Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Animal Production and Health Laboratory

Activities Report

2009

In 2009, the Animal Production and Health Laboratory, within the APH Subprogramme, conducted research on three transboundary animal diseases which are included in the list of economically important animal diseases whose outbreaks have to be notified to the World Organization for Animal Health (OIE):

- Foot and Mouth Disease (FMD). It is a disease of ruminants and pigs and currently it is certainly the most economically important animal disease with a high impact on the trade in animal and animal products. For example, the accidental introduction of this disease into Europe in 2001 cost more than 12 billions US$ for its eradication. With the objective of better managing this disease, in particular to avoid unnecessary culling all animals in infected areas as the only effective control measure, APHL has developed a robust test for differentiating infected animals from vaccinated animals. With this test, it would be possible to vaccinate animals within the outbreak zones to avoid the extension of the disease and then, for the rapid eradication of the disease to cull only those animals that have been truly infected.

- Peste des Petits Ruminants (PPR). This is undoubtedly the most important infectious disease of sheep and goats in countries where it is endemic. In the last few years its enzootic area has been expanding steadily both in Africa and Asia and it is threatening now the production of more than one billion small ruminants. It is caused by a virus whose isolation is problematic and may necessitate in vitro culture for up to 3 weeks if successful. In a major breakthrough for the rapid identification of the pathogen, APHL has developed a cell line that enables the isolation of the virus in just 3 days. In 2009, research was continued to identify a non essential fragment in PPR virus protein that could be used for the production of a marker vaccine that would allow differentiation between vaccinated and infected animals.

- Capripox. This is another important disease of ruminants and is caused by 3 different but very closely related viruses: lumpy skin disease virus, sheeppox virus and goatpox virus, infecting cattle, sheep and goat respectively. In APHL, genes of the genome of many strains have been sequenced to establish an important data bank that can be used for tracing back capripox outbreaks and also studying the evolution of the pathogen. A molecular-based, highly sensitive test has been developed for rapid diagnosis of the disease and genotyping the virus without the need for gene sequencing.

The second main research activity in APHL in 2009 was on the characterization of genetic traits related to inherited resistance to diseases and identifying the genetic markers that could be exploited for the preservation of indigenous animal genetic resources. In this field, two major projects were implemented: the first was to identify chromosomal regions of sheep associated with resistance to intestinal parasites, particularly Haemonchus contortus; the

second was the development and characterization of a goat (Capra hircus) whole-genome radiation hybrid panel. The development of a RH map for the goat will allow researchers that discover a phenotype of interest in the goat to be used as a model for comparative analysis and gene discovery. Because the goat has adapted to virtually every type of environment, it will be a valuable resource for this comparative genomic approach.

APHL has continued the management of the Genetic Repository Bank on small ruminants, repository which can be expanded to include other livestock species. APHL has also worked extensively on the development of other platforms such as an RT-db (Real-Time database) for Quantitative Trait Loci (QTL)/Genes/DNA sequences and genetic characterization in small ruminants. This database will make available the genomic locations of QTL from all known studies on small ruminants. The APHL continued its involvement in fine-mapping a chromosomal region to identify genes responsible for parasite resistance.
The third pillar of APHL activities is in providing the training that will contribute to capacity building in IAEA Member States. In the past year two regional training courses were organized:

- The first was the FAO/IAEA/EC/CIRAD European Regional Training Course on "Molecular Techniques for the Diagnosis of Highly Pathogenic Avian Influenza" (22 September- 2 October 09, Seibersdorf, Austria). This course was supported by the European Commission through the Conflutech project and also by the FAO and the IAEA.

- The second was the Regional Training course on "Genomic DNA preparation, Microsatellite Analyses and Sequencing (December 7-18 2009). This course aimed at enhancing knowledge in genomic DNA preparation, microsatellite analyses and sequencing. The ultimate goal was to train partners in molecular genetic analyses. This course was held with the support of the IAEA Technical Cooperation Programme through the newly initiated TC Regional Project, RER5015 "Supporting Early Warning and Surveillance of Avian Influenza Infection in Wild and Domestic Birds and Assessing Genetic Markets for Bird Resistance".

These courses were attended by over 50 scientists from Africa, Asia and Eastern Europe.

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2. PROGRAMMATIC AND LABORATORY OBJECTIVES

In the common agriculture area, the IAEA and FAO are striving:

(a) To promote sustainable increases in agricultural production and productivity;
(b) To ensure food security worldwide;
(c) To foster management of natural resources
(d) To enhance food quality and safety, protection of the consumer and promotion of agricultural products trade
(e) To contribute to capacity building in FAO and IAEA Member States (MS) in order that they can cope more effectively and quickly to the specific challenges facing to their agricultural economies.

The world's poorest people, some one billion individuals, mostly in Africa and Asia, depend on livestock for ensuring their day-to-day livelihood for food, manure for crop culture, draught power, transport and savings to cope with lean periods that are frequent in the countries in which they live. For livestock researchers, the ultimate goal is a three-pronged approach that aims to have a significant impact on the problems faced by rural economies in MS: reducing poverty and hunger, increasing and sustaining food production and controlling animal diseases. In the past 10 years, the multiplication of international and regional animal health and sometimes public health crises have increased the awareness of different stakeholders about the risks posed by transboundary animal diseases on food security and food safety. The extent of these recurring crises undoubtedly reflects the increase in human and animal movements but must also acknowledge the contribution of environmental alterations caused by climate change. More than 60% of human diseases and 70% of newly emerging diseases are zoonotic, i.e. they cause diseases in both animals and humans. In the face of the as yet unknown consequences of climate changes on livestock production in developing countries, FAO and IAEA have to be prepared to rapidly adjust their agendas to meet the changing needs of vulnerable livestock keepers in the coming decade. Thus, the research activities of the Animal Production and Health Subprogramme of the IAEA are implemented by taking into consideration not only short-term but also long-term objectives. They are focused on the development of nuclear and nuclear-related tools (diagnostic tests, molecular epidemiology tools and improved vaccines) for the control of transboundary animal diseases which pose a major challenge to the production and distribution of food of animal origin and for the preservation of animal genetic resources. Indeed, the world's remaining livestock biodiversity lies mostly in developing countries where small-scale farmers and herders continue to keep a variety of native breeds. It is there that we must look to find and protect animal genetic resources that may prove essential for coping with future global changes in the climate.

3. RESEARCH AND DEVELOPMENT: ANIMAL HEALTH

3.1 FMD Improvement of the stability and robustness of an Enzyme Linked Immunosorbent Assay (ELISA) for Foot and Mouth Disease (FMD) using mutated viral 3ABC non-structural recombinant protein as antigen

Foot and Mouth disease (FMD) is a highly contagious viral disease of ruminants and swine. It constitutes an important threat to livestock production and trade worldwide. Its control would make a major contribution to this international trade. In developed countries, this disease has been eradicated after mass vaccinations and the implementation of a strict stamping out policy involving the culling of susceptible animals in infected zones. This policy was implemented in 2001 in Europe after the accidental introduction of FMD in the United Kingdom (UK) and, for this country alone, its cost was estimated to be at least 12 billions US$. It is obvious that such costs cannot be supported by any of the developing countries where the disease is endemic. Thus those countries where FMD persists are constant threats to other countries where the disease has been eradicated and where vaccination is no longer carried out. An example is the Europe FMD outbreak in 2001 which had an Asian origin. The vigorous implementation of the stamping out policy in which about 2 million animals were culled in the UK, allowed this country to recover very quickly its FMD-free status and thereby the possibility to export animals and animal products. But at the same time this massive animal culling caused a shock to the public with the images of huge piles of carcasses waiting disposal. Since then the stamping out policy is becoming more and more unacceptable politically. This situation has given an impetus to the development of diagnostic tools that would offer the possibility of discriminating between vaccinated animals and those which have been infected. In that case it would be possible to implement vaccination campaigns to control the extension of an outbreak and at the same time to carry out serosurveillance to identify and then eliminate true infected animals. Diagnostic tests that allow such differentiation are named DIVA for ***d***ifferentiation between ***i***nfected and ***v***accinated ***a***nimals.

For many years now, research has been conducted in the Animal Production and Health Laboratory to develop a ***DIVA*** test that could be used in the control of FMD. Initial studies have led to the development of two formats of ELISA, an indirect ELISA (*iELISA)* and a competitive ELISA (*cELISA),* which were developed based on the use of the non-structural protein (NSP) 3ABC of the FMDV. This protein was produced as a recombinant protein in insect cells by a baculovirus, an insect virus, or *in vitro* by a system using the lysate of the bacterium *Escherichia coli* (*E. coli*). During the evaluation process of these tests, it became obvious that the repeatability of the results was subject to significant variations. One explanation for this is the instability of the antigen that is used for coating the ELISA plates. Indeed, the 3ABC NSP itself is highly susceptible to enzymatic degradation because the C part of the protein is a protease, thus it induces its own "self-digestion". To address this instability of the 3ABC NSP, we, in APHL, focussed on the production and use of recombinant proteins which are mutated in such a way that both the protease site of the C portion and also the protease cleavage sites are

inactivated. With this in mind, DNA corresponding to the gene of the FMD 3ABC protein was synthesized with, or without the desired mutation points. By using DNA recombinant technology, the genes were introduced either in the baculovirus for protein production in insect cells or in a plasmid designed for the *in vitro* recombinant protein expression. A long-term stability study with the different recombinant proteins produced in insect cells was carried out over a period of 40 days. They were incubated at 4 different temperatures: +4°C, +37°C, -20°C, -80°C. Aliquots of the different samples were made at day 1, 2, 3, 11, 25 and 40 after incubation and by gel protein electrophoresis followed by the western blot analysis using a monoclonal antibody anti FMD NSP produced in APHL. The three types of proteins that were produced have the following characteristic:

- 3ABC site_pro_mut. The active protease site and the cleavage sites are inactivated;
- 3ABC_mut_optG. The active protease site and the cleavage sites are inactivated and the codons are optimized to improve production of the recombinant protein in insect cells.
- The last protein, 3ABC_WT_Ge, has no modification and thus it is considered as the wild-type protein to be used as a control in studies of the mutated proteins.

The results of this analysis using the proteins which were produced in insect cells (see Figure 1), confirm our hypothesis: the mutated proteins are more stable than the wild type. Indeed, when stored at +4°C, -20°C or even -80°C, the degradation of the wild type protein starts immediately, but the mutated proteins are perfectly preserved when stored at -20°C and -80°C. At +4°C, their degradation is apparent only from day 3 onwards**.** Incubation at +37°C results in complete protein degradation of the samples. With this long term study we have clearly shown that the intrinsic protease activity of the FMDV 3ABC protein plays a key role in its instability. It is well known that insect cells are rich in proteases and those enzymes are almost certainly responsible for the degradation of the mutated 3ABC protein at + 37°C.

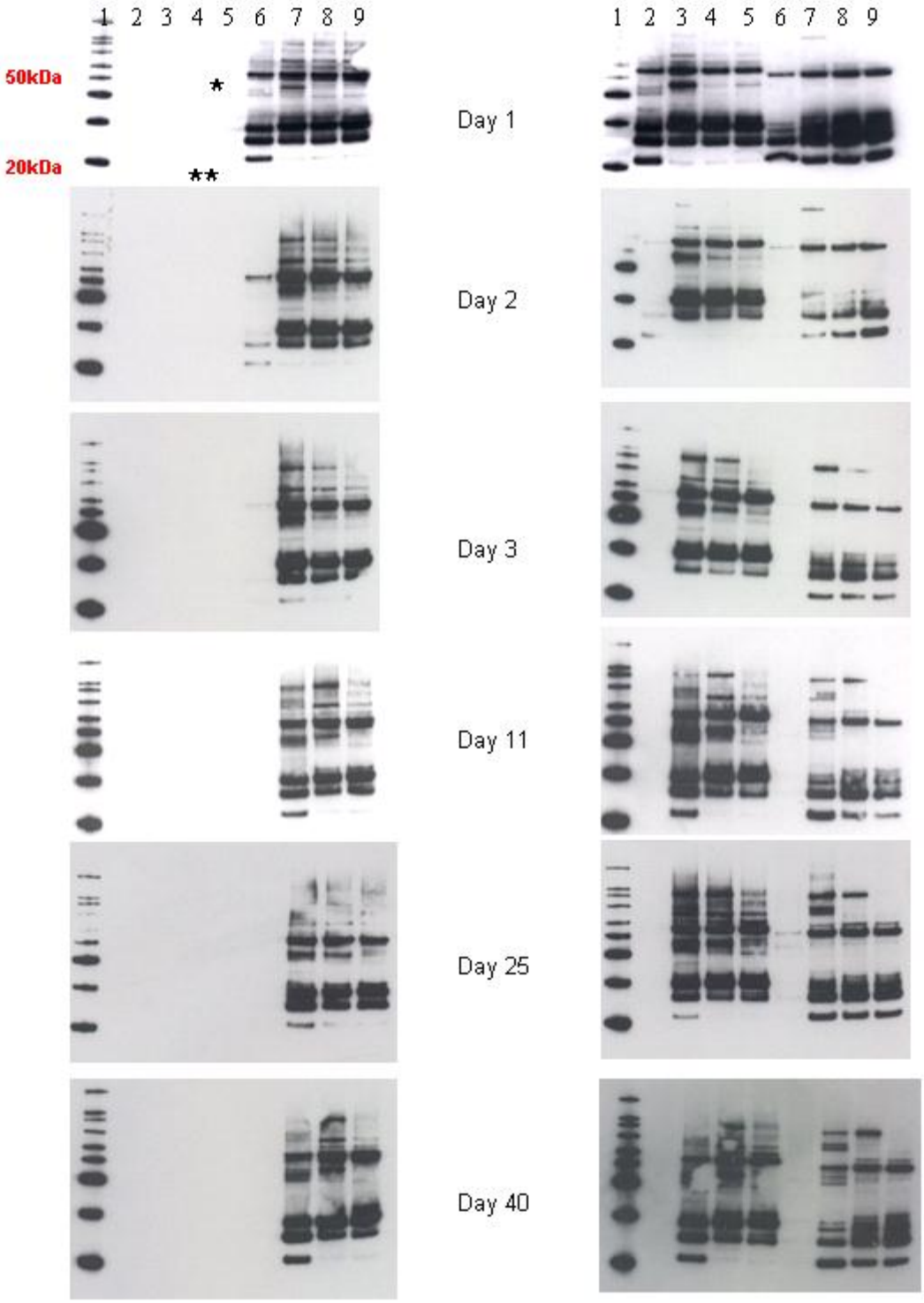

Figure 1: Radiography photos of the different western blots. Left: Western blot results after 1, 2, 3, 11, 25, 40 days of incubation. Lane 1: Protein Molecular Weight Marker MagicMark XP; lane 2: SF21 insect cells, non-infected/+37°C; lane 3: SF21 insect cells, non-infected/+4°C; lane 4: SF21 insect cells, non-infected/-20°C; lane 5: SF21 insect cells, non-infected/-80°C; lane 6:

3ABC_mut_optG/+37°C; lane 7: 3ABC mut_optG/+4°C; lane 8: 3ABC_mut_optG/-20°C; lane 9: 3ABC_mut_optG/-80°C; *: 3ABC NSP 50kDa, **: 20kDa degraded protein. Right: Western blot results after 1, 2, 3, 11, 25, 40 days of incubation. Lane 1: Protein Molecular Weight Marker MagicMark XP; lane 2: 3ABC_site_pro_mut /+37°C; lane 3: 3ABC_site_pro_mut /+4°C; lane 4: 3ABC_site_pro_mut /-20°C; lane 5: 3ABC_site_pro_mut /-80°C; lane 6: 3ABC_WT_Ge /+37°C; lane 7: 3ABC_WT_Ge /+4°C; lane 8: 3ABC_WT_Ge /-20°C; lane 9: 3ABC_WT_Ge / -80°C; *: 3ABC NSP 50kDa, **: 20kDa degraded protein.

