the development of young scientists in NMSs and ACCs and the interaction of laboratory-based research with end-users in the field. The SSA was expanded beyond its original conception to include affiliates in Russia,

Ukraine, Serbia and Macedonia. In addition, extra outputs were achieved over and above those outlined in the technical annex, and included two additional workshops and more training exchange visits for young scientists.

The association and interactions of the PCVD STREP with the NMSACC-PCVD SSA have enabled successful technology transfer and training of young scientists within EU Member States and third countries.

Results generated from this project on training and information dissemination on PMWS and PCVD have contributed significantly to the following outcomes in NMSs and ACCs:

- improved welfare for swine;
- protection of the environment;
- protection of the customer by the much decreased use of antibiotics;
- very significant economical benefits to farmers;
- a platform of young scientists across EU Member States trained in PCV2-related matters.

Keywords

porcine circovirus, virology, swine, wasting disease, postweaning multisystemic wasting syndrome, new Member States

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3.5. Porcine reproductive and respiratory syndrome

[PoRRSCon]

New tools and approaches to control porcine reproductive and respiratory syndrome in the EU and Asia

Summary

Porcine reproductive and respiratory syndrome virus (PRRSV) is the major cause of reproductive and respiratory problems in pigs worldwide. It is endemic in Europe and other parts of the world and in the United States, it is estimated that losses due to PRRSV exceed USD 560 million annually. High abortion rates, mortality of pre-weaned piglets and respiratory disease in fattening pigs are the main features of this syndrome. Controlling this disease is a top priority in pig producing countries. Due to mutations at a high frequency, new variants of the virus appear that are no longer effectively controlled by the commercial vaccines. In addition, highly virulent variants emerge, leading to high losses. With regard to animal welfare and agricultural economics, there is an urgent need to control PRRS. Furthermore, the abusive use of antibiotics to control PRRSV-associated respiratory problems may lead to resistance that may endanger public health. PoRRSCon is an initiative of 14 partners originating from Europe and Asia with strong expertises in virology and immunology. They are doing front-line research on PRRSV and/or vaccine development. Two of these partners are leading European pharmaceutical companies that will guide the consortium in the direction of exploitable results. By joining their strengths, they have an ideal position to be successful in one of the most difficult challenges in pig health: controlling PRRS.

Problem

PRRSV is a positive-sense, single-stranded RNA enveloped virus classified within the genus *Arterivirus*. Nowadays, two genotypes are recognised (1 and 2) that originally were described as European and American because of the geographic origin of their prototypic strains. The European genotype is further separated in three subtypes (I, II, III). It has been clearly shown that the best protection is induced when the vaccine virus belongs to the same genotype. Due to its high genetic variability, highly pathogenic strains of this RNA virus have emerged, causing up to 20–30 % mortality (such as High Fever Disease in China, atypical PRRS in America and disease caused by subtype III European strains). These new strains are difficult to control by currently available

Acronym:
PoRRSCon

Project number:
245141

EC contribution:
EUR 2 999 070

Duration:
54 months

Start date:
1 January 2010

Starting date:
collaborative project

vaccines. PRRS is also undermining the pigs' immunity, leading to extensive bacterial infections in PRRSV infected pigs. Antibiotics are consequently intensively used in order to control these bacterial infections, leading to antibiotic resistance. The only sustainable defence will be the development of adaptable, inactivated, vector and attenuated marker vaccines, which can safeguard the pig industry and animal welfare for the future.

Aim

The aim of this project is to develop new tools and approaches to control PRRS in the EU and Asia. To reach this final goal, the following objectives are forwarded:

- characterise genetically and antigenically current PRRSV isolates in Europe and Asia;
- have a better understanding of the complex pathogenesis of PRRSV infections, immune response against PRRSV and immune modulation by PRRSV;
- define the genetic base of PRRSV virulence;
- identify PRRSV proteins and domains on these viral proteins that are involved in the induction of the immunity against PRRSV and in the immune modulation of PRRSV;
- develop new generation, efficacious and safe marker vaccines that can be adapted to temporary changes and geographical differences;
- develop DIVA assays that allow differentiation of infected from vaccinated animals.

Ultimately, it will be possible to set up a control strategy by combining marker vaccines with DIVA (differentiation of infected from vaccinated animals) assays. The tools and approaches developed in PoRRSCon will be made available to all national and international authorities that want to solve the PRRS problems

Expected results

To control PRRS, a better understanding of the pathogenesis of PRRSV infections is essential. With divergent strains emerging in Europe and Asia, the characterisation of the different PRRSV isolates across the two continents is pivotal, with work being carried out by partners in China, Denmark, Poland and Vietnam. To understand the complex mechanism by which the disease is caused, partners in Belgium, the Netherlands and the United Kingdom are comparing the pathogenesis of low virulence strains with highly virulent or pathogenic ones. Concomitantly, partners in Spain, the Netherlands and the United Kingdom are comparing the immunological parameters of these different strains during infection: the complex factors which cause immune function variation. At the fundamental level, work defining the genetic base of PRRSV virulence is being carried out by partners in China, Belgium and Italy, who are seeking to identify the cellular genes associated with virulence/pathogenicity. The Belgian partner is also studying the entry of high and low virulence strains in its target cell, the macrophage, while partners in Belgium, Spain, the Netherlands and the United Kingdom are analysing the immunobiology of the virus.

The development of a new generation of adaptable inactivated, vector and attenuated marker vaccines which can be adapted for temporary changes and geographical differences is being carried out by partners across China, Belgium, Spain and Switzerland. Concomitantly, the development of DIVA serological assays, which will allow differentiation between infected and vaccinated pigs, is being carried out by one of our Spanish partners (the biotech company Ingenasa). The PoRRSCon consortium consists of European and Asian PRRS experts who are in contact with national and international authorities and farmer organisations. Furthermore, the consortium has very good interactions with PRRS experts in the United States. The final goal is to coordinate PRRS control on a national, European and world level.

Potential applications

Adaptable inactivated, vector and attenuated marker vaccines for PRRSV, DIVA serological assays for PRRSV

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Project website

http://www.porrscon.ugent.be/

Keywords

PRRSV, RNA virus, vaccine, adaptable inactivated vaccine, vector vaccine, attenuated vaccine, marker vaccine, DIVA serological assay, virulence, pig, antibiotics, pathogenesis, virology, immunology, immunobiology.

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CHAPTER 4.

Influenza

Introduction

Influenza infections of animals have recently become a great concern for animal and public health. These include the awareness that a swine-origin influenza virus has become the most recent human pandemic virus and that avian viruses of different subtypes have caused enormous damage to the poultry industry and in some cases human health issues.

Since the late 1990s, outbreaks of avian influenza caused by low pathogenicity (LP) H5 and H7 viruses and also by highly pathogenic H5 and H7 viruses have affected the European poultry population, in some cases causing devastating consequences to the industry. In particular, Italy was affected by HP H5N2, LP H7N1 and H7N3 and by a severe HP H7N1 which caused between 1999 and 2000 death or culling of over 16 million birds. In 2003, the Netherlands was affected by the most devastating H7 HP outbreak recorded to date which caused the death or culling of 30 million birds, approximately 50 % of the entire national poultry population. This outbreak also caused several cases of conjunctivitis in humans and was lethal for one veterinarian involved in the emergency operations to put the outbreak under control. It was a prelude to the more significant and worrisome implications of the H5N1 panzootic.

The first detection of the latter was traced to a goose in Guangdong province in China in 1996. Infection spread to Hong Kong in 1997, causing the death or culling of one and a half million birds. Infection appeared to have been eradicated until 2003 when several south-east Asian countries virtually simultaneously notified infection. As infection continued to spread in Asia, it also spread north, affecting a mixed wild-domestic bird population at the Quinghai Lake in late 2005. This was the dawn of the westward and transcontinental spread of H5N1, which, by spring 2006, had affected eastern and central Europe and Africa, causing enormous direct and indirect losses to the poultry industry, wildlife and a significant number of human infections, approximately 50 % of which were lethal.

The entire scientific community was largely unprepared to manage this outburst of infections, and did not have adequate answers to the questions raised by decision-makers and the industry relating to animal health and public health concerns in an evolving eco-epidemiological situation. Information was lacking on appropriate control strategies since, at the time, the only products which were available were inactivated oil-emulsion vaccines developed for outbreaks in developing countries. In addition, there was the urge to develop vaccines with a companion diagnostic test, which enabled the DIVA (differentiation of infected from vaccinated animals) strategy, essential to map infection within a vaccinated population. EU-funded efforts such as FLUAID, AIV VACC DIAGNOSIS and Novaduck addressed specifically these objectives, also exploring the efficacy of vaccination in diverse avian species.

Direct control measures are always a crucial component of control and eradication procedures, as the resistance of viruses in the environment is a key factor that influences, for example, the duration of the cleaning period before restocking. Rivers and FLURESIST have generated significant data on environment-related aspects of prevention and control, and have highlighted the great variability that exists between viruses, even of the same subtype.

The behaviour of avian influenza within a poultry flock, the transmission dynamics and the effects of vaccination on the spread

of infection were also largely unknown, and this knowledge gap impaired educated decision-making in the face of an outbreak, particularly in areas in which poultry was raised at high densities with multiple species and production categories. The Healthy Poultry consortium addressed these issues, analysing retrospectively data that was generated during the Dutch and Italian outbreaks and laying down guidelines for future management approaches.

The H5N1 crises highlighted that HPAI avian influenza viruses can infect a variety of hosts that were previously believed to be resistant, such as wild birds and some mammals. This awareness triggered international efforts on wild bird surveillance performed by the Directorate-General for Health and Consumers and others supported by the Directorate-General for Research and Innovation. Among these New-Flubird, an extensive and comprehensive effort to monitor influenza viruses in wild bird populations across Europe and experimental studies in wild birds, has shed light on previously unexplored areas. The FLUPATH project was, from certain perspectives, a cross-cutting project which aimed at understanding host-pathogen interactions in a variety of avian and non-avian species, including an immunological component to answer open issues on the host's natural and induced immune response.

The spread of H5N1 to three continents in the western hemisphere, and its circulation in under-resourced countries, prompted the EU to bridge gaps of knowledge and communication with countries that were experiencing massive outbreaks, and required international support. At an EU level, the FLU-LAB-NET effort acted as a means by which EU expertise was made available within the EU-27 and to neighbouring countries, together with ConFluTech. The FLUTRAIN effort was focused on bridging gaps across EU borders, with third countries mainly in Africa and Central/Eastern Asia. Specific training was provided to partners concerning general information, specific areas of interest and transfer of technology.

Over the years, the EU has funded two surveillance efforts for influenza in pigs ESNIP2 and ESNIP3. Although swine influenza was not perceived as a major issue for animal and public health, a forward-looking approach allowed the EU to be able to collect surveillance data on swine influenza, which revealed itself as fundamental when the swine-origin H1N1 (2009) pandemic emerged. The FLUPIG project instead was developed in order to address some scientific questions on the adaptation of influenza viruses to the pig and on the emergence of a pandemic virus for humans.

