

EUROPEAN COMMISSION

Control of infectious diseases

**Catalogue of research projects
in the fifth framework programme
Infectious diseases of livestock and aquaculture animals**

Directorate-General for Research
Key Action 2 — Control of infectious diseases

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Luxembourg: Office for Official Publications of the European Communities, 2005

ISBN 92-894-9696-7

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Printed in Luxembourg

PRINTED ON WHITE CHLORINE-FREE PAPER

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Generic coronavirus vaccine vectors for protection of farm animals against mucosal infections

<i>Project number</i>	QLK2-CT-1999-00002
<i>EC contribution</i>	917.346 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st February 2000

Summary

Coronaviruses cause major diseases in poultry (infectious bronchitis virus, IBV) and swine (porcine transmissible gastroenteritis virus, TGEV). Our recent development of reverse genetics systems for these viruses, and a targeted recombination system for murine hepatitis coronavirus (MHV; a model system), is enabling us to modify the large (~30 kb) RNA genomes of these viruses with the objective of making better vaccines and, in the case of TGEV, using a coronavirus as a vector for the expression of genes of porcine circovirus type 2.

Problem

Conventional coronavirus vaccines, like those against other RNA viruses, are genetically unstable, reversion to virulence by point mutations being a problem. Antigenic variation amongst field viruses reduces the effectiveness of existing vaccines. Also problematic is distinguishing vaccinal virus from very similar virus isolated in the field. Porcine circovirus type 2 (PCV 2), unrelated to coronaviruses, is associated with post-weaning multisystemic syndrome (PMWS). Greater understanding of the disease is needed and a vaccine is required.

Aim

At the heart of this project is the highly specific modification of IBV, TGEV and MHV genomes to generate new information that can be used in the future to produce stable recombinant viruses with potential for vaccinal use. It might also be the case that attenuated TGEV can be used as a vector for the immunity-inducing genes of other pathogens, in this case PCR 2.

Expected results

A system for modifying the IBV genome very specifically, and a recombinant IBV to establish proof of principle.

Recombinant MHVs with knocked-out genes, to identify essential genes, and with gene rearrangements, with assessments of their pathogenicity.

Recombinant TGEV suitable for expressing foreign genes, specifically with PCV 2 genes.

Identification of the role of PCV 2 in PMWS, the immune responses to it and which proteins are the most immunogenic.

Assessment of the potential of recombinant TGEV as a vector vaccine.

Potential applications

The pioneering work of this project will underpin the development of rationally designed vaccines i.e. vaccinal strains based on sound scientific knowledge and highly specific genetic alterations, by vaccine manufacturers. Such new vaccines should be safer (stable), identifiable and appropriate to what is in the field. The results might indicate that TGEV can be used as a vector against other pathogens.

Keywords

Coronavirus, vaccine, vectors, poultry, pigs, circovirus.

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Development of an efficacious vaccine against animal brucellosis that is harmless for humans

<i>Project number</i>	QLK2-CT-1999-00014
<i>EC contribution</i>	1.465.442 €
<i>Duration</i>	60 months
<i>Type</i>	RS
<i>Starting date</i>	1st January 2000

Summary

Currently available live vaccines used for medical prophylaxis of brucellosis present serious side effects which restrict their use and require good managing procedures for sanitary authorities and the farmer to obtain a definitive eradication. The most crucial of these drawbacks is that the live vaccines, whilst attenuated, still remain fully virulent for man. In addition, the vaccination is not fully efficacious and interferes with the diagnosis of field infection. Because of the continuing European incidence of brucellosis in animals and man, the development of a safe and efficacious vaccine that do not possess these disadvantages but which does stimulate protective immunity against the major pathogens of the genus *Brucella* is highly desirable and justified. The objectives of the proposal are to obtain well defined (rationally) attenuated vaccine strains or an efficient DNA vaccine that are immunologically distinguishable from natural infection and do not present the drawbacks of existing vaccines.

Problem

Brucellosis is considered by the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) as the most widespread zoonosis in the world severely affecting livestock and public health. The disease principally affecting cattle, sheep and goats, causes enormous economic loss due to its effect both on animal health but also its effect on international trade in animals and their products. The major practical problems associated with the control and epidemiology of brucellosis in animals and man are:

1. The need for a safe and more efficacious vaccine;
2. The need to distinguish between a naturally infected and a vaccinated animal.

Thus it is necessary to obtain well defined (rationally) attenuated vaccine strains that are immunologically distinguishable from natural infection or an efficient DNA vaccine. We develop a 4 years program of fundamental and applied research to address these questions.

Aim

Brucellosis is considered by the FAO and the WHO as the most widespread zoonosis in the world, severely affecting livestock and public health. In Europe: While nearly eradicated from cattle herds in northern parts of the European Community (EC), brucellosis in sheep and goats caused by *B. melitensis* remains a severe and worrying problem in most of the Mediterranean countries of the EC. The disease is responsible for important economic losses through interference with breeding programs and reduction in milk yield. It also hampers the control of bovine brucellosis. The key to controlling human brucellosis is to reduce the animal disease. It is obvious that to control the disease in the worst affected regions, vaccination is the only effective strategy. The current vaccine for *B. melitensis* (called Rev.1) is a live attenuated strain. Unfortunately, Rev.1 is not fully efficacious, can still cause abortion in animals, is occasionally excreted in milk and is fully pathogenic for humans. The objective of the project is to obtain well defined (rationally) attenuated vaccine strains that can be immunologically distinguishable from natural infection.

Expected results

Identification of virulence factors. Rationally attenuated strains by deletion of the virulence genes. Identification of the type of immune response (Th1/Th2; CD4/CD8; T \square □). Building safeguard that will impair the development of the bacteria in human. Possibility to use the DNA from *Brucella* to obtain DNA vaccines. Construction of a diagnostically distinguishable vaccine. Analysis of the efficiency and safety of the vaccine strains constructed.

Potential applications

The objective of the project is to obtain a new vaccine that will be traded by the industrial partner VETOQUINOL.

Keywords

Brucella, vaccine, attenuation, DNA, immunity.

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Pathogenesis, epidemiology, immunopathology, and diagnosis of post-weaning multisystemic wasting syndrome (PMWS): an emerging disease of swine due to new porcine circovirus (PCV2). Development of recombinant vaccines

<i>Project number</i>	QLK2-CT-1999-00307
<i>EC contribution</i>	1.199.467 €
<i>Duration</i>	42 months
<i>Type</i>	RS
<i>Starting date</i>	1st January 2000

Summary

Postweaning multisystemic wasting syndrome (PMWS) is an emerging disease of pigs, first recognised in 1996, caused presumably by the porcine circovirus type 2 (PCV2). The disease is spreading rapidly in several European countries affected, and is becoming a primary cause of economical loss. Preliminary work has focused on the description of the disease and lesions but detailed studies on the disease, and of the causal agent have not been carried out. These studies are a pre-requisite for development of prototype vaccines, the end-deliverable of this project. The project will help to a better definition of the disease and of its etiological implications, and, in this way, it will provide scientific and technical basis for establishment of Community rules and policies regarding this infection.

Problem

Infectious diseases in farm animals cause severe economic impact due to direct losses and trade barriers, and may represent a risk for human health. The proposed research aims at generating knowledge on pathogenesis, immune response and epidemiology of a newly discovered swine disease, in order to develop a rational design of vaccines against the disease. The final deliverable of the project is the formulation of candidate vaccines against postweaning multisystemic wasting syndrome (PMWS), which is a declared objective to be supported by the action.

Aim

(i) to obtain basic knowledge of the pathogenesis, epidemiology, and immunopathology of PCV infection and PMWS, (ii) to develop tools for diagnosis of the infection, (iii) to identify proper vaccines prototypes by using conventional and DNA-recombinant technology, (iv) to evaluate the possible risk of PCV for humans, and (v) to develop tools and criteria for assure quality and control of porcine reagents currently used in pig industry with regard to PCV.

Expected results

Expected achievements in relationship to the above mentioned objectives are:

- An experimental model of reproduction of PMWS;
- A better definition of the aetiology of PMWS and the implication of PCV2;
- Knowledge of factors that trigger or aggravate the clinical expression of PMWS;
- Identification of ways of transmission and dissemination of PCV1 and PCV2 in farms.

Availability for research and practical diagnostic work of techniques discriminative of PCV1 and PCV2, and establishment of the significance of results in field conditions.

These techniques include:

- A discriminative (PCV1 vs. PCV2) serologic technique, based on ELISA ;
- Discriminative PCR protocols for PCV1 and PCV2;
- Discriminative in situ hybridisation technique;
- Monoclonal antibodies against PCV2;
- Knowledge of the risk and potential presence of PCV in other species, including humans;
- Guidelines for excluding PCV from any biological products for animal and human use;
- Qualitative and quantitative measure of the immunologic dysfunction in PMWS;
- Database with sequences of several PCV1 and PCV2 isolates in Europe;
- Definition of pathogenic determinants of PCV2 at the genomic level;
- Knowledge of the protein expression and functional ORFs of the PCV2 genome;
- Vectors expressing immunogenic peptides of PCV2;
- Explorative research in three prototype vaccines against PCV2 infection and PMWS;
- Guidelines for quality control of presence of PCV in biological products.

Potential applications

The evaluation of risk of presence of PCV in biological products and organs is of interest for xenotransplantation procedures. Techniques developed to distinguish PCV1 and PCV2 will be used for this purpose.

Keywords

Circovirus, swine, pathogenesis, vaccine, xenotransplantation.

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Novel Circovirus infections of pigs: a new target for vaccination

Project number	QLK2-CT-1999-00445
EC contribution	939.956 €
Duration	42 months
Type	RS
Starting date	1st February 2000

Summary

Porcine circovirus type 2 (PCV-2) is a recently identified swine pathogen. This project aimed to characterise the host range and pathogenic mechanisms of PCV2 in vivo and in vitro including its interaction with the host immune system. In addition, potential vaccine candidates have been designed and evaluated. The extent and nature of PCV2 related disease encountered by pig veterinarians and farmers world-wide was also studied.

Problem

PCV2 is an emerging pathogen within all EU member states and elsewhere. PCV2 related diseases, particularly post weaning multisystemic wasting syndrome (PMWS), are of increasing concern in both economic and welfare terms to the EU pig industry. PCV2 has now been strongly associated with other clinical syndromes in pigs, including reproductive disorders and respiratory disease. At initiation of this project, little information was available on the pathogenesis of PCV2, the interaction of PCV2 with the porcine immune system and no vaccines had been developed to combat this emerging pathogen. In addition, little or no information was available regarding the potential host range of this virus.

Aim

Five main objectives were outlined in this project. These were:

1. To define the host range and pathogenesis of PCV2 isolates from Europe and North America;
2. To establish the prevalence of PCV2 infection and PCV2 related disease in pig populations within EU member states and elsewhere;
3. To determine the effect of PCV2 infection on the porcine immune system;
4. The biological and molecular characterisation of PCV2 isolates;
5. Development and testing of candidate vaccines.

Expected results

Objective 1. Intrauterine infections of pregnant sows with abortive isolates of PCV2 has been carried out to determine the role of PCV2 in reproductive failure. PCV2 is now recognised as a reproductive pathogen and the foetal cell types supporting viral replication have been identified. Infections with PCV2, including co-inoculation with immunostimulants, of gnotobiotic, colostrum deprived and seronegative conventional pigs at 1 day of age and 6 weeks of age was carried out to determine the role of immunostimulation and age in the development of PMWS. Immunostimulation in conjunction with PCV2 infection were shown to be the 2 pivotal factors in development of PMWS in gnotobiotic pigs. The replication of PCV2 in species other than pigs was also be examined. No evidence of replication in sheep, humans, horses, cattle or chickens was demonstrated. A challenge model for vaccine studies has been developed.

Objective 2. Serological studies on the extent of PCV2 infections in EU and North America has been carried out and has shown that PCV2 infections are widespread. Studies on the kinetics of PCV2 circulation within pig herds have also been concluded In Belgium and Denmark. A pathotyping model in CD pigs for PCV2 isolates has been established and, to date only one pathotype of PCV2 has been identified. All PCV2 isolates tested in this model, including isolates from non-diseased pigs, have been shown to have the potential to cause PMWS.

Objective 3. The capacity of PCV2 to infect leukocyte sub-populations has been determined. PCV2 has been shown to "infect", but not replicate in porcine macrophages/dendritic cells. Studies on the consequences of PCV2 infection of target cells in vitro in terms of cell function are currently being studied as are in vivo immune function of PCV2 inoculated pigs.

Objective 4. Serological studies on PCV2 isolates from EU and North America have indicated possible differences in PCV2 isolates. Genomic analysis of PCV2 isolates from diseased/non-diseased pigs has shown no consistent, significant genomic differences associated with disease/non-disease.

Objective 5. Studies on recombinant and nucleic acid vaccines have been completed and a prototype vaccine selected. This vaccine has been tested for efficacy and safety in a challenge model.

Potential applications

The work within this project has generated and characterized reagents and expertise in respect of differential diagnosis of PCV2-related diseases. This is important in respect to other pig diseases with similar presenting symptoms, such as ASF and CSF. It is expected that these diagnostic reagents will be commercialised after termination of the project. In addition the biological and molecular characterisation of PCV2 isolates from various geographical locations will generate useful epidemiological data, regarding the past spread of PCV2 and any potential differences in serotypes between countries. This will be of importance in the development of a vaccine for control and/or eradication of PCV2 related disease. The establishment of a consistent experimental model of PCV2-associated disease will be essential for vaccine development. In addition, this model will provide tools for the study of the development of the porcine immune system and the effects of environmental pressure and viral infections on its functions.

Keywords

Porcine, virus, wasting, vaccine, diagnosis.

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Rational design and delivery of vaccines against infectious diseases in pigs: Rapid identification of immunoprotective PRV antigens and optimal antigen delivery systems

<i>Project number</i>	QLK2-CT-1999-00459
<i>EC contribution</i>	902.600 €
<i>Duration</i>	47 months
<i>Type</i>	RS
<i>Starting date</i>	1st February 2000

Summary

This project aims to develop a methodological approach for designing efficacious and safe marker vaccines. It does so by identifying immuno-protective antigens using nucleic acid vaccine technology to screen parts of a viral genome. Furthermore, the project compares the efficacy of different vaccine delivery systems such nucleic acid based and attenuated Orf (parapox) virus based systems and aims to define correlates of vaccine induced immuno-protection by analysing the type of immune responses in relation to the quality of immunity that prevents disease and virus replication.

Problem

Until now, vaccine development has been based on a trial and error approach without clearly defining the needs in terms of the type of immune response and antigens required for optimal protection. Knowledge on the type of immune response required for optimal protection and the antigens involved, would allow for the design of safe and efficacious vaccines, and as these vaccines would be composed of a limited set of antigens, they could serve as marker vaccines to differentiate vaccinated from infected animals. Moreover, knowledge on the requirement in terms of the immune response would allow for the quantitative assessment of the immune status of animals thereby opening the possibility to a more restricted use of vaccines as animals would only be revaccinated in case that the immunity is sub optimal.

Pseudorabies virus (PRV) in pigs is used as a relevant disease model in the pig. PRV in pigs remains of major economical concern within the EU. At present, many countries within the EU are in the process of eradicating PRV using gE deleted modified live marker vaccines. Some EU countries have already eradicated PRV and have ceased vaccination, leading to the creation of non-vaccinated pig populations which are at risk for new PRV introductions. In contrast to inactivated or subunit vaccines, only modified live vaccines have been successfully used in the eradication campaigns but they have the disadvantage of being infectious and therefore pose the potential risk of persistence by circulation in non-vaccinated herds. As such, the availability of a non-infectious, non-spreading marker vaccine which would rapidly induce protective immunity would be an attractive and safe alternative.

Aim

This project aims at the following objectives: 1) a methodological approach to screen genomes for the identification of immunoprotective antigens using nucleic acid vaccine technology; 2) improvement of the efficacy and biosafety of the nucleic acid vaccine delivery system; 3) comparison of the Orf (parapox) based vaccine delivery system versus the nucleic acid delivery system and 4) definition of the quality of the different types of immune responses induced with respect to protection and prevention of virus transmission.

PRV will be used as a relevant disease model in the pig. The anticipated results will be of general importance for the design of efficacious and safe marker vaccines for other diseases present in the European livestock.

Expected results

The expected results are:

- A rational approach to identify immuno-protective antigens for the design of efficacious and safe marker vaccines. This approach can also be used when designing vaccines for other infectious diseases. Identification of novel immuno-protective antigens against PRV;
- Enhancement of efficacy and bio-containment/bio-safety of DNA vaccines. Reduced amounts of DNA necessary for vaccination and bio-safety;
- Validation of the nucleic acid and the Orf (parapox) based vaccination system in relation to the induction of protective immunity. Availability of appropriate delivery systems;
- Characterisation of the immune mechanisms essential for early and long-term prevention of disease and virus transmission. Establishment of correlates of immune protection.

Potential applications

The methodological approach, of identifying immuno-protective antigens and defining the nature of the protective immune responses will be applicable to vaccine development for other infectious agents. The

availability of safe, non-infectious marker vaccines will be of benefit for the successful control and eradication of infectious diseases. Furthermore, the identification of correlates of immune protection will allow for a more restrictive use of vaccines.

Keywords

Vaccines, rational design & delivery, nucleic acid, parapox.

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Vaccination and control of abomasal nematodes in ruminants

Project number	QLK2-CT-1999-00565
EC contribution	939.708 €
Duration	36 months
Type	RS
Starting date	1st January 2000

Summary

The objective of the project was to assess the ability of selected native and recombinant helminth proteases to protect sheep and cattle against co-infecting abomasal nematode species. The ultimate aim is to develop anti-nematode vaccines, the availability of which will minimise reliance on anthelmintic drugs. The nematodes under study were *Haemonchus contortus* and *Teladorsagia circumcincta* in sheep and *Ostertagia ostertagi* in cattle. The species are known to express homologous proteases and these proteases are associated with protective immunity.

Problem

Nematode parasites impair agricultural production and, in heavy infections, cause significant debilitating disease. In sheep and cattle, these parasites are controlled primarily using anthelmintic drugs and drug resistance is an increasing problem. The cost of the disease and control results in a large economic cost within the EU and worldwide. The project was established to evaluate the possibility of developing anti-nematode vaccines based on protease thought to be involved in nematode feeding.

Approach

Preliminary biochemical studies, antibody and DNA probing showed that the nematodes being studied contain and secrete several homologous, presumably digestive, proteases. The procedures used to purify these proteases were unified within the grouping and purified native enzymes tested in vaccine trials in the homologous and heterologous host-parasite systems. Trials were conducted in cattle and sheep using single and trickle infection challenge regimes with the vaccine being delivered using an acceptable adjuvant. The outcome of infection was assessed by comparing faecal egg output and final worm burdens in vaccinates and control animals.

