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PROJECT FINAL REPORT

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1. Executive Summary

Bluetongue virus (BTV) and related, African Horse Sickness virus (AHSV) and Epizootic Hemorrhagic disease virus (EHDV) that are transmitted from animal to animal by biting insect vectors (midges *Culicoides* species), are responsible for livestock diseases with high morbidity and mortality. These viruses are grouped under Orbivirus genus due to their similar overall structures and biology. While bluetongue disease of sheep, cattle and goats is current threat (with an annual global cost ~ 3 billion), African Horse Sickness disease of horses and Epizootic Hemorrhagic disease of deer and cattle are potential future challenges facing European agriculture and animal industry. Moreover, wide spread of *Culicoides* vectors in north, south and central Europe indicate that the whole Community is now at high risk from incursion of these orbiviral diseases. Vaccination as a method of control for these diseases is a realistic possibility. However, the vaccines that are currently in use in Europe (either live attenuated strains, or inactivated tissue culture grown live virus) generate an antibody response to all of the viral proteins, thereby making it impossible to distinguish vaccinated from infected animals (DIVA). In addition, each of the viruses causing these diseases circulates as multiple, serologically distinct types and vaccination generally leads to type specific protection.

The objectives of this consortium were three-fold; the first aim was to develop novel technologies to generate multivalent vaccines for each of these orbivirus species, BTV, AHSV and EHDV. The second aim was to investigate the best vaccination strategy for eliciting multi-serotype protective immunity for these viruses in livestock and to analyze immune responses for each of the novel vaccines developed for breadth of protection against multiple serotypes. The third aim was to develop DIVA compatible diagnostics that will work with the new vaccines developed in order to differentiate between vaccinated and infected animals.

To realise these objectives a coordinated multi-partner approach was undertaken based on specific expertise and reagents only available within the consortium, which included scientists from EU universities, academic institutes, government organisations and commercial entities, both small (SME) and large vaccine manufacturing companies as well as other international institutions (USA and South Africa).

The combination of expertise and the appropriate reagents have successfully allowed us to develop novel designed vaccines and diagnostic reagents. A new class of **replication-defective** vaccine strains (11 serotypes) for multivalent BTV was successfully produced which showed complete protection against virulent virus challenges in sheep and cattle. In addition, protective efficacy of an adenovirus vectors cocktail after homologous and heterologous BTV challenge was performed, demonstrating that with using one vector combination it is possible to protect model mice against different BTV serotypes. In parallel single protein-based AHSV vaccines for all 9 serotypes were produced, each of which had the capability to elicit strong neutralising antibodies when used either individually or in cocktail mixture. Vaccinated horses similarly developed a strong neutralising antibody response that afforded complete protection against virulent virus challenge. A parapox virus as a delivery system for AHSV proteins was also developed and was able to induce an immune response in small model animals. For EHDV, the virus-like particles strategy, that involved the expression of 4 structural proteins, demonstrated immunogenicity in small animal model. As a result, through this project innovative vaccine strategies were developed for the 3 different orbiviruses with a high potential of protection of vaccinated animals.

Together with vaccine development, DIVA compatible diagnostic reagents have been developed which will allow routine testing of vaccinated and imported animals. Diagnosis of orbivirus diseases included microarray, improved real time PCR for BTV serotyping BTV and a new group specific ELISA test that for the first time provides an immunological test for distinguishing EHDV, BTV and AHSV.

In conclusion, the primary outcome is that stable, multivalent, new generation; DIVA compliant vaccines have been produced in this Consortium, which will have a long-term impact both in Europe and elsewhere.

2. Summary Description of project context and objectives

Orbivirus diseases, particularly Bluetongue (BT) of livestock, African Horse Sickness (AHS) of equids and Epizootic Hemorrhagic disease (EHD) of deer, are some of the big challenges facing European agriculture. All three viruses are transmitted by biting midges (*Culicoides* species) from animal to animal. Since 1998 there have been more than 12 separate introductions of bluetongue virus (BTV) into Europe, involving at least ten different virus strains belonging to eight different serotypes (types 1, 2, 4, 6, 8, 9, 16 and a new serotype, type-25). These events have resulted in the deaths of more than two million animals and have caused substantial economic losses to the agricultural economies of Europe. The outbreak caused by BTV-8, which started in Holland and Belgium in 2006, is by itself the largest single outbreak of bluetongue ever recorded. New introductions of the virus into Europe, which have been linked to climate change, have occurred almost every year since 1998, with the identification of four new virus strains in 2008 alone. New *Culicoides* species responsible for virus spread have also been identified in central and northern Europe, confirming that the whole Community is now at high risk from incursion of these diseases.

Since AHSV and EHDV are genetically closely related to, and are transmitted by the same insect vectors as BTV, there is a clear risk of the potential introduction of these other orbiviruses and other related viruses into Europe. The recent detection of BTV-9 in North Africa, EHDV in Turkey and two different strains of AHSV in West Africa, provide further indications of increased risk from these diseases. The continued appearance of new BTV strains in southern, central and northern Europe raises the question of which virus and what serotype will arrive next.

Although there are effective inactivated vaccines for some of the individual BTV serotypes, which are currently used in Europe, these are not currently available for all serotypes. In addition, no effective inactivated vaccines are currently licensed and available for use in Europe, for either AHSV or EHDV.

The live attenuated strains of BTV that have been used successfully for many years in Africa can cause severe clinical signs including high levels of viraemia in European sheep and cattle. Some of these attenuated vaccine viruses can infect adult *Culicoides* and can be transmitted in the field, leading to outbreaks caused by vaccine strains of BTV-2, BTV-6 and BTV-16 in Europe. Consequently, although these live vaccine strains have been used in the Mediterranean region, their use, particularly in northern Europe, is not recommended. Part of the problem with these vaccine strains is that attenuation has been achieved through serial passage of virus in cell culture. The mutations that are associated with attenuation are not described, and the degree of attenuation of any one particular vaccine strain, in terms of attenuation of transmission, is difficult to assess prior to its use in the field.

Vaccination as a method of control for these diseases is a realistic possibility, but current vaccines have limitations in terms of the breadth of protection afforded following a single vaccination cycle. Each of the viruses causing these diseases circulates as multiple, serologically distinct types and vaccination generally leads to type-specific protection. The major outstanding challenge of orbivirus vaccine research is therefore not to identify the antigens which are responsible for protection (this has already been well established) but to develop vaccines that can afford a broad protective immune response against as many serotypes of each virus as possible. The vaccines that are currently in use in Europe (either live attenuated strains, or inactivated tissue culture grown live virus) generate an antibody response to all of the viral proteins, making assays that can distinguish vaccinated from infected animals (DIVA) difficult or impossible to develop. Consequently, the competitive Enzyme-linked immunosorbent assay (ELISA) tests previously used as a front-line

diagnostic assay to detect BTV specific antibodies, for disease surveillance, have been rendered useless by the current vaccination campaigns. A second challenge in Orbivirus vaccine research is therefore to develop effective vaccines, along with a high throughput DIVA assay (e.g. an ELISA).

This EC funded project, ORBIVAC, uses a coordinated multi-partner approach to address these issues, while developing new experimental prototype vaccines and diagnostic approaches. It builds on specific expertise and reagents that are only available within the consortium and links out to other international efforts in USA and South Africa to develop improved vaccines for these diseases where these viruses are also a threat.

Specific objectives of the project are:

1. To develop multivalent vaccines using different approaches for Orbiviruses responsible for livestock diseases, in particular, Bluetongue Virus (BTV), African Horse Sickness Virus (AHSV) and Epizootic Haemorrhagic Disease Virus (EHDV).
2. To understand the best vaccination strategy to elicit multi-serotype protection for these viruses in livestock and analyze immune responses for each of the novel vaccines developed for breadth of protection against multiple serotypes.
3. To develop DIVA compatible diagnostics that will work with the new vaccines developed in order to differentiate between vaccinated and infected animals.

3. Main S&T results/foregrounds

Work packages 1&2: Management of the consortium and appointment of staff
This has been completed.

Work package 3: Generation of Disabled single cycle vaccines to BTV
We have successfully prepared and recovered 11 different serotypes of BTV DISC virus strains; serotypes BTV -1, -2, -4, -8, -9, -10, -13, -16, -21, -23 and -24. Several of these DISC viruses have been tested as vaccine candidates either as a single or in cocktail mixtures. All vaccine trials undertaken using those viruses have shown very promising results with complete protection of all animals challenged with virulent strains. To further analyse the viability of these candidates as vaccine, we performed a range of stability tests including a number of stabilising agents (sucrose, glycerol, etc) and temperature ranges (from RT to -80°C). Cycles of freeze-thaw and desiccation were also tested. The effect of these conditions on virus infectivity was monitored by TCID50 assay. The conditions that demonstrated to be the most effective for preservation of infectivity were desiccation and storage at 4°C. These conditions maintained the virus infectivity with only 0.5 to 1 log10 decrease in virus titre from the original stock over a six week period.

Work package 4: Generation of multivalent subunit vaccine for AHSV
LSHTM: LSHTM successfully completed the aims and objectives outlined in work package 4. In this work package, LSHTM demonstrated the single expression of VP2 from each of the nine serotype of AHSV, as well as generating and demonstrating the expression of VP2 from multiple serotypes in a single recombinant baculovirus (up to six different VP2 expressed). The results showed that a single baculovirus that expressed VP2 from three different serotypes provided optimal expression of VP2. LSHTM also supplied the semi purified soluble form of the singly expressed VP2 to our partners (10 and 11) for testing of immunogenicity in guinea pigs and horses. Immunisation of guinea pigs with a single or cocktail of VP2 proved to be highly successful with all protein eliciting a strong neutralising antibody (see work package 9).

Stability, formulation and vaccination in horses
Partner 1 supplied a number of batches of single VP2 immunogens to Partner 11 for testing in horses. These samples had failed to elicit a neutralising antibody response. Therefore it was decided to formulate optimal conditions to maintain the integrity of the immunogen. AHSV-7 VP2 was used as a model protein and purified according to the method optimised in previous work packages. A number of different stabilising agents (trehalose, sucrose, and glycerol) were added to the purified recombinant protein preparation. The samples were then tested over a range of temperatures (4°C, -20°C and -80°C) and samples were also freeze-dried. Samples from each time point were analysed by coomassie stained SDS-PAGE to examine protein degradation. The results demonstrated that the stability of VP2 was increased with the addition of 10% trehalose when tested. Further, emulsification of the adjuvant with the protein was also highly stable.

Vaccination of horses with AHSV-7 VP2
To investigate whether horses vaccinated with a single serotype vaccine elicited a strong serotype-specific response, AHSV-7 VP2 was partially purified from insect cells infected with a single recombinant baculovirus; mixed with trehalose and adjuvant and supplied to DLTM to study immunogenicity in horses. Vaccination took place in autumn 2013. Early data from the vaccination demonstrated that the addition of the trehalose did not cause any adverse reactions post-

vaccination. Importantly, the stabilising reagent did not affect VP2 immunogenicity in horses with a neutralising antibody response elicited (see work package 9).

Conclusion

LSHTM has successfully completed all objectives outlined in work package 4:

- VP2 from nine serotypes of AHSV was expressed and purified from baculovirus.
- Recombinant baculovirus that can express up to six serotypes was generated.
- Optimal number of AHSV VP2 proteins expressed from a recombinant baculovirus was determined.
- Optimal combination of serotypes to have equivalent protein expression.
- Proven immunogenicity of single VP2 in guinea pigs and horses.
- Formulation for preparing VP2 for transportation.