The significant effort made by the Directorate-General for Research and Innovation, guided by Isabel Minguez-Tudela's tireless, open and flexible approach has placed EU scientists in a position of international leadership. We now have a wealth of knowledge which, in many cases, has been used abroad and by the industry, and is considered as complementary to knowledge generated in the United States and by other countries. Above all, the Directorate-General for Research and Innovation's vision has enabled Europe to consolidate existing networks and expand them to developed and under-resourced countries and thus consolidate its position of excellence in scientific research.

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4.1. Avian flu

[AIV-VACC]

Vaccine, diagnostic test development and immunology aspects of avian influenza

Acronym:
AIV-VACC

Project number:
44141

EC contribution:
EUR 1 372 890

Duration:
48 months

Start date:
1 December 2006

Instrument:
Specific targeted research or innovation project (STREP)

Summary

Avian influenza (AI) is a zoonotic disease and seen as one of the most important emerging diseases with serious economic consequences. Although very useful in the fight against AI, 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 infected from vaccinated animals (DIVA principle), these vaccines have to be administered by labour-intensive and expensive parenteral injections. In view of the worrying spread of epidemic AI H5N1 in the world and the large undertaking to vaccinate billions of poultry 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 greatly needed. In the endeavour to develop an improved vaccine against AI, recombinant DNA technology has been employed to generate vectored, subunit or DNA vaccines. Although a wide range of these vaccines have been experimentally shown to be effective vaccines against AI, only a fowlpox-vectored vaccine with H5 gene insert was commercially available at the start of this project. This recombinant vaccine enables differentiation between infected and vaccinated birds by serological tests, including an agar gel immunodiffusion test, which is based on the detection of antibodies against the nucleoprotein. However, the recombinant fowlpox-H5 vaccine also requires administration by parenteral injection. Another major disadvantage is the fact that in cases of previous fowlpox vaccinations, take-up of the vaccine will be prevented and no immunity against AI will develop. This is another serious drawback since fowlpox vaccination is still widely used.

Problem

Inactivated poultry vaccines have disadvantages related to the application methods and the doses of antigen that needed to be used in the vaccine. For mass application in case of an outbreak, these aspects represent considerable disadvantages that could be overcome with live vector vaccines which could be administered via more practical routes such as drinking water, sprays or eye drops.

Aim

The primary aim of this project has been to develop better AI vaccines through live or vector vaccines that can be mass applicable through sprays, drinking water or eye drops. These vector vaccines would offer considerable advantages:

- being mass applicable;
- with less labour-intensive and more animal friendly application;
- providing protection by local and systemic immunity and less interference with eventual maternal antibodies;
- providing more complete protection through cellular and humoral immunity;
- faster onset of immunity when used in the face of an outbreak;
- cheaper production methods.

The project exploited 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 would be developed to be able to respond very quickly to a change in antigenicity of the field virus. Further optimised additional candidate strains were 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 were planned. Such vaccines would also have marker aspects which will allow differentiation of infected from vaccinated animals (DIVA principle). Sensitive, specific and easy-to-use marker diagnostic tests compatible with the vaccines would also be developed.

Results

The activities of the AIV VACC DIAGNOSIS project have been divided into eight work packages (WPs). The project, originally planned for three years was extended to a fourth and final year.

The objective of the first WP was the construction of optimised Newcastle disease virus (NDV) vectors carrying inserts of recent isolates of H5N1 influenza virus. Several recombinants with an H5 insert were generated at different gene junctions and tested *in vitro*. All expressed H5 well and no clear difference of viral replication was observed. But these recombinants experienced some interference from active or passive NDV immunity and therefore did not qualify as a vaccine virus. Another recombinant with better antigenic features was also created with the potential of inducing a sufficient immune response in maternal antibody-positive animals. Finally, additional recombinants were generated with the aim to obtain an NDV backbone with higher virulence. However, these new NDV/AIV vaccine viruses have yet to be tested in animal experiments.

The objective of WP2 was the characterisation *in vivo* of the vector viruses generated by WP1 and the selection of the best suitable potential vaccine strains based on preliminary safety and efficacy testing. The insertion of the haemagglutinin gene of a highly pathogenic avian influenza virus H5N1 and its expression by NDV did not increase the virulence of recombinant NDV. The intracerebral pathogenicity index (ICPI) was 0 compared to 0.3 of the parental vaccine strain. However, a slight enhancement of virulence would be desirable to overcome maternal-derived antibodies against NDV of MDA+ chickens.

The objective of WP3 was to test the efficacy of NDV-H5 in one-day-old chickens with different active and passive NDV protection levels. Previous active immunisation with NDV does interfere with the take-up of NDVH5 in chickens when given by spray vaccination. Besides, the efficacy of NDVH5 is also negatively influenced by the presence of maternal-derived antibodies against NDV. This interference could be related to the low virulence of the NDVH5 vector virus. So, efforts were undertaken to enhance the virulence of the vector backbone. It was decided to broaden the vector scope, and to include work on Infectious Laryngotracheitis (ILT)

virus, another avian influenza vector virus with proven vector capacities and AI efficacy data. But, later on, ILTV vaccines were abandoned from the market. So other NDVH5 vector viruses were prepared and evaluated but none had the desired properties for a vaccine strain, being too virulent for chickens. However, in the meantime, a new more promising construct (rNDVGu) was developed in WP1 that should have better properties in chickens. The strain NDV Gustav induces a solid protection against challenge with the NDV Beaudette virus, only slightly different from that induced by the wild type NDV. So, vector viruses using NDV Gustav as the backbone and inserting the genes encoding for the high or low pathogenic H5 of the Vietnamese H5N1 isolate, were constructed.

The objective of WP4 was the performance of necessary testing for vaccine development and environmental risk assessment purposes in avian and other species. A NDVH5 master virus seed, working virus seed and production virus seed were produced and NDVH5 is genetically stable on passages through embryonated eggs. This was confirmed by sequencing the sequences flanking the H5 gene and the flanking F and HN genes. However, no further work has been conducted according to the original WP description due to the poor efficacy of available virus vectors in MDA+ chickens and in chickens actively immunised against NDV.

The objective of WP5 was to develop and validate diagnostic marker tests that can be used in conjunction with the marker vaccines or conventional vaccines. A large collection of negative and positive serum samples from different avian species and from different geographical regions has been established. Test reagents (proteins and monoclonal antibodies) were developed and used for assay optimisation. A test, the FlockChek® Avian Influenza MultiS-Screen Antibody Test, was developed, validated and approved by USDA for five species: chicken, turkey, duck, goose and ostrich. The kit is now available on the market in the United States and Europe. In Europe, regulatory approval was obtained in Germany. The test was also validated by GD Deventer (Netherlands).

The objective of WP6 was to develop alternative and optimised tissue culture production systems that would create independency of AI vaccine production from egg-based production. A variety of cell lines was studied and although some cell lines could be used, none tested gave titres comparable to the ones obtained in eggs. Due to the uncertainty regarding the choice of the actual vector, work continued using the parental NDV Clone30, a vaccine strain itself. A proprietary suspension-cell platform was chosen as the preferred cell substrate for production but found to be (much) less potent than egg-based material.

The objective of WP7 was to gain an improved understanding of the immunological pathways after vaccination with AI vaccines or infection with AI in chickens: immunophenotyping of vector-induced immunity against AI. Primers and the corresponding probe sets were designed and partially tested allowing the evaluation of Th-1 and Th-2 immune responses as well as CTL activity. The quantitative PCR procedures were tested on in-house lung tissue materials, isolated from earlier animal experiments involving a respiratory influenza (H9N2) challenge. A new primer and corresponding probe (chicken (ch) CD40) were designed and tested as possible B cell markers. In addition two more sets were developed: ChTGF-β (Th3/Treg marker) and Ch28S (a new reference marker). Finally, the Taqman assay was started for ChIFN-α and ChIFN-β.

The objective of WP8 was to develop methods allowing a quick response to changes in antigenicity of the field-virus, and construction of a set of vectors containing other haemagglutinin subtypes (e.g. H7, H9) for vaccination. A NDVH5 vector backbone in which an H5 can easily be substituted by another haemagglutinin was constructed: transcription cassettes were inserted at three different

NDV gene junctions. The difference of the H5 expression level was unexpectedly low, the insertion site is not crucial for the expression of the foreign gene. However, because of the improved characteristics of the new recombinant NDV (rNDVGu), further constructs shall be generated and experiments performed when rNDVGu excites a sufficient antibody response in MDA+ chickens.

Potential applications

The final aim of this project was to develop a H5N1 vaccine that is mass applicable though sprays, drinking water or eye drops on the basis of recombinant NDVH5 or other recombinant vector viruses. In addition, to be able to respond very quickly to a change in antigenicity of the field virus, a system in which gene cassettes for the foreign proteins can easily be constructed and exchanged, was to be developed. The development of a system that allows propagation of virus vaccine in cell lines instead of eggs was started, in order to contribute to decreasing production costs. Finally, an ELISA-based assay would be developed that would enable discrimination between infected and immunised avian species.

Although the mass applicable H5N1 vaccine has not been developed yet, considerable progress has been made with respect to the development of an effective vaccine vector backbone based on the wild-type NDV Clone30 sequence (i.e. rNDVGu). With its improved properties concerning replication and virulence, this vector will be the focus of experiments continuing after this FP6 project has ended.

The expression cassettes that have been constructed during the course of the project, as well as the ELISA-based assay, which has meanwhile been commercialised, will be used as tools in further investigation and optimisation of its properties. Safety and efficacy testing, which has been delayed due to the lack of proper vaccine vector candidates for testing, will be possible once the appropriate constructs have become available. Thus, promising avenues for further experiments have been identified and will be followed after this FP6 project has ended, including work on improved cell culture systems for the production of sufficient virus titres.

As stated above, avian influenza is seen as one of the most important emerging zoonotic diseases with potentially serious economic consequences, both in the industrialised world and in developing countries. Poultry farming, whether it be in an industrial setting, for example, in Europe or the small-scale backyard poultry farming customary in developing countries, as well as wildlife, must be considered a potential source of a serious worldwide influenza outbreak. Therefore, there is an absolute need for more efficacious measures to combat this virus, for instance measures via more efficient vaccination strategies. A well-vaccinated population provides a proven barricader, preventing transmission of the virus. To date, an efficient and effective, mass applicable, AI vaccine that could be used in either of the settings mentioned above is lacking. In addition, there is demand for vaccines that allow a clear differentiation between infected and vaccinated animals (DIVA principle).

The AIV VACC DIAGNOSIS project has brought together a unique European partnership with expertise in the development of diagnostics (Bommeli-IDEXX), AI vaccine research and development (Intervet) and molecular biology of AI and NDV (FLI). Together, these partners have achieved considerable progress towards the aim of developing an efficient and effective, mass-applicable AI vaccine. In addition, the tools that have been developed thus far will allow the DIVA principle to be put into practice, and include an ELISA-based assay that has meanwhile been commercialised.