Full-length complementary DNA (cDNA) sequences encoding relevant nematode proteases, indicated by data from the experiments above, were isolated and expressed in bacteria to enable the production of recombinant enzymes in quantity. The recombinant proteins were then be tested in vaccine trials in homologous host-parasite systems.

The immune responses elicited by vaccination were evaluated throughout the programme to help identify correlates of protective immunity which will inform future developments.

Results

Practically useful levels of protective immunity (>60% reductions in faecal egg output) were stimulated in sheep (against *H. contortus* and *T. circumcincta*) and cattle (against *O. ostertagi*). In addition, recombinant versions of some of these proteins also stimulated protective immunity. The project confirmed nematode proteases as vaccine targets as well as identifying new antigenic targets. The project also showed that the development of recombinant vaccines was a realistic aim.

Potential applications

This project may pave the way to the production of a commercial vaccine to control some of the most important nematode parasites infecting farmed animals worldwide. The next step would be to commercialise the vaccine in collaboration with a commercial partner. The use of a vaccine in the field would allow parasite control in regions where drug resistance is restrictive, would reduce drug use across the EU and, as a result confer significant economic, production and animal health benefits. The beneficiaries would be the commercial sector involved in vaccine manufacture, the agricultural industry and the consumer because vaccines would eliminate exposure to potentially toxic drug residues in meat and dairy products. The same technology could be applied to vaccine development against human helminth diseases such as hookworm and schistosomiasis, major disease problems in the developing world.

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Development of novel vaccines to control infections with newly emerging highly pathogenic Marek's disease viruses

Project number	QLK2-CT-1999-00601
EC contribution	1.399.971 €
Duration	36 months
Type	RS
Starting date	1st January 2000

Summary

The project aims at the development of novel DNA based and subunit vaccine-based vaccination schedules to prevent losses caused by newly emerging Marek's disease virus strains in Europe. In addition, novel chicken cytokines are defined and their potential use as biological adjuvants is investigated. The investigations shall lead to novel vaccine regimens that protect against economic losses caused by a tumorigenic alphaherpesvirus.

Problem

The problem is that Marek's disease virus strains exhibit gradually increasing virulence and that this increase in virulence is partially – if not entirely – caused by insufficient vaccines with low levels of biological safety.

Aim

Develop novel biologically safe and cost-effective vaccines and vaccine regimens against Marek's disease. The vaccines shall be based on DNA vaccines, recombinant Sindbis viruses, and modified live vaccines constructed from infectious bacterial artificial chromosome clones of Marek's disease virus.

Expected results

The delivery of a novel effective vaccine against Marek's disease virus infections that prevents occurrence of the disease in the European Union.

Potential applications

The potential applications reach from the commercial development of novel vaccines to novel test systems.

Keywords

Marek's Disease, MDV, mucosal infection model, DNA vaccines, Sindbis virus vaccines, modified live vaccines, in-ovo vaccination, immunomodulatory molecules, cytokine, chemokine, biological adjuvants, test systems for chicken cytokines.

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***Intraperitoneal immunopathological reactions following vaccination of farmed fish.
Studies of basic immune mechanisms***

<i>Project number</i>	QLK2-CT-1999-00799
<i>EC contribution</i>	1.165.396 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st January 2000

Summary

This project studies the immune mechanisms of granuloma formation in the peritoneal cavity of vaccinated farmed fish, warm-water (sea bass) and cold-water species (Atlantic salmon and rainbow trout), following the use of oil-adjuvanted vaccines containing *Aeromonas salmonicida*, *Vibrio anguillarum* or *Pasteurella piscicida*. This includes preparation of various vaccine formulations, study of their induction of cell responses at site of injection, the cytokine production and profiles induced and more detail study of some of the key inflammatory cells.

Problem

The efficacy of disease prevention by vaccination of farmed fish against some of the major bacterial and viral diseases today relies on the use of oil-adjuvanted vaccines. The main benefit is the induction of long-lasting protective immunity. The side-effect of using adjuvanted vaccines is the formation of visible lesions at the injection-site – the peritoneal cavity. This may on some occasions also result in down-grading of fish at slaughter or after processing, meaning a negative economical impact.

Aim

The overall challenge in vaccine formulation for fish is to find the balance between inducing immune response and at the same time maintaining an acceptable level of adverse reactions. To address both issues, this project has two main objectives:

1. to elucidate the effect of single and combined bacterial components and vaccine formulations for the initiation, development and maintenance of intraperitoneal immune granulomas;
2. to investigate the key cellular mechanisms related to the development of vaccine granulomas both with regard to cell profile and the cytokine profile.

Expected results

1. Identify the contribution of the individual vaccine components as regards induction and maintenance of immune granulomas at the injection site in commercial aquaculture fish species;
2. Obtain an overview of the kinetics of the inflammatory responses induced at the injection site following vaccination and their development over time;
3. Elucidate the underlying immune mechanisms with emphasis on pro-inflammatory cytokines and their complex interactions.

Studies during the first two years of the project have allowed for characterization of the dynamics of the cell responses in the peritoneal cavity over time showing that the initial response to injection of a vaccine preparation is rapid. In salmonids the response is typically biphasic. The responses in Atlantic salmon persist over extended time periods (increasing up to 4 months and possible longer) while in rainbow trout the responses have waned already at 4 months. Cell responses are diffuse and developed into immune granulomas over time. In sea bass, there is an initial inflammation that develops into a different type of granuloma (foreign-body), and the responses in general are milder in sea bass.

The first two years of the project have also shed more light on the underlying mechanisms of granuloma formation in vaccinated farmed fish. It has been shown that effector cells produce different cytokines. Interleukin 8 and interleukin 1 β (both signal molecules) are produced by cells in the peritoneal cavity following vaccination. These signal molecules attract cells to the site of injection and also give a message to the cell to be activated, e.g. up-regulate their functional capabilities. The production of signal molecules as a response to different vaccines correlates with what is observed in terms of reaction by inflammatory cells. This opens up for using assessment of the signal molecule profile they invoke in vaccinated fish to optimise their antigen content.

Potential applications

1. Results will be of importance for designing oil-adjuvanted vaccines, the aim being to optimise the side-effect profiles.

2. The data obtained can also be of value for the pharmaceutical companies in Europe involved in vaccine development for aquaculture fish species.

Keywords

Vaccination, granuloma, immunological responses, cellular responses, inflammatory cytokines.

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Development of vaccines for sheep pulmonary adenomatosis, an endemic contagious epithelial tumour

<i>Project number</i>	QLK2-CT-1999-00983
<i>EC contribution</i>	1.022.517 €
<i>Duration</i>	48 months
<i>Type</i>	RS
<i>Starting date</i>	1st January 2000

Summary

The overall aim of this project is to develop vaccines to provide an effective tool for the control of sheep pulmonary adenomatosis (SAP), an endemic contagious epithelial tumour. The project will evaluate novel delivery systems with general application that will provide low cost solutions for the livestock industry.

Problem

SPA is caused by a retrovirus known as jaagsiekte sheep retrovirus (JSRV). It is an important infectious disease of sheep because of its high prevalence in several member states, leading to large economic losses. Control of SPA would remove these constraints on production and enhance the export opportunities for EU breeding stock. Unfortunately, there are no diagnostic tests to detect infected sheep, which can be identified only when the disease is clinically apparent. There are no vaccines and current control strategies, therefore, are confined to managerial interventions, such as culling of clinically-affected animals.

Aim

The overall aims of this proposal are to i) characterise the virological and immunological events in sheep during infection and vaccination with JSRV, ii) evaluate several candidate vaccines and iii) provide proof of principle that a protective immune response to JSRV can be induced in sheep by vaccination.

Expected results

- Techniques for the propagation and quantitation of JSRV;
- Assays to measure ovine immune responses to JSRV;
- Optimised challenge system to induce SPA in older lambs. The results will be used to set the best combination of dose and age that can be used for evaluation of candidate vaccines;
- Qualitative and quantitative humoral and cellular immunological comparison of potential JSRV vaccines. The approaches that stimulate the most appropriate immune responses will be selected for further assessment;
- Successful vaccination of lambs to induce a protective immune response to JSRV.

Potential applications

The demonstration that sheep can be vaccinated to provide protection against JSRV infection and development of tumour will offer the first hope of effectively controlling this disease. Novel generic means vaccine delivery that can generate systemic and mucosal immunity will have broad applications.

Keywords

Mucosal immunity, novel vaccines, retrovirus, pulmonary tumour, sheep, quantitative PCR/RT-PCR.

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Phylogenetic sequence analysis and improved diagnostic assay systems for viruses of the family Reoviridae

<i>Project number</i>	QLK2-CT-2000-00143
<i>EC contribution</i>	1.216.087 €
<i>Duration</i>	46 months
<i>Type</i>	RS
<i>Starting date</i>	1st January 2001

Summary

The Reoviridae represents one of the largest families of viruses, containing 10 distinct virus genera, over 60 different virus species, over 200 virus serotypes and many unassigned viruses. These viruses characteristically have a genome composed of 10, 11 or 12 segments of dsRNA. Several of these viruses are the causative agents of economically important disease, affecting humans, crop plants, fish, insects, reptiles or domesticated animals. The project will provide additional RNA sequence data for these viruses, including particularly members of the genera Orbivirus, Seadornavirus, Coltivirus and Aquareovirus. This will allow us to create a database for selected reference strains, making it possible to identify new virus isolates more rapidly and precisely than before, by RT-PCR and sequence analysis. Since these nucleotide sequence data can and will be disseminated via the Web, such diagnostic methods can be used in other laboratories, helping to circumvent the need for the expensive standardised reagents currently required for diagnosis by conventional serological methods. These studies will also provide basic epidemiological data to improve our understanding of the distribution and movement of individual virus strains.

Problem

Correct and rapid identification is essential if we are to understand the threat posed by these viruses and then design and implement appropriate control measures. Precise identification of virus serotype is particularly important for identification of appropriate vaccines and vaccination strategies. The majority of the test systems currently used for the members of the Reoviridae are serologically based. For example, within the genus Orbivirus, several virus species (including Bluetongue virus (BTV), African horse sickness virus (AHSV), Equine encephalosis virus (EEV) and Epizootic haemorrhagic disease virus (EHDV)), can be efficiently and rapidly identified by ELISA. However, these assays depend on the availability of standardised and therefore expensive diagnostic reagents and reference antisera, which are not immediately available within many laboratories around the world. At the outset of this project members of the genera Coltivirus, Seadornavirus and Aquareovirus, were relatively less well characterised, such that relationships between strains and even the numbers of virus species and serotypes within each genus was poorly understood.

Since 1998 Bluetongue virus (BTV) (the prototype Orbivirus species) has been causing disease across much of Mediterranean Europe. These outbreaks have been caused by 5 distinct BTV serotypes (BTV-1, 2, 4, 9 and 16). The precise identification of orbivirus serotype can currently only be achieved by serum neutralisation assays. These techniques, which depend on initial virus isolation and may need repetition to generate reliable results, can be slow (weeks). In addition such assays cannot distinguish between distinct virus lineages within a single serotype and do not therefore provide detailed epidemiological data concerning the distribution and origins of individual virus strains. BTV serotype is controlled primarily by the viral outer capsid protein VP2, which is encoded by segment 2 of the virus genome. Sequence analysis of segment 2 could be used not only to identify virus serotype but also, by comparison to sequences of reference strains, the most likely origins of individual virus strains.

Aim

The project will develop sequencing methods and provide sequence data for selected members of the virus family Reoviridae. These studies will focus on members of the genera Orbivirus, Coltivirus, Seadornavirus and Aquareovirus. For example within the genus Orbivirus many of the 20 virus species are currently have either not been analysed or have been only partially sequenced. The complete genome of representative reference stains of different Orbivirus, Coltivirus, Seadornavirus, and Aquareovirus species and will be analysed and used to identify new virus isolates by phylogenetic sequence analyses and comparison. Genome segment 2 of representative isolates of different BTV serotypes will also be analysed. These data will identify the distribution and relationships of specific virus strains and lineages and will facilitate the design of primers for use in both serotype and virus-species specific RT-PCR based assays.

Expected results

The project will establish a reference collection of BTV, other Orbiviruses and other members of the family Reoviridae. This collection will represent a long-term resource, providing diagnostic reagents and material for further research projects. The nucleotide sequence data generated by the project will be used to create a database for identification of viruses within the genera Orbivirus, Aquareovirus, Seadornavirus, and Coltivirus, more accurately than ever before. The genomes of unassigned and uncharacterised viruses (particularly those genome segments coding for the internal and more conserved T2 or Pol proteins) will be analysed. This will help to determine their relationships with established virus serotypes, species and genera and therefore their correct classification, or to decide if they represent isolates of new virus species or even new genera. By generating phylogenetic trees for different viruses (for example by comparison of genome segment 2 of European BTV isolates) the project will not only help to identify the serotype of specific virus isolates, it will also determine the probable origins of individual virus strains, helping to inform future control strategies. These sequence data will also help us to design primers for the amplification of specific regions (segments) of the viral genomes and thereby the development of more rapid and more sensitive species and serotype specific assays based on RT-PCR.

Potential applications

Sequence data for the virus genomes will be added to the international sequence databases, for general use by the scientific community. Accession numbers for members of individual virus genera, species and serotypes, together with phylogenetic trees illustrating their relationships will be added to those already available on the dsRNA virus web-site. In particular these will demonstrate the relationship of current European isolates of BTV to vaccine and other field isolates of the same serotypes from around the world. The development of RT-PCR and sequencing based methods for the identification of individual virus species and serotypes, will support existing diagnostic capabilities within the laboratories of the partnership. As these methods are validated they will be published and made accessible to other laboratories working with or seeking to identify these viruses. Using such assays, studies will be made of the prevalence of coltiviruses and seadornaviruses in tick populations within Europe and in ticks provided by collaborating laboratories from around the world. Assay systems will also be constructed for aquareoviruses to assess their distribution, prevalence and impact in commercial fisheries. By analysing the genomes of viruses that are currently classified within the genus Orbivirus but as yet unassigned to a specific virus species, the project will either show that they belong to one of the established virus species, or that they represent a new species. These data will be particularly valuable for the identification of new or emerging viruses.

Project web-site: http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/

Keywords

Bluetongue virus, African horsesickness virus, Orbivirus, Coltivirus, Seadornavirus, Aquareovirus, Reoviridae, virus diagnosis, dsRNA, virus taxonomy.

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Development, standardisation and harmonisation of novel multiplex nucleic acid tests for the detection of economically important viruses of farm animals

<i>Project number</i>	QLK2-CT-2000-00486
<i>EC contribution</i>	1.199.888 €
<i>Duration</i>	42 months
<i>Type</i>	RS
<i>Starting date</i>	1st September 2000

Summary

The main aim of the project is the development of multiplex PCR assays for the simultaneous and rapid detection of eight swine viruses of high economic importance: African swine fever virus (ASFV), classical swine fever virus (CSFV), Aujeszky's disease virus (ADV), foot and mouth disease virus (FMDV), vesicular stomatitis virus (VSV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV) and swine vesicular disease virus (SVDV). Multiplex PCR assays will be designed around four clusters of swine viruses based on possible clinical presentation: a) Respiratory; b) Reproductive; c) Haemorrhagic; d) Vesicular. The project incorporates five European-based research laboratories and one EU-based small-size enterprise. This combination allows the consortium to establish the critical mass and distribution of expertise (both scientific and commercial) needed to develop and promote the new technology EU-wide.

Problem

The eight mentioned viruses cause important diseases in the swine populations. Most of them are widespread all over the world and are important pathogens of swine. These viruses cause serious losses in the animal populations, their presence is deleteriously affecting animal health. The detection of the viruses is very difficult due to lack of simple and rapid detection methods. Due to lack of vaccination in many European countries, swine populations are not protected against these viruses, and the disease can easily be introduced into the region. As an example, the last outbreak of FMDV in the United Kingdom in 2001 led up to the numerous losses in farm animals and had heavy economic consequence on agriculture and the rural economy.

Aim

The objectives of the project are: a) The development, standardisation and harmonisation of "conventional" gel-based multiplex PCR tests for detection of virus infections in farm animals; b) The development, standardisation and harmonisation of fluorimeter-based real-time (non-gel-based) multiplex PCR tests for detection of virus infections in farm animals; c) The development of multiplex nucleic acid enrichment procedures to increase sensitivity of multiplex PCR; d) The development of methodology for multiplex detection of viral nucleic acid without thermocycling (i.e., Invader technique) using a DNA model; e) Production and application of a library of internal controls for PCR technology to be applied to the above tests. These controls should allow EU wide standardisation and harmonisation of this technology.

The project is divided into seven work packages: 1) Study of genetic diversity of the targeted viruses; 2) Development of the conventional (gel-based) multiplex PCR tests for detection of the viruses that will be divided into symptomatic clusters; 3) Application of real-time, fluorimeter-based fluorescence resonance energy transfer reaction (FRET) technological approaches to PCR for the multiplex detection of the same clusters of viruses; 4) Development novel methods of multiplex extraction using peptide and nucleic acid capture techniques to enhance the sensitivity and specificity of multiplex PCR; 5) Production of the internal controls for multiplex PCR tests; 6) Generation of standard positive samples by actual animal experiments and from stored clinical specimens of previous experiments in order to assess the applicability of the diagnostic assays; 7) Evaluation of the used methods by performing ring tests, standardisation of the developed novel diagnostic assays in the EU.

Expected results

This project is expected to achieve:

1. The development, standardisation and application of assays for viral infections of farm animals that are practical from a perspective of implementation Europe wide (conventional multiplex assays);
2. Development of novel fluorimeter-based, multiplex PCR assays that may replace conventional assays across the EU in the near future;
3. Achievement of rapid result and dealing with large numbers of samples in a short period of time;
4. The tests developed will offer sensitivity, specificity and internal controls equal to, or better than existing strategies.

5. The novel diagnostic assays will contribute to measures to achieve better animal health, to reduce losses and to obtain cleaner environment by eliminating the incidence of the eight viruses in the swine populations worldwide.

Potential applications

The results will be used by veterinary diagnostic and research laboratories specialising in diagnosis of infectious diseases. This project will apply a multidisciplinary approach by an international consortium to progress and harmonise the technologies involved in the detection of viral nucleic acids in tissue samples and apply these new technologies to the diagnosis of important viral infections.

Project web-site: www.multiplex-eu.org

Keywords

Multiplex real-time PCR, swine viruses, diagnostics of viral diseases.

