Appendix 1

SIXTH FRAMEWORK PROGRAMME
PRIORITY # INCO-2002-A3-1
Food quality and safety
Health of livestock population

Contract for:

SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT

Annex I - "Description of Work"

Project acronym: RP/PPR MARKVAC
Project full title: Development of marker vaccines, companion diagnostic tests and improvement of epidemiological knowledge to facilitate control of rinderpest and peste des petits ruminants viruses
Proposal/Contract no.: 003670
Related to other Contract no.: *(to be completed by Commission)*

Date of preparation of Annex I: 26 October 2004

Operative commencement date of contract: *(to be completed by Commission)*

CONTENT LIST

1. Project Summary:

Development of marker vaccines, companion diagnostic tests and improvement of epidemiological knowledge to facilitate control of rinderpest and peste des petits ruminants viruses

Proposal acronym: RP/PPR MARKVAC

Date of preparation: 26 October 2004

Type of instrument: Specific Targeted research Project

Strategic objectives addressed:

- To define, contain and eliminate the last foci of rinderpest and to improve PPR control,
- To allow the differentiation between vaccinated animals from those infected with the wild type viruses and therefore remove doubts about rinderpest persistence and PPR circulation in vaccinated populations,
- To strengthen rinderpest and PPR surveillance and emergency preparedness.

Proposal abstract:

Rinderpest (RP) and peste des petits ruminants (PPR) control is considered a priority in the countries where these diseases are endemic (Africa, Middle East and West Asia). Because of their high mortality and high morbidity rates, RP and PPR are dreaded animal diseases included in the list A of the Office International des Epizooties, list of dangerous animal pathogens where extremely contagious animal diseases of high economic importance are grouped. Effective control of RP and PPR by vaccination, through international funding, mainly by the EU, contributes to increasing and sustaining livestock and ensures world food security. In the present proposal, the first objective is to develop marker vaccines capable of preventing RP and PPR infections and also companion tests to differentiate between infected and vaccinated animals. This differentiation is of major importance for eradication programmes because it would allow more precise targeting of vaccination while serosurveillance of the disease could be maintained through the use of companion diagnostic tests. This would allow for a quicker lifting of control measures. Through reverse genetics technology it is also aimed to improve the quality and the safety of these marker vaccines derived from current attenuated strains. Another objective of the proposal is to use improved epidemiological information systems for a better analysis of the current situation for rinderpest and PPR and for predicting the impact of vaccination strategies on the control of the two diseases. Thus the expected outputs from this proposal will give additional support to some existing EU developing country programmes.

2. Project objectives

- **General objectives of the project**

Rinderpest (RP) and peste-des-petits-ruminants (PPR) are two important viral diseases of wild and domestic ruminants characterised by high morbidity and mortality rates. These diseases are classified in list A of the Office International des Epizooties (OIE) that contains "Transmissible diseases that have the potential for very serious and rapid spread, irrespective of national borders, that are of serious socio-economic or public health consequence and that are of major importance in the international trade of animals and animal products". Although they produce clinically similar disease, RP and PPR are caused by two distinct viruses, RP virus (RPV) and PPR virus (PPRV), both being members of the *Mobillivirus genus* of the *family Paramyxoviridae*. The two viruses are closely related genetically and antigenically and induce in infected animals, not only the production of antibodies that cross-react, but also cross-protection. RP affects all ruminants although cattle, yak and buffalo seem to be the most susceptible animal species. In contrast, PPR is thought to be more specific to small ruminants, the natural hosts being sheep and goats. Nevertheless, in some circumstances, cattle can show an overt disease and die following PPRV infection (*1*, *2*) and the virus was also isolated from buffalo that died from an RP-like disease in 1995 in India (*3*). Therefore, a common research effort to improve control of these two diseases is desirable and so the present proposal has as its objectives the development of new biotechnological tools and to increase our scientific knowledge to aid control/eradication programmes. In the long-term this will help improve the productivity and sustainability of ruminant herds in the last remaining focus of RP in the world in the Somali ecosystem and in countries where PPR is endemic: Africa, the Middle East and the South and West Asia. Where they occur these ruminant *Morbilliviruses* are of major constraint to increased animal production and international trades. Better control and eradication strategies will, it is hoped, be achieved through the development and use of marker vaccines, with improved safety features, and their accompanying differential diagnostic tests. In addition, the improvement of surveillance information systems for both diseases and the modelling of PPR control strategies by vaccination will greatly facilitate these tasks.

International funding for RP control and eradication has brought the virus close to eradication. At present, its remaining stronghold seems to be confined to an endemic area in Eastern Africa, in countries within the Somali ecosystem (*4*, *5*, *6*). Therefore mass vaccination of national or regional herds is no longer necessary and RPV vaccine will only be required for local eradication campaigns and for emergency vaccination of cattle and buffalo in a previously RP-free zone.

There is at present no corresponding international control/eradication programme for PPR although more than one billion small ruminants are at risk from this disease. However, the negative impact of PPR on small ruminant production is so important that many countries are unilaterally implementing control policies for this disease that involve mass vaccination. Originally this was carried out using the RP vaccine, taking advantage of the strong cross-protection between RPV and PPRV, but now the homologous PPR vaccine, developed with EU funds (*7*), is widely used.

Unfortunately, the use of the current RP vaccine for emergency ring-vaccination to contain RP outbreaks, and similarly the use of the PPR homologous vaccine for PPR control, is not compatible with a simultaneous effective serosurveillance for detection of virus circulation because of the inability to differentiate between infected and vaccinated animals using the available antibody detection tests. This differentiation is of major importance for control and eradication programmes since it would allow the implementation of targeted vaccination while at the same time enabling the continuation of serosurveillance to detect foci of the disease. This would also allow a more rapid lifting of control measures in the vaccinated areas once virus circulation had ceased. Such a policy is only possible to implement through the use of a marker vaccine with its companion diagnostic tests. Specific objectives of the present proposal are the development of such tools. The production of marker vaccines for RP and PPR is now possible using reverse genetics technology, the ability to rescue RNA viruses from copies DNA (cDNA) of their RNA genomes. In the case of RP, candidate marker vaccines have already been produced (*8*) but they have yet to be extensively tested and it may be possible to further improve them by introducing a negative marker.

Morbillivirus vaccine, the attenuated measles vaccine (*12*, *13*, *14*). Therefore, determinants of immunosuppression in the attenuated RPV and PPRV strains need to be characterised and, if possible, deleted. This would not only improve the vaccine quality and safety but could also be used as a characteristic to differentiate serological responses to the vaccine from other virus strains.

Along with improved vaccines it is also important to make available improved and validated diagnostic tests and to broaden our understanding of the epidemiology of RP in the remaining infected areas and also to acquire better knowledge of the epidemiology of PPR. Epidemiosurveillance for RP, PPR and contagious bovine pleuropneumonia (CBPP) is one of the main activities of the Pan-African Programme for Control of Epizootics (PACE) which is funded by the European Union. For RP freedom declaration, the serological test to be used is the one classified as prescribed test by the OIE. However, other RP diagnostic tests exist and some others are in development in the PACE research framework. It is important that all those tests, as well as those for PPR diagnosis, to have their performance validated to ensure their fitness for use, the new basis for animal disease diagnostic tests classification that is expected to be adopted by OIE. This validation process is another objective of the present proposal. It is also necessary to adapt and to improve epidemiological information systems and to develop spatial epidemiology and disease modelling to allow a better analysis of the current epidemiological situation for PPR and to assess the impact of different vaccination strategies for the control of this disease.

In the present proposal, an integrated approach is followed (marker vaccine, companion diagnostic tests, epidemiological tools) in order to achieve better control/eradication of RP and PPR. It covers all relevant disciplines from basic virology to diagnosis, vaccinology, epidemiology and the design of disease control strategies.

- **Specific scientific and technological objectives of the project and state of the art**

a) Development of marker vaccines and improvement of their safety

At present it is not possible to discriminate serologically RP or PPR recovered infected animals from vaccinated animals. Cattle vaccinated with RP marker vaccines under endemic local or emergency situations and small ruminants vaccinated on a large scale with PPR marker vaccines would still act as serological indicators for the presence of wild type virus strains. The availability of rescue systems for generating infectious clones for most of the single-stranded negative sense RNA viruses has enabled the successful development of recombinant viruses through manipulation of their RNA genomes via a cDNA. A number of such viruses have been genetically modified and used as highly effective vaccines in animal models. As indicated above, two candidate vaccines have been recently developed for RP (*8*). Cattle immunised with either of these vaccines were protected against lethal challenge with wild type RPV. Antibody response to the marker protein in a small-scale laboratory trial gave encouraging results and strong confidence that they could be used under field conditions to identify vaccinated animals. It is planned to carry out long-term trials on the RPV marker vaccines under this project, and their effectiveness in establishing a durable antibody response to the marker proteins and to the vaccine itself will be tested in a standard vaccination trial. Using the technology applied to the vaccine strain of RPV (RBOK), the PPR vaccine strain (Nigeria 75-1) will be modified to express marker proteins. In addition to any foreign marker genes that will be introduced into the vaccine infectious clone, a non-essential part of the genome will also be deleted, or modified, in order to develop a double (positive/negative) marker vaccine. The criteria to judge the effectiveness of the candidate markers in the vaccines will be as follows. Ideally there should be different markers in the RP and PPR vaccines in order to avoid interfering antibodies that might cause confusion between the two viruses. They should not be natural antigens that could be found in ruminants, for example gene from a microorganism able to infect ruminants. They should give a strong and durable humoral antibody response that is easily detectable in an ELISA. The choice of the markers, and the site of insertion of the marker genes, will benefit greatly from the knowledge acquired on the prototype RP marker vaccines (*8, 15*). Also work on the structure and biological properties of PPRV proteins investigated by peptide mapping and deletion mutagenesis, will facilitate the choice of deletion/modification sites in the proteins.

Transient immunosuppression is a common feature of *Morbillivirus* infections (*16*, *17*, *18*). Although the

either laboratory infected cattle (*19*) or cohorts of goats maintained in extensive farming systems or in the field (*9*). Therefore, there is a need for a more in depth study of the immunomodulating properties of RP and PPR vaccines. Such studies will require both *in vitro* experiments.

In addition to specific criteria relative to the safety of the vaccines that are also fulfilled in this proposal (i.e., clinical observation, nature and duration of undesirable effects etc.), the potential for environmental spread of genetically modified organisms (GMO) used as vaccines, will be investigated, characterised and compared with the parental non-modified strains. It must be clearly established that no shedding of the live marker vaccines occurs, as is the case for the parent viruses, with possibility for dissemination and transmission to non-target species. This knowledge would strengthen our confidence in the safety of the currently used vaccines and would provide the rationale for the design of the next generation of vaccines with improved and more defined safety features that can be produced using reverse genetics technology.

b) Development of marker vaccine companion diagnostic tests, validation of other assays for fitness for purpose

ELISAs based on the detection of serum antibodies to vaccine markers (positive and negative markers) will be adapted and further developed and their sensitivity and specificity compared with the existing serological tests to identify antibodies to these viruses. Use of marker vaccine in association with such tests would greatly assist PACE in the vital activity of serum surveillance to monitor and eradicate the remaining foci of RP in Africa and to control PPR. Different RP and PPR serodiagnostic tests are available or are in development under the PACE research programme. All these tests will need validation to provide performance data on their use for different purposes. This validation should give information on reliability (specificity, sensitivity and accuracy: referred to the next paragraph), reproducibility (intra and inter-laboratory variability), and relevance to biological status for decision making.

c) Improvement of performances of surveillance systems and decision-making tools for the control of RP and PPR

Surveillance is here defined as "the on-going systematic collection and collation of useful information about disease, infection, intoxication or welfare in a defined animal population, closely integrated with the timely dissemination of relevant outputs to stakeholders, including those responsible for control and preventive measures". The rationale of RP/PPR surveillance is to quickly detect new outbreaks, to reveal changes in the incidence and prevalence in infected and vaccinated areas and to certify disease freedom. Fast detection allows for more effective decision making in relation to animal health protection. It is therefore essential to strengthen and further develop surveillance systems with respect to their early warning and early reaction capabilities in the countries and regions that are at increased risk of RP re-emergence and PPR spread. Thus, the main purpose of this component of the project is to enhance the PPR and RP surveillance systems. The specific objectives are to improve the quality of the data collected, the system management tools and to develop the capability for targeted surveillance in areas at increased risk. In addition, it will be necessary to determine optimum PPR vaccination strategies based on data obtained through the integration of epidemiological studies with modelling approaches. Therefore, three components are to be considered. Data quality assurance and performance of surveillance networks will be investigated by an evaluation of currently available diagnostic tools using Receiver-Operating-Characteristic (ROC) analyses, as well as latent class modelling for the determination of sensitivity, specificity and predictive values. In the same way, new tests developed within the project will also be assessed using similar methods. Spatial epidemiology for targeted PPR surveillance will be carried out to identify geographical areas that require strengthened or reduced surveillance efforts. Modelling of the PPR dynamics will provide a decision support tool for the control of the disease. Furthermore, epidemiological methodologies developed using PPR as a *Morbillivirus*-pattern example could be applied to RP in case of re-emergence.

3. Participants list

Partic. Role*	Partic. no.	PARTICIPANT NAME	Participant short name	Country	Date enter project **	Date exit project **
C01	1	CIRAD-EMVT	CIRAD	FRANCE	Month 1	Month 48
CR2	2	INSTITUTE FOR ANIMAL HEALTH	IAH	UNITED KINGDOM	Month 1	Month 48
CR3	3	THE JOINT FAO/IAEA DIVISION OF THE NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE	FAO/IAEA	AUSTRIA	Month 1	Month 48
CR4	4	INSTITUTO DE BIOLOGIA EXPERIMENTAL E TECNOLOGICA	IBET	PORTUGAL	Month 1	Month 48
CR5	5	ROYAL VETERINARY COLLEGE	RVC	UNITED KINGDOM	Month 1	Month 48
CR6	6	KARI NVRC MUGUGA	KARI	KENYA	Month 1	Month 48
CR7	7	LABORATOIRE CENTRAL VETERINAIRE	LCV	MALI	Month 1	Month 48
CR8	8	NATIONAL VETERINARY INSTITUTE	NVI	ETHIOPIA	Month 1	Month 48
CR9	9	INTER-AFRICAN BUREAU FOR ANIMAL RESOURCES OF THE AFRICAN UNION	AU/IBAR	KENYA	Month 1	Month 48

*CO = Coordinator
CR = Contractor

**Correspond to month 1 (start of project) and month 48 (end of project).
These columns are needed for possible later contract revisions caused by joining/leaving participants.

4. Relevance to the objectives of the specific programmes and/or thematic priorities

- **Relation of project to topics covered by the call for proposal**

At the start of the 21st century hunger remains a major challenge. During the World Food Summit (WFS) organised by the United Nation Food and Agriculture Organisation and held in Rome in November 1996, an action plan that provides for commitments in seven key actions was agreed. The WFS Plan Actions, which are the most pertinent to Emergency Prevention System (EMPRES), are objectives 3.1 of Commitment Three and 5.2 of Commitment Five. They emphasise the continuing threat of animal diseases including those of a transboundary nature such as RP and the critical importance of the Global Rinderpest Eradication Programme (GREP), the main focus for the FAO EMPRES-livestock programme. Along with RP and PPR, animal diseases of transboundary nature are the main targets for the FAO EMPRESS-livestock programme. More recently, at the World Summit on Sustainable Development (WSSD), Johannesburg, 26 August-4 September 2002, Africa was identified for special attention and support by the international community to better focus efforts to address the development needs of the continents and particularly to intensify efforts to decrease poverty and food insecurity. The FAO's Strategic Framework (2000-2015), as the lead agency for agriculture, forestry and fisheries addresses the Challenges of Sustainable Development that include animal production and health. Its contribution is to strengthen its effort and actions to encourage member countries and other partners to build up their capacity to implement this objective. One priority of the Framework can be emphasised which supports this project: "The use of information and new information technologies and existing databases for development planning at all levels". Priority will be given to promote and refine indicators and analytical tools for planning and decision making for food security and sustainable agriculture. These tools will improve access to early warning systems for sustainable food security. The overall objective of the present proposal is to develop tools for a better control/eradication of two important transboundary diseases that compromise poverty alleviation in Africa. Thus, the proposal is in line with the above mentioned World Summits recommendations.

The first specific objective of the proposal is to develop marker vaccines to prevent RP and PPR infections and to examine safety issues relating to the currently used vaccines and, consequently, of the marker vaccines derived from them. The second objective is the development of marker vaccine companion diagnostic tests and validation of the existing RP and PPR assays to provide parameters needed when decisions have to be made on their use for specific purposes (fitness for purpose). Therefore, the application complies perfectly with the request for proposals under the INCO DEV programme section D.3.3: *Research on livestock health protection through development and use of diagnostic tools and vaccines for economically important diseases, namely "rinderpest" (RP) and "peste des petits ruminants" (PPR), in the paragraph entitled "Health of livestock population"*. Another specific objective of the programme is to improve the effectiveness of surveillance and decision-making tools for the control of RP and PPR and this proposal also addresses the part of the call dealing with *"Epidemiological analysis of the above mentioned livestock diseases including..."*.

RP and PPR control is considered as a priority in countries where these diseases are endemic (Africa, Middle East and West Asia) and they have a significant economic impact in African countries. The effect is either direct through animal losses, or indirect, by exclusion of the infected countries from international trade and by the necessity to maintain costly vaccination campaigns to control these diseases. Today, with the success of the Pan African Rinderpest Campaign (PARC), RP is limited to a small number of sites in Africa. In the follow-on programme supported by the EU, the Pan African Control of Epizootics (PACE), RP, PPR and CBPP are the main diseases targeted for further research. Effective control of PPR would contribute to increasing and sustaining small animal production in countries where the disease is endemic and therefore would have a positive effect on livestock farming, food security of small farm holders and rural incomes. Also a final effort is now needed to eradicate RP. To achieve this goal, the immediate challenges of the GREP are:

- To define, contain and eliminate the last foci of rinderpest persistent,

At the GREP Technical consultation held in Rome in May 2000 and at the third meeting of the PACE Advisory Committee held in Addis Ababa, Ethiopia in January 2001, experts unanimously endorsed the need for specific priorities including marker vaccines and their accompanying diagnostic tests. These GREP priorities are clearly addressed in the present proposal.

- **Contribution of the research proposal to addressing the key scientific and technological issues for achieving objectives of the programme**

The present project is designed to present an integrated approach to the control and eradication of two economically important diseases of ruminants, RP and PPR. It will address one of the key factors hampering effective control policies involving vaccination, namely the lack of marker vaccines and companion diagnostic tests for these viruses. The research to develop marker vaccines for RP and PPR will use reverse genetics technology to make the required modifications to the virus genomes and will be used to identify the genetic determinants of the immunosuppressive effects associated with the current vaccines, and consequently of the marker vaccines derived from them. An improved formulation for stable storage of vaccines at higher temperatures will be developed to reduce the stringent requirements for cold chain thus permitting easier vaccine delivery under difficult field conditions. The use of such vaccines, along with the new diagnostic tests to identify their serological signature, will greatly improve our surveillance capabilities for these diseases.

Marker vaccines with improved safety. Vaccination to reduce disease incidence followed by stamping out of remaining foci has long been the preferred strategy for the eradication infectious animal diseases. The recent foot-and-mouth disease (FMD) outbreak in Europe has given indications that the general public will no longer accept stamping out as a means of animal disease management. Immunisation strategies over the last 200 years have contributed greatly, perhaps more than any other tool of veterinary medicine, to the successful management of animal diseases. This success has been possible without fully understanding the pathogenic mechanisms involved in the disease process. However, with the recent advances in our understanding of disease pathogenesis, primarily due to advances in immunology and molecular biology, we are in an era of unprecedented opportunity to develop safer and more effective vaccines to help further reduce diseases caused by infectious agents in both humans and animals. One of the recent advances in veterinary vaccinology is the development and use of marker vaccines, which in conjunction with companion diagnostic tests, has gained wide acceptance in veterinary medicine. Where the eradication of specific diseases is of national/international interest is required, vaccination will be the primary weapon used for disease control, increasingly so in the present climate where the public will no longer accept the huge slaughter of animals demanded by a stamping out policy. While the currently used RP and PPR vaccines are efficacious and are known to provide life long protection against disease, they do not allow the differentiation between vaccinated animals and those infected with wild type viruses. Since with marker vaccines it is possible to differentiate the vaccinated animals from those which have been exposed to pathogenic field strains, this approach can assist in controlling/eradicating disease from a country more rapidly and economically when compared with conventional disease management by vaccination. In the case of an accidental introduction of an infectious disease into an area/country, it would be possible to implement a quarantine policy along with the use of the marker vaccine within and around the periphery of the actual infected area to help prevent disease spread.

Approaches that have been taken to develop marker vaccines are, either deletion of one or more non-essential but immunogenic protein genes from the genome of the pathogen, or the addition of a foreign protein gene as a marker. Gene deletion is more applicable to large DNA viruses that have several dispensable genes whose deletion can be used to mark the vaccine. For negative single-stranded RNA viruses it has not been possible until very recently to manipulate their genomes and, in addition, there is very little dispensable genetic material. The new technique of reverse genetics now allows manipulating the genome of these viruses through cDNA. Although, most of the genes are essential for the replication, it has been possible to recover viable viruses after deletion of part of a gene, or following its replacement with the corresponding gene, or part of the gene, from another virus, the new virus being a chimeric virus (*20*). For RP and PPR, the use of such traceable viruses as vaccines would greatly improve confidence in epidemio-

Safety of the recombinant GMO vaccines. Consideration will be given to the immunosuppressive effect induced by RPV and PPRV, a characteristic common to all *Morbillivirus* infections leading to a secondary bacterial infection. It is likely that the RP/PPR induced immunosuppression, which is certainly transient, is a multifactorial process. Reverse genetics will also be in this case a powerful tool to address the question whether proteins preserve their functionality if the peptides involved in immunosuppression are deleted. This tool will be used under this project to neutralise the short-term immunosuppressive effect of the vaccines for improving the safety of the marker vaccine.

Care is to be taken to the safety of any recombinant GMO vaccine, particularly where extra genes are inserted into the genome, which might alter either the tissue tropism or pathogenicity of the resulting virus. Extensive safety testing *in vitro* and *in vivo* will be carried out to look for altered tissue tropism and to establish if, like the conventional parent vaccines, the recombinant vaccines derived from them are not transmitted by contact from animal to animal. In addition the incorporation, or more likely exclusion, of the marker proteins in the virus envelopes will be studied. If, for example, the marker proteins are shown to be excluded from the virus envelopes, then it is very unlikely that they would alter the virus tissue tropism.

Improvement of the vaccine thermal stability and delivery to animal. Quality of the new generation vaccines in terms of biological activity and stability are important issues that have to be addressed. Stabilisation of vaccines has important implications for extending vaccination to areas lacking a cold chain for transportation and storage. The development of improved formulations for storage of vaccines at higher temperatures may accelerate the development of new immunisation technologies such as lyophilised powder and nanoencapsulated materials for mucosal delivery. Encouraging results were obtained during an ongoing EU project (See annex, A1), in which the vaccine formulation for recombinant vaccines for oral delivery has been studied. The purpose here will be to develop effective formulations to stabilise the current RP and PPR vaccines and later the marker vaccines. The robustness and long-term storage of the vaccines, coupled with cost-effective manufacturing processes, will make them particularly attractive vehicles for parenteral or oral and intranasal vaccination.

Development of tests to be used in conjunction with marker vaccines; validation of assays. Risk assessment is the key component in animal disease management, which is carried out for economic, public health, and environmental reasons. An important factor in risk assessment is evaluation of the sanitary status of animals and their products. Diagnostic testing is an important activity in this process and is useful only if it is applied within specific contexts. The first objective of the present proposal is the development of double marker vaccine to be used to halt disease spread and, at the same time, allow surveillance for the infectious agent. Such vaccines are only useful if the accompanying tests are available to monitor the vaccination (test to detect antibody to the positive marker) and to follow the spatial course of the infection (test to detect antibody directed to negative marker, epitope or whole antigen deleted from the vaccine). Both these tests will be developed within this project as companions for the RP and PPR double marker vaccines. Since in the near future the OIE will classify the animal diagnostic tests according to the fitness for purpose, these new tests, along with the available RP and PPR diagnostic tests, must be validated with respect to variables such as repeatability and reproducibility.