Work package 5: Generation of multivalent antigens to BTV

ANSES: Task one was dedicated to the design of an antigenically optimised BTV VP2 antigen. Because VP2 is the most antigenic and serotype specific protein of BTV, ANSES provided modified VP2 proteins that could provide some cross reactivity between serotypes. *In silico* analysis of VP2 was made by NOKAD SA, a subcontractor of ANSES, based on structural data and sequence alignments of the "first" 24 BTV serotypes. Different mutant VP2 proteins have been designed based on the determination of conserved and sub-antigenic regions. At the end of the *in silico* study, five mutant proteins had been designed based on serotype 1; and four mutant proteins based on serotype 8. All mutants have been chosen to promote cross reactivity between the BTV serotypes present in the Mediterranean area (i.e. 1, 2, 4, and 8).

CIRAD: CIRAD's aim in this project was to develop a gene delivery system derived from capripoxviruses (Cpox) allowing expression of a multivalent BTV-VP2 protein alone or in combination with conserved antigens of BTV such as VP3 and VP7 as a vaccine candidate. Design of four different VP2 epitopes cross-reacting between bluetongue serotypes 1, 2, 4 and 8 was performed based on Pepscan strategy and bioinformatics dN/dS approach. The plan was to *in vitro* test these epitopes through a competition seroneutralisation test (SNT, blocking of neutralization by peptides). Unfortunately, despite repeated assays, only two of the four peptides were chemically synthesised. It was not possible to validate the neutralising activity of these peptides by an assay involving an inactivated BTV strain. Thus, *in vivo* assays have been implemented and did not show any neutralising antibodies induced for two of the chemically synthesised peptides.

IDVet: BTV NS1, NS2 and NS3 were screened as potential markers for DIVA tests (work shared between different partners). NS1 was the best candidate and monoclonals against NS1 were generated. NS1 was expressed as a recombinant protein in bacteria and baculovirus system. Different prototypes were set up and prototype kits were developed and optimised. Two prototypes were promising. The first prototype allowed a good discrimination between naturally infected and naïve animals. But multi-vaccinated animals with currently available vaccines were found positive, probably due to traces of NS1 protein present in the vaccines. This kit could be interesting for the subunit/recombinant vaccines such as ISCOM, DISC, etc. The second prototype was developed to avoid these reactions due to the use of inactivated vaccine. After three injections, partial seroconversion against NS1 on vaccinated animals (n=320) was observed. Prototypes were sent to partners. The kit performance was mainly assessed on sera from seroconversion studies, and a late seroconversion was observed on this format (20-25 dpi).

Work package 6: Multivalent vaccine against BTV derived from capripoxvirus and canine adenovirus

ANSES & Merial: Viral vector based antigen delivery system is able to induce both mucosal and systemic immunity. Moreover, by definition, they can be used as marker vaccines. Vectors based on human adenovirus have been intensively tested for immunisation and have shown their capacity to induce strong immune responses. As an alternative to human serotypes, canine adenovirus appears to be a promising candidate for veterinary vaccine development.

CAV2 vectors have proven their capacity to protect different host species against different viral challenges but not small ruminants. Efficacy study of CAV2 vectors in sheep has been published by ANSES. Due to the availability of different CAV2 constructions expressing BTV-antigens and the rescue of a human adenovirus type 5 (Ad5) expressing VP2BTV8, IFNAR-/- mice were immunised with the cocktail vaccine. This vaccine, corresponding to Ad vectors expressing VP2BTV8, NS1 and VP7, successfully protected mice from lethal homologous and heterologous challenges. Immunised mice presented non-typical BTV-clinical signs and a reduction in the BTV RNAemia was observed.

Moreover, ANSES attempted to test the cross reactivity of commercially available vaccines in collaboration with MERIAL SAS, and planned to test the possibility of heterologous protection after sequential vaccination with different BTV serotype vaccines. The aim of this study was to determine whether a combination of currently available vaccines may provide protection in case of emergency (i.e. pandemic), in order to permit the production of specific serotype vaccine. Observations of cross-protection have been made for some serotypes.

CIRAD: The generation of one single plasmid, containing either BTV2-VP3 or VP7 could not be achieved due to permanent and unexpected technical cloning difficulties. The size of the fragment and its toxicity were some of the encountered problems. Despite the use of several competent bacteria strains, vectors, different approaches have been worked on without any success. Work is still ongoing. The three BTV8-VP2 mutants (mutant 1, mutant 2, mutant 3) generated by Partner 2 (ANSES) through its subcontractor NOKAD were transferred to CIRAD, and efforts were made to clone these mutants in the Capripoxvirus shuttle plasmid. Again technical difficulties did not allow us to get cpox recombinants up to date.

Work package 7: Preparation and testing parapox vectored immunogens for AHSV

Pfizer: VP2 and VP5 proteins from AHSV serotypes 2, 4 and 9 as well as generic NS1 protein coding genes were defined as the target genes to be cloned into the Parapoxvirus Orf virus (ORFV) vector system. Target genes sequences were first analysed and adapted to allow correct expression in this system and then genes were *de novo* synthesised.

Transfer plasmids coding for each one of the target genes were constructed. Recombinant ORFV were generated by inserting foreign genes in place of the viral vegf-e gene. To generate ORFV recombinants, Vero cells were infected with a parental ORFV expressing a marker gene encoding the green fluorescent protein AcGFP. Then, infected cells were transfected with the transfer plasmid. By homologous recombination AcGFP from parental virus was replaced by the target gene.

Replacement of parental AcGFP gene by target gene was checked in rescued recombinant viruses by PCR. Then, insertion of full-length VP2 gene was verified by means of a PCR using a set of primers flanking the insert. For the analysis of transcription, total RNA (clean from DNA) was extracted from infected Vero cells and then transcription verified by RT-PCR using target gene specific set of primers. To confirm proper protein production, positive clones were analysed by means of immunoassays (western blot, IPMA and IFA) in Vero cells infected at a high MOI.

At the end of the project all rORFV-AHSV were obtained (coding VP2 and VP5 from AHSV-2, 4 and 9 and NS1). *In vitro* proper protein expression was demonstrated for AHSV-9 VP5 and NS1 proteins. It was demonstrated that VP2 proteins from AHSV can be cloned and transcribed using Parapoxvirus Orf virus (ORFV) vector system although VP2 protein could not be visualised by immunoassays. AHSV-2 and 4 VP5 protein coding genes were correctly cloned and transcribed but protein expression was not checked. Virus stocks were generated for all generated rORFV-AHSV.

Prior to executing vaccination/challenge studies in mice AHSV experimental infection models using the IFNAR -/- mice model were established. Models for experimental infection in mice were successfully established with AHSV-4 ESP P2BHK 120210 strain, AHSV-9 PMVVD-9/KEN2006/01, AHSV-9 PMVVD-9/SEN1998/01 and AHSV-2 V+/AHSV-2/SV/11-09isolates. However, AHSV-2 PMVRD/AHSV-2 SEN 2007/02 strain failed to infect the animals and was discarded as challenge strain. Exploratory vaccination/challenge studies were carried out using IFNAR -/- mice model.

For homologous vaccination/challenge tests, groups of mice were vaccinated with wild type vector (D1710-V-AcGFP) and rORFV-AHSVs coding for VP2 proteins and then challenged with the homologous AHSV serotype. Clinical signs were prevented in 80% of VP2, and 20% of vector vaccinated animals challenged with AHSV-4. A reduction of viremia was found in animals vaccinated with recombinant expressing AHSV-4 VP2 protein. As no reduction of viremia was elicited by the vector, these viremia results together with the clinical observations confirm that a specific immune response was generated by the AHSV-4 VP2 recombinant vaccine due to the *in vivo* expression of VP2 protein. Therefore, although VP2 protein could not be visualised in vitro, these results prove that ORFV vector system was able to express and to release VP2 protein *in vivo*. However, the vector or recombinants coding for the VP2 protein from AHSV-2 and AHSV-9 were not able to reduce viral replication after a homologous AHSV challenge.

For the heterologous protection test, groups of mice were vaccinated with rORFV-AHSVs coding for NS1 generic protein and VP5 protein from AHSV-9 and then challenged with the different AHSV serotypes. Clinical signs were prevented in 78% of NS1 vaccinated animals challenged with AHSV-4. Vaccination also induced a reduction of viraemia throughout the study. In the case of AHSV-2 challenged mice, reduction of viraemia was only detected in NS1 vaccinated animals at days five and ten post-infection. In animals vaccinated with NS1 or a combination of NS1+VP5 and challenged with AHSV-9 a reduction of viraemia was detected throughout the study. These results prove that ORFV vector system is able to release NS1 protein *in vivo* and that vaccination with NS1 is able to induce a protective effect against AHSV challenge with different serotypes. Therefore, NS1 protein would be a good candidate gene to be included in the development of multivalent cross-protective strategies.

Preliminary-exploratory studies on the efficacy of different adjuvants in horses were conducted. Three different adjuvants (A, B and C) were checked for their feasibility to be used in horses. Results indicated that different adjuvants induced different immune responses. After the second vaccination, the seroneutralising (SN) antibody titers increase was clearly higher for the B-adjuvanted vaccine suggesting that adjuvant B is the most adequate for its use in horses.
Prior to starting the vaccination/challenge studies in horses, AHSV experimental infection in horses for the different serotypes were established. Models for experimental infection were successfully established using AHSV-2 PMVRD/AHSV-2 SEN 2007/02 strain, AHSV-4 ESP P2BHK 120210 strain, AHSV-9 PMVVD-9/KEN2006/01 and AHSV-9 PMVVD-9/SEN1998/01 isolates. However, AHSV-9 V+/AHSV-9/Pak/CH/H/10-10 strain was mild-pathogenic and non useful for challenge-protection studies in horses.

During this period, a vaccination/ challenge study was done in horses to evaluate the ability of rORFV-AHSV coding for AHSV-4 VP2 protein to induce a protective immune response against homologous challenge with AHSV-4 serotype. Vaccination of horses with the recombinant was able to slightly delay the appearance of clinical signs and to confer 25% protection against a highly pathogenic AHSV-4 challenge. Taking into account results from mice experiments, indicative that VP2 protein is expressed, results suggest that VP2 protein is also expressed in horses. However, no specific immune response against VP2 protein was identified before challenge by means of neutralisation or ELISA test. Considering the important role of SN antibodies in protection against AHSV infection, factors causing this lack of specific immune response need to be further investigated. The possibility that CMI could have a role in that protection as well as a non-specific response (derived from the immunostimulating properties of the vector) needs to be considered as well.

In conclusion, although lack of SN antibodies production levels and the contribution of immunostimulating properties of the vector to protection need further investigation, these results validate the ORFV vector system as a potential AHSV immunogen delivery vector for horses.

UCM: Analysis of the optimisation of vaccine components was tested in mice. Finally, the vaccine was evaluated in challenge study in horses. Related to task three, the virus strains of all reference serotypes have been replicated “in vitro” in the UCM facilities. The title of the AHSV strains has been determinated in Vero cells following the OIE protocol. Following task five, UCM performed immunological analyses such as virus neutralisation and flow cytometry. These analyses allow the evaluation of immune stimulation elicited by the vaccine candidate in the horses. The challenge of task six was done in Biosafety Level 3 facilities (Partner 6, Pfizer/Zoetis). Partner 6 sent us the whole blood collected by venopuncture in EDTA vacuum collection tubes and serum samples on day 0 prior to vaccination and at different times of vaccination and challenge. The evaluation of the immune response elicited by the vaccination and infection of the horses was performed by UCM.

Work Package 8: Preparation of VLPs to EHDV-1 and EHDV-2

LSHTM: Virus-Like Particles (VLPs) represent one of the most exciting emerging vaccine technologies for generating effective and long-lasting protection. VLPs consist of viral structural proteins and lack the core genetic material making them non-infectious and unable to replicate. Moreover, BTV VLPs vaccines have been proven to be highly successful in vaccinated sheep by triggering antibodies that protect against virulent virus challenge. In this work package, LSHTM developed VLPs for EHDV-1 and 2 serotypes using baculovirus expression system, then supplied these to Partner 12 for testing immunogenicity in deer.