The project has therefore provided a strong basis for continued research and development in the field of avian influenza vaccine development, both in an academic and an

industrial setting. However, not all the goals of this FP6 project have been achieved: therefore, as is indicated above, work on reaching these goals is ongoing.

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Keywords

avian influenza, viral pathogens of animals, animal immunology, animal diagnostics

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[FLUAID]

Generation of information and tools to support the management of the avian influenza crisis in poultry

Summary

Avian influenza (AI) has become a great risk both for animal and human health. By bringing together both European and non-European laboratories, the FLUAID project aimed to generate data on significant issues relating to AI outbreak management where scientific knowledge at the time of the proposal submission was seen to be lacking.

Problem

Between 2000 and 2005, avian influenza outbreaks caused severe losses to the poultry industry, its stakeholders and, ultimately to the EU taxpayer. It is estimated that 200 million birds died or were culled following infection with influenza viruses subtypes H5 or H7. Approximately 50 million of these birds were from Europe. Most importantly, human infections were also reported in several of these outbreaks. On a global level, H5N1 outbreaks continue to be a serious concern for food security and human health 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 available on several aspects of the disease. This has hampered the adequate management of some of the recent crises resulting in millions of dead animals and raising concerns regarding the loss of human lives and the future management of the pandemic potential of this virus.

Aim

FLUAID's primary goals were:

- to improve scientific knowledge on AI;
- the joint development and application of novel technologies to combat AI infections.

The goals were achieved through the interaction of leading European institutes along with the active collaboration of laboratories in Egypt, Indonesia, Pakistan, South Africa and Vietnam.

Results

In addition to identifying possible strains for an EU vaccine bank and producing new companion diagnostic kits, FLUAID researchers also improved knowledge on pathogenesis and the transmission of the AI virus in individual bird species (e.g. native chickens, turkeys, mule and Muscovy ducks). Of particular note was the age-related association with infection in Pekin ducks, with the data generated suggesting that quail may act as a silent reservoir of AI infection. In generating this data within FLUAID, the consortium

Acronym:
FLUAID

Project number:
022417

EC contribution:
EUR 1 200 000

Duration:
46 months

Start date:
1 January 2006

Instrument:
Specific Targeted Research or Innovation Project (STREP)

promoted European expertise, know-how and scientific achievement globally.

Potential applications

- Using monoclonal antibodies generated within the consortium, three companion diagnostic kits were produced with one of these commercialised successfully.
- Using phylogenetic analysis and serological data, suitable viral candidates for a European vaccine bank were identified.
- Reassortant viruses with rare neuraminidases that could be used as vaccines as part of a DIVA (differentiation of infected from vaccinated animals) strategy were generated.
- Protocols for the identification of the H7 and H5 subtype viruses were successfully transferred to mobile molecular diagnostic platforms.

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Keywords

avian influenza, vaccines, diagnostics

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[Novaduck]

Novel AI DIVA recombinant vaccines for duck

Acronym:
Novaduck

Project number:
044217

EC contribution:
EUR 1 416 380

Duration:
39 months

Start date:
1 January 2007

Instrument:
Specific Targeted Research or Innovation Project (STREP)

Summary

The Novaduck project gathered eight public and private partners that aimed to develop and evaluate new avian influenza live vaccines for ducks based on live vectors and compatible with the DIVA strategy (differentiation of infected from vaccinated animals). Viral vectors have been engineered to express the best protective gene selected in a DNA vaccination model. Two types of vectors (fowlpox and Newcastle disease virus (NDV)) have been evaluated for safety and immunogenicity using newly developed duck-specific immunological tools. Their efficacy was also assessed against highly pathogenic avian influenza (HPAI) H5N1 challenge in both Pekin and Muscovy ducks. The Novaduck project has demonstrated, for the first time, the proof-of-concept of the use of a NDV-based vector vaccine administered once by a non-parenteral route in day-old Pekin or Muscovy ducklings. The level of protection against HPAI H5N1 was further improved by a prime-boost regimen involving two different viral vectors. The compatibility of these vaccines with the DIVA strategy has been confirmed and the effect of vaccination on genetic and antigenic drift of H5N1 has been assessed. The Novaduck project succeeded in achieving most of its initial objectives and valorisation of results is ongoing.

Problem

The ongoing outbreak of H5N1 HPAI has spread from Asia to Europe and Africa and become endemic in several countries, 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 was 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 decreasing AI from the ecosystem.

Aim

The Novaduck project aimed 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 main objectives of the Novaduck project were:

- to identify the optimal avian influenza (AI) immunogenic sequence(s) to be inserted into the selected live vectors;
- to generate and optimise three types of live recombinant vector-based vaccines;
- to 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);
- to assess the safety and immunogenicity of the new vectored vaccine candidates and compare these with those of existing vaccines to select the best vaccine candidate(s);
- to set up a challenge model in ducks for vaccine evaluation of efficacy;
- to measure the efficacy of the most immunogenic vectored vaccine candidates against recent highly pathogenic avian influenza (HPAI) H5N1 and compare it with existing vaccines;
- to study the effect of vaccination on genetic/antigenic drift;
- to 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).

Results

An optimal protective gene was selected using a needle-free DNA vaccination model in SPF Muscovy ducks. The immune response induced by plasmid DNA able to generate retrovirus-based virus-like particles (VLPs) was also evaluated in both mice and Muscovy ducks. This optimal gene was inserted in different viral vectors including poxvirus. The safety of three different poxvirus vectors expressing the same HPAI H5N1 HA (haemagglutinin) gene was found to be excellent in Muscovy ducks. However, the immune humoral response induced by these pox vectors in ducks appeared to be low and transient. A significant increase of immunogenicity was obtained when the pox vector-induced primary immune response was boosted by an inactivated vaccine and the intensity of the boost effect was dependent on the dose of the pox vector.

The optimal protective gene was also expressed in an avian paramyxovirus 1 (APMV1, better known under the name of Newcastle disease virus (NDV)) vector. The HA was shown to be expressed at the surface of NDV virions. The safety of this NDV-based vector vaccine was excellent and the AI HA surface expression did not alter the tissue tropism of the NDV vector in both Pekin and Muscovy ducks. The humoral immune response induced by the NDV vector candidate when administered by a non-parenteral route at day-of-age ducklings was very low or undetectable by the HI test or an H5 blocking ELISA; the

seroneutralisation test was found to be slightly more sensitive but antibody titres were low.

Immunological tools were developed to evaluate the duck immune response. Several monoclonal antibodies recognising the duck interferon gamma (DuIFNγ) were generated but they were unfortunately unable to detect natural DuIFNγ produced by mitogen-activated splenocytes. A real time RT-PCR to quantify DuIFNγ mRNA was then developed and could better detect duck cell-mediated immune (CMI) response than the HD11 cell-based bioassay. AIV-specific IgA, IgM, and IgY ELISAs were also developed to evaluate the humoral as well as the mucosal immune response. These duck-specific immunologicals remained not sensitive enough to reproducibly detect an immune response induced by one administration of the NDV-based vector.

HPAI H5N1 challenge models were developed in Muscovy, Pekin and mule ducks and the Pekin and mule ducks were found to be much more resistant than Muscovy ducks. The fowlpox/inactivated prime/boost vaccination scheme was shown to induce a good level of protection after H5N1 challenge in both Muscovy and Pekin ducks. However, the use of the inactivated vaccine suppressed the possibility to use the DIVA strategy based on commercially available NP-based ELISA. The NDV-based vector vaccine induced a high level and duration of H5N1 protection in all three types of ducks after only one non-parenteral administration at day-of-age in spite of non-detectable or very low antibody response. To our knowledge, this is the first time that a vaccine administered by a non-parenteral route at day-old is shown to induce such level of protection in birds. A heterologous prime-boost regimen using two different vectors further improved the level of protection. In addition, we confirmed that this prime-boost combination of vector vaccines was compatible with the DIVA strategy using internal or commercial NP-based ELISA. The sequence analysis of H5N1 virus recovered from ducks vaccinated and challenged did not show any significant antigenic or genetic drift.

In summary, the Novaduck project succeeded in achieving most of its initial objectives. The NDV-based vector vaccine was shown to be safe and it induced a high level of H5N1 protection in all bird species tested after only one non-parenteral administration at day-of-age. A heterologous prime-boost regimen using two different vectors further improved the level of protection. Further improvement of duck-specific immunological tools will be needed to reproducibly detect the protective immune response induced by one administration of the NDV-based vector.

Potential applications

The work performed within Novaduck has demonstrated for the first time the proof-of-concept of the use of a NDV-based vector vaccine in day-old ducklings. This vector allows the mass application of vaccine at the hatchery and is compatible with the DIVA strategy. Additional studies will be needed to confirm the efficacy profile of the selected vector candidates against other epidemiologically relevant H5N1 isolates and in presence of maternally-derived anti-vector and/or anti-AI antibodies before entering full development. The prime-boost vaccine regimen consisting of a prime with fowlpox vector and a boost with inactivated vaccine can now be evaluated in field conditions since both fowlpox and inactivated vaccines are commercially available. The immunology tools developed for ducks can be used to evaluate the immune response induced by other vaccines in this species. NP-based ELISAs have been thoroughly evaluated on a panel of duck sera and were shown to be valuable as DIVA tests in birds vaccinated with vector vaccines. HPAI H5N1 challenge models have been developed in different duck types and will be useful tools to evaluate current and new AI vaccines for ducks

and to define the standard for evaluation of AI vaccine efficacy.

Overall, the Novaduck project has greatly increased the knowledge of the immune response of ducks, H5N1 challenge models for the different types of ducks and the AI vaccine evaluation and performances to be expected in ducks. Some results of the Novaduck project have already been published in peer-reviewed scientific journals and in another scientific bulletin. Ten abstracts have been presented at seven international scientific congresses and results of the projects have been presented at 13 local scientific meetings and at several seminars with stakeholders. Additional publications containing the latest data on efficacy are being prepared and an exploitation plan has been initiated to valorise the results of the project.

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Project website

http://www.novaduck.eu

Keywords

avian influenza, recombinant vaccines, vectored vaccines, ducks, DIVA, immune response

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[Healthy Poultry]

Development of new integrated strategies for prevention, control and monitoring of epizootic poultry diseases

Summary

When highly pathogenic strains of influenza break out in poultry, the consequences can be devastating. Destruction of infected birds is the primary means of control today but even such drastic measures cannot totally prevent disaster. Given the global spread of the H5N1 AI subtype, policymakers across Europe are looking at revising their strategies to prepare for future outbreaks of epizootic diseases in poultry: that is how to best avoid infection and, if infections occur, how best to limit their spread and impact.