Performance of surveillance system and control methods. Raw data obtained as described in the preceding paragraph to validate the tests will be used here to generate additional information such as test sensitivity, specificity and predictive values in different epidemiological situations (free from disease, endemic with vaccination, endemic without vaccination, outbreak). Precise estimates of these parameters are essential to determine the sampling and testing strategies required for a better understanding of the prevalence of disease through the use of more sensitive and specific epidemiological and diagnostic tools. In order to enhance the surveillance, research on the spatial and statistical methodologies will facilitate defining the geographical areas at risk where field activities have to be concentrated. Modelling the dynamic of the disease will support decisions for vaccination policies and will provide better understanding of *Morbillivirus* infection cycles. Using these approaches, the contribution of this proposal will be to boost the health status of cattle and small ruminants in Africa, allowing higher productivity and therefore an increase in the commercial value of livestock for trade purposes.

5. Potential impact

- **Strategic impact of the proposed measures to solving problems**

a) Background

RP is extremely contagious and probably the most-lethal disease of cattle and buffalo with a case-fatality rate of up to 90% (*21*). All species of the *Order Artiodactyla* are affected although cattle, buffalo and large wild ruminants are the most sensitive. Due to the severity of the disease economic losses can be catastrophic and this endangers food security and the livelihoods of thousands of farmers. As a consequence there is an ongoing campaign to achieve global eradication of the disease. The control of RP has a long history with many international collaborative interventions and these have succeeded in making important advances towards RP eradication from the world (report of the GREP Consultant meeting, 2002). The current RP eradication campaign is mainly funded by the EU with logistical support provided by the FAO and OIE. In Africa, considerable efforts were made by the Pan African Rinderpest Campaign (PARC) which ended in 1999. Owing to its success, it was followed up by continuing surveillance activities under the same authority within the Pan African Programme for the Control of Epizooties (PACE). As a consequence of these international efforts RPV has been brought close to extinction, with only limited foci remaining in Eastern Africa centred in the Somali ecosystem. Global eradication of RP is achievable in the foreseeable future if these efforts are continued and the year 2010 is the date targeted by GREP to declare the world free from RP.

To meet this objective, the OIE exhorted all countries concerned to adopt a time-bound programme leading to verification of RP eradication. This has become known as the OIE Pathway to "Freedom from Rinderpest". The final step requires that no antibody to RPV should be detected in susceptible animals following at least two years of intensive surveillance. The rationale behind this international rule is the inability, when using the current vaccine, to differentiate by serological tests vaccinated animals from those infected with wild type viruses. However, should there be a localised outbreak of the disease due to the re-introduction of RP virus, an emergency vaccination campaign in cattle and buffalo in the previously free zone would be required. This could be more easily managed if marker vaccines were available.

PPR is the other major *Morbillivirus* disease of livestock in Africa. It is highly transmissible and affects mainly sheep and goats. As our knowledge of the epidemiology of PPR improves it is apparent that the disease is not limited to West Africa where it was first described and its presence has now been recognised in many of the sub-Saharan countries, from the Atlantic Ocean to the Red Sea. It is also present in most countries of the Middle East and South and West Asia, including Turkey from where it constitutes a high risk for Europe. Due to the severe economic impact of PPR on small ruminant production, many countries have started to control the disease by vaccination using the homologous live attenuated vaccine developed under two previous EU funded projects (*7*, see annex, A2, A3). This live vaccine has progressively replaced the use of the RP vaccine for the control of PPR in small ruminants. This important shift allows countries free of RP to ban the use of RP vaccine, while still maintaining the ability to carry out PPR vaccination, and thus fulfil the OIE requirements for RP-free status.

b) Development of marker vaccines; safety improvement and development of new vaccine delivery system.

As is the case for RP, the PPR vaccine does not allow distinction between vaccinated and infected animals. Reverse genetics technology now makes it possible to genetically modify the vaccine strain in order to add marker genes. This technology was applied to insert transgenes into the conventional RP vaccine strain (*8, 15*). A gene insertion site has been created which enables foreign genes to be expressed from the vaccine and these have the potential to act as markers in the vaccine. However, these candidate marker vaccines still need to be tested for their ability to induce long-lasting antibodies against the marker. A similar approach can be used to rescue modified PPR attenuated vaccine strain genomes with inserted marker genes from cDNA. Reverse genetics technology already established for RP (and other *Morbilliviruses*) has yet to be applied to PPR. The first step is to rescue infectious virus from the full-length cDNA of the vaccine strain. This will soon be achieved within the framework of a PACE funded Research Project (see annex A4). The

vaccine. This will then be combined with the positive marker protein to produce a double marker (positive and negative) vaccine. The reasoning behind this approach is to allow the specific detection of vaccinated animals through the antibody response to the added marker and also to specifically identify naturally infected animals through their exclusive antibody induction to the deleted epitope of the vaccine. Any vaccinated animals that subsequently become infected and replicate wild type virus will be positive for both the foreign marker protein and the protein (or epitope) deleted from the vaccine strain. Partners CO1 and CR3 will carry out this work on the double marker PPR vaccine. Partner CR2 will produce a similarly positive and negative marker RP vaccine.

The immunological mechanisms underlying the protective effects of the RP and PPR vaccines was addressed in another project supported by EU (see annex, A1). Research carried out under this program have demonstrated that both vaccines strains still retain some residual and transient immunosuppressive effects that are well known to be associated with virulent *Morbillivirus* infections (*10, 11*). It seems that more than one viral protein is involved in the induction of these immunosuppressive effects. Indeed, as is the case for measles virus (MV), a human *Morbillivirus*, coexpression of both the fusion (F) and the hemagglutinin (H) proteins of RPV is sufficient to induce immune suppression *in* vitro (*10*). It was also demonstrated recently that the nucleocapsid (N) protein of MV, PPRV and RPV bind to a cell receptor and this binding can inhibit spontaneous cell proliferation by arresting cells in the G0/G1 phases of the cell cycle (*11*). The region of the N that mediates this binding to the cell receptor is located in the C-terminal end of the protein. The immunosuppressive effects that were demonstrated *in vitro* are supported by other observations made on vaccinated animals (*9, 19*). The N, H and F proteins are all necessary for the virus to replicate, however, the regions of those proteins that are involved in immunosuppression may be dispensable. The present proposal will focus on the transient immunosuppression induced with the aim to modify determinants of the vaccines involved in this effect. The N gene of PPR will be particularly studied to determine the sequences to be deleted. Thus the expected result will be to improve the safety of the vaccine for field use where animals are exposed to many extraneous disease causing agents. Partners CO1 and CR3 will be in charge of this research.

The seed material of the new GMO marker vaccines will be further used to produce manufactured vaccines and should therefore fully comply, during the development process, with the requirements listed in the relevant EU guidelines (Part II E of Directive 81/852/EEC) in conjunction with the European Agency for the Evaluation of Medical Products (EMEA) guidance (EMEA/CVMP/074/95) that deals with environmental risk assessment. The Guidelines of the EMEA outline a compulsory process assessment that should indicate the potential risk of exposure of the environment to the marker vaccines. Thus, the potential negative effects on environment of the GMOs will be investigated and characterised and compared with those posed by the parental non-modified strains.

Partner CR4 will be in charge of the optimisation of the current vaccines formulations and afterwards of the marker vaccines for improvement of storage and delivery. He will benefit from the progress made during the above-mentioned project (see annex, A1) in which different formulations for the maintenance of vector viability during storage were assessed for candidate RP and PPR vaccines based on recombinant viruses intended for oral delivery. Some of the cryoprotectors and lyoprotectant compounds tested yielded promising results. Therefore the valuable information gained during the preceding project will be turned to account for the current and marker RP and PPR vaccines for administration by the classical sub-cutaneous route or by other means.

c) Development of marker vaccines companion diagnostic tests

The strategic value of any marker vaccine is totally dependent on the ability to detect only the vaccinated animals serologically. This will be achieved by the development in a first step of indirect ELISAs using peptides or transgene proteins expressed in prokaryotic (*E Coli*) or eukaryotic (*Baculovirus*) systems as the coating antigen. The ELISAs produced will be evaluated using experimental and field sera available in the partners laboratories. In a second step, competitive ELISA format, with increased specificity, will be developed once monoclonal antibodies specific to the markers can be produced. These assays, along with other RP/PPR assays will be validated in a "round test" exercise to provide data on their performances for fitness for purpose use. It is likely in the future that the OIE will ask for such data for the approval of marker

status of herds. However trade issues are not the only purpose of disease diagnosis. Indeed, an assay can be used, for example, to confirm diagnosis of clinical cases, demonstrate population freedom from infection, or to certify eradication of infection from a defined population etc. The OIE, at its General session held last May, adopted a resolution to provide a much broader recognition of diagnostic tests as fit for a specified purpose, not just for international trade. It is expected that in the near future, the OIE will abandon the test prescribed/alternative classification for one based on fitness for purpose. So in future other animal diagnostic tests will be validated according to fitness for specified use.

d) Improvement of performances of surveillance systems and decision-making tools for the control of RP and PPR

In the final stage of the PACE campaign, serological tools that can accurately discriminate between seropositive and seronegative individuals have become an absolute necessity for countries wishing to be recognised as free from the disease. Therefore highly sensitive and specific tests are required to reliably detect true seropositive animals, especially in herds with a high risk of exposure to wild-type virus, and true seronegative animals. In addition, the antibody titres may vary accordingly to the animal species, RPV and PPRV can infect different species of ruminants and to virus strain (4 lineages for PPRV and 3 for RPV). Therefore it is possible that a single test will not fulfil the needs of high specificity and high sensitivity. It is likely that for a specified purpose, a combination of tests will be needed (fitness for purpose) to provide accurate information on the health status of the population or individuals tested. Those tests should be validated to provide information on different variables of performance. A large database is available from CO1, CR3 and CR6 including ELISA, VNT results and status of the animals using virological results as gold standard to allow computing by ROC analysis, sensitivities and specificities of the RP and PPR tests used in the framework of the PACE surveillance programme. Additionally, further experimental sera, for example from vaccine trials and control challenge animals, will also provide data for computing performance characteristics of the serological tests. The same exercise has to be organised for the validation of the PPR assays with a similar round test. The test performance characteristics, which will be obtained for both RP and PPR, will help in determining testing strategies and planning disease control measures. Development of case definition, systems to record the epidemiological parameters of the disease and consequently systems for collection and recording of these date using modern communication technologies and GIS based systems will be developed and evaluated for their usefulness at the field level. These parameters will be used for modelling the spread and distribution of PPR. Numerous models have been developed for measles and some have been proposed in the past for RP.

- **Innovative features and how the proposal contributes to solving problems at the Third Country level.**

The availability of reverse genetic technology for negative strand RNA viruses now enables production of marker vaccines. Through their marker characteristics they will allow serological differentiation between animals that have been vaccinated and those that have recovered from natural infection. The originality of this project is to develop a double-marker vaccine. A positive marker, an irrelevant transgene not naturally found in ruminants, will allow monitoring the vaccination through the detection of antibodies to the marker protein. A negative marker (deletion) will permit discrimination between vaccinated and infected animals. The combination of the two markers will also permit the specific identification of any vaccinated animals that subsequently become infected since they will be positive for both the marker transgene and for the negative marker. The strategy of using such marker vaccines should have a strong positive impact on RP and PPR control programmes with many downstream advantages not available when using the current vaccines. These advantages will be an improved confidence in sero-epidemiosurveillance results, an acceleration of the steps required to prove RP eradication, and the availability of new tools that will greatly simplify the control of PPR in endemic countries. The programme will also address the potential drawbacks associated with the RP and PPR vaccines in regard to their safety through a comprehensive understanding of the molecular/genetic mechanisms of attenuation and immunoregulation. This integrated approach to understanding and controlling disease-causing agents is an innovative input of the project. It is expected to give valuable data that will also aid the design of new vaccines for other *Morbilliviruses*. Innovation will

5.1. Contribution to Standards

Infectious diseases are the single greatest hindrance to efficient livestock production worldwide. Furthermore there will be ever increasingly meat and meat products consumption in developing countries in the coming years. This will lead to increasingly overall amounts of movement of live animals, meat and meat products. As a result, animal disease issues will play a pivotal role in international trade negotiation. To ensure sanitary international trade in terrestrial animals, the OIE has provided in the Terrestrial International Animal Health Code guidelines for animal trade purposes. The aim of the Code is to assure the sanitary safety of international trade in terrestrial animals and their products. This is achieved through the detailing of health measures to be used by the veterinary authorities of importing and exporting countries to avoid the transfer of agents pathogenic for animals or humans, while avoiding unjustified sanitary barriers. In particular, it is a reference document giving standards, guidelines and recommendations to be followed by veterinary authorities.

In the Code, are listed criteria for determining infected and freedom status for RP and PPR both in List A of the OIE classification of animal pathogens. These standards and recommendations are based on the knowledge and control tools currently available for both diseases: effective vaccines but no test able to differentiate between infected and vaccinated animals.

The test validation that will be carried out in the present project aims to provide information on the performance of the different PPR/RP assays to meet the new criteria that will be adopted by the OIE for the animal disease diagnostic tests classification.

Recommended standards for RP epidemiological surveillance systems are also stipulated. The epidemiological methods described are based mainly on studies already published (*22, 23*). One of the outputs of the present project would be a better understanding of the PPR epidemiology with the development of a similar modelling system for this disease. This will allow proposing standards of procedures for sampling, assessment of PPR disease risk and evaluating the costs of its control. This information, as is the case for RP, would be taken into account in the PPR chapter within The Code.

5.2. Contribution to policy developments

The OIE that serves as a world organisation for animal health has for main objective to inform governments of the occurrence and course of animal diseases throughout the world and of ways to control the diseases. It co-ordinates also, at the international level, studies devoted to the surveillance and control of animal diseases and harmonise regulations for trade in animals and animals products.

Concerning RP policy, OIE's mention is: " Should a localised rinderpest outbreak occur in an infection free country, the waiting period before infection free status can be regained shall be as follows:

1°) 6 months after the last case where stamping-out without vaccination and serological surveillance are applied; or

2°) 6 months after the slaughtering of the last vaccinated animal where stamping-out complemented by emergency vaccination (vaccinated animals should be clearly identified with a permanent mark) and serological surveillance are applied; or

3°) 12 months after the last case or last vaccination (whichever occurs later) where emergency vaccination without slaughter (vaccinated animals should be clearly identified with a permanent mark) and serological surveillance are applied."

In Africa, most of the cattle farmers will not accept the stamping out policy. Therefore, the most realistic and cheapest measure, in case of the occurrence of an outbreak in a RP free country or zone, is the emergency vaccination of susceptible animals with a marker vaccine followed by serological surveillance with companion diagnostic tests. This new control approach proposed will avoid the need to destroy large

6. Project management and exploitation/dissemination

6.1. Project management

Scientific co-ordination: Dr Geneviève Libeau will be the scientific co-ordinator of the project which activity is foreseen to be over a period of four years. For easier decision making, she will be in contact with the head of each team included in the project through electronic communication. All partners have access to e-mail for direct communication with each other.

The most important aspects of the management is to monitor the progress regularly to avoid partners failing to deliver or failing in their project input, and this will be the role of the Workpackage Leaders and the co-ordinator. Regular meetings and, if necessary, on-site visits will be used to ensure that deliverables are on time and of high quality.

Communication flow will be ensured by holding several meetings between all partners including the leader and other scientists of the team and scientists from outside (number limited by the funds available). The dates of the meetings will be set to be convenient to all partners. Another of their aims will be to stimulate the dynamic of the collaboration.

An inaugural meeting, to be scheduled as soon as the project is launched, will allow the different partners to make each other's acquaintance. The other meetings will provide an opportunity to assess the progress made during the past period: it will review the expected and unexpected results, as well as any scientific and technical hitches encountered. The state of the research will be evaluated by comparison with the planned delivery list and the timetable. In order to conform to the workplans, achieve the initial objectives and obtain the agreement of all partners involved, the different tasks, workpackages and protocols, if necessary, will be discussed for the next period. This process will allow possible re-evaluation of some strategies and re-orientation of the research if required.

The main contractors for each task will be responsible for collecting the scientific information from the participants in the workpackage and making a draft report. This will be followed up by an oral presentation at the meeting with any additional relevant information given by the participants. Proceedings will be written by the end of each workshop and will constitute the annual report produced to the European Commission and distributed to all partners as a source of information. The co-ordinator will also take the option to provide the partners with a regular activity reports to strengthen the collaboration.

Financial co-ordination: Supervision of the finances of the project will be undertaken by the Administrative and Financial Services of CIRAD-EMVT. In particular, this department will deal with the receipt, allocation and transmission of the Commission's financial contribution to the project partners, administration of the account held on trust, the control of payments, the provision of financial reports, the analysis and processing of audit results, permanent financial control and comparison of estimates and actual costs. The Administrative and Financial Services benefit from the experience of co-ordination of many former and ongoing EU-projects. It will be the permanent contact point for the Commission concerning payments, cost statements and general questions regarding accounting and financial matters for the project.

6.2. Plan for using and disseminating knowledge

If successful, this project will provide marker vaccines that would allow targeting of vaccination in the infected zones with a much higher precision as the areas where wild virus is circulating can be monitored serologically. In would also allow for a quicker release of control measures in the vaccinated areas once virus circulation has been shown to cease. In addition, new diagnostic tests will be validated following international rules and the results and the reagents made available to all the

A consortium agreement will be drawn up and signed by all partners to specify all their responsibilities and duties concerning this project. The consortium will evaluate the necessity to secure the expected project outputs (vaccines and diagnostic tests) through patent or licence. This protection is primarily foreseen for allowing future utilisation of these outputs by developing countries with no risk of commercial restriction from third-private companies. Indeed, the final beneficiaries of the project are countries where RPV persists and PPRV is endemic. It will be one of the European contributions to helping developing countries to increase their incomes. However, if private companies are interested in the products developed, they will be asked to buy them and to consider the developing countries as privileged customers. As far as it is not interfering with the protection of the project outputs, results will be made freely available to public institutions. Dissemination of scientific results will be by reports, communication of results to at national and international scientific congresses and by publication in high standard international journals. Prior to publication, results obtained by any partner will be made available to other partners within the consortium.

A specific agreement will be made between CIRAD and its sub-contracting partner (NAHRC) to delimit the work to be done on the GMO.

Dissemination of scientific results and techniques will also be achieved through exchange working visits of technicians and researchers between laboratories and, when necessary, by welcoming PhD students or post-doctoral workers for extended periods. At least, one PhD student from each African partner will be expected to visit a European partner's laboratory during the course of the project.

The scientific collaboration between partners is defined according to type workpackages they are involved in. In this context, exchange of reagents, information and methodologies will be implemented between participants. The use of defined standardised methods, defined epidemiological approaches and extensive internal and external quality assurance protocols will ensure the validation and effective use of the project outputs.

6.3. Raising public participation and awareness

There are strong links between agriculture/livestock production, human health and poverty. Prevention and control of infectious diseases, understanding their epidemiology as well as factors that contribute to their spread is essential. Experience suggests that in most of the cases, diseases can be prevented through better knowledge of behaviour and practices of key groups of people. An increase of mutual understanding is needed between professionals interested in that field, and all parties concerned, cattle-owners, veterinary services, policy makers, law enforcement agencies, educational institutions for designing and implementing the most effective communication strategy.

The accompanying actions of the project relative to external communication and exploitation of research results will be of different nature. A number of partners of the project are already providing media materials or education.

For example the Inter-African Bureau for Animal Resources of the African Union (AU/IBAR – Partner CR9), seeks through the PACE programme to eradicate rinderpest from Africa. The human factor for the success of PACE depends on people's co-operation, therefore they cannot be overlooked. Without co-operation from everyone involved, the programme would take longer to complete and would be more costly. Campaign communication already exists. People are informed and motivated about the benefits and are persuaded to conform to its procedure. A number of media and activities are used. These tools of communication are vital to PACE. They include everything from radio programmes to posters, from ministry directives to knowing how to establish a good relationship with cattle-owners. For example, PACE National Co-ordinators are aware that they should win the support of people's attitude. Different target audiences are reached from the nomadic herdsmen, the government policy makers to the non-governmental organisations.

The accompanying communication activities of the project will also aim at strengthening the

community. Most of the partners have indeed a strong involvement in education and training of students in educational institutions such as Veterinary schools and faculties. The dissemination and the promotion of the research results will be naturally carried out by this way.

The other way also used nowadays is a dissemination of knowledge through the World Wide Web. At the level of CIRAD (Partner C01), for example, a project of e-learning is in preparation. This novel method of education will offer to a wide panel of non-specialised auditors the possibility to brake isolation, create a network of knowledge, and prolong their education. The e-learning project will strengthen the efficacy of the traditional training offers already available at CIRAD on epidemiology and tropical diseases.

Most of the partner's institutions have an Internet gate that offers a large panel of information of the institution's activity as well as specialised information, guidelines etc... These sites are benefiting of the permanent input of knowledge derived from research results at all level. They will also gain from this specific project. In particular, work package 9 intends to develop spatial tools for epidemiosurveillance that will improve the understanding of PPR distribution in sub-Saharan African countries and also to develop integrated decision-support tools for reinforcing the surveillance of PPR and similar diseases. In addition, the models will be used to generate hypotheses, which can be tested by future research relating to the maintenance of PPR foci and risk of its expanding into new areas. The gathered data will be integrated using particular software and made available via the Internet. This web-based information system will allow users to view dynamic maps and conduct tailored queries or select specific datasets. To reduce the cost resulting from software purchases, the majority of end users will be able to use softwares that are in the public domain.

In order to integrate the project outputs and transfer of knowledge in a coherent structure, it is also aimed at applying for a Specific Support Action (SSA) in the frame of the FP6. The SSA projects are usually aiming at the organising of stand-alone events (meetings, conferences), to generate shared knowledge. Indeed the specific objective of this proposal will aim at disseminating project achievements, in particular the results from an ongoing INCO-DEV project (see annex, A1) and from this particular proposition to facilitate the participation of a broad scope of laboratories and organisations to a forum on PPR. The SSA proposal, by allowing experts to meet in working groups, will also permit beyond the actual proposal to implement research roadmaps and identify future research activities and policy objectives. The overall objective of the proposal is to strengthen the collaboration between European and non EU research teams to ensure exploitation of research results on an international scale and to built up and propose scientific solutions to better control one important transboundary disease that compromises poverty alleviation in the world, namely PPR.

In this perspective, the SSA-project intends to bring support for the scientific meeting for more efficient collaboration. It will allow improving our communication and to disseminate project achievements in addition to the different type of media, communication activities and means of education carried out as briefly summarised above.

7. Work plan – for full duration of the project

7.1 Introduction – general description and milestones

RP now appears to be confined to an endemic area in Eastern Africa, mainly in Somalia but countries adjoining the Somali pastoral system are at high risk (*4*, *6*). Efforts for the global eradication of RP are now concentrated on eliminating the virus from these areas, however, the current epidemiological situation favours the re-emergence of RP in these neighbouring countries. The formulation of emergency vaccination plans and the availability of marker vaccines and their companion diagnostic tests are essential tools if a more rapid and more economic control of new RP outbreaks is to be achieved. There is also a critical need for PPR marker vaccines and differential diagnostic tools if a feasible control policy for PPR is to be implemented in areas where high levels of infection are found or where there is a high risk of introduction of the disease from neighbouring countries or regions. The project will address this issue by developing new marker vaccines at the same time it will study the immunomodulating capability of the virus with a view to improving the vaccine quality. The strategy proposed is to use reverse genetics to generate marker vaccines *via* genome cDNAs derived from the classical attenuated vaccine strains of RP and PPR. The overall aim is to produce double-marker vaccines by deletion (negative marker) and addition (positive marker). Safety issues regarding attenuated vaccines, specifically the GMO vaccines, by reducing their immunosuppressive characteristics, are particularly important to consider. It must be clearly established that these vaccines will not have any adverse effects in the vaccinated population, particularly in developing countries where animals are naturally exposed to a variety of biological agents that can render them more susceptible to the immunosuppressive effects of vaccine strains. Therefore, improvement of vaccine safety based on rational scientific data is highly desirable. Finally, technology will be developed for the formulation of an inexpensive and robust vaccine easy to administer to animals in areas lacking a cold chain. All these issues will be addressed in Task I through workpackage WP-1 to WP-6.