Generation of recombinant baculovirus expressing core-like particles (CLPs) and triple structural proteins of EHDV-1

Previously, we reported cloning the coding regions of EHDV-1 S2, S5 and S7 into transfer vectors containing a selectable marker that enhances the insertion of EHDV-1 genes at different *loci* in AcMNPV. Each of S5 and S7 genes were inserted at odv-e56 and egt, respectively. In an initial step to develop EHDV-1 VLPs, a recombinant baculovirus expressing the core proteins VP3 and VP7 (CLPs) of EHDV-1 was generated. CLPs synthesis was analysed by coomassie brilliant blue staining and purification conditions were optimised. Furthermore, the formation of CLPs structure was confirmed by negative stain EM grids. Sample of the recombinant proteins were sent to raise EHDV-1 CLPs polyclonal antibodies in rabbit. The next step for EHDV-1 VLPs production was to express the structural proteins VP5, VP3 and VP7 simultaneously in a recombinant AcMNPV. Protein synthesis was detected by coomassie blue staining. Different conditions of sucrose gradients purification were tested for optimisation.

Development of EHDV-1 VLPs

To generate EHDV-1 VLPs, EHDV-1 VP2 coding region was cloned into a number of vectors to increase the insertion possibility at AcMNPV genome which already has EHDV-1 VP5 and VP7. The insertion of VP2 was difficult due to its large size (~3000bp). To solve this problem, VP3 was cloned into transfer vector and inserted at AcMNPV in 39k locus under ph promoter.

Optimisation procedure was carried out to purify EHDV-1 VLPs which were detected by SDS-PAGE. Samples of EHDV-1 VLPs have been sent to raise antisera in mice. The antisera produced were assessed by LSHTM to determine if they have neutralising activities against EHDV-1. Immunogenicity in deer was not possible to evaluate in this time period but we intend to continue collaboration with Partner 12 to complete this work in future.

Development of EHDV-2 VLPs

A recombinant baculovirus expressing each of EHDV-1 VP3, VP7 and VP5 proteins simultaneously was rescued with EHDV-2 VP2. VP2 and VP3 were provided by Partner 12. EHDV-2 gene was cloned into pAcYM1 transfer vector under ph promoter. Several rounds of amplifications were carried out to increase the titer of the virus. For virus purification, same conditions which were optimised for EHDV-1 VLPs were followed and the four proteins bands of EHDV-2 VLPs were visualised on SDS-PAGE gel stained by coomassie brilliant blue.

KSU: Purified RNA from EHDV-2 infected cells cultures was reverse transcribed and PCR amplified. Resulting PCR products were purified and cloned into the pGEM-T easy vector. Positive colonies were sequenced for verification. The VP-2 and VP-5 containing plasmids were shipped to Professor Roy's laboratory for expression in baculovirus systems. An animal room in our BSL-2 facility was refitted with a deer chute in order to be able to house white-tail deer (WTD). These modifications to our animal facility will allow us to test the EHDV-1 and EHDV-2 VLPs produced in this project in a challenge study with EHDV-1 and/or EHDV-2 isolates in both cattle and deer.

Work package 9: Optimal vaccine schedule and immune responses of animals vaccinated with polyvalent AHSV immunogens

LSHTM: LSHTM supplied semi-purified AHSV VP2 vaccines either as a single or as a cocktail of serotypes to our partners to investigate immunogenicity, vaccination strategy and serotype interference/immune-dominance in both guinea pigs and horses.

LSHTM had previously demonstrated that the AHSV VP2 antigen sent to our partners as a single or multiple serotypes vaccine elicited a neutralising antibody response in guinea pigs. The neutralising antibody data generated from LSHTM and Partner 10 was consistent between both groups. Furthermore, the data showed that although there was some cross reaction between serotypes, neutralisation was serotype specific.

Vaccination of horses

AHSV antigens supplied to Partner 11 for horse trial had been unsuccessful due to stability of the antigens during long distance transportation and storage condition. Therefore Partner 1 investigated different stabilising reagents and storage conditions to maintain protein immunogenicity (work package 4). The vaccine was shipped on ice and placed at -80ºC until the day of vaccination. Horses were vaccinated with either AHSV-7 VP2 or baculovirus lysate that had been prepared in a similar manner by Partner 11. There was no adverse reaction at the site of vaccination or post-vaccination to the incorporation of the stabilising reagent in the vaccine preparation. Results indicate that the horses have developed a strong neutralising antibody response. When these horses were challenged with a virulent virus infection, they did not develop any clinical reactions in contrast to the unvaccinated control horses, which showed strong disease symptoms. The data suggested that the

formulation of VP2 and long-distance transportation maintained the immunogenicity of the subunit vaccine as expected.

Conclusion

LSHTM has successfully completed all of their objectives for this work package.

- AHSV VP2 as a single serotype immunogens were supplied to both partners.
- AHSV VP2 as a multi-serotype cocktail was supplied to Partner 10.
- Neutralising antibodies were elicited to the protein in guinea pigs.
- Transport conditions tested to maintain immunogenicity.
- Protective immunogenicity of AHSV VP2 in horses assessed.

DLO-CVI: Baculovirus expressed VP2 proteins of each of the nine AHSV serotypes as well as two different cocktails of VP2 proteins were used to immunise guinea pigs two times with an interval of three weeks to study immunogenicity. All VP2 proteins were immunogenic and induced serotype specific neutralising antibodies. In total, cross-recognition was also very weak in immunostaining assays.

DLTM: Monovalent recombinant VP2-subunit protein of AHSV serotype 7 prepared by Partner 1 of the Orbivac Consortium was used in the initial adjuvant screening studies. After confirmation of immunogenicity in mice, the recombinant antigen was shipped to South Africa, a country where African Horse Sickness Virus occurs endemically, for animal adjuvant screening in horses. Ten different adjuvants originating from various tertiary education institutions, private vaccine developers and commercial adjuvant producers were obtained and evaluated.

Finding a suitable adjuvant proved more difficult than anticipated. Of the ten adjuvants evaluated in fourteen different vaccine formulations, only one adjuvant (for confidentiality purposes identified as “Adjuvant B”) showed promising efficacy in combination with the subunit antigen. The vaccination protocol followed with the experimental AHSV-7 VP2 / Adjuvant B-formulation entailed two subcutaneous inoculations with a four week interval between the primary and the secondary inoculation. Adjuvant B in combination with this antigen was unfortunately moderately irritating to the subcutaneous tissues, resulting in unacceptable fibrosis and permanent scarring in a proportion of the vaccinated animals. Failure to identify alternative adjuvants or vaccine formulations significantly delayed progress in work package 9. Only recently, a modification in the route of administration of the recombinant AHSV VP2 / Adjuvant B-formulation was shown to be efficacious and well-tolerated in the horses. A small group (n = 4) horses vaccinated intramuscularly (instead of subcutaneously) with this experimental vaccine formulation developed significant levels of neutralising antibodies without the undesirable tissue reaction and scarring. Neutralisation titres of these animals were between 64 and 128 TCID50/0.1ml and the site of vaccine administration in these horses healed uneventfully.

A serum neutralisation assay based on the constant-antigen, varying serum dilutions methodology in 96 well-microtitre plates was successful. Standardised neutralisation antigen (AHSV 7 and 4) and guinea-pig serum were prepared as a positive control. The challenge model was established using 5ml intravenously-administrated AHSV-infected horse blood in anti-coagulant (OCG). Body temperature monitoring and clinical observations combined with semi-quantitative PCR (i.e. estimation of the magnitude of the viraemia) enabled timeous euthanasia of more than 90% of the critically-affected animals before fatal lung oedema commenced.

Due to the complications encountered during identification of a suitable adjuvant and vaccine formulation, the evaluation of the potential of multivalent AHSV VPS2 subunit vaccines to induce protective immunity against all nine serotypes of ASHV was not reached. With the recent findings

that a moderately irritant formulation can be safely administered intramuscularly, animal trials aimed at the evaluation of multivalent subunit vaccine formulations will be continued beyond this project period.

Work package 10: Optimal vaccine schedule with polyvalent BTV immunogens

LSHTM: BTV DISC viruses have proven to be highly protective vaccine candidates. In the previous reports it was demonstrated that animals inoculated with DISC viruses were completely protected against a virulent challenge. Two animal hosts, cattle and sheep, that were tested showed neutralising immune response and, further, no replication of BTV after challenge. These successful results led to further study on the level of protection afforded by different vaccine strains cocktails and in different vaccination schedules. BTV DISC vaccines were supplied by Partner 1 as triple or as a cocktail of serotypes to Partner 9 and 10 (FLI and DLO-CVI) to investigate immunogenicity, vaccination strategy and serotype interference or immune-dominance. LSHTM analysed seroneutralisation.

Vaccination of cattle of with triple serotype DISC vaccine

In the second periodic report we showed that cattle single vaccinated with DISC viruses BTV-2, BTV-4 or BTV-8 were able to elicit a protective neutralising antibody response. This result suggested that in cattle DISC viruses can be used as vaccines.

We therefore decided to design an animal trial (Partner 9) to analyse the effectiveness of a cocktail vaccine containing three (triplex) BTV serotypes in cattle. Twelve animals were inoculated with a cocktail vaccine with a mixture of serotypes -2, -4 and -8 DISC viruses and a boost was given 21 days apart. Animals were segregated in three groups and each group was challenged with either BTV-2 or BTV-4 or BTV-8 virulent strains. As control three groups of two animals were mock vaccinated with cell lysate (no virus) and subsequently challenged as the vaccinated animals. Blood samples were taken at regular intervals and neutralisation titre was determined by serum neutralisation assay (SNA) for all animals on challenge day.

Neutralising response was evident in 50% of the vaccinated animals (6 out of 12 animals) although not at very high level and only for BTV-8. No neutralising activity was detected for BTV-2 or BTV-4. As expected none of the control animals showed neutralising response. This result suggested that the dose per animal used in this vaccine trial (7.5×10^7 PFU) was not enough to elicit a strong neutralising response. It also may suggest that there could be some interference between the serotypes included in the vaccine, with BTV-8 behaving as the predominant strain.

Vaccination of a cocktail consisting of multiple BTV serotypes in sheep: optimisation vaccine schedule

During the second reporting period a cocktail vaccine with six serotypes (-1, -2, -4, -8, -13 and -21) of DISC BTV was tested in sheep and showed that it afforded neutralising activity against all serotypes included. Further, all vaccinated animals were completely protected against virulent challenge. Three virulent BTV serotypes of the six included in the vaccine were used as a challenge strain and no replication was detected.

These results encouraged us to test the same cocktail vaccine in long lasting protection protocols. For this protocol, eight animals were vaccinated (Partner 10, DLO-CVI) with the cocktail mix in a prime and boost protocol, 21 days apart. All animals were challenged 133 days post second vaccination (154 days post first vaccination). Blood samples were taken regularly throughout the experiment and the neutralisation titres were analysed by SNA at 21 and 133 days post second vaccination.

All vaccinated sheep presented a neutralising response at 21 days post second vaccination. Six animals presented neutralising activity against all serotypes and two animals' only partial activity. None of the control animal had any detectable neutralising antibodies. To study if this neutralising response was also detectable at longer time, samples taken at 133 days post second vaccination were also analysed. All neutralising titres determined at this time point were lower or under the detection limit with the exception of animal 3 that presented a slightly higher titre against BTV-4. These results supported our previous data indicating that the cocktail DISC vaccine can elicit a neutralising response against BTV at 21 days post vaccination. In this new experiment it was established that this response is still detectable at 133 days post vaccination although at lower level. LSHTM has completed all their objectives in this work package and have demonstrated;

- DISC vaccine as a single serotype elicits a protective immune response in sheep and cattle
- A multi-serotype DISC vaccine cocktail elicits a protective immune response in sheep.
- A single vaccine can elicit a protective immune response in sheep.
- Prime-boost strategy provides greater protection for cattle vaccinated with a single serotype and sheep vaccinated with a cocktail vaccine.