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AEEC infections: pathogenesis, host response and epidemiology of Attaching and Effacing Escherichia coli

<i>Project number</i>	QLK2-CT-2000-00600
<i>EC contribution</i>	1.770.702 €
<i>Duration</i>	42 months
<i>Type</i>	RS
<i>Starting date</i>	1 st October 2000

Summary

Attaching-effacing *Escherichia coli* (AEEC) constitute a group of pathogenic *E. coli* common to human and animals. They include enterohaemorrhagic *E. coli* (EHEC), the causative agent of human haemorrhagic colitis and haemolytic uremic syndrome (HUS), and enteropathogenic *E. coli* (EPEC), an agent of diarrhoea in infants and newborn animals. Public awareness on AEEC, and particularly on EHEC, has recently arisen from their implication in outbreaks of lethal HUS resulting from consumption of contaminated food products, in particular from bovine sources. The project brings together the competence of 12 European, Canadian and Israeli research laboratories from the medical and veterinary sectors to understand the molecular, immunological and epidemiological basis for controlling zoonotic AEEC infections.

Problem

All mammals are colonised by *E. coli* generally at birth and these organisms become part of their intestinal flora for the rest of their lives. This symbiotic occupant of most warm-blooded animal intestines is a tireless workhorse of science and (through procedures such as the presumptive coliform count in water testing) a guardian of public health. However, certain *E. coli* strains have been associated with gastroenteritis, urogenital disease, septicaemia, meningitis, and pleural infections in both humans and animals. *E. coli* is probably the commonest global cause of bacterial diarrhoeal disease affecting both the developing and developed world. The *E. coli* genome is of high plasticity (10 to 20% of the genome of virulent strains is absent in the laboratory K-12 strain). The various disease types and broad host species range of pathogenic *E. coli* are the result of the acquisition of different virulence genes by horizontal transfer during a long co-evolution between the host and the pathogen. Unlike other enterovirulent pathogens such as *Salmonella* spp or *Shigella* spp, *E. coli* belongs to the normal intestinal flora and any eradication strategy should focus on a defined pathotypes. Attaching and effacing *E. coli* (AEEC) are a family of highly adapted enterovirulent *E. coli* strains including both the enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC). These two classes of diarrhoea causing *E. coli* (EPEC and EHEC) produce a characteristic "attaching and effacing" (A/E) lesion in the brush border surface of infected intestinal enterocytes that is characterised by localised destruction (effacement) and intimate bacterial attachment. AEEC constitute a significant risk to human and animal health world-wide. As a human disease, AEEC infections have a high public health importance as defined by world-wide morbidity, mortality (from a mild diarrhoea to haemolytic uraemic syndrome with a fatal infection ulness) and substantial economic burdens. As an animal (and zoonotic) disease, AEEC infections have also a great impact on human food safety (bovine reservoir of human pathogenic EHEC strains), animal welfare, production costs (costly outbreaks of post-weaning diarrhoea, risk of trade barriers), and environmental biosafety (widespread antibiotic use in the livestock industry).

Aim

The overall scientific objectives of this Research and Technological Development (R&D) project is to understand the molecular, immunological and epidemiological basis for controlling zoonotic AEEC infections. The expected achievement of this project is to improve current strategies aimed at assessing and preventing the risk associated with AEEC.

Expected results

The combined results will provide a solid scientific (and technological) basis for the design of effective strategies for the control of AEEC infections in both humans and animals. The novel technical and scientific approaches used in the AEEC project to study AEEC pathogenesis will help to determine which virulence factors are essential for the differentiation of full pathogenic AEEC isolates.

Potential applications

-Development of preventive strategies for AEEC infections including vaccines (a live attenuated vaccine and a DNA vaccine) and immunotherapy (with egg yolk antibodies).

-Development of sensitive and specific diagnostic tests for AEEC (an immuno-magnetic separation method for selective enrichment of AEEC and MAb-based ELISA's).

Project web-site: <http://www.inra.fr/aeec/>

Keywords

Intimin, SLT-VT, type III secretion, Pathogenesis, Zoonosis, Epidemiology.

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Bluetongue & other Culicoides-borne diseases threatening the EU: Identification of vulnerable areas by surveillance & GIS modelling to aid risk assessment

<i>Project number</i>	QLK2-CT-2000-00611
<i>EC contribution</i>	1.099.969 €
<i>Duration</i>	47 months
<i>Type</i>	RS
<i>Starting date</i>	1st December 2000

Summary

Culicoides phylogenetic analysis and studies on population spread is underway and the cytochrome oxidase I gene has been identified as a suitable molecular marker. Survey work is also on target and has confirmed the expansion of the traditional vector, *C. imicola*, into new geographic areas and the involvement of novel vector species of *Culicoides* in eastern Europe. Risk maps based upon environmental requirements of *C. imicola* have been produced that account for over 93% of the observed abundances of this vector in Iberia and Morocco. These maps have been validated and correctly predict the presence of *C. imicola* in the Balearic Is., Tunisia, Sardinia, Sicily, Lazio/Calabria/Puglia and eastern mainland Greece. The models also correctly predict the absence of *C. imicola* from Bulgaria, Macedonia and Yugoslavia (all places where BT has occurred).

Problem

Outbreaks of the OIE list “A” diseases bluetongue (BT) and African horse sickness have occurred several times in Europe in the past resulting in hundreds of thousands of dead sheep and thousands of dead horses. At the present time BT is again devastating sheep industries throughout much of southern and eastern Europe. Both viruses are transmitted by certain species of *Culicoides* biting midge, this makes incursions of the diseases hard to predict in both time and space, and equally difficult to control.

Aim

To undertake field surveys or obtain survey data for *Culicoides* vectors in Portugal, Spain, Morocco, Tunisia, Italy, Bulgaria, Greece and Turkey.

- To identify Greek islands, especially those close to Turkey, and to establish the geographic, climatic, telluric and demographic factors that put an island at risk of vector-BT virus invasion.
- To develop *C. imicola* (the major vector) molecular markers designed to measure gene flow between populations and, thereby, to measure rates of vector population expansion and sources of incursion.
- To assess whether the distributions of vector *Culicoides* are expanding, by resurveying areas found previously to be vector-free but situated close to areas or islands that have vector populations.
- To identify climatic factors favouring large increases in vector population size in certain years, and thereby, significantly increase the risk of epizootics.
- To identify novel vector species of *Culicoides* and assess their levels of vector competence and distributions.
- To attempt isolation of BT virus from *Culicoides* vectors during an outbreak in south-eastern Europe.

Expected results

To reduce or eliminate from the EU and adjoining territories risk of incursion or establishment of internationally important *Culicoides*-borne viral diseases by: identification of the vectors, virus isolation, estimation of vector population distribution and spread, and production of predictive (disease-risk) maps for Europe and surrounding areas.

Potential applications

The results will enable disease incursions to be predicted in time and space and areas “at risk” to be demarcated. This will enable targeted, cost effective control measures to be implemented without delay. The results will be of value to veterinary authorities, international disease control agencies, import-export authorities, legislators and farmers.

Keywords

Culicoides-borne diseases, vector identification, bluetongue, predictive modelling, risk maps, molecular markers, population changes, risk assessment, satellite imagery, GIS.

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Genes Involved in the Pathogenesis and Control of Haemophilus parasuis Infections in Pigs

<i>Project number</i>	QLK2-CT-2000-00726
<i>EC contribution</i>	600.030 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st October 2000

Summary

Haemophilus parasuis is an increasingly prevalent swine pathogen causing significant economic loss in high health herds. Current management and vaccination strategies often break down, and alternative solutions are necessary. The PathoCHIP project is designed to identify genes from the host and pathogen that are critical for disease production by and recovery from this pathogen. This information will be valuable for understanding pathogenesis of other swine pathogens.

Problem

Animal health and welfare are major problems for the livestock industry in Europe. Genomics offers the opportunity to develop the knowledge required for the development of new solutions for these issues.

Haemophilus parasuis is a gram-negative bacterium, isolated from the upper respiratory tract of pigs, which causes Glasser's disease and polyserositis primarily in young pigs. Observed symptoms include pleurisy, pneumonia, arthritis, and inflammation of the membranes of the heart, meninges and abdominal cavity. Progression of the disease leads to reduced appetite, fever, lameness, and death. H. parasuis is becoming increasingly important as a swine pathogen as high-health, multi-site production systems expand. In some parts of the world H. parasuis is a "high health" disease even in conventional health sow herds and can result in significant economic loss. Current treatments include antibiotics and vaccination. Vaccination is of limited success and development of antibiotic resistance is also a concern. Alternative solutions are necessary to alleviate these concerns and to combat the break down in current management and vaccination strategies.

Aim

This project will utilise an animal challenge model to reproduce the disease in vivo. It is hoped that identification of resistant and susceptible individuals will be possible by monitoring the extent of disease using microbiology, PCR and assessment of clinical signs. We aim to identify host and pathogen genes that are important during the disease process that could be used as candidates for novel treatment and management of the disease. This will be achieved by:

- Development of cDNA microarrays from infected host pig tissue and various immune tissues. These will be utilised to identify host pig genes that are differentially expressed during H. parasuis infection.
- Development of a genomic DNA microarray for H. parasuis. This will be utilised to analyse gene expression differences in bacteria isolated from sites of infection compared with those in a control state.

Expected results

1. New resources for the study of H. parasuis infection and other pig diseases:
 - Porcine SSH libraries and normalised cDNA libraries from infected and uninfected pig tissues;
 - Porcine microarrays for host differential gene expression analysis;
 - H. parasuis bacterial microarray for bacterial gene expression analysis.
2. Identification of candidate genes for porcine resistance to H. parasuis infection.
3. Identification of bacterial virulence genes as candidate targets for novel therapeutics.

Potential applications

It is hoped that identification of bacterial virulence genes will lead to development of novel treatments for the disease whilst identification of host candidate genes that are responsible for disease susceptibility may lead to production of animals that are less susceptible to the disease via marker assisted selection.

Project web site: www.pathochipproject.com

Keywords

Pigs, Haemophilus parasuis, Microarrays, Disease resistance, Molecular biology, Pathogenesis, Control of infectious diseases.

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Immunotherapy of Enteric Infections by Rotaviruses and Coronaviruses Using Plantibodies

<i>Project number</i>	QLK2-CT-2000-00739
<i>EC contribution</i>	1.300.000 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st October 2000

Summary

This project proposes the use of novel high-yielding plant expression systems based on mono- and bipartite viruses or on transgenic plants and plant suspension cell cultures for large scale production of recombinant antibodies with therapeutic activity against enteric diseases of farm animals.

Problem

Most pathogens enter the body via mucosal surfaces because their area is about two hundred fold-higher than that of the skin. In consequence, to protect individuals it is important to develop strategies to reinforce their immunity at those highly exposed areas. Frequently, animals have not been vaccinated against certain pathogens that have entered a neighbor farm and are exposed to a high risk. Protection against these enteric infections can be provided by the in situ expression of pathogen neutralizing antibodies using a vector with tropism for gut tissues of by orally providing recombinant antibodies raised in plants.

Aim

The overall objective of this project is the large-scale production in plants of immuno-therapeutic antibodies against enteric diseases of both farm animals and humans at sufficiently low cost for their widescale use to be a practical proposition.

Expected results

- (i) Engineering full-length humanized or porcized monoclonal antibodies and novel multivalent small immuno-proteins (SIPs) specific for animal and human rota- and coronaviruses causing diseases of the enteric tract.
- (ii) Expression of the recombinant antibodies in plants with vectors based on cowpea mosaic virus (CPMV) and plum pox virus (PPV) and on Agrobacterium plasmids.
- (iii) Design of efficient, low cost system, for the production and down-stream processing of the recombinant antibodies.
- (iv) Demonstration of the functionality and biosafety of the plant-expressed recombinant antibodies, both in vitro and in vivo.

Potential applications

Among the applications of the immunotherapy by recombinants antibodies, protection of newborns has a particular interest. Both newborn humans and animals have an immature immune system during the first weeks of life making them highly vulnerable to infections. Recombinant antibodies neutralizing specific viruses or other pathogens expressed in plants can be orally administered to protect newborns against diseases caused by infectious agents.

Keywords

Plantibodies, agroinfiltration, virus vector, transgenic plants, recombinant antibodies, enteric diseases, small immunoproteins, plum pox potyvirus, cowpea mosaic comovirus.

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Sea Lice Resistance to Chemotherapeutants: diagnosis, mechanisms, dynamics and control

<i>Project number</i>	QLK2-CT-2000-00809
<i>EC contribution</i>	900.000 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st January 2001

Summary

Throughout Northern Europe, the salmon louse, *Lepeophtheirus salmonis*, seriously affects the marine phase of Atlantic salmon production. The development of sustainable methods of pest management has been unable to keep pace with the intensification of production, leading to excessive and very fragile reliance on very few chemotherapeutants, a situation that may promote development of resistance in the parasites.

Problem

Resistance against organophosphates has been demonstrated in sea lice in important salmon producing countries, and resistance against pyrethroids and avermectins seems to be emerging. Most reports of clinical failures of control agents are though of an anecdotal nature. Unless they are verified as cases of resistance using validated diagnostic methods, control actions are difficult. Possible actions should be based on knowledge of the underlying mechanism in order to be effective. A thorough knowledge of the dispersion of the genetic material between salmon farms is vital for the assessment of the risk posed by resistance development in sea lice. The main objective of this project is, by a multidisciplinary effort involving scientists and aquaculture industry in Norway, UK, Ireland and Canada, to develop strategies for identification and control of resistance development in sea lice.

Aims

The aims of the project are:

- a. to develop biochemical and toxicological methods (bioassays) for early detection of reduced sensitivity to the pyrethroids cypermethrin and deltamethrin, the organophosphate azamethiphos, the avermectin emamectin and the chitin synthesis inhibitor teflubenzuron;
- b. to study the underlying mechanisms using biochemical and molecular biology techniques;
- c. to monitor sensitivity against the available chemotherapeutants in Norway, Scotland, Ireland and eastern Canada;
- d. to study the dispersal of genetic material between individual farms under different control strategies by developing and applying microsatellite PCR-methods;
- e. to propose control strategies for handling resistance problems based on the knowledge generated through the project.

Expected results

The project is expected to result in:

1. validated protocols for the diagnosis of resistance in sea lice;
2. a thorough description of underlying mechanisms (e.g. altered detoxification, target site mutations);
3. an overview of the sensitivity towards the available control agents in Norway, Scotland, Ireland and eastern Canada;
4. a genetic population structure analysis on lice from adjacent salmon farms within fjords (Norway), in defined single bay management schemes (Scotland and Ireland), and in a location with unusual hydrogeography (Canada).

Potential applications

The basic diagnostic protocols will be established in each of the participating countries, and will provide the industry and the authorities with knowledge-based tools to handle cases where resistance has developed. The project will help to establish strategies that minimize the risk of eroding the agent's effect, and with minimal consequences for the industry and the environment.

Project web-site: <http://www.iacr.bbsrc.ac.uk/pie/search-EU/>

Keywords

Sealice, resistance, pyrethroids, organophosphates, avermectins, teflubenzuron, bioassays, mechanisms, gene-flow.

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Pathophysiology and prevention of Lactococcus garvieae and Streptococcus iniae infections in rainbow trout

<i>Project number</i>	QLK2-CT-2000-01049
<i>EC contribution</i>	579.858 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st January 2001

Summary

Streptococcal infections are an emerging problem in cultured fresh water and marine fish. A few Gram positive cocci have also been associated with human infections and are therefore a risk to public health. The main objective of this project is to explore some basic aspects of the host-pathogen interactions and to generate data that are essential for the future development of efficient preventive tools, which will help reduce the prevalence of these infections in fish farms. The experience acquired would not be limited to *Lactococcus garvieae* and *Streptococcus iniae* but could be applied to other bacteria pathogenic to fish.

Problem

Streptococcal infections are nowadays considered the major cause of rainbow trout losses in several European and Mediterranean countries. Average mortality during the hot season may head to over 50% of the total trout population; due to the stormy character of these diseases, conventional treatments are little effective. Besides being inadequate, massive use of antibiotics is expensive and environmental unfriendly. The definition of valid methods to prevent the onset of *Lactococcus garvieae* and *Streptococcus iniae* infections, through the generation of patho-physiological and immunological data, is therefore essential.

Aim

The aim of the project is to generate information that will enable a more comprehensive understanding of streptococcal infections in fish. The patho-physiological events that lead to the onset of these diseases will be evaluated from two different aspects:

- (a) Bacterial mechanisms of virulence, analyzed through in-vivo, in-vitro and ex-vivo models, and
- (b) The host's response to the pathogens, analyzed through the assessment of the cellular non-specific and specific immune functions.

These data will allow a better understanding of the patho-physiological and immunological events that precede the onset of these diseases and characterize its' course, enabling a rational approach for designing specific vaccines and testing their efficacy in rainbow trout.

Expected results

The results expected from the project consist in:

1. The elucidation of the fundamental aspects of the agents' pathogenetic mechanisms;
2. The definition of the immune mechanisms which might allow fish to overcome *Lactococcus garvieae* and *Streptococcus iniae* infections;
3. The formulation of vaccines eliciting a specific immune response in rainbow trout.

Potential applications

The generation of basic data on *Lactococcus garvieae* and *Streptococcus iniae* infections will lay the basis to the future development of preventive tools.

The availability of efficient vaccines will improve aquaculture production in Europe, diminish the risk to public health and preserve the environment from the undesired impact of antibiotic residues.

Keywords

Aquaculture, fish, streptococcal infections, *Lactococcus garvieae*, *Streptococcus iniae*, epidemiology, pathogenetic mechanisms, virulence factors, immune response, vaccination.

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Stimulation of fish larval defence mechanisms against infectious diseases

Project number	QLK2-CT-2000-01076
EC contribution	999.977 €
Duration	41 months
Type	RS
Starting date	1st February 2001

Summary

Production cycles, especially for Atlantic halibut and sea bass, are at present restricted by large larval mortality within hatcheries due to microbial infections. This problem is also experienced in the production of cod, wolffish and carp eggs and larvae and is encountered throughout the European aquaculture industry. As a consequence it is imperative that a greater understanding of the ontogeny of the defence system within larval fish is developed. In this context, the project aim is to examine the immune defence (e.g. lymphocytes, macrophages, antibodies, complement proteins, pathogen degrading enzymes) status during ontogenic development of different fish species (sea bass, Atlantic cod and halibut, spotted wolffish and carp). This will be achieved using immunological, immunocyto- and histochemical, enzyme histochemical, chromatographic and molecular biological techniques. The effects of immunotherapeutics on these fish larvae will be measured as survival after experimental challenges with pathogenic bacteria. In addition, the immune responses after immunotherapeutic treatments could be monitored as elevation of defence mechanisms and immune cell numbers during the treatments.

Problem

Production cycles, especially for Atlantic halibut and sea bass, are at present restricted by large larval mortality within hatcheries due to microbial infections. This problem is also experienced in the production of cod, wolffish and carp eggs and larvae and is encountered throughout the European aquaculture industry. As a consequence it is imperative that a greater understanding of the ontogeny of the defence system within larval fish is developed.