In parallel to the marker vaccine development, accompanying tests allowing the detection of post-infection antibodies, as well as antibodies to the positive and negative markers, will be developed in Task II, WP-7 and WP-8. Validation of the diagnostic tests to provide information on their performances in term of their epidemiological usefulness, will be addressed in workpackage 9 (WP-9) in Task III. This information will be needed for the improvement of RP/PPR surveillance systems and their control methodologies in sub-Saharan African countries. Surveillance of diseases is the basis for early warning and early reaction in countries and regions that are most exposed to the risk of RP re-emerging and PPR incursions. The detailed objectives are to improve the quality of the epidemiological data collected, with the aim of assessing the performances of the available and newly developed diagnostic tests. In order to enhance the surveillance of RP and PPR in areas at risk, new system management tools coupled with diagnostic tools and spatial epidemiology (WP-10) will be assessed for their performance in improving surveillance and control measures. For PPR, it will be necessary to establish optimum vaccination strategie based on data obtained through the integration of epidemiological studies with a modelling approach (WP-11). Such a model was described for RP (*23*, *22*). It is expected from this approach to demonstrate the usefulness of these tools for improving the rapidity and efficacy of emergency vaccination plans for PPR control in endemic areas.

As indicated above, the proposal is broken down into 3 main tasks that are closely interconnected, the main connection being made through the diagnostic tests. However, the task III will also serve for optimising the conditions of marker vaccine use in the field. Thus, the marker vaccine development (Task I) is useful only if they can be used with reliable and well validated companion diagnostic tests (Task II) and effective epidemiosurveillance systems (Task III).

The project will enable several European and African laboratories to work together to solve problems of mutual interest. The workpackages proposed for each of the groups are complementary and in that way will contributes to promoting technical and scientific co-operation with much mutual benefit. The project will permit senior African researchers to contribute to scientific research on the development and validation of vaccines and differential diagnostic tests and the improvement of epidemiological

scientists and technicians both in their own and in foreign countries through training (PhD, Post-Doc). Partners will therefore strengthen a privileged relationship, which will be very useful in helping to design future co-operative research and development programmes.

a) Task I: Development of marker vaccines for RP and PPR

The main objective of this task is to develop efficient double marker vaccines that allow the easy identification of virus circulation within a vaccinated population. The positive marker will allow a control of vaccination and the negative marker the control of infection. The genome of negative-stranded viruses such as RPV and PPRV can accommodate, without change in virus growth, additional genetic material of up to 3kb, which allows a positive marker gene to be inserted. Depending on the position of the insertion site in the genome, the foreign protein can be expressed either at high or low levels. Deletion of a gene or part of a gene, to produce a negative marker should target a protein eliciting a strong antibody response in animal. This would be absolutely required for the development of sensitive companion diagnostic tests. *Morbillivirus*es have six structural proteins, namely the nucleocapsid protein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F) the haemagglutinin protein (H), the polymerase (L for large protein) and two non-structural proteins, V and C. All serological tests developed so far for the diagnosis of *Morbillivirus* infections are based on two proteins: N or H. Indeed, in infected animals, the majority of antibodies are directed against the N protein although they do not neutralise the virus. The H protein induces a relatively high level of antibody response and, in contrast, these are mainly neutralising. Based on these facts, we have decided to target these two proteins for the deletion purposes to produce negative markers.

Workpackage 1: Duration of humoral immune response to RP positive marker vaccine

To date, two foreign genes, a cell surface anchored version of the green fluorescent protein (GFPanc) gene and a mutated version of the influenza heamagglutinin (flu HA) gene have been inserted into the RPV genome (*8, 15*). Both proteins appear to be good candidate marker antigens. They were shown to be expressed at the surface of virus-infected cells. Virus glycoproteins exposed on the cell surface generally induce a long-lasting immune response. However, nothing is known on the duration of the antibody response against non-glycosylated proteins such as GFP. Therefore, since ELISAs to detect both GFP and flu HA in the original marker vaccines have already been developed and the marker proteins are known to produce good short-term humoral antibody responses, it will be important to determine the effectiveness of the different marker proteins (GFPanc and flu HA) in generating long lasting protective immunity and antibody response to the markers in cohorts of African breeds of cattle. Similar duration of immunity / duration of marker response will be carried out for the new vaccines produced in WP-2 in cohorts of vaccinated cattle in the laboratory of partner CR6 in Africa (WP-1).

For the experimental vaccinations the standard vaccine dose (10^2 $TCID_{50}$) and 10-fold higher and lower doses will be administered to determine if the vaccine dosage affects the strength of the marker immune responses. Positive controls will be animals vaccinated with the classical RP vaccine. Sera from the vaccinated animals will be taken on a weekly basis up to six weeks post infection and then on a monthly basis until the completion of the project. At 1 and 2 years post vaccination a sub-group of vaccinated cattle will be challenged with virulent RPV to check that the vaccine is still effective and to look for evidence of replication of the challenge virus using the newly developed discriminatory test systems. All the conventional serological tests, including the virus neutralisation test (VNT), will be carried out on the sera along with the new marker-specific ELISAs.

All the experimental serological test results will be fed into WP-7, WP-8 and WP-9.

Workpackage 2: Development of a double marker vaccine for RP.

As indicated in the introductory sections, positively marked RPV vaccines (RPV-GFPanc and RPV-fluHA) have already been made and the duration of the humoral immune response to these marker proteins will be assessed in a separate workpackage (see WP-1). In addition to the existing marker vaccine candidates, attempts will be made to produce other positively marked vaccines with smaller

lived. In addition, a negatively marked vaccine will be produced, by deleting a characterised antigenic epitope from the haemagglutinin (H) protein of the vaccine virus. Finally, a vaccine with both characteristics, negatively and positively marked, will be developed using these new strategies.

Deletion of a characterised RPV-specific epitope will be attempted by trying to delete the C1 monoclonal antibody (Mab) binding site in the RPV H gene. The C1 monoclonal is the basis of the current H-specific ELISA to identify RPV seropositive animals. The virus H protein is highly immunogenic and other MAbs are available which bind to other regions of the protein and, if it is possible to delete or alter the C1 binding site, these could be used to develop alternative H-specific ELISAs. The original C1-based test would then only work for animals infected with the normal vaccine or a wild-type virus and not with the C1 deleted vaccine. This would successfully produce a negatively marked RPV vaccine. A 3D structure for the H protein of paramyxoviruses (*24*) has been proposed and the region where the C1 binds to the H protein has been partially mapped using a series of chimeric RPV-measles virus H genes (CR2, unpublished results). This knowledge of the structure and binding site of the RPV H protein will allow us to make rational choices for altering the C1 site. If this proves successful then this marker H gene will be used to replace the original RPV H gene and act as a negative marker in the vaccine.

Secondly, attempts will be made to add epitope tag markers, such as an "HA tag" or a "myc tag", to the C1-deleted region of the RPV H protein. If an epitope tag can replace the C1 epitope then, since the C1 monoclonal is the basis of the current H-specific ELISA, a negative/positive marker vaccine will have been produced with the minimum of foreign genetic material inserted. If it is not possible to obtain a viable virus if the virus C1 epitope is replaced by a tag then attempts will be made to insert the tag in some other region of the H protein gene. If either strategy succeeds this would effectively produce a positive/negative marker vaccine with only the H protein gene altered.

Thirdly, if all these approaches designed to modify the H gene fail, possibly because of severe structural constraints on the H protein, then the N protein gene of RP will be altered in a similar way to that which may prove successful in the case of the PPR vaccine (see WP-3). The work to develop positive/negative marker vaccines for RPV and PPRV by altering virus genes is therefore integrated and data from WP-3 will be fed into WP-2 and *vice versa*. However, if this strategy has to be adopted, then the deleted N gene segment will be replaced with an epitope tag different from the one inserted into the PPR-ΔN-tagged vaccine to facilitate serological differentiation between the two viruses. This vaccine (RPV-ΔN-tag) would give a unique serological signature since vaccinated animals would be negative in a RP-N gene-specific ELISA and positive in a RPV-H-specific ELISA. Any vaccinated animals that subsequently became infected would become positive in the RP N-specific ELISA. The major advantage of having a positive and negatively marked vaccine is that at least two tests can be applied to sera, which are of a disputed or uncertain nature with regard to the animal's RPV exposure status. In addition, the positive marker on its own can be used in the early stages of a control campaign involving vaccination to assess the level of vaccine cover achieved by the various vaccination teams. Experimental vaccinations with the vaccine and subsequent challenge of the animals with virulent virus will be carried out to test the serological responses in small-scale trials in the high security animal facility of partner CR2 to establish their efficacy before any long term experiments are carried out in Africa in partner CR6 laboratory (see WP- 1).

Workpackage 3: Development of a double marker vaccine for PPR.

Since PPR 75-1 vaccine strain has proved to be safe and effective (*25*), this strain will be used for the development of a genetically marked PPR vaccine.

Positive marker: For this work the approach followed for the development of the positively marked RPV vaccine will be used (*8*, *26*). Using a PPR rescue system, an expected output from the PACE research project funded by EU, a recombinant PPR 75-1 vaccine carrying a foreign gene as a genetic marker will be produced. To this end, a DNA cassette, containing *Morbillivirus* protein gene start and stop signals, bracketing a unique restriction site, will be inserted between the P and M genes of the virus. This construction will be made while respecting the "rule of six", a requirement for the correct

that has been used to tag the rinderpest virus (*8*) will be applied to PPRV. However, in order to avoid using the same label in both RPV and PPRV vaccines, another antigen will be tried as an alternative marker. In a first approach, it is proposed to select a protein or an epitope that are not naturally immunogenic for ruminants and for which laboratory or commercial reagents are available.

Negative marker. The generation of a negative marker in the vaccine is required to provide a mean to specifically identify by serology infected animals, previously vaccinated or not, through their exclusive production of antibody to the deleted epitope of the vaccine. N protein encapsidates the genomic RNA and in association with the P and L proteins forms the ribonucleoprotein complex which is the template for genome transcription and replication of the negative-stranded viruses (*31*). Moreover, it seems to regulate these two biological activities within the viral life cycle. Sequence data of the N of different viruses within the *Paramyxoviridae* family have shown that this protein can be divided into two main parts:

- a conserved area in the N-terminal region of the protein sequence; it is involved in N self-association and N-RNA interaction (*32*).
- a C-terminal region which is highly variable from virus to virus within the same genus and which is required for the template function. It is exposed on the surface of the protein and contains a binding site for the P protein (*33*).

Thus it seems that most of the N protein is required for paramyxoviruses replication. Despite this, it has been shown recently with Newcastle Disease virus (NDV) that a B-cell immunodominant epitope in the C-terminal region of N can be deleted to generate an efficient negative-marker vaccine (*34*). This successful result indicates that it is possible to find on the N a sequence that is highly immunogenic but dispensable for virus growth. The deletion of this sequence has not affected the efficacy of the recovered NDV vaccine since an N-specific immune response is not absolutely required for the protection of poultry against a lethal challenge (*35,36, 37*). Furthermore, it has been shown that the N of RPV, delivered into cattle through a recombinant vaccinia virus, did not provide protection against a lethal challenge with RPV (*38*). The same observation has been made with recombinant baculovirus-expressed PPRV N inoculated into goats (CO1, unpublished data). These results provide an indication that it is possibile to delete a sequence in the N of both PPRV and RPV vaccine strains without affecting the protective features of the vaccine if the deletion targets an epitope non-essential for virus growth. Therefore, as in the case of NDV (*34*), it might be possible to generate an effective negative PPR marker vaccine by deleting an epitope from the N protein. The procedure that has been used successfully for NDV will be followed for the development of the negative PPR marker vaccine:

The nucleic acid sequence corresponding to the most reactive peptide (s) as defined in WP-8, will be deleted from the cDNA of the PPRV N gene. The functionality of this modified N will be tested for its ability to rescue a minigenome plasmid containing a reporter gene (either enhanced green fluorescent protein, eGFP or chloramphenicol acetyl transferase, CAT). Any deletion shown not to affect RNA replication, will be introduced into the PPRV full-length antigenome RNA to generate the mutant virus. In a subsequent step, a foreign epitope will replace the deleted PPRV one to generate a double marker vaccine. After each rescue, the modification introduced into the virus will be checked by RT-PCR and sequencing. Each of the modified viruses will be tested in animals for their efficacy in providing protection against a lethal challenge. The absence of antibodies to the deleted epitope but the presence of antibodies against the positive marker in vaccinated animals will be established using the ELISAs whose development is described in WP-8 below.

Workpackage 4: Identification of N sequences involved in immunosuppression

The aim of this Workpackage is to address the immunosuppression effect induced by RPV and PPRV, a characteristic that is common to all *Morbillivirus* infections and which can lead to the establishment of opportunistic infections (*39*). For example, PPR is often associated with lung infection followed by secondary bacterial development, diarrhoea and death in 50 to 80% of acute cases (*40*). Paradoxally, morbillivirus infections induce a strong protective immune response in their hosts, if they recover

immunosuppression induced is transient. In the case of measles (MV) and RP, some of the immunological alterations observed during natural infection can also occur after vaccination with attenuated strains (*13,41,19*). It has been demonstrated recently *in vitro* that the RPV vaccine inhibits the proliferation of bovine peripheral blood cells at the same level as the virulent wild type (*10*). In the same experiment, the PPRV vaccine strain demonstrated a stronger immunosuppressive effect on caprine PBL than RPV. It is likely that many factors contribute to this transient immunosuppressive effect and studies are being carried out to clarify this observation. It is likely that the RP/PPR induced immunosuppression, which is certainly transient, is a multifactorial process, which may be triggered by direct contact of the viral glycoprotein complex with cells, and also by soluble factors through receptors on the host lymphocytes. Indeed, several recent studies suggest that *Morbillivirus* proteins could inhibit the immune response in the absence of viral replication. Recently, it has been shown that *in vitro* replication or RPV or PPRV is not necessary for the induction of immunosuppression (*10*). Indeed inactivated virus efficiently inhibits the in vitro proliferation of bovine and caprine peripheral blood lymphocytes (PBL). This work also indicated that a native complex of the haemagglutinin and the fusion proteins of the virus is obligatory (*10*). Also recent work demonstrated the involvement of the N protein in the immunosuppressive effect induced by MV, CDV, PPRV and RPV by binding to cellular receptors involved in the inhibition of lymphocyte proliferation. Some consequences of this binding are inhibition of antibody production as well as production of interleukin 12 (*14*). Using recombinant expressed proteins the *Morbillivirus*-N-receptor binding domains were localised in the C-terminal domain of the protein. Although this is the least conserved among the morbilliviruses, it contains two conserved short sequences (*42*). As it seems that the domain of the N protein involved in binding to cellular receptors is common to all the morbilliviruses involved in the study (*11*), one can speculate that one of the short conserved sequences in the N C-tail is involved in immunosupression. Therefore this region, or part of it, is a prime candidate to be deleted as a negative marker in the vaccine (Task 1, WP-3) and consequently this region of the protein will be focused on for the design of a companion ELISA test (Task II, WP-8). An additional benefit of the work carried out within WP-4 would be an improvement in the safety of the RP and PPR vaccines by reducing their immunosuppressive characteristics. The full-length N protein of PPRV and a series of deletion mutants expressed as fusion proteins in the baculovirus system, or rapid translation system (RTS), already available in the CR3 laboratory, will be tested for their ability to inhibit spontaneous cell proliferation using the methodology developed by partner CO1. In this workpackage PPR virus will be used as a model to analyse the immunomodulatory role of the N protein and its fragments to demonstrate their participation in the virus induced immunosuppression. The C-terminal part of the N protein of the *Morbilliviruses* in binding the P protein is consequently required for viral transcription (*32,43,44,45*). To address the question of whether the deleted proteins preserve their functionality, they will be used to rescue a minigenome plasmid, containing a reporter gene (eGFP or CAT), in order to determine the effect of deletion. This technology has already been established in the CO1 and CR2 laboratories.

Workpackage 5: Safety of the recombinant GMO vaccines

The new marker vaccines are considered as GMO since their genetic material has been artificially modified and so the potential risks from their release in the environment must be addressed according to the rules governing the voluntary dissemination of GMO in the European Union (2001/18/CE). In the case of rinderpest and PPR viruses, the parental vaccine strains, from which the marker vaccines will be derived, have no negative impact on the environment. For instance, it is well known that animals vaccinated with the current vaccine strains do not secrete the virus and that it remains lymphotropic (*18,46,7*). Therefore it is assumed that the genetically modified PPR and RP strains will retain these parental characteristics. All vaccinated animals will be carefully monitored for unusual clinical signs to ensure vaccine safety. Some of the vaccinated cattle and goats will be sacrificed soon after vaccination to monitor the marker vaccine spread to the different tissues in the animal to ensure that the tissue tropism has not been altered relative to that of the conventional vaccine. Similarly the duration of viraemia and the ability, or inability, of the new vaccines to spread by contact will be determined in contact transmission studies. Eye, nose, mouth and anal swabs will be taken at intervals

experiment for RPV marker vaccines, extensive training of technical staff from CR6 laboratory, leader of this workpackage, will be required for periods of 3-6 months to ensure that they have sufficient mastery of these techniques for transfer to their home institute. More senior staff will be actively involved on 1-3 month visits which will also involve and element of training but which will also allow them to actively participate in the development the new vaccines and their associated serological tests. The safety experiments for PPR marker vaccines will take place in a laboratory in Africa appointed by CO1 as a sub-contractor. This sub-contractor has acquired expertise on animal pathogen transmission studies including contact experiments. It is presently working on foot and mouth disease virus persistence in infected animals previously vaccinated or not. Its expertise in this field will greatly benefit to the work on PPR marker vaccine persistence and transmission. Its task will be limited to GMO safety studies and training regarding specific aspects of PPR will be organised by partner CO1.

Worpackage 6: Marker vaccine formulation for improved stability

Preservation of new generation vaccines during storage is a critical issue. The mechanisms involved in vaccine instability during storage are poorly described and not well understood. There are two main strategies to avoid the decrease in vaccine efficacy during storage: (i) the use of ready to use formulations for storage at 4°, -20° or -80°C and, (ii) the use of concentrated antigen preparations either held at ultra-low temperature or lyophilised for later formulation. Afterwards there is the need for cold chains supported by electrical supply and/or dry ice makes and this can be a difficult task in some developing countries. Within the scope of an on-going EU project (see annex, A2), different formulations, including cryo and/or lyoprotectants (see annex, A5) were shown to enhance the stability at 4 and 20°C for model candidate recombinant RP and PPR vaccines designed for oral delivery. Similar formulations will be tested for the marker vaccines developed under the present proposal.

b) Task II: Diagnostic test development

Workpackage 7: Companion test development for RP marker vaccine

Since very avid monoclonal antibodies are commercially available to the various tag sequences ("HA tag" or a "myc tag") that we propose to insert into the RPV vaccine, it should be relatively easy to develop the necessary ELISAs to detect antibody responses to the tag marker epitopes. It is envisaged that these tests will be in the competitive ELISA format. In order to produce tag antigen to bind to the plates, the tags will be added to a non-viral protein (for example, GFP) and expressed in a bacterial system. The antigens produced will be tested for their reactivity with the tag-specific antibodies. To produce the necessary animal sera to react in the tests the proteins will first be injected into small animals (rats, mice or rabbits) to produce tag-positive sera. Later the tagged marker vaccines (HA-tagged and myc-tagged) produced in WP-2 will be used to infect experimental cattle, in the CR2 laboratory, to produce the required test sera and to see if they generate the expected immune responses in cattle. The sera will also be used in the initial stages of the tests' validation process. The tagged marker vaccines will subsequently be tested in larger numbers of African breeds of cattle in the CR6 laboratory.

The second part of this workpackage will involve the development and validation of a more sensitive and specific ELISA to distinguish RPV and PPRV infections. For this the RPV-specific region of the N protein of RPV has been expressed in a bacterial expression system. This will be used to develop, in conjunction with partner CO1, various ELISA test formats to specifically distinguish sera induced by infection with either of these two viruses.

Workpackage 8: Companion test development for PPR marker vaccine

For the implementation of a targeted and efficient control strategy it is essential to locate disease outbreaks and to monitor the extent and distribution of these outbreaks through clinical and serological surveillance. The serological surveillance is based on the identification and differentiation of antibodies in sera, firstly from animals which were infected by field virus only, secondly from animals which have been vaccinated only, and thirdly from animals which have been infected and subsequently vaccinated or which have been infected after vaccination. All these situations would occur in enzootic areas where disease control by vaccination is implemented. It is worth mentioning that an abortive replication of the challenge virus might occur in vaccinated animals as suggested for RPV (*8*). This limited replication might be sufficient to induce new antibody production in the vaccinated animals. Therefore only a vaccine with a deletion marker will allow distinguishing animals which have been vaccinated only from animals which have been vaccinated and subsequently infected by field virus since any vaccinated animal which becomes infected will also produce antibodies against the epitope/marker which was deleted from the vaccine.

An N protein-based immunoassay has been developed by CO1 for the diagnosis of PPR (*49*). Indeed, because the N proteins of negative-stranded RNA viruses are highly immunogenic in nature this

In a first step, two indirect ELISA will be developed:

- one based on the PPRV N peptide corresponding to the deleted immunodominant epitope as a discriminatory test to differentiate vaccinated animals from infected animals (vaccinated or not);
- a second peptide (or full length antigen) of the positive marker to differentiate vaccinated animals from non-vaccinated animals (test to monitor the vaccination).

The presence or the absence of antibodies to the negative marker will be monitored by an ELISA able to detect antibodies directed against the deleted highly immunogenic region. The two following procedures will be carried out:

1) identification of an immunodominant region. Expression of deleted N representing different regions and analysis of the reactivity of these deleted proteins with a serum from vaccinated animal. Partner CR3 has already produced the different PPRV deleted N antigens within the framework of the PACE research project for the improvement of PPR diagnostic tests. These materials will be used for the identification of the immunodominant region.

2) pepscan analysis of the N protein to identify immunodominant epitope(s). Overlapping 13-15-mer peptides will be synthesised. The reactivity of those peptides with sera raised against different strains of PPRV will be analysed. It is expected to identify peptides allowing the detection of PPR antibodies in the serum of infected animals and not in the serum of the animals vaccinated with negative marker vaccines.

In, a second step, competitive ELISAs, based on monoclonal antibodies produced against each of the markers, will be developed, similar to the one currently in use for PPR serodiagnosis and developed by partner CO1 (*49*).

c) Task III: Improvement of information systems for PPR surveillance and decision making

Worpackage 9: Evaluation of the tests' performance (RP and PPR)

Serological tests for RP and PPR are currently used for confirmatory diagnosis. Until now the assay used to monitor RP was a competitive ELISA based on an anti-haemagglutinin monoclonal antibody (*53*). It has been used successfully throughout PARC and PACE for serological monitoring i.e. to evaluate the level of vaccine cover. More recently, immunoassays based on the use of recombinant N protein were produced and also proved to be effective for differential diagnosis (*49, 54*).

ELISAs are appropriate to large-scale serological surveys, however, the differential cross virus-neutralisation test (VNT) is used to differentiate RP and PPR specific antibodies (*55*). It is still considered to be the most sensitive method and is used as the gold standard in reference laboratories (*56*). During the course of the present proposal a considerable amount of pathological materials will be collected during vaccine trials (WP-2 and WP-3) and virus pathogenicity studies for disease modelling (WP-11). These samples, in addition to other samples collected in the field, will be analysed using the different RP and PPR assays available. These include immunocapture and RT-PCR to analyse swabs and white blood cells for virus nucleic acid and different RP and PPR ELISAs, both the available ones and others which will be developed in WP-8, to analyse the serum samples. Thus it should be possible to ensure that the exact status of each animal with respect to the exposure to each virus can be established (gold-standard features). Afterwards, the performances of the different tests will be established.