Since it is not possible to eliminate completely the action of the insect cell proteases, further studies were conducted with the recombinant proteins that were produced *in vitro* in the bacteria lysate system. In that case the mutated protein is more stable. Its performance was compared with that of the wild type protein produced under the same conditions. Figures 2 and 3 are examples of cELISA results achieved with negative cattle sera that were tested on three different occasions, under identical conditions with two different antigens, the wild type and the mutated proteins. The variation in diagnostic specificity (DSP) with the protein from the wild type gene varied from 66% to 94%, while with the NSP from the mutated gene the results were similar for the three occasions, between 96%, 99% and 100%, reflecting more consistency and greater stability.

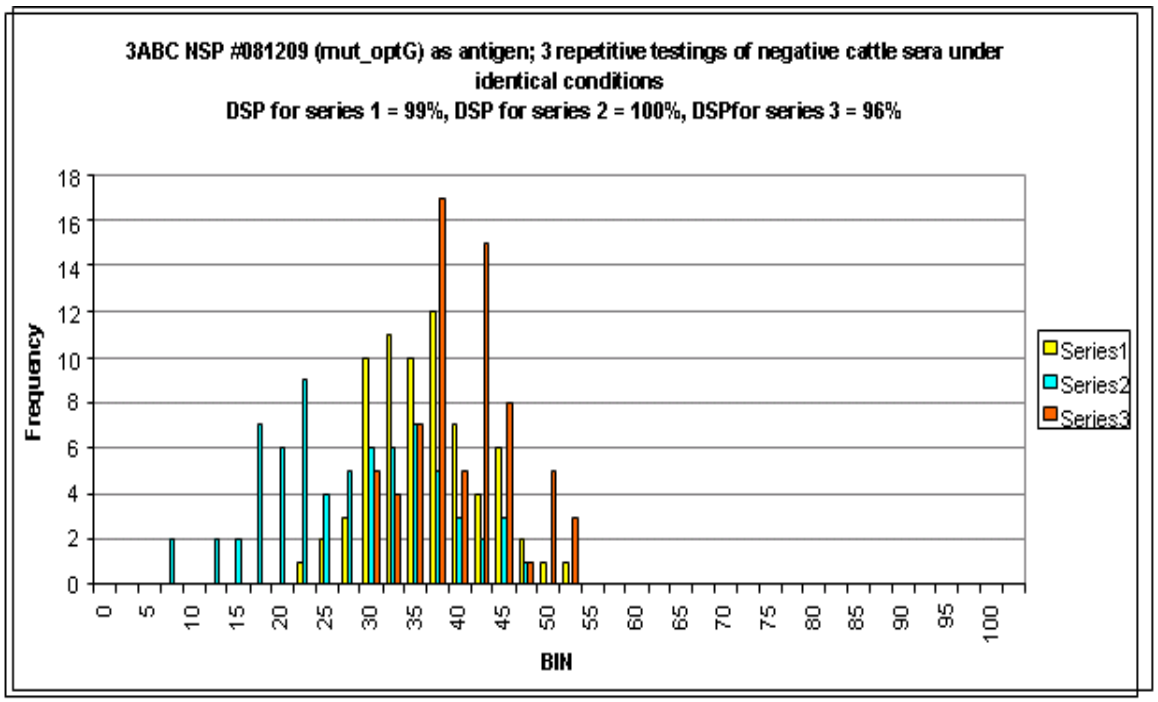

Figure 2: ELISA with 3ABC mutated NSP to evaluate the specificity of the test on FMD negative sera

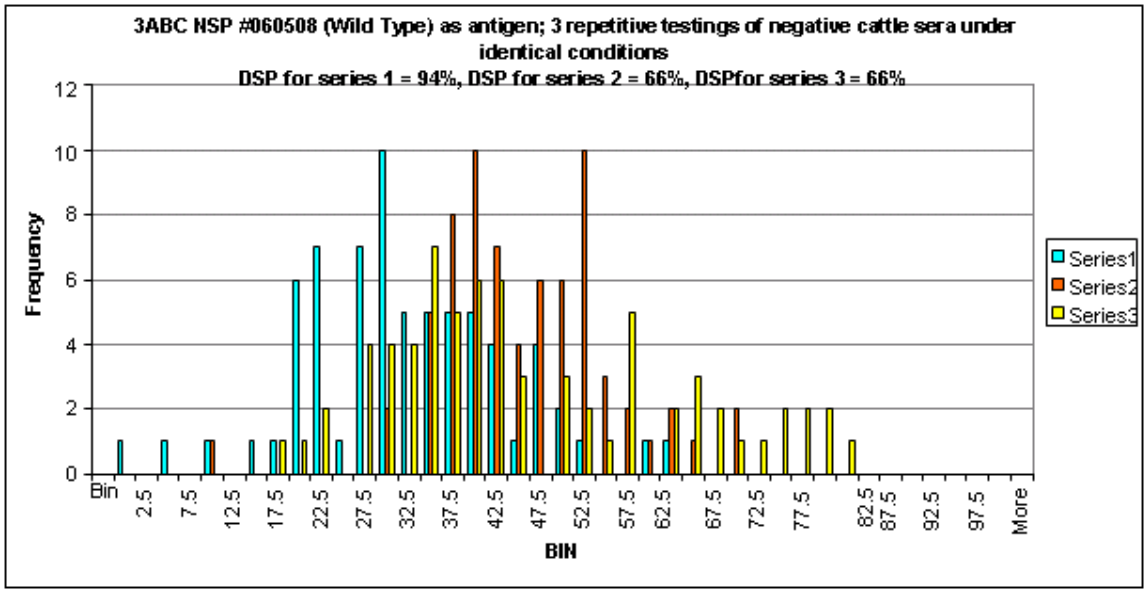

Figure 3: ELISA with 3ABC mutated NSP to evaluate the specificity of the test on FMD negative sera

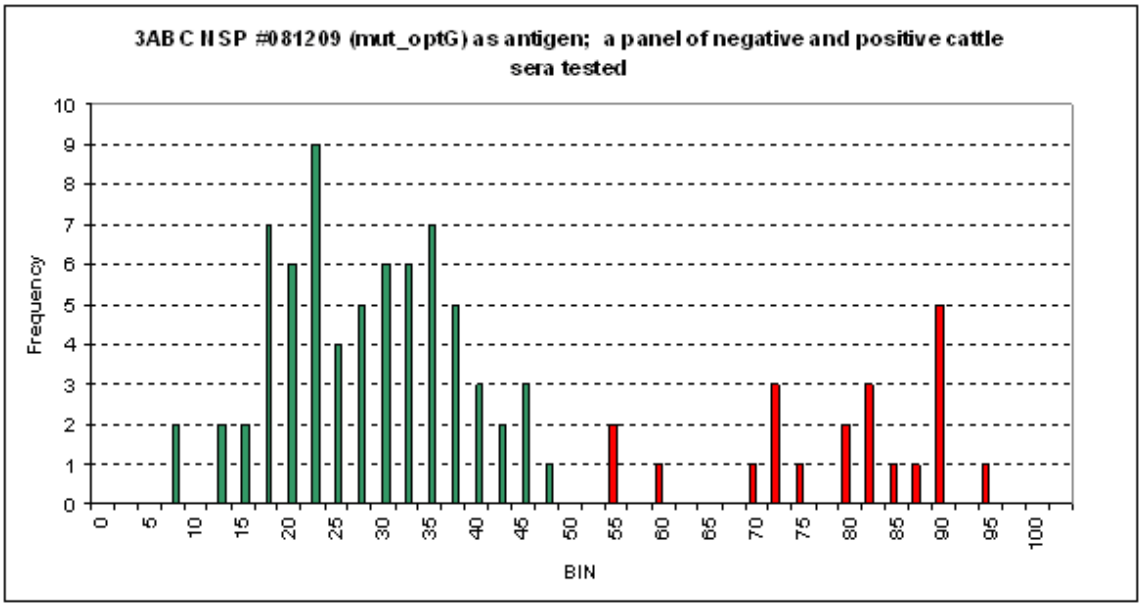

Figure 4: ELISA with 3ABC mutated NSP to evaluate the specificity of the test on FMD negative sera

The Figure 4 here shows the cELISA data of a panel of positive and negative cattle sera tested with the 3ABC NSP as antigen produced from the mutated synthetic gene. Taking 50% as the threshold for the competition between the test serum and the monoclonal antibody for the binding to the antigen, positive sera are well separated from the negative sera.

When the two antigens, mutated and non mutated, are used for testing both positive and negative sera, specificity is more constant with the mutated protein while the wild type protein shows marked fluctuations of the data although the diagnostic sensitivity of both antigens is 100%. A significant difference is observed when comparing data from vaccinated cattle: the 3ABC NSP WT resulted in 73 false positive cases in contrast to 29 false positives for the mutated protein. An ELISA test based on the use of this mutated recombinant 3ABC NSP needs to be evaluated with a large number of sera from both vaccinated and infected animals.

3.2 Peste des Petits Ruminants (PPR)

Peste des Petits Ruminants (PPR) is an acute and highly contagious disease of sheep, goats and small ruminant wildlife. It is the most economically important small ruminant infectious disease in developing countries. The rates of morbidity and mortality are variable but they can be as high as 90-100% and 70-80% respectively in susceptible flock. Described for the first time in Côte d'Ivoire (Ivory Coast) in 1942, PPR was considered for a long time as a West African small ruminant disease. However, since the end of the 1980's, its area of endemicity has been expanding regularly so that by the year 2000 it covered all African countries lying between the Sahara and the Equator, the Middle East, Turkey and the Indian Subcontinent. This expansion has continued in Asia eastwards and westwards to include by 2007 nearly all countries located between Tajikistan and China. In Africa, the spread of PPR south of the equator has been observed since 2005 with cases reported in the Democratic Republic of Congo, Uganda, Kenya and finally Tanzania in early 2009. In the northern part of the continent, this disease was reported on a few occasions only in Egypt. But in June 2008 a PPR outbreak started in Morocco which spread to nearly the whole country in a couple of months. A recent report has mentioned the presence of PPR antibodies in small ruminants in Tunisia. Thus, in the last decade, the geographical distribution of PPR has expanded considerably both in Asia and Africa and the disease is threatening the production of about one billion small ruminants, animals which are an important economic resource relied upon by the poorest pastoralists and farmers. This expansion of the disease endemic area is certainly due to the increase in animal movements worldwide but is also probably brought about by the development and deployment of specific tests allowing better diagnosis. In the light of all these facts, in 2002 an international study identified disease caused by PPR as one of the major factors to be taken into consideration when addressing food security and poverty alleviation in countries where it is endemic. In 2005, with the financial support of the European Commission and the Wellcome Trust Fund, the Animal Production and Health Laboratory embarked on the development and improvement of tools, diagnostic tests and a new vaccine, for the better control of PPR.

3.2.1 Development of diagnostic tests: Improvement of the isolation of PPR virus (PPRV)

PPR is caused by a virus which belongs to a small group named the Morbillivirus genus. This includes also the measles virus affecting human, the canine distemper virus causing disease in dog and the rinderpest virus which was an important threat to cattle and buffalo production until its recent eradication. Central to the pathogenesis of morbillivirus infections is the transient but profound immunosuppression induced in the host. This effect on the host defence favours the establishment and aggravation of the course of opportunistic infections all of which contribute to the severity of the infection. This immunosuppressive effect is in part a result of the virus multiplication in lymphoid cells which are the main targets for its replication. This tropism is determined to a large extent by the presence of a protein on lymphoid cell surface which is used by the virus as a receptor for its entry in the cell. However, morbilliviruses infect and replicate in cells other than lymphoid cells but with very low efficiency. Because those other cells, such as

kidney or lung cells, are easier to maintain in *in vitro* culture than lymphoid cells, they have been preferentially used for morbillivirus isolation even though they are not the most ideal cells. In the case of PPRV, bovine kidney cells, sheep kidney or lung cells, and monkey kidney cells (Vero cells) have been used. But with these cells, isolation is problematic and in most cases the appearance of the virus cytopathogenic effect, i.e. the virus isolation, needs many blind passages and can take 2 to 3 weeks if successful. In 2000, the lymphoid cells surface protein used by the morbilliviruses as receptor was identified. The gene of this protein, the Signalling Lymphocyte Activation Molecule (SLAM) or CD150, of human or canine origins has been inserted into the Vero cell genome. Such modified cells support readily the isolation of measles or canine distemper within 24 hours. Based on that result, in APHL we succeeded at the end of 2008 in modifying another monkey cell line, the CV1 cell, with the sheep SLAM gene. This stable modified cell, named CHS-20, has now been evaluated for its potential in isolating wild type PPRV. In this trial were included the Vero cell, the cell the most used currently for PPRV isolation, the original CV1 cell and finally VeroDogSLAM (VDS) which is a modified Vero cell expressing the canine SLAM. Cells were infected with a 10% ground pathological suspension and incubated at 37°C. They were observed daily for the appearance of the virus cytopathic effect (cpe). One week after infection, cells which do not show any cpe are trypsinised and seeded into another flask, in a blind passage. This is repeated every 7 days up to 3 weeks maximum. After this period, the virus isolation is considered unsuccessful if no cpe is observed and no PPRV nucleic acid is detected by PCR in the inoculated cell medium. In the following table, are summarized the results that were obtained in the first trial with PPR suspected pathological samples that were collected in Nigeria and sent to APHL in 2009. The table shows the results that were obtained with 6 of these samples. It can be seen that PPRV is isolated in CHS-20 in 2 to 5 days after the cell inoculation while at least 9 days minimum with a blind passage are needed before the observation of the cpe in VDS for the sample 43. No cpe was detected in Vero and CV1 cells in a 3 weeks observation period. The culture medium from infected VDS, Vero and CV1 cells were tested for the presence of PPRV nucleic acid by polymerase chain reaction (PCR) amplification assay. As can be seen in figure 5A and 5B the presence of PPRV is detected in VDS after the first passage and also in CV1. For the sample 3, apart from the cell CHS 20 for which the isolation process was successful, the PCR assay has not been positive for the other cells.