Aims

The project aims to examine the immune cell (e.g. lymphocytes, macrophages) and immune defence molecule (e.g. antibodies, complement proteins, pathogen-degrading enzymes) status during ontogenic development of the fish species under study.

Expected results

By achieving the objective, the fish larval health, survival and welfare will be improved and this will contribute to a decrease in the economic losses of the European aquaculture industry.

Potential application

Traditional techniques for delivering immunotherapeutics, such as intraperitoneal vaccination with an oil-based adjuvant are far too traumatic, or even physically impossible, to be attempted on larval animals. The project will present new knowledge about immunotherapeutic strategies to modulate the immune defences of these commercial and potentially commercial species during the delicate larval stages. In addition, the outcome of this project, such as development of strategies to prevent infectious diseases may also be adaptable to other aquacultured fish species kept in the aquaculture industry.

Project web-site: <http://www.hi.is/gadus/>

Keywords

Fish larvae, defence mechanisms, ontogeny, immunotherapeutic treatments.

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Microarray analysis of gene function and host interaction in Salmonella typhimurium

Project number	QLK2-CT-2000-01126
EC contribution	1.399.415 €
Duration	42 months
Type	RS
Starting date	1st December 2000

Summary

This project will exploit the recent sequencing of the genome of *Salmonella typhimurium* to develop a DNA microarray comprising of all of its genes. The microarray will allow us to compare the levels of expression of genes under different nutrient conditions, and while the *Salmonella* are invading or surviving within host cells. We will also analyse changes in the expression of chicken genes likely to affect bacterial infection, and relate changes in host and bacterial expression. The novel gene functions identified by this work will provide the basis for future vaccine development and the identification of resistant animals.

Problem

Despite intensive efforts to prevent or control infection *Salmonella* remains a major public and animal health problem which affects all farm species and is of great significance to human and animal health throughout Europe and world-wide. Host-adapted serotypes of *Salmonella* produce considerable economic loss, especially in countries where production systems are under-developed or where environmental conditions favour environmental contamination. However an even greater problem is the ability of a wide variety of *Salmonella* serotypes to colonise the alimentary tract of domestic animals resulting in carcass contamination with extensive contamination of the food chain. This is particularly the case for poultry and pigs, with *S. typhimurium* and the closely related serotype *S. enteritidis* being the principal serotypes of *Salmonella* involved. Although there have been improvements in poultry hygiene, and in control measures such as vaccines, competitive exclusion and food additives these have not been fully successful in eliminating *Salmonella* infection. So far these approaches have been based on a partial understanding of limited sets of *S.typhimurium* genes which have been individually studied by conventional methods. The recent development of microarray technology, by which the expression of very large numbers of genes can be rapidly studied, coupled with the sequencing of the full genome of *S.typhimurium*, which permits identification of its full gene set, has opened up new possibilities for characterising *S.typhimurium* genes and identifying those which are important to pathogenesis.

Aim

The technology this project will develop will allow us for the first time to analyse global changes in expression of *Salmonella* genes to identify genes and groups of genes which determine its response to external changes.

The project will:

1. Identify genes upregulated under different nutrient conditions which may be important to the survival of *Salmonella* in the chicken gut;
2. Identify the effects on the expression of other genes of mutations in genes affecting growth, to define their relationships and to identify candidate genes for vaccines;
3. Analyse changes in *Salmonella* and host gene expression during its invasion of chicken epithelial cells to identify possible means of preventing its penetration of the gut wall;
4. Analyse changes in *Salmonella* and host gene expression during its growth within host phagocytic cells to identify methods of restricting the survival of the bacterium within the chicken.

Expected results

- The construction of a complete and definitive gene array for *S.typhimurium* based on its genomic sequence.
- The optimisation of methodology for use of the array for the assessment of gene expression of *S.typhimurium*.
- The identification of a selected set of chicken genes involved in host response to *Salmonella* infection.
- Identification of the genes expressed during growth under different nutrient and redox conditions.
- Genes differentially expressed in strains of salmonella carrying mutations in target genes.
- Identification of the genes expressed during adhesion and invasion of host cells.
- Identification of the genes expressed in interactions between macrophages and *S.typhimurium* during intra-cellular infection.

Potential applications

This project will apply novel microarray technology to provide a new holistic means to analyse and understand Salmonella gene functions at different stages of its infection of chickens, including its response to different nutrient conditions in the gut and its interactions with host cells, which are at present poorly understood and which are critical both for colonisation and for pathogenic infection. Study of these poorly explored stages of microbial life cycles will contribute to the more rational design of vaccines, potential probiotics and competitive exclusion which have so far been largely empirical in nature and limited in their success. The project will also give a better understanding of the host response to the Salmonella infection and to identify possible differences in genetic resistance or opportunities for enhancing the host immunological response to limit the extent and persistence of Salmonella infection.

Keywords

Salmonella typhimurium, microarray, expression profiles, gene expression, chicken, bacterial invasion, bacterial infection.

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European surveillance network for influenza in pigs

Project number	QLK2-CT-2000-01636
EC contribution	269.984 €
Duration	36 months
Type	CA
Starting date	1st January 2001

Problem

(State the problem that the project addresses in relation to control of infectious diseases) Swine influenza (SI) causes important losses by lost production in fattening pigs and is probably the major cause of respiratory disease in fattening pigs in Europe. Recently, greater genetic and antigenic changes amongst SI subtypes have been observed in some EU member states in comparison with the period before. These changes seem to coincide with increased virulence and decreased protection by the current SI vaccines. In addition to the economic impact there is of course the public health risk posed by maintenance, evolution and emergence of influenza A virus in pigs.

Aim

The objective of this co-operative surveillance network, that involves 14 partners from 10 countries in Europe, is to standardise and harmonise techniques and protocols for virus isolation and typing and to exchange reference material and information about recent swine influenza virus strains. The available information about recent SI field isolates will be stored in a database and the filed isolates will be stored in a central virus bank to assure free access by all partners. The database will provide a first epidemiological picture about SI in Europe that will be used to define recommendations for SI control.

Expected results

1. Use of standard protocol for virus collection and culture and for antibody detection.
2. Exchange of standardised reference sera among partners.
3. Because standard panels of reference sera will be used, the results of typing of new virus isolates in different Member States will be improved and they will be directly comparable.
4. Standardisation of genetic characterisation of SI virus isolates.
5. Database for future expansion and epidemiological evaluation.

Potential applications

The surveillance network can provide the epidemiological data required to make recommendations to the pig industry and governmental institutions with regard to swine influenza. Furthermore insight in the influenza reservoir in pigs in Europe will provide the background for zoonotic risk assessment. Results may lead to the conclusion that vaccines have to be updated more frequently.

Keywords

Swine influenza, Surveillance, Epidemiology.

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Infectious Salmon Anaemia – Development and Standardisation of Diagnostic Methods and Aspects of the Epidemiology of ISA

<i>Project number</i>	QLK2-CT-2001-00844
<i>EC contribution</i>	1.156.576 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st January 2002

Summary

The objectives of this project are to develop and standardise rapid methods to detect Infectious Salmon Anaemia Virus. A variety of methods will be investigated, including real time nucleic acid amplification (RT-PCR, and NASBA), immunohistochemistry and serology. Antibodies against ISAV will be prepared using phage-display technology. Optimised methods will be used to study the epidemiology of ISA, and to assist in the design of control measures.

Problem

Infectious salmon anaemia (ISA) is a viral disease affecting farmed salmon. Since the first reports of this disease in Norway, ISA has occurred in many salmon producing countries within the European Union and the Americas. Economic losses resulting from ISA can be severe. Rapid and reliable diagnostic methods are essential for the study of the epidemiology of ISA, and for the development of effective control measures. At present, there are no standardised detection procedures for the causative agent of ISA, an orthomyxovirus-like virus (ISAV). Cell culture isolation procedures have been developed for the detection of ISAV, together with molecular based techniques including RT-PCR. However, the sensitivity and specificity of currently available diagnostic procedures is limited, and consequently, the development of improved methods is urgently required.

Aim

The objectives of this project are to develop and standardise rapid methods to detect Infectious Salmon Anaemia Virus. Both molecular and immunological probes will be developed. Methods studied include real time nucleic acid amplification (RT-PCR, and NASBA), immunohistochemistry and serology. Antibodies against ISAV will be prepared using phage display technology. Once established and optimised, these methods will be used to investigate aspects of the epidemiology of ISA, including the role of wild fish as carriers, assessing the risk of vertical transmission, and to determine whether persistent infection with ISAV may occur.

Expected results

1. Optimised nucleic acid amplification procedures for the detection of ISAV (real time RT-PCR; real time NASBA).
2. In-situ hybridization test for ISAV.
3. Optimised immunohistochemical detection method for ISAV.
4. ELISA for detection of ISAV.
5. Standardisation of methods described under 1 –4.
6. Anti-ISAV antibodies produced by phage display.
7. Assessment of the role played by wild fish and vertical transmission in transmission of ISA.

Potential applications

The results of this project will be used by veterinary diagnostic laboratory services throughout Europe, and will contribute to the control of ISA. The results are also applicable to the diagnosis of diseases caused by other pathogens of fishes, such as nodavirus, infectious pancreatic necrosis virus, and viral haemorrhagic septicaemia.

Keywords

Infectious salmon anaemia virus, aquaculture, molecular biology, diagnosis, epidemiology.

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***The role of wildlife in the epidemiology of Mycobacterium avium subspecies
paratuberculosis in domestic ruminants in Europe***

Project number	QLK2-CT-2001-00879
EC contribution	1.295.952 €
Duration	39 months
Type	RS
Starting date	1st October 2001

Summary

The overall aim of the project is to identify key wildlife hosts of Mycobacterium avium subspecies paratuberculosis across Europe and determine their role in the epidemiology of paratuberculosis (Johne's disease) in the domestic livestock and to produce disease control strategies that are pertinent to the range of agricultural systems employed in the EU.

Problem

Paratuberculosis (Johne's disease) is a disease of increasing economic importance across the world wherever domestic ruminant species are reared. Until recently it was considered solely a disease of ruminants, domestic and wild, but recent studies in Scotland have highlighted the potential role that rabbits may play in the epidemiology of this disease. Trying to control or eradicate the disease in the domestic ruminant species has proved to be difficult and this may or may not reflect the potential role of wildlife as a reservoir of the infection. Only by ascertaining which wildlife species which co-exist with domestic livestock are also infected with M.a.paratuberculosis and shed the organism into the environment can sensible control measures be developed for controlling this disease.

Aim

To determine the key wildlife hosts of Mycobacterium avium subspecies paratuberculosis and potential routes of transmission of this organism from wildlife species to domestic animals. This information will be used to develop mathematical models to analyse the dynamics of M.a.paratuberculosis infection between domestic animals and wildlife and to identify potential management tools to control this infection in domestic ruminants.

Expected results

A number of the wildlife species in the different countries involved in the project will be shown to have a potential role in the epidemiology of paratuberculosis in farmed livestock and this will necessitate a revision of control strategies for this disease. Mathematical models will be developed to assist the identification of management tools to control paratuberculosis in the various species of livestock under the different management conditions and this information will be disseminated to the agricultural end users.

Potential applications

The identification of potential wildlife reservoirs of M.a.paratuberculosis has an impact on the practical control strategies which can be implemented by livestock owners. Using mathematical modelling potential management tools will be identified. This information will be made available to agricultural end users and the general scientific community.

Keywords

Paratuberculosis, wildlife, epidemiology, domestic, ruminants, Europe.

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Diagnoses, pathogeneses and epidemiologies of salmonid alphavirus diseases

<i>Project number</i>	QLK2-CT-2001-00970
<i>EC contribution</i>	1.219.016 €
<i>Duration</i>	42 months
<i>Type</i>	RS
<i>Starting date</i>	1 st December 2001

Summary

Sensitive, specific, convenient diagnostic tests will be developed for salmon pancreas disease of farmed Atlantic salmon and sleeping disease of farmed trout, which are caused by closely-related, recently-characterised alphaviruses. Aspects of the pathogeneses and epidemiologies of these salmonid diseases will also be investigated.

Problem

Salmon pancreas disease (SPD) of farmed Atlantic salmon and sleeping disease (SD) of farmed rainbow trout are emerging, economically-important diseases, which have been reported in many European countries. The causal agents of SPD and SD have recently been characterised as closely-related isolates of a novel alphavirus, the first alphavirus reported in fish. Both diseases, which are associated with increased mortality and growth retardation, are thought to be significantly under-diagnosed because histology, the currently-used method of diagnosis, is time-consuming, labour-intensive and requires input from an experienced pathologist. Sensitive, specific and convenient diagnostic tests, based on the detection of virus antigen and virus nucleic acid within tissue samples, are required to provide accurate information on the prevalence, severity and economic importance of these diseases. Such methods are also required to investigate the pathogeneses and epidemiologies of SPD and SD, providing information with which disease control strategies can be formulated.

Aim

The primary aim of this project is to develop and evaluate tests for diagnosing infections caused by salmon pancreas disease virus (SPDV) in farmed Atlantic salmon and sleeping disease virus (SDV) in farmed rainbow trout. Secondary aims are to improve the understanding of the pathogeneses of SPD and SD and to investigate epidemiological aspects of SPDV and SDV infections.

Expected results

1. The first sensitive, specific and convenient diagnostic tests for salmon pancreas disease of farmed Atlantic salmon and more convenient diagnostic tests for sleeping disease of farmed trout.
2. Improved understanding of pathogeneses of salmon pancreas disease and sleeping disease.
3. Improved understanding of epidemiologies of salmon pancreas disease and sleeping disease.

Potential applications

A range of diagnostic tests will be made available to the fish-farming industry as a result of this project. Their application will allow the incidence of the disease caused by SPDV and SDV throughout the EU to be determined. In this way adverse economic effects on farmed fish production can be accurately assessed. This information and that arising from investigations into the pathogeneses and epidemiologies of these diseases will be of use in formulating suitable disease control measures. In the longer-term, the implementation of control measures such as vaccination will reduce adverse economic effects and increase the viability of the fish farming industry.

Keywords

Salmon pancreas disease, sleeping disease, salmonid alphavirus, fish disease, fish virus diagnosis, fish virus pathogenesis, fish virus epidemiology.

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Diagnosis, Epidemiology and Prevention of Neospora caninum-associated Bovine Abortions

<i>Project number</i>	QLK2-CT-2001-01050
<i>EC contribution</i>	1.199.631 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st December 2001

Summary

Infections with *Neospora caninum*, a protozoan parasite discovered only a few years ago, belongs now to the most frequently diagnosed causes of abortion in dairy cattle world-wide. In addition to abortions, *N. caninum* infections in dairy cows may also be associated with premature culling and losses in milk production. The objectives of the project are (1) to identify stage-specific antigens for diagnostic purposes, (2) to standardise and improve diagnostic methods and (3) to conduct epidemiological studies to estimate the prevalence in cattle in different regions of the European Union. These goals will be achieved by collaboration involving a multidisciplinary group of scientists from different member countries (parasitologists, immunologists, biologists, molecular biologists, veterinarians, epidemiologists and biomathematicians) involved in the development and evaluation of diagnostic techniques.

Problem

From the present knowledge about *Neospora caninum*, it is evident that infection with this parasite causes significant problems in terms of animal health and it is the most frequently diagnosed cause of abortion in cattle in several countries of the European Union, resulting in significant economic loss to producers. When the aetiological fraction (AF) of abortions due to *N. caninum* was compared with that of other abortifacient agents, *N. caninum* emerged as the most important single cause of abortion in the England and Wales, where it was even more important than Bovine Viral Diarrhoea Virus as a cause of abortion. *N. caninum* is also the leading diagnosed cause of bovine abortion in The Netherlands. Since *N. caninum* is a relatively recently recognised abortifacient agent in cattle, it has not yet received appropriate attention in many regions. This may in part be due to a lack of knowledge on the benefits and limitations of the available diagnostic techniques, and, in particular, to a lack of scientifically evaluated control strategies.

Aim

The aims of the project are (1) to identify stage-specific antigens of *N. caninum*, (2) to evaluate and standardise diagnostic techniques, (3) to improve diagnostic methods for the detection of antibodies to *N. caninum* (development of methods which can discriminate between acute and chronic infection), (4) to establish of a bank of *Neospora* isolates and to develop tools for the molecular typing of isolates, and (5) to investigate the epidemiological situation in the EU by performing cross-sectional studies in different regions of the European Union.

Expected results

- Standardise diagnostic techniques to allow the identification of infected animals.
- Develop novel diagnostic tests using parasite stage specific antigens.
- Correlation of quality of the humoral immune response and abortion in cattle.
- Develop molecular tools to allow the fine discrimination of parasite isolates.
- Determine the seroprevalence of *N. caninum* in different regions within the European Union.

To standardise diagnostic techniques for the detection of neosporosis, a multi-centred study will be carried out to compare the most widely used tests for specific antibody (IFAT and ELISA) and parasite detection (PCR, immunohistochemistry). To improve diagnostic methods, stage-specific antigens will be identified and analysed for their diagnostic potential. New tools will be developed by testing the immune response to such stage-specific antigens (e.g. tachyzoite vs. bradyzoite) which will be purified from the parasites or expressed as recombinant antigens. In a further approach to improve the currently available serological tools, the quality of the humoral immune response (affinity, isotype of antibodies) and its relationship to abortion will be analysed. The tools for molecular epidemiology will be prepared by developing molecular typing methods (e.g. RAPD, AFLP, RFLP) and establishing a European bank of *N. caninum* isolates. With a standardised set of diagnostic tools, cross-sectional studies will be performed to estimate the prevalence in different regions of the European Union and to identify potential regional and other impact factors (e.g. management system).

Potential applications

Newly developed diagnostic techniques will be published in scientific journals to make them available to the general public. Licenses for the use of new diagnostic tools (e.g. monoclonal antibodies, PCR primers, recombinant antigens) will be offered to industrial enterprises. The group will inform the national veterinary authorities about new epidemiological results, so that they can form the basis for development of rational control strategies for *N. caninum*-associated abortions in cattle.

Keywords

Neospora caninum, abortion, cattle, stage-specific antigens, diagnosis, molecular epidemiology, prevalence, risk factors.

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Development of alternative control methods for the chicken mite (*Dermanyssus gallinae*)

<i>Project number</i>	QLK2-CT-2001-01236
<i>EC contribution</i>	649.984 €
<i>Duration</i>	48 months
<i>Type</i>	RS
<i>Starting date</i>	1st December 2001

Summary

The overall objective of the project is to develop new control systems for the chicken mite, *Dermanyssus gallinae*, a major arthropod pest of increasing importance affecting the health and welfare of poultry. These new methods will be based on the application of naturally occurring semiochemicals (behaviour-modifying chemicals, e.g. pheromones) specifically involved in mite aggregation, mating and feeding behaviour and host selection/location in combination with mite-specific biological control agent (a fungal pathogen), as part of a lure-and-infect strategy.