Healthy Poultry aims to assess current scientific understanding of epizootic AI. New strategies will be suggested for their prevention, control and monitoring. Guidelines will be provided 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 GIS system will allow policymakers 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 cooperation 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 EU 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. Fear amongst the population increased because of a possible impact on human health, 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.

Acronym:
Healthy Poultry

Project number:
513737

EC contribution:
EUR 1 119 404

Duration:
45 months

Start date:
1 November 2004

Instrument:
Specific Targeted Research or Innovation Project (STREP)

Strategies and measures for prevention and control of AI need improvement to fulfil 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 to:

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

Results

The main results of the project can be summarised as follows per task.

Spatial and organisational aspects:

- spatial parameters and conversion tables;
- an organisational and economic database;
- a farm economic analysis of poultry production;
- a spatial, structural and demographic database of poultry production;
- a database on migratory birds issues;
- a descriptive analysis of migratory birds issues;
- a GIS-based toolbox for spatial, structural, demographic and basic disease risk analysis;
- a spatial and geo-statistical analysis of poultry production.

Epidemiological aspects:

- an epidemiological analysis of Italian data on AI;
- an epidemiological analysis of Dutch data on AI.

Strategy and economic aspects:

- an analysis of monitoring systems for AI;
- a qualitative regional risk assessment for AI;
- an epidemiological-economic analysis of prevention and control strategies for AI;
- the provision of guidelines for management of AI.

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. AI) particularly at the level of the EU and of Member States. Some of the results will also be valuable for other stakeholders within the poultry production chains, for example integrated production chains, animal health services, product boards.

References/publications

The full scientific report is available on the project's website: there, a link can also be found to the publications originating from the project and the corresponding authors.

Project website

http://www.healthy-poultry.org/

Keywords

avian influenza, epizootic poultry diseases, disease control and prevention, policy and decision-making

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[FLUPATH]

Avian influenza: Impact of virus-host interactions on pathogenesis and ecology

Acronym:
FLUPATH

Project number:
044220

EC contribution:
EUR 1 915 800

Duration:
42 months

Start date:
1 January 2007

Instrument:
Specific Targeted Research or Innovation Project (STREP)

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 multidisciplinary 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 12 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, microarray 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

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 in 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 aimed to generate data on significant issues linked to AI outbreak management on which scientific knowledge is currently lacking. These issues are all related to the virus-host interactions. Four major tasks (work packages — WP) were identified. WP1 studied the host-pathogen interactions. WP2 was dedicated to the ecology and pathogenesis of the viruses. WP3 focused on receptor specificity and interspecies transmission whereas WP4 was concerned with the immunology/innate immunity response in the infected host.

Expected results

The FLUPATH proposal improved 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.

Summary of most important results

WP1 addressed the issue of pathogen-host interactions and virulence determinants at the molecular level. Little was 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 have been examined *in vivo* in different species in animal experiments as well as *in vitro*. Gene-expression profiling and suppression subtractive hybridisation (SSH) were used to identify host genes specifically involved in the reaction to infection by different avian influenza virus genotypes. The involvement and importance of viral genes as well as host genes were evaluated by cross-validation and *in vitro* experiments.

Chicken and ducks were infected either with highly pathogenic avian influenza virus (HPAIV) strain H7N1 or a low pathogenic avian influenza virus (LPAIV) strain H7N1. LPAIV was able to spread systemically in chickens and was even found in the brain. A ‘cytokine storm’ was not found

in chickens due to H7 HPAIV infection as has been found for H5 HPAIV in some mammalian species. However, the cytokine expression was only investigated within the first 24 hours post-infection in the lungs. Analysis of host responses revealed that chicken and ducks respond differently on infection with LPAIV. The virus preferentially replicated in respiratory tissue in chickens and in intestinal tissue in ducks. A notable difference was observed in the interferon response between ducks and chickens in different organs. Whether, and how, the difference in these responses is also correlated with the difference in clinical outcome is not known. Finally, our results confirm the notion that differences in the outcome of influenza virus infection are determined by multiple genetic determinants.

WP2 studied the ecology and pathology of different avian influenza strains in different host species. Specific attention was given to waterbirds such as ducks which seem to be clinically less susceptible to highly pathogenic avian influenza and, therefore, are implicated in transmission of the virus. The Asian lineage H5N1 (HPAIV) caused large numbers of deaths in both poultry and wild bird populations. Recent isolates of this virus have been reported to cause disease and death in commercial ducks, which has only rarely been reported for other HPAI viruses. Although the classic dogma maintains that HPAI virus infection in duck and waterfowl is limited to the respiratory and enteric tract, the results showed that three of the six classic HPAI viruses are able to replicate in locations different to the respiratory and gastrointestinal tract, which can lead to increased lethality for the host. The identification of genetic markers that determine the pathogenicity of HPAI for ducks requires further characterisation, coupled with pathogenesis studies aimed at the understanding of the innate immune response in varying age ducks that allows them to modulate the outcome of HPAI infection.

Experiments on pigs were also conducted 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 results demonstrated that an avian influenza virus has the capacity to infect pigs under experimental conditions but it is not sufficient to allow transmission to another pig or ferret (model for humans). Such transmission events are thought to be involved in the adaptation of an avian influenza virus to a mammalian host. The replication level of an avian influenza virus in pigs is not necessarily a good parameter for its capacity to transmit to another mammalian host. Because the replication of both swine and avian influenza viruses reached the highest levels in the lungs, future studies will have to be performed to assess if there is a correlation with the generation of reassortants in this location.

The NS1 protein is a multifunctional protein in AIV that counteracts the host immune response by blocking the synthesis of type I interferon (IFN). Recent work has identified truncations and elongations of the C-terminus of the NS1 protein of a number of different avian influenza isolates. The aim here was to determine what role these alterations of the C-terminus could play in the pathogenicity of the viruses that possess them. The analysis has clearly shown that the length variations seen between influenza A viruses isolated from different species is not a random event. The removal or addition of specific amino acid residues at the C-terminal of the protein must confer an advantage or disadvantage on the viruses. The effects on the host immune system of viruses carrying different NS1 truncations was also investigated (see WP4).

WP3 tested the role of receptor specificity and neuraminidase activity of avian viruses in interspecies transmission, pathogenicity and the emergence of potentially new pandemic strains. We characterised the receptor specificity of more than 200 viruses

from different avian species. All H7 subtype viruses, irrespective of host species, displayed a poultry-virus-like binding. This may explain the propensity of aquatic bird H7 viruses to cause outbreaks in poultry. Many also showed weak but significant binding to human-type receptors, consistent with the ability of H7 viruses to cause occasional human infections.

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 were studied by using different cells and tissues including tracheal explants from chickens, turkeys and ducks, and cultures of human airway epithelium. In *ex vivo* porcine organ cultures, we could show a clear, but not absolute, correlation between the receptor distribution and the sensitivity of these tissues to infection with a particular virus. Our findings indicate that changes in the binding tropism towards a more avian-like virus reduce (but do not abolish) the virus replication in nasal, tracheal and especially bronchial organ cultures. These results, for the first time, directly demonstrated that receptor specificity of influenza viruses contribute to their distinctive tissue tropism in swine respiratory tract. Our results also indicate that an exclusive change of the binding tropism of a virus is not sufficient to change its host tropism. Therefore, pandemic risk assessments of newly isolated viruses in the field, should not only be based on a change of binding tropism. Finally, our results suggest that the susceptibility of pigs to avian influenza viruses was overestimated in the past (and therefore also its exclusive role as a mixing vessel for the generation of new pandemic viruses).

Studies of different H5N1 showed marginal but statistically significant differences demonstrating that fine distinctions in the receptor specificity of avian viruses may affect viral replication efficiency in human airway epithelium. These findings highlight the importance of further systematic studies in order to identify avian species and viral binding phenotypes with a high potential for zoonotic transmission to humans.

In WP4, gene expression analyses revealed that H5N1 disseminated to multiple organs where immune responses could be identified. Among those cytokines strongly induced following H5N1 infection were the Th1-associated cytokines but not Th2-associated cytokines. The role of the HA protein in eliciting *in vivo* and *in vitro* cytokine responses was also investigated. The data obtained support the 'cytokine storm' hypothesis to explain the particular virulence of HPAI H5N1 in chicken, placing type I IFN and Th1 cytokine responses at the centre of the immunopathological events.

A method to generate chicken bone marrow-derived dendritic cells (DC) was developed. While infection of chicken DC with LPAIV did not affect their activation, HPAIV induced enhanced DC function. Both LPAIV and HPAIV possess the ability to induce type I IFN in chicken leukocytes, implying the presence of a plasmacytoid-like DC population, which efficiently responds to AIV despite the presence of the IFN antagonist NS1. Altogether, this knowledge on the early immune response against infection with avian influenza viruses may be very important for the development of vaccines. Targeting the early innate immune response which starts within hours after vaccination may be a very effective way of controlling virus replication, thereby limiting tissue damage and promoting adaptive immune responses required for protective immunity.

Human pulmonary microvascular endothelial cells had a clearly higher susceptibility to infection by H5N1 HPAIV than to infection by human influenza viruses. This was related to a relatively higher binding capacity to cellular receptors and associated with endothelial cell activation and apoptosis. Reverse genetics analyses

demonstrated a major role for HA in this cell tropism. Overall, avian H5N1 viruses have a particular receptor specificity targeting endothelial cells that is different from human influenza viruses. This H5N1 receptor specificity could contribute to disease pathogenesis.

Investigations on NS1 revealed that specific C-terminal truncations are shared between different influenza A subtypes indicating a potential role in influenza infectivity and pathogenicity. Additional analysis of recombinant viruses also revealed a potential role of these truncations in altering the expression of iNOS with an associated reduction in NO production.

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 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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Project website

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avian influenza virus, virus-host interactions, virus-receptor interactions, host-specificity, expression profiling, virulence, pathology, ecology, innate immunity

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[New-Flubird]

Network for early warning of influenza viruses in migratory birds in Europe

Acronym:
New-Flubird

Project number:
044490

EC contribution:
EUR 1 855 350

Duration:
36 months

Start date:
1 January 2007

Instrument:
Specific Targeted Research or Innovation Project (STREP)

Summary

The overall objective of New-Flubird — to provide Europe with an early warning and risk assessment system for the threat posed to animal and human health by avian influenza (AI) viruses in migratory birds — has been achieved. With the identification of higher risk species by experimental infection in the laboratory, the development of flyway maps and associated identification of high-risk sites, the upgraded skills and expertise levels, the establishment of a sampling network, the integration of the data and development of mathematical models, a firm basis has been obtained for AI virus (AIV) surveillance in migratory birds. The available data, assembled in the New-Flubird database and the models developed allow for investigating the spread of AIVs in different scenarios, on a scale and depth previously not possible. The future challenge is to maintain a baseline sample flow into the New-Flubird database, whereby targeting certain indicator species in defined high-risk regions and possibly at selected times during the year where appropriate capacity and expertise will also need to be assured.

Problem

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

Aim

The urgent development of early warning and rapid response systems to combat

animal and human influenza threats posed by AI viruses from migratory birds.