Table 1: Isolation of PPRV in CHS-20, VeroDogSLAM (VDS), Vero, and CV-1 cells

sample identification	Virus isolation [a]			
	CHS-20	VDS	Vero	CV-1
Lung n°3	3 days	_	b_	_
Lung n° 27	2 days	-	-	-
Liver n° 28	3 days	-	-	-
Spleen n° 29	5 days	-	-	-
Lung n°43	2 days	9 days	-	_

[a]The day post-infection (dpi) at which time CPE was confirmed in the cell culture
[b]_, unsuccessful virus isolation during 21 days of the observation period after inoculation
ND, non determined

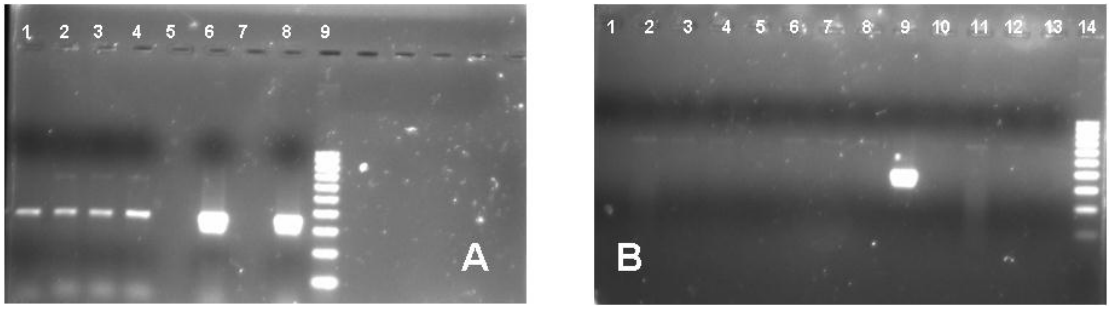

Figure 5: A: PPRV RNA detection by RT-PCR in medium from cells infected with pathological sample lung 43. Lane 1 to 4: CV1 cells corresponding to blind passage 1 to 4. Lane 5: blank. Lane 6: CHS cells without any blind passage. Lane 7: blank. Lane 8: VDS cells corresponding to one blind passage; Lane 9: DNA molecular weight markers.

B: PPRV RNA detection y RT-PCR in medium from cells infected with pathological sample lung number 3: Lane 1 to 4: Vero cells corresponding to blind passages 1 to 4; Lane 5 to 8: CV1 cells corresponding to blind passages 1 to 4; Lane 9: CHS cells corresponding to passage 0 (no blind passage); Lane 10 to 13: VDS cells corresponding to blind passages 1 to 4; Lane 14: molecular marker

In both the CHS-20 and VeroDogSLAM, the cells expressing the sheep and the dog morbillivirus receptor, the cpe is characterized by the development of syncytia, giant cells which result from the fusion of multiple virus-infected cells (see figure 6).

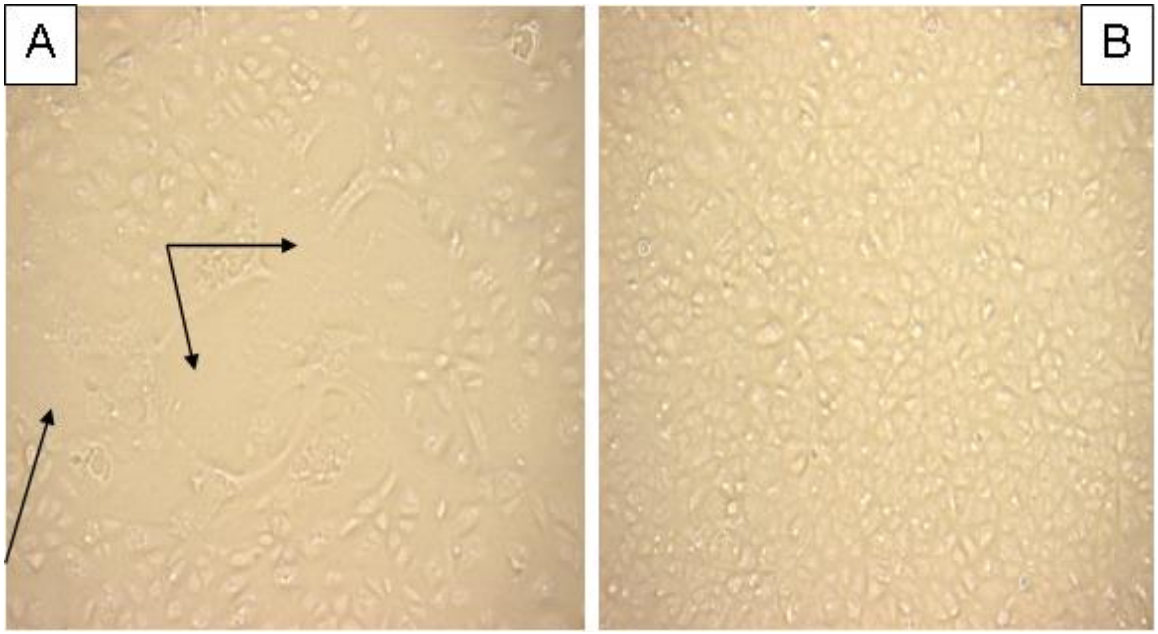

Figure 6: Isolation of PPRV on CV1 cells expressing the PPRV receptor: SLAM protein (CHS cells). Photo A: Syncytia indicated by the arrows in the cell layer infected with PPR suspected

pathological sample. This virus cytopathic effect (cpe) appears 2 days after infection instead of 2-3 weeks for normal cells. Photo B: Control cell, no syncytium is detected.

The fact that PPRV can also be isolated in VeroDogSLAM cells indicates that this virus can bind on the SLAM of animals other than small ruminants but probably at low efficiency since with the latter cells the cpe is evident only after one blind passage.

The preliminary results reported here indicate that the cell line we established, expressing the sheep SLAM, the protein used by PPRV as a receptor, is a promising cell culture system for efficient and rapid isolation of wild type PPR virus. This will be an important contribution to the diagnosis of the disease.

3.2.2 Marker Vaccine Development

Although the treatment of PPR affected animals by the administration of anti-PPR serum or by antibiotics and anti-diarrhoeal has been reported, the only recognized effective control measure is via prophylactic actions. Vaccination to control and reduce the incidence of disease, followed by the stamping-out of the remaining foci, has long been the preferred strategy for the eradication of infectious animal diseases. As indicated about the 2001 foot-and-mouth disease (FMD) outbreak in Europe, the general public is reluctant for the implementation of the stamping-out policies as a means of animal disease control. In addition, in the short term, the stamping-out policy is expensive and most countries where PPR is endemic cannot afford this approach. Immunization strategies over the last 200 years have contributed greatly, perhaps more than any other veterinary intervention, to the successful management of animal diseases. This is also the only strategy adopted by most PPR-infected countries for the control of this disease. A very efficient live attenuated PPR vaccine is available. In sheep and goats it provides protection against the disease for at least three years, i.e. their economic life. The main drawback in using this vaccine is the impossibility of differentiating between vaccinated and infected animals using the currently available diagnostic tests. Thus it is not possible to carry out simultaneously both vaccination and an epidemiological survey for better management of the disease. With the idea of making such a strategy possible, APHL is participating in two projects aimed at developing a PPR marker vaccine with companion diagnostic tests. In these two projects, the role of APHL is to identify the insertion site of the marker in the targeted protein, the major viral protein known as the nucleocapsid protein (N), and to develop the diagnostic tests. The N protein is the “key” element in the biology of the virus since it interacts with other viral proteins and the viral genome for the virus replication. Therefore, identifying the marker insertion site implies the identification of an N protein fragment that is not involved in the interaction with other viral components, i.e. the fragment which is not essential for the multiplication of the virus. In 2006-2008, we reported the results of studies of the protein N self-interactions, its interaction with the virus matrix protein, the protein which drives the maturation of the virus and its interactions with the phosphoprotein (P), the viral protein which is the cofactor of the enzyme responsible for the replication of the viral genome. In particular, in 2008, the binding sites of P on N were identified. In 2009, the N and P interaction studies were continued by the identification of the binding sites of N on P.

The methodology we adopted to study the PPRV N and P interaction is based on the co-expression of N and P followed by the immunoprecipitation of the complex. In earlier studies, the PPRV N protein gene was introduced by recombinant technology into the genome of an insect virus, the baculovirus, to produce this protein, full length and deleted mutants, in high quantities in insect cells. The full length of P was also produced in the insect cells. Monoclonal antibodies specific to PPRV N protein were also generated. In 2009, deleted mutants of P were produced in insect cells through the baculovirus system. A monoclonal antibody anti-P was also produced for use in the N-P interaction study by immunoprecipitation. To better characterize this mAb, mAb 2B11, its binding site on P was identified by western blot analysis. For that, insect cells were infected with baculovirus expressing P full length and also deleted, mutated proteins. Three days after infection, the cells were lysed and the proteins were submitted to gel electrophoresis, transferred onto nitrocellulose membrane and then revealed by the monoclonal antibody. In this study, three controls were included: no infected cells, cells infected with the baculoviruses expressing the N full length and finally cells that were co-infected with baculoviruses expressing both N and P proteins. As can be seen in figure 7, the mAb 2B11 does not react with the insect cell proteins, line 2, nor with the PPRV N protein, line 3. It recognizes the full PPRV P protein and all mutants but with two exceptions: those with the amino-acid deletions 204-284 and 204-324. This result indicates that the binding site of this monoclonal antibody is located in the P protein fragment 284-324.

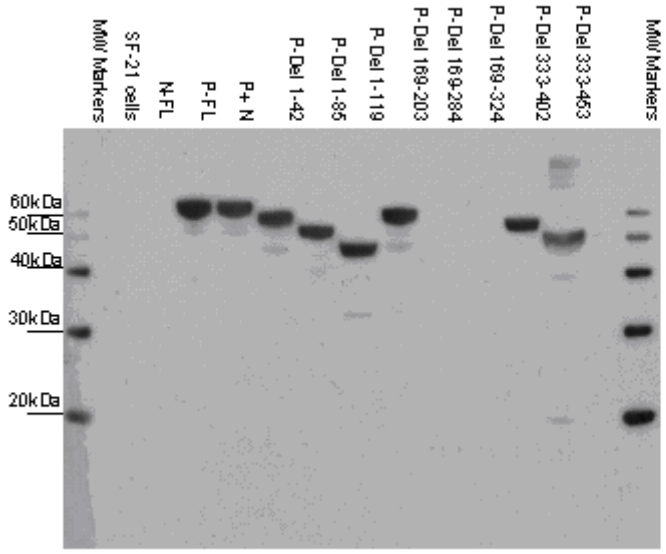

Figure 7: Anti-PPRV P monoclonal antibody mapping

To test which region of PPRV P interacts with PPRV N, the lysates of cells co-infected with the baculoviruses expressing the PPRV N protein and the P protein or its different deleted mutants were immuno-precipitated (IP) using the monoclonal antibody P4A3, which specifically binds to PPRV N. The protein complexes were then separated by SDS-

polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to two nitrocellulose membranes for western blot analysis (WB). One gel was probed with the mAb P4A3 which is specific to PPRV N. The other gel was probed with the hyper-immune sera obtained from mice immunized against PPRV P. The results from the immunoprecipitation/western blot analysis (figure 8) show that PPRV N protein interacts with full-length PPRV P as well as with each of the deleted mutants which were used. Since the mutations were done in a way that the deletions cover all the protein, it can then be concluded that PPRV N interacts with several domains of PPRV P, both at the C- and N-termini. This result has to be confirmed by another study, the binding of N to different P peptides

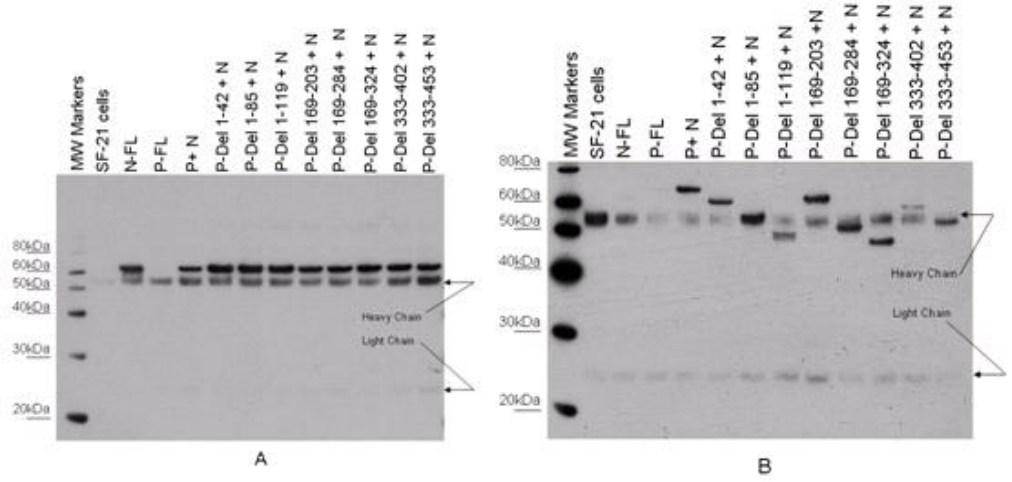

Figure 8: Co-infected SF-21 cells were lysed and subjected to IP/WB analysis.

A. IP using anti-N PPRV N, P4A3. WB using anti-N PPRV N, P4A3. Empty lanes 4 and 13 correspond to PPRV P full-length and SF-21 negative control respectively.

B. IP using anti-N PPRV N, P4A3. WB using anti-N PPRV P serum. Lane 4 indicates that PPRV P by itself cannot be pulled down. It requires the presence of PPRV N (Lane 7). Lanes 11 and 12 are PPRV N and Lane 13 is SF-21 negative control.

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3.3 Development of improved tools for the control of ruminant pox diseases: Capripox

Sheeppox (SPP), goatpox (GTP) and lumpy skin disease (LSD) are viral diseases of sheep, goats and cattle respectively. Collectively, they constitute the most serious poxvirus diseases of domesticated livestock. Lumpy skin disease is endemic only in Africa, apart from one incursion in the Middle East, while SPP and GTP are endemic in Africa, the Middle East and part of Asia extending from the former Soviet Republics to China. In these endemic areas, they have major impact on small ruminant production due to the often high morbidity and mortality in susceptible sheep and goat. These diseases are exotic to most developed countries and their inadvertent or deliberate introduction into such disease-free regions would have substantial economic ramifications due to the disruption to trade in livestock and livestock products, and the costs associated with disease control and eradication. Because of their high negative economic impact, Capripox diseases are categorized as notifiable diseases by the World Organization for Animal Health (OIE). They are also considered to be of significance as potential economic bioterrorism agents.

Sheeppox, goatpox and lumpy skin diseases are caused by three different but closely related viruses, Capripox viruses (CaPV). They are the only members of a genus, *Capripox* genus, within the family *Poxviridae*. The current criterion used for their classification within the genus is based upon the animal species from which the viruses are isolated, i.e, SPPV from sheep, GTPV from goats and LSDV from cattle. This tends to suggest that CaPVs are strictly host-specific. However there are several reports indicating the simultaneous involvement of both sheep and goats in some CaPV outbreaks, and there is an increasing amount of evidence to suggest that various species of game animals may play an important role in LSDV maintenance in the nature. Therefore, this method of classification is inaccurate. In addition, the fact that CaPV infections cannot be distinguished clinically or serologically emphasizes the need to establish more reliable tests for virus identification such as one based on a molecular method. The development of a suitable molecular assay requires the identification of a diagnostic target within the CaPV genome which contains species-specific signatures.

Addressing the need of a more reliable tool for CaPV diagnosis and epidemiological studies is one of the objectives of a four-year project which started in 2005 with the support of the French Ministry for Foreign Affairs and in which APHL was involved with other partners from Africa and Europe. Another objective of that project is to improve the current attenuated capripox vaccines available by identifying, on the genome, genes potentially involved in virus pathogenicity and by rationally deleting them, particularly those that impart a highly suppressive effect on the host immune response. Indeed, poxviruses are well known to encode many proteins that are not essential for the virus multiplication in cell culture but give them an advantage of replication in their host by enabling them to evade the host immune system. These accessory proteins are categorized as immunomodulatory proteins and they play an important role in the pathogenicity of the virus. It has been demonstrated that specific deletions of these proteins contribute to the attenuation of the vaccinia virus, the poxvirus the better studied.