FLI: FLI's main contribution was testing monovalent and trivalent DISC vaccines in cattle, while at the same time collecting data to better understand the immune response to vaccination and challenge. The results of the animal trial showed that cattle vaccinated with monovalent DISC immunogens were all protected against challenge infection with the homologous BTV serotype. Two-thirds of the cattle vaccinated with a trivalent DISC vaccine against BTV-2, BTV-4 and BTV-8 showed RNAemia, although the amount of BTV-genome was considerably reduced compared to the control cattle. Two to four of five cattle in each vaccine group showed positive PCR-results. Therefore, the animal trial of the task one of work package 10 evaluating the safety and immunogenicity of trivalent DISC immunogens was repeated during the third reporting period.

Similar to the first animal trial, individual cattle in the trivalent DISC-vaccine groups that were challenged with BTV-2 or BTV-4 showed low amounts of BTV-genome in their blood. However, the number of PCR-positive animals in each group was lower compared to the first animal trial. Of cattle that were challenged with BTV-2 or BTV-4 after DISC-vaccination 75% (three of four) were protected against challenge infection. All four DISC-vaccinated cattle challenged with BTV-8 were fully protected against this serotype. In both experiments, clinical signs in DISC-vaccinated cattle were generally inconspicuous. No swellings at the injection sites or obvious clinical signs as listed in the CVI score sheet were observed during the immunisation and challenge experiments. A transient hyperthermia was measured in some of the DISC-vaccinated cattle in the first three days after the first or booster vaccination.

In conclusion, although both experiments have demonstrated that some cattle vaccinated twice with the trivalent DISC-vaccine against BTV-2, -4 and -8 were not fully protected against viraemia after challenge infection with BTV-2 and BTV-4, DISC-vaccination induced a considerable reduction of the BTV-genome load in their blood. From experiences with inactivated vaccines, a low level RNAemia might be acceptable and a considerable reduction of viremia is most likely sufficient to have a very strong effect on both the clinical outcome and eradication on the population level. However, whether the blood of those animals could be infectious for *Culicoides*-vectors warrants further investigation.

DLO-CVI: Vaccine properties of a cocktail vaccine consisting of DISC vaccines for serotypes 1, 2, 4, 8, 13, 21 was extensively studied in three subsequent sheep trials. Groups of four sheep were used and received one or two vaccinations with an interval of three weeks. Three weeks after one or two vaccinations sheep were completely protected against clinical disease after infection with virulent

BTV-2, 4, or 8. In the third trial, sheep were completely protected against clinical disease after infection with virulent BTV-2 of 8 at five months post vaccination.

Work Package 11: Comparison of pox vectored, DISC and inactivated vaccines

LSHTM: A number of vaccine trials using the DISC strains for optimising vaccination protocol, antigen dose and cocktail mixture have been undertaken in sheep and cattle. These vaccination-challenge studies demonstrated to be very successful as outlined in earlier work packages. Other vaccine trials using a cocktail of canine adenoviruses (CAV2) were undertaken in mice model. A direct comparison of the efficacy between these two vaccines is currently not possible because of this difference in animal species tested. A valid comparison can be achieved between the DISC vaccine and the virus-like particle (VLP) antigens that have been both generated by LSHTM. VLPs were constructed and tested outside of this current program (EU-Framework programme 6). Both antigens can be equally used in the field with high protection of vaccinated animals, with complete safety. It is predicted that DISC vaccines have strong potential for commercialization.

LSHTM & ANSES: LSHTM supplied DISC vaccine to ANSES to be tested in the host animal sheep. A number of vaccine trials using the DISC strains optimising antigen and deliver were undertaken. These vaccination-challenge studies proved to be very successful as outlined in earlier work packages. ANSES also tested the inactivated vaccine manufactured by Merial (Partner 5). This vaccine also proved to be successful in providing protection against disease in sheep. A direct comparison could not be made between the efficacies of the vaccine as the amount of antigen used in the inactivated vaccine was not supplied.

Work Package 12: Development of diagnostic tools for the molecular typing of the various BTV

VAR: Our goal for work package 12 was the validation of the diagnostic tools developed by Partner 13 (IAH) for BTV typing. In anticipation of these new tools preliminary work has been finalised in order to guarantee the completion of the goals once the new tests have been received from partner 13. This work was also carried out in regards to work package 15.

IAH: During this project we developed a segment-9 based group-specific serotype assay that can detect BTV isolates belonging to all 26 BTV serotypes. This assay was validated against multiple isolates of BTV 1-26. The BTV Seg-2 based Real time RT-PCR assay is commercially available from LSI for all of the European BTV types. The assays for all 26 types are now routinely used as diagnostic reagents by the Non-vesicular Reference Laboratory at the Pirbright Institute, to type BTV samples from various countries around the world (~500-600 samples per year) under ISO1702. We developed EHDV group specific real-time RT-PCR assays which have been validated using multiple isolates of various serotypes. EHDV serotype-specific real time RT-PCR assays were also developed and validated for all seven EHDV serotypes. Furthermore, we developed AHSV group specific real-time RT-PCR assays. Serotype specific real time RT-PCR assays were also developed for AHSV. The serotype-specific primers and probes (for real-time quantitative RT-PCR for all nine AHSV serotypes) and these have been validated against the current nine referenced and filed strains of AHSV. We developed EEV group specific real-time RT-PCR assays which have been validated using multiple isolates of various serotypes. EEV serotype-specific real time RT-PCR assays were also developed and validated for all seven EEV serotypes. Conventional RT-PC assays were developed for BTV and AHSC.

Work Package 13: Development of group specific and serotype specific ELISA tests

LSHTM: LSHTM supplied antigen to our partners when required.

IDVet: A BTV8 serotype-specific ELISA kit is available as a prototype and has been tested by partners. It is based on an anti-VP2 monoclonal antibody generated by IDVET and a recombinant BTV8-VP2 generated by IDVET and ANSES. This competitive ELISA distinguishes BTV8 from all other serotypes tested (24 serotypes), except serotype 23 that is related to serotype 8 in term of seroneutralisation. It provides a very good discrimination in BTV8 naturally infected animals confirmed by VNT (sensitivity: 100%; n=47). The specificity measured was 100% (n=347). The seroconversion appears later than on VP7 ELISAs (15-20 dpi). Weak seroconversions are observed on non-infected animals vaccinated with inactivated vaccine. Despite of several attempts to generate anti VP2-BTV1, and three cell fusions, no VP2-BTV1 Mabs were obtained. Since the initiation of the project, molecular techniques for typing BTV viruses have been developed and are commercially available. Thus, the interest for sertotype-specific ELISAs is more limited, and it was decided to stop this part of the work. An EDVH-specific ELISA was developed. It is based on an EHDV VP7 recombinant protein monoclonal antibody generated by IDVET. An experimental EHDV infection study was done in collaboration with ANSES. This allowed the development of the test and its characterisation. The ELISA has excellent specificity (99.6%, n=240) and sensitivity. It detects all EHDV serotypes tested. Seroconversion is detected between 7 and 15 days post infection.

ANSES: Development of group specific and/or serotype specific ELISA tests was based on the sequence specificities of the VP2 and VP5 viral proteins. These two proteins constitute the outer capsid of the EHD or BT orbiviruses. Specific serotype antibodies against VP2 (and VP5) are presented in the sera from animals infected and/or vaccinated. For this study, structural proteins have been expressed in mammalian and/or insect cells and, after purification (through His-tag), used in ELISA tests. ID VET (Partner 15) developed a specific BTV8 ELISA using BTV8 VP2 tagged protein-derived monoclonal antibody, whose prototype has been sent to ANSES (cf ID Vet report).
ANSES has produced a recombinant EHDV6 VP7 protein in order to develop group specific ELISA for EHDV. After development of this test by LSI, evaluation was performed by ANSES and IAH (Partner 13), proving its specificity and the absence of cross-reactivity with BTV infected or vaccinated sera. Nowadays, the first ELISA kit for EHDV detection is commercially available (LSI).

IAH: The outer capsid protein VP2 has been expressed in a soluble form (in bacteria) for BTV serotype 8 and expression levels have been optimised. This provides a source of type specific antigen that is free of any possible contamination with live BTV and does not therefore represent a disease security risk. Monoclonal antibodies will be generated to the expressed VP2, which will then be assessed for use in direct and competition ELISA to detect BTV-8 VP2-specific antigen and BTV VP2 specific antibodies respectively. We have expressed VP2 of BTV-1, BTV-4 and BTV-8 in bacteria (in soluble form) as well as by baculovirus expression system. Specific polyclonal antibodies have been generated in mice and tested both in western blots against lysates of cells infected by the three BTV serotypes and in ELISAs using the purified recombinant proteins as antigens. Recombinant proteins and antibodies are currently being used to develop serotype specific ELISAs for these BTV types. We will also attempt to use the MAGPIX technology to further develop ELISAs. We have also expressed NS1, NS2, NS3 and NS4 of BTV and raised antibodies in mice and rabbits against the individual proteins. The recombinant non-structural proteins and antibodies are also being currently used to develop group specific ELISAs for BTV.

<u>Work Package 14: Determination of viral load by RT-qPCR in BTV/vaccine challenge studies</u>

VAR: The purpose of this work package was to determine independently the viral load in samples received from different project partners conducting vaccination/challenge experiments in such a way that comparison is possible. The new multivalent DISC vaccine (MultiDisc) provided by LSHTM (Partner 1) was evaluated in cattle (FLI, Partner 9) and in sheep (CVI, Partner 10) by a double vaccination/challenge animal trial. In sheep the new MultiDisc resulted in a 100% protection against

a BTV2 or BTV8 challenge. When the vaccinated animals were challenged with BTV4, one out of the six displayed a transient viremia but became negative at the end of the trial. The viremia was not only transient but the viral load was significantly lower than the control animals, clearly demonstrating a vaccine effect in that animal. With the exception of one vaccinated animal, seroconversion was observed at eight dpv but displayed decreasing titers towards 21 dpv. A clear booster effect upon revaccination was found in all animals which resulted in stable high titers. In cattle however, the protection of the MultiDisc was far less pronounced with 40%, 20% and 80% protected animals against a challenge with BTV2, BTV4 or BTV8 respectively. If this was linked to the more variable serological response, certainly after the primer vaccination is not clear. Simultaneously with the new MultiDisc vaccine, monovalent Disc vaccines were also evaluated in cattle. In contrast to the MultiDisc vaccine, the monovalent vaccine protected all animals against a homologous challenge with BTV2, BTV4 or BTV8 as seen by determining the viral RNA load with real-time PCR. Nevertheless, a certain degree of variability in the serological response was still observed albeit less pronounced.
In the “Early Protection study” a good protection in sheep was achieved against BTV-8 challenge with a single dose of the MultiDisc vaccine. A very limited viremia was seen in only one animal and sterile immunity was observed in an animal without a clear IgG response prior to challenge. Although the protection against a BTV-2 challenge was less pronounced, a clear vaccine effect, with a marked reduction in viremia, was already found after a single vaccine dose at 21 dpv. The potential of the MultiDisc vaccine was further demonstrated in the “Duration of Immunity study” where all of the double-DISC vaccinated sheep were 100% protected against a BTV2 or BTV8 challenge even after 154 days post vaccination. Interestingly, the antibody titers were very high 4 days post revaccination (25 dpv) and remained relatively stable throughout the rest of the animal trial. The latter shows clearly the immunogenic potential of the new DISC vaccine. However, the decrease towards 21 dpv, after the primo-vaccination, similarly observed in both prior sheep trial, indicated the necessity of double vaccination. In both animal trials the presence of the DISC vaccine viral RNA could be demonstrated in the blood shortly after vaccination using serotype specific PCRs. In cattle, a TriDisc vaccine was evaluated. It elicited a more pronounced immune response, certainly after primer vaccination, compared to the MultiDisc vaccine, used in the previous cattle trial, as measured by the Early Detection Elisa and cElisa. The latter is reflected by an improved protection percentage when both vaccines are compared. The TriDisc gave a 100% protection against BTV-8 challenge and 75% against BTV2 or BTV4. Furthermore, the level of viremia observed in the sole PCR positive BTV4 animal was significantly reduced compared to the controls, further emphasising the vaccine effect against BTV4. The cross protection in sheep against virulent heterologous challenge with BTV8 or BTV16 was evaluated by ANSES (Partner 2) using commercial inactivated vaccines (BTV 2, 4, 9). In general, it could be stated that the vaccinated animals were not protected against a heterologous challenge as almost all animals became positive. However, in some cases the observed viremia was significantly lower than the control groups indicating a vaccine effect to some degree.