Problem

Chicken mite infestations are the cause of considerably economic losses and welfare problems in the poultry production. Infested hens may show signs of anaemia and reduced growth and they spend significantly more time carrying out a light feather picking activity. Other effects include decreases in egg production, quality (excretory products of mites on eggs) and hatching success. Mite-induced mortality has also been observed. Chicken mites also generate a serious threat to the health of man and animal by acting as a vector/carrier for important diseases, and they may generate problems for workers in the poultry industry due to the nuisance of mites crawling on the skin. Mite problems have increased considerably in recent years due to national and European policies resulting in a shift from battery systems to more welfare/consumer friendly systems, reduction in number of registered acaricides, and pesticide resistance in mite populations.

Aim

The overall aim of the project is the development of an environmentally acceptable lure-and-infect device for the control of chicken mites. The overall objective will be achieved by three specific objectives : 1) to isolate and identify semiochemicals with the potential of luring the chicken mites into a trap or an autoinoculator, 2) to isolate fungal species present in natural populations of chicken mites in European poultry production, and to select the most suitable isolates for application under field conditions and 3) to develop a prototype lure-and-infect trap based on a combination of the identified semiochemicals and the selected fungal isolates.

Expected results

The development of a lure-and-infect system will facilitate the transition to organic egg production by providing a control system for a pest which – if left untreated - can have significant impact on the health and welfare of poultry. The system will also reduce or eliminate the necessity for broad-spectrum persistent synthetic acaricides in conventional poultry production. The concept of applying mite-specific pathogens in baited traps means that unintentional exposure of livestock and human beings to such organisms will be limited to an absolute minimum.

Potential applications

Where appropriate patents will be applied for before the results of the project can be disseminated. Scientific results will be published in international peer-reviewed scientific journals and commercialization of the control device will be undertaken by the commercial partner. The final product can be used by ordinary poultry farmers in Europe and elsewhere where problems with chicken mites exist. The identified fungal isolates and semiochemicals may have wider application than control of chicken mites.

Keywords

Dermanyssus gallinae, control system, trap, fungal pathogen, semiochemical, pheromone, attractant, *Beauveria bassiana*.

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***Pathology and Ecology of the Genus Clostridium in Humans, Animals and Foodstuffs:
Identification, Epidemiology and Prophylaxis***

<i>Project number</i>	QLK2-CT-2001-01267
<i>EC contribution</i>	387.480 €
<i>Duration</i>	39 months
<i>Type</i>	CA
<i>Starting date</i>	1st October 2001

Summary

The overall aim of this concerted action is to exchange across Europe the scientific knowledge and technical know-how about the genus *Clostridium* to create a standardised basis for new studies and improved techniques in all aspects concerning this bacterial genus. The project workplan includes the organisation of four workshops, the edition of congress proceedings, scientific and a technical book and the creation of a website.

Problem

The Clostridia are a group of ubiquitous, spore-forming anaerobic bacteria. The genus comprises many species. Although some species have been studied in detail, the genus is generally not well characterised.

Some of the species are pathogenic for humans and/or animals among which those causing tetanus, botulism, enteritis, gas gangrene are the most well known.

Clostridial diseases and infections are often underdiagnosed because the isolation and identification of the clostridial species can be uneasy and sometimes inaccurate. Moreover, identification of clostridial species relies, at least in part, on the demonstration of the production in vitro of a specific toxin, or on the demonstration of the presence of their encoding genes, some of which can be lost in vitro.

The epidemiology of the diseases has been barely investigated, because of the lack of standardised techniques for the diagnosis and identification of the Clostridia. This standardisation across Europe and the presence of reference laboratories is a requirement to perform valuable epidemiological studies.

The mechanisms by which many Clostridia cause disease are still not well understood. The toxins produced by the bacteria seem to be often involved in the pathogenesis of the diseases. A knowledge of the structures and modes of action of these toxins has already allowed their therapeutic use in some non-clostridial clinical conditions. However, some of the toxins still need to be further characterized. These toxins also form the basis for vaccines, powerful tools in medicine, and more effective vaccines should be developed with the help of genetic manipulation technologies.

Our increased knowledge of the molecular genetics and cellular physiology of the bacteria will in turn allow improved methods for culturing or for impairing the growth of the bacteria for diagnostic and vaccine production purposes.

Aim

This concerted action aims to gather the scientific and technical know-how of several leading European laboratories in the field of the genus *Clostridium*. Collaboration between human medicine, veterinary medicine, microbiology and food microbiology departments should make possible standardisation of techniques and protocols for epidemiological, bacteriological and genetic studies as well as development of new vaccines. Reviewing and updating the current knowledge on the genus *Clostridium* is the main purpose of this project.

Expected results

Information in all the fields concerning the genus *Clostridium* (ecology and pathogenesis, molecular genetics and taxonomy, virulence factors and vaccination, classical and molecular identification and epidemiology, antibiotic resistance, food safety and spoilage, sporulation) will be disseminated in various forms:

1. Direct exchange of information in the four meetings.
2. Extended exchange of information through scientific booklets summarizing the topics of the meetings.
3. Collection of protocols and technical information in the technical book.
4. Website for permanent and easy exchange of information.

Potential applications

The proceedings of the workshops and the scientific booklets will be disseminated to all partners and external participants, to veterinary and medical practitioners and routine diagnostic laboratories, to food microbiologists, and to private companies and stock breeders, to some extent. The technical booklets will be disseminated to researchers, diagnosticians and laboratory personnel, working with *Clostridium* sp. The advertisement of the events and the dissemination of the results will also happen via the website.

It is also the aim of the Concerted Action to disseminate the newly acquired knowledge to non scientific communities, such as stock breeders in the veal, beef, porcine, and poultry sectors and such as meat supermarkets, during their national and regional meetings and via seminars organized by pharmaceutical companies. Popular works may also be published in appropriate newspapers from and for the “sectors”. Links with other websites dealing with Clostridia in health, disease and food safety will be created.

Keywords

Clostridium, taxonomy, toxins, pathology, molecular genetics, vaccines, food safety, diagnosis, sporulation, epidemiology.

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Oral vaccination of fish with plant-derived protein vaccines

Project number	QLK2-CT-2001-01288
EC contribution	1.127.605 €
Duration	36 months
Type	RS
Starting date	1st December 2002

Summary

Viral haemorrhagic septicaemia (VHS) and spring viraemia of carp (SVC) are two devastating viral diseases of salmonids and carp, respectively. FISHOV is a collaborative effort of virologists, immunologists, plant-biotechnologists and feed industry to develop a new class of oral vaccines for the prevention of VHS and SVC and delivery in feed. The methodologies developed will allow vaccination with a minimum of stress and handling at mass scale and from the moment young fish are immunocompetent.

Problem

In Europe and elsewhere, VHS and SVC are serious problems in farm reared salmonids and carp respectively. Several experimental vaccines comprising the G protein of VHS virus (VHSV) resulted in the production of protective antibodies upon parenteral immunization of rainbow trout and in protection in case of a DNA vaccine developed by one of the partners. Commercial vaccines are limited to some bacterial diseases and vaccination is mainly by labour-intensive injection or immersion methods. The major advantages of oral vaccines is that they will enable the farmer to immunise fish with a minimum of stress and handling at mass scale and from the moment that young fish are immune competent. Earlier fundamental research by one of the partners have shown the potency of oral vaccination. However, the procedures necessary to overcome digestion of the vaccine in the digestive tract are too expensive for commercial application.

In FISHOV the complementary skills of five research groups will be combined to develop a cost-effective oral vaccination method for the prevention of VHS and SVC. To this end, the G proteins of VHSV and SVCV will be produced in plants and combined with gut adhesion (carrier) molecules. Their transport, uptake and immunogenic capacity will be studied in rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*). Plant-derived vaccines giving best results will be incorporated in fish feed and formulations not harming the antigenic capacity of the G proteins will be evaluated. Vaccination by feeding and challenge by viral infections will ultimately proof whether these new vaccines are successful and will be strongly beneficial for a healthy aquaculture.

Aim

The central focus of FISHOV is the development of oral vaccination protocols with the objectives:

- Production of viral G proteins in plants and evaluation of immunogenicity;
- Estimation of most effective carrier molecule for gut delivery;
- Investigation of antigen processing in rainbow trout and carp gut and subsequent immune responses;
- Production of most promising vaccine in tubers and formulation in fish feed and
- Evaluation of protection against VHS and SVC by challenging.

Expected results

- The expected results will be:
- Production of viral G protein antigens and carrier complexes in potato tubers;
- Knowledge about antigen transport, uptake and immunogenicity in carp and rainbow trout;
- Detailed characterisation of antigen processing and aspects of immunity (systemic and mucosal immune responses, cell mediated immunity) in trout and carp;
- Method for the incorporation of plant-derived protein vaccines in fish feed;
- Knowledge about the relation between immunity and protection.

Potential applications

The results obtained in FISHOV can lead to a safe and cost-effective innovative procedure for oral vaccination. Methodologies and findings will be published in peer-reviewed journals and our website (www.fishov.org) and through presentations at international scientific meetings. The technology developed and tested on farm reared fish can be extended much further and used for the development of edible vaccines for veterinary and human purposes. A marketing plan will assess further commercialisation of the technology.

Project web-site: <http://www.fishov.org>

Keywords

Rainbow trout, carp, oral vaccination, fish feed, viral G proteins, VHS, SVC, gut adhesion molecules, genetically modified plants, viral challenges.

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Identification of efficacious delivery systems for recombinant and nucleic acid construct vaccines

<i>Project number</i>	QLK2-CT-2001-01346
<i>EC contribution</i>	1.175.204 €
<i>Duration</i>	42 months
<i>Type</i>	RS
<i>Starting date</i>	1st September 2001

Summary

Prevention of classical swine fever virus (CSFV) infection, its spread and the occurrence of disease in naive swine is the aim of the project. Development and application of novel-type CSFV marker vaccines linked to qualitative and quantitative analysis of the immune response, the innate as well as the acquired are employed. The differentiation of low from high efficient vaccine types and the definition of the immunologic requirements for a rapid protection in intervention strategies are ensured by a high degree of technical standard in a consortium of experts.

Problem

Re-emerging epidemics of classical swine fever (CSF) in 1997/98 and 2000 and recently foot-and-mouth-disease (FMD) have demonstrated the particular vulnerability of an immunological naive livestock population for highly contagious viral diseases. In the modern eradication programs of the EU marker vaccine technology is demanded and in addition potent rapidly protective novel type vaccines are needed to be used in intervention strategies to avoid disastrous spread of infection. Besides the fulfilling of existing high standard safety criteria vaccination intervention strategies require a completely new quality of efficacy different from that accepted for repeated vaccine applications in time consuming basal immunisation programs. It seems, therefore, reasonable to investigate and exploit the capacities of the porcine immune system in response to potent CSFV antigens and to enhance its reactivity as well as to limit the time in the onset of protection against CSF.

Aim

This project is focussed on the comparative evaluation of newly developed classical swine fever virus marker vaccines in combination with rapid and potent immuno-modulatory activation of defence mechanisms leading to solid protection in swine. Four prototype novel vaccines (2 nucleic acid vaccines and 2 vector delivery systems) containing two immuno-dominant proteins of CSFV will be generated. After generation of eukaryotic expression vectors (pcDNA4-E2/NS3) these constructs will be supplemented by DNAs encoding the interleukins IL-12, IL-18 and the CD40 ligand for efficacy tests. As a new subunit recombinant protein vaccine the *Pseudomonas aeruginosa* outer membrane fusion lipoprotein (E2/NS3OprI-CSFV) will be used. Recombinant parapoxvirus ORF virus (ORFV) is developed as a safe and immuno-stimulatory vector expressing the CSFV proteins E2/NS3. Representing nucleic acid vaccines cDNA and RNA encoding CSFV-E2/NS3 are prepared and combined with DNAs encoding the above mentioned cytokines when an enhancement of the immune response is desirable. Characterisation of the immune responses and their beneficial modulation in terms of potency improvement and shortening of the onset period for protection will be followed by the application of i) cytokine encoding genes or cytokine protein ii) the CD40 ligand and iii) a dendritic cell (DC) delivery system. The latter consists of monocyte- and bone marrow derived DC, which are loaded with antigen for in vivo injection. The detailed analysis of the immune response encompasses cell culture systems, flow cytometry, cytokine assays for the assessment of the capacity of selected vaccine formulations to rapidly induce protective immunity against CSFV challenge infection.

Expected results

The partnership in this project allows the generation of new vaccine formulations and their efficacy testing with up-to-date methods in porcine immunology. The results of the detailed comparative immunological read-out will differentiate low from high efficient new generation marker vaccines against CSF. Possible improvement of such vaccines by addition of immuno-stimulatory components will be revealed. The major focus in evaluating of vaccine potency is the rapid onset of protective immunity in naive animals. Comparative potency tests will provide the information of the general requirements for vaccine-induced protection against invasion by CSFV. This virus can also be considered as a model for single-stranded RNA viruses of positive polarity, particularly in context of T helper lymphocyte reactivity patterns.

Potential applications

The project contributes to community social objectives, since the gained knowledge of these subjects can lead to improved control strategies for CSFV by providing essential information on the design of intervention

vaccines and on vaccine trials. Application of 2nd generation vaccines during an outbreak can reduce the number of pigs slaughtered, the number of animals in farms that have to be culled, and might limit the duration of the outbreak. This, in turn, reduces the costs of an outbreak. It can also reduce welfare problems for pigs. Moreover, when trade remains allowed after vaccination, it also prevents problems for pig holders in other regions, and it reduces the amount of uninfected meat that has to be destructed because of trade restrictions. The most important benefit of this project will be the potential improvement of control of a disease of economical importance, which is still a threat for Europe as shown by recent outbreaks in countries of the EU.

Keywords

Classical swine fever, novel type marker vaccines, intervention vaccination, efficacious delivery, rapid onset immunity.

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Immunological mechanisms of protection against classical swine fever virus: Towards the development of new efficacious marker vaccines.

<i>Project number</i>	QLK2-CT-2001-01374
<i>EC contribution</i>	998.114 €
<i>Duration</i>	39 months
<i>Type</i>	RS
<i>Starting date</i>	1st November 2001

Summary

Outbreaks of Classical Swine Fever, which still occur in countries of the EU, have both an immense economic and social impact, and cause economic losses in the farming and food industry. This project focuses on understanding the immune mechanisms underlying protection against CSF. The mechanisms involved in (early) protection against clinical signs of CSF after vaccination will be investigated. Improved knowledge of these subjects contributes to the development of new diagnostics, new efficacious vaccines and immuno-stimulants to improve the quality of vaccine-induced immune responses. This might result in improved control strategies for CSFV during outbreaks or eradication campaigns.

Problem

The high costs of a Classical Swine Fever (CSF) - outbreak, the social impact and resistance against destruction of pigs have led to reconsideration of emergency vaccination against CSF. The application of marker vaccines that can provide early protection might contribute to improved intervention and control strategies and might limit the duration and costs of an outbreak.

Aim

The objective of this project is to understand the immune mechanisms underlying protection against CSF, in particular the mechanisms involved in early protection against clinical signs of CSF. First, the innate immune response involved in the early phase of protection will be characterised. This will include the analyses of the interaction of antigen presenting cells (APC) with the vaccine virus with respect to cytokine profile, and functional activity of APC relevant to the induction of a rapid and protective immune response. In addition the role of NK cells in innate immunity to CSFV will be analysed. The second step will be to determine the involvement of Major-histocompatibility-complex (MHC) class II restricted T helper cells in the immune responses induced upon vaccination. Thirdly, the CSFV specific, cytotoxic MHC-I restricted immune response will be characterised. The quantitative analysis of antigen specific CD8⁺ T cells has been revolutionised recently by the introduction of MHC-I class tetramer technology. MHC-peptide combinations will be identified which allow us to design MHC tetramers able to identify and quantify populations of virus specific CTLs produced after vaccination and infection. Furthermore, by constructing suitable viral mutant C strain viruses, viral proteins and epitopes may be defined that are involved in the protective immune response. Therefore, suitable viral expression products will be constructed and synthetic peptides will be used. To this end in vitro generated antigen presenting dendritic cells will be of high value. The new techniques, mutant C strain viruses and reagents will be used to further characterise the kinetics and properties of the innate and CSFV-specific protective immune response. The knowledge about relevant viral components, and about the qualitative and quantitative aspects of the vaccine-induced immune response may contribute to the future development of new and safe marker vaccines that can induce a fast protective immune response. Viral components important for (early) induction of protection may be defined.

Expected results

1. Characterisation of the protective immune response against CSFV; defining of correlates of clinical protection. Identification of immunomodulatory elements of CSFV interfering with protective innate and specific immune responses. Once identified, immunosuppressive activities of the virus can be specifically removed from the vaccine candidates.
2. Identification of viral B cell, T helper and cytotoxic T cell epitopes relevant for early and efficient protection against CSFV. Identification and characterisation of antigen uptake, processing and presentation by APC of vaccine candidates leading to efficient MHC class I and MHC class II restricted presentation.
3. Construction of MHC I tetramer prototyped, prediction of MHC I binding peptides based on anchor residue of MHC I motifs. Isolation of MHC II, defining MHC II peptides, construction of MHC II liposome prototype.
4. Construction of C strains mutants, expression products of viral proteins.

5. Development of the methodologies permitting the measurement of immunological correlates of protection.

Potential applications

Improved knowledge on understanding the immunopathogenesis and the mechanism of the protective immune response against CSF contributes to improved vaccines and improved control strategies for CSFV during an outbreak. Application of (marker) vaccines during an outbreak can reduce the number of pigs slaughtered, the number of farms that have to be culled, and might limit the duration of the outbreak. This, in turn, reduces the costs of an outbreak. Therefore, the most important benefit of this project will be the potential improvement of control of a disease of economical importance. Furthermore, newly developed products or techniques of high value for vaccinology in general will become available. Pharmaceutical companies will be contacted for commercialisation of new vaccines and diagnostic tools.

Keywords

Classical Swine Fever Virus (CSFV), vaccination, innate immune response, (early) induction of protection.

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Paratuberculosis Epidemiology And Risk Assessment: Novel Approaches To Identify Strain-Specific Markers

<i>Project number</i>	QLK2-CT-2001-01420
<i>EC contribution</i>	719.224 €
<i>Duration</i>	39 months
<i>Type</i>	RS
<i>Starting date</i>	1st December 2001

Summary

The principle aim of this project is to exploit new technologies to identify strain-specific markers for *Mycobacterium avium* subsp. *paratuberculosis*, the causative agent of paratuberculosis. The strain-specific markers identified will encompass both genotypic and phenotypic traits and for the first time will enable differentiation of isolates from different host species. New improved typing assays will be developed using the strain-specific markers to permit epidemiological monitoring, forecasting, and risk assessment.