Results

The waterbird migration in and to Europe was described in maps to facilitate risk assessment and selection of sites for AI sampling in wild birds. Furthermore, a list of higher risk species was developed: a review of the current situation of highly pathogenic avian influenza (HPAI) H5N1 in and around Europe was initiated, leading to a preliminary list in 2007 and resulting in a finalised list, which all ornithological partners within the New-Flubird consortium finalised in 2008. A list of 82 higher risk species was issued and integrated into the New-Flubird database with the technical tools (coding systems) required.

Based on data from the International Waterbird Census (IWC, Wetlands International) and the Important Bird Areas database (Birdlife International), New-Flubird established and published combined migratory flyways allowing for the selection of important sites for inclusion in the early warning system network and for forecasting and modelling movements of birds in the event of an outbreak. All flyway maps were finalised and made available through GIS shape files to be introduced in the New-Flubird database and published in the *Wader Atlas* in 2009.

Also based on the IWC, the high numbers of higher risk species counts per site were mapped for each species and then combined. This analysis allowed the identification and the targeting of sites for strategic surveillance and for establishing a network of semi-continuous sites.

The *Wader Atlas* was officially launched during the African-Eurasian Waterbird Agreement's 15th Anniversary in The Hague, the Netherlands, providing Europe with comprehensive information on 561 populations and 294 waterbird species from 3 020 wetland sites. It has been designed to help people easily obtain information on the most important sites for migratory waterbirds at both national and international level.

At the basis of the New-Flubird activities and results lies the creation and maintenance of a network of sampling sites for sampling AIVs in wild birds as a component of an early warning system. Concerning the mortality monitoring, New-Flubird decided, after consultation with a selected group of advisors coordinating waterbird counting in the framework of the International Waterbird Census (Wetlands International), to reconsider its efforts in relation to the development of a mortality-monitoring programme because this activity was covered by others. Concerning live bird monitoring, New-Flubird has collected over 25 000 samples in over 10 countries across Europe and Africa during the course of New-Flubird, covering the higher risk species sites. The sampling effort was accompanied by a range of on-site capacity-building activities in wild bird capturing and sampling (e.g. duck traps, duck decoys, swan pipes and cannon netting, sample collection, storage and transport, personnel health and safety and animal welfare).

The collected samples were screened for the presence of influenza A virus, the influenza viruses subtypes characterised by haemagglutination inhibition (HI) assays and/or nucleotide sequence analysis. The information on the prevalence of low pathogenic avian influenza (LPAI) and HPAI viruses in wild birds in Europe was integrated into the New-Flubird database, together with over 145 000 samples from the active and passive monitoring of the EU Member States.

New-Flubird contributed to the standardisation of field and laboratory methodology and training of the technical and scientific personnel in three consortium workshops, establishing and validating methods that are robust, repeatable and safe for personnel and wildlife, thereby ensuring a highly qualitative process from sample in the field

to result in the participating laboratories. In addition, New-Flubird has built capacity for influenza surveillance in wild birds by organising training courses and workshops at key regions in Africa and Europe on the crucial skills sets for safe and effective wild bird surveillance.

A large set of representative virus isolates has been characterised genetically by full genome sequencing. This set of virus genome sequences serves as a reference set in the public sequence databases that are used by the scientific community.

Next to the surveillance and capacity-building activities, New-Flubird has also focused on identifying migratory and other wild bird species that could act as 'spreaders' or 'sentinels' for HPAI (H5N1) virus infection and the assessment of the contribution of contacts with migratory and other wild bird species to the overall risk posed by this virus infection to poultry, other birds, and mammals (including humans). To test the hypothesis that wild waterbirds can excrete HPAIV (H5N1) in the absence of debilitating disease and so potentially act as long-distance virus vectors, groups of six species of wild ducks were infected experimentally with HPAIV (H5N1). HPAIV (H5N1) infection caused clinical signs of disease only in tufted ducks (*Aythya fuligula*) and common pochards (*Aythya farina*), both of which are diving duck species. The main clinical signs were neurological and were caused by viral encephalitis. In contrast, the remaining four species of dabbling ducks were clinically unaffected. Pharyngeal excretion of HPAIV (H5N1) varied significantly among the six duck species and cloacal excretion was uncommon. Of the six wild duck species studied, the mallard (*Anas platyrhynchos*) is the prime candidate for being a long-distance vector of HPAIV (H5N1) because it was the only species to show abundant virus excretion without clinical or pathologic evidence of debilitating disease. Pochards and tufted ducks are more likely to act as sentinels for HPAIV (H5N1) in wild bird populations. However, pochards cannot be ruled out as potential vectors because some birds excreted abundant virus in absence of severe clinical signs.

The stress hormone corticosterone contributes to regulating physiological changes that prepare wild birds for migration, but also may lead to higher susceptibility of migratory birds to infection. The effect of these physiological changes on the spread of HPAIV H5N1 in migratory birds is unclear. Therefore, we infected red knots (*Calidris canutus*) with HPAIV H5N1 before the migration period, just prior to migration, at the time of migration, and after the migration period and measured both clinical signs and virus excretion. Red knots infected at the time of migration had pharyngeal virus titres that were strongly positively correlated with plasma concentration of corticosterone. Furthermore, birds that developed severe disease had higher pharyngeal virus titres than those that remained asymptomatic. Therefore, wild birds in migratory condition may shed more HPAIV H5N1 and may be more susceptible to developing severe disease, which may shorten or delay migratory flights, slowing yet not necessarily abrogating the geographical spread of HPAIV H5N1 by wild migratory birds.

At the heart of New-Flubird was the establishment of a web-based database managing surveillance data of AIVs in wild birds

as a central data repository describing and analysing the epidemiology of AIV infection in wild bird populations.

For this purpose, laboratory data are combined with ornithological and environmental data. As a central instrument, a database system has been developed to store, manage and analyse data from the different disciplines. Flanking environmental data are provided as well. Data access rights can be configured independently for different users, and different data types, respectively. Interaction by project participants is possible via a secured Internet connection and a web interface, which provides the different tools and modules for data processing. An exchange of data and information with related initiatives, for example the wild bird monitoring campaigns in the European Union or the Global Avian Influenza Network for Surveillance (GAINS) of the World Conservation Society (WCS), is easily achieved since data structures and coding systems were implemented and designed to preserve compatibility. Emphasis is placed on the integrative process of combining the interdisciplinary data for analysis, which is realised on different levels. Interactive software modules allow for the creation of database queries and target parameters which are shared by the different types of data. Basic and advanced features of the New-Flubird database have been introduced to New-Flubird partners in two workshops.

New-Flubird has developed an agent-based, stochastic epidemiological model assuming a three-species scenario with intra- and interspecies transmissions in mallards, mute swans (*Cygnus olor*) and common pochards at fixed geographic localities and during migration. For all species, juvenile animals were modelled to have a twofold higher susceptibility compared to adults. Course and outcome of HPAIV H5N1 infection for the individual bird was modelled along species-dependent characteristics based on findings from experimental infection studies partly conducted within the framework of the New-Flubird project.

Characteristic migration patterns for different species were also included in the model.

Burn-in runs highlighted parameters to which the model reacted sensitively. By defining rule sets in accordance with H5N1 outbreaks observed in the wild (e.g. regarding observed lengths of outbreaks and measured prevalences), thresholds were defined for these. Changes in the overall length of the epidemic course, as well as amplitude and time point of the number of maximally infected birds were used as readouts.

New-Flubird has achieved the vast majority of its planned results. In some cases, New-Flubird has gone beyond its original plans (e.g. the New-Flubird database contains data from the International Waterbird Census, with millions of records, to provide background on waterbird distribution with time series) whilst in other areas, it did not meet expectations. This includes monitoring the occurrence of dead birds in the field as part of the International Waterbird Census, which has not proven feasible and another approach will be needed. The overall objective of New-Flubird — to provide Europe with an early warning and risk assessment system for the threat posed to animal and human health by avian influenza (AI) viruses in migratory birds — has been achieved.

Potential applications

The knowledge gained from the experimental infection studies has several implications for surveillance in wild ducks. Active surveillance should give priority to mallards and, to a lesser degree, pochards. Sampling should not be limited to cloacal swabs, but should include pharyngeal swabs. Passive surveillance should pay extra attention to tufted ducks and pochards. Sampling of wild duck carcasses should not be limited

to cloacal, pharyngeal, and tracheal swabs and should include internal organs.

The developed agent-based, stochastic epidemiological model allows for investigating the spread of HPAIV H5N1 in various scenarios. Starting with a single-location scenario, each modelling step, corresponding to one day, simulates randomised contacts between individuals of the different model compartments: Susceptible, Exposed, Infectious, Removed (SEIR). By varying the effective contact rates within and between model species, differences in feeding habits and seasonal gregariousness are respected.

When considered in relation to the onset of migration, the chance of migration-capable birds propagating the virus and spreading over larger distances can be examined. Furthermore, the current surveillance schemes in wild birds using the model and data from the New-Flubird database were re-evaluated.

With the identification of higher risk species, the development of flyway maps and associated identification of high risk sites, the improved capacity and expertise levels, and the establishment of a sampling network, a firm basis has been obtained for the AIV surveillance in migratory birds. The available data, assembled in the New-Flubird database and the models developed allow the investigation of the spread of AIV in different scenarios, on a scale and depth previously not possible. The future challenge is to maintain a baseline sample flow into the New-Flubird database, focusing on certain indicator species in certain — high risk — regions and possibly (at certain times) target species at key sites where appropriate capacity and expertise will also need to be assured.

If this network is extended and strengthened, geographic gaps in the surveillance (Caspian Sea, Lake Chad and other areas) can be covered, surveillance of dead birds can be implemented, existing pathology networks can be involved and other new techniques such as blood sampling, feather collection and water sampling can be implemented in the capacity-building activities.

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Project website

http://www.new-flubird.eu

Keywords

avian influenza, virus infections, flyway, database, early warning system, migratory birds, poultry, H5N1, highly pathogenic

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[FLURESIST]

Avian influenza virus survival in poultry commodities, poultry manure and the environment

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 highly pathogenic avian influenza (HPAI) H5N1 is not only spreading through trade but is also carried by wild birds. H5N1-infected wild birds, mainly waterfowl, have been detected in the EU in Germany, Greece, France, Italy, Austria, Poland and Sweden. These findings raise our awareness that H5N1 is becoming ever more endemic in wild birds. The discovery of infected mammals such as cats, leopards, stone martens and raptors that died as a result of infection with H5N1 has uncovered the consequences of this development.

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 in the Middle East and Africa could represent the origin of a pandemic virus for humans, and many 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 on 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, virus survival will be determined in a standardised manner in different environments.

Acronym:
FLURESIST

Project number:
044311

EC contribution:
EUR 870 000

Duration:
36 months

Start date:
1 March 2007

Instrument:
Specific Targeted Research or Innovation Project (STREP)

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 the survival of different AI 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 such commodities at ambient temperatures and at temperatures used to treat poultry products.