Part of the test evaluation based on field and experimental work will involve six African laboratories including partners CR6, CR7 and CR8 for the validation of the PPR ELISA assays currently available or under development and expected to be available in the course of the present project. This validation will be undertaken in a round test organised by CR3. All the data generated will be used to determine the characteristics of each test and this will be needed when decisions have to be taken on which test is most suitable for a specific use (fitness for purpose).

The present proposal aims to improve our knowledge of the prevalence of the diseases as a result of a

and VNT results and reference disease status of the animals, will be used to conduct ROC analyses. These analyses will provide information about optimum cut-off values and associated sensitivity and specificity values for the RP and PPR tests to be used in the surveillance framework. Sensitivity and specificity are conventionally used to evaluate assays on a given cut-off. However, they should not be considered as a fixed threshold but rather as a flexible parameter adjustable to the circumstances under which the test is used. The ROC curve analysis will evaluate the performance of the assays independently of their threshold values. A test can be evaluated on the complete measurement range to obtain a better idea of its discriminating potency of seropositives and seronegatives (*57*, *58*). Predictive values of given test results will be calculated for PPR using prevalence estimates from the different sub-Saharan African countries. In the case of RP low prevalence values will have to be assumed, and expected predictive values of positive/negative test results will be calculated for different typical scenarios.

The main expected outcomes of the WP will be standardised multistage sampling procedures including time and type of samples for the different laboratory tests in different species (goat, sheep, cattle), animal breeds and virus strains and finally a guideline document setting out plans for PPR sero-surveillance and cross-sectional surveys.

Worpackage 10: Spatial epidemiology of PPR

Disease occurrence is influenced by proximity between infected and susceptible animal populations (closeness in space and time) and environmental factors (spatial overlapping of animal disease and environmental risk factors). The methods that are to be used and developed for spatial epidemiology consist of data mapping, cluster detection and disease risk modelling.

Data from recent outbreaks and serological results will be collated from existing surveillance systems and data sources. Complementary field surveys will be undertaken in the context of this project with the African partners. Based on these and other spatial and non-spatial information (environmental features, animal density completed by expert opinion), risk models will be generated using "knowledge-driven" methods (spatial regression analysis, multi-criteria decision making models). These methods are expected to identify geographical areas that require strengthened or allow reduced surveillance effort. In addition, the models will be used to generate hypotheses for future research relating to the persistence of PPR foci in selected African agro-systems. This WP will ask for the investigation and the development of new statistical tools and will strongly associate partner CR5.

Workackage 11: Modelling and the dynamic of PPR

In 1989 an epidemiological model of RP in cattle and wildlife populations was described (*22*, *23*). In this model, fixed parameters for different hypothetical strains of RPV and different susceptible populations were described together with details of their derivation. Simulations were then carried out in a computer model to determine the effects that varying these parameters would have on the behaviour of RPV in the different populations. The results indicated that virulent strains were more likely to behave in epidemic fashion whereas milder strains would tend towards persistence and the establishment of endemicity. High herd immunity levels prevent virus transmission and low herd immunity levels encourage epidemic transmission. Intermediate levels of immunity assist the establishment of endemicity. The virus is able to persist in large populations for longer periods than in small populations. Different vaccination strategies were also investigated. In areas where vaccination is inefficient annual vaccination of all stock may be the best policy for inducing high levels of herd immunity.

The model that was proposed for RP epidemiology and for defining a vaccination strategy took into consideration the existence of virus strains with well-defined degrees of pathogenicity. Unfortunately, no such information is yet available for PPR. Sequencing data from the F protein gene and the N gene of different PPRV strains have allowed their classification into 4 lineages, which reflect their geographical distribution, however, there is no relationship either to their pathogenicity or the speed with which the virus diffuses in nature (*59*, CO1, unpublished data). Information gathered from the

sheep are, but it often happens in some endemic areas that the disease does only affect the later species, goats in contact remaining apparently healthy (*2*). It is not known if this variation is due to the animal species, to the breed (dwarf goats are know to be more susceptible to PPRV than Sahelian breeds) or to the virus strain. In the sub-Sahelian zone, small ruminants graze freely during the rainy season. Although widespread, this livestock production gives additional incomes to the farmers. To allow prediction of the impact of preventive programmes for PPR and the advantages obtained in term of nutrition and incomes, a socio-economic study related to the field application of the current PPR vaccine has to be assessed. In this WP-11, animal experiments and a socio-economic study will be carried out to provide data related to all these variables:

1°) Experiments with different virus strains will be carried out by CR7 and CR8 laboratories to provide information on disease evolution including incubation period, morbidity and mortality rates, delay for seroconversion and risk factors (species, breed, season). Indeed, for each strain, goats, sheep and cattle will be inoculated by intramuscular route. Although it is generally admitted that cattle are not susceptible to PPRV, it is not clearly established if they can excrete the virus or not after infection and thereby participate or not into its maintenance in endemic areas. Partner CR8 will also test a strain in animals during the cold dry season and also at the beginning of the raining season. During the proposed experiments all animals will be submitted to clinical examination, nasal and ocular swabbing and blood sample collection for serum antibody detection and white blood cell analysis. To complete this, it is intended to perform the same experiments in Côte d'Ivoire, which is not a partner in the present project. The experiments in Côte d'Ivoire will be supported by funds from another project. In that way data will be collected from a third country that will allow all ecological zones in Africa where PPR is endemic to be investigated.

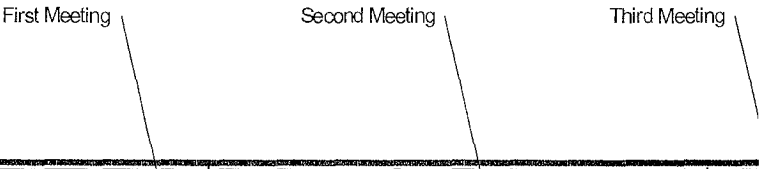
2°) According to the results from previous experiments, partner CR8 will implement experiments to determine the delay necessary for an infected animal to infect an in-contact susceptible animals. This experiment will be carried out with 2 different strains (results of previous experiments). One animal will be infected by the intranasal route, the natural route of infection. It will be put in direct contact with goats, sheep and cattle. The in-contact animals will be examined daily for clinical signs of disease. Handling of animals (record of temperature, collection of samples etc.) will be carried out while wearing disposable gloves and overalls and changing them between each animal.

All samples collected will be analysed in WP-9 using the PPR diagnostic assays currently available: ICE, PCR and c-ELISA.

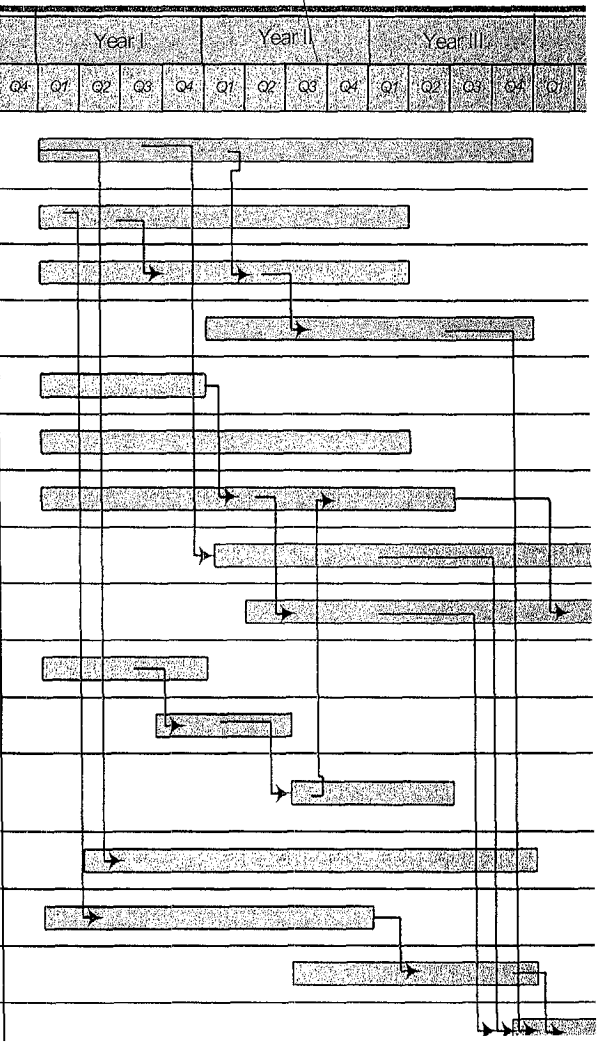
With the information obtained from these experiments, in addition to the existing knowledge about PPR and particularly the fact that it is an immunising infection (as well as previous models on RPV, MV etc.), a mathematical model of the "SEIR" ("susceptible – infected - infectious – recovered/immune") type will be developed. It will be a deterministic model that allows prediction of the 'average' expected impact of different control strategies in the animal population. In SEIR models, the transmission dynamics are expressed in continuous time using differential equations. We will assume that the animals are randomly mixed within a herd and between age levels, but will allow for heterogeneous mixing at the species level (livestock and wildlife) and between herds. The model will be set up using stratification to allow for heterogeneity, and the vaccination coverage will be included in the equations defining the flows between the susceptible and immune compartments. The required parameters (transition parameters, birth and mortality rates and probability of effective contact between infectious and susceptible individuals per unit time (beta parameters) will be determined from field data, if possible, but may also require use of expert opinion. The beta parameters need to be stratified so that non-random mixing between species and herds can be accommodated in the model. The beta values will be obtained by estimating the force of infection using sero-prevalence data (catalytic model) and computing with "Whom acquire infection from who?" (WAIFW) matrix equations. Data from past serological prevalence surveys conducted in various areas prior to vaccination will be used. Supplementary field surveys will be undertaken. The basic reproduction number (R0) and the "herd immunity threshold" (HIT) will be displayed.

3°) Mortality is a major constraint to goat flock productivity in extensive farming systems in sub-Saharan Africa. Pneumonic pasteurellosis, peste des petits ruminants and parasitism are frequently cited as major causes of mortality. The estimate of the overall impact of preventive programmes for PPR will be fully assessed if the key epidemiological questions are addressed such as: pattern of mortality of small ruminants by age, season and year, but also if a socio-economic component of the impact is taken into account. This additional component of the expected impact related to the field application of the current PPR vaccine will be a measure of the added value generated by the introduction of the vaccine in the PPR endemic zones. It will be evaluated as the economic and the social transformations induced at the level of the rural society or at the level of the national economy in the African agro-ecosystem context. The sociologic analysis will principally be focused on the fitting of the animal husbandry in the production system and the advantages in term of nutrition and incomes. A reference situation must be established – the situation before the intervention – in order to display the neat results of the vaccine effect.

'lanning and timetable: Work planning, showing the different WPs and their components

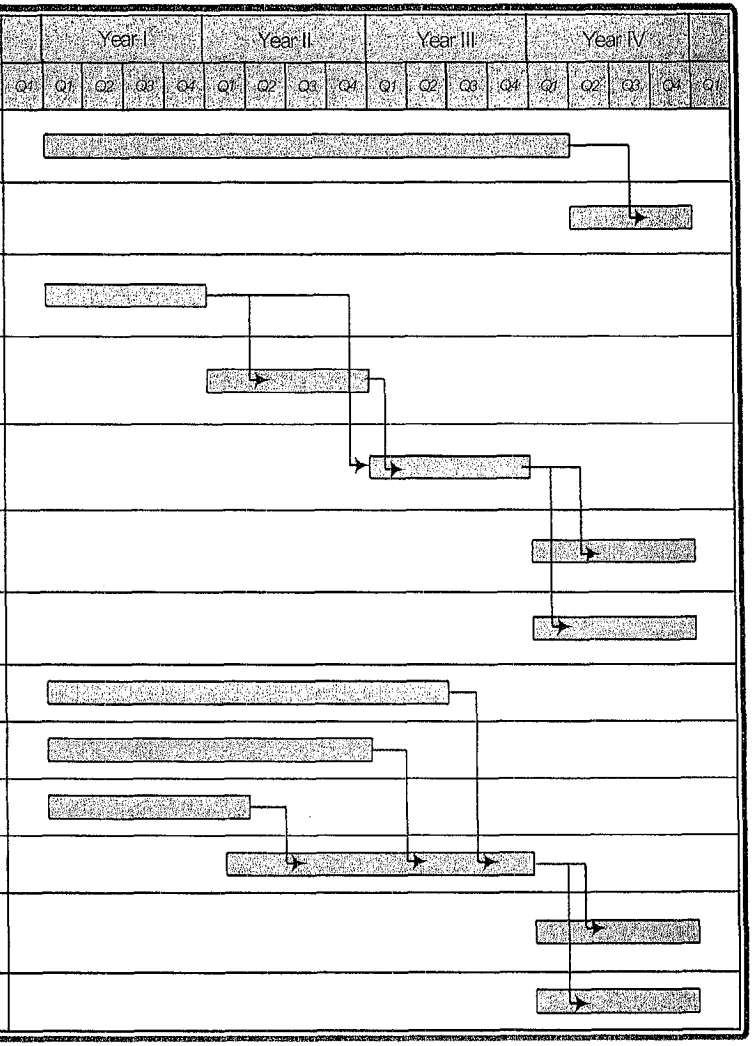


k /	WP N0	Deliverable N0	Name of the Deliverable	Start date	Delivery date
	WP1	D1	Establishment of the duration of humoral immune response to the RPV GFPanc and flu HA marker vaccines.	Month 1	Month 36
	WP2	D2	A negative RPV marker vaccine (RPV?-H).	Month 1	Month 27
		D3	A positively "tag" marked RPV vaccine (RPV-H-tag).	Month 1	Month 27
		D4	A positive and negative RPV marker vaccine (RPV?-H-tag).	Month 13	Month 36
	WP3	D5	Deleted N protein tested to support minigenome amplification	Month 1	Month 12
		D6	A positively (genome insertion) marked PPRV vaccine tested for efficacy	Month 1	Month 27
		D7	A negatively marked PPRV vaccine (PPRV?-N) tested for efficacy.	Month 1	Month 30
		D8	A positive (genome insertion) and negative marked PPRV vaccine tested for efficacy	Month 13	Month 42
		D9	A positive (N-tag) and negative marked (PPRV?-N) PPRV vaccine tested for efficacy.	Month 16	Month 42
	WP4	D10	Identification of sequences on the N protein inducing immunosuppression	Month 1	Month 12
		D11	Identification of critical residues on the N protein that are vital for the viral replication.	Month 7	Month 18
		D12	N protein with deleted immunosuppressive sequence (possibly candidate for negative marked vaccine).	Month 19	Month 30
	WP5	D13	Establishment of the vaccines' safety for subsequent field use	Month 4	Month 36
	WP6	D14	Process flow sheet for marker vaccine production	Month 1	Month 24
		D15	Formulation for injection and oral delivery.	Month 19	Month 36
		D16	Testing for long term storage (up to one year) of marker vaccines.	Month 35	Month 48

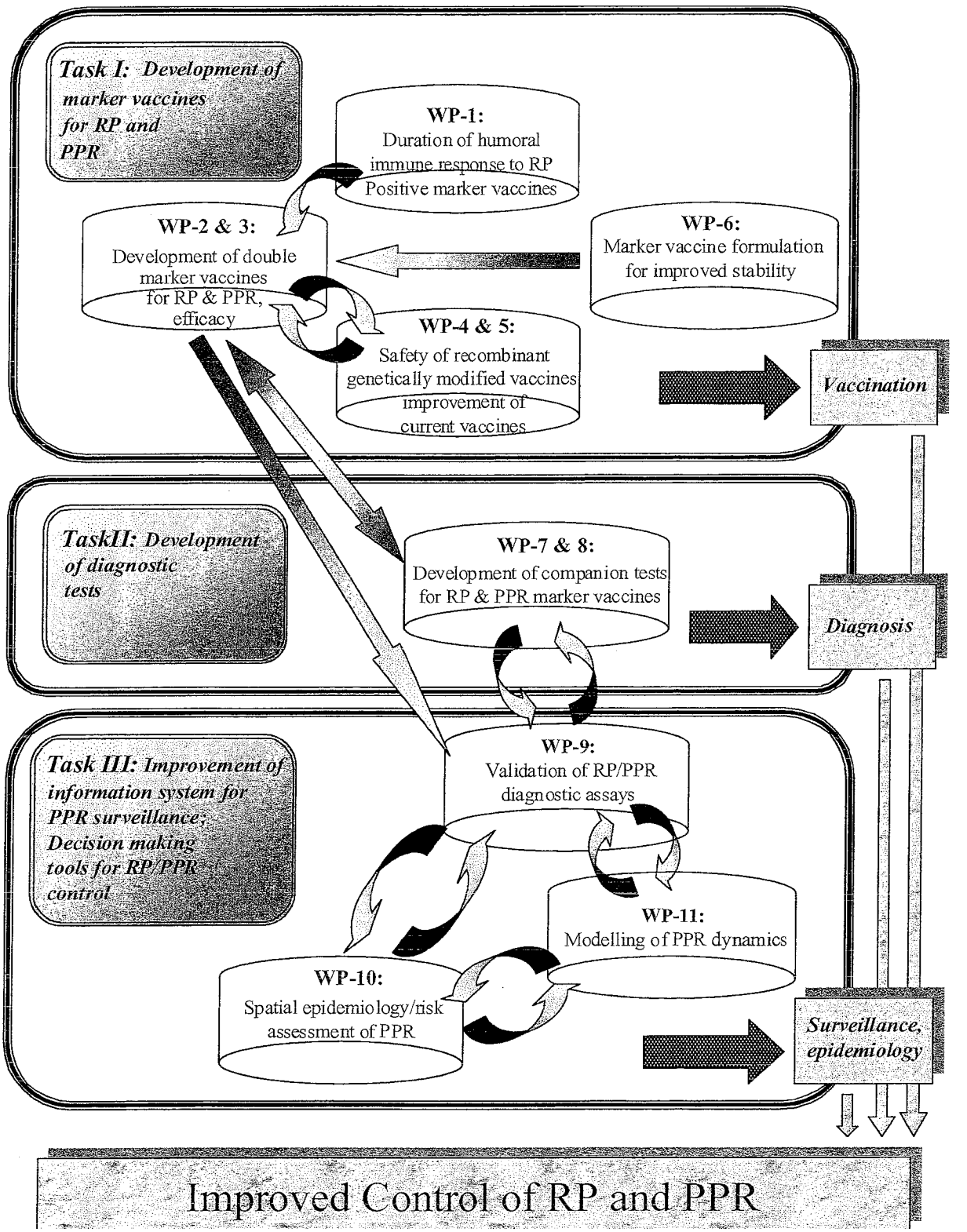


Task #	WP NO	Deliverable NO	Name of the Deliverable	Start date	Delivery date	Year I Q4 Q1 Q2 Q3
1	WP7	D17	Bacterially expressed antigens that have the epitope tags for use in RP serological tests.	Month 3	Month 12	
2		D18	Sera containing antibodies to the epitope tags incorporated into the bacterially expressed antigens (RP).	Month 13	Month 16	
3		D19	Validation of test to distinguish RP-vaccinated, wild type RP virus infected and vaccinated/infected animals.	Month 16	Month 21	
4		D20	ELISAs to distinguish virus specific anti-N antibodies induced by either RPV or PPRV infection of animals.	Month 3	Month 27	
5		D21	Serological test results from vaccinations with the new RP marker vaccines (positively and negative and double marker vaccines)	Month 28	Month 42	
6	WP8	D22	Baculovirus (or bacterial) expressed positive marker protein for use in PPR serological tests.	Month 1	Month 9	
7		D23	Recombinant N-PPRV protein antigen (deleted proteins or oligopeptides) and anti-N monoclonal antibodies.	Month 1	Month 9	
8		D24	ELISA test for the detection of antibodies to the positive PPR mark.	Month 7	Month 28	
9		D25	ELISA test for the detection of antibodies to the negative PPR mark.	Month 7	Month 31	
10		D26	ELISA test for the specific detection of antibodies to PPRV.	Month 1	Month 27	

able N0	Name of the Deliverable	Start date	Delivery date
28	Quantitative performance characteristics of current and newly developed cELISA tests (RP and PPR).	Month 1	Month 39
29	Guideline documentation for setting up standardised sampling plans for PPR sero-surveillance.	Month 40	Month 48
30	Geographical mapping of available PPR data at the pan-African, regional and national level.	Month 1	Month 12
31	Estimated spatial and temporal risk profiles for PPR at the continental level; and at the national levels for the countries included in the project.	Month 13	Month 24
32	Strategy for strengthening national, regional and global PPR surveillance.	Month 25	Month 36
33	Development of working hypotheses in relation to control of PPR.	Month 37	Month 48
34	Development of a web-based for a computerised PPR surveillance system.	Month 37	Month 48
35	Information on the susceptibility of animal species and breed to PPRV.	Month 1	Month 30
36	Information of the virus strain parameters in the epidemiology of PPR.	Month 1	Month 24
37	Socio-economic study related to the field application of the PPR vaccine	Month 1	Month 15
38	Mathematical model on PPR-spread within the African agro-ecosystems context.	Month 13	Month 36
39	Establishment of the large-scale basic reproduction number (R0) and Herd Immunity Threshold (HIT).	Month 37	Month 48
40	Ro (and HIT) mapping per agro-ecosystem.	Month 37	Month 48



7.3. Graphical presentation of workpackages



7.4. Work package list

Workpackage list (full duration of project) (Annex I)

Work-package No[1]	Workpackage title	Lead contractor No[2]	Person-months[3]	Start month[4]	End month[5]	Deliverable No[6]
WP- 1	Duration of humoral immune response to RP positive marker vaccine	CR6	37	1	36	D1,
WP- 2	Development of a double marker vaccine for RP	CR2	42	1	36	D2, D3, D4,
WP-3	Development of a double marker vaccine for PPR	CO1	104	1	42	D5, D6, D7, D8, D9
WP-4	Identification of N sequences involved in immunosuppression	CO1	30	1	30	D10, D11, D12
WP-5	Safety of the recombinant GMO vaccines	CR6	66	3	36	D13,
WP-6	Marker vaccine formulation for improved stability	CR4	56	1	48	D14, D15, D16,
WP- 7	Companion test development for RP marker vaccine	CR2	38	3	42	D17, D18, D19, D20, D21,
WP-8	Companion test development for PPR marker vaccine	CR3	76	1	46	D22, D23, D24, D25, D26, D27
WP-9	Evaluation of the tests' performances (RP and PPR)	CO1	52	1	48	D28, D29,
WP-10	Spatial epidemiology of PPR	CR5	54.5	1	48	D30, D31, D32, D33, D34,
WP-11	Modelling and the dynamic of PPR	CO1	93	1	48	D35, D36, D37, D38, D39, D40
	TOTAL		638.5			

[1] Workpackage number: WP 1 – WP n.
[2] Number of the contractor leading the work in this workpackage.
[3] The total number of person-months allocated to each workpackage.