Work Package 15: Development of tests to differentiate BTV infected or vaccinated animals

CIRAD: CIRAD participated in BTV diagnosis improvement and tests. Group and serotype specific ELISAs based on BTV VP2 and/or VP5 have been developed and a specific BTV8 ELISA has been tested.

ANSES: ANSES participated with ID VET (Partner 15) in the evaluation of antigenic properties of non structural proteins (NS1, 2 and 3) in ELISA test. In conclusion of the different tests made, it appears that infected animals as well as vaccinated ones show antibodies against NS2 and NS3, proving their non efficacy as discriminatory antigen for DIVA tests. On the contrary, NS1 protein seems to be a good candidate for such tests (cf ID Vet report). A RT-qPCR based on the segment 9

of EHDV was developed by LSI. This method was validated by ANSES and IAH, proving the specificity of this real-time RT-PCR for EHDV. The kit is available and commercialised by LSI.

VAR: Our goal for this work package was the validation of the newly developed primers (by partner 2 and 4) using currently available primers for BTV in our lab. Anticipated progress was made to determine the feasibility of the non-structural proteins as a potential DIVA test. It was found that both NS2 and NS3 cannot be used as antigen for DIVA BTV tests. Although, NS2 antigen was not a good candidate for a DIVA test, it was found that it could be used as group specific BTV antibodies similar to VP7. Furthermore, a real time RT-PCR for the detection of EHDV genome in blood and spleen samples was developed and is currently being validated by the Pirbright Institute (European Reference Laboratory for EHDV) and commercially available.

Work Package 16: Development of tests to evaluate immunoresponse for AHSV vaccine

UCM: The focus of this work package was to improve serological, molecular and immunological techniques to obtain a better knowledge on the immunological response elicited upon the infection and vaccination with AHSV, since many aspects of the immunology of AHS remain unknown. For these purposes, the work package has been divided in five tasks. Within the framework of task one, different tests for the serological and molecular detection of ASHV have been validated in the UCM laboratory.

These tests have been used to analyse the AHSV viraemia and antibody response in infected and vaccinated horses. In addition, evaluation of other tests has been performed to improve the diagnostic test. In order to analyse the levels of cytokines produced by vaccinated and infected animals UCM developed, optimised and validated a set of RT-PCRs to quantify cytokine expression (Interleukin (IL)-1, IL-2, IL-4, IL-10, IL-12, TNFα, IFNγ and IFNβ). These RT-PCRs based on SYBR Green (Sánchez-Matamoros et al. 2013) have been used to study the profile of the cytokines produced by infected and vaccinated horses. In order to identify the lymphocyte population involved in immune response of vaccinated and infected horses, UCM tested a blood stabiliser (conservation of cell morphology) with antibodies against CD4, CD8, CD14 and lymphocyte B (CD21) on horse blood cells to be able to perform the flow cytometry assays in the Partner 3 facilities. This protocol has been used to determine possible variations in immune cells populations elicited by vaccination and infection.

The results of all the immunological/virological analyses, cytokine RT-PCRs, AHSV RT-PCR, virus neutralisation, ELISA, PENSIDE and flow cytometry; have allowed to enhance our understanding of the horse immune response to AHSV infection. Finally, task 5 had the aim to express of AHSV non-structural protein NS3 in order to use it in a DIVA ELISA test.

INGENASA: During the project, Partner 16 has developed and evaluated a DIVA ELISA for AHSV based on the non-structural protein NS3. Since this protein is not present in the virus, the level of concentration of this protein in the vaccines should be low and therefore the horses will not induce an immune response against NS3. Recombinant NS3-AHSV4 protein has been expressed and used to produce specific monoclonal antibodies (MAbs). These MAbs have been characterised by ELISA, Western blot and IPMA with different AHSV serotypes. Those MAbs able to recognize the NS3 protein of all the serotypes tested have been used to develop a blocking-ELISA in order to detect specific antibodies against the AHSV NS3 protein. This new DIVA ELISA has been validated with positive and negative field horse sera and with all the experimental sera produced by Partner 6 during the project. This new ELISA detects specific NS3 antibodies, which are only present in the sera of the infected animals. Therefore, this DIVA test for AHSV will be an important tool for the control of the virus and the disease.

4. The potential impact **(including the socio-economic impact and the wider societal** implications of the project so far) and the main dissemination activities and exploitation of results

Orbivirus diseases, particularly Bluetongue (BT) of livestock, African Horse Sickness (AHS) of equids and Epizootic Hemorrhagic disease (EHD) of deer, are some of the big challenges facing European agriculture.

Based on the latest developments in vaccine and orbivirus research, there are a number of exciting approaches that offer the potential to produce effective multivalent vaccines to orbivirus diseases.

However, none of the current commercial vaccine approaches, inactivated and attenuated virus vaccines, are compliant with DIVA principles, and therefore it is necessary for a new generation of vaccines that overcomes this limitation to be developed. The approach that was used is based on proven observations that highly attenuated viruses, VLPs and the ***viral neutralisation protein VP2*** of BTV and AHSV are protective.

During this highly successful project, one of the consortium partners (Partner 1 - LSHTM), who is a leader in orbivirus reverse genetics, was responsible for generating a new class of **D**isabled **S**ingle **C**ycle (DISC) vaccine for BTV. This vaccine promises improved immunogenicity over non-replicating vaccines and avoids the problems associated with replicating attenuated vaccines. A complementary, protein based, AHSV multivalent subunit vaccine based on VP2 has also been produced by LSHTM. In addition, Partner 1 also developed VLP vaccines for EHDV, similar to the BTV VLPs of 4 different viral structural components. Other partners have specific expertise in the use of Canine Adenovirus and Capripox viruses as delivery systems for orbivirus antigens and were responsible for evaluating these systems in order to deliver optimised multivalent immunogens (Partners 2 - ANSES, 4 - CIRAD, 5 - MERIAL). Another partner, in collaboration with one of the industrial partners, used parapox virus as an expression system for the VP2 protein of AHSV (Partners 3 - UCM & 6 - PFIZER).

The project also generated new reagents and methods for rapid diagnosis and typing of orbivirus outbreaks and to distinguish between infected and vaccinated animals. Current typing of orbivirus diseases is based on RT-PCR approaches. The consortium developed real-time RT-PCR assays for all 26 BTV serotypes as well as microarrays to provide high throughput and improved sensitivity for the detection and rapid typing of diagnostic BTV samples. The new molecular (microarray) assays have been independently validated against current RT-PCR based systems and reference samples from previously identified BTV strains for speed and accuracy.

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

BTV

Two types of licensed BTV vaccine are currently available; live attenuated vaccines and inactivated (killed) vaccines but each has associated risks. The live attenuated BTV vaccines have been used for many years in Africa but can cause high levels of viraemia and severe clinical signs in European sheep breeds. Moreover, teratogenic effects have been reported in newborn following vaccination of pregnant ewes and cows. In addition, they can infect feeding *Culicoides* insect and be transmitted in the field leading to outbreaks of BT disease in non-vaccinated areas. Several cases of infection by BTV-2, BTV-6 and BTV-16 in Europe have been linked to vaccine strains and as a consequence the use of these vaccines in Europe is not recommended. Effective inactivated vaccines for some BTV serotypes are available as relatively crude preparations and ~120 million animals were vaccinated against BTV-8 in Europe in 2009 and 2010 at a cost of >€150M. However, killed vaccines also have drawbacks including a large initial dose required to induce immunity and the possibility that the vaccine batch has not been fully inactivated and may contain live virus leading to a vaccine associated outbreak. A safe vaccine that will protect against all currently circulating European types of the virus is therefore a priority; and the market for an improved vaccine is significant. Crucially, there are 26 serotypes of the virus potentially able to cause disease yet currently available BTV vaccines are limited to only a few serotypes. The potential for a rapid, scalable and controlled multi-serotype vaccine cocktail is very significant.

Impact of research: During the project, a Vaccine technology based on Reverse Genetics (RG) System was developed to generate replication-deficient vaccine strains (DISC) for BTV that can only be propagated on specific engineered cells, but not in any normal cells or animal species. In total, 11 BTV DISC viruses for serotypes BTV -1, -2, -4, -8, -9, -10, -13, -16, -21, -23 and -24 were successfully generated and shown to be unable to propagate in normal tissue culture cells. A range of stability tests including a number of stabilising agents and temperatures range was also performed.

These DISC virus strains have been tested as vaccine candidates both as single or cocktail mixture. It was found that:

- The DISC vaccine as a single serotype elicits a protective immune response both in sheep and cattle, the main hosts of BTV replication.
- Multi-serotype DISC vaccine cocktail elicits a strong protective immune response in sheep.
- Prime-boost strategy provides greater protection for cattle vaccinated with a single serotype and sheep vaccinated with a cocktail vaccine.

In parallel, protective efficacy of an adenovirus vectors cocktail after homologous and heterologous BTV challenge was performed, demonstrating that with using one vector combination it is possible to protect model mice against different BTV serotypes. Furthermore, four peptides cross-reacting with the four Mediterranean BTV serotypes have been identified, two of the four were synthesised and evaluated for their neutralising activities in sheep, but sera samples did not show the induction of any neutralising activity for any of the peptides.

AHSV

African horse sickness (AHS) is a highly fatal viral disease causing up to 90% death in a naïve equine population within one week of infection. Death of the horse results from severe pulmonary oedema and cardiac arrest in horses and also infect (without disease symptom) other animals (mules, donkeys, zebras, goats), which act as reservoirs. AHSV, a member of the *Orbivirus* genus (family *Reoviridae)* is closely related to Bluetongue virus (BTV), discussed above. AHSV and BTV are the most economically important members of the genus.

AHSV is an insect transmitted virus, spread primarily by blood sucking midges (*Culicoides* species) that are also responsible for the transmission of BTV. The virus emerged in the UK in 2008 but did not become endemic due to wide spread vaccination. The spread of AHSV and BTV is influenced by climatic conditions that favour the survival of insect vectors (warm, moist weather, high rainfall), that are also spread via wind dispersal. In the last decade, global distribution of *Culiciodes* transmitted orbiviruses has changed dramatically. This is highlighted by the emergence of multiple serotypes of AHSV in West and sub-Saharan Africa, multiple serotypes of BTV into northern Europe, USA, Middle East, and high activity of Epizootic hemorrhagic disease of deer virus (EHDV) in the Middle East and USA.

It is likely that climate change will increase the distribution and severity of arthropod-borne virus (arbovirus) disease of animals and humans. The sudden outbreaks of a number of arboviruses (e.g. West Nile virus, Chikungunya virus, Dengue virus) in unexpected areas illustrate the capacity of the spread of vector-borne viruses into naïve areas. The biting midges already exist in UK and Europe and BTV outbreaks have changed from sporadic in the Mediterranean basin to endemic in northern Europe.