Problem

Mycobacterium avium subspecies *paratuberculosis* (*M.a.paratuberculosis*) is an enteric pathogen of ruminants responsible for significant economic losses to cattle, sheep and goat industries worldwide. Recently there has been an upsurge of interest in *M.a.paratuberculosis* as a potential hazard to human health (Crohn's disease) due to its presence in commercial milk supplies and survival of pasteurisation procedures. Despite this alarming observation, the zoonotic potential of *M.a.paratuberculosis* is unclear because it has not been possible to demonstrate that the same strains causing disease in ruminants are found in patients with Crohn's disease. Conventional typing procedures such as restriction fragment length polymorphism and pulsed-field gel electrophoresis detect limited genetic diversity among *M.a.paratuberculosis* isolates and it has not been possible to resolve the problem of inter-species transmission and risk assessment. The aim of this project is to identify *Mptb* strain-specific markers correlating with host preference, which is of paramount importance for epidemiological studies, eradication measures, risk assessment and vaccination strategies.

Aim

The focus for this project is to identify *M.a.paratuberculosis* strain-specific markers correlating with host preference. These strain markers should detect genetic differences (pathogenicity islands), differences in protein expression and differences in surface carbohydrate structure. The scientific objectives are:

- Assemble a reference panel of *M.a.paratuberculosis* strains representative of different host groupings (bovine, ovine, caprine and humans);
- Determine genotypic and phenotypic markers for *M.a.paratuberculosis* strains from the different host groupings;
- Develop new typing assays based on the identified strain-specific markers;
- Perform a controlled evaluation of new *M.a.paratuberculosis* strain markers in a field situation.

Expected results

1. A reference panel of *M.a.paratuberculosis* strains representative of four different host groupings reflecting the host species most important for inter-species transmission.
2. A panel of new strain-specific markers that will differentiate *M.a.paratuberculosis* isolated from different host species that is relevant for risk assessment, epidemiological studies, eradication measures and control strategies.
3. New *M.a.paratuberculosis*-specific typing assays evaluated using clinical isolates from all over Europe.

Potential applications

Use of the *M.a.paratuberculosis* specific markers correlating with host preference will allow for the first time

- Epidemiological studies evaluating the frequency of interspecies transmission.
- A risk assessment of contracting Crohn's disease from contaminated milk supplies.
- An economically feasible strategy for *M.a.paratuberculosis* control in dairy, sheep, and goat industries emphasising consumer safety.
- The development of plausible EU guidelines for *M.a.paratuberculosis* testing of animals and milk with respect to consumer health.

Keywords

Mycobacterium avium subsp. paratuberculosis, paratuberculosis, Crohn's disease, strain-specific markers, host preference, inter-species transmission, epidemiology.

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The Genetic Basis of Gyrodactylus salaris Resistance in Atlantic salmon

<i>Project number</i>	QLK2-CT-2001-01631
<i>EC contribution</i>	1.085.333 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1 st September 2001

Summary

The overall aims of the proposed project are to elucidate the molecular basis for variation in resistance to *Gyrodactylus salaris* in Atlantic salmon, the mechanisms involved in host-parasite interactions, and to provide the scientific basis for new options for controlling the impact of the parasite on susceptible salmon stocks.

Problem

Gyrodactylus salaris is a monogenean ectoparasite of fish that occurs in a number of European countries, and has had devastating effects on Atlantic salmon stocks in rivers, most notably in Norway. The usual method of control in Norway is treatment of the affected rivers with the chemical, Rotenone. This is a very severe treatment, resulting in the death of all vertebrates in the river, and so is unsuitable for rivers with high biodiversity. It is also not feasible for use in treatment of complicated river systems. Alternatives to this approach are therefore required. The current project is based on the results of challenge experiments using *G. salaris*, which have shown that salmon stocks from the Baltic area appear to be resistant to the parasite, while Atlantic salmon stocks are highly susceptible. This difference in resistance is thought to have a genetic basis. Examination of differences in fish response at the gene expression level, and isolation of resistance associated DNA markers, will provide alternative approaches to control of this dangerous fish pathogen.

Aim

The main aim of the project is to gain an understanding of the interaction between *G. salaris* and its salmon host through investigation of the host immune response, the molecular basis for variation in host resistance to the parasite, and differences in genetic makeup of the parasite populations. Understanding the genetics of host resistance and parasite pathogenicity will be integrated and used to develop novel management options in relation to: 1) limiting the potential for inadvertent introductions of pathogenic strains of *G. salaris* into areas with susceptible salmon stocks, and 2) for genetically enhancing susceptible wild salmon stocks for increased resistance to *G. salaris* in rivers where inadvertent introductions take hold.

Expected results

- Isolation of molecular markers or quantitative trait loci (QTLs) linked to resistance to *G. salaris* infection.
- Development of molecular marker based selection methods that could be applied to introducing heritable resistance to parasites into wild salmon populations.
- Isolation of markers for discrimination of *G. salaris* at the population level for use in the identification of pathogenic strains and epizootiology studies.
- Elucidation of the immune response of salmon to *G. salaris* infection.
- Elucidation of specific differences in host/parasite response that confer resistance to *G. salaris*.

Potential applications

The project aims to enhance animal welfare by increasing survival of salmon infected by *G. salaris*. Isolation of genetic markers linked to *G. salaris* resistance provides the potential for developing appropriate breeding programmes where the desired markers and associated genes can be increased in the population with the minimum of loss in stock genetic diversity. Knowledge of the salmon immune response to *G. salaris* infection may provide information that can be used in the development of vaccines or immune enhancement. These may have limited use in the wild, but may reduce the potential of farmed fish to act as reservoirs for *G. salaris*. Knowledge of the salmon immune response to *G. salaris* will in turn provide insights into other fish/parasite interactions.

Improved control of *G. salaris* will protect the aquatic environment by preventing future loss of salmon populations and reducing the need for management approaches based on harmful chemicals such as rotenone, which eradicate *G. salaris* but also have a negative impact on the biodiversity of treated areas. Therefore, the management of the natural resources of salmon and the aquatic environment will be improved and sustainable fisheries will be protected through the control of this infectious disease.

The results of the project could potentially be used by legislators, fisheries managers and disease control authorities in planning and implementing methods for detection and prevention of disease, thus conserving at-risk populations of an already endangered species, the salmon.

Keywords

Gyrodactylus salaris; resistance; immune response; DNA markers; gene expression; pathogenicity.

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Development of a safe, efficacious bluetongue virus vaccination strategy in Europe

Project number	QLK2-CT-2001-01722
EC contribution	1.698.569 €
Duration	36 months
Type	RS
Starting date	1st December 2001

Summary

Bluetongue virus (BTV) causes an internationally important disease of sheep. Control is difficult because the virus is transmitted by certain species of biting insects that can spread the disease widely and without warning. The only available vaccines are live preparations that are perceived to have significant drawbacks so that many authorities refuse their use. This project will test the existing vaccines for safety, will develop molecular methods to enable the detection of virus incursions and vaccine breakdown, and will attempt the development of efficacious, inherently safe, inactivated vaccines, thereby enhancing control.

Problem

Outbreaks of bluetongue (BT) have occurred several times in Europe and the disease is once again causing severe animal health and trade problems throughout much of the southern half of the continent. During the period 1998-2002 well over 300,000 sheep have died or been culled but the disease is still continuing. Throughout the current series of outbreaks the affected countries have attempted to control and eradicate the virus by traditional zoosanitary measures but sadly this has not halted its spread. Vaccination is a vital element in the control of many infectious diseases however this is a problem fraught with difficulty in the case of BT and the use of the existing live virus vaccines is associated with a number of concerns and limitations so that many countries prohibit them. This project addresses many of the problems associated with the existing vaccines and also seeks to develop novel vaccines that are inherently safe and confer enhanced protection thus improving control. In addition, the project seeks to develop methods to enable backtracking of virus incursions into the EU and to enable differentiation between vaccinated and field-infected animals so that control strategies can be more effectively targeted.

Aim

To test the ability of live vaccine viruses to re-assort with wild-type viruses both in the insect and mammalian hosts.

- To attempt the colonization of European and other BTV vectors and, subsequently, to assess reversion to virulence of vaccine viruses on passage through the vectors.
- To develop safe, efficacious inactivated whole virus, sub-unit or recombinant baculovirus expressed vaccines, and to evaluate them for possible commercial production.
- To develop assays enabling the differentiation between animals infected with a live BTV (field or attenuated vaccine viruses) and those vaccinated with non-replicating vaccines.
- To develop molecular epidemiological methodologies for backtracking BTV incursions to source, and for differentiating infection with vaccine viruses, from infection with field strains, thus enabling vaccine breakdown to be detected.

Expected results

- The reduction or elimination of BTV from the EU and adjoining territories by:
- Assessing the safety of using the existing live virus vaccines and recommending their use or otherwise.
- The development of inherently safe non-replicating virus vaccines of enhanced efficacy.
- Enabling the detection of sources of virus incursions into Europe thus facilitating the early deployment of targeted control measures.

Potential applications

The results will allow a safe, effective and coherent BTV vaccination strategy to be developed for the first time in Europe and will enable "outside" sources of infection to be identified and monitored. They will also facilitate the manufacture of BTV vaccines in Europe enabling a more rapid and specific response to future virus incursions into the EU.

Project web-site: http://www.iah.bbsrc.ac.uk/dsRNS_virus_proteins/ReoID/vaccine.htm

Keywords

Bluetongue virus, attenuated and inactivated vaccines, vaccine testing, Culicoides vectors, molecular epidemiology, vector passage, reversion.

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African swine fever (ASF): improved diagnostic methods and understanding of virus epidemiology and virus-host interactions

<i>Project number</i>	QLK2-CT-2001-02216
<i>EC contribution</i>	1.467.035 €
<i>Duration</i>	39 months
<i>Type</i>	RS
<i>Starting date</i>	1st November 2001

Summary

African swine fever (ASF), is a highly contagious disease and it is a limiting factor for the pig industry, causing severe economical losses and serious social impact in areas where it occurs. In Europe ASF is enzootic in Sardinia (Italy) but a confined and disturbing outbreak has occurred recently (1999) in Portugal. Lack of knowledge on the role of relevant viral components on the activation of protective immune mechanism of pigs, has impaired the development of efficient and safe vaccines. ASFV also infects soft ticks of the genus *Ornithodoros* that parasite the pig thus creating a cycle of infection that maintains the virus.

Problem

ASF outbreaks in pigs have often been a chronic form of disease from which many pigs recover and may remain persistently infected. Diagnosis of this form of the disease is difficult as it can be overlooked or confused with other diseases. Persistently infected recovered pigs also provide a virus reservoir and may infect healthy pigs. The virus genome encodes several proteins that interfere with host defence systems and this may explain how ASFV can persist in recovered domestic pigs and in its natural hosts in Africa, the warthog and bushpig. It may also help to explain why attempts to develop a vaccine have failed. A better understanding of the role of these genes in the modulation of pig immune responses and the definition of the molecules involved in determining host cell tropism are needed to evaluate the variable pathogenesis of virus isolates and mechanisms of persistence. This information will be relevant for understanding the epidemiology of disease and in the longer term will aid in development of a rational scheme to produce an effective and safe vaccine.

Aim

The major goal of this proposal is to develop basic scientific knowledge in order to: i) Improve diagnostic capabilities of African swine fever (ASF), ii) Gain better understanding on the molecular epidemiology of the etiological agent, iii) Characterize virus genes important for immune evasion and persistence in pigs and soft ticks (*O. erraticus*), and iv) Characterize the potential role of these argasids as reservoirs in EU countries, free of the disease.

Expected results

1. The development and evaluation of ELISA assays to detect antibodies against ASFV in pig sera, using a) mixtures of recombinant proteins, b) non-infectious virus-like particles and the assessment of recombinant ASFV proteins in delayed type hypersensitivity (DTH) tests.
2. The development of ELISA assays using recombinant *Ornithodoros* proteins to test for the presence of these soft ticks in farms, trough detection of specific antibodies in pigs.
3. The development of improved PCR tests to detect virus during a) early stages of infection of pigs b) non-apparently infected pigs and c) soft ticks.
4. The establishment of a sequence database from conserved and variable genome regions that can be used to help trace the source of disease outbreaks.
5. The characterisation of virus ligands involved in cell attachment and of the critical binding domains in those ligands. Identification of the putative cellular receptor(s).
6. The construction of virus deletion mutants lacking genes involved in immune evasion.
7. Evaluation of the role of the genes mentioned in 6) by assessment of infection of pigs, pig macrophages, ticks and tick cells with deleted mutant virus.
8. The evaluation of the potential role of *Ornithodoros erraticus* in maintenance and transmission of ASF by: a) survey of soft ticks in Spain and Portugal for the presence of ASFV, b) experimental infection of laboratory colonies of *O. erraticus* to establish infection rates, persistence of infection and tick-to-tick and tick-to-pig transmission.

Potential applications

The availability of efficient and safer methods to diagnose ASF as well as the acquisition of knowledge on fundamental aspects of the biology of the virus and its relations with the natural host (domestic pig) and with its vector or potential reservoir (*Ornithodoros erraticus*) will contribute for the prevention of the disease and will

open new insights for the development of new strategies for its immunogenic control. Approaching prevention and control of ASF using the rational of this proposal will contribute for the preservation of natural resources in Community countries.

Keywords

ASF, diagnosis, molecular epidemiology, virus-cells, viral genes, *Ornithodoros erraticus*.

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Epidemiological and transmission studies in sheep and moufflons naturally infected by jaagsiekte retrovirus, the causative agent of sheep pulmonary adenomatosis

<i>Project number</i>	QLK2-CT-2001-02380
<i>EC contribution</i>	574.967 €
<i>Duration</i>	48 months
<i>Type</i>	RS
<i>Starting date</i>	1st December 2001

Summary

The project comprises 9 integrated workpackages that will deliver the tools and information to describe the epidemiology of jaagsiekte sheep retrovirus (JSRV) the causative agent of sheep pulmonary adenomatosis (SPA, ovine pulmonary adenocarcinoma). Convenient and reliable protocols to obtain genomic DNA for JSRV PCR from large numbers of blood and milk samples will be established. Parallel studies will develop high throughput PCR procedures, including development of multiplex PCR assays that retain the sensitivity of the existing JSRV PCR, as well as new techniques for detection of PCR products, such as hybridisation ELISA, real time PCR and molecular beacons. Following validation of the JSRV PCR protocols, prospective longitudinal surveys will be conducted in three flocks of sheep with a well-established history of SPA. These flocks comprise three different breeds, representative dairy and meat production systems, different pathological forms of SPA and an endangered species of wild sheep. Cohorts of JSRV viremic and JSRV negative ewes and their progeny will be sampled at 3-4 month intervals over 30-33 months and tested for JSRV infection by PCR. Most of these sheep will be killed at the end of the study to determine the precolostral blood and fetuses for JSRV. A similar epidemiological survey will be performed in wild moufflon. Finally, transmission of JSRV through colostrum/milk will be investigated experimentally. Groups of lambs will be fed with colostrum/milk from JSRV viremic mothers. Blood from these lambs will be tested for 6 months after exposure for JSRV, after which they will be necropsied to determine their disease status. A group of lambs will be fed milk spiked with JSRV as positive control.

Problem

Current control strategies for sheep pulmonary adenomatosis (SPA) are ineffective. SPA is only identifiable by clinical signs or lesions. There are no serological or other blood tests available. Epidemiological features of the disease, particularly its routes of transmission in affected flocks are not known.

Aim

The overall aim of this proposal is to characterise the epidemiological features of the infection of the retrovirus (JSRV) causing sheep pulmonary adenomatosis (SPA), using new PCR blood tests, particularly its routes of transmission in naturally affected flocks. Milk transmission experiments will be also developed. This can be used to identify the risk factors for transmission and maintenance of the SPA in affected flocks.

Expected results

1.- Development of robust, high throughput and specific PCR test to detect JSRV in blood and milk; 2.- Determination of the epidemiological features of the JSRV infection (ranges of JSRV prevalence, influence of breed or management system, determination of the time and ages which infections first occurs, evaluate the evolution of JSRV infection, etc) , 3.-Determination of the occurrence and epidemiological relevance of the maternal transmission of JSRV infection. 4.-Estimate the prevalence of JSRV in free-living moufflon.

Potential applications

The identification of the risk factors for transmission and maintenance of SPA, will be able to apply in the design of control and eradication programs in flocks affected by the disease. As SPA is on the B list of the IOE and it is considered by UE (directive 91/68: intracommunity trade in ovine and caprine animals) as an important infectious disease of sheep because of its high prevalence in several member states, the development of a high throughput and specific blood test for SPA will be able to apply in the intracommunity and international control of sheep commercial movements. Control of SPA will reduce economic losses caused by the disease and enhance the export opportunities for UE breeding stock.

Keywords

Contagious neoplasia, retroviruses, sheep, PCR, lung tumour, epidemiology, blood test, jaagsiekte, pulmonary adenomatosis, ovine pulmonary adenocarcinoma.

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Evaluation of lentivirus DNA vaccination strategies in sheep

Project number	QLK2-CT-2002-00617
EC contribution	1.701.759 €
Duration	36 months
Type	RS
Starting date	1st September 2002

Summary

Lentivirus vaccines have proven difficult to develop to date. We propose to evaluate DNA vaccination strategies in sheep challenged with maedi visna virus (MVV). Sheep will be immunised with MVV core or envelope genes or a combination of these via the skin (by gene gun) or mucosal tissues (using Salmonella vehicles). The effects of including cytokine genes encoding during priming will also be investigated. Animals will be boosted with recombinant Adenovirus encoding the MVV genes. Immunological variables as well as virus load and disease will be determined before and after challenge. The project will help to identify protective virus antigens, provide useful information on immune correlates of protection, and indicate whether mucosal or cutaneous immunisation correlates with protection.

Problem

MVV and caprine arthritis-encephalitis virus (CAEV) infections in sheep and goats cause significant economic losses. It has, however, proven difficult to develop successful lentivirus vaccines in either man or animals to date. The reasons for this include a lack of information on target antigens, protective immune responses, and effective vaccine strategies. Important questions that need to be addressed include:

1. Do the virus core proteins, or envelope proteins, or a combination confer protection after immunisation?
2. Which immunisation route confers better protection - cutaneous or mucosal?
3. Does priming in the presence of cytokine immunomodulators alter the character of the immune response to MVV proteins, and influence the degree of protection?