Genome sequence analyses have shown that some of these virulent genes are disrupted in the genome of live attenuated virus strains.

3.3.1 Molecular Epidemiology of CaPVs Based on the virus RNA polymerase subunit 30 kD (RPO30) and the viral Late transcription factor 4 (VTLF4) genes

The need to improve our knowledge of the epidemiology of CaPVs and to search for species specific markers for a molecular based method for CaPV strain differentiation has led us to investigate several CaPVs genes. In 2007 work was initiated on CaPVs molecular epidemiology using the chemokine receptor gene which guides the synthesis of a protein potentially involved in the process of evading the host immune system. This collaborative work, carried out with the French Research Institution, CIRAD, was successfully completed with the identification of several species specific markers and the findings were published in a peer-reviewed journal in 2009.

In 2008 a similar approach targeted the CaPVs RNA polymerase subunit 30 kD (RPO30) gene. This was consolidated in 2009 with the sequencing of this gene from 8 additional CaPV isolates. The sequence data that were obtained were compiled with those of 29 other viruses obtained in 2008. The phylogenetic analysis based on these sequences has strengthened our previous findings that CaPVs can be divided into 3 groups using their RPO30 gene (Fig. 9).

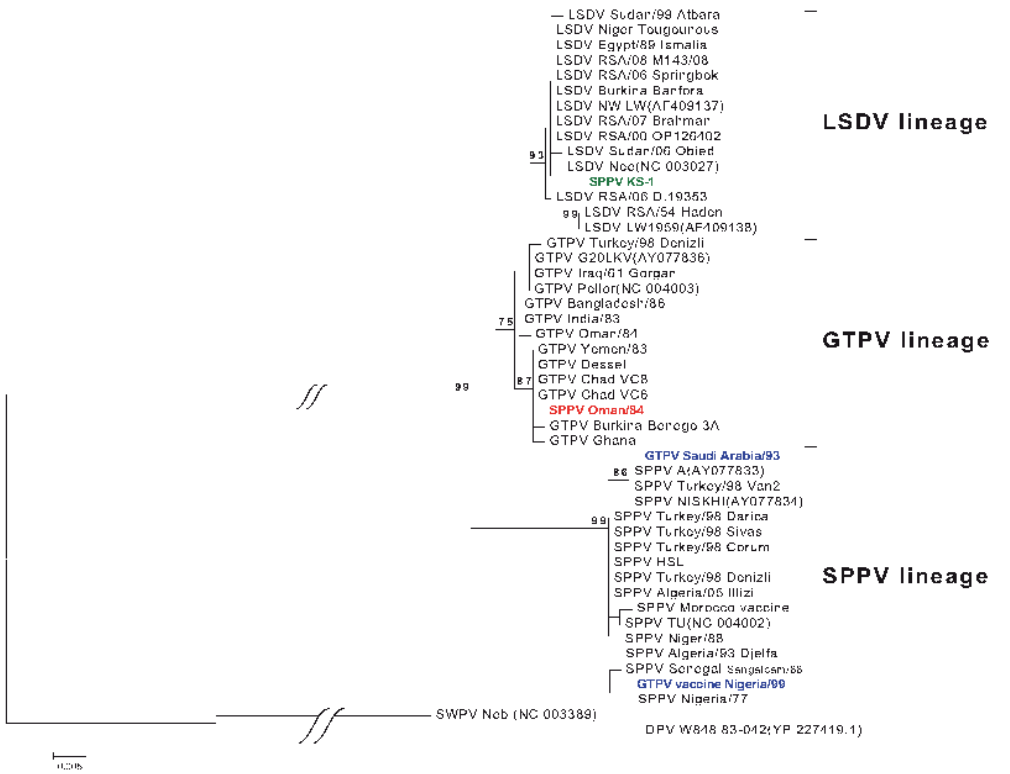

Figure 9: Phylogenetic analysis of 46 Capripoxviruses including 8 retrieved from GenBank and based on the RPO30 gene. One Deer poxvirus and one Swine poxvirus retrieved from GenBank were used as out-groups.

A most important finding was the identification of a species specific signature for SPPV. It consists of a 21 nucleotides deletion which is found only in the sheeppox RPO30 gene. Another CaPV gene, the viral late gene transcription factor 4 (VLTF-4), was also studied in 2009. The VTLF 4 genes of 22 CaPVs were sequenced at APHL and the obtained sequences were used to study the relationship between the CaPV strains together with 8 CaPVs sequences that were retrieved from GenBank. The nucleotide consensus tree shows three tight genetic clusters of SPPV, LSDV and GTPV lineages (Fig. 10). Careful sequence data analysis suggests a strong co-adaptation of the strains and their respective hosts. Further analysis of this VTLF 4 phylogenetic tree suggests that SPPV and LSDV are more closely related to each other than GTPV (Fig. 10). This is surprising since with the two other genes that were used in our previous studies, GTPVs were always more similar to LSDV than SPPV. However, this may suggest a closed performance of this transcription factor in LSDV and SPPV. Both the SPPV and GTPV lineages showed more intra-group diversity than the LSDV cluster. There were two subgroups within the GTPV lineage: the first group contained Middle Eastern and West African isolates, while the second group comprised Middle Eastern and Asian isolates. The SPPV lineage contained three subgroups: subgroup S1 is comprised of Turkish and Algerian isolates, subgroup S2 is comprised of Asian strains and subgroup S3 of African strains. Three

isolates in this study fall outside their designated lineages: GTPV Saudi Arabia falls within the SPPV cluster; SPPV Oman falls within the GTPV cluster and SPPV KS-1 appears to be closely related to the LSDV strains. This is consistent with our previous findings on the RPO30 gene and the Chemokine gene.

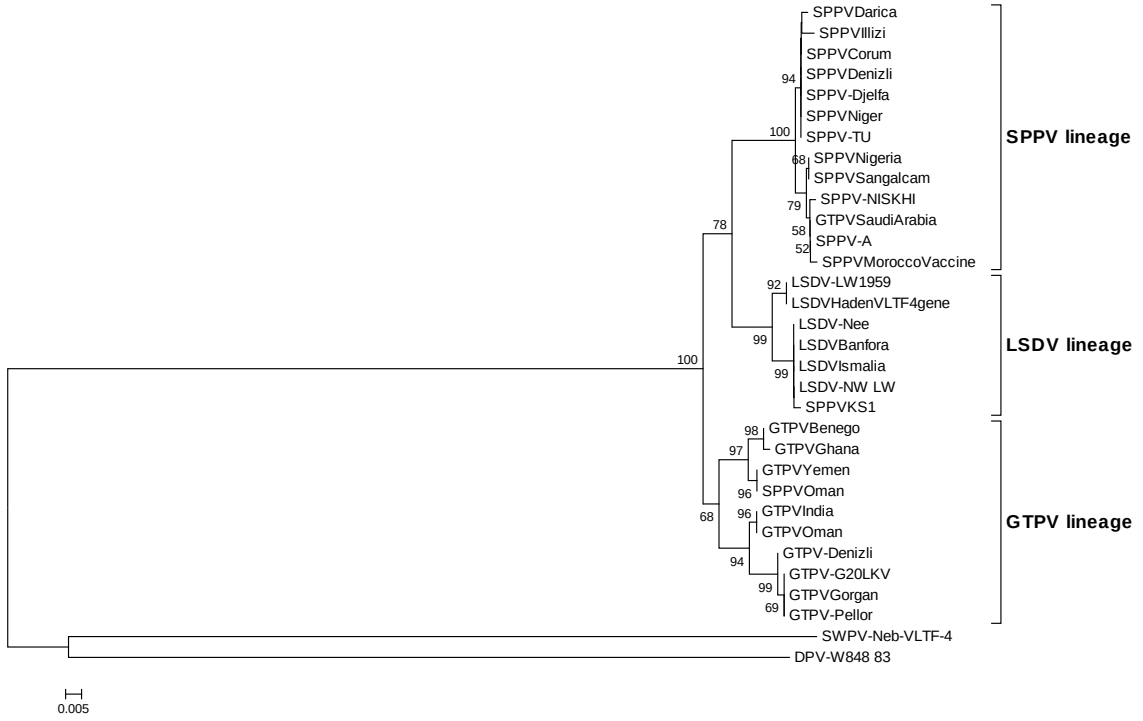

Figure 10: Phylogenetic analysis of 30 Capripoxvirus including 8 retrieved from GenBank and based on the VLTF-4 gene. One Deer poxvirus and one Swine poxvirus retrieved from GenBank were used as out-groups.

3.3.2 Validation of the different molecular based methods developed at APHL with clinical specimen

The complexity of capripoxviruses host specificity and the problem associated with strain designation show that CaPVs identification can be reliable only by using molecular based methods. To date, CaPVs are differentiated via genomic restriction fragment pattern analysis which requires viral multiplication facilities and time-consuming processing. Alternative methods such as gene amplification by PCR followed by gel analysis of DNA fragments generated by restriction nuclease digestion, or by sequencing the amplified DNA were also proposed for CaPVs genotyping. However, these methods also require time-consuming post-PCR processing that are expensive and can increase the risk of contamination that should be avoided in all diagnostic settings. In addition, each of these methods was validated on only a small number of strains which does not guarantee their robustness.

To contribute to the better diagnosis and differentiation of CaPVs, our approach at APHL, was:

- To identify the appropriate diagnostic targets by using molecular epidemiology data for a robust test design;

- To create tools that can be used for simultaneous detection and genotyping without the need for DNA sequencing and post-PCR processing;

- To design methods that can be used by other partners in MS according to the equipment platform available in their laboratories.

3.3.2.1. Classical PCR for differentiating GTPV from SPPV

Based on the SPPV specific 21-nucleotides deletion identified in the RPO30 gene, a classical PCR was developed at APHL for differentiating GTPV from SPPV: the amplified fragment is 172 base-pair long in the case of goat poxvirus but 151 for sheeppox.

Follow-up studies were made in 2009 for its optimization and validation. The optimized method can detect as few as 250 copies of CaPVs genome per reaction. The specificity of the PCR method in detecting only CaPV DNA was tested by attempting to amplify DNA extracted from three parapoxviruses, or cDNA from PPRV: in each instance no amplification was obtained for these viruses (Fig. 11).

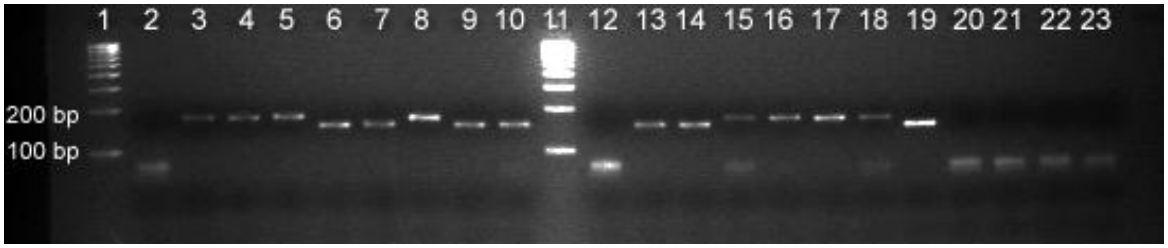

Figure 11: Classical PCR for differentiating GTPV from SPPV: SPPV and GTPV PCR products have different lengths due to a deletion in SPPV RPO30 gene. The PCR products were separated on a 3% high resolution agarose gel. This gel shows the PCR results of different isolates of GTPV and SPPV as well as the negative results obtained with parapoxvirus and peste des petits ruminants virus. GTPV Saudi Arabia/93 and GTPV vaccine Nigeria/99 have a SPPV-like fragment size and SPPV Oman/84 has a GTPV-like fragment size. 1 = Molecular marker; 2 = water; 3= GTPV Turkey/98 Denizli; 4 = GTPV Oman/84; 5 = GTPV Iraq/61 Gorgan; 6= SPPV Turkey/98 Denizli; 7 = SPPV Algeria/93 Djelfa; 8 = SPPV Oman/84; 9 = GTPV Saudi Arabia/93; 10 = GTPV vaccine Nigeria/99; 11 = Molecular marker; 12 = water; 13 = SPPV Nigeria/77 ; 14 = SPPV Turkey/98 Corum; 15 = GTPV Ghana; 16 = GTPV Yemen/83; 17 = GTPV India/83; 18 = GTPV Bangladesh/86; 19 = SPPV Turkey/98 Darica; 20 = ORF Virus D1701; 21 = Stomatitis pAPHLlosa virus M1; 22 = ORF Virus CE030ODV; 23 = Peste des petits Ruminants Virus.

The test was further evaluated for its ability to detect CaPVs in clinical specimens. Ocular and nasal swab samples were collected from a goat experimentally infected with GTPV at different days post infection. The results show that the viral DNA is detected in the swab samples from day 8 until day 20 (Fig. 12).

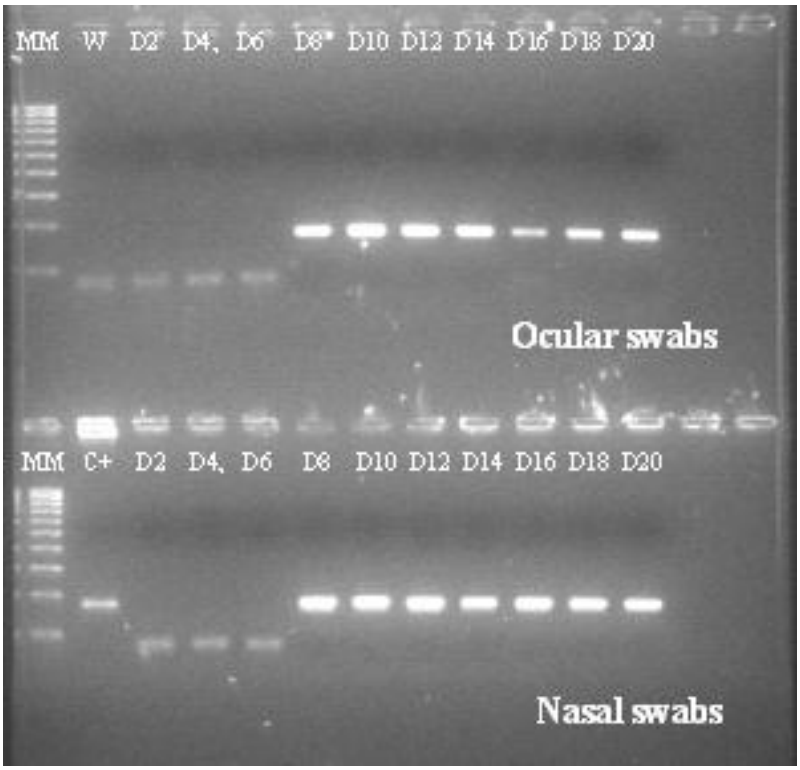

Figure 12: Detection of capripoxviruses in ocular and nasal swab samples by PCR. Samples were taken from a goat experimentally infected with GTPV Denizli at two days intervals from day 2 post inoculation.

This classical PCR method can detect CaPVs in swabs as of 8 days post-infection, i.e. many days before the pox symptoms are obvious. This assay was transferred to Laboratoire Central Vétérinaire (LCV) in Mali.