7.5. Deliverables list

Deliverable No[7]	Deliverable title	Delivery date[8]	Nature[9]	Dissemination level[10]
D1	Establishment of the duration of immunity to the RPV GFPanc and flu HA marker vaccines and the validity of the test assays	36	R	PU
D2	A negative RPV marker vaccine (RPVΔ-H).	27	P	RE
D3	A positively "tag" marked RPV vaccine (RPV-H-tag).	27	P	RE
D4	A positive and negative RPV marker vaccine (RPVΔ-H-tag).	36	P	RE
D5	Deleted N protein tested to support minigenome amplification (functional ΔN).	12	P	RE
D6	A positively marked PPRV vaccine (genome insertion) tested for efficacy.	27	P	RE
D7	A negatively marked PPRV vaccine (PPRVΔ-N) tested for efficacy.	30	P	RE
D8	A positive (genome insertion) and negative marked PPRV vaccine tested for efficacy	42	P	RE
D9	A positive (N-tag) and negative (PPRVΔ-N) marked PPRV vaccine tested for efficacy.	42	P	RE
D10	Identification of amino-acid sequences on the N protein inducing immunosuppression.	12	P	PU
D11	Identification of critical residues on the N protein that are vital for the viral replication.	18	P	RE
D12	N protein with deleted immunosuppressive sequence (possibly candidate for negative marked vaccine).	30	P	RE
D13	Establishment of the vaccines' safety	36	R	PU
D14	Process flow sheet for marker vaccine production.	24	R	PU
D15	Formulation for injection and oral delivery.	36	R	PU
D16	Testing for long term storage (up to one year) of marker vaccines.	48	R	PU
D17	Bacterially expressed antigens that have the epitope tags for use in RP serological tests.	12	P	PU
D18	Sera containing antibodies to the epitope tags incorporated into the bacterially expressed antigens (RP).	16	R	PU

[7] Deliverable numbers in order of delivery dates: D1 – Dn

[8] Month in which the deliverables will be available. Month 0 marking the start of the project, and all delivery dates being relative to this start date.

[9] Please indicate the nature of the deliverable using one of the following codes:

R = Report
P = Prototype
D = Demonstrator
O = Other

[10] Please indicate the dissemination level using one of the following codes:

D19	Validation of test to distinguish the 3 categories of vaccinated and infected animal (RP-vaccinated, wild type RP virus infected and vaccinated/infected).	21	R	RE
D20	ELISAs to distinguish virus-specific anti-N antibodies induced by either RPV or PPRV infection of animals.	27	P	RE
D21	Serological test results from vaccinations with the new RP marker vaccines (positively and negatively marked and double marked).	42	R	PU
D22	Baculovirus (or bacterial) expressed positive marker protein for use in PPR serological tests.	9	P	RE
D23	New recombinant N-PPRV protein antigen (deleted proteins or oligopeptides) and new monoclonal antibodies.	9	P	RE
D24	ELISA test for the detection of antibodies to the PPR positive mark.	28	P	RE
D25	ELISA test for the detection of antibodies to the PPR negative mark.	31	P	RE
D26	ELISA test for the specific detection of antibodies to PPRV.	27	P	PU
D27	Serological test results from vaccination with the new PPR marker vaccines (positively and negatively marked and double marked).	46	R	PU
D28	Quantitative performance characteristics of current and newly developed cELISA tests (RP and PPR).	39	R	PU
D29	Guideline documentation for setting up standardised sampling plans for PPR sero-surveillance.	48	R	PU
D30	Geographical mapping of available PPR data at the pan-African, regional and national level.	12	R	PU
D31	Estimated spatial and temporal risk profiles for PPR at the continental level; and at the national levels for the countries included in the project.	24	R	PU
D32	Strategy for strengthening national, regional and global PPR surveillance.	36	R	PU
D33	Development of working hypotheses in relation to control of PPR.	48	R	PU
D34	Development of a web-based for a computerised PPR surveillance system.	48	P	PU
D35	Information on the susceptibility of animal species and breed to PPRV	30	R	PU
D36	Information of the virus strain parameters in the epidemiology of PPR.	24	R	PU
D37	Socio-economic study related to the field application of the current PPR vaccine	15	P	PU
D38	Mathematical model on PPR-spread within the African agro-ecosystems context.	36	P	PU
D39	Establishment of the large-scale basic reproduction number (R0) and Herd Immunity Threshold (HIT).	48	R	PU
D40	Ro (and HIT) mapping per agro-ecosystem.	48	R	PU

7.6. Work package descriptions

Workpackage description (full duration of project) Annex 1

Workpackage number	1	Start date or starting event:				Month: 1	
Activity type	RTD/Innovation activities						
Person-months per participant:		CR6 / 36	CR2/1				

Duration of humoral immune response to RP positive marker vaccine

Objectives

- To establish the duration of immunity / duration of marker response to RP positive marker vaccines that are already available.
- To validate the C-ELISAs developed to detect antibodies to both GFP and flu HA marker proteins incorporated into the marker vaccines.

Description of work

For the experimental vaccinations the standard vaccine dose (10^2 $TCID_{50}$) and 10-fold higher and lower doses will be administered to determine if the vaccine dosage affects the strength of the marker immune responses. Sera from the vaccinated animals will be taken on a weekly basis up to six weeks post vaccination and then on a monthly basis until the completion of the project. At 1 and 2 years post vaccination a sub-group of vaccinated cattle will be challenged with virulent RPV to check that the vaccine is still effective and to look for evidence of the challenge virus using the newly developed discriminatory test systems. All the conventional serological tests, including the virus neutralisation test (VNT), will be carried out on the sera along with the new marker-specific ELISAs.

All the experimental serological test results will be fed into the epidemiological WP-9.

Deliverables

- **D 1** – Establishment of the duration of immunity to the RPV GFPanc and flu HA marker vaccines and the validity of the test assays.

Milestones[11] and expected result

- Establishment of the duration of immunity to the RPV GFPanc and flu HA marker vaccines and the validity of the test assays: results available by month 6, 12, 18, 24 and 36.

Workpackage description (full duration of project) Annex 1

Workpackage number	**2**	**Start date or starting event:**				**Month: 1**	
Activity type	**RTD/Innovation activities**						
Person-months per participant:		**CR2/ 24**	**CR6/18**				

Development of a double marker vaccine for RP

Objectives

To produce several positive and negative candidate marker vaccines for rinderpest with the ultimate aim of combining these to create a single positively and negatively marked RPV vaccine to allow serological identification of vaccinated animals and differentiate them from naturally infected ones.

Description of work

The C1 monoclonal antibody binding site in the RPV H gene will be deleted. If this proves successful then this marker H gene will be used to replace the original RPV H gene and act as a negative marker in the vaccine (RPVΔ-H).

Next, attempts will be made to add tag markers, such as an "HA tag" or a "myc tag", to the C1-deleted RPV H protein gene. Since the C1 monoclonal is the basis of the current H-specific ELISA, a positive and negatively marked vaccine will have been produced with the minimum of foreign genetic material inserted (RPVΔ-H-tag).

If the C1 site cannot be deleted then attempts will be made to delete another immunogenic region of the H protein gene and to insert a tag epitope into another region of the H protein gene (RPV-H-tag).

If all these approaches designed to modify the H gene fail, possibly because of severe structural constraints on the H protein, then the N protein gene of RP will be altered in a similar way to that which may prove successful in the case of the PPR vaccine (see WP-3). The deleted gene segment will be replaced by an epitope tag different from the one inserted into the PPR-ΔN-tag vaccine to facilitate serological differentiation between the two vaccines.

Experimental vaccinations with these vaccines and subsequent challenge of the vaccinated animals with virulent virus will be carried out to test the serological responses in small-scale trials in CR2 laboratory. All the experimental serological test results will be fed into the epidemiological WP-9.

Deliverables

- **D 2** – A negative RPV marker vaccine (RPVΔ-H).
- **D 3** – A positively "tag" marked RPV vaccine (RPV-H-tag).
- **D 4** – A positive and negative RPV marker vaccine (RPVΔ-H-tag).

Milestones[12] and expected result

- A negative RPV marker vaccine (RPVΔ-H): at the end of year 1 it should be possible to say if the strategy to delete the C1 region is feasible.
- A positively "tag" marked RPV vaccine: Concurrently attempts will be made to add a "tag epitope" in a region of the H protein that would not be considered to alter its structure too drastically. Initial results should indicate feasibility at the end of year 1(RPV-H-tag).
- A positively and negatively marked RPV vaccine: During the second year work to make the positive / negative marked vaccine should be undertaken with a result expected by the end of year 3 (RPVΔ-H-tag).
- During the final year extensive studies on the growth characteristics, efficacy and safety of the final choice of negative / positive marker vaccine will be undertaken.

Workpackage description (full duration of project) Annex 1

Workpackage number	**3**	**Start date or starting event:**				**Month: 1**	
Activity type	**RTD/Innovation activities**						
Person-months per participant:		**CO1/40**	**CR3/20**	**CR7/18**	**CR8/26**		

Development of a double marker vaccine for PPR

Objectives

- To introduce a foreign marker gene in the vaccine infectious clone and to delete a non-essential part of the genome in order to develop a double-marker vaccine for PPR which would allow serological identification of vaccinated animals and differentiate them from naturally infected ones

Description of work

Positive marker: to insert an additional gene in the viral genome, the first activity will be to rescue a clone of the vaccine strain of PPR 75-1. Then, between P and M genes, to insert new gene start and stop signals bracketing a short polylinker to act as a gene insertion site. This clone will be used in the subsequent stages for the easy introduction of foreign protein genes. The green fluorescent protein (GFP) has been used to mark the RPV vaccine and the same approach, i.e. by adding a sequence anchoring the marker protein to the cell membrane, will be applied to the GFP protein gene and any other protein gene selected as a marker for PPR vaccine.

Negative marker: mutants of the N protein gene will be obtained by deleting the sequence encompassing a variable area of the gene using PCR mutagenesis. To replace the gap by a foreign sequence of the same length, compatible cohesive ends supplied during the amplification process will be linked with an inert sequence. The functionality of the modified N clones will be tested in a minigenome rescue system using plasmid containing a reporter gene. In this way it will be possible to determine the effect of the gene modifications on the protein functioning before it is incorporated into a full-length PPRV cDNA. In a second step the infectious PPRV clone will be recovered from a cDNA of its genome containing the desired N modifications while also allowing the cloning of the tailored N gene into a positively marked version of the PPRV genome copy.

Experimental vaccinations with these vaccines and subsequent challenge of the vaccinated animals with virulent virus will be carried out by CR8 to test the serological responses in small-scale trials. All the experimental serological test results will be fed into the epidemiological WP-9.

Deliverables

- **D 5** – Deleted N protein tested to support minigenome amplification (functional ΔN)
- **D 6** – A positively marked PPRV vaccine (genome insertion) tested for efficacy
- **D 7** – A negatively marked PPRV vaccine (PPRVΔ-N) tested for efficacy
- **D 8** – A positive (genome insertion) and negative (PPRVΔ-N) marked PPRV vaccine tested for efficacy.
- **D 9** – A positive (N-tag) and negative (PPRVΔ-N) marked PPRV vaccine tested for efficacy.

Milestones[13] and expected result

- Identification of a sequence on N protein dispensable for critical functions of the N (self-association, association to P and L for transcription and replication). Different deleted N proteins are available. It is expected to finish the protein-protein interaction studies which have already been initiated . Therefore it is expected that by month 12 of the project, it will be possible to identify a dispensable sequence on the N protein if it exists.
- A positive PPRV marker vaccine with foreign gene inserted at the P-M junction should be available at month 27: functional PPRV minigenome, and cDNA clones representing the different PPRV genes are available with CO1. The main milestone here will be the assembling of the different CdnA clones to make a full-length copy of the PPRV genome. Expected date for the positive marker vaccine: month 27
- A negatively marked PPRV vaccine (PPRVΔ-N) should be available at month 30 if a dispensable sequence is identified on the N protein by month 12. If such a sequenced has not been identified, then the strategy adopted for RPV negative marked vaccine based on the H protein (see WP-2) will be investigated for PPRV.
- A positive and negative PPR marker vaccine should be available with short time and an efficacy test could be completed by month 42
- During the final year extensive studies on the growth characteristics, efficacy and safety of the final choice of negative / positive marker vaccine will be undertaken

Workpackage description (full duration of project) Annex 1

Workpackage number	4	Start date or starting event:				Month: 1	
Activity type	RTD/Innovation activities						
Person-months per participant:		CO1/15	CR3/5				

Identification of N sequences involved in immunosuppression

Objectives

The N protein, the major protein of the *Morbilliviruses* participates in the virus-induced immunosuppression. Paradoxically, in response to RPV or PPRV infection or vaccination, the host develops a rapid and strong immune response against the protein. The N protein has also significant role in viral replication cycle. The purpose of WP-4 is:

- I*n vitro* assessment of the immunosuppressive role of the N protein of PPRV and analysis of determinants of the protein contributing to this effect using deleted mutants.
- To use a functional assay in order to establish if the deleted sequences are not vital for virus replication. This will help to define the role of particular amino-acid deleted by mutagenesis.

The protein to be used as a model in this study will be the N protein of the PPRV vaccine strain Nigeria 75/1. It is expected that this study will generate fundamental information on the immunosuppression induced during RPV/PPRV infections. These results will help with the development of safer vaccines by deleting the sequence involved in the immunosuppression if it is not vital for the virus growth.

Description of work

Proliferation assays. The inhibitory effect of the N protein and deleted mutants (made available by CR3), on the PHA stimulated proliferation of peripheral blood lymphocytes (PBL) freshly isolated from goats will be examined. The proliferation assay will also be performed on PBL from other species especially of ovine, bovine, porcine and murine origin. Inhibition of cell proliferation of PBL from uninfected animals will be examined using a non-radioactive flow cytometry (FCM) method. To examine this mechanism of suppression in more depth, the *in vitro* measurement of the production of interferon-γ in culture supernatants from stimulated cells will be quantified by capture ELISA (IFNγ).

Plasmid-based replication assay. The plasmid based PPR minireplicon that express a reporter gene, for example the enhanced green fluorescent protein (eGFP) or the chloramphenicol acetyltransferase (CAT), established by CO1 will be used as a template for intracellular replication assays. The activity of the N proteins from the PPR 75-1 vaccine strain, and the deleted mutants, will be evaluated by cotransfection of plasmids carrying each of the protein genes involved in the replication process of the virus, namely the L and the P, and with the plasmid encoding the minigenome. By matching the results obtained with the deleted N mutants, the influence of critical residues situated in these regions will be examined to delineate the minimal functional part of the N protein..

Deliverables

- **D 10** – Identification of amino-acid sequences on the N protein inducing immunosuppression.
- **D 11** – Identification of critical residues on the N protein that are vital for the viral replication.
- **D 12** - N protein with deleted immunosuppressive sequence (possibly a candidate for a negatively marked vaccine)

Milestones[14] and expected result

- Assay construction of functional minigenomes with deleted N proteins: by month 6
- Availability of C-terminal N-PPR deletion mutants or oligopeptides for use in lymphoproliferation tests: by month 12.
- Identification of immunosuppressive regions on the N protein: by month 18 .
- Assessment of the deletion strategy of the immunodominant region: by month 30

Workpackage description (full duration of project) Section B1-Anonymous

Workpackage number	5	Start date or starting event:			Month: 3		
Person-months per participant:		CR6/30	CO1/4	CR2/2	SUB.C/30		

Safety of the recombinant GMO vaccines

Objectives

Marker vaccines are considered as GMOs and an environmental risk assessment is needed to comply with the EU requirements. Cattle and goats will be vaccinated with the RP and PPR marker vaccines:

- To establish the safety of the new vaccines by seeing if the markers alter the tissue tropism of the vaccines.
- To establish the safety of the new GMO vaccines with regard to their potential to contaminate the environment.
- To establish the safety of the new GMO vaccines with regard to their potential to transmit to non-target species that could be exposed to the vaccines and/or the vaccinated animals.

Description of work

Cattle devoid of antibodies to RP will be vaccinated with a standard vaccine dose (10^2 $TCID_{50}$). Sera from the vaccinated animals will be taken on a weekly basis up to six weeks post vaccination. All vaccinated animals will be carefully monitored for unusual clinical signs to ensure vaccine safety. Some cattle will be sacrificed soon after vaccination to monitor the vaccine spread to the different tissues in the animal to ensure that the tissue tropism has not been altered relative to that of the conventional vaccine. Similarly the duration of viraemia and the ability, or inability, of the new vaccines to spread by contact will be determined in contact transmission studies including non-target species. Eye, nose, mouth and anal swabs will be taken at intervals of two days following vaccination to detect virus secretion, both by co-cultivation with cells (B95a cells are highly sensitive to RPV infection), and to look for evidence of virus secretion by sensitive nested RT-PCR assays. Sera from the vaccinated animals will be taken on a weekly basis up to six weeks post infection. The safety features of the new GMOs will be compared with those of the conventional live vaccines.

The same experiment will be carried out on goats with PPR vaccines by the sub-contractor of CO1.

Deliverables

D 13 – Establishment of the vaccines' safety for subsequent field use.

Milestones[15] and expected result

- Outcome of the marked vaccine in the host and possibly shedding into the environment: results expected by month 36.

Workpackage description (full duration of project) Annex 1

Workpackage number	6	Start date or starting event:			Month: 1		
Activity type	RTD/Innovation activities						
Person-months per participant:		CR4/52	CO1/2	CR2/2			

Marker vaccine formulation for improved stability

Objectives

- To develop simple, robust production processes for marker vaccines; to develop formulations enhancing stability and efficacy of marker vaccine for administration by injection or other means.

Description of work

Production of candidate marker vaccines

An appropriate preliminary production set up for double marker vaccines will be performed by partner 4. Testing different parameters such as cell growth, viral infection kinetics and viral harvesting time as well as simple purification processes will define a minimum number of steps to be used for future vaccination.

Formulation: effect of storage upon vaccine stability/efficacy

The aims of this task are to identify the main factors involved in vaccine stability and to improve thermostability at non-freezing temperatures of candidate vaccines by adding cryo- and lyo-protectors. Previous studies (see annex, A1) performed by partner CR4 show that the shelf life of several vaccines, namely candidate adenovirus based rinderpest vaccines, can be increased using stabilizer compounds and different formulation buffers during storage. These compounds include sucrose, or mannose, trehalose or new compatible solutes such as hydroxyectoin and mannosylglycerate patented by partner CR4. Formulations which have proven successful under the previous project will be tested first with the traditional and currently available attenuated PPR vaccine and later with the double marker vaccines obtained from WP-3 (obtained from partner CO1). Lyophilisation will also be evaluated as an alternative strategy to the liquid formulation during storage; although more expensive, lyophilisation might be the most appropriate approach for some climatic and general conditions found in the regions where the vaccines will eventually be used. Optimization of lyophilisation protocols for these particular vaccines will be done (cooling and heating temperature profiles, vacuum profiles and residual humidity content). The effect of storage upon vaccine efficacy will be monitored using traditional viral titration in tissue culture. Stability during storage up to one year will be assessed.

Formulation – route of administration

The formulations developed will be explored for different vaccine delivery routes, in particular muscular injection as well as oral and nasal delivery; for these mucosal deliveries lyophilised powder and nanoencapsulated materials will also be produced for testing in cattle (partner CR2) and sheep and goats (partner CR1).

Deliverables

- **D 14** – Process flow sheet for marker vaccine production (month 24)
- **D 15** – Formulation for injection and oral delivery (month 36)
- **D 16** – Testing for long term storage (up to one year) of marker vaccines – (month 48)

Milestones[16] and expected result

- Simple robust production process established (month 22).
- Injection delivery established (month 28)
- Oral formulation established (month 34)
- Simplified and optimised strategy for storage of vaccines (month 48)

[16] Milestones are control points at which decisions are needed; for example concerning which of several technologies

Workpackage description (full duration of project) Annex 1

Workpackage number	7	**Start date or starting event:**				**Month: 3**	
Activity type	**RTD/Innovation activities**						
Person-months per participant:		**CR2/ 20**	**CR6/18**				

Companion test development for RP marker vaccine

Objectives

- To develop ELISAs specific to the new RPV positive and negative marker vaccines.
- To establish the specificity and sensitivity of the tests for detection of the tag-specific antibodies.
- To validate the new anti-tag assays for distinguishing the three possible categories of animal: vaccinated, wild type virus infected and vaccinated/infected.
- To develop ELISAs to specifically distinguish RPV and PPRV infected animals.

Description of work

Since very avid monoclonal antibodies are commercially available to the various "tag epitope" sequences that we propose to insert into the RPV vaccine, these will be used to develop the necessary ELISAs. The approach will be to use a competitive ELISA format to detect antibody responses to the tag marker epitopes. In order to produce tag antigen to bind to the plates, the tags will be added to a non-viral protein (for example, GFP) and expressed in a bacterial system. The antigens produced will be tested for their reactivity with the tag-specific antibodies. To produce the necessary animal sera to react in the tests the proteins will first be injected into small animals (rats, mice or rabbits) to produce tag-positive sera. Then the tagged marker vaccines (HA-tagged and myc-tagged) produced in WP-2 will later be used to infect experimental cattle in the CR2 laboratory to generate the necessary test sera and to see if they generate the expected immune responses for the initial stages of the validation process.

The RPV-specific region of the N protein of RPV has been expressed in a bacterial expression system. This will be used to develop, in conjunction with partner CO1, various ELISA test formats to specifically distinguish sera induced by infection either of these two viruses.

Deliverables

- **D 17** – Bacterially expressed antigens that have the epitope tags for use in RP serological tests
- **D 18** - Sera containing antibodies to the epitope tags incorporated into the bacterially expressed antigens
- **D 19** – Validation of test to distinguish the 3 categories of vaccinated and infected animal (RP-vaccinated, wild type RP virus infected and vaccinated/infected)
- **D 20** – ELISAs to distinguish virus-specific anti-N antibodies induced by either RPV or PPRV infection of animals.
- **D 21** – Serological test results from vaccinations with the new RP marker vaccines (positively and negatively marked and double marked)

Milestones[17] and expected result

- Bacterially expressed antigens that have the epitope tags for use in serological tests: by month 12.
- Availability of sera containing antibodies to the epitope tags incorporated into the bacterially expressed antigens: by month 16.
- ELISAs to detect antibodies specific to the two types of epitope tag that will be introduced into the RPV vaccine: by month 21.
- ELISAs to specifically anti-N antibodies induced by either RPV of PPRV infection of animals: results by month 27.

[17] Milestones are control points at which decisions are needed; for example concerning which of several technologies

Workpackage description (full duration of project) Annex 1

Workpackage number	8	Start date or starting event:				Month: 1	
Activity type	RTD/Innovation activities						
Person-months per participant:		CR3/ 16	CO1/24	CR7/18	CR8/18		

Companion test development for PPR marker vaccine

Objectives

- To develop ELISAs specific to the new PPRV positive and negative marker vaccines.
- To develop ELISAs to specifically distinguish PPRV and RPV infected animals.

Description of work

The three tests will be developed in the competitive ELISA format adaptable to the different species of ruminants. However, for convenience, an indirect ELISA format will be first tested.

The identification of an immunodominant region on the N protein is a prerequisite to the development of an ELISA specific to the PPR negative marker vaccine. The reactivity of truncated N proteins (partner CR3) and pepscan analysis of overlapping peptides (CO1) will be established with known sera. This information will be also valuable for the development of ELISAs that specifically distinguish PPRV from RPV infected animals.

The positive marker will be selected according to the availability of a specific monoclonal antibody and/or a competitive ELISA. These positive marks will be different from the ones used for RP positive marker vaccines. The gene corresponding to the positive marker antigen will be cloned in an expression plasmid to be used as an antigen in ELISA as already described in WP-7.

Deliverables

- **D 22** – Baculovirus (or bacterial) expressed positive marker protein for use in PPR serological tests.
- **D 23** – New recombinant N-PPRV protein antigen (deleted proteins or oligopeptides) and new monoclonal antibodies.
- **D 24** – ELISA test for the detection of antibodies to the PPR positive mark.
- **D 25** – ELISA test for the detection of antibodies to the PPR negative mark.
- **D 26** – ELISA test for the specific detection of antibodies to PPRV
- **D 27** – Serological test results from vaccination with the new PPR marker vaccines (positively and negatively marked and double marked)

Milestones[18] and expected result

- Selection and expression of positive marker antigen for use in PPR serological tests: by month 9.
- Production of new recombinant N-PPRV antigens (deleted proteins or oligopeptides) and new monoclonal antibodies: by month 9.
- Development of a prototype ELISA test for the detection of antibodies to the PPR positive mark: by month 28.
- Development of a prototype ELISA test for the detection of antibodies to the PPR negative mark: by month 31.
- Development of a prototype ELISA test for the specific detection of antibodies to PPRV: by month 27
- Serological test results from vaccination with the new PPR marker vaccines (positively and negatively marked and double marked): by month 46.
- ELISAs to specifically detect anti-N antibodies induced by either RPV or PPRV infection in animals: by month 24

[18] Milestones are control points at which decisions are needed; for example concerning which of several technologies

Workpackage description (full duration of project) Annex 1

Workpackage number	9		Start date or starting event:				Month: 1	
Activity type	RTD/Innovation activities							
Person-months per participant:	CO1/6	CR2/6	CR3/7	CR5/2.5	CR6/10	CR7/10	CR8/10	CR9/0.5

Evaluation of the tests' performance (RP and PPR)

Objectives :

- To evaluate the performances of the serological tests for improving the confidence in diagnostic data from RP/PPR surveillance and epidemiological studies.