Impact of research: During this research consortium, immunogenicity of all 9 VP2 was tested individually in guinea pigs, which showed that each VP2 has the capability to elicit strong neutralising antibodies against AHSV. Formulation for long-distance transportation conditions of VP2, which maintains immunogenicity capabilities, was determined and optimised. Immunogenicity of AHSV VP2 in horses was assessed and showed good level of virus neutralisation. Most excitingly, results indicate that the horses have developed a strong neutralising antibody response. When these horses were challenged with a virulent virus infection, they did not develop any clinical reactions in contrast to the unvaccinated control horses, which showed strong disease symptoms. The data suggested that the formulation of VP2 and long-distance transportation maintained the immunogenicity of the subunit vaccine as expected. In sum, this study confirmed that VP2 based multivalent vaccines have potential to be used in Europe and elsewhere.

Evaluation of the cellular and antibody responses of pre and post challenged small model animals that were vaccinated with Parapox-based vaccines was performed. The immune response elicited by the vaccination and infection of horses did not show any differences between vaccinated and infected animals, indicating that parapox-vectored vaccine may be suitable for horses.

EHDV

Epizootic hemorrhagic disease is widespread in North American white-tailed deer and periodically causes serious epidemics in wild populations. Until recently, it was believed that EHDV (7 different serotypes, EHDV-1 to EHDV-7) did not cause significant clinical signs in cattle, with the exception of the Ibaraki virus, a Japanese isolate, which caused several outbreaks in Japan and Korea wherein thousands of herd of cattle were affected. Recently, however, outbreaks of EHDV in dairy cattle were reported from the island of Reunion, Israel, Morocco, Algeria and Turkey. The latter 3 outbreaks were caused by EHDV serotype 6. A reassortant strain with this particular serotype was recently isolated from several states in the USA. This was parallel to EHDV-associated morbidity in cattle that was reported in 2007 in USA. During these epidemics, the economic impact of EHD disease in cattle was characterised by the significantly reduced milk production and high mortality. In addition to the economic losses associated with this, the possibility of infection means that there is a restriction of international movement of livestock from countries suspected to harbour the disease. Such a restriction could also lead to significant economic losses for EHDV-endemic countries.

Impact of research:

- During the project, VLPs for EHDV-1 and -2 serotypes were developed using Baculovirus expression system.
- An EDHV-specific ELISA has been developed.
- A serotype-specific ELISA allows for a distinction between BTV, EHDV and AHSV infected sera from the 23 other serotypes that were developed.
- EHDV group specific and serotype-specific real-time RT-PCR assays have been developed and validated.

Socio-economic Impact

Socially, immediate beneficiaries include farmers, traders, exporters, meat producers, wool producers and the public end user. Wider beneficiaries include the agricultural community in general and the government bodies charged with ethically acceptable control of animal disease. Since the Foot and Mouth disease virus outbreak of 2001 it has been widely acknowledged that animal disease has great empathy with the public and those methods of control that prevent mass slaughter with the associated distressing views of carcass disposal would have huge public appeal.

The current and future impact of the Innovation relates to the seriousness and economic consequences of Bluetongue disease and the other BTV related diseases, African Horse Sickness (AHSV) and Epizootic Hemorrhagic Disease of Deer (EHDV).

BTV: Clinically BTV, which is transmitted by *Culicoides* midges to sheep, can result in weight loss, wool break and death with examples such as Cyprus in 1943 and Portugal and Spain in 1956 where 60-70% of flocks were lost. Since 1998 BTV has been active on the continent every year causing the deaths of more than 2 million animals with associated economic losses to the agricultural economies of Europe. In 2006 a highly pathogenic BTV-8 strain emerged for the first time in Northern Europe spreading very rapidly and affecting thousands of herds. The same serotype re-emerged in 2007 and 2008, causing devastating disease not only in sheep but also in cattle with high morbidity and mortality. The emergence of BTV-8 in France alone in 2007 cost more than $1.4 billion (US). In 2008 Belgium, France, Germany, Luxembourg, the Netherlands and Switzerland declared to OIE that they considered BTV-8 to be endemic within their borders. As a result BTV is an Office International Des Epizootics (OIE) list A pathogen and the economical loss attributed to BTV outbreaks is significant. Vaccination is the only control measure that has been shown to impact

the disease and its spread to neighbouring locales. The vaccines developed in this Consortium will have a long-term impact both in Europe and elsewhere.

AHSV: The economic and social impacts are significantly different between agricultural and equine industry. The control measures including animal slaughter, vaccination, movement restrictions and vector control methods are well understood for most agriculturally important disease where as outbreaks of equine diseases have a degree of extra complexities, as animal slaughter would be unacceptable and restriction in horse movement could potentially have a more serious impact on the industries than the disease. The economic impact of an AHSV outbreak in Europe could amount to several billions. The increase in horse movement for racing carnivals, sporting events (polo, dressage, etc) and importation of semen and embryos has increased the risk associated with the introduction of exotic viruses, such as AHSV. A worst-case scenario is the rapid spread of the virus once introduced, by wind borne spreading of midges into a susceptible naïve horse population in which 75-95% could die. In the past, vaccination was used to control and eradicate AHSV in Spain and Iberian Peninsula outbreak, there are currently no AHSV vaccine licensed for use within the EU to prevent its spread and minimize economic impact due to safety issues associated with the vaccine. The subunit AHSV vaccines that have been developed during this project, as well as those that are still in the developmental phase, are all very safe, efficacious and affords protection would have a major impact in preparing the EU for any potential outbreak.

EHDV: The social impact of EHDV outbreaks in comparison to BTV and AHSV is harder to gauge as they tend to occur mainly in wild animals in addition to occasionally in cattle with severe economic consequences. Since EHDV predominantly occur in wild deer, the nature enthusiast and social/professional hunters are initially affected. Sick and dying deer congregate around water sources, this in turn may pollute the water making it unsafe for human consumption as well as creating a distressing scene. This results in park having restricted access or closing certain routes. During outbreaks, hunting permits and licences (antler/antlerless) are either limited or not issued depending on the severity of the outbreak. Reducing permits and licenses can have consequences including less income from licenses means less spending toward wildlife and park management. This has a flow on effect to the local communities which provide service to wild life enthusiast and hunters as the displaced hunters may hunt in other area which would impact the expected harvest and population of deer in those areas. There is also social hardships caused rural communities due to decrease income associated decreased milk production/productivity in asymptomatic EHDV infection in cows. VLP vaccines that have been developed through this project are protein-based vaccines, completely void of genetic materials. Therefore these vaccines pose no risk for animal to animal transmission. Further, like other subunit vaccines, VLPs are DIVA compliant, thus potentially acceptable vaccines for all stake holders including vaccine manufacturing industries.

Overall the global population of ruminants is estimated at **several billion**, with 40% of these likely to receive veterinary care such as vaccination. The fragmented vaccination policies of the past, the result of 26 distinct serotypes in separate geographical regions, could now be unified. Prophylactic immunization of sheep and cattle alone has the potential to provide complete control of BT disease with a potential annual market of **~£900 million**. The partnerships formed during the research project were strategic in that the potential for the commercial companies to develop and market these vaccines in the very near future has been realised.

Potential applications

The primary outcome is that stable, multivalent, new generation; DIVA compliant vaccines have been produced.

The project produced and tested new prototype vaccines to BTV (DISC and multi-antigen types), AHSV (vector based multiprotein and protein based multivalent subunit) and EHDV (virus-like particles). All of the new vaccines are multivalent and designed to elicit cross protection against multiple strains of 3 different orbiviruses.

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

The consortium comprises several industrial partners, which including both SME and the major industrial companies currently manufacturing vaccines for BTV in Europe. Inclusion of these partners in the same consortium facilitates commercialisation of any of the new vaccine approaches developed by the consortium. The consortium also includes active participation from non-EU countries (USA and Republic of South Africa), with partners who brought specific expertise to the consortium and enhanced its scientific and technical excellence, the aim being to transfer patented vaccines for BTV, AHSV and EHDV into full scale production.

5. Website and contact details

The website is live at www.orbivac.eu.

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6. Use and dissemination of foreground

6.1 Section A (public)

This section includes two templates

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

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[1] (if available)	Is/Will open access[2] provided to this publication?
1	*Bluetongue Virus*	*Bhattacharya, B.; Roy, P.*	*Manual of Security Sensitive Microbes and Toxins (Liu, D. Ed.)*		*CRC Press*	*UK*	*2014*	*Part V: Microbes affecting Animals: Viruses*	*ISBN 97814 66553965*	*No*
2	*Immunogenicity of recombinant VP2 proteins of all nine serotypes of African horse*	*Kanai, Y.; Roy, P. et al*	*PLoS One*	*In press*	*Public Library of Science*	*UK*	*2014*	*In press*		*Yes*

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

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

	sickness virus									
3	*Development of safe and efficacious Bluetongue virus vaccines*	*Stewart, M, Roy, P.*	*Molecular Vaccines Volume 1 (Giese, M. Ed.)*		*Springer-Verlag Wien*	*Vienna, Austria*	*2013*	*pp313-327*	*ISBN: 978-3-7091-1418-6*	*No*
4	*Virus-Like Particles*	*Roy, P*	*In: Bionanotechnology: Biological self-assembly and its application (*B.H.A. Rehm ed*)*		*Caister Academic Press*	*UK*	*2013*	*Chapter 7*	*ISBN: 978-1-908230-16-4*	*No*
5	*Bluetongue virus serotype 8 virus-like particles protect sheep against virulent virus infection as a single or multi-serotype cocktail immunogen*	*Stewart, M.; Roy, P. et al.*	*Vaccine*	*31(3)*	*Elsevier*	*UK*	*2013*	*pp. 553– 558*	*doi: 10.1016/j.vaccine.2012.11.016*	*No*
6	*Multiple large foreign protein expression by a single recombinant baculovirus: A system for production of multivalent vaccines*	*Kanai, Y.; Roy, P. et al*	*Protein Expression and Purification*	*91(1)*	*Elsevier*	*UK*	*2013*	*pp. 77–84*	*doi: 10.1016/j.pep.2013.07.005*	*No*
7	*Recovery of African horse sickness virus from synthetic RNA*	*Kaname, Y; Roy, P. et el.*	*Journal of General Virology*	*Volume 94*	*Society for General Microbiology (SGM)*	*UK*	*2013*	*pp. 2259–2265*	*doi:10.1099/vir.0.055905-0*	*Yes*
8	*Rapid generation of replication-deficient monovalent and multivalent vaccines for bluetongue virus: protection against virulent virus challenge*	*Celma, C.; Roy, P. et al.*	*Journal of Virology*	*Volume 87, No 17*	*American Society for Microbiology*	*USA*	*2013*	*pp. 9856–9864*	*doi: 10.1128/JVI.01514-13*	*Yes*

	in cattle and sheep									
9	*Control of bluetongue in Europe*	*Zientara, S., Sánchez-Vizcaíno, J. M.*	*Veterinary Microbiology*	*Volume 165, Issues 1–2*	*Elsevier*	*Amsterdam, The Netherlands*	*2013*	*pp. 33-37*	*doi: 10.1016/j.vetmic.2013.01.010*	*No*
10	*Development and validation of a SYBR Green real-time RT-PCR assay for quantification of cytokine gene expression in equines.*	*Sánchez-Matamoros; Sánchez-Vizcaíno, J. M. et al.*	*Cytokine*	*Volume 61, Issue 1*	*Elsevier*	*Amsterdam, The Netherlands*	*2013*	*pp. 50-53*	*doi:10.1016/j.cyto.2012.10.004*	*No*
11	*Comparative study of clinical courses, gross lesions, acute phase response and coagulation disorders in sheep inoculated with bluetongue virus serotype 1 and 8*	*Sánchez-Cordón; Sánchez-Vizcaíno, J. M. et al.*	*Veterinary Microbiology*	*Volume 166, Issues 1–2*	*Elsevier*	*Amsterdam, The Netherlands*	*2013*	*pp. 184-194*	*doi: 10.1016/j.vetmic.2013.05.032*	*No*
12	*The use of infrared thermography as a non-invasive method for fever detection in sheep infected with bluetongue virus*	*Pérez de Diego; Sánchez-Vizcaíno, J. M. et al.*	*The Veterinary Journal*	*Volume 198, Issue 1*	*Elsevier*	*Amsterdam, The Netherlands*	*2013*	*pp. 182–186*	*doi: 10.1016/j.tvjl.2013.07.013*	*No*
13	*Bluetongue Virus RNA Detection by Real-Time RT-PCR in Post-Vaccination Samples from Cattle.*	*De Leeuw, I; De Clercq, K et al.*	*Transbound and Emerging Diseases*	*2013*	*Blackwell Verlag GmbH*	*UK*	*2013*	*Epub ahead of print*	*DOI: 10.1111/tbed.12100*	*No*
14	*Serosurveillance of orbiviruses in wild cervids from Spain.*	*Arenas-Montes AJ; Sánchez-Vizcaíno, J. M. et al*	*Vet Rec*	*11;172(19)*	*Journal of the British Veterinary Association*	*UK*	*2013*	*508-9*	*doi: 10.1136/vr.f2932*	*Yes*