3.3.2.2. Real-Time PCR for CaPVs based on classical Fluorescence Resonance Energy Transfer (FRET)

A real time PCR assay based on Fluorescence Resonance Energy Transfer (FRET) chemistry was developed, optimized and evaluated in 2008 for the CaPVs strain differentiation. The assay was found to be specific with high sensitivity. For 2009 our objective for this assay was to validate it on clinical specimens. Clinical samples from experimentally-infected animals were used to evaluate the ability of the method to simultaneously detect and genotype CaPVs in pathological samples that will be collected

from the field. Figure 13 illustrates the ability of the FRET assay to detect the CaPVs in such type of clinical specimen.

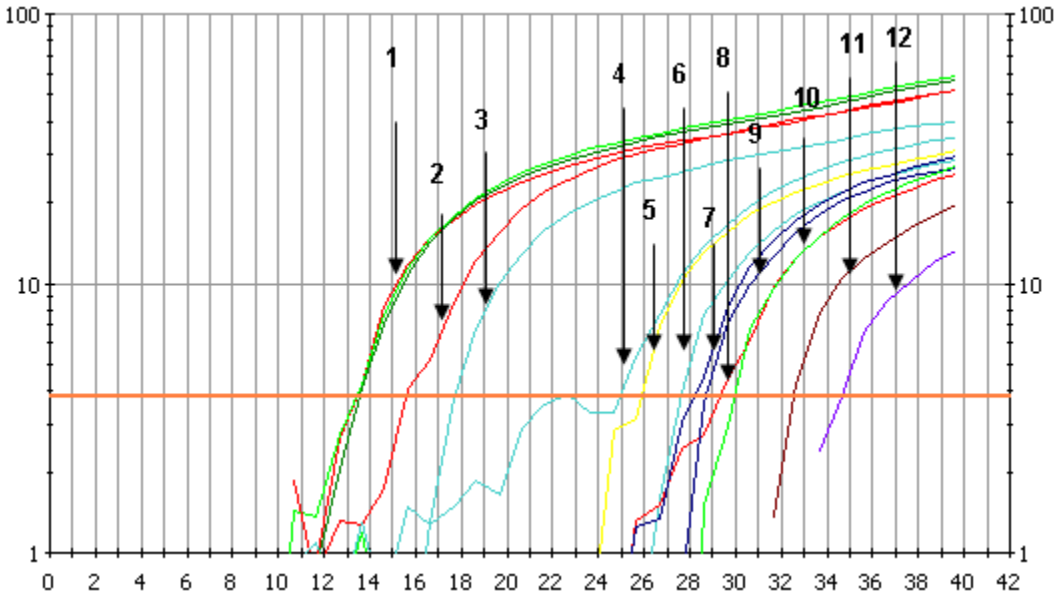

Figure 13: Detection of capripoxviruses in clinical samples from experimentally infected goat with GTPV by FRET real time PCR: 1 = Standard 10^7 copies/uL, 2 = skin nodule, 3 = nasal swab sample, 4 = Mesenteric ganglion, 5 = Tongue, 6 = Trachea, 7 = Bronchial ganglia, 8 = spleen, 9 = Lung, 10 = Liver, 11 = Intestine, 12 = Rumen

From this figure, it can be seen that the swabs are rich in virus as the nodules.

3.3.2.3. Real time PCR for CaPVs using Black Hole Quencher (BHQ) on the 3' end of the FRET donor probe.

The DNA amplification reported above, using FRET, can be performed only on a platform which is equipped with a specific channel for measuring fluorescence. This requirement is a limitation on its wider application in MS. To enable the genotyping of CaPVs without this restriction, we developed a new realtime PCR methodology that can be performed on any platform. Preliminary results that were obtained with the BioRad CFX machine, were reported last year. In 2009, the assay was tested using three other different real time PCR platforms: BioRad Chromo4, BioRad Mini Opticon and Stratagene Mx 3005P. In this report, we are giving the amplification plots on the Stratagene Mx 3005P (Fig. 14) that was used in our collaborating laboratory (the High security laboratory of the AGES) and the plots from the BioRad Chromo 4 (Fig. 15) available at the LCV in Mali.

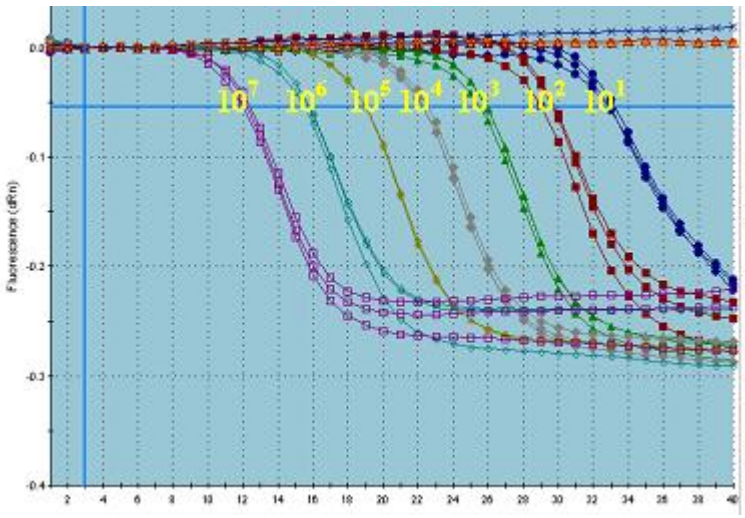

Figure 14: Amplification curves in BHQ-FRET assay as displayed with the Stratagene Mx 3005P

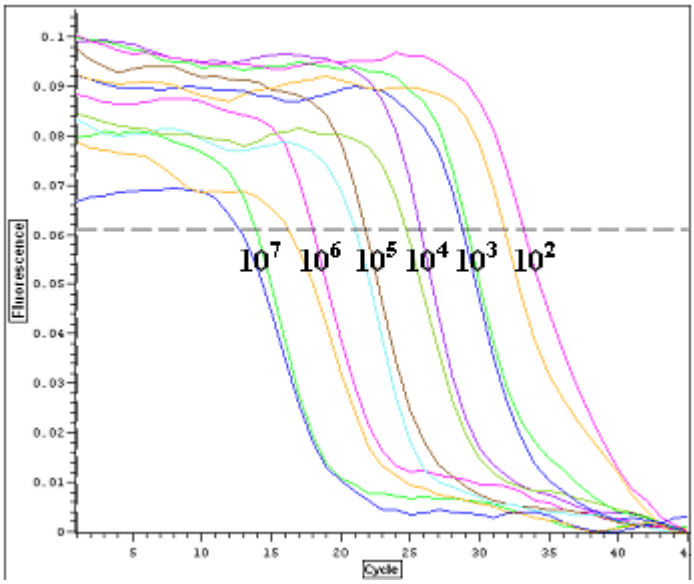

Figure 15: Amplification curves in BHQ-FRET assay as displayed with the BioRad Chromo 4

This method was transferred to veterinary diagnostic laboratory in Mali in November 2009. The assay was able to detect the virus in several type of clinical specimen: skin nodules, mesenteric ganglion, tongue, trachea, bronchial lymph nodes, spleen, lung, liver, intestine and rumen. It was successfully tested also on nasal swab and oral swab samples without the need of preliminary DNA extraction and thereby it offers the possibility of reducing the cost of the assay.

3.3.3. Full genome comparison of Capripoxviruses

In endemic areas, infections due to CaPVs are controlled by the use of vaccination with live attenuated vaccines. However, there are a few problems associated with the use of these vaccines such as vaccination failures and adverse clinical effects. The main reasons could be the emergence of new strains, or the fact the virus strains used in the vaccine are not compatible with the pathogenic strains that are present in the region, and also the possibility that the vaccine is not efficiently attenuated. To better understand the problems associated with CaPV vaccination failures/adverse effects, it is important to have sufficient data on their full genome sequence. This is the key to improving CaPVs vaccine because it will allow identification of the genes involved in the CaPV pathogenicity and their host specificity. By a rational deletion of some of these genes, it will be possible to produce more efficient and safer vaccines.

Currently, the genomes of three LSDV (2 pathogenic and 1 vaccine strains), three SPPV (2 pathogenic and 1 vaccine strains) and two GTPV (1 pathogenic and 1 vaccine strains) are available in the GenBanK. However, there are some limitations regarding these data:

- The selected strains are too close to each other in each of the three CaPV groups.

- The strains are not selected within those that are known to have complex behaviour.

Strategies for the selection of isolates for full genome sequencing

For a better assessment of CaPVs genomic data, APHL started in 2007 sequencing the full genome of several strains. At the end of 2008, the genome of 9 CaPVs strains were sequenced. In 2009, the full genome of 2 additional isolates was sequenced. The criteria for the choice of the isolates for full genome sequencing are summarized in table 2: they included strains of different geographical origins, known epidemiological characteristics and pathogenicity profiles and the specific characteristics of the chemokine gene and RPO30 gene sequencing data.

Table 2: Criteria used for CaPV strains selection for full genome sequencing

Name	*Country*	*Epidemiology/ pathogenicity*	*Molecular data (CaPVs chemokine and RPO30 gene)*
GTPV Denizli	Turkey	**From outbreak where only goat were affected**	GTPV like profile
GTPV Gorgan	Iraq		GTPV like profile
GTPV Oman	Oman	**Pathogenic in goat only**	GTPV like profile
GTPV Yemen	Yemen	**Equally pathogenic for both goats and sheep**	GTPV like profile/ **deletion in the GPCR gene**
GTPV Saudi Arabia	Saudi Arabia		**SPPV like profile**
SPPV Oman	Oman	**Equally pathogenic for both goats and sheep**	**GTPV like profile/ deletion in the GPCR gene**
SPPV Denizli	Turkey	**From outbreak where only sheep were affected**	SPPV like profile
SPPV Corum	Turkey		SPPV like profile
SPPV Djelfa	Algeria	**From area where only sheep pox disease if frequent**	SPPV like profile
SPPV vaccine Morocco	Romania	**Use as vaccine strain in Morocco**	SPPV like profile
SPPV KS-1	Kenya	**Vaccine for small ruminant, adverse effect in cattle**	**LSDV like profile**

General features of the two CaPVs genomes that were sequenced in 2009

Regarding the general features of the genomes, the two CaPVs isolates, attenuated SPPV Romanian and GTPV Saudi Arabia, which were sequenced in 2009 did not show any variation in comparison to the previously sequenced isolates. The length of the SPPV Romanian was 146883 base pairs (bp) but 147865 bp for GTPV Saudi Arabia. The annotation of the two newly sequenced virus genome against data of reference strains, the SPPV A and the GTPV Pellor, revealed that both have a full SPPV profile. This was expected for SPPV Romania which is the vaccine strain currently used in Morocco. For the GTPV Saudi Arabia, the annotation against the reference GTPV strain, the GTPV Pellor, revealed that only 19 out of 150 protein genes are 100% conserved between the two strains, while 138 out of 150 genes are 100% conserved between GTPV Saudi Arabia and the reference SPPV strain, the SPPV A.

This is clearly showing that, at the genetic level, the GTPV Saudi Arabia is a SPPV strain that was isolated from goat. This is also, for the first time, giving clear evidence, based on the full genome data, that SPPV may infect and cause disease in goats.

Together with our previous findings, that SPPV KS-1 had a full LSDV profile and SPPV Oman a GTPV profile, this study is giving more evidence on the complexity of the CaPVs host specificity.

The incorporation of the two newly sequenced genomes to the analysis of the CaPV genomes does not change our previous conclusion that the CaPVs genomes are more variable than foreseen. The GTPVs are more heterogeneous with only 22/150 genes that are entirely conserved between the different strains.

Indeed, the GTPV genomes comparison has revealed the presence of two sub-groups at the genetic level:

Sub-group 1: more than half of their genes displaying 100% similarity to GTPV Pellor and which contains Pellor, G20LKV, Denizli, Gorgan and Oman.

Sub-group 2: GTPVs with less than half of their genes showing 100% similarity to GTPV Pellor and which contains GTPV Yemen and SPPV Oman.

The SPPVs are highly similar to SPPV A, with more than 110 genes out of 150 being fully conserved between each of them and SPPV A. Furthermore, 93 genes, out of 150, are fully conserved between the different isolates of SPPVs, including those that are used for vaccine production. It is important to note that because the GTPV Saudi Arabia has a full SPPV profile, it was analyzed as a SPPV. Also, the SPPV KS-1 was analyzed as a LSDV.

Multiple alignment and phylogenetic analysis of CaPVs using their full genome nucleotide sequences.

To further characterize the genetic relationship amongst CaPVs, we have aligned the full genome nucleotide sequence of 11 CaPVs produced at APHL together with those of 8 others retrieved from the gene bank.

The phylogenetic tree derived from the multiple alignments of 19 CaPVs full genome sequences (Fig. 16) shows the existence of 3 separated clades composed of GTPV (Clade A), LSDV (Clade B) and SPPV (Clade C). Careful observation of the phylogenetic tree shows that GTPVs are more related to LSDV than SPPVs, confirmation of one of the conclusions that was drawn from the CaPVs GPCR gene and their RPO30 gene sequence analysis and reported above.

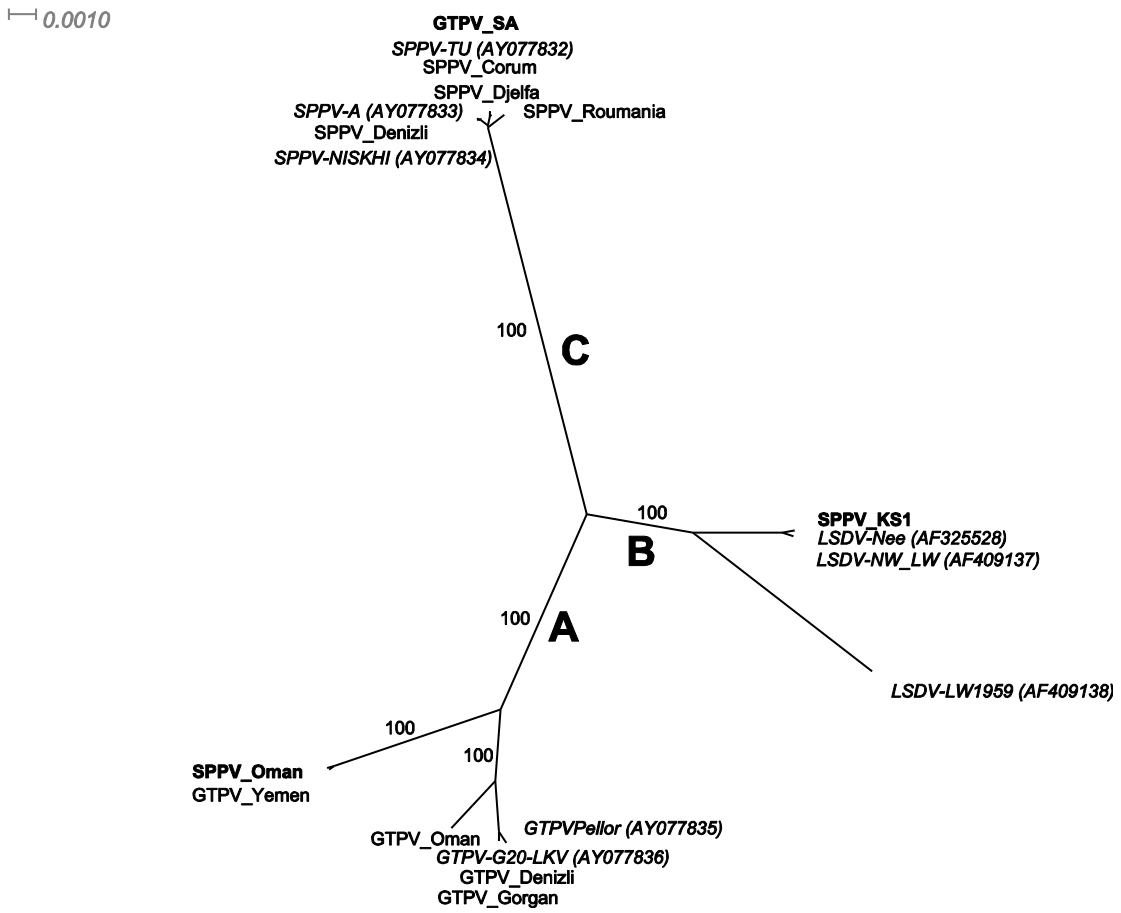

Figure 16: Phylogenetic reconstruction of CaPVs based on their full genome sequences

The phylogenetic tree analysis has also shown the subdivision of GTPVs into two sub-groups. In these phylogenetic analyses, it has been confirmed that GTPV Saudi Arabia is part of the Clade C grouping which comprises SPPVs, SPPV Oman falls into the grouping of Clade A that is designated GTPVs, and SPPV KS-1 is assigned into Clade B that contains LSDV isolates (Fig. 16), confirming our previous results on the CaPVs GPCR and RPO30 genes sequence data. This evidence of isolates being reclassified to a group but not the host from which they were originally isolated is also in agreement with the CaPV comparison based on their gene similarity to the corresponding reference strains. This supports the argument that some CaPV strains can infect multiple hosts. It is also showing why the identification of the CaPVs should rely on genomic data, rather than the current method that is based on the host from which the virus has been isolated for the first time. Therefore, it is crucial to develop tools that can be used for simultaneously detecting and genotyping CaPVs, such as those described above in this report.