Description of work

Serological tests (VNT, cELISA) developed for RP/PPR are currently used for confirmatory diagnosis.

The test evaluation will be based on both field and experimental serum samples. Clinical and post-mortem data, virology and molecular results collected will be used as a "gold-standard" for test assessment. In addition, advanced modelling methods (e.g. TAGS, generalised mixed modelling) that do not require a reference standard will be used to define test sensitivity and specificity.

The existing databases on diagnostic RP/PPR testing (e.g. CO1, CR2, CR3) which include ELISA, SNT results and reference disease status of the animals will be used to perform ROC analyses. The results will provide information about optimum cut-off values and associated sensitivity and specificity values for the RP/PPR tests to be used in the surveillance framework. Predictive values of given test results will be calculated for RP/PPR using prevalence estimates from the different sub-Saharan African countries. In the case of RP/PPR low prevalence values will have to be assumed, and expected predictive values of positive/negative test results will be calculated for different typical epidemiological scenarios. Analogous studies will be undertaken for the tests developed within this project (WP-7 and WP-8).

Deliverables

- **D 28** – Quantitative performance characteristics of current and newly developed cELISA tests (RP and PPR)
- **D 29** – Guideline documentation for setting up standardised sampling plans for PPR sero-surveillance

Milestones[19] and expected result

Year 1and 2: Compilation of available data and computation of cut-off values, sensitivity (Se) and specificity (Sp) of the existing cELISA tests using ROC analysis.

Year 2 and 3: Data from field surveys carried out with the African partners will be used for the ROC analyses. Publication of more accurate values of Se and Sp (including confidence intervals) than are currently available. ROC analysis on the newly developed tests based on experimental and field data collected during the project

Year 4: Computation of positive and negative predictive values based on calculated test performances characteristics and prevalence estimates from the different countries and production systems. Production of guideline documentation for multistage sampling, taking the diagnostic test performance and the characteristics of the African livestock production systems into account.

Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project

Workpackage description (full duration of project) Annex 1

Workpackage number	10	Start date or starting event:				Month: 1	
Activity type	RTD/Innovation activities						
Person-months per participant:		CR5/4.5	CO1/2	CR7/24	CR8/24		

Spatial epidemiology of PPR

Objectives :

- To develop spatial tools for epidemiosurveillance that will improve the understanding of PPR distribution in sub-Saharan African countries.
- To develop integrated decision-support tools for reinforcing the surveillance of PPR and similar diseases.

Description of work

Data from outbreaks and surveys will be collated from existing surveillance systems and data sources. Additional field surveys will be conducted in the context of this project with the African partners to obtain data for areas where currently only limited data are available. Studies will be carried out at different levels - pan-African, regional, national or provincial – according to the available and newly produced data. Molecular data will be integrated and also analysed with respect to spatial and temporal patterns

Based on this and other spatial and non-spatial information (environmental, animal density etc., as well as expert opinion) a preliminary geographical analysis will be performed with the aim of identifying risk factors which will be later used to generate risk models based upon the data available. "Knowledge-driven" methods (spatial regression analysis, multi-criteria decision making models) will be also used to identify geographical areas that require increased or reduced surveillance efforts.

In addition, the models will be used to generate hypotheses, which can be tested by future research relating to the maintenance of PPR foci and risk of its expanding into new areas.

Data will be integrated using GIS software and made available via the Internet (e.g. ArcIms, ESRI). This web-based information system will allow users to view dynamic maps and conduct tailored queries or select pecific datasets. To reduce the cost resulting from software purchases, the majority of end users will be able to use ArcExplorer, which is a public domain map-viewing tool

Deliverables

- **D 30** – Geographical mapping of available PPR data at the pan-African, regional and national level
- **D 31** – Estimated spatial and temporal risk profiles for PPR at the continental level; and at the national levels for the countries included in the project
- **D 32** – Strategy for strengthening national, regional and global PPR surveillance
- **D 33** – Development of working hypotheses in relation to control of PPR
- **D 34** – Development of a web-based for a computerised PPR surveillance system

Milestones[20] and expected result

Year 1 and 2: Compilation of information from existing datasets. Construction of a standardised database consisting of data on outbreaks and serological results (geo-referenced using accessible locations (e.g. district, village etc.) by means of geographical thesaurus). Investigation for the presence of the disease clusters in time and space, using space-time scan statistic. Analyses will be carried out at pan-African, regional as well as sub-national levels where appropriate. Production of descriptive maps, including spatial probability distribution maps (e.g. kernel smoothing approach).

Year 3: Extension of analyses at pan-African and regional levels, after incorporating census and environmental data. Commencement of field surveys for collection of geo-referenced data about infection and disease occurrence. Integration of active and passive surveillance data for cluster detection analysis at national or provincial level

Year 4: Integration of environmental data and analyses. Development of statistical models for production of risk maps. Validation of distribution maps with independent datasets

Milestones are control points at which decisions are needed; for example concerning which of several technologies will

Workpackage description (full duration of project) Annex 1

Workpackage number	11	Start date or starting event:					Month: 1
Activity type	RTD/Innovation activities						
Person-months per participant:		CO1/6	CR3/4	CR5/3	CR7/40	CR8/40	

Modelling and the dynamic of PPR

Objectives :

To develop a mathematical model for predicting the impact of the vaccination coverage on the disease pattern and on circulation of PPRV strains.

Description of work

Taking into account existing knowledge about PPR and in particular that it is an immunising infection, a mathematical model of the "SEIR" type ("Susceptible-Infected – Infectious-Recovered/Immune") will be developed. It will be a deterministic model that allows prediction of the 'average' expected impact of different control strategies in the animal population.

In SEIR models, the transmission dynamics are expressed in continuous time using differential equations. We will assume that the animals are randomly mixing within a herd and between age levels, but will allow for heterogeneous mixing at the species level (livestock and wildlife) and between herds. The model will be set up using stratification to allow for heterogeneity, and the vaccination coverage will be included in the equations defining the flows between the susceptible and immune compartments.

The required parameters – transition parameters, the birth and mortality rates and the probability of effective contact between infectious and susceptible individuals per unit time (beta parameters) will be determined from field data (cross-sectional survey) and experimental reproduction (inoculation of PPRV strains in the different susceptible species; all the serological test results will be fed into the epidemiological WP-9). The beta parameters have to be stratified so that non-random mixing between species and herds can be accommodated in the model. The beta values will be obtained by estimating the strength of infection using seroprevalence data (catalytic modelling) and computing with WAIFW ("Whom Acquire Infection From Who") matrix equations.

The most important purpose is to establish the Ro. Ro is the "basic reproduction number", which is the "average number of secondary infectious cases resulting from each infectious case following its introduction into a totally susceptible population". Ro determines the rates at which a disease will spread through a population, the equilibrium level of infection, the age at which hosts acquire infection and the level of effort required to control the disease (Herd Immunity Threshold, HIT). Separate Ro have to be computed for each agro-ecosystems taking into account the different PPRV strains.

The development of a model is an iterative process, which requires permanent interactions between field work and development work.

T evaluate the socio-economic impact of vaccination with the current PPR vaccine, required economic indicators determined in the African agro-ecosystem will be as follow: the animal production, the meet consumption, the incomes of the production, the added value to the rural sector, to the balance of trade and the final food balance sheet. The sociologic analysis principally focused on the fitting of the animal husbandry in the production system requires criterions of the social impact: improved nutrition, an increase of employment and a better distribution of the incomes.

Deliverables

- **D 35** – Information on the susceptibility of the tested animal species and breed to PPRV
- **D 36** – Information of the virus strain parameters in the epidemiology of PPR
- **D 37** – Socio-economic study related to the field application of the current PPR vaccine
- **D 38** – Mathematical model on PPR-spread within the African agro-ecosystems context
- **D 39** – Establishment of the large-scale basic reproduction number (R0) and Herd Immunity Threshold (HIT)
- **D 40** – Ro (and HIT) mapping per agro-ecosystem

Milestones[21] and expected result

Year 1:
Compilation of literature data on prevalence surveys conducted in various areas prior to vaccination and first estimation of the model-input parameters
Implementation of cross-sectional survey by CR7 and CR8 (One site in each country).
Setting up of a preliminary mathematical model to be challenged with field data:
In-station inoculation of PPRV strains (sheep, goats and cattle):
- Cold dry season, test in goats only with 3 strains of lineage 1 and 2 and isolated from goats to select 2 strains with different degrees in virulence (CR8)
- Beginning of rainy season: test with a high virulence strain of lineage 1 or 2 selected in the preceding experiment. Test on sheep, goats and cattle (CR8)
- Cold season, test with a strain of lineage 3 isolated from goat (CR7). Test on sheep, goats and cattle.

Collection in west African countries of the socio-economic indicators that define the impact of the current PPR vaccine.
Socio-economic study related to the field application of the vaccine.

Year 2:
In-station inoculation of PPRV strains (sheep, goats and cattle):
- Cold dry season: Repetition of experiment carried out by CR8 in rainy season in the first year with the same virus (determination of the season parameter).
- Cold dry season: test with a strain of lineage 3 isolated from sheep to study the virus origin parameter (CR7). Test on sheep, goats and cattle
- Rainy season: test on goats only to study the rate of the virus diffusion in a susceptible population (highly virulent strain) CR8.

Field cross-sectional surveys – second site in each country: CR7 and CR8

Year 3 and 4:
Rainy season: test only on goats to study the rate of the virus diffusion in susceptible population (low virulent strain) CR8
Data analyses
Model validation with independent datasets
Prediction and optimisation of the model
Advanced-model transfer for decision making

Milestones are control points at which decisions are needed; for example concerning which of several technologies will

'roject resource and budget overview

8.1. Efforts for the full duration of the project

STREP Project Effort Form –full duration of the project

RP/PPR MARKVAC

	CIRAD		IAH	FAO/IAEA	IBET	RVC	KARI	LVC	NVI	AU/IBAR	TOTAL PARTNERS
Research/innovation activities	CO1	N AHRC Sub-contr.	CR2	CR3	CR4	CR5	CR6	CR7	CR8	CR9	
WP – 1			1				36				37
WP – 2			24				18				42
WP – 3	40			20				18	26		104
WP – 4	15			5							20
WP – 5	4	30	2				30				66
WP – 6	2		2		52						56
WP – 7			20				18				38
WP – 8	24			16				18	18		76
WP – 9	6		6	7		2.5	10	10	10	0.5	52
WP – 10	2					4.5		24	24		54.5
WP – 11	6			4		3		40	40		93
Total research/innovation	99	30	55	52	52	10	112	110	118	0.5	638.5

8.2 Overall budget for the full duration of the project

Form A3.1 and A3.2 from CDFs and detailed description of the budget, A3.3 (three pages).

osal Number	*003670*	*Proposal Acronym*	*RP/PPR MARKVAC*

Financial Information (Specific Targeted Project)								
		RTDor innovation-relate activities		*Demonstration a*		*Management activities*		*Total*	
i	*Cost model*	*Costs*	*Requested grant to the budget*	*Costs*	*Requested grant to the budget*	*Costs*	*Requested grant to the budget*	*Costs*	*Requested grant to the budget*
	FCF	596 650	298 325	0	0	17 500	17 500	614 150	315 825
	AC	199 175	199 175	0	0	7 500	7 500	206 675	206 675
	AC	107 000	107 000	0	0	0	0	107 000	107 000
	AC	116 000	116 000	0	0	0	0	116 000	116 000
	AC	50 000	50 000	0	0	0	0	50 000	50 000
	AC	230 250	230 250	0	0	6 000	6000	236 250	236 250
	AC	232 000	232 000	0	0	0	0	232 000	232 000
	AC	236 250	236 250	0	0	0	0	236 250	236 250
	AC	34 500	0	0	0	7 500	0	42 000	0
Total		1 801 825	1 469 000	0	0	38 500	31 000		1 500 000

e use additional copies of form A3 if the number of lines is not sufficient

osal Number	003670	Proposal Acronym	RP/PPR MARKVAC

al Information (Specific Targeted Project) part ½

pant n°	Number of person/months	Personnel Costs	Durable Equipment	Laboratory and Animal Consumables	Travel and Subsistence	Other Specific Project Costs	Receipts (contribution in kind)	Subcontracting	Partners' management activities	Subtotal part 1/2
O1	120	348 396	2 100	107 330	17 150	0	/	30 000		504 976
anageme ctivities	9	17 500	/	/	/	/	/			17 500
al co-inator osts	129*	365 896	2 100	107 330	17 150	0	Trainee***	30 000		522 476
R2	55	118 145	/	44 000	6 000	0	Trainee***	/	7 500	175 645
R3	52	36 000	/	57 270	7 750	0	Trainee***	/	0	101 020
R4	52**	43 200	/	47 400	8 000	0	/	/	0	98 600
R5	10	30 215	/	5 000	8 000	0	/	/	0	43 215
R6	112	11 513	25 500	71 800	6 000	80 000***	/	/	6 000	200 813
R7	110	10 000	39 200	65 000	8 000	75 000***	/	/	0	197 200
R8	118	11 513	37 300	60 000	12 000	80 000***	/	/	0	200 813
R9	0.5	23 800	0	0	4 400	0	/	/	7 500	35 700
Total	638.5	650 282	104 100	457 800	77 300	235 000	/	30 000	21 000	1575 482

nprising 30 pm from the sub-contractor
which only 30 pm are requested from EC
vers the research training in Europe of a PhD student (see description in the technical Annex, from page 50)

osal Number	003670	Proposal Acronym	RP/PPR MARKVAC

ipant n°	Subtotal of part ½	Computing	Protection of Knowledge	Overhead costs	Total Costs	Cost Basis: FCF/AC	% Requested from the Community	Requested Contribution from the Community	Advance payment
CO1	504 976	0	0	91 674	596 650		50	**298 325**	/
anagement tivities	17 500	0	0		17 500		100	**17 500**	/
tal co-ator costs	522 476	0	0	91 674	**614 150**	FCF	/	**315 825**	/
CR2	175 645	0	0	31 030	**206 675**	AC	100	**206 675**	/
CR3	101 020	0	0	5 980	**107 000**	AC	100	**107 000**	/
CR4	98 600	0	0	17 400	**116 000**	AC	100	**116 000**	/
CR5	43 215	0	0	6 785	**50 000**	AC	100	**50 000**	/
CR6	200 813	0	0	35 437	**236 250**	AC	100	**236 250**	/
CR7	197 200	0	0	34 800	**232 000**	AC	100	**232 000**	/
CR8	200 813	0	0	35 437	**236 250**	AC	100	**236 250**	/
CR9	35 700	0	0	6 300	**42 000**	AC	0	**0**	/
b-) Total	1 575 482	0	0	264 843	**1 840 325**			**1 500 000**	

8.3. Management level description of resources and budget. Description of each participating organisation

- **Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Département d'Elevage et de Médecine Vétérinaire Tropicale (CIRAD-EMVT) – Partner C01**

Dr Geneviève LIBEAU, CIRAD-EMVT - Programme santé Animale - TA 30/G - Campus International de Baillarguet – 34398 Montpellier Cedex 5.

Involvement in the project

CIRAD-EMVT will be leader of WP-3 and WP-4, which deal with the development of a double marker vaccine for PPR and its associated diagnostic tests. In addition CIRAD will input into WP-6, WP-8 and WP-10. On the present proposal CIRAD will also leader of the work packages WP-9 and WP-11 devoted to the epidemiology of RP and PPR.

Ability of the research team to execute the proposed project

CIRAD-EMVT is a French public Research Institute. CIRAD scientists have contributed to scientific knowledge on both diseases. In the recent years, the Virology team has carried out several projects aiming at producing and testing recombinant vaccines against PPR virus. The group has also expertise in recombinant DNA technology (baculovirus, capripox and adenovirus vectors) and reverse genetics. The group has been involved in the development and transfer of diagnostic tools, which are used for virus identification and for serological analysis and has established a sequence database to study the phylogenetic relationship between PPRV isolates. It is heavily involved in the epidemiological study of other tropical diseases, including PPR. The extensive experience in these scientific fields for RP and PPR study will ensure that the best possible conditions for carrying out this type of work will be made available for the project. Facilities include laboratories furnished with all the necessary equipment needed to carry out up to date molecular research, animal experimentation facilities and a P3-level laboratory for handling live exotic pathogens.

The CIRAD's own resources that are used in the project are as follow:

- personal attributed: 3 researchers (total 28 pm), two technicians (40pm), entirely paid by CIRAD.
- other estimated technical support (10pm)
- equipment used: 40 000€.
- administrative and finance (20pm)

Scientific and management team

- Dr Geneviève Libeau (M.Sc, PhD senior scientist): co-ordination of the proposed project and responsible of the research on the diagnostic and on the molecular work.
- Dr François Roger (DVM, PhD): co-ordination of the epidemiological work, supervision of animal studies in Africa
- Dr Emmanuel Albina (DVM, PhD, head of the Virology group): supervision of the reverse genetic work.
- Miss Cécile Minet, technician: reverse genetics work.
- Mr Olivier Kwiatek, technician: molecular biology and diagnostic tool development.
- A Technician specially appointed to work full time on the project: help with reverse genetics, molecular biological and immunological work.
- A PhD student from Ethiopia hosted by CIRAD: development of the PPR marker vaccines and its companion diagnostic test.
- A PhD European student appointed by a Marie-Curie training application: ROC and probabilistic approach to evaluate the performance of the RP/PPR tests.

- Institute for Animal Health (IAH) – Partner CR2

Prof. Tom Barrett - Morbillivirus group - Pirbright laboratory - Surrey GU24 ONF – UK.

Involvement in the project

The Institute of Animal health will be leader of WP-2 and WP-7, which deal with the development of a double marker vaccine for RP and its associated diagnostic tests. In addition he will input into WP-1 and WP-6. Dr. John Anderson and members of his group in the World reference laboratory for RP will be responsible of developing and testing the various ELISAs for the marker RP vaccine in WP-7.

Ability of the research team to execute the proposed project

The Morbillivirus group at the IAH, Pirbright, headed by Prof. Barrett (M.Sc., Ph.D.), is carrying out extensive research to determine the molecular basis of RP virus pathogenicity using reverse genetics and is also heavily involved in developing and testing recombinant vaccines for RP and PPR. Other aspects of his work include molecular epidemiological studies on RP, PPR and on various other morbilliviruses found in wildlife, including those found in marine mammals. In collaboration with the World reference Laboratory for RP and PPR at Pirbright, the group has developed molecular diagnostic techniques for these viruses and established a sequence database to study the phylogenetic relationship between different virus isolates.

The Laboratory is furnished with all the necessary equipment to carry out modern molecular biological research. In addition, the Pirbright laboratory is unique in Europe in having extensive animal facilities enabling vaccination and challenge experiments on large numbers of domestic farm animals (cattle, sheep, goat and pigs).

The IAH's own resources that are used in the project are as follow:

- personal attributed: 2 researchers (total 16 pm), two technicians (23pm), entirely paid by IAH.
- other estimated technical support (10pm)
- estimation of the equipment used: 40 000€.
- administrative and finance (3pm)

Scientific and management team

- Prof. Barrett (head of the Morbillivirus Group): co-ordination of the research and supervision of the animal experiments.
- Dr Anderson (head of the World reference Laboratory for RP and PPR): supervision for the development and validation of ELISA tests.
- Mr Dalan Bailey, currently a Ph.D student.
- Mr Daniel Rayner: designated technician appointed to the project.
- Two animal technicians for the animal experiment allocated on the project.
- A PhD student from Kenya hosted by IAH: development of the RP marker vaccines and its companion diagnostic test.

- **Joint FAO/IAEA Division – Partner CR3**

Dr Adama Diallo – Animal Health Product Unit – FAO/IAEA Agriculture and Biotechnology Laboratory – IAEA Laboratories – A – 2444 Seibersdorf – AUSTRIA.

Involvement in the project

The FAO/IAEA Joint division will be responsible for WP-8 (Companion test development for PPR marker vaccine) and will contribute to WP-3, WP-4, WP-9, WP-11 (Development of the double marker vaccine for PPR; identification of sequences involved in immunosuppression and test development and epidemiology of PPR).

Ability of the research team to execute the proposed project

The FAO/IAEA Joint Division is carrying out extensive research to determine the biological function of the PPR viral proteins. It is also heavily involved in developing and validating a variety of diagnostic tools and others are currently being validated. Dr Adama Diallo is a world-renowned expert in the field of PPR and contributed greatly to our knowledge of the disease. He was responsible for the development of specific diagnostic tests and development recombinant vaccines against PPR virus, especially of capripox-based PPR recombinant vaccines. In Seibersdorf Laboratories, the routine operation of an external quality assurance by enabling test comparison between different countries and regions of Asia and Africa will be an important component of the validation of diagnostic tools to be developed/validated within this project. The extensive experience in the field of RP and PPR molecular biology and in the approach of standardisation of assays must be considered a key element for the success of the project.

The FAO/IAEA's own resources that are used in the project are as follow:

- personal attributed: 1 researchers (total 5 pm) 90% paid by FAO/IAEA, two technicians (40pm), researcher 90% paid by FAO/IAEA.
- other estimated technical support (5pm)
- estimation of the equipment used: 35 000€.
- administrative and finance (3pm)

Scientific and management team

- Dr Adama Diallo (DVM, PhD): co-ordination of the molecular biology research and diagnostic tool development.
- Dr Mamadou Lelenta, DVM: development of PPR ELISA tests, co-ordination of the test evaluation with other partners in Africa.
- A technician specially appointed to the project: molecular biologist, working under the supervision of Dr Adama Diallo, on the development of the PPR negative marker vaccine.
- A PhD student from Mali will be hosted by IAEA for the development of the PPR diagnostic test.

- **Instituto de Biologia Experimental e Technologia (IBET) – Partner CR4**

Dr Paula Alves – Animal Cell Biotechnol Lab - Av. da República – EAN – Qta. do Marquês – Oeiras – PORTUGAL.

Involvement in the project

The Animal Cell Biotechnology Laboratory at IBET will be leader of WP-6 (Marker vaccine formulation for improved stability) where a robust production process for marker vaccines will be developed, new formulations evaluated and vaccine stability/efficacy during storage assessed. The formulations to stabilise the current RP and PPR vaccines will be first developed then later the marker vaccines.

Ability of the research team to execute the proposed project

IBET, under Prof. Manuel J.T. Carrondo, are very active in animal cell technology processes for biomedical applications. IBET showed from their earlier work on virus production that they can optimise the kinetics of infection and viral degradation. Previous work has shown that the final optimisation of the culture system is essentially dependent upon the specific product and affects directly its quality. The final formulation of the product, also studied at IBET, depends upon the specific product characteristics and on its final application. IBET is thus in a perfect position for the production of the vaccines and for quality control making the jump into a larger programme for control faster and cheaper.

The IBET's own resources that are used in the project are as follow:

- personal attributed: 3 researchers (total 11 pm) entirely paid by IBET, one technician (10pm), 70% paid by IBET.
- other estimated technical support (10pm)
- equipment used: 25 000€.
- administrative and finance (3pm)

Scientific and management team

- Prof. Carrondo (Head of Biochem Eng. Group): supervision of the Animal Cell Technology Group.
- Dr Paula Alves (PhD): co-ordination of the research on vaccine production and purification.
- Dr Pedro Cruz (PhD): participation in WP-6 for the formulation and optimisation of bioprocesses steps.
- C. Peixoto (laboratory technician): carrying out the product evaluation and characterisation.
- One PhD Student will assist them for cell growth, virus infection, production and purification and checking vaccine stability and efficacy during storage.