15	*Full genome sequence of a Western reference strain of bluetongue virus serotype 16 from Nigeria.*	*Mertens, PPC.*	*Genome Announc.*	*1(5)*	*American Society for Microbiology*	*USA*	*2013*	*e00684-13*	*doi: 10.1128/genomeA.00684-13*	*Yes*
16	*Use of Bacterial Artificial Chromosomes in Baculovirus Research and Recombinant Protein Expression: Current Trends and Future Perspectives*	*Noad, R. and Roy, P.*	*In: ISRN Microbiology*	*Volume 2012*	*Hindawi Publishing Corporation*	*USA*	*2012*	*Article ID 628797*	*doi: 10.5402/2012/628797*	*Yes*
17	*Protective efficacy of Bluetongue virus-like and subvirus-like particles in sheep: presence of the serotype-specific VP2, independent of its geographic lineage, is essential for protection*	*Stewart, M.; Roy, P. et al*	*Vaccine*	*30(12)*	*Elsevier*	*Amsterdam, The Netherlands*	*2012*	*pp. 2131-9*	*doi: 10.1016/j.vaccine.2012.01.042*	*No*
18	*Bluetongue virus, a double-stranded RNA virus, induces type I IFN in primary plasmacytoid dendritic cells via a MyD88 dependent TLR7/8 independent signalling pathway*	*Ruscanu, S.; Roy, P. et al*	*Journal of Virology*	*86(10)*	*American Society for Microbiology*	*USA*	*2012*	*pp. 5817-28*	*doi: 10.1128/JVI.06716-11*	*Yes*
19	*Complete genome sequence analysis of a reference strain of bluetongue virus serotype 16.*	*Maan S.; Mertens, P. et al*	*Journal of Virology*	*86(18)*	*American Society for Microbiology*	*USA*	*2012*	*10255-6*	*doi: 10.1128/JVI.01672-12*	*Yes*
20	*Genome sequence of a reassortant strain of bluetongue virus serotype 23 from western India.*	*Maan S.; Mertens, P. et al*	*Journal of Virology*	*86(12)*	*American Society for Microbiology*	*USA*	*2012*	*7011-2*	*doi: 10.1128/JVI.00671-12*	*Yes*
21	*The genome sequence of a reassortant bluetongue virus serotype 3 from India.*	*Maan S.; Mertens, P. et al*	*Journal of Virology*	*86(11)*	*American Society for Microbiology*	*USA*	*2012*	*6375-6*	*doi: 10.1128/JVI.00671-12*	*Yes*
22	*The genome sequence of*	*Maan S.; Mertens, P.*	*Journal of Virology*	*86(10)*	*American*	*USA*	*2012*	*5971-2*	*doi:*	*Yes*

	bluetongue virus type 10 from India: evidence for circulation of a western topotype vaccine strain.	*et al*			*Society for Microbiology*				*10.1128/JVI.00596-12*	
23	*The genome sequence of bluetongue virus type 2 from India: evidence for reassortment between eastern and western topotype field strains.*	*Maan S.; Mertens, P. et al*	*Journal of Virology*	*86(10)*	*American Society for Microbiology*	*USA*	*2012*	*5967-8*	*doi: 10.1128/JVI.00536-12*	*Yes*
24	*Complete genome sequence of an isolate of bluetongue virus serotype 2, demonstrating circulation of a Western topotype in southern India.*	*Maan S.; Mertens, P. et al*	*Journal of Virology*	*86(9)*	*American Society for Microbiology*	*USA*	*2012*	*5404-5*	*doi: 10.1128/JVI.00420-12*	*Yes*
25	*Full genome sequence of bluetongue virus serotype 1 from India.*	*Maan S.; Mertens, P. et al*	*Journal of Virology*	*86(8)*	*American Society for Microbiology*	*USA*	*2012*	*4717-8*	*doi: 10.1128/JVI.00188-12*	*Yes*
26	*Identification and differentiation of the twenty six bluetongue virus serotypes by RT-PCR amplification of the serotype-specific genome segment 2.*	*Maan S.; Mertens, P. et al*	*PLoS One*	*7(2)*	*Public Library of Science*	*USA*	*2012*	*e32601*	*doi: 10.1371/journal.pone.0032601*	*Yes*
27	*Characterization of Protection Afforded by a Bivalent Virus-Like Particle Vaccine against Bluetongue Virus Serotypes 1 and 4 in Sheep*	*Perez de Diego, A.C.; Roy, P.; Sánchez-Vizcaíno, J. M. et al*	*PLoS ONE*	*6 (10)*	*Public Library of Science*	*USA*	*2011*	*e26666*	*doi: 10.1371/journal.pone.0026666*	*Yes*
28	*Generation of Replication-Defective Virus-Based Vaccines That Confer Full Protection in Sheep against Virulent Bluetongue Virus Challenge*	*Matsuo, E.; Roy, P. et al.*	*Journal of Virology*	*85(19)*	*American Society for Microbiology*	*USA*	*2011*	*pp. 10213-10221*	*doi: 10.1128/JVI.05412-11*	*Yes*

29	*Contamination in bluetongue virus challenge experiments*	*Eschbaumer, M.; Zientara, S. et al.*	*Vaccine*	*29(26)*	*Elsevier*	*Amsterdam, The Netherlands*	*2011*	*pp. 4299-4301*	*doi: 10.1016/j.vaccine.2011.04.049*	*No*
30	*Serotype specific primers and gel-based RT-PCR assays for 'typing' African horse sickness virus: identification of strains from Africa.*	*Maan S.; Mertens, P. et al*	*PLoS One*	*6(10)*	*Public Library of Science*	*USA*	*2011*	*e25686*	*doi: 10.1371/journal.pone.0025686*	*Yes*
31	*Validation of a novel approach for the rapid production of immunogenic virus-like particles for bluetongue virus*	*Stewart, M; Roy, P. et al.*	*Vaccine*	*28(17)*	*Elsevier*	*Amsterdam, The Netherlands*	*2011*	*3047-3054*	*doi: 10.1016/j.vaccine.2009.10.072*	*No*
32	*Bluetongue virus coat protein VP2 contains sialic acid-binding domains, and VP5 resembles enveloped virus fusion proteins.*	*Zhang , X.; Roy, P. et al*	*Proceedings of the National Academy of Sciences USA*	*107 (14)*	*National Academy of Sciences*	*USA*	*2010*	*pp. 6292–6297*	*doi: 10.1073/pnas.0913403107*	*Yes*
33	*RT-PCR assays for seven serotypes of epizootic haemorrhagic disease virus & their use to type strains from the Mediterranean region and North America.*	*Maan S.; Mertens, P. et al*	*PLoS One*	*5(9).*	*Public Library of Science*	*USA*	*2010*	*e12782*	*doi: 10.1371/journal.pone.0012782*	*Yes*
34	*Full genome characterisation of bluetongue virus serotype 6 from the Netherlands 2008 and comparison to other field and vaccine strains.*	*Maan S.; Mertens, P. et al*	*PLoS One*	*5(4)*	*Public Library of Science*	*USA*	*2010*	*e10323*	*doi: 10.1371/journal.pone.0010323*	*Yes*
35	*The role of wildlife in bluetongue virus maintenance in Europe: Lessons learned after the natural infection in Spain*	*Ruiz-Fons, F.; Sánchez-Vizcaíno, J. M. et al*	*Virus Research*	*In review*	*Elsevier*	*Amsterdam, The Netherlands*		*Epub ahead of print*	*doi: 10.1016/j.virusres.2013.12.031*	*No*

36	*Evaluation of immune mechanisms in horses exposed to two different serotypes of African Horse Sickness Virus (AHSV) and their relation to the clinical form.*	*Sánchez-Matamoros, A., Sánchez-Vizcaíno, J. M. et al*	*Veterinary Microbiology*	*In review*	*Elsevier*	*Amsterdam, The Netherlands*				*No*

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

NO.	Type of activities[3]	Main leader	Title	Date/ Period	Place	Type of audience[4]	Size of audience	Countries addressed
1	*Conference: Keynote speaker*	*P. Roy*	*Structure-based designer vaccines for Orbiviruses*	*October 2013*	*Seventh Vaccine & International Society for Vaccines (ISV) Annual Global Congress, Spain*	*Scientific Community*	*300*	*International*
2	*Conference: Poster*	*J.M. Sánchez-Vizcaíno et al*	*Cytokine and antibody responses in horses showing different clinical forms of African horse sickness virus*	*October 2013*	*Seventh Vaccine & International Society for Vaccines (ISV) Annual Global Congress, Spain*	*Scientific Community*	*300*	*International*
3	*Congress*	*T. van den Berg*	*Epizone Meeting 2013*	*October*	*Brussels,*	*International*	*300*	*Europe, U.S., Africa,*

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

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

				2013	Belgium	scientists		Australia
4	Consortium meeting: NA array development: Validation of the NA array for BTV detection	P. Mertens et al	WildTech	September 2013	Windsor, UK	Scientific Community	25	International
5	Conference: Presentation	P. Roy et al	Development of reverse genetics systems for African Horse Sickness Virus with T7 RNA transcripts	July 2013	American Society for Virology: 32nd Annual Meeting	Scientific Community	300	International
6	Conference: Presentation	P. Roy et al	Newly developed in vitro and in vivo assembly assays for investigating Bluetongue Virus RNA packaging order and signals	July 2013	American Society for Virology: 32nd Annual Meeting	Scientific Community	300	International
7	Other – Training AHSV	J.M. Sánchez-Vizcaíno et al	Diagnosis and control of African Horse Sickness Virus	June 2013	SUAT-VISAVET (UCM) Research center. Madrid, Spain	Scientific Community	3	Bulgaria
8	Conference: Keynote speaker	P. Roy	Non-Replicating Viral Vaccines and Novel Antigen Delivery System	May 2013	Vaccine Delivery Systems for the Future, Euroscicon	Scientific Community/ Commercial	200	International
9	Meeting	K. De Clercq	Communication group	May 2013	Coda-Cerva, Belgium	National and local labs	50	Belgium
10	Lecture	P. Roy	Phased replication by Bluetongue virus and its application to vaccine design	April 2013	The Scripps Research Institute	Scientific Community	100	International
11	Meeting: NA array development and pathogen sequencing -Validation of the NA array for BTV – EHDV detection.	P. Mertens et al	WildTech meeting AHVLA – UK	April 2013	UK	Scientific Community	25	International
12	Conference Presentation	P. Roy et al	Development of virus-like particle vaccines for Epizootic Hemorrhagic Disease Virus (EHDV)	March 2013	SGM Annual Conference 2013	Scientific Community	100	International