By inspecting the multiple alignments of the full genome sequence of 19 CaPVs, we have identified some features that could be used to characterize the different strains according to their group or sub-group.

A region of 127-nucleotide deletion corresponding to the position 5811 to 5936 of SPPV TU (intergenic area between ORF06 and ORF07), is present in all GTPV sub-group 1 members except GTPV Oman. Other exceptions in the GTPV group (Clade A) are:

- SPPV Oman does not possess the deletion of 41 nucleotides at position corresponding to SPPV TU 122114 to 122161 (within the VARV B22R homologue, SPPV ORF128), that is present in all other GTPVs.

- all GTPV subgroup members have insertions of 125, 122 and 58 nucleotides in GTPV Yemen, SPPV Oman, and GTPV Oman respectively within positions corresponding to SPPV TU 127778 and 127779.

- a 110-nucleotide deletion region, corresponding to position 17519 to 17629 of GTPV Pellor (intergenic between ORF022 and ORF023), is found in SPPV Oman only which differentiates it from all other CaPVs. A 24-nucleotide deletion, corresponding to SPPV TU positions 12540-12564 (within the kelch-like protein, ORF016), is a marker for all GTPV group members (Clade A) in contrast to SPPV (Clade C) and LSDV (Clade B). Another deletion (of 61 nucleotides), corresponding to GTPV Pellor 18152 to 18213, (intergenic between ORF022 and ORF023), is a SPPV-specific signature. All GTPVs and LSDV group members and a few SPPV group members (SPPV A, Niskhi, GTPV SA, SPPV Romania) present a deletion of 126 nucleotides at position corresponding to SPPV TU 109872 to 109998 (within the RNA polymerase subunit RPO132, ORF SPPV111).

CaPVs virulence and host specificity genes

The inclusion of SPPV Romania, the vaccine strain used in Morocco to control sheeppox, has allowed the identification of 6 new potential virulence factors for CaPVs (Table 3). These genes are likely to affect the virulence because they are highly disrupted in the attenuated SPPV Romania strain but well conserved in SPPV virulent field strains and in the SPPV NISKHI (a vaccine strain). The ankyrin repeat gene (SPPV-A 138) which was believed to affect the virulence of CaPVs is highly disrupted only in the vaccine strain SPPV NISKHI but is well conserved in the SPPV Romania strain and in all the virulent field strains. The kelch-like protein gene (GTPV_gp 016) of GTPV G20LKV (GTPV vaccine strain) is the only virulence factor that was identified within GTPVs. Finally, 4 SPPV genes and 6 GTPV genes do not seem to play and important role in the virulence, nor to affect the viral multiplication in the host, because they are poorly conserved or highly variable between the virulent strains.

Table 3: Capripoxviruses virulence genes

SPPV A	GB	TU	Niskhi	Djelfa	Denizli	Corum	Mar	GTPV SA	Ortholog Group Name
SPPV-A-002	2	x	x	x	x	x	27.9	X	Virulence factor (Cop-B9R)
SPPV-A-003	3	x	x	x	x	x	24	X	IL-10
SPPV-A-004	4	x	x	x	x	x	49	X	IL-1 receptor (LSDV-N-006)
SPPV-A-007	7	x	x	x	x	x	54.7	X	Alpha-amanitin sensitivity
SPPV-A-017	17	x	x	54.2	x	x	X	X	Ribonucleotide Reductase small subunit
SPPV-A-080	79	x	x	57.6	x	x	X	x	NTPase, DNA replication
SPPV-A-084	83	x	x	x	34.8	x	X	X	mutT motif/NPH-PPH/RNA levels regulator
SPPV-A-092	91	x	x	x	x	x	X	11.8	Core protein (Cop-A4L)
SPPV-A-097	96	x	x	x	54.9	x	X	X	Membrane protein (Cop-A9L)
SPPV-A-115	113	x	x	x	x	39.3	X	X	IMV MP/Virus entry (Cop-A28L)
SPPV-A-127	124	x	x	x	x	x	44	X	Unknown (MYX-L-m130R)
SPPV-A-128	125	x	x	x	x	x	48	X	Unknown (LSDV-130)
SPPV-A-141	138	x	58.8	x	x	x	X	X	Ankyrin (SPV-N-144)
SPPV-A-149	146	x	x	x	x	x	27.9	X	Virulence factor (Cop-B9R)

Two case studies:

Outbreaks in the Sultanate of Oman and the Arab republic of Yemen between 1983-1985

GTPV Oman, SPPV Oman and GTPV Yemen are three capripoxviruses that were isolated in the early 1980s. The current phylogenetic study is indicating that GTPV Oman differs from GTPV Yemen and SPPV Oman which are clustering into the same GTPV sub-group 2.

To compare further GTPV Yemen and SPPV Oman which are within the sub-group 2 of GTPVs, we have carefully inspected the alignment of the two genomes. Major differences, consisting of single nucleotide polymorphism (SNP), deletions and insertion, were found between them. The most important difference between them is a 107-nucleotide deletion within SPPV Oman genome in an intergenic region. The role of such a deletion outside the coding sequences remains to be clarified. However it is clearly indicating that SPPV Oman is genetically distinct from GTPV Yemen, and both are distinct from GTPV Oman. Taken together, this is showing that, at the time of these outbreaks, 3 genetically distinct CaPVs were circulating in Oman and Yemen region, both capable of infecting goats, but only SPPV Oman and GTPV Yemen are highly pathogenic in sheep. GTPV Oman, GTPV Yemen and SPPV Oman are currently being investigated by our partners at the Laboratoire Central Vétérinaire (LCV) in Mali to assess their host selectivity in local African breeds of small ruminants.

Capripox outbreaks in Turkey in 1998

GTPV Denizli and SPPV Denizli are two CaPVs that were isolated from two different outbreaks in the Denizli city of Turkey. The outbreak with GTPV Denizli had involved only goats and that with SPPV Denizli had involved only sheep. This epidemiological information was implying that the two strains are very specific and different from each other. The phylogenetic tree shows that the GTPV Denizli is located into the GTPV group while the SPPV Denizli is within the SPPV group, confirming that the two strains are not related to each other. To investigate further the genomic differences between the

two strains, we aligned their respective nucleotide sequences. The alignment shows that the two strains differ by SNP, deletion and insertion, with the most important deletion being located into the GTPV Denizli genome in an intragenic region corresponding to GTPV Pellor ORF05 and ORF06. Also, all GPTV specific features were found in GTPV Denizli and absent in SPPV Denizli and vice-versa. An experimental study carried out in our partner laboratory in Mali has clearly shown that GTPV Denizli and SPPV Denizli produce severe diseases in only goats and sheep respectively, showing that they are highly adapted for goats and sheep.

The main implication of the information derived from these case studies is the need to use molecular tools for a better management of CaPVs diseases. In areas where 3 or more different strains are circulating, the risk of vaccine failure can increase as the vaccine may not be adapted for protection against the CaPVs strains responsible for disease. The second implication is that it would be probably of more interest to attenuate strains that have multiple host specificity, and evaluate them for their immunogenicity. Such vaccines could be used universally to protect sheep, goats and cattle against CaPV infections.

An important conclusion that derives from this genomic study together with the epidemiology and pathogenicity data acquired is that the sub-group1 members of the GTPV (Clade A) tend to be highly selective for goat, while those in the sub-group 2 behave in an intermediate manner, between GTPV and SPPV, with the ability to infect the two hosts.

4. RESEARCH AND DEVELOPMENT: ANIMAL GENETICS

Small ruminants are a principal source of animal protein in many developing countries. Their biodiversity is often expressed in well-adapted traits that enable them to survive harsh, local environmental conditions or show resistance to endemic diseases where other, exotic breeds, cannot thrive. Unfortunately, since such traits are not sufficiently characterized, they are underutilized in conventional breeding programmes and there is insufficient research on the ways to select breeds or individuals carrying the most advantageous traits. The characterization and mapping of genes controlling such traits, "quantitative trait loci" (QTL), and the subsequent use of this information in selection and breeding programmes, should make it possible to facilitate significant increase in small ruminant productivity.

Comparative analyses among livestock species is one of the more promising mechanisms for identification of the underlying causes for disease susceptibility. The goat is an important species worldwide with thousands of years of phenotypic observations. The cumulated information provides an opportunity for scientists to identify and understand the underlying causes for a multitude of traits. However, our understanding of the goat genome is far behind that of other livestock species, such as cattle, pigs, chickens and sheep.
So in collaboration with some other institutions, APHL has started studies for mapping the genome of both sheep and goats.

4.1 Radiation Hybrid Mapping for Goat: Construction of a goat (Capra hircus) whole-genome radiation hybrid panel

To improve our understanding of the genetic components of traits related to goat health, production and biology, there is an urgent need to develop a detailed goat genome map. Some progress has been made on the construction of a linkage and cytogenetic maps, although the density of mapped loci is low compared to that available for other domesticated livestock species. Radiation hybrid (RH) mapping is a method for producing high-resolution maps that can be used for integrating linkage maps and also that serves as a link across species for comparative mapping. Therefore, it is of critical importance to construct a RH panel providing a resource for rapid, large-scale physical mapping of the goat genome. This will facilitate the resolution of genetic and physical distances prior to designing strategies for positional candidate cloning of the gene(s) that are involved in economically important traits. A goat RH map will help to understand the chromosome assignment of genes and markers and will contribute to positional candidate cloning of the gene(s) which are linked to important traits. Most importantly, the RHMap will lead to the development of new breeding strategies for genetic improvement. A schematic overview of goat radiation hybrid map development is illustrated diagrammatically in Figure 17.

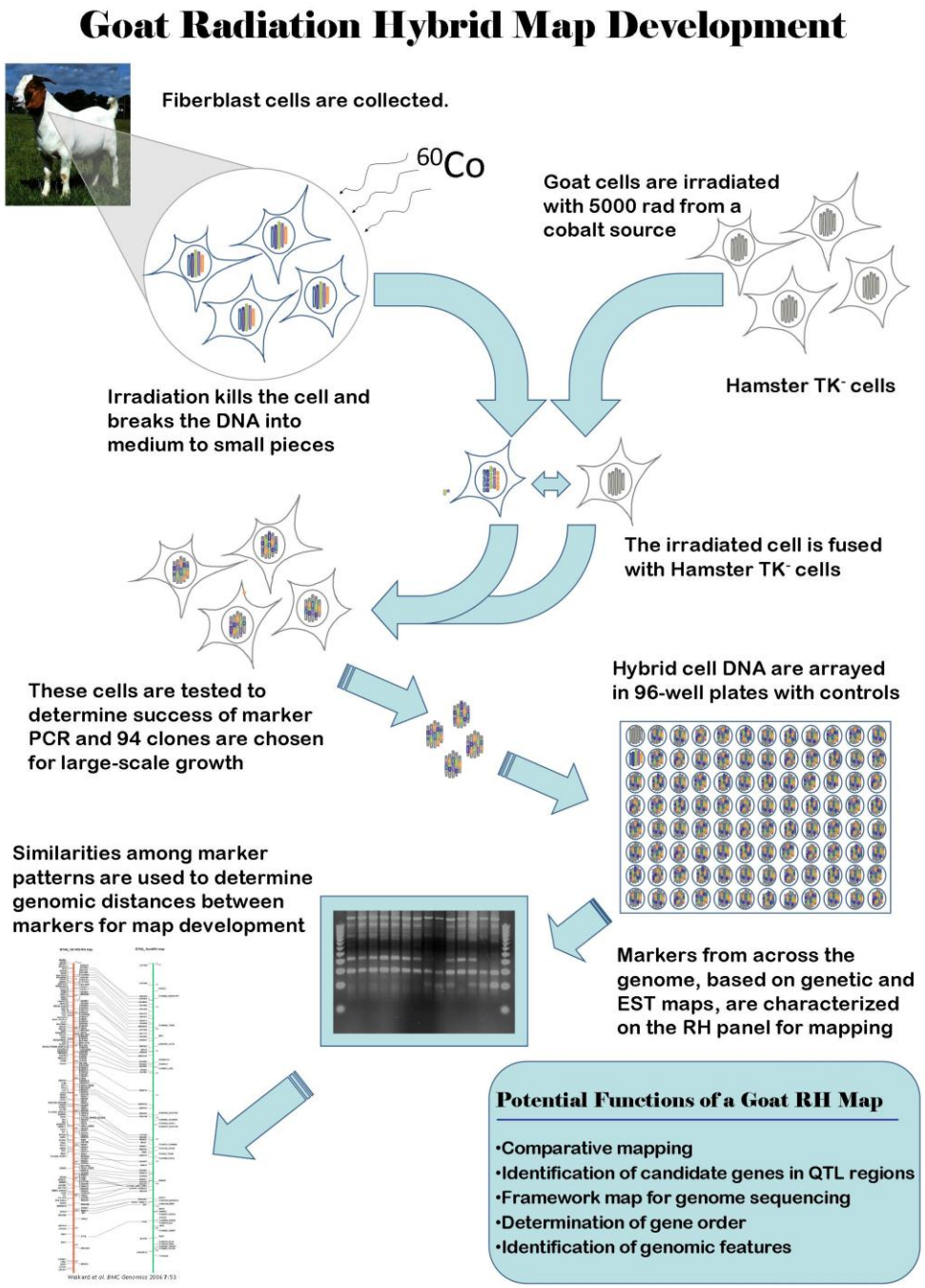

Figure 17: Schematic overview of goat radiation hybrid map development

The development and characterization of a goat (*Capra hircus*) whole-genome radiation hybrid panel (Goat RH5000) has been carried out by APHL in collaboration with other institutes. Our aim is to (1) develop and characterize a whole-genome radiation hybrid panel (GoatRH5000) in the goat; (2) develop an initial radiation hybrid map for the goat using SNP markers; (3) develop a goat RH mapping server allowing the user to map goat markers relative to a framework of previously mapped markers; (4) provide a unique tool

for the study of goat genomics and potentially to utilise important traits in the genetic improvement the goat; (5) train researchers, graduate students and technicians to conduct genetic research in the goat.