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 AI 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 biosecurity measures. Any such response must be driven by an understanding of the behaviour of the virus. Threats to industry include the introduction of the 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 the virus in different materials, particularly avian materials and products, and thus help calm public concerns.

Project website

http://www.fluresist.eu

Keywords

avian influenza, poultry commodities, virus survival, biosecurity measures, disinfection, cleansing operations, public health risk

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[Rivers]

Resistance of influenza viruses in environmental reservoirs and systems

Acronym:
Rivers

Project number:
044405

EC contribution:
EUR 1 395 000

Duration:
36 months

Start date:
1 February 2005

Instrument:
Specific Targeted Research or Innovation Project (STREP)

Summary

The surge of the global avian influenza (AI) epizootic mainly caused by the genotype Z highly pathogenic avian influenza virus (HPAIV) has posed numerous questions, in particular to risk managers and policymakers. Scientific knowledge is scarce 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 is even contradictory. The results obtained with various subtypes of influenza A viruses cannot be extrapolated to the current A (H5N1) viruses without careful consideration. Furthermore, little information is provided regarding the survival of IVs in the air and on 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 routinely applicable before or after a standardised sampling method, that does or does not include concentration.

For 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 the time of restocking. More specific objectives are to: (a) understand the basis of virus survival from a virological viewpoint; (b) understand the impact of physical and chemical elements on virus survival; (c) evaluate the role of environmental reservoirs; (d) propose standardised protocols for the concentration and detection of AIVs in waters, including waste waters, and in different matrices including food; and (e) 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 (HPAI) epizootics associated with zoonotic human cases.

Aim

The overall aim of this project is the prevention and control of AI type A (H5N1) in the animal population through the following specific objectives: (a) gathering data on the survival of AI viruses (AIVs) in natural environments; (b) generating scientific knowledge about the survival of AIVs 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; and (e) elaborating models about

the survival of AIVs in natural environments to demonstrate, in connection with projects relevant to tasks 3 and 4 of the SSP-5B-Influenza call, their perpetuation in nature both in biological and environmental reservoirs.

Expected results

- Criteria for bio-equivalence in relation to virus survival between IV strains.
- 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.
- Data on the prevalence of AIVs in aquatic environments (lakes, ponds and rivers) throughout the year and along the stream of rivers, data on gastropods and bivalve molluscs regarding their potential role as concentrators of AIVs in aquatic biotopes.
- Data (database) on IV survival in the air and on various kinds of surfaces and under various conditions, data on the prevalence of AIVs in the surroundings of farms with present and past outbreaks throughout the year.
- Descriptive, data-driven, low-level simulation models of AIV perpetuation, viability and deactivation in: (a) various water environments, laboratory-controlled and natural; (b) in air in laboratory-controlled environments; (c) in avian faeces and farm manure.
- Multi-scale agent-based simulation model of possible determinants for AIV stability, perpetuation and deactivation.

Potential applications

- Recommendations for the prevention and control of current and future AI 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 European Commission to allow evidence-based policymaking.
- 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.

Project website

http://www.rivers-project.eu

Keywords

influenza, avian, pathogenic, zoonotic, resistance, inactivation, virus, environment, reservoirs, systems, models, restocking

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[FLUTEST]

Improved diagnosis and early warning systems for avian influenza outbreak management

Acronym:
FLUTEST

Project number:
044429

EC contribution:
EUR 1 502 880

Duration:
48 months

Start date:
1 February 2007

Instrument:
Specific Targeted Research or Innovation Project (STREP)

Summary

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

Studies were 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 models include criteria for harmonised diagnostic tests for on-farm outbreak investigation.

A range of diagnostic tools were developed and evaluated alongside a range of commercially available tests. We examined 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 were particularly focused on the validation of tests for use on clinical materials derived from Anseriformes, other wild bird species and selected mammalian species.

Problem

In recent years, AI outbreaks have caused severe losses to the poultry industry, its stakeholders and the EU taxpayer. The ongoing Asian H5N1 outbreak is a serious concern for food security and human health. It is estimated that between 2000 and 2007, more than 200 million birds died or were culled following infection with influenza

viruses subtypes H5 or H7. Approximately 50 million of these birds were from Europe. Human infections have also been reported in several of these outbreaks. In Asia, the crossing of the species barrier represents a serious risk of a new human pandemic virus emerging.

AI is a highly contagious trans-boundary animal disease. Thus, the prompt identification of infected animals is crucial for control and eradication. 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

The project aims were to generate data on issues linked to AI surveillance and outbreak diagnosis and management, on which scientific knowledge is lacking. We also planned to 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.

Results

Mathematical models have been developed that can serve as a blueprint for the design of a surveillance programme for HPAI as well as for LPAI. These models have been parameterised using data from experiments and epidemics. Moreover, a risk assessment model has been developed.

FLUTEST addressed issues of molecular diagnostics, antigen detection and novel technologies. Focusing 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.

Many factors can influence the size, spread and duration of HPAI outbreaks, and also the impact that control measures have on these parameters. Time to initial detection and the time taken to perform tracings of potentially infected premises can have a strong influence. Delays to the initial detection can be exacerbated by problems with the differential diagnosis of poultry diseases that present with similar clinical signs in the early phases of disease.

We developed and evaluated a range of novel molecular based tests for the rapid detection and differential diagnosis of suspected cases of HP avian influenza. Some offered no improvement over EU-recommended methods, for example multiplex PriProET assays, but others offered promise for pen-side testing. A range of control reagents was also developed and validated, potentially leading to better standardisation and harmonisation of testing in the EU Member States. A rapid (compared with sequencing) and sensitive pathotyping method showed notable promise and an international patent application has been filed on the method.

Domestic ducks play a pivotal role in LP and HP AI epidemiology and tools for detection of prior infection by antibody detection were lacking. We developed a range of ELISAs for the detection of antibodies against the H5, H7, N2, N3 and N9 subtypes. Assays for the neuraminidase antibodies are important where vaccination and the DIVA (differentiation of infected from vaccinated animals) principle are applied. We also assessed the performance of four commercial antibody detection ELISAs against the 'gold standard' haemagglutination inhibition (HI) test for poultry sera. The best ELISAs had high sensitivity but lower specificity than HI, although all were > 90 %. An ELISA and sampling regime was developed using egg yolk as a sample. This test has applications for the automated high-volume surveillance of layer flocks at reduced cost to current protocols.

Label-free AI nucleic acid and/or antigen detection was a novel approach we pursued

with the aim of using electrochemical detection sensor devices. However, the sensitivity of these devices currently does not improve on the current PCR-based approach, although we demonstrated the potential for this technology in the future.

The use of microarray for AI sample subtyping was successful both in sensitivity and specificity and could offer advantages over other tests, however the cost of this technology is currently prohibitive.

Potential applications

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

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 impact of FLUTEST is the development and provision of validated diagnostic tests tailored to complement surveillance and outbreak management programmes. The results have indicated that some technologies are not currently suited to AI detection and cannot currently be recommended, and the project has provided confidence that current surveillance protocols are largely 'fit for purpose'.

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Project website

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[FLU-LAB-NET]

Development and enhancement of laboratory networks for avian influenza

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 European Union. This contributes to the strengthening, 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 have been key areas of exploitation, contributing to a European laboratory task force capability for AI in animal species. Rapid, formal interactive communications are addressed through web-based forums. Laboratories involved in influenza research in domestic mammals also participate. FLU-LAB-NET has fostered formal links 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 continue to be 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 global H5N1 HPAI 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. Spread to humans has also highlighted real concerns of the pandemic potential of H5N1 and, more recently, the emergence and global spread of the pandemic H1N1 (2009) virus in humans has reinforced the need for closer integration between veterinary and public health laboratories and demonstrated the benefits in real time.

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, policymakers, 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

- Enhancement and reinforcement of the existing Community Reference Laboratory and National Reference Laboratory network for avian influenza (AI) within the European Union Member States (EU MS) — achieved and ongoing.
- Development and implementation of web-based, global interactive communities facilitating rapid, formal interactive

Acronym:
FLU-LAB-NET

Project number:
044453

EC contribution:
EUR 930 000

Duration:
48 months

Start date:
1 March 2007

Instrument:
Coordination action

communications forums — achieved and ongoing.
- Strengthening harmonisation and development of laboratory and diagnostic methods, coordination of research efforts, and sharing of expertise — achieved and ongoing.
- Facility for rapid responses to national and global emergencies, with data sharing — achieved and ongoing.
- Extension of knowledge sharing and laboratory support to strategically important third-country and INCO partner laboratories — achieved and ongoing.
- Participation of laboratories involved in influenza research in domestic mammals — achieved and ongoing.
- Fostering of formal links and coordination with corresponding human, swine and equine influenza networks — achieved and ongoing.

Potential applications

By reinforcing and enhancing the existing Community Reference Laboratory and National Reference Laboratories network for AI within the EU, FLU-LAB-NET continues to 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 have facilities to optimise rapid, formal interactive communications forums. In turn, the network continues to be extended to participating laboratories involved in influenza research in domestic mammals, and provide a hub fostering formal links and coordination between corresponding human, swine and equine influenza networks.

In addition, FLU-LAB-NET allows 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, are key areas of exploitation, contributing to a European laboratory task force capability for AI in animal species. FLU-LAB-NET provides opportunities for the identification and development of the complementarities of both EU and global multidisciplinary influenza research programmes. This promotes 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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Project website

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avian influenza, laboratory, network, FLU-LAB-NET

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[FLUTRAIN]

Training and technology transfer of avian influenza diagnostics and disease management skills

Summary

The ongoing worldwide avian influenza (AI) crisis has highlighted the need for comprehensive training and the transfer of technology to accession and International Cooperation Countries (INCO) with the clear goal of aiding these countries in combating AI with the provision of the most up-to-date diagnostic and disease management procedures. The FLUTRAIN project aimed to fulfil this requirement at two levels.

- It resolved the need for training by providing two workshops over the duration of the project that allowed experts in the AI field to pass on their expertise in the diagnosis and management of AI to participants from accession and INCO countries. Follow-on training opportunities were provided in partner laboratories in order to consolidate the information and practical experience gained during the workshops. In addition, FLUTRAIN identified and supplied funds for one-off specific support missions that targeted specific AI problems in recipient countries. The project partners have also developed an e-learning course (E-Flu) for AI that was designed to support veterinary administrations in training newly recruited staff involved in AI prevention, diagnosis and control activities.
- The transfer of technology to accession and INCO countries via the provision of new, simplified and cost-effective diagnostic methods and reagents. It also involved the transfer of deliverables, both for serological and virological diagnosis that were developed in three European projects namely AVIFLU, LAB-ON-SITE and FLUAID. In 2009, the global outbreaks of pandemic H1N1 highlighted the unpreparedness of many laboratories to deal with such a crisis particularly if the virus infected pig populations. In order to address this issue, the project was amended so that important data on the immunity of pig populations to pandemic H1N1 could be generated and transferred to laboratories in developing countries.