Aim

The overall aim of the project is to test hypotheses relevant to the immune response against MVV in vaccinated sheep, thereby providing useful information for the rational design of an efficacious vaccine. The specific objectives are to: (i) construct and test plasmids encoding MVV gp110, gp46, p25, p16, and p14 under the control of the CMV immediate early gene promoter; (ii) immunise sheep with plasmids encoding the MVV genes with and without a plasmid encoding IFN γ via the skin (using a gene gun) or via mucosal tissues (using salmonella vehicles), followed by boosting with adenovirus recombinants encoding the same MVV genes; (iii) measure humoral and cell-mediated immune responses in vaccinated and control sheep; (iv) determine the degree of protection from infection and disease.

Expected achievements

1. Construction of all the vectors for priming and boosting.
2. Production of all the recombinant MVV proteins required for measuring immune responses.
3. Determination of whether MVV core proteins, envelope proteins, or a combination of both core and envelope proteins confer protection after DNA vaccination.
4. Determination of whether cutaneous or mucosal immunisation is a better strategy for inducing protection.
5. Determination of whether co-administration of IFN γ gene with MVV genes modifies the type of immune response generated and the degree of protection.

Potential applications

If an efficacious vaccine is identified, it will be patented and commercialised. In addition, the recombinant virus proteins produced may have commercial potential as diagnostic reagents. The results will provide detailed information relating to DNA vaccination strategies for MVV infection, which will enable future developmental and optimisation work on a vaccine as well as cytokine immunomodulation to be performed. The results will be relevant not only to MVV but also to other lentiviruses such as HIV. In addition, the questions being addressed and the approaches being adopted will be of interest in transdisease vaccinology fields.

Keywords

Lentivirus, small ruminant, DNA vaccine, immune responses, virus load.

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Diagnosis, epidemiology and control of an enteric myxosporosis of commercial Mediterranean fish

<i>Project number</i>	QLK2-CT-2002-00722
<i>EC contribution</i>	899.954 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1 st October 2002

Summary

The present project intends to evaluate the epidemiological status of the enteric disease caused by *Myxidium leei* (Myxozoa) in cultured sparids and in wild fish, by validating and using a diagnostic PCR-based test. PCR will also be used to screen samples from invertebrates that might act as alternate hosts for the parasite, and will be useful to control the introduction and spreading of the disease in culture facilities. The mechanisms involved in transmission, pathogenesis and immune response will also be studied in order to implement adequate prophylactic measures.

Problem

Myxidium leei (Myxozoa) is an important pathogen of sparid fish cultured in the Mediterranean and causes high mortality in net-pens and in land-based mariculture systems, with a notable economical impact for Mediterranean mariculture. In spite of the relevance of this myxidiosis, there are important gaps in the knowledge of the parasite life cycle, transmission, epidemiology, mechanisms of infection and immunology. Reliable, high-throughput diagnostic methods and efficacious control measures are currently unavailable.

Aim

The overall aim of this project is to generate tools and knowledge useful to develop efficacious measures for the prevention and control of this enteric myxosporosis. The detailed objectives are:

- To provide and validate a reliable PCR-based test for the specific diagnosis of the parasite, including sampling methods non-lethal for the fish.
- Application of this PCR assay in field epidemiological studies involving diverse sparid systems in the different countries.
- Use of this PCR assay to evaluate the role of wild fish and invertebrates as reservoirs of the parasite.
- Study of the mechanisms involved in transmission, pathogenesis and infective process in sparid fish, in the course of experimental infections.
- Study of relevant aspects of the innate and adaptive immune responses to the parasite, and evaluation of immunomodulation strategies for the prophylaxis of the infection.

Expected results

- Validated PCR diagnostic tests and methods.
- Data on the epidemiological situation of the disease in cultured and wild fish.
- Data on the role on invertebrates as reservoirs or true alternate hosts.
- In situ hybridisation methods for detecting all parasite stages.
- Knowledge of parasite/host interaction, parasite development, and pathogenic mechanisms.
- Knowledge of key factors involved in the immune response and determination of the existence of antibody response.
- Evaluation of the adequacy of levamisole for disease prevention.

Potential applications

1. The PCR- based test for the specific diagnosis of *Myxidium leei* will be exploited by the partners, after an adequate agreement. It could be applied by the partners' laboratories, or by other licensed laboratories, including national and European reference laboratories. End users will be the fish farmers, veterinarians and diagnostic services on fish health. The use of this test will allow:

- to know the epidemiological status in the different countries, coastal areas and farms and to evaluate the risk of infection;
- to control the introduction and dispersion of the disease in national and international fish transactions.

2. Eventually, a protocol for the use of levamisole for disease prevention. Its exploitation will depend on the results obtained and the criteria of the laboratories involved, but the end users would be the farmers.

3. The generated knowledge on the parasite epidemiology, life cycle, transmission, and pathogeny will facilitate the design of management recommendations for the prevention and control of this disease. Such

recommendations will be disseminated among the involved entities, and some of them will be exposed in a final meeting with end users and, eventually, using a web site (to be developed).

Keywords

Myxozoa, Myxidium leei, PCR, Diagnosis, Life cycle, Epidemiology, Immunology, Aquaculture, Sparidae.

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Novel coronavirus vector-based vaccine for prevention of foot-and-mouth disease

Project number	QLK2-CT-2002-00825
EC contribution	1.554.598 €
Duration	42 months
Type	RS
Starting date	1st September 2002

Summary

A previously developed biosafe transmissible gastroenteritis virus (TGEV)-based vector will be engineered to amplify foot-and-mouth disease capsid proteins, to serve as a marked vaccine against FMD. In addition to the biosafety features of the TGEV-based vector, constructions will include exclusively FMDV protease 3C and P1-2A (to produce virus-like particles in situ) and or a fragment of 3D so that no FMDV can be produced. The TGEV chosen replicates in the enteric and respiratory tracts which are very adequate replication sites to evoke mucosal immunity. Antigen expression, immune responses, protection against challenge with virulent FMDV, and possible virus transmission after challenge will be examined using swine, one of the major animal hosts for FMDV.

Problem

Foot-and-mouth disease is the economically most important animal disease world-wide. No safe and effective anti-FMD vaccines that do not require the handling of infectious virus: are available.

Aim: To develop a general strategy for preparation of safe, marked and effective anti-FMD vaccine that evokes a strong systemic and mucosal immune response and that prevents the establishment of the carrier state.

Expected results

The TGEV-based vectors available provide the means to express complete FMDV capsids, without FMDV RNA, and, together with T-cell epitopes, are expected to evoke a protective response in swine.

Potential applications

This new vaccine design would allow the manufacturing of an anti-FMD vaccine without the need to handle live virus or to administer inactivated FMDV, a major drawback of classical vaccines. Safe and effective anti-FMD vaccines have a potential world-wide market, and the industrial partner of the consortium is prepared to guide the commercial exploitation of a new vaccine that may result from the development of this project.

Keywords

Foot-and-mouth disease, coronavirus, vector, delivery, immune response, viral disease, marked vaccine.

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Development of a pathogen epitope prediction program, and evaluating its usefulness in designing fish vaccines

Project number	QLK2-CT-2002-00838
EC contribution	1.600.043 €
Duration	36 months
Type	RS
Starting date	1st September 2002

Summary

Peptide based vaccination is an approach that has been proven successful in warm-blooded vertebrates in particular relating to viral pathogens. In this project we will generate sufficient knowledge to evaluate this technology in a cold-blooded vertebrate (Atlantic salmon, *Salmo salar*). The peptide-binding specificity for MhcSasa class I alleles will be established providing the basis for peptide-binding assays, a pathogen-epitope prediction programme, and a prototype viral peptide vaccine. Byproducts generated throughout the project will be immunological reagents and cellular model-systems adapted to fish.

Problem

Presently, no vaccines are available that successfully protect against viral infections in fish.

Aim

The objective of the project is to develop a pathogen epitope prediction programme and to design a viral pathogen peptide - vaccine. This will be accomplished by determining the interaction of the Atlantic salmon Major Histocompatibility Complex (MHC) class I molecule with peptides derived from viral pathogens.

Expected results

The project will provide a broader understanding of the interactions between the immune system and viral pathogens. The approach of using peptide vaccines targeted at the animals immunological content will be tested and evaluated as an approach for the future.

Potential applications

The project will develop reagents for use in immunological research in Atlantic salmon. A pathogen epitope prediction programme will enable easier detection of viral peptides exploitable for vaccine production. Additionally, a viral peptide vaccine will be developed and tested. If successful, it represents a new approach to developing vaccines against viral agents in fish and may be further developed into a commercial product available to the public.

Project web-site: <http://www.farmasi.uio.no/forskning/PeptidEx/index.html>

Keywords

Viral infections, fish, peptide vaccines, Mhc, immunological reagents.

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Animal brucellosis: Genetically engineered live vaccines against B. melitensis

Project number	QLK2-CT-2002-00918
EC contribution	1.163.346 €
Duration	48 months
Type	RS
Starting date	1st September 2002

Summary

The objective of the proposal is to develop new vaccine(s) useful for the prophylaxis of Brucella melitensis brucellosis of sheep (and goats) which will not interfere in the serological tests already implemented in the EU and will pose no threat to human health (both are important drawbacks of the currently used vaccine, B. melitensis strain Rev 1). The new vaccine will be developed on the basis of a complete understanding of the genetics and immunochemistry of the main sections (i.e. core oligosaccharide and O-polysaccharide) of the Brucella surface lipopolysaccharide. This lipopolysaccharide is a key virulence-factor of brucellae and removal of its O-polysaccharide will both eliminate the epitopes eliciting the antibodies which interfere in the diagnosis and reduce virulence. This approach has been followed before and produced rough B. abortus vaccines that had controversial results in cattle and have seldom tested or failed to protect sheep. However, these vaccines were developed from B. abortus (rather than B. melitensis) and were obtained empirically. This means that, in addition to their inappropriate genetic background, their defects are unknown, and that selection was not based on a systematic study of the effects of the several possible LPS structural deficiencies on Brucella virulence. Since attenuation by LPS deficiencies can be so severe so as to reduce immunogenicity excessively, such a systematic study is undertaken in this project by a combination of bacteriological, genetic, immunochemical, chemical and biological analyses. These analyses will help to select a short set of vaccine candidates that will be tested in sheep for the ability to colonize and persist in lymph nodes and to protect against a challenge of virulent B. melitensis in comparison with the Rev 1 vaccine. The study will be completed by the definition of the interference of the new vaccine in the standard serological tests for B. melitensis diagnosis, as well as in those for B. ovis diagnosis.

Problem

Brucellosis is a zoonotic disease causing heavy economical losses and human suffering. Sheep and goat flocks under extensive breeding conditions are often infected by B. melitensis. This species is also the commonest cause of human brucellosis, a serious disease which may have important sequelae and which represents a serious problem in Mediterranean countries and world-wide. Brucellosis can be eradicated by vaccination, serological testing, culling and appropriate animal management. There is a vaccine available (B. melitensis Rev. 1) for sheep and goat immunization. However, Rev. 1 has several drawbacks: it is abortifacient in pregnant animals, virulent in humans and resistant to streptomycin, one of the antibiotics of choice to treat brucellosis. Moreover, like field strains, Rev 1 carries a surface lipopolysaccharide whose immunodominant section (the O-polysaccharide) induces an antibody response difficult to distinguish from that resulting from infection, particularly in adult sheep. This complicates considerably the diagnosis because the best diagnostic tests detect antibodies to the O-polysaccharide. Because of this, Rev 1 vaccination is limited to a single dose early in life and adult vaccination or revaccination is not used in eradication programs. Rev.1 also interferes in the diagnosis of B. ovis. This species is not dangerous for humans, but it causes significant economical losses to sheep breeders.

Aim

To develop new vaccine(s) for the prophylaxis of sheep (and goat) brucellosis which will not interfere in the serological diagnosis, could be used repeatedly and will pose no threat to humans.

Expected results: Definition of the genetics and immunochemical structure of the core oligosaccharide and polysaccharide of B. melitensis lipopolysaccharide; a set of lipopolysaccharide mutants with adequate attenuation/immunogenicity for sheep vaccination; a serological test to differentiate B. ovis infection from post-vaccine response to the mutant vaccine.

Potential applications

Vaccination of sheep and goats for brucellosis eradication.

Keywords

Brucellosis, Brucella, sheep, goats, lipopolysaccharide, gene, rough, mutant, vaccine.

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Targeting Immunostimulating-structure Production in Plants

<i>Project number</i>	QLK2-CT-2002-01050
<i>EC contribution</i>	1.050.000 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1 st September 2002

Summary

The goal of this project is the development of generic systems for oral immunisation. Immunostimulating structures will be designed which will consist of an enteric targeting molecule fused to the antigen of interest. The targeting molecule will be based on the S protein of Transmissible gastroenteritis coronavirus (TGEV), a protein with proven enteric targeting properties. This targeting molecule will be a generic vehicle for the oral immunisation of pigs, and the first application will be focused on Porcine circovirus (PCV). Subsequently other antigens will be expressed. The production of the high levels of the immunostimulating-structures required for oral immunisation will be achieved through expression in plants.

Problem

For oral immunisation to become a practical approach to vaccination, 3 essential requirements must be met: (i) a method must be devised for targeting antigens capable of conferring protective immunity to the enteric tract; (ii) a system must be developed which permits the large-scale production of the vaccine at low cost; and (iii) the immunogenic material produced at low cost must be shown to be as effective as the equivalent material produced by more conventional routes.

Aim

We will address the requirements described above in the following ways: (i) To develop method of targeting antigens to the enteric tract we will identify the domains of the TGEV spike protein essential for binding to the M cells of the enteric tract of pigs. These domains will be fused to antigenic proteins from the animal pathogens PCV, Porcine reproductive and respiratory syndrome virus (PRRSV) and *M. pneumoniae* to create targeting immunostimulating-structures; (ii) To produce the targeting immunostimulating-structures at sufficiently low cost for their use to a practical method of vaccination, we will express them in plants using recently developed high-yielding plant expression systems. (iii) To demonstrate the effectiveness and safety of the targeting immunostimulating-structures expressed in plants, we will compare the efficacy of plant-expressed structures with the equivalent material expressed in mammalian cells using coronavirus vectors.

Expected Results

The development of a targeting molecule, based on the spike protein of TGEV, capable of directing antigens to the enteric tract of pigs and its fusion to antigenic sequences from porcine pathogens which can confer protective immunity. This will lead to the creation of targeting immunostimulating-structures for the oral immunisation of pigs. (ii) Expression of large quantities of the immunostimulating-structures in plants (iii) the demonstration that plant-expressed targeting immunostimulating-structures can immunise pigs via the oral route.

Potential applications

The immediate goal of the project is to develop a cheap and effective method for the oral immunisation of farm animals. The use of plants for the production of cheap and reliable vaccines that can be directly fed to pigs would make a significant contribution to combating diseases of farm animals. Thus the project is of considerable importance in the sphere of veterinary medicine. In addition, the experience gained in the development of novel vaccines for animal health will be of great help in the development of similar materials for human health.

Keywords

Immuno-complex/Transmissible gastroenteritis virus (TGEV)/Spike protein/Porcine circovirus (PCV) Porcine reproductive and respiratory syndrome virus (PRRSV)/*Mycoplasma pneumoniae*/Plant expression systems/coronavirus vector.

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Antibiotic resistance in bacteria of animal origin – II

Project number	QLK2-CT-2002-01146
EC contribution	392.500 €
Duration	36 months
Type	CA
Starting date	1st December 2002

Summary

This concerted action will create a network of national veterinary reference laboratories in Europe and establish a surveillance system for monitoring the occurrence and emergence of antibiotic resistance among bacteria from food animals. It involves 19 laboratories in 18 European countries. An external quality control for the capability of laboratories to perform susceptibility testing of bacteria correctly will be performed. Different bacterial strains with known susceptibility patterns will be sent four times each year to the different laboratories for testing and the results entered into a central database. Each year the data generated in the individual laboratories will be collected centrally and a report on the occurrence of antibiotic resistance among the different bacterial species isolated from food animals will be generated and published.

Problem

The development of antimicrobial resistance among bacteria from food animals is considered a major public health problem in the European Union. Knowledge of the occurrence of antimicrobial resistance in the different countries is requirements to do something about the problem. There is currently no central collection of susceptibility data from the different veterinary diagnostic laboratories in Europe. In addition, there is a lack of standardisation of susceptibility testing methods between the different laboratories.

Aim

The main objective of this concerted action is to:

Improve the quality of the data obtained with the monitoring of antimicrobial resistance among food animals in the European Union and utilise the results already available.

The specific objectives are to:

1. Create a network of national veterinary reference laboratories in the EU Member States.
2. Harmonise the susceptibility testing of bacteria from food animals in European veterinary reference laboratories.
3. Collect and evaluate the susceptibility data from these laboratories.
4. Make comparable results available for the public and decision-makers.

Expected results

1. An external quality assurance system for all national veterinary reference laboratories in the EU countries ensuring harmonisation of the susceptibility testing performed.
2. A network of these laboratories.
3. Comparable and quality controlled information on the resistance situation among food animals in the different EU countries will be known.

Potential applications

The ability to point out new and emerging problems with antimicrobial resistance at an early stage could assist in performing successful interventions resulting in a higher food safety.

A reduction of the prevalence of resistant bacteria in food of animal origin will be a benefit for the export of high safety food from the EU countries. Furthermore, reduction of the occurrence of antimicrobial resistant bacteria will also have beneficial consequences for the European citizen because of reduced mortality and morbidity.

Keywords

Antibiotic resistance, susceptibility testing, monitoring, bacteria, animals.

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Optimizing DNA based vaccination against FMDV in sheep and pigs

Project number	QLK2-CT-2002-01304
EC contribution	1.299.687 €
Duration	36 months
Type	RS
Starting date	1st September 2002

Summary

The final objective of this proposal is to develop optimized strategies of DNA vaccination to foot-and-mouth disease (FMD) in sheep and pigs, for potential use as vaccines including those for “emergency”, depending on EU policy of FMDV eradication programmes. One of the major advantages of DNA vaccination over current inactivated vaccines is the avoidance of the costly manipulation of infectious virus in high containment facilities and the risk of infection in the animal due to insufficient inactivation. The ways by which the FMDV DNA vaccine strategy will be optimized will include a comparison of the immunogenicity in sheep and pigs of different FMDV antigens, of a new generation of plasmids, of promising adjuvants for DNA vaccines, of parenteral versus nasal routes of administration and of prime-boost regimens. The protocols giving the best immune responses will be used to evaluate protection to FMDV challenge in sheep and in pigs.

Problem

To generate a new generation of FMDV vaccines based on improved efficacy of DNA vaccination, which would avoid manipulations of infectious virus and would allow differentiation of infected and vaccinated animals.