The construction of the panel began in October 2009 at the laboratory of the collaborating institute at Texas A&M University. Fibroblasts, cultured from a male Boer goat with a normal karyotype, were irradiated with 5000 rad from a cobalt-60 source prior to PEG-facilitated fusion with A23 Chinese Hamster cells. About one hundred and forty hybrid cell lines were grown and submitted initial testing for the presence of the goat DNA.

4.2 Identification of Host Candidate Genes for Parasite Resistance in Small Ruminants

4.2.1. Sheep Genome Mapping (Genome Scan Linkage Analysis) for Identification of Parasite Resistance Markers

The development of DNA-based markers has had a significant impact on gene mapping. They make it possible to exploit the entire diversity in DNA sequences that exists in any crossbreed. For this reason, high resolution genetic maps are being developed.
APHL has been collaborating with ILRI and the USDA since 2004 in a project to map the Red Maasai and Dorper sheep breeds to identify QTLs associated with parasite resistance and related traits.

Within this project, APHL was in charge of the genotyping of four sheep chromosomes (OAR21, OAR23, OAR25 and OAR26). QTL expression was used to find the linkage map for a genome scan including these four chromosomes.

Comparative mapping was used to fine-map a chromosomal region and to improve the ability to identify genes responsible for parasite resistance. A total of 809 genes were selected based on the cattle BTA5 region sharing synteny with markers on the OAR3 QTL region associated with parasite resistance in previous scans.

The selected genes were assessed for their putative effects. In this step, gene function, and biochemical pathways were annotated for all genes. After clearance, 149 genes involved in immune response pathways were selected as candidate genes for further analysis. The next objective was the development of a test to detect Single Nucleotide Polymorphisms (SNPs) associated with parasite resistance in sheep for the 149 selected genes as an input into the current CRP on small ruminant genetic characterization in Asia (CRP.D.3.10.25).

PCR primers from selected genes were developed for sheep based on published sequences of cattle. The primers were standardised with genomic DNA from a local Austrian breed of sheep. After this standardization, over 200 primers were optimised for PCR to amplify DNA from the same Austrian breed for sequence verification. After this verification, a subset of samples from the most representative breeds from different populations were pooled, amplified, and then sequenced again. Analysing this data

allowed identification and selection of 100 SNPs on different genes.

4.2.2. Sequence variation and genetic marker

Restriction enzyme cut sites were selected from the polymorphisms following digestion of amplified DNA in order to genotype the alleles by PCR-RFLP in agarose gels. A primer set was used to amplify ten selected candidate genes. PCR-RFLP tests for selected genes were genotyped for 144 individual sheep from different populations in order to confirm these markers. Restriction endonuclease digestion of total genomic DNA was followed by hybridization with a P^{32}-radiolabelled probe which revealed different-sized hybridising fragments. An additional eight genes have been directly sequenced.

Several candidate genes were chosen for establishing the Southern Blot method. DNA amplification by PCR followed by digestion with restriction enzymes (if available) and gel electrophoresis on selective DNA samples was carried out.

In figure 18, 19 and 20, are shown some of the results that were obtained. For case 1 (weak bands) the gene PLXNC was chosen following digestion by the Tsp509I restriction enzyme (Figure 18). After employing the ^{32}P labelled probe all previously weak signals were significantly intensified allowing a clear-cut interpretation of the results.

As an example for multicuts, the gene CSF2RB-2 was used after digestion with the enzyme MspI (Figure 19). This enzyme cuts PCR products not only in the SNP position (340) but also in two other positions, resulting in several DNA fragments after the DNA-RLFP investigation. The special located ^{32}P labelled probe attaches specifically to the 340 bp long DNA fragment in case the initial nucleotide is present, and to the 462 bp long DNA fragment in case the other nucleotide shows up in this position. In the case of heterozygotes two bands were detected.

For testing of “no cut” genes, the gene ARHGAP6 was used (Figure 20). No restriction enzyme was found at the SNP position (318) thus all PCR products showed the same gel electrophoresis pattern. The SNP evaluation was therefore carried out by sequencing. From the six investigated ^{32}P labelled probes only those with an “A” (Figure 20 B) or “G” (Figure 20 C) in the middle attached specifically to the corresponding complementary sequences. In case of heterozygotes both of the above-mentioned probes showed positive signals, thus the sequencing results could be confirmed. The probes with A and G at the 3`or 5`ends bound to all PCR products independently of the SNP.

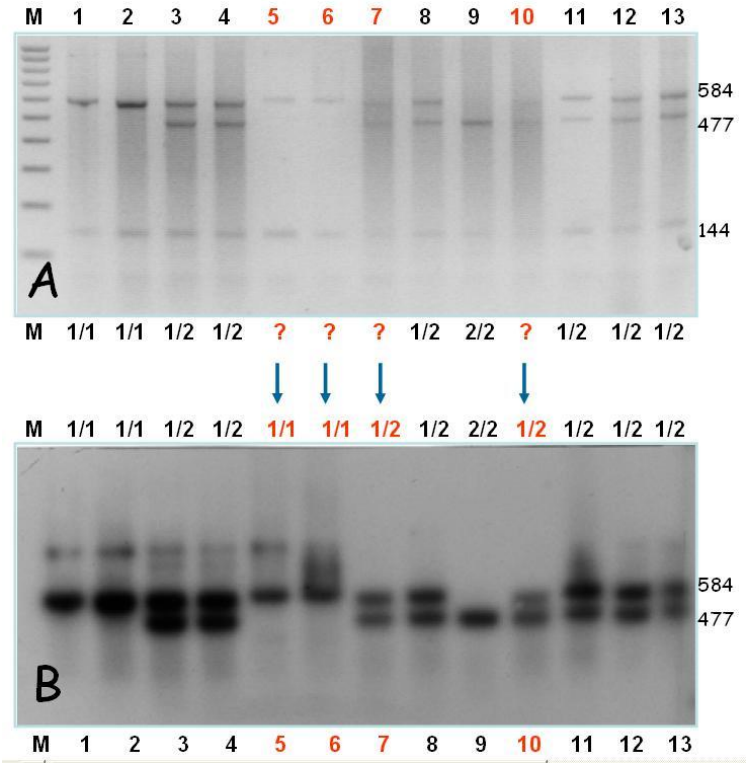

Figure 18: (Weak signal). Figure 17A shows the DNA-RLFP picture of the gene PLXNC after digestion with the Tsp509I enzyme. The samples no. 5, 6, 7 and 10 are difficult to analyze. In Figure 10B it is demonstrated that Southern blotting leads to the intensification of the initially weak signals, leading to improved interpretation of the results

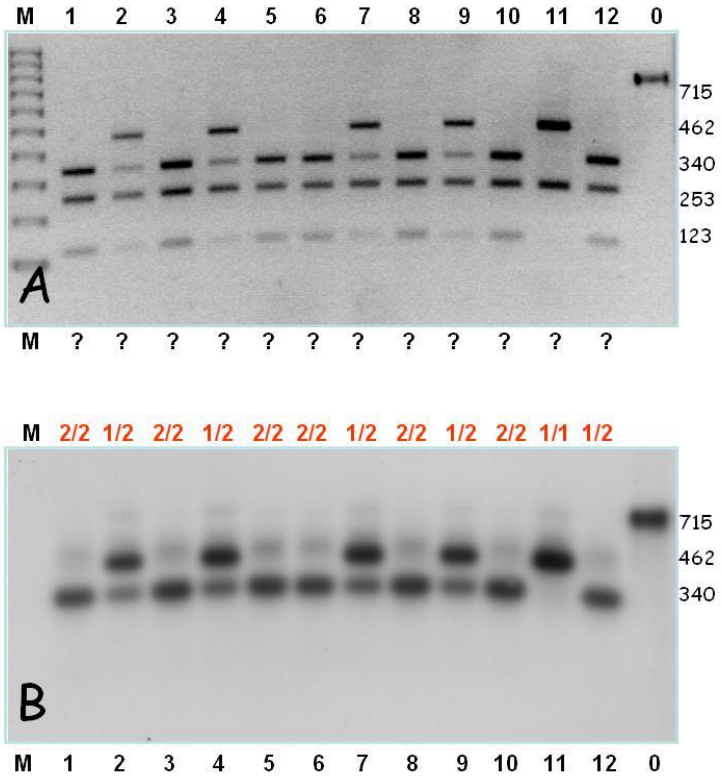

Figure 19: (multicuts). Figure 19A shows the PCR-RLFP photograph of the gene CSF2RB-2 after digestion with the enzyme MspI. This enzyme cuts PCR products not only in the SNP position (340). The Southern blot assay illustrated in Figure 19B leads to the selection of important bands and allows the correct interpretation of results. Sample no. 0 constitutes an undigested PCR product.

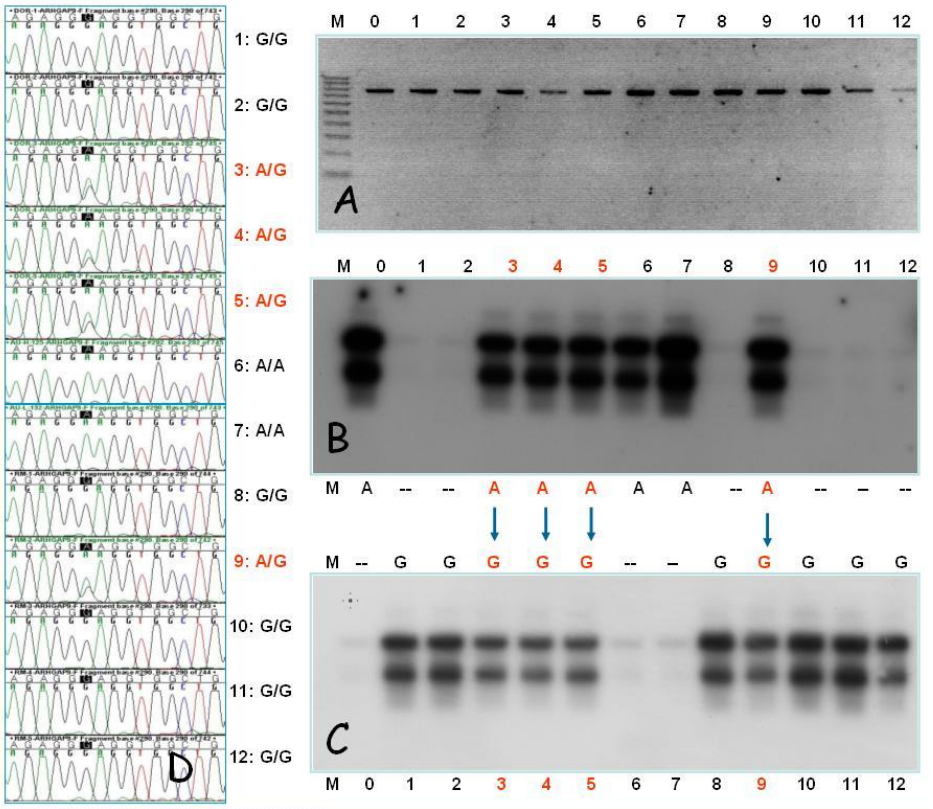

Figure 20: (no cut). In Figure 19A the PCR photo of the gene ARHGAP6 is shown. No restriction enzyme was found at the SNP position (318). The selected samples (selection was performed by sequencing, figure 20D) were Southern blotted and hybridized with 3 probes containing each SNP: in the middle as well as at the 5`and 3`end (altogether 6 probes). Only the probes with an "A" (Figure 20B) or "G" (Figure 20C) in the middle attached specifically to the corresponding complementary sequences. In case of heterozygotes (samples no. 3, 4, 5 and 9) both mentioned probes showed positive signals, confirming the sequencing results. Sample no. 0 presents a control Austrian sheep DNA

Conclusions

A new Southern blot assay using isotope labelling was successfully established to confirm supplement the candidate gene result studies. This method can be used for signal intensification if the PCR-RLFP bands are too weak, for detection of specific restriction fragments in case of multicut, and for detection and differentiation of SNPs if restriction enzyme cleavage sites are not identified in the SNP position. In the last case only DNA probes which contain a corresponding SNP in the middle are able to generate the expected results. By using this assay, sequencing can be supplemented or even replaced.

4.3 Creating a DNA Bank at the Agency's Laboratory for Use by Member States

An objective for several current and planned TCs and CRPs in the Animal Production and Health Programme is to transfer the technical capacity for DNA analysis and marker assisted selection to Member States (MS). In order to identify SNPs for parasite

resistance in sheep and to develop assays to detect these, each SNP needs to be verified in different populations. Arrangements were made to acquire samples of blood and DNA from representative breeds from different populations. Blood samples were sent in two ways: blood mixed with Magic buffer in an evacuated tube, and blood deposited on Whatman FTA Classic cards. Both methodologies allow the long-term storage of the blood sample. Each MS was provided with material for blood collection, and samples from indigenous breeds were isolated and stored. These data were entered into a Genetic Resource Database (GR-*db*) that was created specifically for this purpose. At present this bank contains over 1000 samples from 32 breeds of small ruminants from 15 countries.

4.4 Bioinformatics and Genomics

4.4.1. Development of New modules and databases required for the web-based Laboratory Information Management System (ABL-LIMS)

The current Laboratory Information Management System of the FAO/IAEA Agriculture & Biotechnology Laboratory (ABL-LIMS) is based on an open-source, independent platform that consists of a large number of customised databases designed according to the specific needs of each organisational entity within ABL. The new platform is a transition between a traditional web application and a format that will provide improved usability and faster application. APHL has expanded their existing LIMS with some modifications and new modules. A central database inventory of laboratory chemicals, serum bank management and appropriate records, for health, reproduction and genetics (phenotypic and genetic) data, will be gathered from different MS who have given their consent to their use in research and testing at the IAEA. This database is web-accessible and housed at APHL so it can be integrated with other available information.

4.4.2. Extending the existing web-based Laboratory Information Management System (LIMS) into an interactive Research and Management Platform (RaMP)

APHL has also developed an interactive research and management platform as a follow-up application for LIMS users. The objective of this project is to facilitate a transition from ABL-LIMS into a fully-fledged interactive research and management platform (RaMP) that provides an efficient and quality-assured management tool for defining, assigning and reserving tasks and activities, for monitoring progress to task and report levels at all stages and for assuring the quality of tasks and data. To ensure these capabilities the RaMP will provide also an interactive interface with the multitude of databases and data contained within the current LIMS. RaMP, the new platform is a transition between traditional web application to Ajax (Asynchronous JavaScript and XML) in order to improve usability and faster applications.

4.4.3. Genetic Repository Bank (GRB) Database of genetic materials and Gene Profiling (GP) for candidate gene information

APHL has developed a program to manage a Genetic Repository Bank on sheep, which can be expanded to include other livestock species. This facility will allow users to

graphically view the location of samples with links to Google Map. In addition, the site will allow registered users to insert their own information into the database, so that the site can remain continually up-to-date. This Genetic Repository Bank database (GRB-db) is a first step in providing MS with genomic information on small ruminants in a user-friendly format. The application does not require software installation on user computers and can work on all web-browsers. This tool will be used for sample traceability at DNA Repository Bank at Seibersdorf Laboratories of IAEA.