The consortium was made up of 11 members (including two SMEs) in addition to eight associated partners, many of whom were recipients of training.

Acronym:
FLUTRAIN

Project number:
044212

EC contribution:
EUR 1 809 133

Duration:
51 months

Start date:
1 March 2007

Instrument:
Coordination action

Problem

Avian influenza, and more recently pandemic H1N1, infections have increased in relevance both from animal and human health perspectives. With the extension of the H5N1 epidemic from Asia to eastern Europe and Africa and the global spread of H1N1, notwithstanding the efforts of international organisations, there is clear evidence that in the current situation, attempts to stop the infection's progress are insufficient. 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, and possibly H1N1 in swine populations,

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 technologies. The objective of FLUTRAIN was therefore to bridge the gap of knowledge between EU scientists and colleagues in AI-affected countries. This project also had the objective of supporting countries affected by AI infections through training and the transfer of knowledge and technology.

As a result of the global H1N1 (2009) pandemic, FLUTRAIN also covered important aspects of swine influenza. In 2009, it was not known to what extent pigs were immune to infection with the pandemic H1N1 virus. Indeed, many developing countries do not have the expertise or facilities to answer such questions and so, using the European situation as a model, data were generated to determine the cross reactivity of the EU swine population to pandemic H1N1 (2009).

Results

1. Data on alternative methods for virus isolation and detection.
2. Development of alternative methods for reagent production and improvement.
3. Training of diagnosticians and scientists in EU laboratories.
4. International training workshops addressing general and specific topics related to AI.
5. Two international meetings in collaboration with OIE, FAO and WHO to discuss AI at the human-animal interface.
6. Ad hoc support and training missions in Egypt, Senegal and Sierra Leone.
7. Generation of online course on avian influenza management and control (E-Flu).
8. Generation of important tools and information on the immunity of pig populations to pandemic H1N1.
9. Generation of important tools and information on avian H1, H2 and H3 subtype viruses

Project website

http://www.flutrain.eu

Publications

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Keywords

avian influenza, training, technology transfer, INCO

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[ConFluTech]

Capacity-building for the control of avian influenza through technology transfer and training

Acronym:
ConFluTech

Project number:
44462

EC contribution:
EUR 547 255

Duration:
60 month

Start date:
1 July 2007

Instrument:
specific support action

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).

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. To fill 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 Iraq, Turkey and Vietnam. 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 project 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.

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.

Activities

A number of activities, including technical workshops, courses, training and collaborations were organised at both national and regional levels.

In the context of collaboration, links were established to:

- authorities in charge of veterinary and human public health, research centres and universities in many countries;
- a number of other EU-funded projects such as FLUTRAIN, LAB-ON-SITE, Income, ASEM-Dialog, EPIZONE, etc., dealing with avian influenza or other infectious diseases;
- FAO, European Society for Veterinary Virology, CIRAD and the FGI-ARIAH in the Russian Federation.

Through these links, it was possible to extend the activities of ConFluTech to central and eastern Europe, the Middle East,

Central Asia and North Africa. Thus, seminars and technical workshops for veterinary staff from Croatia, Czech Republic, Estonia, Hungary, Lebanon, Lithuania, Macedonia, Mauritania, Moldova, the Palestinian National Authority, Serbia, Slovakia, Slovenia were successfully organised. The seminars and technical workshops held by ConFluTech covered areas of great relevance for the control of the diseases including:

- biosecurity at farm and diagnostic laboratory level;
- epidemiology, monitoring and surveillance tools;
- sampling and transport of biological material;
- preparation of awareness booklets in different languages.

To cover these fields, experts in the fields of management of poultry and poultry diseases, laboratory diagnostics, biosecurity, outbreak management, epidemiology and disease monitoring and surveillance were invited. These included Prof. Hafez Ahmed Hafez, Head of the Institute of Poultry Diseases from the Free University of Berlin and President of the World Society for Poultry, Dr Abdulwahab from the same institute, Dr Filip Claes, Institute of Tropical Medicine, Antwerp and Dr Mohammed El-Nator, Veterinary Faculty, Jordan Technical University, Irbid.

In summary, a total of 22 technical workshops and workshops were held on a national or regional basis in the following countries: Armenia, Austria, Bulgaria, Georgia, Germany, Greece, Iraq, Jordan, Mauritania, Romania, Russia, Slovakia, Sweden, Syria and Turkey.

Dissemination activities

The following information has been disseminated.

- Distribution of a booklet, containing information about the project aims and activities as well as six standard operation procedures (SOP).
- Distribution of CDs containing presentations and protocols of the workshops.
- Distribution of a special issue of *Transboundary and Emerging Diseases*, Vol. 55, number 5–6, August 2008, containing the proceedings of the Income project held at the Lisbon meeting, 2007; supplemental issue of *Vaccine*, Vol. 26, Suppl. 6, December 2008 containing proceedings of the ICTTD-3 meeting held in Borstel, 2007.
- Maintenance of a specific website for this purpose (http://www.conflutech.net).

Keywords

avian influenza, polymerase chain reaction, epidemiology, disease outbreak

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4.2. Swine flu

[ESNIP 2]

European Surveillance Network for Influenza in Pigs 2

Acronym:
ESNIP 2

Project number:
022749

EC contribution:
EUR 300 000

Duration:
36 months

Start date:
1 January 2006

Instrument:
Coordination action

Summary

The European Surveillance Network for Influenza in Pigs (ESNIP) 2 maintained and expanded a surveillance network that was established during a previous EC concerted action (ESNIP 1, QLK2-CT-2000-01636). The main aim was to gain a better understanding of the epidemiology and evolution of swine influenza virus (SIV) in Europe through an organised surveillance programme and extensive antigenic/genetic characterisation. The data were used to improve the diagnosis of SIV by updating the reagents used in classical techniques and by the development of a rapid molecular test. The virus bank and electronic database of ESNIP 1 were expanded. In addition, we performed a serological monitoring of swine for avian influenza (AI) viruses. Influenza viruses currently circulating in European swine were compared with those in avian species and humans as well as with influenza viruses circulating in southern China and the United States.

Problem

The epidemiology of swine influenza in Europe has become particularly complex. At least three SIV subtypes (H1N1, H3N2 and H1N2) are circulating and new reassortants between these subtypes have been detected occasionally. The heterogeneity in SIVs has important implications for diagnosis and control. The strains used in serodiagnostic tests need to be matched to the current epidemic viruses, and inactivated SIV vaccines should contain all prevailing subtypes to ensure broad protection. So far, there had been little surveillance for SIVs and there was a lack of standardised reagents and protocols for subtyping and antigenic/genetic characterisation of SIV. A rapid and simple test would be valuable. Finally, pigs are known to be susceptible to infection with both human and AI viruses, but the true public health risk of this was unknown, due to a lack of screening of pigs for influenza virus from other hosts.

Aim

The first objective of ESNIP 2 was to expand our knowledge of the epidemiology and evolution of SIVs in Europe, and to apply this knowledge to optimise diagnostic techniques for swine influenza. Therefore, we aimed to:

- keep track of major changes in the epidemiology of SIV in Europe;
- study the extent of antigenic and genetic evolution of SIVs;
- improve the diagnosis of SIV;
- expand the SIV bank and electronic database of ESNIP 1.

The second objective of ESNIP 2 was to provide insights into the public health risk of influenza in swine by monitoring swine for AI viruses and by comparison of influenza viruses in swine and in human populations. Therefore, we aimed to:

- screen European swine populations for the circulation of AI viruses;

- compare the influenza situation in swine with that in humans and birds, and ensure dissemination of information to human and AI researchers.

Major outcomes are:

- maintenance and expansion of the pig surveillance network;
- data about the prevalence and circulation of SIV subtypes in swine in Europe and comparison with the situation in China and the United States;
- antigenic and genetic characterisation of the isolated SIVs;
- recommendations for the reagents to be used in classical diagnostic tests for SIV;
- standardisation and validation of rapid tests for the diagnosis and characterisation of SIVs;
- maintenance and expansion of the SI virus bank and electronic database;
- first approaches in the development of a serologic assay for AIVs in swine;
- formal interaction between the swine, avian and human surveillance networks.

Potential applications

ESNIP 2 improved and facilitated 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, it provided insight into the SIV subtypes that are currently circulating in Europe and their antigenic characteristics in comparison with vaccine strains. These insights are crucial in the decision whether changes in the vaccine strain composition may be required. Furthermore, ESNIP 2 guaranteed transparency of animal disease status worldwide. It helped to collect, analyse and disseminate veterinary scientific information to Member States in order to improve their methods for control of influenza.

Project website

http://www.esnip.UGent.be

Keywords

influenza, swine, surveillance, evolution, diagnosis, public health risk

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[ESNIP 3]

European Surveillance Network for Influenza in Pigs 3

Acronym:
ESNIP 3

Project number:
00259949

EC contribution:
EUR 1 000 000

Duration:
36 months

Start date:
1 November 2010

Instrument:
Coordination and support action

Summary

This European Surveillance Network for Influenza in Pigs (ESNIP) 3 will maintain and expand surveillance networks established during previous EC concerted actions (ESNIP 1, QLK2-CT-2000-01636; ESNIP 2, SSPE-022749). Three work packages (WP2, 3, 4) aim to increase the knowledge of the epidemiology and evolution of swine influenza (SI) virus (SIV) in European pigs through organised field surveillance programmes (WP2). Virus strains detected in these programmes will be subjected to detailed characterisation both antigenically (WP3) and genetically (WP4) using standardised methodology. Specifically, this will involve timely information on genomic data and generation of antigenic maps using the latest technology. These analyses will provide significant and timely added value to knowledge of SIV. A strong focus will be monitoring spread and independent evolution of the pandemic H1N1 (2009) virus in pigs. All these data will, in turn, be used to improve the diagnosis of SI by updating the reagents used in the recommended techniques (WP2). The virus bank and electronic database that were established during ESNIPs 1 and 2 will also be expanded and formally curated with relevant SIV isolates and information for global dissemination within and outwith the consortium (WP5). ESNIP 3 represents the only organised surveillance network for influenza in pigs and seeks to strengthen formal interactions with human and avian surveillance networks previously established in ESNIP 2. A timely and transparent interaction with these networks will be a key output. These approaches are entirely consistent with improved pandemic preparedness and planning for human influenza whilst providing an evidence base for decisions in relation to veterinary health. The project consortium consists of 24 participants, which contribute a blend of different specialisms and skills ensuring multi-disciplinary cutting-edge outputs. The vast majority of the partners are actively working with SIV, some in a field setting. Twenty-one participants are from 11 EU Member States, seven of which were actively involved in ESNIP 2. Cooperation with partners in China and North America will continue to promote a greater understanding of the epidemiology of SIVs at a global level.