- **Royal Veterinary College (RVC) - Partner CR5**

Prof. Dirk Pfeiffer - University of London - Hawkshead Lan - North Mymms - AL9 7TA, UK

Involvement in the project

The RVC will be leader of the following WP:

- WP-10 that assesses the performance of the new system management tools coupled with diagnostic tools and spatial epidemiology in improving surveillance and control measures.

And will collaborate in the two followings:

- WP-9 in which will be addressed the validation of the diagnostic tests to provide information on their performances in term of their epidemiological usefulness;

- WP-11 will give a modelling approach for PPR to establish optimum vaccination strategy based on data obtained through the integration of epidemiological studies.

Ability of the research team to execute the proposed project

The Epidemiology Division within the RVC currently consists of 3 academic staff members, and in the research domain sees its strength particularly in the epidemiological investigation of spatial aspects of disease patterns, and assessment of diagnostic tests. Current activities include projects concerning *M.bovis* in cattle, risk analysis and modelling, spatial analysis of animal health data and design of animal health programmes at farm and national level. The experience in these fields applied to RP and PPR will ensure that the best possible conditions for carrying out this type of work will be made available for the project.

Scientific and management team

- Prof. Dirk Pfeiffer: co-ordination of the research on epidemiology.
- One PhD Student will work on the modelling approach of PPR epidemiology.

- **Kenyan Agriculture Research Institute, (NVRC) Muguga – Partner CR6**

Dr Rosemary N. Ngotho – P.O. Box 32, Kikuyu

Involvement in the project

The KARI will be leader of the workpackages WP-1 (Duration of the immunity against the RP marker vaccines) and will be also involved in WP-9 devoted to the evaluation of the test's performance.

Ability of the research team to execute the proposed project

The Division of Virus Research at NVRC Muguga, headed by Dr Henry Wamwayi, has a long history of carrying out research on rinderpest and related diseases. In recent years, the NVRC team has carried out four large-scale recombinant vaccine trials and has sufficient experience in conducting such trials. The team has also conducted a large number of experiments to study the pathogenicity and transmission of a number of viral diseases including RP, malignant catarrhal fever (MCF) and lumpy skin disease. NVRC has also collaborative links with other institutions carrying out related work and is thus able to draw on expertise it may not have among its own staff. The Center is thus well suited to carry out its role under this project.

Scientific and management team

- Henry Wamwayi, DVM, MSc, PhD (Virology): supervision of the Virology group in KARI.

- Dr Rosemary N. Ngotho, DVM, Msc (Epidemiology and economics): will co-ordinate the research at the level of her group. She will be seconded by

- Dr Eunice K. Ndungu, DVM, MSc: (Bacteriology): participation to the different WP.

- **Laboratoire Central Vétérinaire (LCV) - Partner CR7**

Dr Oumou Sangaré - P.O. Box - Bamako – MALI

Involvement in the project

LCV will participate to the work packages WP-9 devoted to the evaluation of the test's performance and will be also involved in WP-10 and WP-11: Spatial epidemiology of PPR, Modelling and the dynamic of PPR.

Ability of the research team to execute the proposed project

LCV is a public institution devoted to veterinary vaccine production diagnosis and research on animal health for Mali. In the housing facilities that enable experimental infection of sheep, goat and cattle, LCV has conducted experimental infection of small ruminants with PPR. As well in the recent past, cattle were naturally infected by CBPP for a previous INCO project devoted to the study of the immune responses. The facilities include virology laboratories which are equipped with up to date equipment (centrifuges, ELISA readers, laminar flow cabinets, CO2 incubators, microscopes, PCR equipment...) thanks to a previous INCO project. A specific standby generator and water reservoir ensures that the laboratory does not suffer from any power or water cuts.

Scientific and management team

- Dr Oumou Sangaré, DVM: co-ordinate the research at the level of her group.
- She will be seconded by a DVM, MSc (Virology).

• **National Veterinary Institute (NVI) – Partner CR8**

Dr. Berhe Gebreegziabher - P.O. Box 19 - Debre-Zeit - ETHIOPIA.

Involvement in the project

The NVI will be involved in WP-3 (development of a double marker vaccine), WP-9, WP-10 and WP-11 related to the evaluation of tests, spatial epidemiology and modelling of PPR).

Ability of the research team to execute the proposed project

NVI has many years of accumulated experience in conducting collaborative research activities mainly with the French research centre, CIRAD-EMVT. Dr Berhe Gebreegziabher, now at the head of the NVI has worked in the area of vaccinology, and has strongly contributed to the development of recombinant vaccine development (PPR-capripox recombinant vaccine) and DNA vaccine manipulation as part of his Masters and PhD research work.

NVI has implemented the required and internationally accepted laboratory working practices with proper GMP. It has also large and small laboratory animal accommodation and procedures for proper handling of dangerous pathogens under safe conditions. Regarding the manpower status, the Institute has about 11 veterinarians and more than 26 laboratory technicians at different levels of research experience. The laboratories are equipped with materials and equipment necessary for the project. This infrastructure was established on an area of about 30 hectares of land, side by side with the Veterinary Institute, Addis Ababa University. The availability of the latest laboratory equipment will facilitate the adoption of new technologies. The NVI erected several standby generators for power supply independence and established its own water supply. Moreover, new laboratory animal breeding and quarantine facilities, with high security accommodation were constructed.

Scientific and management team

- Dr Berhe Gebreegziabher (Director): co-ordination of the research at the level of the NVI.
- Dr Fekadu Kebede (DVM), will be the manager of the scientific team
- Dr Aschelew Zeleke (DVM), will assist them for the animal experiments.
- Mrs. Martha Yami (Quality Control laboratory head): collaboration for animal experiments, serology and test evaluation.
- One technician, Mr Berhanu Beyene will assist them.
- One PhD Student and Lab technicians will assist them.
- Two animal technicians for the animal experiment allocated on the project.

- **Inter-African Bureau for Animal Resources of the African Union (AU/IBAR) – Parner CR9**

Dr Gavin Thomson - P.O. Box 30786 - Nairobi – KENYA

Involvement in the project

AU/IBAR will be involved in WP-9 (Evaluation of tests' performance).

Ability of the research team to execute the proposed project

PACE is associated with the project as it is a key player concerned with epidemiological studies and control strategies for RP and PPR in Africa. An Epidemiology unit, including a wildlife component, is at the heart of the PACE Programme. Three subunits deal with the situation regarding RP and other major epizootic diseases. One is located in Nairobi and deals with the area where RP is still endemic Another is in Bamako for countries of West and Central Africa that have ceased ant-RP vaccinations and embarked on the OIE pathway towards official recognition of RP-free status. The third is in N'djamena and is responsible for monitoring the Western ex-cordon sanitaire, which protected West Africa against possible re-infection from East Africa.

The PACE programme is consisting of the epidemiological unit (PEU) and the Community-Based Animal Health and Participatory Epidemiology Unit (PACE-CAPE). The specific contributions of the AU-IBAR PACE to the project will be the following: because it has the mandate from the Heads of State and Government of AU member countries, it is the only institution in Africa able to liaise with appropriate authorities of member states, regional groups, inter-governmental and international organisations. Accordingly the PEU from AU-IBAR with the African partners, Ethiopia, Mali and Kenya will be of great help for collecting, collating, and disseminating information on the aspects of RP and PPR. The PACE programme with the African partners will play a key role since they will provide a great deal of information about the epidemiological patterns of RP/PPR-disease and infection.

Scientific and management team

The technical staff of epidemiology Unit comprises:

- Dr Gavin Thomson, DVM, PhD: Main epidemiologist:
- Dr Bidjeh Kebkiba, DVM, Msc, PhD: Counterpart of the main epidemiologist;
- Dr Karim Tounkara, DVM, M.Sc, PhD: laboratory expert;
- Dr Gijs Van't Klooster, DVM: epidemiologist for East Africa, CIRAD contract of employment;
- Dr Fatah Bendali, DVM, PhD: Epidemiologist for Central Africa, CIRAD contract of employment;
- Dr Richad Kock, DVM: Wildlife experts, CIRAD contract of employment.

9. Ethical issues

The present project will develop genetically modified organisms (GMOs) by reverse genetics. In Europe, GMOs constitute a public concern because they are believed to represent a risk to the environment or human health when they are cultivated or incorporated into human or animal food. On the other hand, the potential benefits of these GMOs are not fully understood or appreciated by a part of the population. GMOs designed for use in human medicine, for example vectors for gene therapy, are considered to be more acceptable to the public. The recent successful use of recombinant adenovirus for gene therapy in human has engendered great hopes for the control of genetic diseases for which there is no other treatment available at present. Although efficacious, these GMOs may, however, have shown side-effects several months after their administration, thus leading to a provisional moratorium on their use and again this generates a collective fear and suspicion of these promising medical tools. This illustrates the next big challenge for science that is to increase our efforts to ensure the safety of medical GMOs. Safety issues regarding live attenuated vaccines are a major concern as well. Many scientific teams have proven the usefulness of GMO vaccines for the induction of an immune response that can protect against disease, however, only in a few cases have they addressed the question of safety.

Establishing the safety together with the demonstration of efficacy of the new RP and PPR GMO vaccines will be part of the work carried out under this project. Several of the safety issues raised in different European documents and regulations will be addressed by *in vitro* and *in vivo* experiments. The aim is to show by preliminary studies that GMOs obtained as outputs of the present project will have no detrimental effect on the animal's health and will not be capable of transmission to non-vaccinated animals. In particular we will focus on the transient immunosuppression with the aim of modifying this effect by deleting the genetic determinants responsible and thus reduce further the impact on the vaccinated animal's health. The main European documents that will be taken into account are:

- Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro-organisms,
- Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC

It must be first stressed that GMOs produced within this project will be negative-sense RNA viruses. These viruses do not replicate through DNA intermediates, so that integration of their genomes into the host cell genome is a very remote possibility.

Secondly, these GMOs will be derived from well-established live attenuated vaccines. These vaccines induce a long-lasting immunity and they have been used for many years with no evidence of reversion to virulence. In addition, it was clearly demonstrated that the vaccine viruses are not excreted following vaccination. These vaccines do not induce any significant side-effects, apart from a mild and transient immunosuppression after vaccination. A work-package in this project will focus on the genetic determinants responsible of this transient immunosuppression. Theses results will be taken into account for the development by reverse genetics of the new RP and PPR GMO .

The transgenes that will be used for the positive marker in our GMOs will be carefully selected to avoid any possibility of conferring a new biological advantage to the marker vaccine. For instance, the transgene will not encode a known micro-organism receptor or any protein that could be integrated in the virus structure. In other words, the positive marker should be a protein only expressed in the infected cells and which is not incorporated into the virion.

Once these criteria have been fulfilled, the project will implement *in vitro* studies to check the safety of the new GMOs generated in comparison with the conventional live attenuated vaccines. By these means we will ensure that the new vaccines will not cause any additional sanitary problems in the vaccinated population, particularly in the developing countries where animals are naturally exposed to

In addition, for the whole project, animal experiments (vaccination trials) in Europe and in Africa will be conducted in compliance with national and international regulations regarding ethical and safety provisions applicable in the countries in which the research will be conducted. This concerns mainly the conditions under which the GMOs are manipulated and the protection of vertebrates used for scientific experiments. For instance, in the country of the project coordinator, the use of GMOs requires an official agreement of the "Commission du Génie Biologique". Animal trials are only carried out in authorised animal facilities by authorised people (both are subject to an agreement from the Ministry of Agriculture).

The project will involve the use of goats and cattle (the target hosts for the final product). The modified vaccines will be used for vaccination experiments in secure laboratories. Measures will be taken to avoid accidental release of GMO and pathogenic strains (disinfecting work areas, infected material being autoclaved, etc..). In all partner's laboratories, the protocols will be designed and the work will be processed according to the same European directives and good laboratory practices (OECD principles on good laboratory practice, ENV/MC/CHEM(98)17, unclassified).

A. Research activities related to ethically sensitive issues

Does the proposed research raise sensitive ethical questions related to:	YES	NO
• Human beings		X
• Human biological samples		X
• Personal data (whether identified by name or not)		X
• Genetic information		X
• Animals	X	

B. The proposed research does not involve:

- Research activity aimed at human cloning for reproductive purposes,
- Research activity intended to modify the genetic heritage of human beings which could make such changes heritable[1].
- Research activity intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer;
- Research involving the use of human embryos or embryonic stem cells with the exception of banked or isolated human embryonic stem cells in culture[22].
- Research involving the use of non-human primates;
- Research activity intended to create genetic modification of animals;
- Research activity intended to create genetic modification of plants.

C. The proposed research does involve:

- Research involving the use of transgenic animals*,
- Research involving the use of other animals.

* Expression plasmids transferred to animals via bacteria will lead to transient somatic transgenosis of the host.

[22] Applicants should note that the Council and the Commission have agreed that detailed implementing provisions concerning research activities involving the use of human embryos and human embryonic stem cells which may be funded under the 6th Framework Programme shall be established by 31 December 2003. The Commission has stated that, during that period and pending establishment of the detailed implementing

10. Other issues

In many countries, women's participation in planning and decision-making process in social, economic and political aspects of life is still minimal. For this reason, a gender and development approach has brought to the fore some issues related to social roles and responsibilities of women and men and their access to control over resources. In the last three decades, the EU has been at the forefront of efforts to promote the status of women. The proportion of women students obtaining a diploma in science and in engineering is increasing in EU. In 2002, women graduates represented 41% of those receiving diplomas in science, mathematics and informatics in the EU. Evidently women represent a significant reserve fund for recruitment. However, they stand for only one third of the researchers in universities and in public institutions and the situation is worse in the private sector. Indeed only 15% of the researchers employed in industry in EU are women. In the member states the proportion varies from 9% (Germany) to 28.2% (Ireland).

The gap between human capital investment in men and women is highest in low-income regions, which currently invest the least in education of women compared to men. Gender bias persists in Africa. Although efforts have been made to close the gap with respect to gender disparities, the continent still lags behind the rest of the developing world in reducing the gap (*60*). However, in Africa, contrary to Europe, gender inequity is due to a difference in access to education with a primary school enrolment rate higher for boys. Fortunately, we can observe a narrowing of this gap with time.

The proposed research does involve:
A total of 21 scientists.,

Women in an overall proportion of 41%,
A proportion of 27% of women for the European partners,
A proportion of 71% of women for the African partners ,
The co-ordinator is a woman and three women are leaders of the partner's staff. In addition, women will lead 5 out of 11 workpackages (45%).

Appendix A- Consortium description

A.1 Participants and consortium

a) Description of the consortium

The consortium consists of 5 European partners, 4 African partners and one African sub-contrator. For its general and long-standing expertise in the field, as well as for the number of staff devoted to the project, CIRAD-EMVT will be the co-ordinator. All partners, with the exception of one, have strong national and international commitments with the diagnostic and/or vaccine development and in the control of RP and PPR. However, the exception, The RVC-London holds a leading international position in spatial disease epidemiology and modelling. The main areas involving the expertise of the consortium are defined as follows: **development of marker vaccines, assessing their safety issues, and improving the effectiveness of surveillance and decision-making tools for epidemiologists.** The ultimate objective is an improved control of RP and PPR. The scientific approach in each discipline is based on up-to date or innovative methodologies. According to the specific expertise of individual staff members, CIRAD will be the link between these different disciplines. By including two partners (AU-IBAR and RVC-London) with epidemiology expertise a new dimension will be brought to the overall project. By strengthening the scientific relationships between laboratories in European and Developing Countries the project will allow partners in the South to have access to international research and enable them to work in less isolation. Finally, the project will have a positive impact on all the institutions involved by strengthening their scientific programmes and their research capacities.

Among the European partners, the joint FAO/AIEA Division, located in Austria has been providing diagnostic support to the RP eradication programme in Africa (PARC) from 1988 to 2000 and since then to the GREP. It has contributed extensively to RP control by facilitating technology transfer and the use and distribution of diagnostic tools in the national veterinary laboratories sited in the endemic areas of RPV and PPRV and more particularly in Africa. The Animal Production Unit Head appointed in 2001 at the FAO/IAEA joint Division has been involved in the development of different PPRV vaccines, homologous attenuated and recombinant vaccines, since 1985. IAH in Pirbright (UK) is the FAO World reference laboratory for RP. The laboratory plays a leading role in vaccine development and has pioneered the use of reverse genetics for animal morbilliviruses while CIRAD-EMVT is well known for its research on PPR, recombinant vaccine and diagnostic tool development. CIRAD-EMVT is an FAO reference laboratory for PPR. IBET, in Portugal has long experience in controlled release formulation using bioprocesses for the development of pharmaceutical final product formulations. Therefore the expertise of scientists from CIRAD, IAH, IBET and FAO/IAEA joint Division complement each other with respect to the different aspects of vaccine development, formulation for field use and quality assurance. Since 1988, the FAO/IAEA joint Division has been collaborating with IAH and CIRAD in the development, validation and transfer of animal disease diagnostic technology. The association with the 3 African laboratories (KARI, Kenya, LCV, Mali and NVI, Ethiopia) with the development of the marker vaccines and the validation of the diagnostic tools will greatly improve their research expertise and capabilities. KARI in Kenya is one of the OIE reference laboratories for RP/PPR and also one of the three laboratories selected by PACE as regional reference laboratories in Africa. Ethiopia is already associated with CIRAD-EMVT and IAH on a RP/PPR project supported by the EU or directly by France with bilateral development funds. The Laboratory of NVI has for a long time been the home of the PANVAC, a FAO laboratory for veterinary vaccine control for Africa. Thus, these two African laboratories, where most of the vaccine efficacy tests will be carried out, play a crucial role in vaccine development. The purpose of the other main component of this project is to enhance the PPR and RP surveillance systems in African countries. The specific objectives are to improve the quality of the data collected and of the system management tools. To this end CIRAD, FAO/IAEA Joint Division and IAH by collaborating with RVC in London, will give them access to a large database of diagnostic testing information. An international organisation AU/IBAR/PACE has been associated in the project although it is not entitled to receive grants through this call. The overall objective of AU-IBAR is to improve the animal resources of Africa so as to enhance the nutrition and

Epizootics (PACE) is the ability to correctly diagnose and monitor the major epizootics, in particular rinderpest, PPR, CBPP and FMD and to carry out differential diagnosis of rinderpest and PPR. The PACE programme and its epidemiological unit (PEU) and the Community-Based Animal Health and Participatory Epidemiology Unit (PACE-CAPE) have a special interest in the researches about the persistence of RP foci in the Somali ecosystem. PACE is a key player concerned with epidemiological studies and control strategies for RP and PPR in Africa. The specific contributions of the AU-IBAR PACE to the project will be the following: because it has the mandate from the Heads of State and Government of AU member countries, it is the only institution in Africa able to liaise with appropriate authorities of member states, regional groups, inter-governmental and international organisations. Accordingly the PEU from AU-IBAR with the African partners, Ethiopia, Mali and Kenya will be of great help for collecting, collating, and disseminating information on the aspects of RP and PPR. They will provide therefore a great deal of information about the epidemiological patterns of RP/PPR-disease and infection as a result of a better understanding of the predictive values of the tests available or developed within the project. The involvement of AU-IBAR in the project will ensure that the research objectives, which are considered priorities in this project, have a direct application once the results are obtained. The African laboratories concerned are located in the different African agro-ecological zones that will be investigated using spatial analysis. Cross-sectional field surveys will be carried out and complemented with existing serological data (CIRAD). CIRAD, FAO/IAEA Joint Division will validate the diagnostic tests, established with the aim of recommending a testing strategy for the surveillance of RP and PPR and evaluating the usefulness of these tests in a potential outbreak situation.

The National Animal Health Research Centre (NAHRC) in Sebeta, Ethiopia will be a sub-contractor of CIRAD. Its tasks will be delimited to GMO safety studies on the PPR marker vaccine versus the conventional attenuated vaccine. NAHRC has the laboratory and animal facilities to undertake this part of the project. It has also the expertise in diagnostic and molecular biology of PPR. This centre has acquired expertise in persistence and transmission of foot and mouth disease virus and vaccines. NAHRC is also developing a quality assurance system for research and diagnosis using the good laboratory practice standard and ISO/17025 (General requirements for the competence of testing and calibration laboratories). It is also considered as the National Reference Laboratory for RP and PPR by the Ethiopian authority whereas NVI is much more considered as a vaccine developer and manufacturer for the country and the Eastern African region. Therefore, it is important to generate independent results on the GMO safety at NAHRC because the data they generate will serve to facilitate future agreements of African governments for more extensive trials on these new vaccines. Since NVI will be extensively involved in the marker PPR vaccine development and testing in this project and since NAHRC will only be responsible for a limited investigation on safety, it was decided to appoint NVI as a regular partner and to sub-contract NAHRC.

b) Description of each participating organisation

- **Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Département d'Elevage et de Médecine Vétérinaire Tropicale (CIRAD-EMVT) – Partner C01**

Dr Geneviève LIBEAU, CIRAD-EMVT - Programme santé Animale - TA 30/G - Campus International de Baillarguet – 34398 Montpellier Cedex 5.

CIRAD-EMVT is one of the leading organisations recognised internationally in the field of tropical animal health and production. It is a French public Research Institute. Due to the leading position of the virology group on PPR disease research, CIRAD-EMVT was appointed by OIE as reference laboratory for RP and PPR and by FAO for PPR. The staff working on the virological and epidemiological aspects of the project are employed under a single hierarchical programme called "Animal Health". This will strengthen the unity of the collaboration.

Dr Geneviève Libeau (M.Sc, PhD) is the senior permanent staff member who will co-ordinate the proposed project. She has been working on RP and PPR for at least 15 years. Her research activities include development and validation of ELISAs and molecular diagnostic techniques for both viruses and studies on the genetic and antigenic diversity of RPV and PPRV. She was heavily involved, as the head of the reference laboratory, in the analysis of sera from wildlife during the "African wildlife veterinary project" a EU project initiated by OAU/IBAR, to better understand the epidemiology of rinderpest in wildlife. She has experience in managing EU projects and she is currently co-ordinating the INCO project entitled "Oralvac" for the development of oral vaccines for RP and PPR. Her group also has expertise in recombinant DNA technology and has established a sequence database to study the phylogenetic relationship between PPRV isolates and is carrying out extensive research on the immunosuppressive activity of the virus using flow cytometry and reverse genetics. This technology will be made available to the project.

Dr François Roger (DVM, PhD) has been heavily involved in the epidemiological study of major tropical diseases, particularly PPR and African swine fever. On the present proposal he will be responsible of the work packages devoted to the epidemiology of RP and PPR.

Dr Emmanuel Albina (DVM, PhD) has been working on the virology and immunology of swine for 10 years. He was a partner in the Fair PL96 1317 project (DNA vaccination of pigs) and is currently a partner in the Quality of Life QLRT-2000-02216 project on "Integrated control of African swine fever". He will participate in work packages devoted to reverse genetics and supervise the work of Miss Cécile Minet, technician.

Mr Olivier Kwiatek, technician, will carry out the molecular biology work and will help with the diagnostic tool development. A technician will be appointed on the project to help with the reverse genetics and immunological work. A PhD student from Ethiopia will be hosted by CIRAD for the development of the PPR marker vaccine and its companion diagnostic test.

List of relevant publications:

1. **Libeau G,** Prehaud C, Lancelot R, Colas F, Guerre L, Bishop DH, Diallo A. (1995) Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. *Res Vet Sci.* 58(1):50-5.
2. Rousset D, Randriamparany T, Maharavo Rahantamalala CY, Randriamahefa N, Zeller H, Rakoto-Andrianarivelo M, **Roger F.** (2000) African Swine Fever introduction into Madagascar, history and lessons from an emergence. *Arch Inst Pasteur Madagascar.* 167(1-2):31-3.
3. Diallo A, Minet C, Berhe G, Le Goff C, Black DN, Fleming M, Barrett T, Grillet C, **Libeau G.** (2002) Goat immune response to capripox vaccine expressing the hemagglutinin protein of peste des petits ruminants. *Ann N Y Acad Sci.* 969:88-91.
4. Bastos AD, Penrith ML, Cruciere C, Edrich JL, Hutchings G, **Roger F**, Couacy-Hymann E, R Thomson G. (2003) Genotyping field strains of African swine fever virus by partial p72 gene characterisation. *Arch Virol.* 148(4):693-706.
5. David Laine, Marie-Claude Trescol-Biémont, Sonia Longhi, **Geneviève Libeau**, Julien C. Marie, Pierre-Olivier Vidalain, Olga Azocar, Adama Diallo, Bruno Canard, Chantal Rabourdin-Combe and Hélène

6. Institute for Animal Health (IAH) – Partner CR2
Prof. Tom Barrett - Morbillivirus group - Pirbright laboratory - Surrey GU24 ONF – UK.