4.4.4. Development of Real -Time Databases (RT-db) for Genetic Information on Small Ruminants

APHL has worked extensively on the development of an RT-db (Real-Time database) for Quantitative Trait Loci (QTL)/Genes/DNA Sequences and Genetic characterization in small ruminants. The database will make available the genomic locations of QTL from all known studies on small ruminants. It will allow users to view graphically the positions of the QTL, filtered according to a number of criteria, such as trait name, chromosome number and statistical significance. RT-db will be the first step in providing the MS with up-to-date genomic information on small ruminants in a user-friendly format. The database is now available online and is fully functional. This work has been presented at an international conference (http://www.intl-pag.org/16/abstracts/PAG16_P08a_852.html).

4.4.5. Development of Genetic Characterization Databases (GC-db) of cattle breeds

Genetic characterization is one of the areas in which we promote coordination and collaboration of scientists in member states. For this purpose, we developed on-line database of microsatellite genotypes for characterization of cattle, sheep and goats breeds. This will allow users to compare their breeds with those of others. In addition, we intend to promote a global "meta-analysis" of characterized species, which is essential for breed conservation activities.

To accomplish this task, we are asking scientists who have done molecular characterization of breeds to share their data. The data can be uploaded in a database and made available to interested users. Providers of data would also have free access to available data of others.

The sensitivity of the data in terms of confidentiality, integrity varies for different sections of this database and it has been taken into account in developing the resource.

5. TRAINING ACTIVITIES

5.1 Training course on Avian Influenza diagnosis (September 22- October 2, 2009)

A training course, entitled "Advanced Molecular Diagnosis and Characterization of Avian Influenza (Bird Flu)" was organized at the FAO/IAEA Biotechnology Laboratory from September 22 to October 2 2009 as part of the Conflutech project activity. Conflutech is one of the EC-supported projects for the control of the deadly avian influenza. Its objective is to contribute to the improvement of technical as well as scientific skills of staff in charge of the control of animal diseases through technology transfer and training. This training course of September-October 2009 benefited also from the financial support of FAO and the International Atomic Energy Agency (IAEA). It was a theoretical and practical course with the aim to enhance participants' knowledge on highly pathogenic avian and also on swine influenza caused by the virus H1N1. The participants were from East Europe, Asia and Africa.

5.2 Regional Training Course (RTC) on Genomic DNA Preparation, Microsatellite Analyses and Sequencing Dec 7- Dec 18, 2009)

Under the IAEA Technical Cooperation Programme TC Regional project RER5015 "Supporting Early Warning and Surveillance of Avian Influenza Infection in Wild and Domestic Birds and Assessing Genetic Markets for Bird Resistance" a regional training course was organized on genetics at the Agency's laboratory, Seibersdorf from 7 to 18 December 2009. This course aimed at enhancing knowledge in genomic DNA preparation, microsatellite analyses and sequencing. The ultimate goal was to train partners in molecular genetic analysis.

The course was attended by 18 participants of 12 member states from RER5015 participating countries (Albania, Armenia, Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Hungary, Kazakhstan, TFRMR of Macedonia, Montenegro, Moldova, Romania, Russian Federation, Turkey, and Serbia) which are considered at risk regarding avian flu outbreaks. In addition, one participant from Ghana also joined the training course with support from relevant national project of the TC programme for Africa. Two additional participants from India and Azerbaijan were able to participate with the support of FAO. The course developed methodologies, generated information and formulated decision support systems for defining phenotypic and molecular genetic diversity, using microsatellite DNA marker and related technologies, and enabled the development and implementation of national, international and regional strategies for optimum use, improvement and conservation of poultry genetic resource.

5.3 Fellowships/Internships

Ms. *Violeta Dicusara (*From 1 April 2009 to 30 June 2009)***:*** Ms. ***Violeta Dicusara,*** DVM, from Moldova was awarded an internship for three months. She received training in the identification of immune response genes involved in parasite resistance in sheep.

Ms. *Anahita Daryabeigi (*From 1 April 2009 to 30 June 2009)**:** Ms. ***Anahita Daryabeigi,*** student at the University of Vienna***,*** was awarded an internship for three months. She was trained to optimize and validate protocols for using isotopes in southern blot as a follow up to detecting single nucleotide polymorphisms (SNPs) for disease resistance for small ruminants.

Mr. *Christian Schwarz* (From 1 April 2009 to 30 June 2009)**:** Mr. ***Christian Schwarz*** was an intern from Austria. He worked on the identification of resistance genes involved in immune responses to helminth parasites in sheep. He also received training on the optimization and validation of protocols for using isotopes in Southern blot techniques.

- ***Mr. Turshintulja Bumduuren (TCPMon/08004):*** Mr. Bumduuren, from Mongolia, spent 3 months, 19 Jan 2009-18 Mar 2009, in APHL for learning gene sequencing and sequence data analysis in collaboration with the Plant Breeding Unit.

- ***Mr. Esayas Gelaye Leykum (TCP ETH5/014:)***
Mr Esayas, from Ethiopia, spent 3 months, Sept 1 - Nov 30, 2009, in APHL for training on the generation of hybridomas for the production of monoclonal antibodies (mAb) anti Peste des petits ruminants virus (PPRV).

- ***Ms. Brigitte Mbuze Balobake-So (TCP ZAI/08006):*** – (Ms Balobake was in APHL for 6 months training, March 1 - Sept. 1 2009, on the monoclonal antibody production technology and the ELISA technique.

- ***Ms Metchilda Byamungu***: Ms Byamungu, a Tanzania national, was in APHL from June 2009 to February 2010 for a training on on molecular technique for the detection of trypanosomes. She was sponsored by the Rothamsted International in UK.

- ***Mrs Kimberley Alexis Schiller:*** Mrs Kimberley, Sweden national, is student at the Royal Veterinary College (RVC) in London. She spent one month and half in APHL as intern (22 June- 6 August, 2009). She was involved in the capripox virus molecular epidemiology study. She cloned, sequenced and analysed the capripoxvirus VTLF 4.

6. CONSULTANTS

Ms. *Jolanta Kolodziejek:* Ms. Kolodziejek is from the Institute of Virology, at the University of Veterinary Medicine, Vienna (VUW). In APHL she worked for nine months on the development of SNP markers and their validation in sheep as part of the implementation of an IAEA CRP. She successfully established a Southern blot assay using isotope labelling to confirm or complete the candidate gene results from RLFP analysis. She also provided training and support for Fellows and Interns as well as carrying out several administrative duties.

- ***Mr. Abdallah Traoré (Mali):*** Mr Traoré is scientist working in virology section of the Laboratoire Central Vétérinaire (LCV) at Bamako in Mali. LCV is a partner of APHL in

three research projects on capripox and PPR. Mr Traoré was awarded a three-month consultancy contract to work on the detection of PPR virus in pathological samples by a new molecular technique developed in APHL, the realtime PCR.

- ***Ms. Linda Fuga:*** Ms. Fuga is an MD from Albania. She worked for five months on "Development of a test to detect Single Nucleotide Polymorphisms (SNPs) associated with nematode resistance in sheep". She contributed to protocols and method development activities for quantification of total DNA by spectrometroscopy, oligonucleotide primer design, PCR optimization, digestion by restriction enzymes, multiplex PCR, DNA sequencing and bioinformatics based analysis.

7. ACKNOWLEDGMENT

The Animal Production and Health Laboratory (APHL) gratefully acknowledges the helpful collaboration of Dr Roland Silber and his colleagues at the Austrian Agency for Health and Food Safety (AGES) and for enabling staff of APHL to work on exotic pathogens in secure isolation premises in their high security laboratory.

8. APPENDICES

8.1 Staff Publications

-Publication in refereed journals

1) Ramos, A.M.; Pita, R.H.; **Malek, M**.; Lopes, P.S.; Guimarães, S.E.F and Rothschild, M.F, Analysis of the mouse high-growth region in pigs. *Journal of Animal Breeding and Genetics*, **126**, Number 5, pp. 404-412

2) Silva, M. V. B., Sonstegard, T. S., Hanotte, O., Mugambi, J., Iraqi, F., Garcia, J. F., Boettcher, P., Nagda, S., Gibson, J., McClintock, S., Kemp, S., **Malek, M**, Van Tassell, C. P., and Baker, R. L. Identification of quantitative trait loci affecting resistance to gastro-intestinal parasites in a double backcross population of Red Maasai and Dorper sheep**.** *Journal of Animal Breeding and Genetics*, (in press).

3) Le Goff, C., **Lamien, C.E.**, Fakhfakh, E., Chadeyras, A.L., Aba-Adulugba, E., Libeau, G., Tuppurainen, E., Wallace, D., Adam, T., Silber, R., Gulyaz, V., Madani, H., Caufour, P., Hammami, S., **Diallo, A**. and Albina, E. Capripoxvirus G-Protein-Coupled-Chemokine Receptor: A Host-range Gene Suitable for Virus Animal Origin Discrimination**.** *Journal of General Virology*, 2009, **90**, 1967–1977

4) Minet, C., Yami, M., Egzabhier, B., Gil, P., Tangy, F., M. Brémont, G. Libeau, **A. Diallo**, E. Albina. Sequence analysis of the large (L) polymerase gene and trailer of the peste des petits ruminants virus vaccine strain Nigeria 75/1: Expression and use of the L protein in reverse genetics. *Virus Research*, 2009, **14**, 9-17.

5) C. Minet; O. Kwiatek; D. Keita; **A. Diallo**; G. Libeau; E. Albina. Morbillivirus infections of ruminants: the nearly eradicated rinderpest and the "peste des petits ruminants", an expanding disease in the south and a threat for Europe. *Virologie*, 2009, **13**, 1-11.

Oral presentations in Symposium/congress

Lamien C.E., Lelenta M., Silber R., Le Goff C., Wallace D., Gulyaz, V., Tuppurainen E., Luckins A.G., Albina E., Diallo A., 2009. Genotyping Capripoxviruses using real

time PCR. International Meeting on Emerging Diseases and Surveillance (IMED), 13-16 February 2009, Vienna, Austria.

Posters presentation Symposium/congress

1) C. E. Lamien, M. Lelenta, C. Le Goff , R. Silber, A. G. Luckins, E. Albina and A. Diallo. Phylogenetic Grouping and Real Time PCR Method for Differentiating Capripoxviruses. International Meeting on Emerging Diseases and Surveillance (IMED), 13-16 February 2009, Vienna, Austria

2) Lamien C.E., Lelenta M., Silber R., Le Goff C., Wallace D., Gulyaz, V., Tuppurainen E., Luckins A.G., Albina E., Diallo A., 2009. Phylogenetic Analysis of the Capripoxvirus RPO30 Gene and its Use in a PCR Test for Differentiating Sheep Poxvirus from Goat Poxvirus. International Meeting on Emerging Diseases and Surveillance (IMED), 13-16 February 2009, Vienna, Austria.

8.2 Staff Travel

Massoud Malek

- 10-14 January, 2009. San Diego (USA): To attend the Plant Animal Genome (PAG) international conference and the Sheep Geno Consurtium meeting to discuss sheep HAP-MAP project
- 18-20 March, 2009, Bydgoszcz, Poland; To discuss at the GLOBALDIV meeting collaboration among GLOBALDIV, IAEA and FAO on hosting genetic characterization data from scientists in member states
- 22-23 June 2009, Paris (France). To attend Joint FAO/INRA workshop on "Animal genetic resources and their resistance/tolerance to diseases, with special focus on parasitic diseases in ruminants
- 06-10 July 2009, Sofia, Bulgaria. To provide technical support on molecular genotyping (TCP BUL5012) and evaluate progress of RER5015
- 27-31 July 2009, Gaborone, Botswana. To advice on the establishment of LIMS in animal diseases diagnostics and research laboratory practices. (TCP BOT5005)
- 07-11 September 2009, Piacenza, Italy. To discuss at the GLOBALDIV meeting collaboration among GLOBALDIV, IAEA and FAO on hosting genetic characterization data from scientists in member states
- 16-20 November 2009, Bamako, Mali. To develop ICT-based training materials in the field of veterinary emergencies, such as Avian Influenza (TCP RAF/5/057). Participants will introduce internet based data management software for application as veterinary lab info.

Lamien Charles

- 15 to 21 November 2009, Bamako, Mali: To transfer to the Laboratoire Central Vétérinaire (LCV) in Bamako the real time PCR technology applied to animal disease diagnosis.

8.3 Coordinated Research Projects and Technical Cooperation Projects

CRP Title	Technical Officer
Gene-based Technologies in Livestock Breeding: Characterization of Small Ruminant Genetic Resources in Asia (2005 – 2009)	Malek Massoud
Technical Meeting on The Early and Sensitive Diagnosis and Control of Peste des Petits Ruminants **(**D32026)	Diallo Adama

TCP Title	Technical Officer
Genomics of the Alpaca: Identification of Expressed Genes and Genetic Markers Associated with Productivity and Embryonic Mortality – Peru (2007 – 2009)	Malek Massoud
Maximizing Productivity on Goat Farms through Cost-Cutting and DNA-Based Technology in Selection for Breeding – Sri Lanka (2007 - 2009)	Malek Massoud
Improving Sheep and Goat Production in Morocco through Genomic and Reproductive Physiology Characterization with the Help of Radio-immunoassay and Molecular Techniques – Morocco (2007 – 2009)	Malek Massoud

8.4 External Collaborations and Partnerships

Institution	Topic
Austrian Agency for Health and Food Safety (AGES), Vienna, Austria	Use of the high security laboratory by APHL for handling exotic pathogens
Centre International en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France	Partner in the European Commission (EC) supported research project, '*Development of marker vaccines, companion diagnostic tests and improvement of epidemiological knowledge to facilitate control of rinderpest and peste des petits ruminants viruses (MARKVAC)*' and the project funded by the French Ministry of Foreign Affairs, '*Renforcement de cinq laboratoires de recherche vétérinaire en Afrique pour la surveillance et le contrôle de maladies animales*' for the work on capripox viruses.
Institute of Animal Health (IAH), Pirbright, UK	Partner in the Wellcome Trust supported research, '*Peste des Petits Ruminants (PPR) a serious and emerging plague of small ruminants in Developing countries: Epidemiology, transmission, host range and pathogenicity of the virus in West Africa'*. Collaboration also on the capripox research (provision of capripox viruses isolates).
Institut National de Médecine Vétérinaire (INMV), Algeria	Partner in the project supported by French Ministry of Foreign Affairs ''*Renforcement de cinq laboratoires de recherche vétérinaire en Afrique pour la surveillance et le contrôle de maladies animales*' for the work on capripox viruses.
Laboratoire National d'Appui au Développement Agricole (LANADA), Bingerville, Côte d'Ivoire	Partner in the Wellcome Trust supported research, '*Peste des Petits Ruminants (PPR) a serious and emerging plague of small ruminants in Developing countries : Epidemiology, transmission, host range and pathogenicity of the virus in West Africa*'

Laboratoire National Vétérinaire (LANAVET), Garoua, Cameroun	Partner in the project supported by French Ministry of Foreign Affairs ‘‘*Renforcement de cinq laboratoires de recherche vétérinaire en Afrique pour la surveillance et le contrôle de maladies animales*’ for the work on capripox viruses.
National Animal Research Health Center (NAHRC), Sebeta, Ethiopia	Partner in the project supported by by French Ministry of Foreign Affairs ‘‘*Renforcement de cinq laboratoires de recherche vétérinaire en Afrique pour la surveillance et le contrôle de maladies animal”* for the work on capripox viruses.
Pendik Veterinary Institute , Turkey	Collaboration with APHL on the work on Capripox and PPR viruses