Problem

SI is enzootic in the major swine-producing countries of Europe and the epidemiology of SI in Europe is recognised to be different from that in Asia or North America. However, unlike human or equine viruses, organised surveillance for SIVs only began relatively recently. Gaps in surveillance in pigs have been criticized by public health officials in the light of the emergence of the pandemic H1N1 (2009) virus putatively from pigs. This large European consortium is critical in ensuring that uncertainties over the epidemiology of SI in European pigs are thoroughly addressed.

Three antigenic and genetically distinct swine influenza virus subtypes (namely avian-like swine H1N1; human-like swine H3N2 (reassortant of human and avian viruses); and swine H1N2 (reassortant of human and avian viruses)), have

co-circulated for many years within the swine population in Europe. However, the completed ESNIP 1 and ESNIP 2 coordinating actions showed that the prevalence and incidence of individual subtypes may vary from one country or region to another. For example, the H3N2 virus seems to have disappeared from some regions, whereas the H1N2 virus is becoming one of the most prevalent subtypes in others. Furthermore, new reassortant viruses, not only between the three endemic SIV subtypes, but also between SIV and seasonal human influenza viruses, have occasionally been detected during the last 10 years. Recently, isolated outbreaks of infection with the pandemic H1N1 (2009) virus (pH1N1) have been reported in several pig herds in the world, including Europe. The continued spread of this pandemic virus of potential swine origin in the human population and the demonstrated high susceptibility of pigs to the virus makes it likely that the risk of it entering pig farms in Europe will increase in the foreseeable future. In fact, endemnicity can be expected based on the ease of transmissibility between pigs and parallels with previous human pandemic strains that became established in global pig populations. Expansion and consolidation of the detection and identification of swine influenza viruses in pig herds in Europe is necessary to provide new data about potential changes in the epidemiology of the three endemic European SIV subtypes, as well as adaptation and circulation of novel reassortant viruses in European pig herds and the introduction, and possible ongoing transmission, of the pH1N1 virus into European pigs. Recent reports of a novel H3N2 human-avian reassortant virus emerging in pigs and spreading to mink, despite no reported detection in swine, demonstrates the importance of a coordinated surveillance network for monitoring pigs in Europe for influenza viruses.

Aim

To build on the achievements of ESNIP 1 and 2 which were:

1. the standardisation of protocols for swine influenza (SI) virus (SIV) isolation, serology, antigenic and genetic typing of SIV isolates;
2. the selection and production of reference virus strains and (hyperimmune) sera — these were made available to all participants for preliminary subtyping of SIV isolates;
3. the establishment of a central SIV bank with a collection of recent isolates from various geographical areas in Europe;
4. the establishment of an electronic database with relevant information on the SIV isolates that were obtained in different countries during the life of the network;
5. the antigenic and genetic characterisation of a number of recent H1N1, H3N2 and H1N2 SIV isolates from different European countries;
6. the organisation of a serological survey to obtain preliminary data on the prevalence of different SIV subtypes in various European countries.

Expected results

ESNIP 3 represents the largest structured consortium delivering coordinated surveillance for influenza in pigs in Europe to date. This will be an invaluable resource to officials responsible for veterinary and public health alike. The coordination action will directly impact on the diagnosis and control of SI in Europe and thus enhance the welfare of swine and the profitability of swine farmers. In addition, it will increase our understanding of the public health risks of influenza in swine. Comprehensive information relating to the epidemiology and evolution of swine influenza in pig populations across Europe will be made available. This, in turn, will enable a robust scientific evidence base to be available when assessing public health risk from SI, directly contributing to the production of policy documents and risk assessments prepared by ECDC. Such enhanced interaction will be timely following the emergence of the pandemic

H1N1 (2009) virus that has already been detected in pigs in Europe. The continued evolution of this virus in pig populations will be a key output from the project and will be of strategic benefit especially for public health. Furthermore, the authorities' position will be strengthened when handling these issues in the knowledge of a coordinated surveillance network addressing influenza virus in pigs. Context at a global level will be facilitated through interaction with key global bodies (i.e. WHO) and institutes furthering the EU preparedness and know-how in this subject area.

Potential applications

Strategic impact: ESNIP 3, as the only organised surveillance network for influenza in pigs, will strengthen formal interactions with human and avian surveillance networks. Specific innovation-related outputs are the improvements to and validation of rapid tests for the detection of SIVs. Significant improvements to the way SIVs are characterised both antigenically and genetically will be realised through the application of the latest technologies by international experts in their respective fields. This project will deliver the first complete antigenic maps of European SIVs using cutting-edge technologies whilst whole genome characterisation of these viruses will be carried out on a scale not achieved previously. The latest tools for understanding virus evolution will be used to analyse the genetic data sets.

Contributions to standards: Standardised protocols (conforming to those specified in the OIE Terrestrial Manual) and reference reagents, for both antigenic and genetic characterisation agreed during ESNIP 2 will continue to be used and updated where applicable.

Policy developments: Epidemiological data on the circulation of avian/human influenza viruses in swine and their comparison with viruses circulating in avian/human populations will increase our understanding of the zoonotic potential of influenza in swine. These data will be communicated to decision-makers and authorities in both veterinary and public health spheres. Continued evolution of the pandemic H1N1 (2009) virus, should it become established in European pigs, will provide an evidence base to policymakers on the need for enhanced disease control measures for this non-notifiable disease. Policy documents have already been produced at EU level and are subject to continual review in the light of developments in the field. This project will directly inform the decision-making process through determining the level of threat for both swine and human health as continued evolution of the virus is monitored. Recommendations on reagents to be used for SI diagnosis and on SIV vaccine strain composition may have an impact at national or international level. Data from WP2 (serological screening of swine for avian influenza viruses) may have a significant impact on the control of avian influenza if developments indicate changes in the normal host range of notifiable avian influenza viruses. This, in turn, could have a significant influence on the design of control strategies for avian influenza even though some provision is made in Council Directive 2005/94/EC.

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Project website

http://www.esnip3.eu/

Keywords

swine influenza virus, surveillance, serology, antigenic cartography, genetic characterisation

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[FLUPIG]

Pathogenesis and transmission of influenza in pigs

Summary

Pandemic influenza viruses come from wild birds, but must adapt to efficient replication and transmission in humans to cause a pandemic. Pigs are considered important intermediate hosts in which avian viruses can adapt to mammals before they transmit to humans. However, the exact role of the pig and the nature of the genetic changes required for efficient replication of avian viruses in pigs and for subsequent virus transmission between pigs, from pigs to humans and between humans is still largely unclear. FLUPIG aims to study the role of adaptive mutations, genetic reassortment, host and environmental factors in the adaptation.

The occurrence and severity of a pandemic also depends on the immune status of the human population. FLUPIG will study the extent and mechanism of cross-protection between antigenically different H1N1 influenza viruses (heterovariant cross-protection), and between influenza viruses belonging to different haemagglutinin subtypes (heterosubtypic cross-protection). We will also evaluate the capacity of novel generation vaccines to broaden cross-protection.

Problem

The mechanisms by which influenza viruses gain the capacity to abandon the animal reservoir and become widespread in human beings are largely unknown. The pig is believed to play an essential role since pigs are susceptible to all subtypes of influenza A viruses, including those of avian origin, and have receptors for both avian and mammalian origin viruses. As such, they represent an ideal vessel for viral reassortment or adaptation. However, there is no direct evidence that pigs played a role in any of the three pandemics of the 20th century. Only the 2009 pandemic (H1N1) influenza virus almost certainly comes from pigs. It is still unclear why and how this novel H1N1 virus obtained the capacity for human-to-human spread. It is also unclear what role is played by genes of avian origin in the generation of pandemic viruses, particularly in view of the widespread infection of poultry with H5N1 and H9N2 viruses.

Another aspect in the influenza ecology is that pigs support the endemic circulation of viruses that belong to the same subtype as viruses that have been shown (to date) to cause pandemics in humans. Understanding how the immune system of pigs responds to infection and the extent of cross-protection within and between virus subtypes is crucial to limiting the degree of circulation of these viruses in pigs, as is the development of novel vaccination strategies. Cross-protection studies in both pigs and ferrets will also help us to understand the susceptibility of humans to swine-origin influenza viruses.

Aim

We aim at gaining new insights into the role of pigs in overall influenza ecology, with particular reference to the generation of human pandemic viruses. In order to allow us to more accurately predict, respond to, and control such events, in-depth research

Acronym:
FLUPIG

Project number:
258084

EC contribution:
EUR 4 854 452

Duration:
54 months

Start date:
1 July 2010

Instrument:
collaborative project

on the pathogenesis and the transmission of influenza viruses between pigs and from pigs to other relevant species is essential. In particular, we want to improve our knowledge on the gene constellation and genetic interactions that are necessary to generate pandemic viruses in combination with an improved understanding of host-dependent variables such as receptor distribution and immune response. Combined with improved surveillance for influenza in animals, effective vaccines and antiviral drugs, this knowledge will be critical to the control of future influenza pandemics.

Expected results

The use of state-of-the-art technologies will allow us to develop advanced and innovative knowledge on virus-host interactions, specific factors that determine species barriers and replication efficiency of influenza viruses of various origin, and immune mechanisms that generate protection against homologous and heterologous influenza virus subtypes. This knowledge is of critical importance to assess the probability and risk of transmissibility of influenza viruses from swine to other mammalian hosts, and further spread within mammalian hosts.

The knowledge generated by FLUPIG will provide clear insight into the role of pigs in overall influenza ecology and, in particular, in the zoonotic potential of SIVs. Consequently, it will allow us to improve prevention and control strategies for human influenza pandemics.

Potential applications

Various approaches will be used to design and test experimental live-attenuated influenza virus vaccines. The results of these studies will allow us to conclude on a rational vaccine design. Furthermore, a large collection of genetically modified influenza virus mutants will be generated. The primary goal is to use these mutants to study the effect of certain mutations on pathogenesis and transmission of influenza virus. However, certain mutants may turn out to be potential vaccine candidates.

Project website

http://www.flupig.ugent.be/index.html

Keywords

pig, influenza, pathogenesis, transmission, cross protection, pandemic, H1N1, genetic adaptation

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This publication gathers information on initiatives funded by the European Commission over the last decade regarding animal health challenges. There are some general actions which have been developed for the harmonisation of European funding schemes, for the prioritization of research topics or for implementing improved surveillance systems on emerging and re-emerging diseases. Several projects concern notifiable diseases, which are often highly epidemic and are frequently regulated by the culling of infected animals. The goal pursued is to achieve the capacity to react more quickly by using fast and reliable diagnostics tools, but also to develop alternative disease eradication protocols through new generation vaccines. Regarding endemic diseases, which decrease the efficiency and profitability of the livestock sector and are difficult to phase out, the aim is to progressively reduce their incidence. Finally, there is a chapter which gives information on projects targeting the influenza viruses, for controlling animal infection and improving our knowledge on cross-species infection within a “one health” vision.

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