The IAH, Pirbright, is funded by the Biotechnology and Biological Research Council (BBSRC) in the United Kingdom. The Morbillivirus group, headed by Prof. Barrett (M.Sc., Ph.D.), is carrying out extensive research on rinderpest and PPR viruses using reverse genetics and is also heavily involved in developing and testing recombinant vaccines. In collaboration with the FAO World reference Laboratory for RP and PPR, the group has also played a role in molecular characterisation and phylogenetic relationship of RP, PPR and of various other morbilliviruses found in wildlife, and in the development of diagnostic tests for these diseases.

Prof. Barrett is a world-renowned expert in the field of rinderpest. Prof. Barrett has been working for 15 years in the area of rinderpest and more recently on the development of recombinant vaccines. He has heavily contributed to our knowledge on reverse genetics of Morbilliviruses. He has published several publications in the subject area, and run international courses in reverse genetics of Morbilliviruses in the past years. He will co-ordinate the research at the level of the Morbillivirus group and will be leader of the work packages which deal with the development of a double marker vaccine for RP and its associated diagnostic tests.

Dr. John Anderson and members of his group in the World reference laboratory for RP will collaborate in developing and testing the various ELISAs for the marker RP vaccine. Dr Anderson is a renowned expert in the field and was responsible for the development and transfer of the H-based C-ELISA which was used exclusively for serological analysis to monitor vaccination cover under the PARC programme to eliminate RP from Africa. The test has also been widely used in Asia as part of the RP elimination campaign on that continent.

List of relevant publications:

1. Baron, M.D. and **Barrett, T**. (1997) Rescue of recombinant rinderpest virus from cloned cDNA. *Journal of Virology* 71, 1265-1271.
2. Baron, M.D. Foster-Cuevas, M., Baron, J. and **Barrett, T**. (1999) Expression in cattle of epitopes of a heterologous virus using a recombinant rinderpest virus. *J. Gen. Virol.* 80, 2031-2039.
3. Das, S.C., Baron, M.D. and **Barrett, T**. (2000) Recovery and characterisation of a chimeric rinderpest virus with the glycoproteins of peste des petits ruminants virus: Homologous F and H proteins are required for virus viability. *Journal of Virology* 74, 9039-9047.
4. Walsh, E.P., Baron, M.D., Rennie, L., Monahan, P., Anderson, J. and **Barrett, T.** (2000) Recombinant rinderpest vaccines expressing membrane anchored proteins as genetic markers: evidence for exclusion of marker protein from the virus envelope. *Journal of Virolog,* 74, 10165-10175.
5. Heaney, J., **Barrett, T**. and Cosby, L. (2002) Inhibition of leucocyte proliferation by morbilliviruses. *Journal of Virology* 76, 3579-3584. 6.

- **Joint FAO/IAEA Division – Partner CR3**

Dr Adama Diallo – Animal Health Product Unit – FAO/IAEA Agriculture and Biotechnology Laboratory – IAEA Laboratories – A – 2444 Seibersdorf – AUSTRIA.

The Animal Production Unit within the FAO/IAEA Joint Division is the Collaborating Centre of the OIE for ELISA and Molecular Techniques for the Diagnosis of Animal Diseases. The FAO/IAEA support programme in animal health aims to assist national and regional authorities to carry out disease surveillance as part of completing the OIE Pathway and ensuring final eradication of RP and to assist in ensuring reliability and assurance of results generated from the above activities. In providing this support, the FAO/IAEA Joint Division, in collaboration with some other Institutions, has validated a variety of diagnostic tools and others are currently being validated.

As the Head of this collaborating centre, Dr Diallo sits in the OIE Biological Standards Commission. Dr Adama Diallo (DVM, PhD), was the Head of the Virology Section in CIRAD-EMVT until December 2000. His work in that institute contributed greatly to our knowledge of PPR: This included the development of the current attenuated PPR vaccine, sequencing of the different virus protein genes, development of specific diagnostic tests and development of capripox-PPR recombinant vaccines. Since January 2001, he has been the head of the Animal Production Unit within the FAO/IAEA Joint Division in Vienna. He is an FAO expert consultant for PPR. At CIRAD he was co-ordinator of 3 EU projects and was a partner in two others. Dr Adama Diallo will be responsible for the work package that will develop the companion test for PPR marker vaccine and will contribute several others: development of the double marker vaccine for PPR; identification of sequences involved in immunosuppression and test development and epidemiology of PPR.

Dr Mamadou Lelenta, DVM, has been working in the FAO/IAEA (Animal Production Unit) on test validation and transfer since 1988. He will be involved in the present project in the development of PPR ELISA tests and will be co-ordinating test evaluation with other partners in Africa.

A technician will be appointed for the project. He will be a molecular biologist, working under the supervision of Dr Adama Diallo, on the development of the PPR negative marker vaccine. A PhD student from Mali will be hosted by IAEA for the development of the PPR diagnostic test.

List of relevant publications:

1. W.P. Taylor, **A. Diallo,** S. Gopalakrishna, P. Sreeramalu, A.J. Wilsmoree, Y.P. Nanda, G. Libeau, M. Rajasekhar, A.K. Mukhopadhyay. (2002) Peste des petits ruminants has been widely present in southern India since, if not before, the late 1980s. , *Preventive Veterinary Medicine* 52, 305–312
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3. G. Berhe, C. Minet, C. Le Goff, T. Barrett, A. Ngangnou, C. Grillet, G. Libeau, M. Fleming, D. N. Black and **A. Diallo.** (2003) Development of a Dual Recombinant Vaccine To Protect Small Ruminants against Peste-des-Petits-Ruminants Virus and Capripoxvirus Infections *J. Virology* 77, (2), 1571-1577
4. David Laine, Marie-Claude Trescol-Biémont, Sonia Longhi, Geneviève Libeau, Julien C. Marie, Pierre-Olivier Vidalain, Olga Azocar, **Adama Diallo,** Bruno Canard, Chantal Rabourdin-Combe and Hélène Valentin. (2003) Measles Virus Nucleoprotein Binds to a Novel Cell surface Receptor Distinct From FcγRII via its C-Terminal Domain: Role in MV-Induced Immunosuppression. *J. Virolog, (in press).*
5. Madhuchhanda Mahapatra, Satya Parida, Berhe G. Egziabher, **Adama Diallo** and Tom Barrett. (2003) Sequence Analysis of the Phosphoprotein Gene of Peste des Petits Ruminants (PPR) Virus and Editing of the Gene Transcript. *Virus Research, (in press).*

- **Instituto de Biologia Experimental e Technologia (IBET) – Partner CR4**

Dr Paula Alves – Animal Cell Biotechnol Lab - Av. da República – EAN – Qta. do Marquês – Oeiras – PORTUGAL.

IBET is a private-not-for-profit institute that acts as an interface to bridge the interests of both fundamental and applied research with those companies interested in using bioprocesses and in developing bioproducts. At IBET, R&D projects can be expanded up to pilot plant scale working under cGMP (current Good Manufacturing Practices) rules for phase I/II clinical trials, for proteins, cells, virus and antibiotics. The Animal Cell Biotechnology Laboratory at IBET, under Prof. Manuel J.T. Carrondo (Head of Biochem Eng. Group), Professor of Chemical and Biochemical Engineering and Head of Animal Cell Technology Group at IBET, has been very active in animal cell technology processes for biomedical applications. Under his supervision, Dr Paula Alves (PhD), will co-ordinate the research at the level of her group. She holds a 5-year degree in Biochemistry and a Ph.D. in Biochemistry on Cell Metabolism and Physiology and is currently responsible for vaccine production and purification at IBET, including adenovirus and VLP- based vaccines. She will be leader of the work package where a robust production process for marker vaccines will be developed, new formulations evaluated and vaccine stability/efficacy during storage assessed.

In addition Dr Pedro Cruz (PhD, 5-year degree in Chemical Engineering, branch of Biotechnology and a Ph.D. in Chemical Engineering on the optimisation of bioprocesses) will also participate in the WP for the formulation steps.

One laboratory technician, C. Peixoto, will be involved in the project. She holds a 5-year degree in Applied Engineering, branch of Biotechnology. Her task will be to carry out the product evaluation and characterisation.

One PhD Student will assist them for cell growth, virus infection, production and purification and checking vaccine stability and efficacy during storage.

List of relevant publications:

1. Cruz, P.E., Maranga, L., Carrondo, M.J.T. (2002) "Integrated process optimization: lessons from retrovirus and virus like particle production", *Journal Biotechnology* 99:199-214.
2. Marang, L., Rueda, P., Antonis, A.F.G., Vela, C., Langeveld, J.P.M., Casal, J.I., Carrondo, M.J.T. (2002) "Large scale production and downstream processing of a recombinant porcine parvovirus vaccine", *Applied Microbiology Biotechnology* 59: 45-50.
3. Fonseca, L.L., **Alves, P**.M., Carrondo, M.J.T., Santos, H. (2001) "Ethanol effects on the metabolism of primary astrocyte culture studied by multinuclear NMR spectroscopy", *Journal Neuroscience Research.* 66(5):803-811.
4. Cruz, P.E., Peixoto, C.C., Devos, K., Moreira, J.L., and Carrondo, M.J.T. (2000). Purification and Characterisation of HIV-1 Core and Virus-Like-Particles Produced in Serum Free Medium. *Enzyme and Microbial Technology* 26: 61-70.
5. **Alves, P**.M., Moreira, J.L., Rodrigues, J.M., Aunins, J.G., Carrondo, M.J.T. (1996) Two Dimensional versus Three Dimensional Cultures Systems: Effects on Growth and Productivity of BHK Cells", *Biotechnology and Bioengineering* 52, 429-432.

- **Royal Veterinary College (RVC) - Partner CR5**

Prof. Dirk Pfeiffer - University of London - Hawkshead Lan - North Mymms - AL9 7TA, UK

The Epidemiology Division within the RVC currently was created in 1999, following the appointment of Prof. Dirk Pfeiffer to the Chair in Veterinary Epidemiology and consists of 3 academic staff members. The research domain sees its strength particularly in the epidemiological investigation of spatial aspects of disease patterns, and assessment of diagnostic tests. The current activities include risk analysis and modelling, spatial analysis of animal health data and design of animal health programmes at farm and national level (projects concern *M.bovis* in cattle). The Division currently has 7 PhD and 4 MSc students. It has strong collaborative links to the London School of Hygiene & Tropical Medicine where Prof. Pfeiffer is a Visiting Professor. Prof. Pfeiffer has been working for 15 years in the area of spatial analysis of epidemiological disease problems. He has published several peer-reviewed publications in the subject area, and run 5 international training courses in spatial analysis since 1997. He has worked on epidemiological problems in developing countries since 1988, including projects in Malawi, South Africa, Somalia, Mongolia, Thailand and Indonesia.

List of relevant publications:

1. Greiner,M., **Pfeiffer,D.** and Smith, R.D. (2000) Principles and practical applications of the receiver-operating characteristic analysis for diagnostic tests. *Preventive Veterinary Medicine* 45, 23-41;
2. Norstrom,M., **Pfeiffer, D.U**. and Jarp,J. (2000) A space-time cluster investigation of an outbreak of acute respiratory disease in Norwegian cattle herds. *Preventive Veterinary Medicine* 47, 107-119;
3. **Pfeiffer, D.U.** (2000) Spatial analysis – a new challenge for veterinary epidemiologists. In: M.V. Thrusfield and E.A. Goodall (eds.) Proceedings of a meeting held at University of Edinburgh from March 29th to 31st 2000. Society for Veterinary Epidemiology and Preventive Medicine. p86-106;
4. **Pfeiffer, D.U.** and Hugh-Jones, M. (2002) Geographical information systems as a tool in epidemiological assessment and wildlife disease management. *Revue scientifique et technique Office international des Epizooties* 21 (1), 91-102;
5. Stevenson, M.A., Wilesmith, J.W., Ryan, J.B.M., Morris, R.S., Lawson, A.B., **Pfeiffer, D.U.** and Lin, D. (2000) Descriptive spatial analysis of the epidemic of bovine spongiform encephalopathy in Great Britain to June 1997. *Veterinary Record* 147 (14), 379-384.

- **Kenyan Agriculture Research Institute, (NVRC) Muguga – Partner CR6**

Dr Rosemary N. Ngotho – P.O. Box 32, Kikuyu

The Division of Virus Research at NVRC Muguga, is a public institution devoted to diagnosis and research on animal health in Kenya. The NVRC was appointed by FAO as Regional Reference Laboratory for RP and PPR and support programmes in animal health to assist national and regional authorities to carry out disease surveillance and ensure final eradication of RP. Headed by Dr Henry Wamwayi, the Center has a long history of carrying out research on rinderpest and related diseases. In recent years, the NVRC team has carried out large-scale recombinant vaccine trials.

Under the supervision of Henry Wamwayi, DVM, MSc, PhD (Virology), a Senior Research Officer with more than 25 publications, Dr Rosemary N. Ngotho, DVM, Msc (Epidemiology and economics), will be responsible of the work programme of the project at KARI and will co-ordinate the research at the level of her group. She will be leader of the work packages on the duration of the immunity against the RP marker vaccines and will be also involved in the work package devoted to the evaluation of the test's performance. She will be seconded by Dr Eunice K. Ndungu, DVM, MSc (Bacteriology).

List of relevant publications:

1. Markine-Goriaynoff N, Georgin JP, Goltz M, Zimmermann W, Broll H, **Wamwayi HM**, Pastoret PP, Sharp PM, Vanderplasschen A. (2003) The core 2 beta-1,6-N-acetylglucosaminyltransferase-mucin encoded by bovine herpesvirus 4 was acquired from an ancestor of the African buffalo. *J Virol.* 77(3):1784-92
2. Stolte M, Haas L, **Wamwayi HM**, Barrett T, Wohlsein P. (2002) Induction of apoptotic cellular death in lymphatic tissues of cattle experimentally infected with different strains of rinderpest virus. *J Comp Pathol.* 127(1):14-21.
3. Ngichabe CK, **Wamwayi HM**, Ndungu EK, Mirangi PK, Bostock CJ, Black DN, Barrett T. (2002) Long term immunity in African cattle vaccinated with a recombinant capripox-rinderpest virus vaccine. *Epidemiol Infect.* 128(2):343-9.
4. Verardi PH, Aziz FH, Ahmad S, Jones LA, Beyene B, Ngotho RN, **Wamwayi HM**, Yesus MG, Egziabher BG, Yilma TD. (2002) Long-term sterilizing immunity to rinderpest in cattle vaccinated with a recombinant vaccinia virus expressing high levels of the fusion and hemagglutinin glycoproteins. *J Virol.* 76(2):484-91.
5. Kock RA, Wambua JM, Mwanzia J, **Wamwayi H**, Ndungu EK, Barrett T, Kock ND, Rossiter PB. (1999) Rinderpest epidemic in wild ruminants in Kenya 1993-97. *Vet Rec.* 145(10):275-83.

- **Laboratoire Central Vétérinaire (LCV) - Partner CR7**

Dr Oumou Sangaré - P.O. Box - Bamako – MALI

LCV is a public institution devoted to vaccine production for disease control, investigates livestock diseases, and gives diagnosis services and finally carries out research on the prevalence of the main animal diseases in Mali. The Virology Unit works since 1988 on rinderpest and PPR: survey in domestic animals and contribution to the development of homologous PPR vaccine (current vaccine). The laboratory has a very high expertise in the handling of animals for virus and vaccines studies and has been partner in a previous INCO project.

Dr Oumou Sangaré, DVM, will be responsible of the work programme of the project at LVC and will co-ordinate the research at the level of her group. She will participate to the work packages devoted to the evaluation of the test's performance and will be also involved in two others on spatial epidemiology of PPR and on modelling and the dynamic of PPR. She will be seconded by a DVM, MSc (Virology).

List of relevant publications:

1. **Sangaré O**, Bastos ADS, Venter EH, Voslo W. (2004) A first molecular epidemiology study of ST-2 type foot-and-mouth disease viruses in West Africa. *Epidemiol.Infect.* 132:525-532.
2. **Sangaré O**, Bastos ADS, Marquart O, Venter EH, Vosloo W, Thomson GR (2001). Molecular epidemiology of serotype O foot-and-mouth disease virus with emphasis on West and South Africa. Virus Genes 22: 345-351.
3. Bastos ADS, **Sangaré O** (2001). Geographical distribution of SAT-2 type foot-and-mouth disease virus genotypes in Africa. *Proceedings of the southern African Society for Veterinary Epidemiology and Preventive Medicine, Onderstepoort, South Africa* 10-11 May, pp 20-26.
4. **Tounkara K**, Traore A, Traore AP, Sidibe S, Samake K, Diallo BO, Diallo A. (1996) Epidemiology of rinderpest and cattle plague in Mali: serological surveys. *Rev Elev Med Vet Pays Trop.* 49(4):273-7.
5. **Tounkara K**, Maiga S, Traore A, Seck BM, Akakpo AJ. (1994) Epidemiology of bovine brucellosis in Mali: serologic investigation and initial isolation of strains of Brucella abortus. *Rev Sci Tech.* 13(3):777-86.

- **National Veterinary Institute (NVI) – Partner CR8**

Dr. Berhe Gebreegziabher - P.O. Box 19 - Debre-Zeit - ETHIOPIA.

The National Veterinary Institute (NVI) was established at Debre-Zeit in 1964 by the Ethiopian Government, under the Ministry of Agriculture in collaboration with the French Government. The current status of the NVI is a public enterprise with the mandate to produce vaccine to be used for the control or eradication of prevalent livestock diseases, investigate livestock diseases and give diagnostic services to veterinary field work and, finally, to carry out or apply research on vaccine development. The Institute is identified as a reliable and potential supplier of veterinary vaccines to countries in Africa and other developing regions. Indeed, it has been possible to export to more than 23 African, Asian and Middle East countries, in addition to satisfying the national demand. This indicates that the quality of its vaccines is of a high standard and competitive in international markets. At present it has many years of accumulated experience in conducting collaborative research activities mainly with the French research centre, CIRAD-EMVT. Headed by Dr Berhe Gebreegziabher (Director), the NVI carries out research on large-scale recombinant vaccine trials. In the present proposal, he will co-ordinate the research at the level of the NVI. He previously worked as Research and Technical Dept. Head at NVI. He completed his DVM and MVSc. He is currently conducting his PhD studies at CIRAD in France under a sandwich programme. He has worked in the area of vaccinology, specifically cell culture mediated recombinant vaccine development (PPR-capripox recombinant vaccine) and DNA vaccine manipulation as part of his Masters and PhD research work. He will be involved in the work package devoted to the development of a double marker vaccine and those related to the evaluation of tests and epidemiology). He will be seconded by Mrs. Martha Yami (Quality Control laboratory head). She is a senior scientist in the group and worked as a vaccine technologist at the NVI and the Pan-African Vaccine Centre (PANVAC). She obtained her DVM, MVSc degrees. One PhD Student and Lab technicians will assist them.

List of relevant publications:

1. **Berhe G**, Minet C, Le Goff C, Barrett T, Ngangnou A, Grillet C, Libeau G, Fleming M, Black DN, Diallo A. (2003) Development of a dual recombinant vaccine to protect small ruminants against peste-des-petits-ruminants virus and capripoxvirus infections. *J Virol.* 77(2):1571-7.
2. Diallo A, Minet C, **Berhe G**, Le Goff C, Black DN, Fleming M, Barrett T, Grillet C, Libeau G. (2002) Goat immune response to capripox vaccine expressing the hemagglutinin protein of peste des petits ruminants. *Ann N Y Acad Sci* 969:88-91.
3. Madhuchhanda Mahapatra, Satya Parida, **Berhe G. Egziabher**, Adama Diallo and Tom Barrett. (2003) Sequence Analysis of the Phosphoprotein Gene of Peste des Petits Ruminants (PPR) Virus and Editing of the Gene Transcript. *Virus Research* (in press).

- **Inter-African Bureau for Animal Resources of the African Union (AU/IBAR) – Partner CR9**

Dr Gavin Thomson - P.O. Box 30786 - Nairobi – KENYA
The PACE Programme is one of the major development programmes co-ordinated by the Inter-African Bureau for Animal Resources (IBAR) of the African Union (AU). The PACE is an ongoing five-year programme financed with EDF regional funds. This development programme is in the field of animal health in Africa, covering 32 African countries. It was initiated in November 1999, and the various countries started the implementation of their national components from 2000. The PACE programme aims to build upon the success of the PARC campaign against RP in order to establish lower-cost national and continental epidemiological surveillance networks for the main animal diseases affecting the continent. It also provides the countries with the capacity needed to organise economically and technically justified control programmes and develops effective and sustainable distribution of veterinary products and services. It insures the Technology Transfer in the field of animal production and health in the following key areas:

- Establishment of a network of laboratories for the diagnosis and surveillance of rinderpest and PPR using the nuclear and related techniques such as ELISA and PCR
- Establishment of a regional capability for the supply of reagents and materials for animal disease diagnosis.
- Capacity Building, leading towards sustainability, through the organisation of fellowships, workshops and training courses.

To support the implementation of activities at national level, the PACE programme is divided in six units comprising the Epidemiology Unit that includes a wildlife component.

The technical staff of epidemiology Unit comprises: Main epidemiologist (Dr Gavin Thomson, DVM, PhD); Counterpart of the main epidemiologist (Dr Bidjeh Kebkiba, DVM, Msc, PhD); laboratory expert (Dr Karim Tounkara, DVM, M.Sc, PhD); epidemiologist for East Africa (Dr Gijs Van't Klooster, DVM); Epidemiologist for Central Africa (Dr Fatah Bendali, DVM, PhD); Wildlife experts (Dr Richad Kock, DVM).

AU/IBAR:

1. Kock ND, **Kock RA**, Wambua J, Mwanzia J. (1999) Pathological changes in free-ranging African ungulates during a rinderpest epizootic in Kenya, 1993 to 1997.Pathological changes in free-ranging African ungulates during a rinderpest epizootic in Kenya, 1993 to 1997. *Vet Rec.* 145(18):527-8.
2. **Kock RA**, Wambua JM, Mwanzia J, Wamwayi H, Ndungu EK, Barrett T, Kock ND, Rossiter PB. (1999) Rinderpest epidemic in wild ruminants in Kenya 1993-97. *Vet Rec.* 145(10):275-83.
3. **Kock R, Kebkiba B**, Heinonen R, Bedane B. (2002) Wildlife and pastoral society--shifting paradigms in disease control. *Ann N Y Acad Sci.* 969:24-33.
4. Taylor WP, Roeder PL, Rweyemamu MM, Melewas JN, Majuva P, Kimaro RT, Mollel JN, Mtei BJ, Wambura P, Anderson J, Rossiter PB, **Kock R**, Melengeya T, Van den Ende R. (2002) The control of rinderpest in Tanzania between 1997 and 1998. *Trop Anim Health Prod.* 34(6):471-87. Trop Anim Health Prod. 2002 Nov;34(6):471-87.
5. **Thomson GR,** Vosloo W, Bastos AD. (2003) Foot and mouth disease in wildlife. *Virus Res.* 91(1):145-61.

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ANNEX

PREVIOUSLY SIGNED CONTRACT WITH EU

A1: No: ICA – CT –2000 –30027

A2: Project No TSD-A-091

A3: TS2-A-0178-F)

A4: REG/5007/005 EDF VII & VIII)

EUROPEAN PATENT

A5 : European patent n° 97670002.1

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