13	*Conference Presentation*	*P. Roy et al*	*The use of reverse genetics to identify the functional domains of NS2 during Bluetongue Virus replication*	*March 2013*	*SGM Annual Conference 2013*	*Scientific Community*	*100*	*International*
14	*Workshop – Oral presentation*	*J.M. Sánchez-Vizcaíno et al*	*Epidemiological situation and control of African horse sickness.*	*February 2013*	*CEI Campus Moncloa UCM-UPM. Madrid, Spain*	*Scientific Community*	*40*	*International*
15	*Symposium: Keynote speaker*	*P. Roy*	*Bluetongue virus molecular biology*	*November 2012*	*Eleventh International Symposium on Double-Stranded RNA Viruses, Puerto Rico*	*Scientific community*	*500*	*International*
16	*Conference presentation*	*P. Roy et al*	*Bluetongue Virus VP4: Structure Function relationship*	*November 2012*	*Eleventh International Symposium on Double-Stranded RNA Viruses, Puerto Rico*	*Scientific community*	*300*	*International*
17	*Conference presentation*	*P. Roy et al*	*Use of reverse genetics systems for rational designing of highly attenuated BTV vaccines*	*November 2012*	*Eleventh International Symposium on Double-Stranded RNA Viruses, Puerto Rico*	*Scientific community*	*300*	*International*
18	*Podcast*	*P. Roy*	*Science Weekly podcast: Engineering living tissue*	*August 2012*	*The Guardian*	*Scientific community*	*General Public*	*International*
19	*Workshop – Oral*	*J.M. Sánchez-Vizcaíno*	*New Advances in The Diagnosis*	*July 2012*	*Ministry of Agriculture,*	*Multiple*	*50*	*Spain*

	presentation		*and Vaccination of African Horse Sickness*		*Food and Environment, Madrid, Spain*			
20	*Symposium: Keynote speaker*	*P. Roy*	*Bluetongue Virus: replication, assembly and egress*	*May 2012*	*Seventh International Virus Assembly Symposium, Spain*	*Scientific community*	*150*	*International*
21	*Conference Presentation*	*J.M. Sánchez-Vizcaíno et al*	*Serotypes of African horse sickness with highest risk to Spain*	*April 2012*	*VII Complutense Conference, VI National Congress of Research for Undergraduate Students in Health Sciences and XI Congress of Veterinary and Biomedical Sciences. UCM*	*Students*	*50*	*Spain*
22	*Conference Presentation*	*J.M. Sánchez-Vizcaíno et al*	*Conventional RT-PCR and real-time RT-PCR with SYBR Green dye for the diagnosis of African Horse Sickness.*	*April 2012*	*VII Complutense Conference, VI National Congress of Research for Undergraduate Students in Health Sciences and XI Congress of Veterinary and Biomedical Sciences. UCM*	*Students*	*50*	*Spain*
23	*Press coverage*	*P. Roy*	*Innovator of the Year 2012*	*March 2012*	*Innovator of the Year 2012*	*Scientific Community*	*General Public*	*UK*
24	*Conference Presentation*	*P. Roy*	*Bluetongue virus: the global spread, genetic diversity and structural constraints*	*March 2012*	*SGM Annual Conference 2012*	*Scientific Community*	*200*	*International*

25	*Conference: Keynote speaker*	*P. Roy*	*From atomic structure to safe vaccines for a viral disease*	*January 2012*	*Women in Science Ninety Ninth Indian Science Congress*	*Scientific Community*	*500*	*International*
26	*Conference: Keynote speaker*	*P. Roy*	*Bluetongue virus in post-genomic era*	*Sept ember 2011*	*International Congress of Virology*	*Scientific Community*	*1000*	*Japan*
27	*Press coverage*	*P. Roy*	*Improved Bluetongue vaccine in works*	*September 2011*	*Online – producer.com*	*Farming community*	*General Public*	*UK*
28	*Press coverage*	*P. Roy*	*Scientists take a step towards developing better vaccines for Bluetongue*	*August 2011*	*Farming UK*	*Scientific and farming community*	*General Public*	*UK*
29	*Press coverage*	*P. Roy*	*Breakthrough In Bluetongue Vaccine*	*August 2011*	*Online*	*Farming community*	*General Public*	*UK*
30	*Press coverage*	*P. Roy*	*A Step Closer To Developing Better Vaccines For Bluetongue*	*August 2011*	*Online*	*Scientific community*	*General Public*	*International*
31	*Conference: Keynote speaker*	*P. Roy*	*Recognising Emerging pathogens through their Structure-Function*	*July 2011*	*First One Health Conference in Africa*	*Scientific Community*	*400*	*South Africa*
32	*Poster*	*P. Pourquier et al*	*IDScreen Bluetongue Range extended: New tools for new challenges*	*April 2011*		*International scientists*	*300*	*Europe, Africa, Asia*
33	*Conference Presentation*	*J.M. Sánchez-Vizcaíno et al*	*Evaluation of the immune response in horses: Quantification of*	*April 2011*	*V National Congress of Research for*	*Students*	*50*	*Spain*

			cytokines and flow cytometry.		*Undergraduate Students in Health Sciences and X Congress of Veterinary and Biomedical Sciences. UCM*			
34	*Other – master class*	*J.M. Sánchez-Vizcaíno*	*Bluetongue and African horse sickness*	*February 2011*	*Master in Virology. Viral diseases of animals. Complutense University of Madrid*	*Scientific Community*	*50*	*Spain*
35	*Workshop of the EU Bluetongue and African Horse Sickness National Reference Laboratories 2010*	*J.M. Sánchez-Vizcaíno*	*Risk factors of AHS entry into Europe*	*December 2010*	*Ministry of Agriculture, Food and Environment, Madrid, Spain*	*Scientific Community*	*100*	*European community*
36	*Conference presentation*	*P. Roy*	*High Resolution Structure of Bluetongue Virus outer capsid and its role in cell entry*	*July 2010*	*American Society for Virology – 29th Annual Meeting*	*Scientific community*	*200*	*International*
37	*Conference: Keynote speaker*	*P. Roy*	*Bluetongue Virus: from structure to vaccine*	*June 2010*	*Centre of Excellence for Emerging and Zoonotic Animal Diseases*	*Scientific community*	*250*	*USA*
38	*Conference: Keynote speaker*	*P. Roy*	*Bluetongue virus: Structure to reverse genetic based vaccine*	*May 2010*	*Seventh Meeting of 'Immunology of domestic animals'*	*Scientific community*	*300*	*France*
39	*Conference Presentation*	*P. Roy*	*Bluetongue virus Proteins: their roles in virus replication, assembly & egress*	*April 2010*	*Marburg*	*Scientific community*	*200*	*Germany*

40	*Conference Presentation*	*P. Roy*	*Three dimensional structure of bluetongue virus and its implications in virus entry*	*April 2010*	*European Congress of Virology*	*Scientific community*	*500*	*Italy*
41	*Symposium: Organiser and Keynote speaker*	*P. Roy*	*Bluetongue virus replication and the molecular basis for rational vaccine development*	*February 2010*	*Bluetongue Virus Symposium – LSHTM*	*Scientific community*	*200*	*National and International*
42	*Lecture*	*P. Roy*	*New approaches to bluetongue virus vaccine development*	*February 2010*	*Ecole Normale Superieure (ENS) EU Virus and Immunity lectures, Lyon*	*Scientific Community*	*150*	*International*
43	*Conference Presentation*	*Sánchez-Vizcaíno, J.M et al*	*Characterization of the network of equine movements and its application to the prevention of diseases such as African Horse Sickness in Castile and Leon.*	*February 2010*	*II Iberian Congress of Veterinary Epidemiology. Veterinary School, Autonoma University of Barcelona.*	*Scientific Community*	*100*	*Spain*

6.2 Section B (Confidential[5] or public: confidential information to be marked clearly)

Part B1

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

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

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.

Type of IP Rights[6]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	No		WO2009068870 (corresponding European application no. EP08854770.8)	Method for Producing Vaccinial Viral Strain of a Virus of the ReoViridae Family	Professor Polly Roy
Patent	No	24/08/2011	WO2010055292 (corresponding European application no. EP2358883 (A2))	Baculoviral vectors	Professor Polly Roy

[5] Not to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

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

Part B2

Type of Exploitable Foreground[7]	**Description of exploitable foreground**	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	**Exploitable product(s) or measure(s)**	**Sector(s) of application**[8]	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**
Vaccine	*VLP based vaccines for: BTV DISC vaccine EHDV AHSV subunit vaccine*	*Yes*	*2015/2016*	*Vaccine for EHDV*	*Veterinary biologics*	*2015*	*Other vaccine, therapeutics and diagnostics*	*LSHTM*
DIAGNOSTIC	*Blocking ELISA for AHSV-NS3 Antibodies detection. DIVA ELISA*	*Yes*	*2015*	*ELISA KIT*	*Veterinarian (1)*	*2015*	*Other vaccine, therapeutics and diagnostics*	*INGENASA*

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

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

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

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

7. Report on societal implications

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

A General Information *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	245266
Title of Project:	Development of Vaccines for BTV, EHDV and AHSV
Name and Title of Coordinator:	Professor Polly Roy, LSHTM

B Ethics

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

RESEARCH ON ANIMALS	
• Did the project involve research on animals?	✓
• Were those animals transgenic small laboratory animals?	-
• Were those animals transgenic farm animals?	-
• Were those animals cloned farm animals?	-
• Were those animals non-human primates?	-
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	-
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	✓
DUAL USE	-
• Research having direct military use	-
• Research having the potential for terrorist abuse	-

C Workforce Statistics

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

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	0
Work package leaders	0	5
Experienced researchers (i.e. PhD holders)	20	18
PhD Students	3	1
Other	15	5

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**4**
Of which, indicate the number of men:	3

D Gender Aspects

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

No	Yes
○	✓

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

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

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

○ Yes- please specify

✓ **No**

E Synergies with Science Education

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

○ Yes- please specify

✓ **No**

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

○ Yes- please specify

✓ **No**

F Interdisciplinarity

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

○ Main discipline[9]: 4.2, 1.5

○ Associated discipline[9]:

○ Associated discipline[9]:

G Engaging with Civil society and policy makers

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

○	Yes
✓	**No**

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

○ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

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

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?

○	Yes
○	No

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

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

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

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

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

13b If Yes, in which fields?

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

13c If Yes, at which level?

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

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**25**
To how many of these is open access[10] provided?	**20**
How many of these are published in open access journals?	**20**
How many of these are published in open repositories?	
To how many of these is open access not provided?	**5**
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ✓ no suitable open access journal available ❑ no funds available to publish in an open access journal ✓ lack of time and resources ❑ lack of information on open access ❑ other[11]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		**n/a**

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

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

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
Difficult to estimate	Approx. 40-50

[10] Open Access is defined as free of charge access for anyone via Internet.

[11] For instance: classification for security project.

I Media and Communication to the general public

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

○ Yes ✓ **No**

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

✓ Yes ○ **No**

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

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

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

❑	Language of the coordinator	✓	**English**
❑	Other language(s)		

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

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

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

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

1.3 Chemical sciences (chemistry, other allied subjects)

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

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

2 ENGINEERING AND TECHNOLOGY

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

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

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

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

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

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

4. AGRICULTURAL SCIENCES

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

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

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

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

6. HUMANITIES

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

6.2 Languages and literature (ancient and modern)

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