Aim

Our objectives will be to identify the best ways of optimizing the efficacy of DNA vaccines to FMDV in sheep and in pigs. Efficient DNA vaccines to FMDV could replace current inactivated vaccines and emergency vaccines, depending on EU policy of FMDV eradication programmes. As a potential emergency vaccine, the optimized DNA vaccination strategies will be tested for their ability to induce early protective immunity, following preferably only one DNA injection. The various parameters tested will include several FMDV antigens, a new generation of plasmids, promising adjuvants, several routes of administration and homologous (DNA/DNA) versus heterologous (DNA/protein) prime-boost regimens. Protective immunity will be evaluated in two major target species for FMD: sheep and pigs.

Expected results

1. Definition and production of the most immunogenic FMDV antigens and of the best plasmid constructs when administered as DNA in sheep and pigs;
2. Definition of the most effective adjuvants and of the best vaccination strategy to generate a specific immune response in target animals, including the route of administration, the number of injections, the benefit of a heterologous prime-boost regimen;
3. The evaluation of protective immunity to challenge in target species.

Potential applications

The possible exploitation of the results of this project, in terms of emergency vaccination of cattle, pigs and sheep, will be subject to EU policy of FMDV eradication programmes.

Keywords

FMDV; foot and mouth disease; sheep; pigs; DNA vaccination.

Project co-ordinator

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Pathogenesis and improved diagnosis and control of avian influenza infections

Project number	QLK2-CT-2002-01454
EC contribution	1.839.294 €
Duration	36 months
Type	RS
Starting date	1st October 2002

Summary

Avian Influenza (AI) is a devastating disease of poultry that has recently also been a concern for human health. The last AI epidemic in the EU occurred in Italy in 1999/2000, caused direct and indirect economic losses of over 600 million euros, and ultimately resulted in the depopulation of approximately 14 million birds. The disease is highly contagious and has its natural reservoir in wild birds. The aims of this project are to develop EU validated rapid diagnostic tests for the identification of infected flocks, natural reservoirs and potentially infected meat. Furthermore, the molecular basis of pathogenicity and the mechanisms involved in host susceptibility will be investigated in animal models, to clarify the pathogenetic aspects of this potential zoonosis.

Problem

Highly pathogenic avian influenza (HPAI) is an exotic disease to the EU, which if introduced to poultry would be of major economic importance (the last outbreak in the EU resulted in the loss of 14 million birds - mainly chickens and turkeys). The disease is therefore the subject of legislation aimed at maintaining freedom and control of any outbreaks that occur. The EU Council Directive 92/40/EEC, imposes slaughter of poultry flocks infected with HPAI and restrictions on movements of birds and trade in poultry products in specified areas around the infected premises.

Wild aquatic birds provide the reservoir for the maintenance and dissemination of all influenza A viruses. Periodically viruses cross over from the wild-bird reservoir to poultry, including chickens, turkeys, gamebirds, domestic ducks, ratites, and other commercially raised birds. In poultry, influenza A viruses may cause asymptomatic infections or a range of disease symptoms from mild respiratory disease to severe systemic infection with high mortality. Particular emphasis is placed on the H5 or H7 haemagglutinin (HA) subtypes of AI viruses because these are the only subtypes clearly shown to cause HPAI in poultry, although most viruses of these subtypes are low pathogenicity avian influenza (LPAI). HPAI viruses are not of a single evolutionary lineage but apparently arise from non-pathogenic strains, the change to high pathogenicity seems to occur after transmission to poultry.

Preventing the introduction and spread of HPAI in poultry requires an understanding of the mechanisms involved in the evolution of highly pathogenic strains and techniques for the rapid detection and characterisation of the viruses. This project aims to address these issues.

Aim

1. To develop rapid diagnostic tests for the prevention of spread of avian influenza infection due to contact with infected animals in the sub-clinical phase and to identify potentially infected meat and wild bird reservoirs.
2. To develop a transmission model to elucidate the dynamics of influenza A virus infection within chickens and to use this model to examine the emergence of highly pathogenic viruses from those of low pathogenicity, and to measure the efficacy of vaccines and vaccination strategies.
3. To develop and evaluate the use of avian influenza marker vaccines to be applied in case of an emergency.
4. Study the pathogenesis of AI infections in animal models and the molecular mechanisms of virulence.

Expected results

It is envisaged that at the end of the project the consortium will have developed a range of diagnostic tests and reagents useful for the detection and study of AIV infections in birds. We will have an EU validated RT/PCR protocol for avian influenza virus (AIV) detection: Monoclonal antibodies (Mabs) to H5, H7 and NP of AIV that will be available to all member states for research purposes or for use in diagnostic tests: ELISAs for the rapid detection of AIV group antigen (NP): ELISAs for the detection of AI A virus antibodies in sera and meat juice from a range of bird species (NP), and for the detection H5 and H7 and M antibody: an assay for the rapid isolation of virus from infected birds.

We will have developed a mathematical infection model that will quantify the transmission of AI viruses in chickens. This will be used to measure the effect of different intervention strategies e.g. vaccination.

Novel viral like particle (VLP) technology will have been used to development and test the efficacy of a marker vaccine. Reverse genetics will have given us insights into the molecular basis for host barriers and pathogenicity.

Potential applications

All the diagnostic tests and reagents developed in this project will be available for use in the national avian influenza reference laboratories of the EC.

The antigen detection ELISA should be useful a) during an outbreak of HPAI to identify infected but pre-clinical flocks within restriction zones, thus allowing the elimination of infected flocks without delay, b) to be used as a general surveillance tool. The test will be simple enough for use in non-specialist laboratories and possibly at the flock side. There should be potential for this test to be commercialised. The serum ELISA test will be valuable for surveillance programmes. It could also be used to detect antibodies in poultry meat products that are imported into the EU, and which may potentially introduce AI.

Differences in the transmission of highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) strains may play a role in the mechanism of selection by which HPAI viruses arise in the field. The mathematical infection model should contribute to an understanding of these processes. It will also enable the effect of different intervention strategies to be measured e.g. vaccination. VLP marker vaccines could have potential role in the control of HPAI outbreaks.

Reverse genetics techniques will enable us to allocate the determinants for host range and pathogenicity to the individual functional domains of the viral proteins.

This work will lead to the identification of host range/organ tropism and pathogenicity markers. Such markers will be highly relevant for the development of new diagnostic tests and for the surveillance of avian influenza.

Keywords

‘Avian Influenza’, Poultry, ‘Highly Pathogenic’, pathogenicity.

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Appraisal of the zoo-sanitary risks associated with trade and transfer of fish eggs and sperm

<i>Project number</i>	QLK2-CT-2002-01546
<i>EC contribution</i>	300.000 €
<i>Duration</i>	36 months
<i>Type</i>	CA
<i>Starting date</i>	1 st September 2002

Summary

In this project, the scientific basis for current zoo-sanitary control requirements imposed upon transfer and trade in seeds for international aquaculture will be reviewed and future research needs in this field will be identified. The results will be disseminated towards national, European Union and international governments and organisations, towards stakeholders in the European aquaculture industry, and towards the international scientific community for use during revision and further improvement of legislation and industrial practices.

Problem

As the fish farming industry matures and becomes more competitive on an international level, there is a growing need to increase productivity by use of genetically improved stocks. In the resulting international trade, import requirements to prevent transfer of diseases is still mostly based on a “zero-risk” approach, and there is generally a discrepancy between less stringent regulations being applied to movements inside states versus more strict regulations applying to transfers across state borders. Knowing of the paucity and advanced age of scientific data forming the basis for current zoo-sanitary legislation in this field, a scientific appraisal of hazards and quantitative risks associated with the transfer of fish eggs and sperm has now become timely. A further motivation for the project is current work to revise European Community legislation on fish disease control.

Aim of the project

Aim of this concerted action is to scrutinise the scientific basis for current zoo-sanitary control requirements imposed upon transfer and trade in seedstocks for international aquaculture, and to identify future research needs in this field. The quantification of risks for transferring certain infections when moving eyed eggs and fish sperm, and the potential of new techniques for improving the efficacy of disease risk management will be especially addressed.

Work and expected results

During a series of project meetings, disease hazards associated with the transfer of fertilised eggs or gametes from cultured finfish, and scientific documentation relevant to risk assessment will be subjected to scientific scrutiny and in-depth discussions. Particular focus will be laid upon the potential for “true” vertical transfer (inside the egg), of studies allowing for a quantitative assessment of risks, and of studies on disease transfer risks with fish sperm. Pathogen survival and resistance to disinfection, and the quality of diagnostic tests for surveillance will be addressed in separate work packages.

In the outcome of the project, reports and scientific opinion documents will be produced, giving comprehensive and up-to-date background data for use in risk assessment and risk management procedures according to the sanitary and phytosanitary (SPS) agreement governing world trade. Scientific dissemination and publication based on the results of the project will be pursued.

Potential applications

Reports and scientific documents from this project will give comprehensive and up-to-date background data for use in risk assessment and risk management procedures according to the sanitary and phytosanitary (SPS) agreement governing world trade. The reports from the project will also be utilised during the revision of current European Community legislation in this specific field, for the update of zoo-sanitary recommendations made by the OIE, and for the update on national and industrial rules and practices.

Through dissemination activities directed towards the international aquaculture industry and regulatory authorities, the project will contribute towards a commonly accepted risk perception, and stimulate the settlement of current disputes between European and non-European aquaculture trading partners.

Project web-site: Established under URL: www.veso.no/fisheggtrade/index.html

Keywords

Aquaculture, diagnosis, diagnostic test, disease control, disinfection, eggs, fish diseases, fish health, pathogen, risk assessment, risk management, sperm, SPS agreement, trade, zoo-sanitary.

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Bovine virus diarrhoea virus (BVDV) control

Project number	QLK2-CT-2002-01573
EC contribution	314.988 €
Duration	30 months
Type	TN
Starting date	1st October 2002

Summary

The network will be based on the establishment of an international group of European specialists, summarise the current knowledge regarding diagnosis, epidemiology, vaccination and socio-economic aspects of BVDV in a position paper.

Through identifying the gaps in our knowledge, guidelines for future development will be suggested and support will be provided for policy decisions regarding BVDV control in Europe.

Problem

The main problems addressed in the project are: 1) the qualities of current diagnostic tools and need for improved diagnostics tools and techniques, 2) epidemiological aspects especially focusing on the risk of re-infection in control areas, 3) the quality of current vaccines and use of vaccination in future control and 4) socio-economic aspects of BVDV control (e.g. cost-benefit) in different European livestock and social settings.

Aim

1) Harmonise diagnostic tools used in BVDV control and suggest future improvements, 2) identify the risk of re-infection for European livestock and suggest control measures, 3) harmonise vaccines and suggest improvements and 4) identify the socio-economic benefits of and suggest systems for BVDV control in different regions of Europe.

Expected results

- A position paper on different aspects of BVDV control in Europe.
- Integrated project and research proposals.
- Seminars and press releases on BVDV control.
- Policy suggestions for BVDV control in Europe.

Potential applications

The results of the project will be made available to the European society, providing the official authorities, industrial bodies and institutions and personnel working with animal disease control with crucial information needed for making decisions regarding BVDV control.

Project web-site: www.bvdv-control.org

Keywords

BVDV, animal disease control, diagnostics, epidemiology, socio-economics.

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Anti-viral innate immunity in cultured aquatic species

Project number	QLK2-CT-2002-01691
EC contribution	1.252.514 €
Duration	36 months
Type	RS
Starting date	1st September 2002

Summary

Despite the impact that viruses have on aquatic organisms, relatively little is known about how fish and invertebrates fight these infections. Thus, the project aims to characterise anti-viral innate immunity in fish, bivalves and crustaceans in order to provide new approaches for the control of viral infections in aquacultured species. Some molecules and genes involved in anti-viral innate immunity will be defined and compared in different species. Selected anti-viral effectors will be monitored by *in vitro* and *in vivo* assays to assess potential anti-viral effects in different cellular and animal models. Furthermore, expression of genes encoding selected molecules will be analysed in healthy and virus-infected individuals. Candidate molecules and genes will be supplied for further exploitation in aquaculture and to aid in the development of tools to control viral diseases of humans and other animals.

Problem

Viral infections are among the most destructive diseases that affect vertebrate and invertebrate species in aquaculture. Despite the impact that these diseases have on aquatic organisms, we know relatively little about what farmers can do to prevent and treat viral infections. The problems in controlling viral infections in aquaculture come mainly from the lack of commercial vaccines and specific therapeutic agents. Moreover, the use of currently available drugs in aquaculture is highly regulated in order to avoid risks to public safety and to prevent the development of resistant pathogen strains. Consequently, farmers are left with few resources other than the use of basic preventive measures. In the long term, alternative treatments using anti-viral drugs may be developed in combination with the production of animals selected for disease resistance.

Aim

Non-specific anti-viral defence mechanisms (innate immunity) are important because they constitute the first line of defence in vertebrates, and the only one in invertebrates. Therefore, innate immunity will be investigated in fish, molluscs and crustaceans. Through this project, we hope to identify conserved mechanisms and pathways of innate immunity. The project will be part of the research and technological development activities of Key Action 2 (Control of Infectious Diseases), Topic 2. 2. (Strategies to identify and control diseases) and Subtopic 2. 2. 1. (Treatment of, and protection against, human and animal infectious diseases). The main objective of this project is to identify and characterise new targets for anti-viral interventions.

Expected results

The expected achievements of this research project are :

1. to characterise new genes and molecules involved in anti-viral innate immunity in bivalves, crustaceans and fish;
2. to correlate functional data obtained in this project with genetic information already available through ongoing genomic projects in vertebrate and/or invertebrate species;
3. to assess the efficacy of candidate genes and molecules in the control of viral diseases of aquatic species.

Potential applications

In turn, the programme will be of benefit to the design of more potent vaccines in fish and anti-viral therapeutic agents, and to the identification of new targets for preventive actions in different cultured aquatic species.

Project web-site: <http://www.ifremer.fr/latremblade/en/europeanprojects/Avinsi/avinsi.htm>

Keywords

Viral infections, infectious diseases, fish, crustaceans, bivalves, innate immunity, aquaculture, anti-viral molecules, virus-induced genes, apoptosis.

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Foot and Mouth Disease: the molecular basis of tissue tropism and persistence

<i>Project number</i>	QLK2-CT-2002-01719
<i>EC contribution</i>	964.092 €
<i>Duration</i>	36 months
<i>Type</i>	RS
<i>Starting date</i>	1st September 2002

Summary

Foot-and-mouth disease virus (FMDV) causes a highly infectious, vesicular disease in cloven-hoofed animals. The risk posed by asymptomatic carrier animals is the crucial barrier to the use of vaccines in Europe for emergency disease control. In recent years we have learnt much about where the virus persists in carriers, and about mechanisms of host-cell recognition *in vitro*. By linking and extending these two areas of research, this project aims to elucidate the molecular mechanisms underlying viral tropism and persistence in the natural host. This project, which combines molecular virology, immunology, and state-of-the-art histopathology, will provide vital intelligence in the war against FMD by revealing targets for therapeutic or immune intervention aimed at preventing, or curing, the carrier state.

Problem

Foot and Mouth Disease is a highly contagious disease of ruminants and pigs, and is one of the OIE Category list A diseases. The EU currently follows a non-vaccination policy, and outbreaks in any of the member states of the EU are controlled by restrictions on animal movements and culling of infected and suspected herds. The disease causes enormous economic losses resulting both from the imposition of these drastic control measures, loss of trade in animals and animal products and very heavy collateral damage to tourism. Furthermore the destruction of millions of mainly uninfected animals is becoming unacceptable politically as well as economically.

Current vaccines fail because vaccinated animals are prone to re-infection. Most important, ruminants infected with FMDV, either before or after vaccination, can develop a persistent infection (carrier state) that is resistant to further vaccination. In many individuals the persistent virus fails to elicit any detectable serological response, yet such carrier animals still have the potential to spread the disease amongst vaccinated or unvaccinated animals.

It is clear that better methods of disease control are needed. Inhibitors of cell entry and replication offer an alternative means of controlling virus spread. At present the rational design of such inhibitors is compromised because there is insufficient knowledge concerning the nature, specificity and distribution of cellular receptors for FMDV in the natural ruminant host.

Aim

This project will study how the virus enters and replicates in epithelial cells 'in vivo' and will exploit new technologies such as laser microdissection (LMD) and manipulation of recombinant viruses to cross the bridge between molecular virology and veterinary pathology. LMD allows individual cells and microanatomical features to be dissected from histochemical preparations and assayed for gene expression. LMD will be used to gain quantitative estimates of FMDV genomes produced in specific epithelial cells during acute and persistent infection. These genomes will be sequenced to follow changes that may occur during the transition to persistent infection. FMDV uses the integrin family as receptors for cell entry. Mutant viruses will be constructed to identify crucial amino acids that determine the specificity of FMDV for specific integrins. LMD will be used to gain a quantitative estimate of specific integrin expression at sites of FMDV infection and persistence. The ability of integrins expressed by monocytic cells to bind FMDV and traffic the virus to sites of persistence 'in vivo' will also be determined, as will the effects of FMDV on monocytic cell function.

Expected results

The results will provide quantitative data concerning viral load in specific epithelial cells of the ruminant soft palate during acute and persistent infection, and determine if the genome of FMDV changes during the transition to persistent infection. The results will also provide quantitative estimates of specific integrin expression in specific epithelial cells in the ruminant dorsal soft palate, particularly those at sites of persistence. The project will provide the sequences of amino acids in the virus capsid that are required for FMDV to bind specific integrins, and determine if these sequences change during transition from acute to persistent infection. The project will also determine if integrins are required for the binding of FMDV to monocytic cells, and whether these cells carry FMDV to sites of persistence 'in vivo'. The ability of FMDV to modulate the immunological effector functions of monocytic cells will also be established.

Potential applications

An understanding of FMDV receptor interactions and receptor distribution 'in vivo' will provide the starting point for the development of peptide blocking agents for preventing infection and/or curing persistence. The work will also lead to the establishment of cell lines expressing high levels of the natural receptor for FMDV with the potential to provide 'in vitro' assays for the diagnosis of FMDV, and for the screening of peptide inhibitors of virus binding and entry. In that respect, this project complies with the objectives of The Specific Program "Quality of Life: Control of Infectious Diseases", since improved knowledge of these subjects might contribute to improved control strategies for FMDV by providing essential information for rapid diagnosis and development of peptide blocking agents and drug trials. Such reagents could be applied as additional tool to control an outbreak of FMDV which might reduce the social, ethical, and economic impact of an outbreak.

Keywords

FMDV, tropism, persistence, carrier state, laser micro-dissection, epithelial cells, antiviral, vaccine.

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European Commission

EUR 21712 — Control of infectious diseases — Catalogue of research projects in the fifth framework programme — Infectious diseases of livestock and aquaculture animals

Luxembourg: Office for Official Publications of the European Communities

2005 — 98 pp. — 21 × 29.7 cm

ISBN 92-894-9696-7